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Frontiers in Dermatology and Venereology

- A series of theme issues in relation to the 100-year anniversary of ActaDV

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ACTA DERMATO-VENEREOLOGICA

The journal was founded in 1920 by Professor Johan Almkvist. Since 1969 ownership has been vested in the Society for Publication of Acta Dermato-Venereologica, a non-profit organization. Since 2006 the journal is published online, independently without a commercial publisher. (For further information please see the journal's website https://www.medicaljournals.se/acta)

ActaDV is a journal for clinical and experimental research in the field of dermatology and venereology and publishes high-quality papers in English dealing with new observations on basic dermatological and venereological research, as well as clinical investigations. Each volume also features a number of review articles in special areas, as well as Correspondence to the Editor to stimulate debate. New books are also reviewed. The journal has rapid publication times.

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Frontiers in Dermatology and Venereology

- A series of theme issues in relation to the 100-year anniversary of ActaDV

Series Editors:

Olle Larkö, MD, PhD, and Anders Vahlquist, MD, PhD

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This book – issued on the occasion of the 100-year anniversary of *Acta Dermato-Venereologica* (ActaDV) in 2020 – reiterates a recent publication about the history and current status of Acta DV together with 44 invited papers already appearing online in the centenary theme issues of ActaDV (https://www.medicaljournals.se/acta/content). Though the overall title of the theme issues, "Frontiers in Dermatology and Venereology" is ambitious, it goes without saying that the issues do not cover all aspects of this vast subject. The selection of themes largely reflects the scope of topics appearing in ActaDV over recent years, except psychodermatology which was recently covered in a supplement of ActaDV (https://www.medicaljournals.se/acta/content/issue/96-217). About the content list of this book, it should be noted that the order in which the theme issues appear is given by their dates of completion and online publication. The organization of the papers within a theme was however decided by the theme editors.

Incidentally, "Frontiers in Dermatology and Venereology" was also the name given to ActaDV's jubilee symposium, originally planned to be held on May 15, 2020, but later cancelled because of the corona pandemic. Faced with this force majeure, we were indeed pleased to know that the invited speakers also appear as co-authors of the centenary theme issues.

We wish to take this opportunity to sincerely thank all the invited authors (who kindly contributed for free), the Theme Editors (recruited among the editorial board), the peer reviewers of the papers (selected by the editors), and the staff of the editorial office of ActaDV. In particular, we wish to acknowledge the expert assistance of two staff members: Ms Anna-Maria Andersson, who speedily and skilfully managed the editorial processing of the theme issues, and Mrs Agneta Andersson, the Editorial Manager of our journal, who with great competence and enthusiasm helped to make the centenary project come true.

Gothenburg and Uppsala in June 2020

Olle Larkö
Editor-in-Chief of ActaDV

Anders Vahlquist
Chairman of the Society for Publication of ActaDV

ActaDV 100 Years – An Incomplete History and Current Status of the Journal

Anders Vahlquist
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The advance of medical science during the early 1900s inspired many clinical disciplines to issue new multi-lingual periodicals in Scandinavia. To highlight an international ambition, several of the journals were given Latin names, Acta Chirurgica, Acta Paediatrica, etcetera. Following this trend, Acta Dermato-Venereologica (ActaDV) was founded in 1920 by Professor Johan Almkvist at Karolinska Institutet. Below, important milestones in Acta DV´s development over 100 years are described together with fresh information about the journal´s performance and planned centenary activities.

Fig. 1. The changing face of Acta Dermato-Venereologica. *Left to right:* Year 1920, 1936, 1985, 1989, 2002, and 2019.

Examples of ActaDV's front pages during one hundred years are shown above. From the start in 1920, ActaDV was privately owned by Johan Almkvist (Fig. 2), who occupied the only Swedish Chair in Dermatology and Syphilidology at the time. The journal was initially trilingual (English, French, German) with appointed co-editors from Finland, the Netherlands, Norway, Sweden, Switzerland and the USA. For over a decade the journal content was dominated by syphilis and its skin manifestations, later outweighed by papers on more general topics of Dermatology and Venereology.

In 1936, Sven Hellerström, professor *in spe* at Karolinska Institutet, took over as editor (see Fig. 2). Except for an understandable decline during the war (volume 23 combines papers from both 1942 and 1943), the journal subsequently prospered with many contributions now coming from outside Europe. Hence English became compulsory for ActaDV.

After an astonishing 33 years as editor, Hellerström handed over to Nils Thyresson, the new professor at Karolinska in 1969 (see Fig. 2). Concurrently, ActaDV was donated to a newly started, non-profit Swedish society with a sole responsibility of publishing the journal. Together with his wife Inga-Lisa (as editorial secretary) and a board consisting of the chairpersons of Dermatology and Venereology in the Nordic countries, Nils Thyresson further developed ActaDV into an esteemed and steadily growing journal. On retirement, he moved the editorial office to his private home in Uppsala and continued as editor for another 6 years.

In 1988, Professor Lennart Juhlin of Uppsala University took over as editor and recruited Ms Agneta Andersson as editorial assistant & manager, a position she has now held for 32 years(!). Together they modernized the journal both in terms of lay-out (see Fig. 1) and handling of manuscripts, all in co-operation with Scandinavian University Press and later Taylor & Francis (TF) as publisher. In parallel, the ActaDV society started Forum for Nordic Dermatology and Venereology. By attracting more advertisements from industry, this journal could partially balance the falling revenues from subscriptions seen also for many other medical journals in the 1990s (this was largely due to economic constraints imposed on public libraries and an increase in digital reading).

In 1999, when I took over as editor of ActaDV, the economy was steadily problematic and our long-standing contract with TF was felt like a straight-jacket, financially as well as publicity-wise. Hence, in 2003 the Board decided that ActaDV should

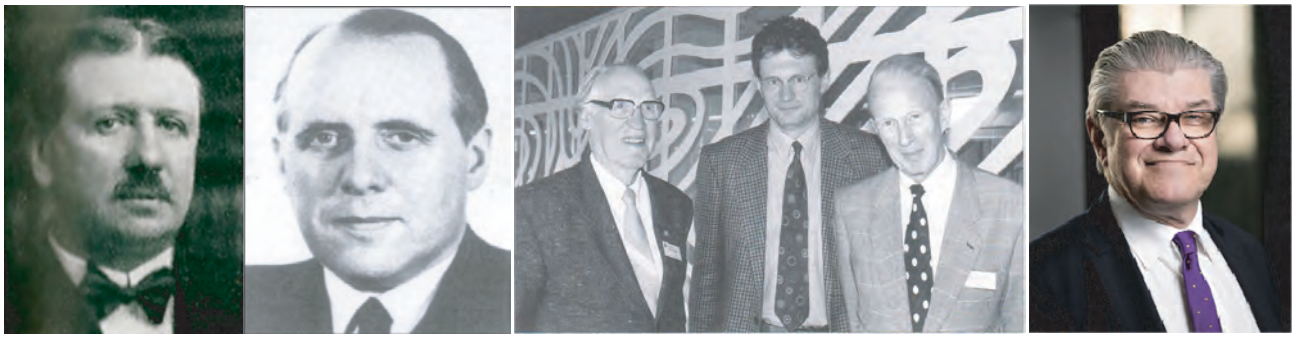

Fig. 2. . Six of the Editors over 100 years (from left to right: Johan Almkvist, Sven Hellerström, Nils Thyresson, Anders Vahlquist, Lennart Juhlin (jointly portraited in 2000) and Olle Larkö.

continue on its own, i.e. without a commercial publisher, and to move rapidly toward open-access (OA) and digital publication, requiring our economy to be based on publication fees rather than subscriptions. This major undertaking had not been possible without the superb help of Agneta Andersson, the ActaDV Board members, including Ove Bäck, Mona Ståhle, Inger Rosdahl, and the co-editors Torbjörn Egelrud and Artur Schmidtchen, and several other domestic as well as international colleagues.

These and other important moments in the history of ActaDV are summarized in Fig. 3.

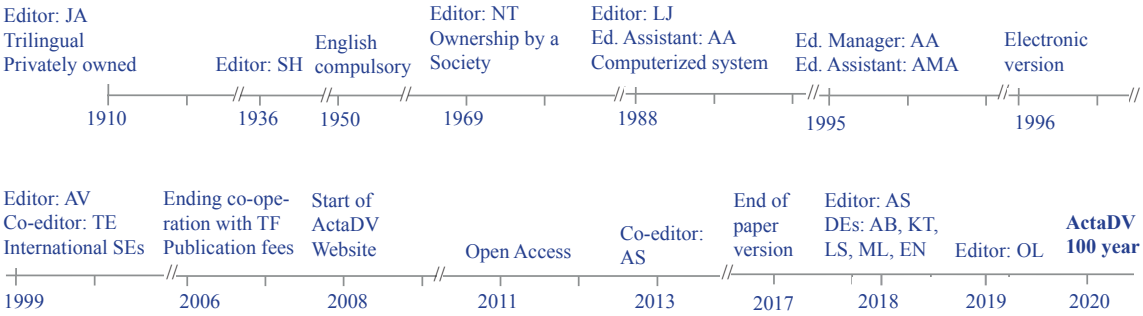

Fig. 3. Time-line of Acta DV´s development and significant mile stones. AA: Agneta Andersson; AB: Anette Bygum; AMA: Anna-Maria Andersson; AS: Artur Schmidtchen; AV: Anders Vahlquist; DE; Deputy Editors; EN: Elisabet Nylander; JA: Johan Almkvist; LJ: Lennart Juhlin; KT: Kaisa Taasanen; LS: Lone Skov; NT: Nils Thyresson; OL: Olle Larkö; SE: Section Editors; SH: Sven Hellerström; TE: Torbjörn Egelrud; TF: Taylor & Francis.

Fig. 4. The Editorial Office and two of its staff, Agneta Andersson (Editorial Manager) and Anna-Maria Andersson (Senior Editorial Assistant.).

Today, ActaDV is thriving as an on-line OA journal, produced entirely in-house by an editorial team (Fig. 4), which shares office and staff with Journal of Rehabilitation Medicine. The editorial board, now led by Professor Olle Larkö, Sahlgrenska Academy, Gothenburg (see Fig. 2), also consists of 4 Deputy Editors (3 women; 1 man), 17 Section Editors (8 women; 9 men) and an Advisory Board embracing distinguished colleagues from around the world.

For many years, the journal has also benefitted from a collaboration with two international societies, IFSI (International Forum for Study of Itch) and ESDaP (European Society for Dermatology and Psychiatry), which *inter alia* has added valuable scientific expertise to the peer-review process.

Presently, ActaDV publishes 250–300 papers per year with manuscripts submitted from all corners of the world, though

mostly from Europe and Asia. In addition, nearly 300 supplements have been published since 1920. The current acceptance rate is around 50% and the impact factor, which has markedly risen since 2000, is now in the order of 4.

On the occasion of ActaDV´s 100-year anniversary, a series of centenary issues will be published in volume 100 and a one-day symposium will be arranged in Stockholm, collectively called: "Frontiers in Dermatology and Venereology".

Hopefully this initiative will further spur the research interest in our specialty and contribute to a continued success of our journal.

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The Challenge of Basic Itch Research

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Basic mechanisms and pathways of itch signaling are reviewed, with an emphasis on the progress to date as well as remaining challenges in translating current knowledge to the clinical treatment of chronic itch. Recent studies reveal 3 subsets of pruriceptive sensory neurons highly expressing itch-related genes. Their fibers project into the spinal cord to activate neurons expressing gastrin releasing peptide (GRP) and its receptor (GRPR), which connect to neurons that express the substance P (NK-1) receptor and project to the parabrachial nucleus and thalamus. Spinal inhibitory interneurons release GABA, glycine and dynorphin to modulate segmental itch transmission. However, nearly all pruriceptive neurons also respond to algogens such as capsaicin. Alternative theories of itch-pain discrimination, such as intensity or spatial contrast, are based on the observation that focal stimulation of nociceptive nerve endings elicits itch while more widespread stimulation elicits pain. These findings cloud the issue of a labeled line for itch- a long-debated but currently unresolved challenge. In higher primates there is a dichotomy of histaminergic and non-histaminergic itch-signaling pathways which is less demarcated in rodents, suggesting species differences. A cardinal symptom of chronic itch is alloknesis, i.e., mechanical or touch-evoked itch. Recent evidence indicates that low-threshold mechanosensory afferents can access the spinal itch pathway, but are normally kept in check by inhibitory interneurons expressing neuropeptide Y (NPY). In chronic itch, NPY-mediated inhibition is reduced, allowing touch to excite itch-signaling pathways. These recent advances provide novel targets for development of therapeutic strategies to relieve chronic itch.

Key words: itch; pain; labeled-line coding; gastrin releasing peptide; alloknesis.

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SIGNIFICANCE

This paper reviews the basic mechanisms and pathways of itch signaling, emphasizing the progress to date as well as remaining challenges in translating current knowledge to the clinical treatment of chronic itch. Major questions that are addressed include: is itch signaled by a labeled-line pathway separate from that for pain; can alternative theories explain the ability to distinguish between itch and pain; are there specific markers of itch (such as gastrin releasing peptide and its receptor); are there histaminergic and non-histaminergic itch-signaling pathways? We also address challenges in understanding touch-evoked itch (alloknesis) as a symptom of chronic itch.

Like pain, acute itch provides a warning signal for the organism to scratch away insects or plant spicules from the skin surface or to dig out invasive parasites. However, chronic itch lasting >6 weeks does not serve a useful function but instead imposes suffering, high socioeconomic costs, and reduces the quality of life. It has been estimated that itchy skin conditions such as atopic dermatitis or psoriasis affect upwards of 10% or more of the general population with associated annual health care and economic costs in the billions of dollars (1–6). Most types of chronic itch are resistant to antihistamines, so there is a pressing need to develop novel drugs and other treatment strategies. This is one of the great challenges for translational itch research. Optimism is warranted based on recent research that has led to new effective treatments for chronic itch (7).

OVERVIEW OF ITCH PATHWAY

Huge strides have been made in the past decade in our understanding of how itch is transduced and transmitted from the periphery into the central nervous system. A schematic overview of itch processing is shown in **Fig. 1**. A wide variety of itch mediators interact with their cognate receptors that are expressed in the free nerve endings of pruriceptive afferents in the skin. Fig. 1 provides a partial list. Histamine is the most well-known itch mediator, acting at histamine H1 and H4 receptors linked to TRPV1, the heat- and capsaicin-sensitive ion channel (8, 9), which opens to depolarize the nerve ending and thereby activate voltage sensitive sodium channels (Nav 1.7, 1.8) to initiate action potentials in the afferent fiber. Many non-histaminergic itch mediators act via TRPA1 (10), and recent reports implicate TRPV4 in histamine, serotonin and chloroquine itch transduction (11–13). Single-cell RNA sequencing has been used recently to categorize 11 subpopulations of dorsal root ganglion

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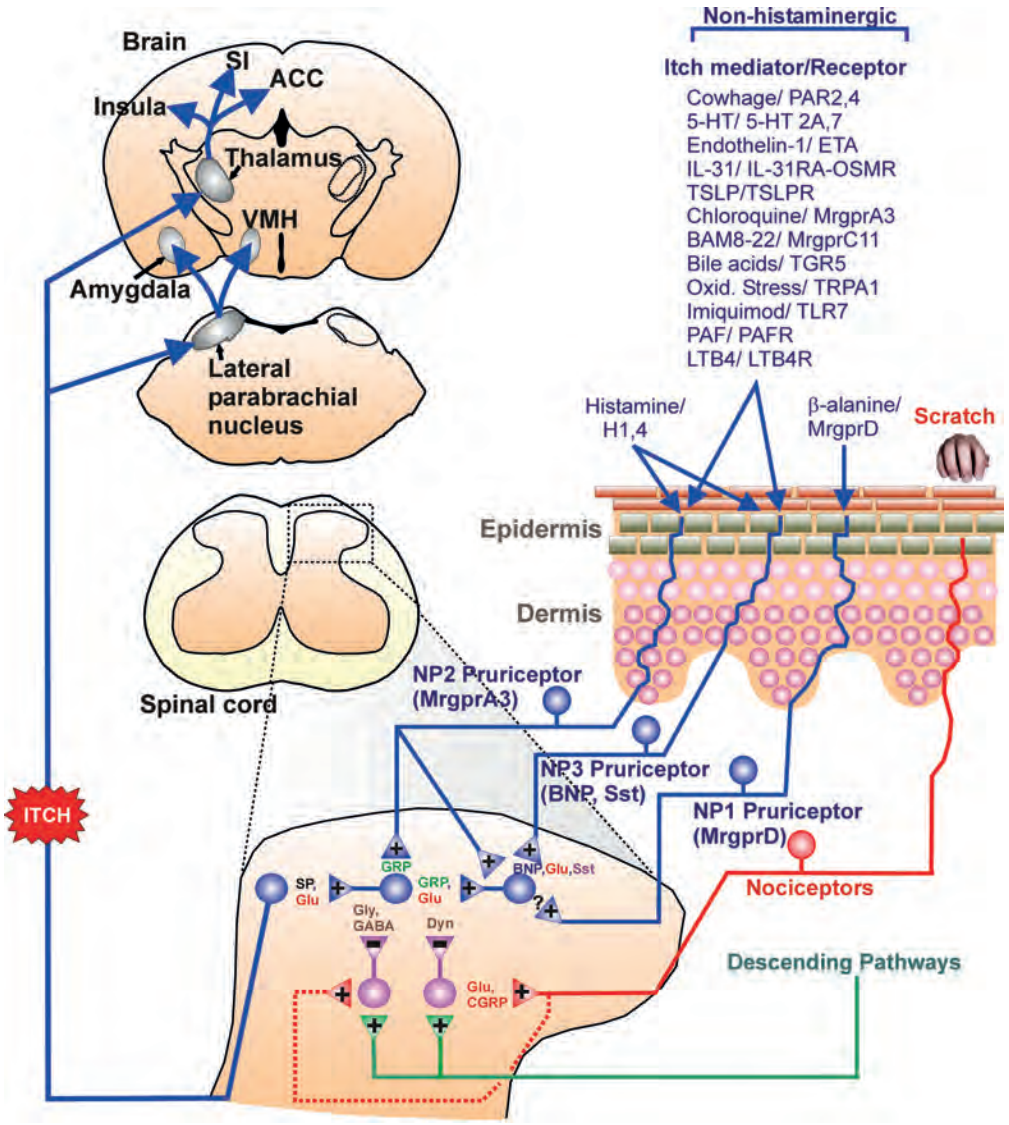

Fig. 1. Schematic of itch-signaling pathways. 5-HT: 5-hydroxytryptamine (serotonin); ACC: anterior cingulate cortex; BNP: brain natriuretic peptide; CGRP: calcitonin gene related peptide; Dyn: dynorphin; Glu: glutamate; Gly: glycine; GRP: gastrin releasing peptide; IL: interleukin; LTB4: leukotriene B4; Mrgpr: Mas-related G-protein coupled receptor; PAF: platelet activating factor; PAR: protease-activated receptor; SI: primary somatosensory cortex; SP: substance P; Sst: somatostatin; TLR: toll-like receptor; TLSP: thymic stromal lymphopoietin.

(DRG) cells, with 3 largely nonpeptidergic (NP) groups expressing genes associated with itch: NP1 (*MrgprD*), NP2 (*MrgprA3*), and NP3 (brain natriuretic peptide [BNP] and somatostatin) (14). *MrgprD*, *MrgprA3*, BNP and somatostatin have all been implicated in itch (15–18). In addition, neuroimmune interactions have been implicated in chronic itch. Recent studies have implicated IL-31 (19, 20), IL-4 and IL-13 (21) in itch and itch sensitization, leading to the development of biologics and antagonists that block activation of sensory neurons by cytokines (7). Clearly, improved understanding of the peripheral transduction of itch and immune function is already addressing the challenge of translating basic research into more effective treatments for chronic itch.

Pruriceptive afferent fibers transmit signals into the spinal cord dorsal horn, where they release neuropeptides including BNP (17), possibly gastrin releasing peptide (GRP) (22, 23), substance P (23), neuromedin B (24), and somatostatin (25) as well as the neurotransmitter glutamate (see below). The spinal circuitry includes excitatory interneurons that express GRP and substance P (26, 27), as well as itch-inhibitory interneurons expressing GABA, glycine and dynorphin (28–31) (Fig. 1). Projection neurons ultimately give rise to ascending itch-signaling pathways to the parabrachial nucleus and somatosensory thalamus. A high percentage of ascending projection neurons express the NK-1 receptor (32, 33). A majority of antidromically identified spinothalamic and spinoparabrachial projection neurons in rats respond to intradermal injection of pruritogens, with most also responding to the algogens capsaicin and mustard oil (34, 35). Using a double-label strategy, we observed similar

proportions of retrogradely labeled spinothalamic and spinoparabrachial neurons that co-express the activity marker, c-fos, following intradermal injection of histamine, chloroquine or capsaicin (36). Finally, the spinal itch-signaling circuitry is very likely under descending modulatory influences from the brainstem, although this has only begun to be experimentally addressed (37, 38).

IS THERE A LABELED LINE FOR ITCH?

On the one hand, there is evidence that spinal transmission involving the neuropeptide GRP provides a specific pathway for itch transmission (discussed further below). On the other hand, based on neural recordings from peripheral and second- or higher-order neurons in the spinal cord and brain, it is evident that neurons that respond to pruritogens invariably also respond to algogens such as capsaicin, mustard oil, and other noxious stimuli. Thus, there appear to be few if any itch-specific neurons, implying that itch must be distinguished from pain (and other dysesthetic sensory qualities) by some mechanism that can decode activity in non-selective neurons. A great challenge of basic itch research is to reconcile these seemingly disparate observations to understand how itch is conveyed to the brain and how it is discriminated from pain and other sensory qualities.

There is much evidence that activation of pruritogen-sensitive primary afferent fibers elicits a sensation of itch via a specific "labeled line" pathway. An older study using electrical stimulation at discrete sites on the skin surface reported that the intensity of the evoked itch increased as a function of increasing stimulus frequency but never transitioned to pain (39). A seminal observation was that mechanically insensitive C-fibers recorded by microneurography responded to cutaneous application of histamine, such that the action potential firing pattern closely paralleled the time course of concomitant itch sensation (40). More recent studies suggest that activation of specific primary afferents elicits itch, even though the afferents respond to algogenic as well as pruritogenic stimuli. For example, MrgprA3 is a Mas-related G-protein-coupled receptor expressed in sensory nerve endings that respond to the itchy antimalarial drug chloroquine (15). When TRPV1 (the capsaicin and heat-sensitive receptor) is genetically engineered into sensory neurons expressing MrgprA3 in otherwise TRPV1-null mice, capsaicin activation of these neurons elicits itch (scratching) rather than the pain behavior that is normally elicited by capsaicin (41). Moreover, MrgprA3-expressing sensory nerves responded not only to chloroquine but also capsaicin and other chemicals, indicating that they are not itch-specific (41). Optogenetic activation of MrgprA3-expressing peripheral afferents also elicited itch-related scratching behavior (27). These findings indicate that activation of MrgprA3-expressing nerve endings elicits itch, regardless of whether they are activated by pruritic, algogenic or artificial stimuli. This implies that although the MrgprA3-expressing afferents are not exclusively activated by itchy stimuli, they access circuits at higher levels of the nervous system that selectively signal itch but not pain.

This concept is also reflected in a "population coding" theory, which is similar to the selectivity theory (**Fig. 2**A). Using calcium imaging of DRG sensory neurons, we found that most if not all neurons that responded to itch mediators additionally responded to capsaicin and mustard oil (42, 43). The same was true for superficial dorsal horn neurons (44). Similarly, high percentages of neurons in the ventral posterior medial and posterior triangular thalamic nuclei responded to pruritogens as well as capsaicin (45). We postulated that pruritogen- and algogen-sensitive DRG and spinal dorsal horn neurons signal itch, whereby these non-selective spinal neurons access itch-specific central mechanisms (Fig. 2A). In contrast, pruritogen-insensitive but algogen-sensitive neurons signal pain. Note that this idea still embodies

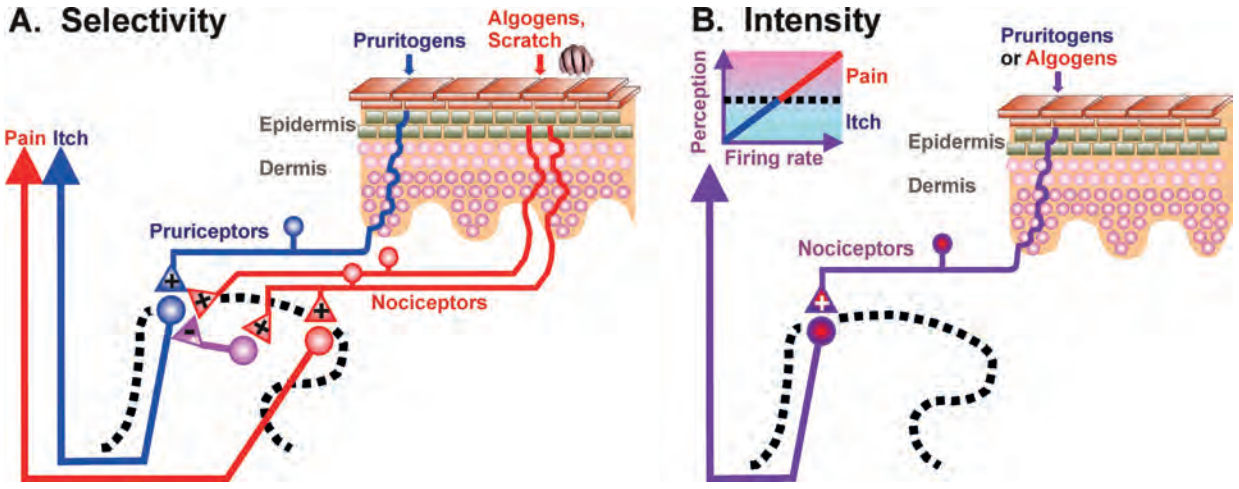

Fig. 2. Itch theories. A: selectivity (similar to population coding). B: intensity theory. See text for explanation. +: excitatory synapse; –: inhibitory synapse.

separate, central labeled line mechanisms for itch and pain. Pain-evoking stimuli would activate both populations of neurons, implying that itch and pain are elicited simultaneously. In this case, pain sensation dominates due to the ability of nociceptive spinal input to activate itch-inhibitory interneurons (Fig. 2A) (46), consistent with the selectivity theory of itch. Itch perception may also be masked by stronger pain.

IS GASTRIN RELEASING PEPTIDE AN ITCH-SPECIFIC MARKER?

A role for GRP in itch was first demonstrated by a significant reduction in pruritogen-evoked scratching, but not pain behavior, in transgenic mice lacking the GRP receptor (GRPR) (22). Neurotoxic destruction of GRPR-expressing spinal neurons also significantly attenuated pruritogen-evoked scratching but not pain behavior (47). These findings were recently corroborated by the report that chemogenetic activation of GRP-expressing dorsal horn neurons elicited behavioral signs of itch but not pain (48). These data strongly support GRP and GRPR expressed in spinal neurons as itch-specific markers.

However, another recent study reported that selective activation of GRP-expressing spinal neurons elicited behavioral signs of both itch and pain (27). The authors genetically engineered TRPV1 into GRP-expressing spinal neurons in otherwise TRPV1-null mice. Intrathecal administration of capsaicin dose-dependently elicited behavioral signs of itch (scratching) as well as pain (licking). At higher doses of capsaicin (1–20 μg), pain (but not itch) responses decreased and were rescued by administration of the μ-opioid antagonist naloxone. The authors suggested that high-dose capsaicin triggered an opioid mechanism that reduced pain but not itch, and that a common population of GRP-expressing spinal neurons signals both itch and pain. This is consistent with the intensity theory of itch (Fig. 2B), which postulates that itch is signaled by a lower firing rate and pain by a higher firing rate in a common population of spinal neurons. Indeed, capsaicin elicited much higher firing rates in GRP-expressing spinal neurons compared to those elicited by the pruritogens SLIGRL, chloroquine or histamine (27). In general, capsaicin and mustard oil elicited consistently higher firing rates compared to pruritogens in spinal dorsal horn neurons, including spinothalamic projection neurons (49). Thus, there is conflicting evidence as to whether GRP- and GRPR-expressing spinal neurons are itch-specific or signal both itch and pain, a challenge to theories of itch-pain discrimination that requires future studies to resolve.

Besides GRP, neuromedin B (24), brain natriuretic peptide (BNP) (17), glutamate (50) and substance P (51) have also been implicated in the spinal transmission of itch. We found that individual intrathecal delivery of receptor antagonists of GRP (RC-3095), substance P (L-733060) or the AMPA glutamate receptor (CNQX), partially reduced scratching behavior and spinal neuronal responses to chloroquine, while a combination of all 3 antagonists completely inhibited these responses (52). This is supported by another study reporting that of dorsal horn neurons responsive to intradermal histamine or chloroquine, some responded to intrathecal delivery of BNP or GRP, but less commonly to both (53). These findings suggest that there may be parallel spinal pathways for itch, each utilizing these neurotransmitters/neuropeptides to different extents. CNQX almost completely abolished scratching and neuronal responses to histamine, implicating glutamate as the primary spinal neurotransmitter in histaminergic itch (52). These studies provide points of intervention in the spinal cord to block the transmission of itch signals.

SPATIAL CONTRAST THEORY OF ITCH

A further complication to our understanding of itch mechanisms is the finding that a dominant sensation of itch, together with sub-dominant nociceptive sensations (burning, stinging, pricking), were elicited by insertion of either a single histamine-loaded, or capsaicin-loaded, or native cowhage spicule into the skin (54). This implies that highly localized activation of a minimal number of nociceptive nerve endings in the skin by either pruritogenic or algogenic chemicals is sufficient to elicit a dominant sensation of itch. This supports the "spatial contrast" theory of itch, which holds that limited activation of nociceptive nerve endings is itchy, while activation of a greater number of nerve endings over a broader area (e.g., by intradermal injection of capsaicin) is painful, possibly due to disruption of a specific pattern for itch via the activation of many nociceptors. This concept was suggested as a possible mechanism of neuropathic itch following nerve injury that results in degeneration of most but not all C-fibers, such that activation of the few spared fibers elicits a sensation of itch (55). The challenge remains to explain how either itch or pain results from localized patterns of activation of nociceptive C-fibers.

HISTAMINERGIC VS. NON-HISTAMINERGIC ITCH: SPECIES DIFFERENCES?

It is a dogma that there are two types of itch, histaminergic and non-histaminergic. In humans, histaminergic itch is mediated by the histamine-sensitive, mechanically insensitive C-fiber afferents mentioned above (40, 56). In contrast, non-histaminergic itch can be elicited by spicules of cowhage, which contain proteases (57, 58). Cowhage excites mechanically sensitive polymodal nociceptors (56, 59). The duality of histamine- and cowhage-sensitivity applies to non-human primates as well, since intradermal injections of histamine or placement of cowhage spicules activated largely separate po-

pulations of spinothalamic tract neurons (60). However, in rodents there appears to be greater overlap in primary and secondary sensory neurons responsive to histamine and non-histaminergic itch mediators. Using calcium imaging of mouse DRG and trigeminal ganglion (TG) cells, it was variously reported that 100% (15), 50% (61) or 17–23% (62) of chloroquine-responsive cells also responded to histamine. Using *in vivo* recording from identified MrgprA3-expressing DRG cells in mice, 78% (7/9) responded to both histamine and chloroquine (41). Recordings from mouse spinal dorsal horn neurons revealed that 47–71% of chloroquine-responsive neurons also responded to histamine (63). In rat somatosensory thalamus, all 7 chloroquine-responsive neurons that were additionally tested with histamine responded, although it is noted that a large number of histamine-responsive thalamic neurons did not respond to chloroquine (45). These data imply that histaminergic and non-histaminergic pathways may be more segregated in humans and non-human primates compared to rodents. A challenge for the field is to understand the limitations of rodent models for translation to human itch.

THE CHALLENGE OF CHRONIC ITCH AND ALLOKNESIS

Cardinal symptoms of chronic itch are ongoing ("spontaneous") itch, alloknesis (mechanical or touch-evoked itch), and hyperknesis (increased itch to a normally itchy or punctate mechanical stimulus). In healthy normal mice, lightly touching the skin does not elicit any behavioral signs of itch. However, following intradermal injection of histamine and other pruritogens, light touch elicits immediate scratch bouts – a model of alloknesis (64). Chemogenetic silencing of spinal neuropeptide Y (NPY) – expressing neurons led to increased touch-evoked scratching (65), and intrathecal delivery of NPY-1 receptor agonists reduced touch-evoked scratching (66), implying that the NPY-expressing neurons normally inhibit itch elicited by low-threshold mechanoreceptors. Scratching elicited by intradermal chloroquine, but not mechanical stimulation, was attenuated by antagonizing or ablating GRPR-expressing neurons, implying that mechanical itch is independent of, or converges downstream of GRP-GRPR signaling in the spinal itch circuit (**Fig. 3**). A reduction in the number of cutaneous Merkel cells and reduced expression of the mechanotransduction channel piezo2, as occurred in aged mice or under dry skin conditions, was associated with increased alloknesis, while chemogenetic activation of Merkel cells prevented alloknesis in dry skin (67). This suggests that Merkel cells connected to slowly adapting type I (SAI) afferents excite NPY-expressing interneurons to inhibit spinal itch transmission. It was very recently reported that activation of neurons expressing the NPY-1 receptor promotes mechanical itch (68). Mechanical itch was not affected following ablation of spinal neurons expressing the NK-1 receptor, implying that mechanical itch is transmitted via a pathway independent of that for chemical itch (Fig. 3). NPY-mediated inhibition can be overcome by mechanoreceptor activation of NPY-1 receptor-expressing neurons to drive the mechanical itch-signaling pathway under conditions in which Merkel cell-SAI input is reduced (Fig. 3).

A number of animal models have been developed to mimic various types of chronic itch and alloknesis, including atopic dermatitis, psoriasis and others (69, 70). Repeated topical application of ovalbumin induced an atopic dermatitis-like condition in mice, characterized by skin hyperplasia and lesions, increased IgG and Th2 cytokines, and importantly, increased spontaneous scratching behavior, alloknesis and hyperknesis (32). Alloknesis, but not hyperknesis or spontaneous scratching, was nearly abolished in OVA-treated mice that received intrathecal injection of substance P-saporin but not bombesin-saporin. This implies that the effect of low-threshold

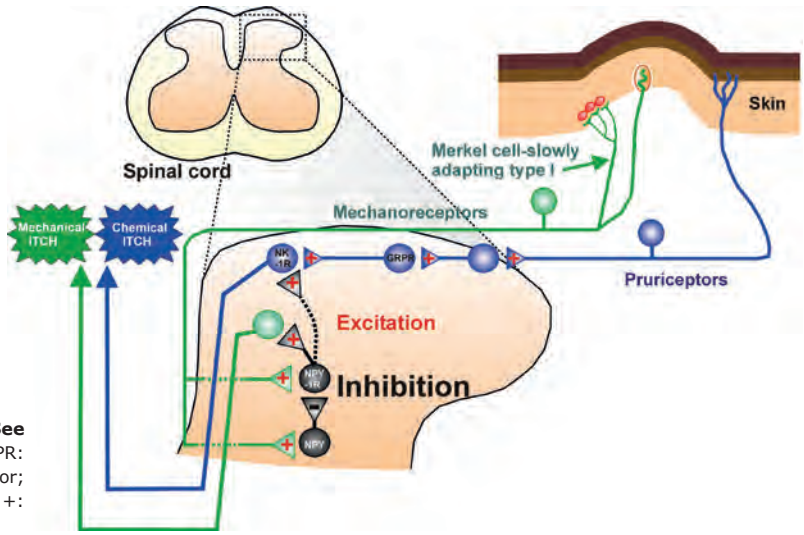

Fig. 3. Schematic of mechanical itch (alloknesis). See text for explanation. GRP: gastrin releasing peptide; GRPR: gastrin releasing peptide receptor; NK-1R: neurokinin-1 receptor; NPY: neuropeptide Y; NPY-1R: neuropeptide Y-1 receptor; +: excitatory synapse; –: inhibitory synapse.

mechanoreceptor input to inhibit itch signaling neurons occurred downstream of GRPR-expressing neurons but requires NK-1 receptor-expressing neurons, supporting the idea that mechanoreceptive input converges onto the chemical itch-signaling pathway (Fig. 3; dashed line connecting NPY-1R to NK-1R). Consistent with this, intrathecal NPY agonists suppressed both chemically- and mechanically-evoked itch behavior (66).

Given that alloknesis is quite bothersome to patients suffering from many types of chronic itch, a challenge to the field is to better understand how low-threshold mechanosensory input interacts with spinal itch-signaling pathways and potential anti-alloknesis interventions targeting spinal NPY1 receptors.

CONCLUSIONS

The preceding text has identified a number of challenges arising from basic itch research to explain how itch can be discriminated from pain, and to translate our increasing knowledge of itch signaling into clinical treatment. Given the remarkable progress of the past decade and the current strong interest in itch research, several novel approaches to the treatment of chronic itch are already being used and more can be expected in the near future. Nevertheless, it has been debated for more than 100 years whether itch and pain are signaled by separate labeled-line pathways or by a common population of non-specific neurons. This debate continues unresolved up to the present, with arguments favoring both concepts.

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Centenary theme section: ITCH AND PRURITIC DISORDERS

REVIEW ARTICLE

Mechanisms and Management of Itch in Dry Skin

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Chronic itch is a burdensome clinical problem that often accompanies pathological dry skin-based conditions, such as atopic dermatitis, and systemic disorders, such as kidney diseases, with an unclear pathomechanism and treatments. One of the basic mouse models to investigate mechanisms of itch associated with dry skin is a mixture of acetone and ether followed by water. Animal studies using the acetone and ether followed by water model have revealed that many mediators and receptors, e.g. mas-related G protein-coupled receptor family, transient receptor potential, and chemokines, are responsible for itch and its hypersensitivity, supporting the hypothesis that dry skin-induced itch is a histamine-independent pathway. New insights have been acquired into the interplay between neurones and non-neuronal cells in the initiation, modulation, and sensitization of itch. Several therapeutic options for itching have thus been developed. This review summarizes the updated pathogenesis and therapeutic strategies for itch in dry skin conditions.

Key words: dry skin; hypersensitivity; itch; sensory neuron; mouse model.

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SIGNIFICANCE

Itch is an unpleasant sensation that may disturb quality of life, and for which the pathomechanism and appropriate treatments are unclear. Chronic itch, which lasts more than 6 weeks, often accompanies pathological dry skin-based conditions, such as xerosis, atopic dermatitis, liver and kidney diseases. A decline in skin barrier function is thought to be the primary cause of itch induced by dry skin. Many kinds of mediators, receptors, and channels are involved in itch signalling among the skin nervous system, skin cells, and central nervous system. Several therapeutic options for itching have thus been developed, such as phototherapy, phospholipids, antioxidants, and emollients.

Skin, the body's largest organ, serves as a first physiological barrier against the external environment. The barrier function of the skin is exerted by the epidermis, the most superficial layer of the skin, of which the stratum corneum (SC) is largely responsible for the barrier function. There are 2 elements important for the maintenance of SC humidity: intercellular lipids, which form the main barrier against diffusion of water across the SC, and natural moisturizing factor, which has a key role in the absorption of water in the SC. Impaired skin barrier integrity causes excessive water loss and leads to skin dryness (1, 2).

DRY SKIN

Dry skin is characterized by a scaly, rough, cracked, and fissured surface, and is closely associated with the somatosensory sensation of itch, especially chronic itch (3). Dry skin with chronic itch is the most common clinical manifestation of dermatoses, such as xerosis, atopic dermatitis (AD), and psoriasis, and is a common cutaneous manifestation in pruritic systemic diseases, such as chronic kidney disease (CKD), chronic liver diseases (CLD), and diabetes mellitus (DM) (4).

Histamine is a well-known substance that induces itch; however, antihistamines (histamine H_1-receptor blockers) are not fully effective in many dermatological and systemic diseases characterized by dry skin, suggesting that dry skin is an important feature of antihistamine-resistant (histamine-independent) itch (2). The underlying condition of dry skin is impaired function of the skin barrier, which can be caused by environmental factors, such as sun exposure, temperature, humidity, and genetic factors, such as filaggrin mutations (1, 5, 6). To assess skin barrier function, transepidermal water loss (TEWL), SC hydration, and pH are commonly used (1). The signs and clinical manifestations of dry skin are not only physically uncomfortable, but also affect patients psychologically (7).

Disease-related dry skin

Aged skin. Xerosis is one of the most prevalent dry skin conditions in the aged population worldwide (8), affecting over 50% of individuals aged ≥65 years (9). Multiple skin changes in the elderly are related to xerosis: (*i*) alterations in the barrier function of SC, including cellular and intercellular lipid matrix changes; (*ii*) pH variations; (*iii*) alterations in SC proteases; (*iv*) reduced activity of sebaceous and sweat glands; and (*v*) decreased oestrogen levels. All of these factors may lead to itch induction (10).

Inflammatory skin diseases. Dry skin itch is a common symptom in dermatoses characterized by dysfunction of the skin barrier, such as AD and psoriasis. In these diseases, pruritogens, such as cytokines and chemical mediators, are released from the affected area (5, 11). Pruritogens induce itch mainly by acting on the sensory nerves, and the affected area is scratched, then further aggravates dermatitis (12). This vicious cycle is called the "itch-scratch cycle". Skin hyperesthesia (a skin condition that involves an abnormal increase in sensitivity to stimuli) occurs in inflammation, such as AD (5). Elongation of the sensory nerve in the epidermis to immediately underneath the SC, due to drying and inflammation, is considered to be a cause of skin hyperesthesia. Nerve growth factor (NGF), amphiregulin (AR), and artemin (ARTN), which are nerve elongation factors (NEFs), and semaphorin 3A (Sema3A, a nerve repulsion factor (NRF)), are related to this aberrant nerve elongation and sprouting in AD (13).

More recently, Pogatzki-Zahn et al. (14) reported skin hyperesthesia in patients with chronic pruritus, such as AD, but it was not related to hyperinnervation in the epidermis, observed as a decreased number of cutaneous nerves crossing the basement membrane. The authors speculated that, although the nerves crossing the basement membrane were reduced, increased intraepidermal sprouting of nerves is possible. Another possibility is that the density, structure, and functional properties of intraepidermal nerves fluctuate in different skin disease states, especially in acute and chronic phases.

Systemic diseases. Dry skin is also a common cutaneous manifestation in pruritic internal diseases, such as CKD, CLD, and DM (4). Skin dryness may appear at different stages of CKD, but it is more frequently diagnosed in dialysis subjects (45%) (15). The functional abnormalities of eccrine sweat glands may account, at least in part, for dry skin in uraemic patients (16). It has been suggested that dry skin can cause itch in CKD; however, objective measurements of the barrier function of the skin, such as the degree of hydration of the skin, lipid bilayer abnormalities, and dryness of the skin, do not always correlate with pruritus (17).

The pathogenesis of pruritus in CLD is poorly understood and often refractory to treatment, with a prevalence of 40.3% (18). Several potential itch-causing substances may be involved, including bile salts, endogenous opioids, histamine, serotonin, and steroids (19). We reported recently that the plasma dynorphin A level of endogenous opioids correlates with the severity of pruritus and may reflect its degree in patients with CLD (20).

Skin disorders are common complications and comprise a broad spectrum of disorders in both type 1 and type 2 DM, e.g. cutaneous infection, dry skin, and pruritus (21). Clinical observations are supported by a reduced hydration of the SC and reduced sebaceous gland activity in patients with DM. Even in the absence of clinically apparent dry skin, patients with diabetes have an impaired desquamation process (22). Pruritus is more common in patients with diabetes who have dry skin or diabetic neuropathy (21). Higher postprandial glucose levels were reported to result in a higher probability of having generalized pruritus (23).

Dry skin mouse models

Acetone-treated model (acute dry skin model with no itch). One mouse model to induce dry skin uses acetone application. The hair of mice is shaved over the rostral part of the back at least 3 days before acetone treatment. The shaved area was treated with acetone-soaked cotton balls for 5 min. In the control group, the shaved area was treated with sterile water (3, 24).

Analyses of experimental animals treated with acetone demonstrated that intraepidermal innervation-related factors, such as *NGF* and *ARTN* gene expression, were increased in the epidermis, and the artificial restoration of the barrier immediately following barrier disruption by acetone treatment inhibited the increase in these mRNA levels (3, 24). Others observed the release of histamine from mast cells in the skin of acetone-treated mice (25). We found that acetone-treated mice displayed a rapid increase in TEWL and a decrease in SC hydration during the first hour after treatment, which returned to normal by 48 h after the treatment. Thus, the acetone-treated mice manifest the characteristics of dry skin and have altered cutaneous barrier permeability. No scratching behaviours or epidermal hyperplasia were observed in the acetone-treated mice, although there was an increase in nerve fibre density in the epidermis (**Fig. 1**A). Of note, we found that the expression of epidermal NGF and AR (which promote nerve growth) was increased (3), but Sema3A (which inhibits nerve growth) expression was decreased (Tominaga et al., unpublished data) before the penetration of nerve fibres into the epidermis (Fig. 1B and C). The increase in intraepidermal nerve fibres may be an important factor for the regulation of itch in dry skin (3).

Acetone/ether/water (AEW)-treated model (chronic dry skin model with itch). The AEW-treated mouse model is one of the most well-known mouse models for the study of dry skin-induced itch (26). The hair of mice was shaved over the rostral part of the back at least 3 days before the start of the experiment. To disrupt the cutaneous barrier, cotton (2 × 2 cm) soaked in a mixture of acetone and ether (1:1) was placed on the shaved area for 15 s. Immediately after AE treatment, cotton soaked with distilled water was placed on the same area for 30 s. Treatments were performed twice daily under ether anaesthesia for 5–7 consecutive days. TEWL and scratching behaviour were increased, and SC hydration was decreased, under this treatment. The histopathological analysis showed that the AEW-treated mice had marked epidermal hyperplasia, parakeratosis, and infiltration of nerve fibres into the epidermis, but no infiltration of inflammatory cells in the dermis (26, 27). Overall, the AEW treatment produces

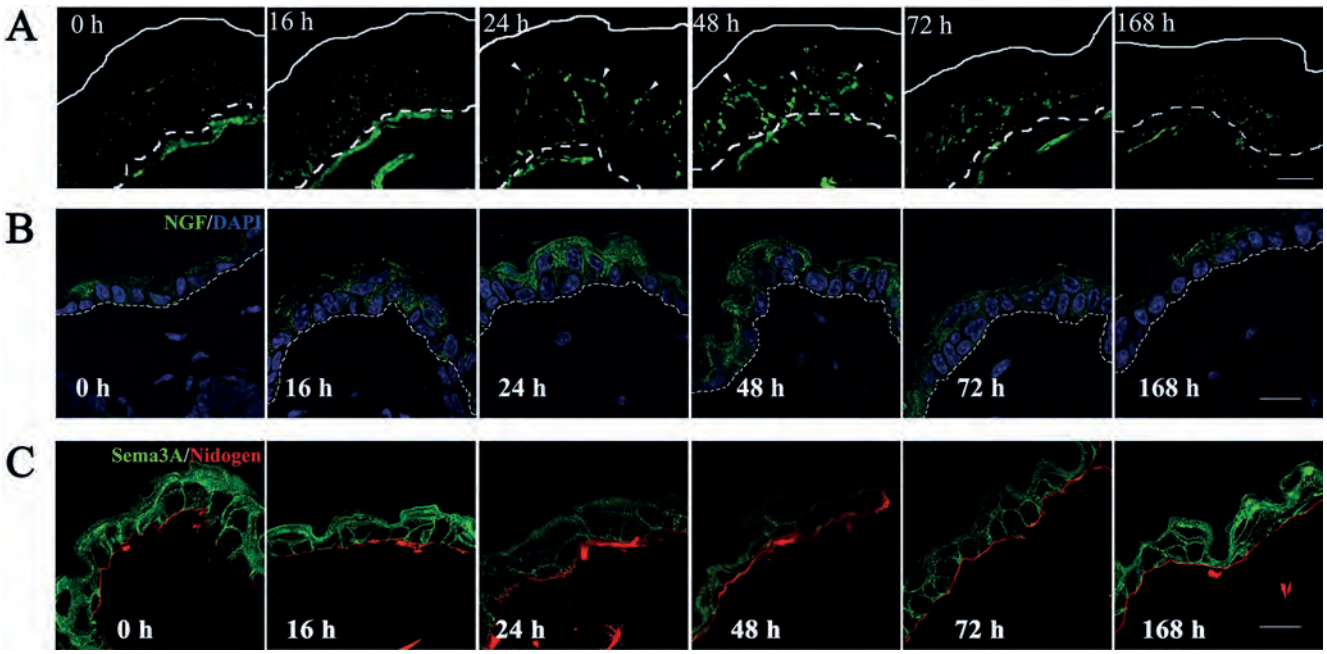

Fig. 1. Alterations in nerve fibre distribution, and nerve growth factor (NGF) and Sema3A expression in the epidermis of acetone-treated dry skin model mice. (A) Sequential alteration of intraepidermal nerve growth in acetone-treated mice was examined by immunohistochemistry using an anti-PGP9.5 antibody. (B, C) Maximum expression of NGF (*green*) was noted 16–24 h after the treatment (B). In contrast, the expression level of Sema3A (*green*) was decreased 24 h after acetone treatment (C). These expression levels gradually returned to normal by 168 h after the treatment. Nuclei are counterstained by DAPI (*blue*). The *broken lines* in panel A indicate the border between the epidermis and dermis (basement membrane). The basement membrane in panel B was stained with an anti-nidogen antibody (*red*). *White* and *broken lines* indicate the skin surface and the border epidermis and dermis (basement membrane), respectively. *Arrowheads* indicate epidermal nerve fibres (green). Scale bars: 15 μm.

marked skin barrier dysfunction, robust scratching, and changes in gene expression in sensory nerves and the skin (26), which recapitulate the dry skin symptoms present in many chronic itchy conditions in humans (28). There was no apparent difference in AEW-induced spontaneous scratching between mast cell-deficient mice (WBB6F1-W/W^{v}) and normal litter-mates (WBB6F1-$^{+/+}$) (26).

This dry skin model also exhibits alloknesis (scratching behaviour evoked by a stimulus that is normally non-pruriceptive) and hyperknesis (the abnormal pruriceptive state, in which a normally pruritic stimulus elicits a greater than normal duration and/or magnitude of itch) (27, 29), as described later in this review.

Special diet food model. HR-1 hairless mice fed a special diet (HR-AD) is one of the dry skin-based experimental mouse models. Mice were fed HR-AD for 48 days. These mice exhibited severe dry skin symptoms accompanied by a decrease in skin water-holding capacity, increase in TEWL, and prolonged scratching bout duration. Marked epidermal hyperplasia, and increase in circulating T cells and serum IgE are observed (30). Lipid composition analysis revealed that HR-AD is an essential fatty acid (EFA)-deficient diet. Feeding HR-AD with EFA inhibits the symptoms of dry skin (31). EFA deficiency was reported to depress skin barrier function due to structural changes in ceramides, and reduced elaboration and deposition of epidermal intercellular lipids (32). Therefore, HR-AD causes deterioration of the skin barrier function due to EFA deficiency.

MECHANISMS OF ITCH IN DRY SKIN

The sensation of itch is generated by the binding of itch-inducing substances to their cognate receptors on peripheral sensory afferents, e.g. unmyelinated C-fibre afferents and thinly myelinated Aδ-fibre afferents. The evoked action potential is transmitted through the ascending sensory pathway to the somatosensory cortex, resulting in the perception of itch (**Fig. 2**).

Sensory neurones

Nerve elongation and repulsion factors. In healthy skin, most cutaneous nerve fibres terminate under dermoepidermal junctions. An increased intraepidermal nerve density has been observed in the skin of patients with pruritic dermatological diseases, such as senile xerosis and AD (13), as well as in dry skin mice models (3, 33). The controlling mechanism of cutaneous nerve density is regulated by the balance of NEFs, such as NGF, ARTN and AR, and NRFs, such as Sema3A, produced by keratinocytes (5, 13). These axonal guidance molecules may also act on keratinocytes, immune cells, and vascular endothelial cells, and may be indirectly involved in the regulation of itching (34).

Matrix metalloproteinase (MMP)-2 and MMP-8. The process of cutaneous nerve growth in dry skin requires several MMPs for growth cones to penetrate the 3-dimensional extracellular matrix (ECM) barriers (Fig. 2). Using *in vitro* models of ECM, we found that MMP-2

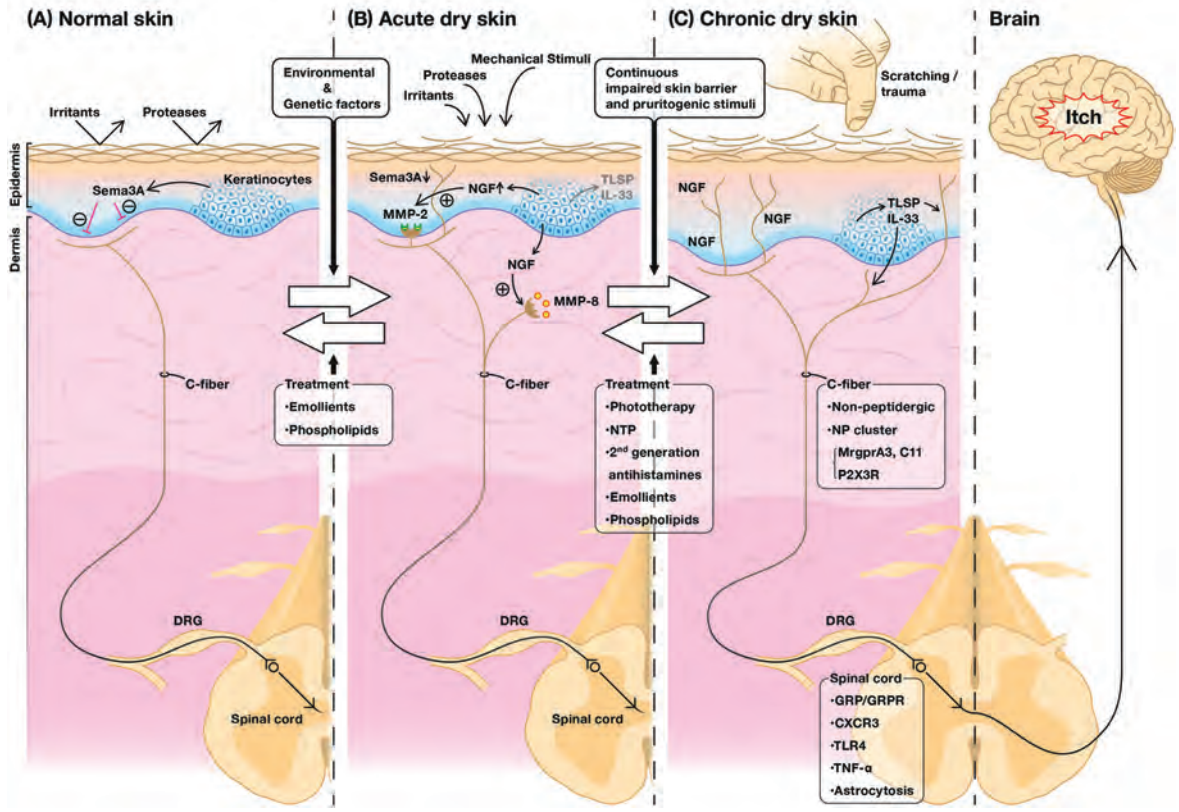

Fig. 2. Mechanisms and management of dry skin-induced itch. The perception of itch starts when endogenous and exogenous itch mediators activate their respective receptors/channels expressed in peripheral sensory afferents. Electric signals generated in the peripheral nerve endings are transmitted to the somatosensory cortex in the brain through the spinal cord, resulting in the recognition of itch. (A) In healthy skin, Sema3A, a nerve repulsion factor (NRF) produced mainly by keratinocytes (KCs), is dominant. It maintains the cutaneous nerve fibres under the dermo-epidermal junction. (B) During environmental stimuli in acute dry skin conditions, nerve growth factor (NGF), an epidermal nerve elongation factor (NEF) produced by KCs, is prominent and induces the elongation of cutaneous nerve fibres into the epidermis. This elongation may also be affected by thymic stromal lymphopoietin (TSLP) and interleukin (IL)-33 released from KCs. NGF also promotes matrix metalloproteinase (MMP)-2 and MMP-8 production in sensory nerve fibres, which leads to the penetration of nerve fibres into the basement membrane and their growth. Emollients and phospholipids are effective at alleviating the symptoms in this phase. (C) In chronic dry skin accompanying the itch-scratch cycle, such as in systemic or inflammatory skin diseases, more sensory nerve fibres penetrate the epidermis. In addition to substances released from KCs, the non-peptidergic C-fibres (NP cluster) are also involved in itch signalling, along with astrocytosis in the spinal cord. More treatments have been confirmed to be beneficial in this condition.

localized on the growth cone functioned in penetration into the basement membrane (35). In addition, MMP-8 secreted by nerve fibres was reported to be involved in nerve growth within the dermis (36). The levels of expression of MMP-2 and MMP-8 were upregulated by NGF and down-regulated by Sema3A. The selection and up-regulation of MMPs corresponding to the ECM components surrounding the growing nerve fibres may be required for efficient nerve fibre penetration, suggesting that the coordinated activation of neurotrophin and ECM-integrin signalling is necessary for efficient and long-distance axon extension (37). As class 3 semaphorin signalling inhibits integrin-mediated adhesion signalling, Sema3A stimulation of growing nerve fibres may provide a reverse signalling pathway for these events (38).

Peptidergic fibres. Substance P (SP) and calcitonin gene-related peptide (CGRP) are neuropeptides produced by sensory nerves in the dermis to communicate with different cell populations in the different layers of the skin, which in turn stimulate nerve fibres. An increase in the elongated epidermal peripheral nerve fibres consist of SP/CGRP-containing C-fibres, which usually represent epidermal peptidergic nerve fibres, has been reported in the AEW model (39, 40).

The neuropeptide gastrin-releasing peptide (GRP) is characterized as a neurotransmitter that specifically relays itch signals and specifically expressed in a small subset of peptidergic dorsal root ganglion (DRG) neurones. Genetic ablation of GRP receptor $(GRPR)^{+}$ neurones resulted in significant reduction in the scratching response to multiple pruritogens (41). The transcription factor Tlx3 in the spinal cord was demonstrated to be essential for the development of $GRPR^{+}$ neurones (42). Huang et al. (43) reported that Tlx3 conditional knockout (*Tlx3*$^{F/F}$; *Nav1.8-Cre* mice specifically lost Tlx3 expression in most TrkA-lineage DRG neurones) mice scratched much less compared with controls in the dry skin model, suggesting impairment of dry skin-induced chronic itch in these mice.

Non-peptidergic fibers. C-fibres have been divided into peptidergic and non-peptidergic subsets mainly on the basis of neurochemical criteria. The peptidergic neurones are

mostly marked by neuropeptides, including SP and CGRP, whereas non-peptidergic neurones are commonly labelled by the purinergic P2X3 receptor and the plant lectin isolectin B4 (IB4) (44). On the contrary to previous reports of SP and CGRP involvement in the AEW model, a recent study reported that AEW treatment increased non-peptidergic intraepidermal fibres, but not $CGRP^+$ fibres, suggesting that a specific subset of non-peptidergic fibres function in dry skin itch (45), as is observed in itch behaviour of the imiquimod-induced psoriasis mouse model (46).

Protease-activated receptors (PARs). PARs consist of 4 members: PAR-1, PAR-2, PAR-3, and PAR-4. PARs other than PAR-3 are expressed in cutaneous nerve fibres, keratinocytes, mast cells, and macrophages, and are considered involved in itch (27, 47). Spontaneous scratching behaviour in dry skin-treated animals was significantly attenuated by a PAR-2 antibody either delivered locally to the dry skin area or systemically. In addition, DRG cells from AEW-treated mice exhibited significantly larger responses to the PAR-2 agonist, implicating a role for endogenous agonists of this receptor in chronic itch (27).

Mas-related G protein-coupled receptor family (Mrgpr). The Mrgpr family in mice can be grouped into several subfamilies: MrgprA, MrgprB, MrgprC, and MrgprD-G (48). MrgprA3, MrgprC11, and MrgprD in mice, which are expressed only on small-diameter sensory neurones in the DRG and trigeminal ganglia (TG), and were recently suggested to be involved in the transmission of itch (49–51). The expression of mRNAs encoding MrgprA3 and MrgprC11 was found to be higher in AEW-treated dry skin model mice than in water-treated controls (52). Moreover, the ablation of $MrgprA3^+$ DRG neurones reduced chronic itch induced by AEW treatment, suggesting that MrgprA3 functions in dry skin-related itch (53). The increases in expression of MrgprA3 and MrgprC11 were inhibited in acid-sensing ion channel 3 (ASIC3) knockout mice (52), suggesting that fluctuations in skin pH are involved in dry skin-related itch.

Transient receptor potential family (TRP). The TRP channels are known as polymodal cellular sensors. The ion channel TRP subfamily A member 1 (TRPA1) was previously reported to mediate acute histamine-independent itch, e.g. sensory neurone activation and itch behaviour downstream of 2 histamine-independent pruritogens, chloroquine and BAM8-22 (49, 54). Wilson et al. (55) found that functional TRPA1 is required for the dry-skin-evoked phenotypes, including AEW-evoked scratching, epidermal hyperplasia, and expressional changes in the skin. Among the human disease genes, TRPA1 regulates both scratch-dependent and scratch-independent changes: AQP3, IL-33, chemokine receptor CXCR2, lipocalin, Slc9a3r1, and S100A9 require TRPA1, and are independent of the itch-scratch cycle, whereas CCL27 and Tenascin C (TNC) are scratch- and TRPA1-dependent. These genes play diverse roles in the initiation and maintenance of chronic itch (55).

TRP cation channel subfamily V member 1 (TRPV1) is a heat-sensitive cation channel that is selectively expressed in a population of primary sensory neurones in TG and DRG, which plays an important role in thermal and pain sensations (56). Yu et al. (28) reported an increased innervation density of TRPV1-expressing sensory fibres in the skin of AEW model mice due to expansion of this channel. This may also be partly involved in the induction and/or enhancement of itch in dry skin.

NP clusters of sensory neurones. Usoskin et al. (57) reported 4 neuronal clusters (further divided into 11 fundamentally distinct types of sensory neurones) in single cells of sensory neurones from mouse lumbar DRGs. The first cluster is the NF cluster (including NF1-5), which expresses neurofilament heavy chain (*Nefh*) and parvalbumin (*Pvalb*), and was previously associated with myelinated DRG neurones. The second, the PEP cluster (including PEP1-2), expressed SP (*Tac1*), TRKA (*Ntrk1*) and CGRP, which were previously associated with peptidergic nociceptors. The third, the NP cluster (including NP1-3), expressed *Mrgprd* and *P2rx3*, which were previously associated with non-peptidergic nociceptors. The fourth, the TH cluster, exhibited distinct expression of tyrosine hydroxylase (*Th*) and has been described in a distinct subclass of unmyelinated neurones. Furthermore, NP1, NP2, and NP3 neuronal types were reported to function in itch, and NP3 is likely to sense and transduce inflammatory itch. They detected lysophosphatidic acid–responsive neurones (*Lpar3* and *Lpar5*) in the NP1 class, chloroquine-responsive neurones (*Mrgpra3* and *Mrgprx1*) in NP2, and interleukin (IL)-31 (*Il31ra* and *Osmr*)- and cysteine leukotriene (*Cysltr2*)-responsive neurones, neuropeptides natriuretic peptide, neurotensin, and somatostatin (*Nppb, Nts*, and *Sst*) markers, and a low level of P2X3 in NP3. Histamine receptors (*Hth1*) were found in NP2 and NP3, and serotonin receptors (*Htr1f*, *Htr2a*) were found in NP3 and PEP2. Thus, their data support the existence of at least 3 classes of itch responsive neurones with unique response profiles: lysophosphatidic acid associated with cholestatic disorders may be tuned to NP1 neurones, chloroquine and histamine associated with acute itch may be tuned to NP2 neurones, and mediators, such as IL-31 and cysteine leukotrienes, which are linked to chronic states of inflammatory itch, as well as histamine and serotonin, may engage NP3 neurones (57). These new types and classification of neurones may be closely related to the pathomechanism of dry skin-induced itch.

Keratinocytes

Transient receptor potential cation channel subfamily V member 4 (TRPV4) was reported to be involved in acute itch elicited by exogenously applied histamine and 5-hydroxytryptamine (5-HT) (58, 59). Luo et al. (60) revealed that TRPV4 is selectively expressed by epi-

dermal keratinocytes in mice. Lineage-specific deletion of TRPV4 in keratinocytes reduced itch in AEW-treated mice. Moreover, TRPV4-dependent chronic itch requires 5-HT signalling secondary to activation of distinct 5-HT receptors in AEW as downstream signalling.

AD and allergic contact dermatitis (ACD) are cutaneous diseases characterized by dry skin and chronic itch. Previously, we demonstrated that, possibly through PAR-2 activation in keratinocytes, the cytokine thymic stromal lymphopoietin (TSLP) produced by keratinocytes plays an important role in the development of AD (61). Of note, it was further reported that keratinocytes communicate directly with cutaneous sensory neurones via TSLP to promote itch. Wilson et al. identified the ORAI1/NFAT calcium signalling pathway as an essential regulator of TSLP release from keratinocytes, and TSLP acts directly on a subset of TRPA1-positive sensory neurones to trigger robust itch behaviours (62).

Liu et al. (63) also reported that IL-33 produced and released by keratinocytes is a key cytokine up-regulated in the skin of urushiol-challenged ACD model mice. In this study, IL-33 and its receptor ST2 (expressed in DRG neurones, which innervate the skin) were functionally present in primary sensory neurones and found to lead to pruritus in this model. Studies revealed that hypo-osmotic stress to keratinocytes, such as that noted in AD, and trauma to the skin, such as tape-stripping, promote IL-33 production from keratinocytes (64, 65). Considering the close relationship between itchy-dry skin and AD or ACD, it is highly possible that TSLP and IL-33 produced by keratinocytes play an essential role in the mechanism of dry skin itself.

Spinal cord

Gastrin-releasing peptide system. The GRP and its receptor (GRPR), a $G_{\alpha q}$-protein-coupled receptor (GPCR), were reported as itch-specific signalling molecules and expressed in the spinal cord. Intrathecal GRP acts via GRPR to induce scratching behaviour (41). PI3Kγ, a member of lipid kinases that participate in the intracellular signalling cascade, is activated downstream of GPCRs and is related to itch. In a dry skin model of itch, GRPR blockade or PI3Kγ inhibition by intrathecal or systemic route, attenuated the scratching behaviour, suggesting that GRPR is expressed by the central terminals of DRG nociceptive afferents, which transmit itch via the PI3Kγ pathway. These data suggest that the spinal GRP/GRPR system is partly involved in the induction of itch in dry skin (66).

Chemokines. Chemokines are expressed in the central nervous system, where they regulate its function under both physiological and pathological conditions, including neuronal development, synaptic transmission, and disease-associated neuroinflammation (67). Qu et al. (68) reported that C-X-C motif chemokine ligand 10 (CXCL10) and C-X-C motif chemokine receptor 3 (CXCR3) are increased in the DRG in an ACD model, and CXCL10 directly activates a subset of cutaneous DRG neurones through neuronal CXCR3. Of note, AEW treatment induced the expression of CXCR3 and CXCL10 in the spinal cord, and $CXCR3^{-/-}$ mice had fewer scratching responses than control mice. In addition, AEW-induced astrocyte activation was reduced in $CXCR3^{-/-}$ mice, suggesting that the spinal CXCR3 plays an essential role in the pathogenesis of chronic dry skin-induced itch (69).

Toll-like receptors (TLR). TLR are type I transmembrane proteins that can mediate innate and adaptive immunity via recognition of exogenous and endogenous ligands produced after tissue injury. There is increasing evidence that primary sensory neurones express TLRs, e.g. TLR3 and TLR4 (70, 71), and their important roles, such as spinal cord glial activation in neuropathic pain (72, 73). The AEW mouse model exhibited persistent upregulation of TLR4 mRNA and increased TLR4 expression in GFAP-expressing astrocytes in the spinal dorsal horn. $TLR4^{-/-}$ mice exhibited substantial reductions in scratching and alloknesis, a touch-elicited itch in wild-type mice, after AEW. This model also induced TLR4-dependent astrogliosis (GFAP upregulation) in the spinal cord. Intrathecal injection of astroglial inhibitor L-α-aminoadipate reduced AEW-induced itch and alloknesis. Scratching plays an essential role in spinal astrogliosis because AEW-induced astrogliosis was abrogated by placing collars on the neck to prevent scratching. Intrathecal injection of lipopolysaccharide from *Rhodobacter sphaeroides* (LPS-RS), a TLR4 antagonist, suppressed AEW-induced itch and alloknesis. These findings suggest that spinal TLR4 signalling is important for spinal astrocyte activation and astrogliosis, which may underlie chronic itch and alloknesis (74).

Tumour necrosis factor-α (TNF-α). Emerging evidence suggests that cytokines and chemokines also serve as key itch mediators and/or modulators (75). TNF-α was reported to play a central role in regulating synaptic plasticity in the spinal cord and chronic pain via its receptors, TNFR1 and/or TNFR2 (76). Dry skin itch induced by AEW was reduced by the administration of thalidomide (TNF-α-synthesis inhibitor) and etanercept (TNF-α antagonist), and in TNFR1/R2 double-knockout mice. AEW treatment induced TNF-α expression in the skin, DRG, and spinal cord, and TNFR1 expression only in the spinal cord. Thus, these findings suggest that TNF-α/TNFR1 signalling is partly required for the full expression of chronic itch in dry skin via peripheral and central mechanisms (77).

Others

Zeta chain-associated protein kinase 70. The T-cell signal pathway was reported to function in dry skin pruritus (78). Zeta chain-associated protein kinase 70 (ZAP70),

as a T-cell receptor, may induce IL-2 secretion and promote NGF secretion in skin (79). After AEW treatment, 22-month-old AEW mice exhibited increased spontaneous scratching compared with 5-month-old AEW mice. ZAP70 expression was significantly increased, in addition to the secretion of IL-2 and NGF in 22-month-old AEW mice compared with 5-month-old AEW mice. This study revealed that increased ZAP70 is involved in dry skin in pruritus in elderly people, probably due to increased secretion of IL-2 and NGF (80).

Toll-like receptor 3. TLR3 was found to be an important receptor in murine itch signalling, and is expressed by sensory nerves and DRG in mice. TLR3 is also expressed by mast cells and keratinocytes (81). AEW treatment elicited a marked 25-fold increase in TLR3 expression in the skin, but not in the DRGs. Moreover, AEW treatment induced marked NGF upregulation in the skin, which was TLR3-dependent. Spontaneous itch was eliminated in TLR3$^{-/-}$ mice. Thus, TLR3 and its upregulation in the dry skin are important for the induction and sensitization of chronic itch (82).

Opioids. Previous studies have identified 4 major types of opioid receptors, μ-type (MOR, a receptor for β-endorphins), κ-type (KOR, a receptor for dynorphins), δ-type (a receptor for enkephalins), and nociception (a receptor for nociceptin/orphanin FQ). Activation of μ-opioid receptors is thought to induce pruritus, whereas activation of κ-opioid receptors is believed to have suppressive effects (18, 83). We previously reported that the κ-opioid system was downregulated in the epidermis of patients with AD, and that psoralen-ultraviolet A (PUVA) therapy downregulated the μ-opioid system and restored the κ-opioid system, concomitant with a decrease in the visual analogue scale (VAS) score (84). Spontaneous scratching after AEW treatment was significantly suppressed by subcutaneous injection of μ-opioid antagonists, such as naloxone and naltrexone (26), and the κ-opioid agonist nalfurafine (85), presumably via both peripheral and central mechanisms.

Hyperknesis

The term "hyperknesis" was proposed as an umbrella term encompassing the state in which there is enhanced itch to normally itch-provoking stimuli or lowered itch threshold to a given stimulus (86). The mechanisms of hyperknesis are not clear, and it remains unknown which type of afferents mediate the mild itch resulting from punctate stimuli (87). Hyperknesis may be mediated by type-I Aδ fibres through a central mechanism when secondary to itch provocation or an actively itchy skin lesion (88).

Akiyama et al. (27) reported a significant increase in the number of scratching bouts evoked by intradermal injections of a PAR-2 agonist and 5-HT under dry skin conditions. Moreover, DRG cells from AEW-treated mice exhibited significantly larger responses to the PAR-2 agonist and 5-HT. Furthermore, enhanced responses of lumbar superficial dorsal horn neurones to intradermal PAR-2 agonist in this model have been reported (89). This implies that acute itch elicited by certain pruritogens, such as 5-HT and PAR-2 agonists, is enhanced in chronic itchy skin. This reflects hyperknesis, which is consistent with the sensitization of itch-signalling pathways.

Alloknesis

Innocuous mechanical stimuli were reported to elicit itch when delivered within a region of normal skin surrounding a site of experimental itch induced by the intradermal injection of histamine, a phenomenon known as itchy skin or alloknesis (90). Alloknesis may reflect a central mechanism in which the activation of low-threshold mechanoreceptors excites sensitized itch-signalling neurones in the spinal cord. Innocuous mechanical stimulation elicited scratching when delivered at the edge of a region of AEW treatment in mice, suggesting the presence of alloknesis in this animal model of chronic dry skin itch (29).

Merkel cells, the touch receptors in the skin, were reported to make "synapse-like" contacts with type I slowly adapting afferents (91). Feng et al. (92) reported that alloknesis in dry skin is associated with a loss of Merkel cells. Targeted genetic deletion of Merkel cells and its associated mechanosensitive Piezo2 channels produced alloknesis. Chemogenetic activation of Merkel cells protected against alloknesis in dry skin. These data suggest that cutaneous Piezo2 channel-Merkel cell signalling is critical in modulating the conversion of touch to itch.

ITCH OF DRY SKIN AND ANXIETY

Chronic itch is clinically correlated with the development of mood disorders, such as anxiety and depression, predominantly in dermatological patients (7). The psychological burden produced by chronic itch was reported with high incidences of suicidal motivation (21.1%) and psychiatric illnesses (70%) (93). Zhao et al. (94) reported that AEW mice developed anxiety-like symptoms 2–3 weeks and depression-like phenotypes 3–4 weeks after AEW treatment, suggesting that mood impairment due to chronic itch evolves over time. The mood impairment behaviours were significantly related to the itch-associated behaviour. They also demonstrated primary disturbance of the hypothalamic pituitary adrenal (HPA) axis function in AEW-treated mice with chronic itch.

The amygdala is the key brain region for the generation of anxiety (95). Recently, Sanders et al. (96) reported that acute itch stimuli, such as histamine, induced anxiety-like behaviour and increased neurone activity in a subpopulation of the amygdala in adult mice. These results highlight the importance of itch-responsive amygdala

neurones in the regulation of itch-related effects and behaviour, which may also apply to chronic itch conditions due to dry skin.

MANAGEMENT OF DRY SKIN-INDUCED ITCH

Since the mechanisms of dry skin-induced itch in animal models were reported, there have been many studies on the management of dry skin-induced itch (Fig. 2 and **Table I**).

Antihistamines

Second-generation H_1-antihistamines (e.g. bepotastine) were reported to be beneficial for pruritus in patients with AD (97). We recently demonstrated that bepotastine downregulated NEFs (NGF and ARTN) mRNA in normal human epidermal keratinocytes. The alteration was mediated by the transcription activity of AP-1- and/or NF-kB-dependent mechanisms via the histamine H_1 receptor. These results provide therapeutic evidence that second-generation H_1-antihistamines may be effective for controlling itch associated with epidermal nerve density in dry skin conditions (98). Another report found that topical application of H_1 (diphenhydramine) and H_2 (famotidine)-antihistamines prevented epidermal hyperplasia in mice whose skin barrier was disrupted by acetone treatment (99). Similar to many intractable pruritic conditions, AEW-induced itch is thought to be histamine-independent; however, antihistamines may partly improve skin barrier function and epidermal hyperinnervation in dry skin conditions.

Emollients

In our previous study, immediate and delayed application of emollients, e.g. hydrophilic petrolatum and heparinoid cream, onto acetone-induced dry skin reduced the number of penetrated intraepidermal nerve fibres and NGF levels in the mouse skin (33). In addition, application of gel-like moisturizing lotion (TSG), which contained water, glycerin, urea, methyl paraben, propyl paraben, and agar, reduced the number of infiltrated intraepidermal nerve fibres, and induced higher expression of Sema3A in the epidermis of AEW-treated mice. This suggests that topical application of TSG attenuates itch induced by chronic dry skin through a mechanism involving the inhibition of epidermal hyperinnervation (100).

Phototherapy

UV-based therapies, such as PUVA and narrowband-ultraviolet B (NB-UVB), are efficacious in the treatment of chronic pruritus in patients with AD (101) and psoriasis (102). In our previous study, PUVA therapy reduces epidermal hyperinnervation in patients with AD (103). Furthermore, in the acetone induced-dry skin mice model, PUVA, PUVA+betamethasone valerate ointment (BV), NB-UVB, and excimer lamp treatments significantly reduced the intraepidermal nerve growth induced in this model. PUVA+BV and NB-UVB also normalized the abnormal expression of NGF and Sema3A in the epidermis (104).

In addition, we reported that excimer lamp irradiation of nerve fibres formed by cultured DRG neurones induced degenerative changes in these fibres. We demonstrated that attaching a cut-off excimer filter (COF) to the lamp, thus decreasing cytotoxic wavelengths, reduced hyperinnervation and the production of cyclobutane pyrimidine dimer, a DNA damage marker, in the acetone-induced dry skin mouse model. This suggests that the antipruritic effects of excimer lamp irradiation with COF are due to the induction of epidermal nerve degeneration and reduced DNA damage (105).

Opioids

Dry skin-related itch in animal models was suppressed by μ-opioid receptor antagonists (26) and κ-opioid receptor agonists (85). Clinically, μ-opioid receptor antagonists and κ-opioid receptor agonists were found to inhibit itch in dry skin-related cutaneous or systemic diseases (106, 107).

Table I. Therapies for dry skin-induced itch

Therapeutic method	Mechanisms of antipruritic effects
Topical treatment	
Emollients • hydrophilic petrolatum, heparinoid cream, gel-like moisturizing lotion	• Reduction in epidermal nerve density
Film dressings	• Reduction in epidermal nerve density • Prevention of mechanical stimulus
Phototherapy • Psoralen ultraviolet A • Narrowband ultraviolet B • Excimer lamp	• Normalization of expression levels of nerve elongation factors and nerve repulsion factors ⇨ Reduction in epidermal nerve density • Induction of cutaneous nerve degeneration
Systemic treatment	
Neurotropin	• Reduction in epidermal nerve density
μ-receptor antagonist & κ-opioid agonist	• μ-receptor antagonist and κ-opioid agonist in the central nervous system
Collagen tripeptide	• Reduction in epidermal nerve density, normalization of axon-guidance factors
Antioxidants	• Inhibition of oxidative stress in the periphery
Fish oil	• Improvement of skin barrier function
Adjunctive treatment	
Second generation of H1-antihistamines, e.g. bepotastine	• Reduction in nerve elongation factors and epidermal hyperplasia

Nalbuphine is a synthetic opioid analgesic, a mix of κ-opioid receptors agonist-μ-opioid receptors antagonist, clinically indicated for moderate to severe pain (108). A systematic review suggested that nalbuphine is a superior treatment option for opioid-induced pruritus because of its antagonistic effects and high affinity to the μ-opioid receptors (109).

Neurotropin

Neurotropin, a non-protein extract isolated from the inflamed skin of rabbits inoculated with vaccinia virus, was reported as an effective treatment for antihistamine-resistant pruritus in a multicentre, open-label, small sample study (110). We found that neurotropin inhibits NGF-induced neurite outgrowth of DRG neurones *in vitro* (111). Moreover, the intraepidermal nerve density in acetone-treated mice was reduced by the intraperitoneal administration of neurotropin, probably through the expression of Sema3A in the epidermis (112).

Phospholipids

Eicosapentaenoic acid (EPA, 20: 5n-3) and docosahexaenoic acid (DHA, 22: 6n-3) are representative omega 3 (n-3) polyunsaturated fatty acids (PUFA). Previous studies suggested that n-3 PUFA and related monohydroxy metabolites play an essential role in skin homoeostasis because their content within the skin regulates the skin barrier function (113, 114). Supplementation of fish oil, a well-known source of n-3 PUFA, in an acetone-induced dry skin rat model restored the skin barrier defects and improved scratching behaviour (115).

Dietary milk-derived phospholipids (MPLs) and milk-derived sphingomyelin have been reported to have beneficial effects on epidermal functions (116, 117), such as increased SC hydration in normal hairless HR-1 mice (116), and improved skin barrier function in the HR-AD mouse model (117). Recently, we reported that dietary MPLs attenuate the penetration of nerve fibres into the epidermis by reducing epidermal NGF levels and increasing the Sema3A level in a mouse model of acetone-induced dry skin. Thus, dietary MPLs may have beneficial effects for the prevention and/or alleviation of dry skin-induced itch by reducing intraepidermal nerve fibre density (118).

Collagen tripeptide

Collagen tripeptide is a highly purified, non-antigenic, low allergenic collagen fraction that is known to have many biological effects, such as enhancing hyaluronic acid production in human dermal fibroblasts *in vitro* and in murine skin *in vivo* (119). Oral administration of collagen tripeptide to acetone-induced dry skin model mice improves dry skin and normalizes axon-guidance factors in the epidermis, in addition to reducing pruritus (120).

Antioxidants

Oxidative stress has long been proposed to play a role in the pathogenesis of itch-related skin and systemic diseases, including AD, psoriasis, and chronic renal failure (121). Oxidants were demonstrated to induce histamine-independent itch via the activation of TRPA1 in mice (122). Zhou et al. (123) reported that antioxidants were systematically effective in reducing the scratching bouts of AEW-treated mice, possibly through the inhibition of oxidative stress in the periphery (affected skin) and suppression of p-ERK activation in the spinal cord. Thus, antioxidants, such as N-acetyl-L-cysteine and N-tert-butyl-a-phenylnitrone, may have therapeutic effects on dry skin-induced itch.

Film dressings

More recently, we reported that the application of film dressings, which are used for wound treatment to provide an appropriately moist environment and act as a barrier to contamination, alleviated the epidermal hyperinnervation and alloknesis in the AEW-induced dry skin model mice. Film dressings may reduce itch hypersensitivity of the skin (124). Consistent with this, we and others found that the level of NGF in the mouse epidermis significantly decreased by occlusion with emollients (33) or a vapour-impermeable membrane (24) after skin barrier disruption by tape-stripping or acetone. This suggests that skin moisturization prevents epidermal hyperinnervation induced by barrier disruption and mechanical stimuli to the skin.

CONCLUSION

This review presented recent knowledge regarding the mechanisms of dry skin-induced itch and its management. A decline in skin barrier function is thought to be the primary cause of dry skin-induced itch, as observed in the AEW model, the most well-known mouse model of dry skin. Many kinds of mediators, receptors, and channels are involved in itch signalling among the skin nervous system, skin cells, and central nervous systems, including Mrgprs, TLR, cytokines, and TRP channels. Continued studies are required to better understand these complex interactions and to develop antipruritic drugs to improve the quality of life of the patients.

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Centenary theme section: ITCH AND PRURITIC DISORDERS

REVIEW ARTICLE

Non-dermatological Challenges of Chronic Itch

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Chronic itch occurs in many skin diseases, but also in a variety of systemic, neurological, and psychogenic/psychosomatic disorders, or is caused by drug intake. When several diseases or causes co-exist, chronic itch is categorized as "mixed origin". These patients present with unaltered skin or with chronic scratch lesions including chronic prurigo. Precise diagnostics are necessary to evaluate the underlying aetiology, to enable identification of the best treatment available, and to improve patients' quality of life. This is of particular relevance in elderly people in whom chronic itch is often of systemic or mixed origin. Xerosis cutis is a frequent cofactor contributing to chronic itch of non-dermatological origin. Treatment is frequently multimodal, considering age, comorbidities, current drug intake, quality and intensity of itch. With regard to the demographic situation of the population, characterized by increasing life expectancy and polypharmacy, itch of non-dermatological origin will represent an increasing medical challenge in the future.

Key words: cholestasis; chronic kidney disease; liver; pruritus; systemic disease; uraemic itch.

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SIGNIFICANCE

Itch (medical term: pruritus) is an unpleasant sensation occurring in many skin diseases, but also in a variety of systemic, neurological, and psychogenic/psychosomatic disorders, or is caused by drug intake. These patients usually present with normal-looking skin or chronic scratch lesions of variable degree, including the clinical picture of chronic prurigo. This review focusses on the systemic causes of chronic itch, in particular liver disease, chronic renal failure, haematological disorders and adverse reactions of drug use. Furthermore, a diagnostic approach is presented, and effective, multimodal treatment options for the different systemic causes are summarized.

Pruritus or itch is an unpleasant sensation that can evoke scratching even in patients who do not currently have a skin disease. These patients usually present with normal-looking skin or chronic scratch lesions of variable degree, including the clinical picture of chronic prurigo (CPG; **Fig. 1**). For a long time it was assumed that itching and scratching must be associated with a skin disease. This may result from previous conditions, in which scratching aimed at extracting mites or other skin infestations. It took medical doctors and researchers some time to realize that chronic itch (CI), aside from dermatological disorders, can also be caused by a number of other diseases/origins. This comprises chronic kidney disease, a variety of hepatobiliary, haematological, and endocrine diseases, as well as so-called drug-induced itch (**Table I**). To date, it is now known to what extent drug intake and, in particular, polypharmacy contributes to CI. The underlying mechanisms of drugs causing pruritus as adverse events are only partially understood, ranging from mainly type-I and type-IV allergy to several other possible mechanisms (1, 2).

Advanced technical and medical care for chronically ill patients prolongs their life expectancy, albeit with an increased number of patients experiencing non-dermatological pruritus. Chronic pruritus further significantly reduces the quality of life in chronically ill patients. These observations represent an important incentive to increase itch research, with the aim of improving medical care for patients with CI with no underlying skin disease (3).

This review summarizes major non-dermatological aetiologies of CI. It also describes neuropathic itch secondary to brain and spinal cord injuries or peripheral nerve damage, as seen in brachioradial itch and notalgia

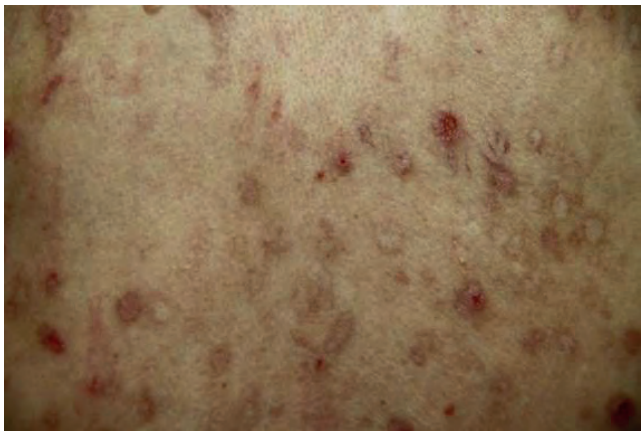

Fig. 1. Chronic prurigo on the upper leg in a patient with haemochromatosis.

Table I. Systemic diseases associated with chronic pruritus

Frequency	Organ system	Diseases
Common	Renal disorders	Chronic kidney failure
	Hepatobiliary disorders	Primary biliary cholangitis, primary / secondary sclerosing cholangitis, IgG4-related cholangitis, drug-induced toxic cholestasis, liver cirrhosis, benign or malignant obstructive cholestasis
	Haematopoietic diseases	Polycythaemia vera, essential thrombocytosis, Hodgkin and non-Hodgkin lymphoma, hypereosinophilic syndrome, mastocytosis
Rare	Endocrine diseases	Hyperthyreoidism, hypothyreoidism, hyperparathyreoidism, carcinoid syndrome, diabetes mellitus
	Malassimilation syndromes	Lactose intolerance, coeliac disease, anorexia nervosa, iron deficiency, vitamin B/D deficiency
	Infectious diseases	Chronic HBV-/ HCV-/ HIV-/ HSV-infection, H.p. infection, VZV-reactivation (post-herpetic), parasitoses
	Solid tumours	Carcinoma of the thyroid gland, ears, nose and throat, breast, lungs, stomach, pancreas, colon, prostate, uterus and sarcoma

paraesthetica. Finally, this review emphasizes that diagnosis and treatment is challenged in daily clinical routine by the fact that several origins frequently contribute to the occurrence of CI of non-dermatological origin.

DIAGNOSING NON-DERMATOLOGICAL ITCH

Identifying the underlying disease or itch-causing drug often represents a clinical challenge for the treating physician, but is a major need for the affected patient. A recent cohort analysis of more than 3,000 patients with CI illustrated that it occurs mainly in older patients and in those with many comorbidities (4). In contrast to previous doctrines, there is no correlation between generalized pruritus and a systemic disease as underlying cause. Patients with generalized pruritus suffer comparably often from systemic as from dermatological disorders (5). Thus, careful and detailed medical history taking, clinical examination and interdisciplinary, laboratory and radiological diagnostics are of significant importance (6). For this purpose a structured pruritus questionnaire has been developed (7).

Pruritus on primarily affected skin hints at a dermatological disease. In addition to clinical skin investigations, bacterial, mycological, allergic and autoimmune-serological analyses should be performed. Skin biopsy might further help to establish a clinical diagnosis (6). Pruritus on primarily unaffected skin is often caused by systemic, neurological or psychiatric/somatoform disorders or drug intake (8, 9). If medical history taking and clinical examination are inconclusive a step-wise approach for diagnosis is recommended, including various laboratory diagnostics (6).

It should be noted that CI may occur prior to manifestation and/or clinical diagnosis of the underlying disease. This so-called premonitory itch may present up to several years prior to diagnosis, as seen in polycythemia vera (10).

In rare cases malignancy is responsible for CI (Table I). Two large cohort studies of 8,744 and 12,813 patients with CI without primary skin changes, respectively, unravelled solely increased rates for haematological and bile duct malignancies (11, 12). In case of CI of unknown origin (PUO) the diagnostic approach should focus on these 2 cancer entities. If no underlying diagnosis could be established an annual repetitive work-up may be performed.

HEPATOBILIARY DISEASES

Chronic pruritus is commonly reported by many patients with hepatobiliary diseases, in particular those with cholestatic features. Prevalence of hepatic itch varies considerably between the different underlying diseases, with 100% as defining symptom in intrahepatic cholestasis of pregnancy, up to 70% in primary biliary cholangitis, primary and secondary sclerosing cholangitis, 15–45% in benign and malignant biliary disorders, and 5–15% in chronic viral hepatitis C infections (13). Affected patients often report the highest intensity on their limbs, in particular the palms of the hands and the soles of the feet, albeit CI may often be generalized (14). In female patients pruritus typically worsens premenstrually, during hormone replacement, and in the last trimester of pregnancy. Hepatic itch presents independently of the severity of cholestasis or liver function.

The pathogenesis of hepatic itch is not yet fully understood. In the past, bile salts, histamine, progesterone metabolites and endogenous opioids have been discussed as culprits, albeit a correlation with itch intensity could not be established (15). Interestingly, the semi-synthetic bile salt obeticholic acid, which has been licensed for the treatment of primary biliary cholangitis (PBC) dose-dependently worsens pruritus. Recent data have suggested that bilirubin and bile salts may mediate cholestatic itch via the mas-related G protein-coupled receptor X4 (MRGX4), albeit human data supporting this observation is lacking (16, 17). Cholestatic patients do not present with histamine-induced skin alternations, such as erythema, urticaria or wheals, and antihistamines are largely ineffective (18). Recently, lysophosphatidic acid (LPA) and its forming enzyme autotaxin (ATX) have been identified as potential mediators of hepatic itch (19). However, drugs inhibiting the ATX-LPA axis have not yet been investigated in hepatic itch to prove this pathophysiological concept.

Treatment should focus primarily on adequate therapy for the underlying hepatobiliary disease, which may

result in relief of hepatic itch. In this regard, itch due to obstruction of the extrahepatic biliary tree is often efficiently ameliorated by endoscopic biliary stenting, transcutaneous or nasobiliary drainage. In contrast, itch due to intrahepatic cholestasis may represent a significant clinical challenge.

Ursodeoxycholic acid (UDCA) is used as anticholestatic baseline treatment in many cholestatic disorders, such as PBC, primary sclerosing cholangitis, and intrahepatic cholestasis of pregnancy (20). UDCA is a safe and effective anti-pruritic therapy in women with intrahepatic cholestasis of pregnancy (21). However, in randomized placebo-controlled trials UDCA did not significantly improve itch intensity. Topical treatment with rehydrating and cooling (e.g. menthol-containing) ointments may mitigate hepatic itch if mild in intensity. If insufficient, cholestatic itch is recommended to be treated with cholestyramine (4–16 g/day) as first-line therapy, followed by rifampicin (150–600 mg/day), naltrexone (25–50 mg/day) and sertraline (75–100 mg/day) according to the current guidelines (13, 20) (**Table II**). Bezafibrate or fenofibrate may be used as alternative approaches (22, 23). Future therapies could be based on inhibitors of the ileal bile acid transporter (IBAT) in the terminal ileum, which are currently investigated in placebo-controlled trials (24). In refractory cases invasive procedures, such as plasmapheresis, albumin dialysis (e.g. MARS®, Prometheus®), transcutaneous or nasobiliary drainage, may be performed. After liver transplantation most patients experience relief of CI.

RENAL DISEASES

Itch in renal disease, also referred to uraemic pruritus (UP), affects patients with advanced stages of chronic kidney disease (CKD), mostly those on dialysis. Epidemiological studies indicate that up to 50% of patients on haemodialysis have CKD-associated pruritus (CKD-aP) depending on the investigated country (25, 26). The underlying pathological mechanism remains elusive. Increased levels of uraemic toxins and parathormone, as well as xerosis and subclinical skin inflammation have been suggested to play a role in the pathophysiology. Furthermore, the endogenous opioid system may play a role, possibly through upregulation of μ-opioid and/or downregulation of κ-opioid activity (27). This may, at least partially, explain the efficacy of μ-opioid antagonists and κ-opioid agonists in the treatment of CKDaP. Recent experimental data suggested central neuropathic and neuroplastic changes in patients with CKD-aP, which may explain the good response to calcium-channel-blockers in these patients (28).

CKD-aP greatly impacts the quality of life of affected patients (25). Retrospective analyses identified risk factors for UP in dialysis patients even before dialysis has begun. These include male sex and certain comorbidities, such as congestive heart failure, chronic hepatitis C virus infection, neurological diseases, depression and higher serum calcium/phosphorus levels (29). When uraemic itch occurs, skin appearance is normal in most patients, except for common changes in skin colour and a frequently observed xerosis. Scratch lesions, such as excoriations with or without impetigo, may be observed in some patients and, in some cases, chronic prurigo (Fig. 1). Medical therapy for CKD-aP remains a clinical challenge. Emollients for skin care and hydration are essential. In early stages gabapentin and pregabalin, although not licensed for this indication, may be helpful (30). Ultraviolet phototherapy may ameliorate itch in uraemic patients (31). Detailed information about drugs and dosages is given in **Table III**.

NEUROPATHIC DISEASES

Neuropathic itch is caused by neuronal or glial damage to peripheral neurones, either localized (e.g. nerve compression) or generalized (e.g. nerve degeneration). Damage to the central nervous system, such as by tumours of the brain or in the spinal cord, rarely causes pruritus.

Several entities can be discerned. Patients with notalgia paraesthetica (NP) perceive itch in the subscapular region, associated with a slightly painful or burning character. The pathophysiology of this entity is unclear, but NP is presumed to be a mononeuritic disease affecting thoracic nerve fibres. Patients with brachioradial itch have localized itch within the dorsolateral parts of

Table II. Therapeutic recommendations for hepatic pruritus

Approach	Drug[a]	Dose
1st line	Colestyramine	4–16 g/day (po)
2nd line	Rifampicin	150–600 mg/day (po)
3rd line	Bezafibrate	400 mg/day (po)
4th line	Naltrexone	25–50 mg/day (po)
5th line	Sertraline	100 mg/day (po)
6th line	Experimental approaches, e.g.	
	Gabapentin	300–3,600 mg/day
	UVB light	1–2×/week

[a]Solely colestyramine is licensed for the treatment of hepatic pruritus; all other drugs are off-label use.
po: per os.

Table III. Therapeutic recommendations for chronic kidney disease associated pruritus

Approach	Drug[a]	Dose
1st line	Gabapentin	After dialysis: 100 mg 3×/week or 300 mg 3×/week or 400 mg 2×/week (po)
2nd line	Pregabalin	75 mg 2×/week–75 mg/day (po)
3rd line	UVB light	1–3×/week
4th line	Capsaicin	3–5×/day (topical)
5th line	Experimental approaches, e.g.	2.5–5 μg/day (po) or 5 μg (iv) after dialysis
	Nalfurafine	2×/day (topical)
	Tacrolimus	500 mg 3×/day
	Curcuma	

[a]All drugs are off-label-use.
po: per os; iv: intravenous.

the forearms and, less frequently, around the shoulders. As in NP, patients report mixed itch and pain sensations. Cervical cord compression or radiculopathies have been observed in these patients (32).

The small fibre neuropathy is a systemic neuropathy of peripheral nerves accompanied by severe pruritus in some patients. This sensory disorder leads to a variety of symptoms, including pain, tingling, numbness, deranged thermoregulation and signs of malfunction of the autonomic nervous system, such as gastrointestinal dysmotility and orthostatic hypotension. The diagnosis is difficult to establish, requiring thermoregulatory sweat testing and skin biopsy with reduced small fibre density (33).

Apart from these entities, neuropathic itch may occur in the course of herpes zoster infections as so-called post-herpetic itch, polyneuropathies and scars induced by trauma or burns.

Treatment of neuropathic itch is difficult. Local treatment with capsaicin or systemic treatment with calcium-channel-blockers, such as gabapentin or pregabalin, may alleviate pruritus (3, 34).

ENDOCRINE DISEASES

CI may occur in association with several endocrine diseases. Whether patients with diabetes mellitus are more frequently afflicted than those without remains a matter of debate. In one study investigating almost 400 patients with diabetes 27.5% reported on generalized itch (35). In another study truncal itch was most prominent in diabetic patients. Of 2,656 patients with diabetes mellitus, 11.3% reported itch located on the trunk, whereas this symptom was present in only 2.9% of 499 age-matched patients without diabetes (36). Further endocrine diseases associated with the occurrence of CI include Grave's disease and multiple endocrine neoplasia type II.

HAEMATOLOGICAL DISEASES INCLUDING AQUAGENIC ITCH

Itching is commonly reported in patients with haematological diseases. Aquagenic pruritus is a typical feature in many of these patients, with a pungent itchy character after contact of the skin with water. Polycythaemia vera is a rare myeloproliferative disease with a clonal dysfunction of pluripotent hematopoietic cells. Affected patients report itch in 30–65% of cases. Aquagenic itch is most commonly observed in patients with a homozygous JAK2 617V mutation (37). Inhibitors of the JAK-STAT pathway, such as ruxolitinib, strongly attenuated pruritus in addition to improving the underlying disease (38, 39). Similarly, patients with essential thrombocytosis and primary myelofibrosis often report aquagenic itch.

Hodgkin's disease belongs to the class of B-cell lymphoma. CI on primarily unaffected skin is reported by 15–50% of patients (40). Severe itch may precede the outbreak of the disease by many years. CI worsens at night, often starts at the lower limbs, and may generalize. After successful anti-tumour therapy, CI may also indicate a relapse of Hodgkin's disease. In patients with non-Hodgkin lymphoma pruritus may affect up to 30% of patients. In leukaemia patients CI is more commonly observed in lymphatic compared with myelocytic leukaemia and in chronic compared with acute forms. Recommended therapy consists of the calcium channel blockers gabapentin (300–2,400 mg/day) and pregabalin (75–600 mg/day), acetylsalicylic acid (300 mg/day) and mirtazapine (7.5–30 mg/day) (**Table IV**) (6).

PARANEOPLASTIC DISEASES AND CANCER

In daily clinical practice the term "paraneoplastic itch" (PI) is used to describe itch in patients with cancer. Itch caused by haematological diseases is described above. In general, PI is considered as a rare disorder. It occurs most commonly in lymphoreticular malignancies, while being rarely reported in patients with solid tumours. Its true frequency remains unknown, as epidemiological data is limited. This may mainly be due to other symptoms receiving more attention and being regarded as more important in cancer patients with paraneoplastic diseases. According to the literature and our own clinical studies CI further impairs quality of life in patients with malignant diseases. In 2012, an interdisciplinary study interest group (SIG) of physicians and researchers was founded, with the aim of generating a clear definition of PI (40).

Previously, several terms have been used to describe the different types of paraneoplastic itch. The SIG states that the term paraneoplastic itch should be used in case itching occurs as a systemic, but not local, reaction in the presence of a solid tumour or haematological malignancy. This term excludes itch induced either by the local invasive growth of cancer cells or by anti-tumour therapy. PI usually disappears with remission of the tumour and may return with its relapse (40).

Diagnosing PI represents a clinical challenge, as it remains difficult to exclude other aetiologies and reasons for CI, such as paraneoplastic skin diseases, skin or non-dermatological diseases occurring in chronically ill patients, as well as adverse drug reactions. The me-

Table IV. Therapeutic recommendations for pruritus of hematopoietic origin

Approach	Drug[a]	Dose
1st line	Gabapentin	300–2,400 mg/day (po)
2nd line	Pregabalin	75–600 mg/day (po)
3rd line	Acetylsalicylic acid	300 mg/day (po)
4th line	Mirtazapine	7.5–30 mg/day (po)
5th line	Experimental approaches, e.g.	
	Aprepitant	125-80–80 mg/week or 80 mg/day
	Naltrexone	50–150 mg/day
	Nalfuraphine	2.5–5 µg/day (po)

[a]All drugs are off-label use.
po: per os.

chanisms of PI are still not understood.

Baseline therapy of PI comprises the treatment of the underlying malignancy. In many cases, cytoreductive therapies are effective. There are no randomized controlled trials (RCTs) for the treatment of PI, which may be explained largely by the rarity and diversity of PI. Topical therapies, including cooling agents, may lead to symptomatic relief. H1-antihistamines are mostly ineffective. PI in lymphoma can improve with oral prednisone (see above) acting via different mechanisms, and longer systemic treatment with prednisone may be considered in PI (3). Serotonin reuptake inhibitors (SSRI), such as paroxetine, up to 20 mg daily, calcium alpha (2)-γ-channel blockers, such as gabapentin and pregabalin, can be used for treating PI as well as thalidomide 50–200 mg daily. Opioid receptor antagonist, such as naloxone (0.8–2 mg i.v./day), naltrexone (50–100 mg/day orally) or neurokinin (NK)-1-receptor-antagonists, e.g. aprepitant, have been used for itch, for example in T-cell lymphoma, solid tumours and itch-related biological cancer treatment (40). It should be considered that patients with cancer frequently receive analgetics that may induce CI as a side-effect.

DRUG-INDUCED ITCH

Itch associated with systemically or locally applied drugs is a common phenomenon (41). Almost every drug may cause localized or generalized itch. Drug-induced itch may emerge as a hypersensitivity reaction towards the drug or, as in many cases, due to another, mostly unknown, pathophysiological process. There is evidence that, with some drugs, the MRGX2-receptor is activated, resulting in a different release of pruritogenic compounds than in IgE-mediated hypersensitivity reactions (42). Identifying the responsible compound remains, for many cases, a clinical challenge, as many patients are taking multiple drugs and there might be a considerable delay between the start of drug intake and the development of itch.

Chloroquine, which is mainly used as an antimalarial drug, can induce itch, probably by activating the MrgprX1-receptor. Interestingly, chloroquine provokes itch mainly in black Africans, while Caucasians are less commonly affected potentially by a strong binding capacity to melanin with significantly higher skin concentrations in Africans (43).

Itching provoked by hydroxylethyl starch (HES) infusion is thought to be caused by HES depositions in peripheral nerves (44). Treatment is cumbersome and mostly frustrating (45).

Opioidergic drugs may cause strong itch, especially when applied epidurally or intrathecally (46) which may be mediated by activation of the isoform D of the μ-opioid receptor (47). It can be treated effectively by μ-receptor-antagonists, such as naloxone.

Along with the expansive development and use of targeted tumour therapies, numerous reports have emerged about therapy-associated chronic pruritus (48–52). The neurokinin-1-antagonist aprepitant seems to have antipruritic potential in such cases (53).

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Itch and Psyche: Bilateral Associations

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Beginning from embryological development, skin and psyche are closely related to physiological state regardless of age. Altering the homeostasis of one of these components impacts on the other, thereby substantiating that the relationship between itch and psyche is bilateral. Itch has a complex pathogenesis, which involves the peripheral and central nervous systems, as well as various inflammatory mediators. This paper reviews key aspects of itch pathogenesis, relevant associations with stress, the contagiousness of itch, psychological and psychiatric considerations related to itch, and the burden of itch with respect to impairment of health-related quality of life (HRQoL) and stigmatization. Despite the fact that itch–psyche associations still pose many questions, current knowledge supports the role of a holistic, interdisciplinary approach to these patients in order to improve their well-being.

Key words: itch, psyche, pathogenesis, stress, psychiatry, burden of disease.

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SIGNIFICANCE

The relationship between itch and psyche is complex and bilateral. Increasing interest in itch and its associations with psyche is indicated by the abundance of experimental and clinical articles published in this field. This review covers the pathogenesis of itch, associations with stress, the contagiousness of itch, psychological and psychiatric aspects related to itch, and the burden of itch with respect to impairment of health-related quality of life and stigmatization.

Beginning with human embryogenesis, skin and brain are organs that are closely connected due to their ectodermal origins (1). These associations continue to unfold after birth, and constitute a foundation for the normal psychosocial development of an individual. According to the *moi-peau* concept (2) (**Fig. 1**) (as extensively reviewed by Dutray & Misery (1)), skin possesses a wide psychological meaning, reflecting various needs of the psyche. Firstly, skin has the function of a "bag" or "container", as it embraces the positive stimuli experienced by a baby during nursing and being cared for. Secondly, skin is an "interface" with the outside world, which serves as a protective barrier against external aggression. Lastly, skin may be considered as a specific "place" or "means of communication" in order to establish relationships, and as a "surface" on which others may leave their trace. It seems substantiated that chronic skin diseases with their rich symptomatology, especially acquired during early childhood, ensue in altering both physical and psychological homeostasis of an affected individual. The impact is always reciprocal: the psyche may predispose to cutaneous complaints (e.g. itch, chronic scratch lesions), whereas dermatological signs and symptoms "scar" the psyche.

Itch is defined as an unpleasant sensation leading to scratching, further classified as acute or chronic (lasting less or more than 6 weeks, respectively) (3). Chronic itch (CI) is a feature of various dermatoses, although it may also stem from systemic, neurological, or psychiatric disorders. Occasionally, the diagnosis of pruritus of unknown origin (PUO) is established. The presence of CI is associated with significant morbidity, mortality, reduction in quality of life (QoL), feelings of stigmatization, stress, impairment of mood, lack of concentration,

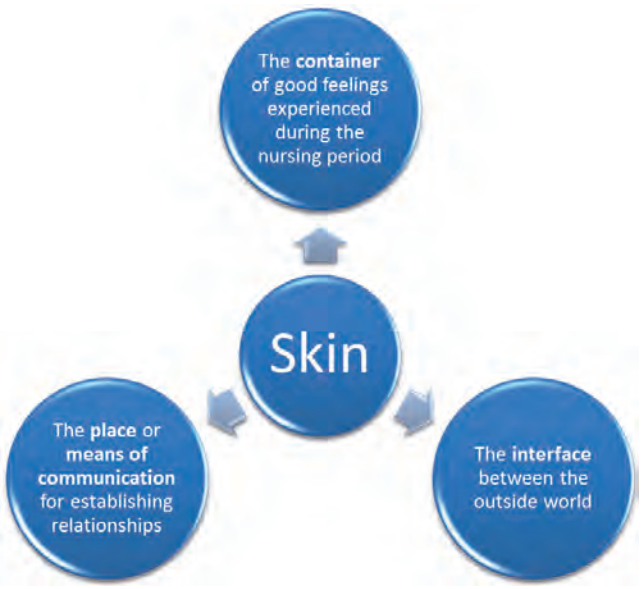

Fig. 1. The moi-peau concept (The Ego-Skin concept) (according to (1)).

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reduced sexual desire and appetite, as well as inability to express emotions (alexithymia) (4–9). On the other hand, clinicians are aware that various psychological and psychiatric alterations may contribute to the occurrence or exacerbation of itch. One of the models that aims to explain CI mechanisms in the course of cutaneous disorders is the Biopsychosocial Model, proposed by Verhoeven et al. (10). The occurrence of itch is a result of various factors: internal (associated with personality), external (e.g. stressful environmental stimuli), mediating (e.g. cognitive, behavioural and social) as well as physiological. Tey et al. (11) have clinically classified the itch–psyche interactions as pruritic disorders with psychiatric sequelae (I); pruritic disorders aggravated by psychosocial factors (II); and psychogenic disorders causing itch (III). These complex bilateral associations between itch and psyche support the need for multidisciplinary approach to itch and the associated conditions in the clinical setting (12–14).

This review covers the pathogenesis of itch, cerebral regions associated with pathogenesis of itch, the interplay between itch and stress, the contagiousness of itch, psychological and psychiatric aspects associated itch, and the burden of itch with respect to impairment of QoL and stigmatization.

ITCH PATHOGENESIS

There is growing data concerning the pathogenesis of itch, as this topic is gaining increased attention in the medical literature. From the historical point of view, itch has been perceived as a subtype of pain (15), and it is thought that the symptom might have developed as an evolutionary defence mechanism against various potentially dangerous stimuli, such as parasites, insects, sharp objects, irritants, and allergens (16). However, itch is currently deemed a separate entity from pain. The role of peripheral (PNS) and central nervous system (CNS) is crucial in eliciting CP, starting from free nerve endings in the epidermis. The statement "it is the brain that itches, not the skin" (16) remains valid, as different cortical areas are included in processing itch intensity, location, associated unpleasant feelings, generating the need to scratch, and preparing and executing scratch behaviour (17–20). In a positron emission tomography (PET) study among human participants histamine injection resulted in major activation in the left primary sensory cortex, as well as the primary motor cortex, supplementary motor area and premotor cortex (17). Another study utilizing PET and regional cerebral blood flow (rCBF) revealed that a skin prick test with histamine resulted in activation of the contralateral somatosensory cortex; in addition, both ipsilateral and contralateral motor areas were involved. Itch unpleasantness was associated with the activation of contralateral sensorimotor cortex, prefrontal cortex, posterior insula and ipsilateral supplementary motor area (18). A functional magnetic resonance imaging (fMRI) study demonstrated itching and pain both activated the anterior cingulate cortex, the anterior insula, the basal ganglia and pre-supplementary motor area (20). However, the activity in posterior cingulate cortex and the posterior insula was more prominent in itching than in pain. Moreover, it was proportional to itching sensation. In an experiment with cowhage-induced itch, as demonstrated by fMRI, itching was associated with increased activity in supplementary motor area, premotor cortex, primary motor cortex, and midcingulate cortex (21). The role of the caudate nucleus was also substantiated, as this region is involved in the reward system. Papoiu et al. (22) demonstrated that active scratching was accompanied by higher pleasure and deactivation of anterior cingulate cortex and insula compared with passive scratching. In addition, the activation of ventral tegmentum area (VTA) of the midbrain, as well as deactivation of periaqueductal grey matter (PAG), are associated with the itch-scratch reward system.

ITCH AND STRESS

Stress is a concept frequently mentioned nowadays, yet despite its widespread appearance in various situations it may be described in different ways, e.g. as "the non-specific response of the body to any demand made upon it" (23) or "a relationship with the environment that the person appraises as significant for his or her well-being and in which the demands tax or exceed available coping resources" (24). The bilateral associations of itch and psyche are perfectly reflected by the interplay of itch and stress. Similarly to the vicious itch-scratch cycle, chronic pruritic conditions (e.g. psoriasis, urticaria, atopic dermatitis) generate huge stress levels, which may subsequently perpetuate exacerbation of the disease. Aberrant parasympathetic response may possibly link chronic stress and itch (25), while stress-induced itch is associated with activation of the hippocampus and subcortical regions (corpus callosum and putamen) (26). The itch-stress association does, in fact, pose a certain therapeutic implication towards first-generation H_1-antihistamines and GABA-ergics (gabapentin and pregabalin) (11, 26).

In a large population-based study among Norwegian adolescents mental distress (assessed by the Hopkins Symptom Checklist-10) was correlated with the presence of itch (27). Moreover, the severity of itch correlated with the level of mental distress, regardless of sex. Subsequently, another report linked the presence of itch with low self-efficacy in individuals under higher stress (28). The perceived self-efficacy is a concept of people's beliefs that they can exert control over their motivation, behaviour and social environment (29). Individuals lacking the sense of self-efficacy are not able to manage

demanding situations effectively, despite knowing what to do and possessing the necessary skills. The increase of self-efficacy in psychological interventions may exercise control over stressors due to its immunomodulating properties (30). Recently, Schut et al. (31) employed cognitive behavioural stress management programmes in patients with atopic dermatitis (AD). The study revealed that individuals subjected to these interventions demonstrated diminished cortisol awakening response, while maintaining calm and presenting lower salivary cortisol levels under acute stress. Thereby, various psychological interventions (reviewed in detail by Schut et al. (32)) should be considered as an adjunctive therapy in patients with itch of various origins. The associations between stress and itch in patients with AD constitute a problem of particular complexity and importance and involve the components of the so-called neuro-endocrino-immunocutaneous system (NEICS) and the hypothalamo-pituitary-adrenal (HPA axis). The relevant aspects of this issue are covered in detail elsewhere (33–38), although there is evidence that acute or chronic stress have different impacts on the HPA axis (39).

Regarding patients with psoriasis, our group has demonstrated that patients under heavy or extremely heavy stress more commonly suffer from itch, with the severity of stress and intensity of itch being positively correlated (40). Similar results were observed by Amatya et al. (7). Another study revealed that the self-reported stress reactivity was moderately correlated with the degree of itching (41). Stress-related exacerbation of itch in psoriatic subjects may also be associated with higher expression of substance P receptor, tropomyosin receptor kinase A and calcitonin gene-related peptide receptor in keratinocytes of psoriatic plaques (42). Other reports mentioned the role of stress in pruritic disorders, such as chronic urticaria (43, 44), acne vulgaris (45, 46), hand dermatoses (47) and post-burn itch (48). Recent research reported that patients with generalized CI reported more tension and subjective stress than healthy controls, with the expectation of the acute stress test (49). Notably, "variations of intensity associated with stress" (50) constitute an optional criterion for the diagnosis of functional itch disorder (FID; psychogenic itch).

Itch-stress associations are complex, yet they serve as the foundation of psychological interventions aimed at enhancing coping abilities of the affected individual, e.g. cognitive restructuring (32). A Dutch study reported that a nursing programme "Coping with itch" successfully targeted catastrophizing and helpless itch-related coping (51). Subsequently, it was proven by Evers et al. (52) that patients with AD may benefit from multidisciplinary itch-coping group training. The programme focused on skin care, itch-triggering factors, stress management, long-term goals, relapse prevention, habit reversal and scratch-triggering factors. The training ensued in reducing itch-scratch behaviour, improving skin status, decreasing the need for dermatological visits and treatment. Coping with itch was improved, as the itch-related self-efficacy increased, with decline in catastrophizing. These benefits were regarded both as short-term and long-term.

NOCEBO EFFECT: IS ITCH A CONTAGIOUS PHENOMENON?

The nocebo effect is the negative counterpart of the placebo effect (53). In essence, an individual receives an inert substance or undergoes a neutral procedure, which is intended to induce negative expectations. Interestingly, outbreaks of itch among schoolchildren attributed to epidemic hysteria have been described in the literature (54, 55). Acknowledging the relevant role of psyche in eliciting itch, the nocebo-related concept of "itch contagion" or "contagious itch" was conceived and investigated in different studies. Based on the experience that lectures about pruritic dermatoses induce itching in the listeners, Niemeier et al. (56) proved that a public lecture entitled "Itching – what's behind it?" caused the participants to feel itch and exert scratching behaviour. Subsequent research revealed that patients with AD, when compared with healthy controls, were more prone to scratch themselves after being subjected to histamine or saline injection, followed by watching a short video of people scratching (57). Holle et al. (58) reported that itch contagion is a normative response experienced by most people and its degree may be associated with neuroticism as a personality trait (the impact of personality traits on itch is reviewed below). No association between itch contagion and sex or empathy was established in this study. The fMRI examination revealed that itch intensity correlated with the activation of the left Brodmann area (BA) 44, primary somatosensory cortex and BA6. Lloyd et al. (59) revealed that solitary visual stimulants provoke itch in healthy individuals. Another study demonstrated that the combination of conditioning and verbal suggestion may result in relevant nocebo and placebo effects on itch in healthy individuals (60). It was subsequently proved that nocebo effects regarding the itch sensation may be minimized or reversed via conditioning with verbal suggestion (61). Recently, increased contagiousness of itch in children with autism spectrum disorder was demonstrated (62).

PSYCHOLOGY OF ITCH

The personality of an individual may be defined as a characteristic pattern of behaviours considered in the broad sense, also including thoughts, feelings and motivation (63). One of the most popular models used for the description of personality structure is The Big Five model, which encompasses 5 bipolar dimensions (extraversion, agreeableness, conscientiousness, neuroticism and open-

ness to experience) (64). The impact of personality traits on itch sensation was explored in several reports and supports the role of psychological interventions in aiding affected individuals. In a Swedish study (65) the persistence of post-burn itch was associated with lack of assertiveness, as assessed by the Swedish Universities Scales of Personality (SSP). Moreover, the Coping with Burns Questionnaire (CBQ) scores revealed that itch was more persistent among individuals who sought more instrumental and less emotional support. Patients with prurigo nodularis (PN) exhibited higher neuroticism and lower extraversion traits than controls when examined via the revised Eysenck Personality Questionnaire (EPQ-R) (66). Notably, among subjects with psoriasis, severe itch was significantly associated with somatic trait anxiety, embitterment, mistrust, and physical trait aggression (assessed via SSP) (67). Conversely, Janowski et al. (68) found no differences in basic personality traits regarding psoriatic patients with various frequency of itch (assessed via NEO-Five Factor Inventory; NEO-FFI). However, resignation and self-blame were more common coping strategies among patients experiencing itch more frequently (assessed via the Ways of Coping Questionnaire; WCQ). In a German study, individuals with AD and healthy controls were exposed to videos featuring crawling insects and skin disorders (69). Compared with healthy controls, agreeableness (NEO-FFI) and public self-consciousness (the Self-Consciousness Scale; SCS) were significant predictors of scratching behaviour in subjects with AD. These findings were subsequently replicated (70). Kini et al. (71) investigated patients with CI (recruited from the National Eczema Association and US Veterans Health Administration National Patient Care Database). The authors observed that the lethargic personality style (defined as low extraversion and conscientiousness) (NEO-FFI) was associated with greater mean total ItchyQoL score. On the other hand, higher ItchyQol symptom score was observed both in overcontrolled (high neuroticism and conscientiousness) and undercontrolled (high neuroticism and low conscientiousness) patients.

Another concept that has evolved as a potential paradigm for understanding the influence of emotions and personality on physical illness and health is alexithymia (72). In general, this personality construct defines the inability to identify and verbalize emotions. Our group has investigated alexithymia using the Bermond-Vorst Alexithymia Questionnaire (BVALQ-40) among patients with end-stage renal disease on maintenance haemodialysis (9). It was observed that patients with uraemic itch exhibited lower scores on the fantasizing subscale score. Another group assessed alexithymia via the Toronto Alexithymia Scale (TAS) among individuals with chronic urticaria (73).

PSYCHIATRIC PERSPECTIVE ON ITCH

Taking into account the widespread relationship between itch and psyche, one cannot omit the obvious psychiatric background of itch in certain cases, whereas the presence of itch may frequently ensue in a wide spectrum of psychiatric comorbidities. In a study by Mazeh et al. (74) among a cohort of patients ($n=111$) hospitalized in the psychiatric ward, CI affected 32%. Of those, 45% stated that stress was one of the major aggravating factors of itch. Similarly, our group enrolled inpatients ($n=40$) who were hospitalized with depression (75). Itching was experienced by 17.5% of patients during the depressive episode. Notably, itching disappeared in all affected individuals after the depressive symptoms markedly decreased, whereas recurrent itching was associated with recurrent depressive episodes.

A different approach was presented by Schneider et al. (76), who examined 109 dermatology inpatients with itch and observed that in over 70% of them 1–6 psychiatric diagnoses could be established. In over 60% of patients psychotherapeutic or psychiatric treatment was advised. Ferm et al. (77) evaluated the medical records of 139 patients with CI, among whom 31 (22.3%) had an underlying psychiatric disorder. A recent study among 560 patients with CI who were referred by the dermatologist for a psychosomatic consultation demonstrated that 77.1% had at least one psychosomatic/psychiatric comorbidity (78). The most common comorbidities encompassed psychological/psychosomatic cofactors in itch (F54 according to ICD-10) (74.5%), depression (F32–F34) (30.7%), adjustment disorder (F43.2) (17.8%), dissociative/somatoform disorder/hypochondria (F44–F45) (11.2%), anxiety/compulsive disorder (F40–F42) (6.6%) and others (17%). Notably, patients with the psychiatric/psychosomatic comorbidities presented higher intensity of itch, longer duration and coexistence of chronic scratch lesions. Dermatologists and psychiatrists often utilize psychoactive drugs in order to alleviate itch of different origins; however, itch may also be induced by the use of selective serotonin reuptake inhibitors or neuroleptics (79, 80).

There are also reports in the literature concerning "classic" pruritic disorders, which were also evaluated with regard to psychiatric comorbidities. Gupta et al. (81) linked alleviation of itch in psoriasis with changes in depression scores. Subsequently, Conrad et al. (43) executed a complex study of 41 patients with chronic idiopathic urticaria (CIU) and 44 patients with psoriasis. The investigators assessed the relationship between itch and several domains, including emotional distress and anger (assessed via the Symptom Checklist 90-R (SCL-90-R) and State Trait Anger eXpression Inventory (STAXI) tools, respectively). In patients with chronic

idiopathic urticaria (CIU) anger was a predictor of itch severity, whereas depression seemed to influence itch severity in patients with psoriasis. Regarding patients with CIU, the authors discussed possible pathway involving anger and stress, which stimulate corticotrophin-releasing hormone, subsequently leading to mast cell activation and degranulation of mediators, such as histamine. These aspects might, at least, partially account for the presence of itch accompanying urticarial wheals. In a study by Dazzi et al. (66) 20 subjects with PM were compared with healthy controls with regards to scores in EPQ-R, the Beck Depression Inventory second edition (BDI-II) and the State Trait Anxiety Inventory – form Y (STAI). It was observed that patients with PN exhibited higher scores for the T-anxiety scale (STAI – form y-2; describing how subject feel in general), depression and neuroticism, while lower than the controls concerning extraversion. Subsequently, PN was linked to depression (adjusted odds ratio (OR) 2.82; $p<0.001$), the use of antidepressants (adjusted OR 2,6; $p<0.001$), anxiety (adjusted OR 2.06; $p<0.05$) and the use of anxiolytics (adjusted OR 4.64; $p<0.001$) compared with healthy controls (82). Similar relations were reported in a recent study concerning PN burden with respect to depression and anxiety. In addition, patients with PN more often had suicidal ideation (83). In a previously mentioned study by Remröd et al. (67) ($n=101$) subjects with plaque psoriasis with severe itch presented higher scores for depression and anxiety (as assessed via STAI and BDI-II). A study among 27 patients with AD reported that there is a connection between high scores on the depression scale (Hospital Anxiety and Depression Scale; HADS-D) and higher increase in itch intensity compared with controls (69).

Interestingly, a study by Weisshaar et al. (84) recounted that affective reactions, such as depression and aggression, were more common in German individuals with CI due to dermatological diseases than those with CI associated with underlying systemic disorders ($p=0.04$ and $p=0.03$, respectively). A comparison between German and Ugandan patients with CI was also performed in terms of emotional reactions, revealing that German patients tend to be significantly more aggressive ($p<0.0001$) and more often do not have any drive ($p<0.0001$). In a cohort of patients with end-stage renal disease, the severity of CI (4IIQ) was correlated with depressive symptoms (assessed by BDI) (85). The complicated itch and psyche interplay is elegantly embraced in functional itch disorder (FID; also termed psychogenic itch). This entity was defined as "an itch disorder, where itch is at the centre of the symptomatology, and where psychological factors play an evident role in the triggering intensity, aggravation or persistence of the pruritus" (50, 86) and can be diagnosed according to several criteria. The 3 compulsory criteria encompass: (*i*) localized or generalized itch without primary skin lesions, (*ii*) chronic pruritus of at least 6 weeks' duration, and (*iii*) no somatic cause. In addition, at least 3 out of the following 7 additional criteria have to be found: (*i*) a chronological relationship of pruritus with 1 or several life events that could have psychological repercussions, (*ii*) variations in intensity associated with stress, (*iii*) nocturnal variations, (*iv*) predominance during rest or inaction, (*v*) associated psychological disorder, (*vi*) pruritus that could be improved by psychotropic drugs, and (*vii*) pruritus that could be improved by psychotherapies. Regarding the International Forum for the Study of Itch (IFSI) classification according to its aetiology, FID is associated with the 4th category (psychogenic/psychosomatic origin) (3). Unfortunately, the detailed aspects associated with psyche and well-being of patients with FID in particular have rarely been investigated (87, 88).

Finally, considerations concerning psychiatric associations with itch are nowhere near complete without mentioning the risk of suicide. In a study by Halvorsen et al. (89), 3,682 adolescents responded to a special questionnaire focusing on itch, pain and suicidal ideation. Severe itch was strongly associated with suicidal ideation (OR 3.0). Among the individuals reporting itch, suicidal ideation was reported by 21.1%, in contrast to 8.4% among subjects denying itching. In a large meta-analysis, patients with AD (in which itch is generally considered a constant feature) were 44% more likely to have suicidal ideation and 36% more likely to die by suicide that those without the disease (90).

HEALTH-RELATED QUALITY OF LIFE IMPAIRMENT: THE BURDEN OF SCRATCHING

QoL may be defined as a measure of the goodness of several life aspects, e.g. reactions to life occurrences, disposition, sense of life fulfilment and satisfaction, as well as satisfaction with work and personal relationships (91). Not infrequently, this term is confused with HRQoL, which has multiple definitions. One of the most relevant encompasses "how well a person functions in their life and his or her perceived well-being in physical, mental and social domains of health" (92). The impairment in HRQoL in dermatological patients can be measured with multiple tools, e.g. the Dermatology Life Quality Index (DLQI) (93) or Skindex (94). Impairment in HRQoL stems from various disease-related signs and symptoms, with a special emphasis on CI. Recently, an itch-specific instrument (ItchyQoL) for assessing HRQoL has been validated in several languages (95).

There is recent literature focusing on itch and HRQoL, both in cutaneous and primarily extracutaneous disorders. These issues were studied in detail in subjects with psoriasis. According to Yosipovitch et al. (96), itch bothered 84 (84%) patients, among whom 35% became more agitated, 24% became depressed, 30% had trouble concentrating, and 23% changed their eating habits.

Remarkably, two-thirds of the patients were bothered by difficulties falling asleep and night awakenings due to itch. Moreover, 40% of pruritic subjects reported decreased or non-existent sexual desire, whereas 35% reported decreased or non-existent sexual functions. Subsequently, our group found that HRQoL, assessed via the DLQI, was significantly decreased in patients with itch (6). In addition, the DLQI score correlated with itch intensity assessed via the 4-Item Itch Questionnaire (4IIQ) and visual analogue scale (VAS). The impact of itch on HRQoL in AD is well-documented (35, 97); it is imperative to acknowledge its detrimental influence on children's and their parents' sleep (98). In our study among patients with hidradenitis suppurativa (HS), itch was reported by 62.1% of patients (99). Its presence did not correlate with DLQI scores, whereas its intensity did. Other researchers proved impaired HRQoL in cutaneous T-cell lymphoma (100), dermatomyositis (101), systemic sclerosis (102) and itch following exposure to sulphur mustard (103). Notably, in a large cohort of dermatological outpatients ($n=3,485$), the presence of itching was associated with sexual dysfunction (assessed by the 9th question of the DLQI) (104).

The HRQoL issues associated with itch have also been investigated in relation to underlying systemic disorders. It was observed among German individuals that, compared with dermatological disorders, systemic disorders causing CI were more commonly associated with decreased HRQoL ($p=0.003$) (84). Nevertheless, various systemic conditions afflicting different organs have been associated with CI and impairment in HRQoL. A prominent example is CI due to end-stage renal disease. In a study by Weiss et al. (105), the authors evaluated 860 patients on haemodialysis, revealing that the point prevalence of CI was 25.2%, while the 12-month prevalence and lifetime prevalence were 27.2% and 35.2%, respectively. The SF-12 questionnaire was used to assess HRQoL, revealing that the physical component subscale was significantly more affected among those with CI ($p<0.05$). A subsequent study on the same cohort demonstrated that the mean severity of CI correlated with the total score of the ItchyQoL (106). The strongest correlation with the mean itch severity was observed with respect to the emotions subscale, followed by self-efficacy, functionality and symptoms. Our group has also evaluated CI in 200 patients with end-stage renal disease, among whom CI concerned 38% (85). Patients with uraemic pruritus had significantly lower quality of life according to the 36-item Short Form Health Survey (SF-36) (93.0 ± 20.4 vs. 99.6 ± 19.9 points, $p=0.03$). Among the SF-36 dimensions, general health perception was markedly worse among pruritic subjects ($p=0.0003$). In addition, we found significant negative correlations between the total SF-36 score and itch intensity. The debilitating impact of CI on QoL has also been investigated in other systemic conditions (chronic venous insufficiency (107), Sjogren's syndrome (108), primary sclerosing cholangitis (109), polycythemia vera (110) or HIV infection (111)), although detailed considerations are beyond the scope of this review.

THE SOCIAL WOUNDS: ITCH AND STIGMATIZATION

Stigmatization may be defined as an awareness of social disapproval, discrediting or devaluation, based on an attribute or physical mark and on social rejection (112). Unsurprisingly, stigmatization has been studied in the context of various cutaneous disorders, such as psoriasis, vitiligo, leprosy or acne, to name just a few (113). The role of itch and fatigue in experiencing stigmatization in patients with AD or psoriasis may be associated with higher levels of stress (114). In 1989 Ginsburg & Link (115) explored stigmatization in a cohort of 53 psoriatic subjects via an original questionnaire containing 33 questions focusing on 6 factors (anticipation of rejection, feelings of being flawed, sensitivity to the opinions of others, guilt and shame, positive attitudes, and secretiveness). Ninety-three percent of participants reported itch; it was observed that the extent of bleeding at the time of the study (followed by itching) were the strongest predictors of stigmatization. The possible explanation involves itch as an elicitor of scratching behaviour, which may ensue in bleeding. To the best of our knowledge, this was the first experimental study relating itch to stigmatization. In a study by Lu et al. (114) 131 outpatients with psoriasis and 139 outpatients with AD were evaluated by several tools, including the 6-Item Stigmatization Scale (6ISS) regarding the perceived stigmatization on a 4-point Likert scale. Subsequently, our group investigated the well-being of 102 patients with plaque-psoriasis, among whom itch affected 89.2% (6). The intensity of itch correlated significantly with the level of stigmatization assessed via the 6ISS, as well as the Feelings of Stigmatization Questionnaire. Regarding the latter, the domains "feeling of being flawed", "sensitivity to other attitudes" and "secretiveness" were mostly influenced. Another study among Arabic subjects with psoriasis ($n=108$) revealed that itching (present in 78.7%) predicted stigmatization according to the Feelings of Stigmatization Questionnaire, whereas the intensity of itching significantly correlated with stigmatization level assessed via the 6ISS (116).

CONCLUSION

Despite a constantly increasing volume of data, there are still many unresolved questions about the phenomenon of itch. The known associations between itch and psyche are bilateral and multidimensional, posing challenges for clinicians. Taking into account the abundance of both ex-

perimental and clinical findings, coupled with increasing experience and involving psychiatrists, psychologists and other specialists in the field, is the basis of the holistic approach to the patient. This is a sure recipe for better management of both skin and psyche, as they constitute an unusual union that lasts a lifetime.

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REVIEW ARTICLE

A New Generation of Treatments for Itch

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For decades, antihistamines have been the mainstay of treatment for chronic pruritus, yet they often only work by making patients drowsy and forgetful of their itch. A new era of antipruritic drugs is quickly approaching, presenting more effective treatments for patients suffering from chronic itch. Several treatments have been developed targeting specific receptors in the nervous system, such as the transient receptor potential channels, sodium channels, neurokinin-1 receptors, opioid receptors, and many more. Additionally, antipruritic therapies developed to work on the immune system have become more targeted, leading to greater safety and efficacy measures. These include crisaborole, several interleukin antagonists, and janus kinase inhibitors. The promising results presented with these new antipruritic therapies allow physicians to be better equipped to treat their itchy patients.

Key words: pruritus; antipruritics; cytokines; unmyelinated nerve fibers.

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Chronic itch can negatively impact sleep, mood, and quality of life (1), causing patients to become desperate for relief. For decades, clinicians have resorted to antihistamines as a primary treatment for itch. However, the majority of chronic pruritus cases do not respond to antihistamines. In fact, they are not at all effective, and only make patients drowsy, forgetting that they are itchy.

With a more profound understanding of the pathophysiology of itch, newer and better targets for treatment have arisen. Medications working on the nerves, such as gabapentin and pregabalin, have improved symptoms, especially in cases of neuropathic itch such as brachioradial pruritus and notalgia paresthetica. Likewise, cases of inflammatory itch, such as psoriasis and recently atopic dermatitis (AD), have been dramatically improved by the advent of immunomodulating therapies.

New treatments for itch are continuously being developed. Herein, we will first discuss new antipruritic therapies working on the nervous system, and next we will discuss the antipruritic therapies targeting the immune system.

SIGNIFICANCE

Itch is a pesky sensation that can be difficult to eliminate. Although a mainstay of anti-itch therapy for many decades, antihistamines are not an effective therapy for patients with chronic, unrelenting itch. With a greater understanding of itch, newer treatments have been developed that are much more effective. These include drugs targeting the neural system and drugs that affect the immune system.

TREATMENTS TARGETING THE NERVES

The sensation of itch is transmitted by unmyelinated C nerve fibers originating in the skin, synapsing in the spinal cord, and traversing the spinothalamic tract to the thalamus, before being further projected to various areas in the brain (2). Yet, the sensation of itch is not that simple. At the levels of the skin, spinal cord, and brain is an additional mechanism referred to as neural sensitization. This phenomenon causes the itch-selective neurons to become hypersensitive to pruritic stimuli (2). In the skin, neuronal sensitization is the result of inflammation, abnormal epidermal innervation, and dysfunction of cutaneous touch receptors. Dysfunction and attenuation of the inhibitory spinal circuits lead to neural sensitization at the level of the spinal cord. Finally, in the brain, chronic pruritus can lead to functional and structural changes in brain connectivity and activation, causing neural sensitization (2–4).

At each step of this pathway is an array of receptors involved in transmitting this pesky symptom. Discovery of the involvement of these receptors and their ligands in itch has led to development of novel targeted therapies. **Fig. 1** diagrams where these antipruritic drugs targeting the nerves act in the skin, spinal cord, and brain.

Neurokinin-1 inhibitors

Neurokinin-1 (NK-1) serves as a receptor for substance P (SP), a known pruritic mediator. NK-1 is located throughout the central nervous system and skin. Activation of NK-1 by SP leads to pro-inflammatory cytokine production and mast cell release of pruritic mediators such as histamine, tumor necrosis factor (TNF)-α, prostaglandin D2, and leukotriene B4 (5).

Aprepitant, an NK-1 inhibitor originally developed to treat chemotherapy-induced nausea, is effective for treatment-refractory pruritus, as well as prurigo nodularis

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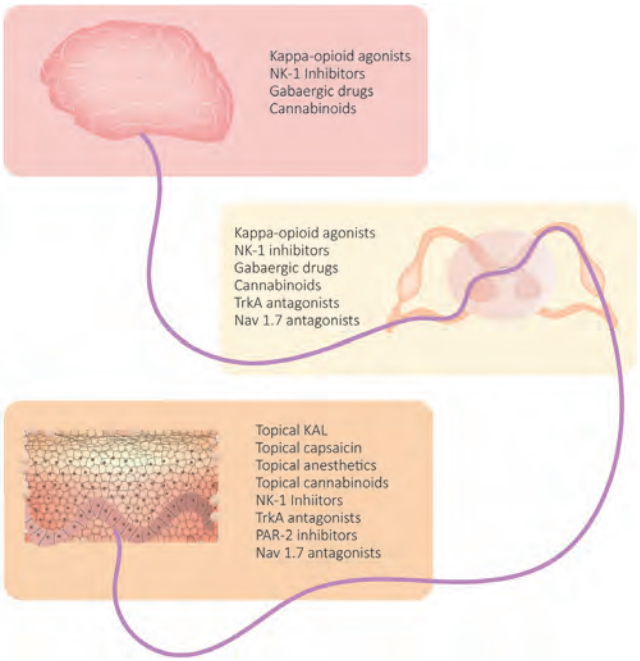

Fig. 1. Site of action of new antipruritic drugs targeting the neural system. NK-1: meurokinin-1; TrkA: tropomyosin receptor kinase A; PAR-2: protease-activated receptor-2; KAL: ketamine-amitriptyline-lidocaine; Nav1.7: voltage-gated sodium channel 1.7.

and cutaneous T-cell lymphoma (5, 6). Unfortunately, aprepitant is expensive and has a multitude of potential drug interactions, making it difficult to administer to patients (2, 7)

Serlopitant and tradipitant, newer NK-1 inhibitors, may be better alternatives and are currently being assessed in clinical trials (**Table I**). In randomized, placebo-controlled, phase II clinical trials, serlopitant exhibited a statistically significant decrease in pruritus in patients with treatment-refractory itch as well as prurigo nodularis (8, 9). In patients with AD, tradipitant showed a statistically significant decrease in itch in a randomized, placebo-controlled, phase II trial (10).

Opioids

Opioids are classically thought of as highly effective pain medications, but more recently, opioids have also been shown to play a significant role in the treatment of pruritus. μ-, κ-, and δ-opioid receptors exist throughout the central and peripheral nervous systems, including the peripheral nerve fibers in the skin (11). In the spinal cord, imbalance in the activation status of μ- and κ-opioid receptors result in neuronal sensitization, which can lead to chronic itch (2). Similarly, an imbalance can occur in the periphery, for example a decrease in the expression of κ-opioid receptors seen in the epidermis of patients with AD (12).

μ- and κ-opioid receptors have been well-studied as they relate to pruritus, while the role of δ-opioid receptors in itch remains poorly understood (11). Specifically, μ-opioid antagonists and κ-opioid agonists are effective in treating itch.

Mixed μ-opioid antagonists and κ-opioid agonists. Butorphanol, both a μ-opioid antagonist and κ-opioid agonist, treats pruritus of varying etiologies with high efficacy. It is administered intranasally and has a rapid onset of action. Most importantly, it has little abuse potential (13). Butorphanol presents a great treatment option especially in cases of refractory chronic itch. However, its mode of intranasal administration is not something that dermatologists feel comfortable to use.

More recent developments are similar drugs like nalbuphine, a mixed μ-opioid antagonist and κ-opioid

Table I. New antipruritic drugs targeting the neural system with corresponding ongoing clinical trials

Category	Drug name	Indication	Phase	Administration	NCT #
Neurokinin receptor-1 inhibitor	Serlopitant	Prurigo nodularis Atopic dermatitis Psoriasis	3	Oral	NCT03540160
		Chronic pruritus of unknown origin	2	Oral	NCT03841331
		Prurigo nodularis	3	Oral	NCT03677401 NCT03546816
		Epidermolysis bullosa	2	Oral	NCT03836001
	Tradipitant	Atopic dermatitis	3	Oral	NCT03568331
μ-opioid antagonist/κ-opioid agonist	Nalbuphine	Prurigo nodularis	2/3	Oral	NCT03497975
κ-opioid agonist	Nalfurafine	Uremic pruritus		Oral	
	Asimadoline	Atopic dermatitis		Oral	
	CR845	Uremic pruritus	3	IV	NCT03998163 NCT03636269 NCT03281538
		Chronic kidney disease	2	Oral	NCT03617536
		Cholestatic pruritus	2	Oral	NCT03995212
		Atopic dermatitis	2	Oral	NCT04018027
	MR13A9	Pruritus in hemodialysis patients	2	IV	NCT03802617
	SHR0410	Pruritus in hemodialysis patients	1	IV	NCT03857568
TrkaA antagonist	CT327	Psoriasis		Oral	
PAR-2 inhibitor	Doxycycline	Hives	2	Topical	NCT03968562
γ-aminobutyric acid analog	Pregabalin	Recessive dystrophic epidermolysis bullosa	3	Oral	NCT03928093
Ileal bile acid transporter inhibitor	GSK2646264	Cholestatic pruritus	2	Oral	NCT02966834

agonist. Clinical trials measuring its efficacy in treating uremic pruritus and prurigo nodularis have shown encouraging results (Table I) (14, 15).

K-opioid agonists. Nalfurafine, a κ-opioid agonist available in Japan, is an effective antipruritic agent in patients with uremic pruritus (16). In the United States, no κ-opioid agonists have yet been approved by the U.S. Food and Drug Administration (FDA) for the treatment of pruritus; however, clinical trials are underway. In a phase II clinical trial (17), intravenous (IV) CR845, a κ-opioid agonist, had significant antipruritic effects for pruritus associated with end-stage chronic kidney disease. Specifically, patients receiving CR845 three times a week after dialysis had a 68% greater reduction from baseline in worst itch scores compared to those receiving placebo (17). Clinical trials evaluating use of CR845 in other pruritic conditions are currently underway (Table I).

Asimadoline, a κ-opioid agonist originally developed for irritable bowel syndrome, completed phase II trials for pruritus associated with AD, but results have not yet been published (18). Likewise, SHR0410 and MR13A9 are undergoing phase I and II clinical trials, respectively, for treatment of pruritus in hemodialysis patients.

Tropomysin receptor kinase A antagonists

Epidermal keratinocytes and eosinophils release nerve growth factor (NGF), which binds to its receptor, tropomyosin receptor kinase A (TrkA). This leads to neural sensitization of transient receptor potential vanilloid 1 (TRPV1) and increased nerve sensitivity to SP, calcitonin gene-related peptide (CGRP), and brain-derived neurotrophic factor. Furthermore, NGF causes sensitization of the skin to nonhistaminergic cowhage-induced itch. Together, the TrkA-NGF pathway leads to hypersensitivity of peripheral sensory nerves to pruritic stimuli, and therefore presents a compelling target for antipruritic treatment (2).

In a phase IIb clinical trial of patients with psoriasis, CT327, a topical TrkA antagonist, demonstrated a statistically significant reduction in pruritus (19). Another Phase IIb trial with the same TrkA antagonist (SNA-120) also showed a robust reduction of pruritus, with 58% of patients receiving a meaningful itch reduction of 4.3 in Numerical Rating Scale (NRS). However, the vehicle also had significant anti-pruritic effects with 53% of patients receiving reduction in their itch, and the difference was not statistically significant (20).

Protease activated receptor-2 inhibitors

Protease-activated receptor-2 (PAR-2) is a type of G-protein coupled receptor activated by proteolytic cleavage of its extracellular N-terminus. For example, cowhage, the well-known inducer of nonhistaminergic itch, contains the protease mucunian, which activates PAR-2, as well as PAR-4, causing pruritus. Several other proteases can similarly activate PAR-2, causing symptoms of itch and making it a good target for treatment (2).

PZ-235, a pepducin that inhibits PAR-2, showed efficacy in the reduction of itching behaviors in a mouse model of AD (21). In humans, a one-time application of a different topical PAR-2 inhibitor led to a significant reduction in ratings of cowhage-induced itch intensities in a placebo-controlled study (22). Randomized, placebo-controlled clinical trials are warranted to determine whether the efficacy of PAR-2 inhibitors in treatment of various chronic itch conditions.

Interestingly, doxycycline, an antibiotic, has shown antipruritic properties in the treatment of acne vulgaris. In addition to its ability to reduce inflammation, its antipruritic mechanism is most likely due to its attenuation of the PAR-2 interleukin (IL)-8 pathway (23).

GABAergic drugs

Gabapentin and pregabalin, analogs of γ-aminobutyric acid (GABA) (an inhibitory neurotransmitter), have proven effective in treating various types of neuropathic itch (24). More specific GABAergic drugs are currently in development. In mice, targeting inhibitory α2 and α3 $GABA_A$ receptors reduced acute histaminergic and non-histaminergic pruritus. Furthermore, this α2/α3 $GABA_A$ modulator reduced chronic pruritus in a mouse model of AD and in dogs who were sensitized to house dust mites (25). Most importantly, these antipruritic effects seemed to come without any unwanted adverse effects (25).

Nav 1.7

An antibody inhibiting voltage-gated sodium channel (Nav) 1.7 with high selectivity suppressed chronic and acute itch in mice (26). This study indicated that Nav 1.7 is in fact involved in both histamine-dependent and -independent pruritus, and modulates spinal cord synaptic transmission for both itch and pain (26). Currently, Nav 1.7 antagonists are still in clinical development. Neu-P12, a Nav 1.7 antagonist, is currently being studied in phase I clinical trials for neuropathic pain (27), and will be interesting to see if it has an effect on itch.

TREATMENTS TARGETING THE IMMUNE SYSTEM

The immune system plays an important role in itch, especially in inflammatory pruritic conditions. Classically, systemic immunosuppressive agents, such as glucocorticoids, methotrexate, cyclosporine, and azathioprine were the most effective therapeutic agents available, and although still often used as first-line treatment, can come with some potentially serious adverse effects.

Recently developed immunosuppressive treatments have a more specified mechanism of action, producing a

higher level of efficacy and safety. Moreover, the era of biologic therapies is still ongoing and vastly expanding to include various itchy skin conditions, with clinical trials steadily underway (**Table II**) (28).

Phosphodiesterase-4 inhibitors

Crisaborole is a topical non-steroidal phosphodiesterase-4 (PDE4) inhibitor approved for the treatment of moderate-to-severe AD. Applied as an ointment, crisaborole has proven to be effective in rapidly reducing pruritus in these patients. Pruritus relief was observed in significantly more patients receiving crisaborole ointment than vehicle in a *post hoc* analysis of two phase III clinical trials (29). This study showed that this rapid antipruritic effect was seen as early as day 2, and that 20% of patients receiving crisaborole experienced complete relief of their pruritus by day 6 (29).

Furthermore, a significant and strong link has been seen between pruritus and dermatology-specific quality of life scores. In a *post hoc* analysis of two phase III clinical trials, as patients' itch improved with the help of crisaborole, so did their quality of life scores (30).

Similarly, improved quality of life scores and greater reductions in pruritus as measured by a visual analogue scale (VAS) were achieved in patients with plaque psoriasis receiving apremilast, an oral PDE4 inhibitor (31). The efficacy of apremilast as an antipruritic therapy in patients with scalp psoriasis is currently being studied in phase IV trials (Table II).

Interleukin antagonists

Interleukins (IL) are cytokines which help mediate immune responses and inflammation. Cytokines can act on a number of different targets including immune cells,

Table II. New antipruritic drugs targeting the immune system with corresponding ongoing clinical trials

Target	Drug name	Indication	Phase	Vehicle	NCT #
Phosphodiesterase-4	Apremilast	Scalp psoriasis Psoriasis vulgaris	4	Oral	NCT03553433
		Plaque psoriasis	3	Oral	NCT03721172
	Crisaborlole	Atopic dermatitis		Topical	
Interleukin-4Ra	Dupilumab	Chronic spontaneous urticaria	2	Subcutaneous	NCT03749135
		Cholinergic urticaria	2	Subcutaneous	NCT03749148
	Pitrakinra	Atopic dermatitis		Subcutaneous	
Interleukin-13	Tralokinumab	Atopic dermatitis	3	Subcutaneous	NCT03587805 NCT03761537 NCT03526861
	Lebrikizumab	Atopic dermatitis		Subcutaneous	
	Nemolizumab	Prurigo nodularis		Subcutaneous	
Interleukin-31RA	Nemolizumab	Atopic dermatitis	2	Subcutaneous	NCT03921411
		Atopic dermatitis	3	Subcutaneous	NCT03989206 NCT03985943 NCT03989349
Oncostatin M receptor-β	KPL-716	Prurigo nodularis	2	Subcutaneous	NCT03816891
		Chronic idiopathic urticaria Chronic idiopathic pruritus Lichen planus Lichen simplex chronicus Plaque psoriasis	2	Subcutaneous	NCT03858634
Interleukin-17A	Secukinumab	Atopic dermatitis	2	Subcutaneous	NCT03568136
	Ixekizumab	Psoriasis, genital pruritus		Subcutaneous	
Janus kinase 1/JAK 2 or 3	Baricitinib	Atopic dermatitis	3	Oral	NCT03435081 NCT03733301 NCT03334435 NCT03428100 NCT03952559
	Ruxolitinib	Atopic dermatitis	3	Topical	NCT03745651 NCT03745638
	Tofacitinib	Psoriasis Atopic dermatitis		Oral Topical	
Janus kinase 1	Upadacitinib	Atopic dermatitis	1	Oral	NCT03646604
		Atopic dermatitis	3	Oral	NCT03607422 NCT03569293 NCT03568318 NCT03738397
	Abrocitinib	Atopic dermatitis	2	Oral	NCT03915496
		Atopic dermatitis	3	Oral	NCT03575871 NCT03627767 NCT03422822 NCT03720470 NCT03796676
IgE	Ligelizumab	Chronic spontaneous urticaria	3	Subcutaneous	NCT03580356 NCT03580369
Histamine 4 receptor	ZPL389	Atopic dermatitis	2	Oral	NCT03948334 NCT03517566

keratinocytes, and even sensory nerves (**Fig. 2**) (32). Some of the newer cytokines used as targets in regard to treating itch are discussed below and include IL-4, IL-13, IL-31, and IL-17.

IL-4. Dupilumab is a monoclonal antibody targeting the α subunit of the IL-4 receptor, blocking the signaling of cytokines IL-4 and IL-13, key cytokines involved in T-helper (Th) 2 immunity. Dupilumab has revolutionized the treatment of AD, significantly improving clinical symptoms of AD, rapidly reducing itch, and improving patients' quality of life (33, 34).

Currently, the use of dupilumab in other pruritic conditions is of great interest. Case reports have shown that dupilumab can be helpful in the treatment of patients with prurigo nodularis (35–37), uremic pruritus (38), and bullous pemphigoid (39). However, randomized, placebo-controlled trials are necessary to evaluate its true efficacy in other pruritic conditions. Clinical trials assessing its efficacy in chronic spontaneous urticaria and cholinergic urticaria are currently underway (Table II).

Similar to dupilumab, pitrakinra also targets the IL-4 receptor α subunit, inhibiting IL-4 and IL-13 signaling (40). Pitrakinra has mostly been studied in the treatment of asthma (41). Subcutaneous administration of pitrakinra in moderate to severe AD was investigated in a phase II clinical trial, however results have not been published (40, 42).

IL-13. This cytokine produced by Th2 lymphocytes, is implicated in the pathway of AD. Development of therapies targeting this cytokine are of great interest in treating AD. Two biologic drugs targeting IL-13 that are under investigation for treatment of AD include lebrikizumab and tralokinumab.

Lebrikizumab targets soluble IL-13 and binds with high affinity, preventing binding to the IL-4 receptor α subunit and subsequent signaling (28, 43). In a phase II randomized, placebo-controlled trial (43), patients receiving lebrikizumab in combination with topical corticosteroids showed a significantly greater achievement of 50% reduction in eczema area and severity (EASI) score when compared to placebo. As for pruritus, mean percent reductions in baseline itch as assessed by VAS were not statistically significant when compared to placebo in this study (43). However, in a phase IIb study, lebrikizumab showed dose-dependent improvements in pruritus as early as day two, continuing until day 16 (44).

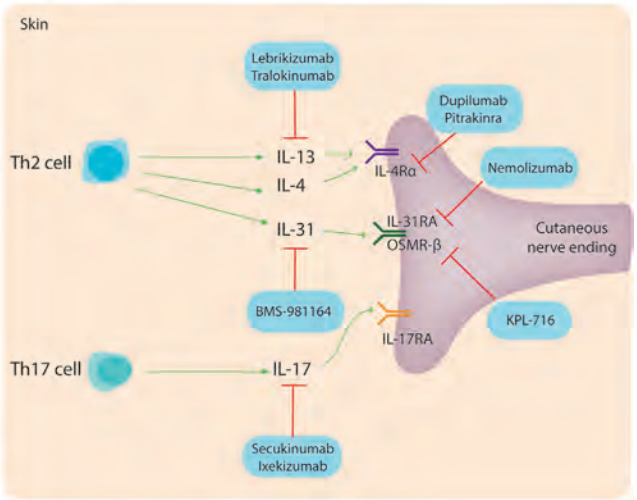

Fig. 2. Effect of interleukin (IL) antagonists on sensory nerves in the skin. OSMR: oncostatin M receptor.

Tralokinumab potently binds to IL-13, prohibiting its binding to IL-13 receptor subunit α-1 and IL-13 receptor subunit α-2, neutralizing its effects (45). Results from a phase II study have indicated promising results for patients with moderate to severe AD treated with tralokinumab (46). At a dose of 300 mg administered subcutaneously every other week, patients received clinically significant improvements in EASI score, Scoring of Atopic Dermatitis (SCORAD), and the Dermatology Life Quality Index (DLQI). Additionally, a significant decrease in pruritus assessed by NRS was seen in patients treated with tralokizumab compared to placebo (46). Phase III trials are currently underway (Table II).

IL-31. This cytokine is heavily implicated in the pathophysiology of chronic pruritus. Increased levels of IL-31 have been associated with a variety of itchy conditions such as AD, prurigo nodularis, cutaneous T-cell lymphoma, mastocytosis, chronic spontaneous urticaria, and bullous pemphigoid, making it a desirable pharmacological target for treatment of these patients (4, 47).

Nemolizumab, the first drug developed to inhibit IL-31 signaling, works by binding to IL-31 receptor A, which is located on a variety of cells including neurons, keratinocytes, macrophages, dendritic cells, and basophils (48). Thus far, nemolizumab has only been studied as a treatment for AD and prurigo nodularis. Pruritus was significantly improved in patients with moderate to severe AD in a phase II, randomized, double-blind, placebo-controlled clinical trial (49). As for prurigo nodularis, a phase II trial was recently completed with positive results (50).

BMS-981164, a monoclonal antibody targeting circulating IL-31, has completed a phase I clinical trial in AD, but no results have been published to date (51).

KPL-716, a monoclonal antibody against oncostatin M receptor beta (OSMR-beta), interferes with IL-31 and oncostatin M (OSM) signaling and has shown an antipruritic effect in patients with AD (52). A phase II clinical trial is currently recruiting patients with prurigo nodularis to assess the efficacy of KPL-716 in reducing their itch (53). Additionally, a pilot phase II study is assessing the efficacy of KPL-716 in reducing pruritus associated with chronic idiopathic urticaria, lichen planus lichen simplex chronicus, plaque psoriasis, and chronic idiopathic pruritus (Table II) (54).

IL-17. This is a pro-inflammatory cytokine that is primarily produced by Th17 cells, along with neutrophils and possibly mast cells. Keratinocytes are stimulated by IL-17A to secrete other pro-inflammatory mediators, which recruit neutrophils, Th17 cells, dendritic cells, and lymphoid cells (55). Drugs in this category are currently FDA-approved for the treatment of plaque psoriasis, and based on current studies, have shown the largest magnitude of effect in reducing psoriatic itch (5).

Secukinumab is a monoclonal antibody that selectively binds and neutralizes IL-17A (55). Two phase III, double-blind clinical trials assessing the efficacy of secukinumab in psoriasis showed that secukinumab was significantly more effective in reducing itch compared to placebo and etanercept, a TNF-α inhibitor (55, 56). In fact, in a pooled analysis of these two phase III trials, patients taking secukinumab achieved a significantly greater reduction in itching as early as the second week of treatment, demonstrating its rapid effect (57).

Phase II clinical trials assessing the role of secukinumab in treating AD are currently ongoing (Table II) (58, 59).

Ixekizumab is a high affinity monoclonal antibody also targeting IL-17A. Ixekizumab has proven to be effective in treating psoriasis, especially psoriatic itch in which phase III trials showed rapid, significant improvements. As early as the first week of treatment with ixekizumab, a significantly greater percentage of patients receiving the drug reported improvement of pruritus compared to those receiving etanercept or placebo (60).

In a long-term extension study of this trial, patients receiving ixekizumab maintained improvements in itch severity through the end of the study. By week 60, 48.2% of patients receiving ixekizumab every 4 weeks throughout the study achieved an itch NRS of 0. Similarly, after being switched to ixekizumab at 12 weeks, 45.1% and 45.3% of patients originally receiving placebo or etanercept, respectively, achieved an NRS of 0 at week 60 (61).

Additionally, ixekizumab is also effective in rapidly reducing genital pruritus. In a phase III, randomized, double-blind, placebo-controlled trial, a greater percentage of patients with genital psoriasis receiving ixekizumab achieved a significantly greater clinically meaningful itch reduction (greater than or equal to 3 point reduction on a numerical rating sale) than patients receiving placebo (59.7% versus 8.3%, $p < 0.001$) (62). Furthermore, a significant improvement in genital itch was seen as early as week 2 in those treated with ixekizumab (62).

Janus kinase inhibitors

Pruritus induced by cytokines is mediated at least partially by the janus kinase (JAK)/signal transducer and activation of transcription (STAT) pathway (2). JAK inhibitors block the JAK/ STAT pathway, which mediates signal transduction of cytokines and growth factors. When ligands bind to their receptors, JAKs are activated which lead to phosphorylation of STATs, which enter the cell nucleus to regulate transcription of target genes (63).

JAK inhibitors are commonly used in the treatment of inflammatory conditions such as rheumatoid arthritis. More recently, these medications are being investigated for use in chronic inflammatory skin conditions, such as AD and psoriasis (Table II).

Tofacitinib, an oral JAK inhibitor, works by blocking JAK1 and JAK3. It has been investigated for the treatment of psoriasis, with results from two randomized phase III trials showing that tofacitinib improved itch in patients with psoriasis, as soon as one day after treatment initiation (64, 65). Patients receiving tofacitinib also achieved a significant improvement in health-related quality of life, which were maintained through the end of the study at week 52 (64). Topical tofacitinib has also been effective for treating pruritus in both patients with psoriasis as well as AD (66, 67).

In a mouse model of psoriasis, tofacitinib significantly decreased mRNA expression of itchy cytokines IL-22, IL-23, and IL-31. This study also demonstrated that tofacitinib increased peptidergic epidermal nerve fiber density, which may aid in rescuing inhibitory itch mechanisms, proposing a novel mechanism for itch reduction by tofacitinib (68).

Additionally, JAK inhibitors may show promise in treating other cases of itch, even non-inflammatory causes of pruritus. In five patients with refractory, chronic idiopathic pruritus, oral tofacitinib led to marked improvement in their itch after only one month (69).

Baricitinib selectively inhibits JAK1 and JAK2. In a phase II, double-blind, randomized trial, baricitinib significantly improved AD and resulted in decreased pruritus (70). Phase III studies are currently underway (Table II).

Upadacitinib is a newer JAK inhibitor whose mechanism of action is selective for JAK1. In a phase II, randomized, placebo-controlled trial, upadacitinib significantly decreased ratings of itch in patients with AD (71). Phase III trials measuring the efficacy of upadacitinib in AD are currently in progress (Table II) (72).

Abrocitinib, another JAK inhibitor specifically targeting JAK1, has demonstrated excellent results in the treatment of AD. In a phase III randomized, double-blind, placebo-controlled trial, a statistically significant greater proportion of patients taking abrocitinib achieved a 4 point or larger reduction in itch NRS versus those taking placebo. Likewise, patients taking abrocitinib achieved a statistically significantly greater magnitude of decrease in the Pruritus and Symptoms Assessment for Atopic Dermatitis (PSAAD) compared to patients receiving placebo (73).

Histamine-4 receptor antagonists

Antihistamines, most of which are antagonists to the histamine-1 receptor, are often given to patients endor-

sing itch despite no clear evidence of their effectiveness as antipruritic therapies. Antihistamines specifically targeting the histamine-4 receptor (H4R), however, have shown some promise in treating itch (74). In a mouse model of AD, pretreatment with an H4R antagonist attenuated scratching responses in a dose-dependent manner (75).

ZPL389, an oral H4R antagonist, is currently being studied as a treatment for AD (Table II). However, results from a phase II randomized, double-blind, placebo-controlled study did not show a significant difference in pruritus reduction between patients taking ZPL389 and those receiving placebo (76).

Anti-IgE

A monoclonal anti-IgE antibody, ligelizumab, is undergoing phase III clinical trials to investigate its efficacy in treating chronic spontaneous urticaria (Table II). Studies have shown that ligelizumab has a much higher affinity to bind IgE in comparison to omalizumab (77), and thus it will be interesting to see its effect on pruritus in these patients.

CONCLUSION

New therapies for itch are continuously being developed. A new era of antipruritic drugs targeting specific neural receptors, itchy cytokines, and small molecules is swiftly approaching. Now that the pathophysiology of pruritus is better understood and research into new targets and mechanisms is perpetually underway, discovery and development of newer and better treatments for itch is ongoing. Unfortunately, treatment for chronic itch is not always simple and every patient requires individualized therapy. With education and development of new targets, clinicians can obtain a greater arsenal of treatment for their patients, to successfully treat them and improve their quality of life.

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Centenary theme section: ITCH AND PRURITIC DISORDERS

REVIEW ARTICLE

Challenges in Clinical Research and Care in Pruritus

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Chronic pruritus is a frequent global condition. The pathophysiology, underlying aetiology, clinical manifestation, associated burden and response to therapy of chronic pruritus varies from patient to patient, making clinical research and management of this condition challenging. There are still several unmet needs, such as the need to standardize translational research protocols, diagnostic and therapeutic procedures and to enhance the knowledge of the humanistic and economic burden associated with chronic pruritus. Basic and clinical research is of the utmost importance to target these matters. Clinical research has the potential to identify new relevant mechanisms in affected patients, which may lead to identification of novel therapy targets. This article discusses in depth current shortcomings in the daily care of patients with chronic pruritus and the challenges clinical researchers and physicians treating chronic pruritus face in addressing these matters.

Key words: itch; patient-reported outcome; guideline; clinical trials; clinical research.

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Chronic pruritus (CP), defined as pruritus lasting for 6 weeks or longer, is highly prevalent, affecting approximately one-sixth of the population in Germany (1). Population-based analyses of the nationwide search volume of pruritus on Google suggest an even larger number of individuals suffering from CP within Germany and the USA (2–4). Those affected often report a substantial burden and a relevant impairment of their quality of life (QoL) (5, 6). The aetiology, clinical manifestation, diagnostic measures and therapeutic response to CP vary from patient to patient, which contributes to the challenging management of CP (7). Although recent years have witnessed a marked increase in basic and clinical research efforts in this field, CP researchers and physicians treating CP currently face several challenges when targeting unmet needs that impact on clinical routine. The aim of this review is to highlight and discuss methodologies used in CP research, as well as current challenges and unmet needs in clinical care.

SIGNIFICANCE

Itch lasting for 6 weeks or more is considered chronic and represents a high burden for those affected. Various aspects of chronic itch, including the underlying origin of the itch, symptoms, skin manifestations, response to therapies and impairment of quality of life vary from patient to patient, constituting a challenge for clinicians and clinical researchers. Unmet needs, such as the standardization of experimental and clinical research protocols, diagnostic procedures and therapeutic regimens, as well as a better understanding of associated burdens and the development of novel effective therapies should be targeted by physicians and researchers dealing with chronic itch.

CHRONIC PRURITUS: A MULTIDIMENSIONAL CONDITION

CP is a heterogeneous condition in terms of demographics, clinical presentation and underlying origin. Although the prevalence of CP increases with age, patients of all age groups, including children, may be affected (8). Moreover, patients of both sexes and all ethnic backgrounds may suffer from CP. According to the International Forum for the Study of Itch (IFSI), CP may present on inflamed skin (IFSI I), on normal appearing skin (IFSI II), or accompanied by chronic scratch lesions, such as chronic prurigo or lichen simplex chronicus (IFSI III) (9). As for the underlying aetiology, CP may arise from dermatological, systemic, neurological or psychiatric/psychosomatic conditions. Some patients show multiple causes for the pruritus (multifactorial CP), while in few cases the origin of the pruritus remains unknown despite extensive diagnostic work-up (9). Recent studies have further suggested a substantial impact of climate and weather on the prevalence of pruritus, especially involving specific localizations on the body, but these issues have yet to be examined in epidemiological studies (2–4). Adding to these factors, patients with CP vary in terms of medical history, comorbidities, co-medication, socioeconomic backgrounds and therapy goals, contributing to the complexity of the management of these patients.

UNMET NEEDS IN CLINICAL RESEARCH

Due to the complexity and multidimensional nature of CP, clinical research investigating several aspects of pruritic diseases and its management is needed. In particular, clinical research contributes to a better understanding of pathophysiological mechanisms involved in the development and chronicity of CP in humans. Moreover, clinical researchers should focus on various aspects of clinical management, such as standardizing diagnostic and therapeutic procedures, and assessing the humanistic burden and impact of CP on affected patients. Another important area is the performance of longitudinal translational studies, clinical trials investigating novel agents for the treatment of CP, and psychometric research. It should also be investigated how the care of patients with CP is integrated into the health system in order to identify shortcomings and to adopt innovative strategies, as, for example, digital tools to improve care. The next section of this article discusses the challenges and difficulties with regard to these issues.

CHALLENGES IN CLINICAL RESEARCH AND CARE

Understanding the pathophysiology

Basic and clinical research has led in recent decades to a better understanding of the mechanisms underlying CP. In clinical-translational research, different methods investigating specific pathophysiological features that augment data obtained from morphological and molecular biological research have been established and validated. The collaboration with biostatisticians and experts of medical informatics is essential for the interpretation of data and for obtaining a comprehensive understanding of the results. An overview of these methods is given in **Table I**.

Several methodologies have been developed in order to investigate the functions of peripheral nerves. Skin stimulation with pruritogens (e.g. histamine, capsaicin, cowhage, β-alanine) has led to the characterization of several subpopulations of C-fibres. Histamine activates mechano-insensitive C-fibres (CMi-fibres), while cowhage and β-alanine activate heat and mechano-sensitive C-fibres (CMH-fibres) (10). Hyperknesis related to cowhage-induced pruritus, but not histaminergic pruritus, is present in several types of CP compared with controls, arguing for sensitization of CMH-fibres in CP (11). One limitation of this methodology is the high inter-individual variability, while intra-individual variability is low (12). Another method, transcutaneous electrical stimulation, allows the selective activation of subpopulations of peripheral nerve fibres. For example, using the Neurometer® C, Aδ and Aβ fibres are activated by electrical stimulation at a frequency of 5, 250 and 2,000 Hz, respectively (13). Higher itch intensities could be induced upon stimulation at 5 and 2,000 Hz in patients with CP of different origins compared with healthy individuals, arguing for hyperknesis in patients with CP (14). Of note, patients are often anxious when undergoing experimental procedures with electrical stimulation, which leads to biased ratings of evoked sensory symptoms, thus constituting a limitation of this method. Another functional well-established diagnostic tool that is used for clinical itch research is quantitative sensory testing (QST). It is a battery of psychophysical tests, in which response to graded thermal and mechanical stimuli is assessed (15). This procedure, originally developed for determination of neuropathic pain, informs about gain or loss of function of different peripheral nerve fibre subpopulations and hints at possible signs of central sensitization. In itch research it could be shown that patients with CP of different origins have an altered QST profile, arguing for disordered peripheral neuronal mechanisms (11). Although QST is widely used in neurological routine care, it has some limitations. It is time-consuming, requires highly qualified personnel, as well as the collaboration of patients. Morphological methodologies for investigation of the anatomy of cutaneous cells and expression of receptors and mediators are essential in clinical itch research. In particular, determination of the intraepidermal nerve fibre density is useful in order to reveal potential epidermal neuroanatomical alterations (16). In this examination, a skin biopsy is obtained and intraepidermal nerves are stained with the axonal marker protein gene product 9.5 (17). This simple method, which is also used in routine care, has some limitations.

Table I. Clinical research methodologies used in itch research

Methodology	Mechanism
Cutaneous stimulation with histamine	Chemical-evoked itch via activation of mechano-insensitive C-fibres (CMi-fibres), mast cell degranulation
Cutaneous stimulation with cowhage or ß-alanine	Chemical-evoked itch via activation of mechano and heat-sensitive C-fibres (CMH-fibres) as well as A-fibres.
Hyperknesis	Increased itch in response to (chemical, electrical) pruritogens or pinprick stimulation
Alloknesis	Itch response to non-pruritogenic stimuli: touch/brush strokes. Functional testing of itch sensitization phenomena with punctuate or dynamic mechanical stimulation (von Frey filaments, cotton swab)
Transcutaneous electrical stimulation	Electrical-evoked itch via selective activation of peripheral nerve fibre classes
Quantitative sensory testing	Psychophysical testing of subpopulations of peripheral nerve fibres (C-fibres, Aδ-fibres, Aβ-fibres) and signs of central sensitization using graded thermal and mechanical stimuli
Functional magnetic resonance imaging/positron emission tomography	Identification of activated and deactivated cerebral centres upon pruritic stimulation
Conditioned pruritic modulation	Central descending inhibition of pruritus
Intraepidermal nerve fibre density	Morphological determination of epidermal nerve fibre numbers (skin biopsy)

The skin probe is sensitive to the fixative and needs to be processed in a timely manner in the laboratory. In addition, reference values are available only for the distal lower leg (18, 19), thus interpretation of values from other body sites is difficult. It is a purely morphological examination, which does not inform on functional alterations of peripheral nerve fibres.

Central processing of pruritus is very challenging. Most current knowledge on spinal transmission of itch was obtained from animal studies (20). Functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies have been used to investigate transmission and processing of pruritus in upper centres. Most studies have been performed on healthy volunteers exposed to pruritic stimulation during the scans. Brain regions associated with sensory recognition, cognition, motor response and emotional states are activated in response to pruritus (21). Interestingly, both overlapping and distinct brain areas are activated upon histaminergic and non-histaminergic pruritic stimulation, pointing to differences in cerebral processing of distinct pruritus types (22). Patients with CP also show differences in brain activation upon stimulation with histamine compared with healthy individuals, arguing for central sensitization in CP (23). Also, structural changes in grey matter have been observed in patients with CP, showing the neuroplasticity in chronic states (24). Although functional imaging studies have enhanced our understanding of cerebral processing of pruritus, this methodology has some pitfalls. fMRI and PET are very expensive methodologies, which are available only in specialized centres. In addition, patients with claustrophobia, ferromagnetic prostheses or implanted devices (e.g. defibrillator) cannot undergo an MRI examination, while others (e.g. children) have difficulty lying still during the procedure, leading to poor imaging quality. More imaging studies, enrolling patients with CP of different origins, are needed in order to establish similarities and differences in cerebral brain processing of pruritus across distinct CP conditions.

Upper centres exert a descending inhibitory modulation via a noradrenergic and a serotonergic pathway, as shown by imaging studies (21, 25). The paradigm of conditioned pain modulation (CPM), in which a noxious stimulus inhibits pain elsewhere in the body, can be used experimentally to assess descending inhibition (26). In patients with CP, as in those with chronic pain, CPM is impaired, which may contribute to the chronicity of the pruritus (11). An analogous paradigm of conditioned pruritic modulation, in which a pruritic stimulus inhibits pruritus elsewhere in the body, has also been investigated, with conflicting results regarding its effect (27, 28). More studies are needed to enhance the understanding of the mechanisms of descending modulation in CP and its role in the chronicity of the disease.

Standardization of assessment instruments

Since pruritus has a subjective dimension, patient-reported outcomes (PRO) play a pivotal role. A plethora of standardized questionnaires and scales have been developed in order to gather information on several aspects of CP, including pruritus characteristics, course of the disease, QoL, reactive disorders, such as anxiety, depression and sleep impairment, and therapy goals.

Pruritus characteristics (e.g. onset, duration and distribution of pruritus, as well as accompanying sensory symptoms and scratching behaviour) can be assessed using general patient-oriented itch questionnaires (29, 30). Mono-dimensional scales, namely the visual analogue scale, the numerical rating scale and the verbal rating scale, have been validated to assess the intensity of pruritus (31). Intensity scales are also validated for the use in electronic diary applications (32). The interpretation of itch intensity scales is challenging, since scores vary from patient to patient according to external factors, such as sex or ethnic background (1, 33). Some efforts have been put into understanding the minimal clinical relevant difference of itch intensity change, e.g. after initiating a therapy (34). However, many factors (e.g. the aetiology of the pruritus, recall period, socioeconomic background) may influence the minimal clinical relevant difference, making the interpretation of results difficult. An overview of other instruments for the assessment of itch is shown in **Table II**.

As for secondary conditions arising due to CP, standardized questionnaires are used to screen for anxiety and

Table II. Examples of instruments for itch assessment

Instrument	Description
Visual analogue scale (VAS)	Monodimensional scale for assessment of itch intensity; used in clinical trials and routine care (electronic and paper versions available)
Numerical rating scale (NRS)	Monodimensional scale for assessment of itch intensity; used in clinical trials and routine care (electronic and paper versions available)
Verbal rating scale (VRS)	Categorial scale for assessment of itch intensity; used in clinical trials and routine care (electronic and paper versions available)
Labelled magnitude scale (LMS)	Monodimensional scale for assessment of itch intensity; used in experimental research (paper version)
Itch Severity Scale (ISS)	Multidimensional scale for assessment of itch intensity; used in clinical trials (paper version)
Dynamic Pruritus Score (DPS)	Patient global impression of change for assessment of itch improvement; used in clinical trials and routine care (electronic and paper versions available)
5-D itch scale (5 dimensions itch scale)	Multidimensional questionnaire for assessment of itch characteristics; used in clinical trials (paper version)
ItchyQuant	Monodimensional scale for assessment of itch intensity; combination of VAS and cartoon depictions of scratching; developed for populations with cognitive limitations (elderly, children); used in routine care (paper version)

depression (e.g. Hospital Anxiety and Depression Scale (35)), to assess sleep disorders (36, 37) and to measure the impairment of QoL (e.g. Dermatological Life Quality Index (38) and pruritus-specific ItchyQol (39)).

PRO measures are usually collected via paper and pencil questionnaires, but electronic PRO systems are emerging. Computerized collection of PRO measures offers a couple of advantages compared with paper questionnaires: reduction of errors emerging through typewriting, reduction of missing data by requiring completion, and reduction of invalid data by implementation of skip patterns (40). Furthermore, electronic assessment can increase the compliance of completing questionnaires at home by up to 70%, using, for example, computerized reminder functionality (40). To overcome the drawbacks of paper questionnaires, an electronic PRO system, enabling the patient to complete the PRO measures via digital survey, may be implemented (41–43). Since most PRO measures have been validated as paper tools, it cannot be assumed that the electronic version of the same PRO measures deliver the same results for a patient encounter. Gwaltney et al. (40) showed that "as long as substantial changes are not made to the item text or response scales, equivalence studies should not be necessary to demonstrate anew the equivalence or validity of a computerized measure". However, in order to ensure the validity of electronic measures, it would be preferable if the concrete implementations were examined using test–retest or alternate-forms reliability.

Standardization of itch assessment instruments across centres treating patients with CP is of great importance, since it would allow the comparability of data. A first step was already taken by the Task Force Pruritus of the European Academy of Dermatology and Venereology. In a consensus conference, it was agreed that pruritus intensity and QoL should be regarded as the 2 most important parameters to be assessed in routine care. The visual analogue scale and the ItchyQol were regarded as the instruments of choice (44). Another important task is the validation of the assessment tools into various languages in order to be used in clinical trials and routine care across different countries. Future clinical research should focus on showing which instruments have better utility for the clinical practice and which tools are better accepted by patients and physicians.

Understanding the humanistic and economic burden

CP, similarly to chronic pain, can lead to a severe impairment of QoL (45–47) and has negative effects on mood, ability to concentrate, quality of sleep, everyday life and work productivity (48, 49). Some patients have mental health problems, such as depression, in response to CP, and are at significantly higher risk of suicide (50, 51). QoL is a highly subjective construct and is influenced by various factors, such as pruritus intensity, but also duration, frequency and localization of pruritus. Another challenge in understanding the impact of pruritic conditions on affected patients is that cross-cultural factors influence the reported intensity of pruritus and QoL rather than the specific dermatological diagnosis. This could be found in a study in 9 European countries enrolling more than 500 patients with CP due to different dermatoses (6). Clinical research should take the cultural background of patients with CP into account when investigating the humanistic burden of pruritic conditions.

In addition to limiting QoL, there are often psychiatric complications. Patients with CP and with a psychosomatic/psychiatric comorbidity suffer more from CP and its consequences. Interestingly, even if their symptoms improve, psychological suffering is higher in these patients (52). In order to cope with these associated disorders, individual multimodal medical care is needed, not only for diagnosing the underlying disease of CP and for symptomatic antipruritic therapy, but also for adjunct therapy against, for example, sleep disorders or mental health conditions. An important challenge for clinical researchers is to detect risk factors for developing mental diseases in patients with CP and thus identify patients at risk. This would aid attending physicians in redirecting vulnerable patients to mental health professionals and ultimately to improve care.

Definition of treatment goals

As a multifactorial chronic disease, CP requires complex and cost-intensive therapy. Knowledge of the treatment goals of patients with CP is therefore of great importance in order to plan an efficient therapy regimen. Therapy goals were recorded and evaluated in 2,474 patients with CP using the Patient Benefit Index – Pruritus (PBI-P), which was validated in 2009 for patients with CP and consists of 27 items, including various aspects, such as physical and mental well-being, professional and everyday performance, social and leisure activities, and QoL in general. The relevance of these items is calculated on the basis of a 5-step Likert scale (0="not at all important" to 4="very important") (53). It was shown that, in addition to demographic data such as sex and age, the CP IFSI group, pruritus intensity and QoL had a significant impact on different patient needs. Women considered the reduction in physical and psychological symptoms, such as depressive feeling, nervousness, or burning sensations, as more important (54). For men, an important goal was the improvement in aspects of social life, such as social contacts, partnership, and sex life. Patients with CP on inflamed skin (IFSI I) or pruritus with chronic scratch lesions (IFSI III) considered the healing of the skin lesions as a very important treatment goal (54). Overall, it has been shown that therapeutic goals relate to the diagnosis and medical therapy. Reduction in pruritus or confidence in therapy were considered important or very important

by the majority of patients with CP. However, patients with CP focus not only on symptom relief, but also on trustworthy and efficient medical action. Their overall need level seems to be higher than in other dermatological patients as, for example, patients with atopic dermatitis (54). The complexity of patients' needs constitute a challenge for clinical researchers, since patients' needs and goals influence how they perceive their disease and the efficacy of a therapy. Patients' needs should thus be taken into consideration when planning studies involving patients with CP.

Standardization of diagnostic and therapeutic procedures

Owing to the heterogeneity of CP conditions, it is difficult to achieve a standardized diagnostic and therapeutic approach across specialized centres and physicians treating patients with CP. The first guideline addressing CP was published in Germany in 2006 (55) and constituted an important first step in this regard. Since then, the German guideline has been regularly updated (56), while a European guideline was also developed and recently updated (57).

There is still a lack of randomized controlled trials investigating anti-pruritic drugs, and therefore many of the recommendations of current guidelines are based on case series, case reports and expert opinion. Another challenge that guidelines face is the heterogeneity of CP aetiologies. For instance, while antihistamines are effective in the treatment of urticaria, they do not reduce pruritus in other diseases, such as atopic dermatitis or systemic pruritic conditions (58). Therefore specific therapeutic recommendations are needed for the various pruritic conditions. Clinical trials targeting different pruritic diseases (e.g. atopic dermatitis, uraemic pruritus, cholestatic pruritus, paraneoplastic pruritus) are of the utmost importance in order to generate quality data on which to base therapeutic recommendations.

Dermatologists are usually the first specialists consulted for CP and play a key role in the management of CP. Their job is not only to treat the CP, but also to assign the patient to local physicians of other specialties for specific diagnostic procedures (e.g. medical imaging). However, due to time constraints, taking a comprehensive medical history in patients with CP is challenging in a dermatological practice. Reactive disorders of CP, such as depression and anxiety, need to be considered, as possible causative factors and skin findings, comorbidities and medication must be well documented. The consultation of patients with CP in specialized pruritus centres can offer an important advantage in the management of CP, especially when refractory to basic therapeutic measures, such as antihistamines, topical steroids and the use of emollients. However, the centres are scarce and more are needed for the current demand (7). Pruritus centres should work on an interdisciplinary basis together with medical specialties other than dermatology, such as internal medicine, neurology, pain medicine, psychosomatics, radiology, and medical informatics (59). In addition to outpatient care, specialized centres should also offer the possibility of inpatient care for highly complex patients who require extensive diagnostic procedures or who have relevant psychosocial factors, such as suicidal ideation or severe sleep impairment (59).

Guideline-based treatment of patients with CP at specialized centres can significantly reduce costs, both in the inpatient and outpatient sectors. This is the result of a recent study in which data from 300 patients with CP were analysed regarding their QoL, health economic burden and therapeutic benefits. Six months after the start of treatment at a specialized pruritus centre there was not only a significant improvement in the pruritus intensity, QoL and therapeutic benefit (PBI-P), but also a significant reduction in all costs (60).

Need for novel effective therapies

Owing to a better understanding of the mechanisms underlying CP, new promising agents with an anti-pruritic effect have been identified and are being tested in randomized controlled trials. These include monoclonal antibodies (e.g. nemolizumab, tralokinumab), neurokinin-1 receptor antagonists (e.g. serlopitant, aprepitant), opioid modulators (e.g. nalbuphine, nalfurafine), phosphodiesterase-4 inhibitors (e.g. apremilast, crisaborole), janus kinase inhibitors (e.g. tofacitinib) among other novel agents.

The development of innovative drugs faces important challenges. So far, clinical trials have been performed only for a few indications, especially atopic dermatitis, chronic prurigo, uraemic and cholestatic pruritus. For many other pruritic conditions clinical trials are lacking, and thus no novel drugs are available.

Since these new agents represent a high cost for the healthcare system, it is important to select the patients who can profit the most from them. Clinical trials and observations from routine care should inform which target population is suitable for each drug.

At present, innovative drugs being tested in clinical trials are available in only a few centres and thus not all patients have access to them. Licensing of these drugs is needed in order to extend the availability of these promising agents to patients in need. Hence, clinical research should focus on producing high-quality data on the safety and efficacy of novel anti-pruritic drugs, so that regulatory agencies can approve these medications.

CONCLUSION

Clinical research efforts in CP have increased in recent years, leading to better understanding of this condition, and ultimately to better care. However, the multidimen-

sional nature of this condition and the heterogeneous population affected by CP pose a challenge for clinical researchers and attending physicians. Unmet needs, such as the shortage of knowledge on chronicity mechanisms, insufficient standardization of a diagnostic and therapeutic approach to CP patients, and the development of novel promising drugs for refractory CP, ought to be targeted by researchers and physicians dealing with CP.

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REVIEW ARTICLE

Psoriasis and Genetics

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Psoriasis is a common inflammatory skin disease caused by the interplay between multiple genetic and environmental risk factors. This review summarises recent progress in elucidating the genetic basis of psoriasis, particularly through large genome-wide association studies. We illustrate the power of genetic analyses for disease stratification. Psoriasis can be stratified by phenotype (common plaque versus rare pustular variants), or by outcome (prognosis, comorbidities, response to treatment); recent progress has been made in delineating the genetic contribution in each of these areas. We also highlight how genetic data can directly inform the development of effective psoriasis treatments.

Key words: psoriasis; genetics; precision medicine; disease progression; treatment outcome.

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SIGNIFICANCE

Psoriasis has benefited greatly among dermatological conditions from genome-wide association studies (GWAS) of increasingly large, clinically well-described samples. Sixty-five regions of the genome have been linked to psoriasis risk in Europeans, with the largest contribution due to *HLA-C*06:02*, a variant of an important gene involved in immunity. Other regions implicate numerous immune and skin barrier processes in psoriasis development. Recent GWAS-based research has shown that genetics can help distinguish subgroups of psoriasis patients characterised by type (pustular vs. plaque psoriasis), development of joint disease or response to various drugs. This may help inform future tailored treatment strategies for individuals with psoriasis.

Dermatological research has made extraordinary progress over the past 100 years. This has been matched – if not exceeded – by advances in the field of genetics, particularly in the two decades since the initial mapping of the human genome (1, 2). Recent insights into the genetic basis of the common skin disease psoriasis illuminate the translational potential of genetic studies, having directly informed the design of several powerful biologic therapies and small molecule inhibitors.

Psoriasis is a chronic immune-mediated inflammatory disease that affects around 2% of the world's population (3). It has been designated a serious non-communicable disease by the World Health Organisation and its increasing prevalence represents a substantial global public health burden (4). Genetic research has delivered critical insights into the biology of psoriasis. We now know that psoriasis is a multifactorial disease caused by the interplay between multiple inherited alleles (**Box 1**) and environmental risk factors. Indeed, it has a particularly strong genetic component among complex diseases, with heritability estimated to exceed 60% (5).

Unlike other biological features, the genome is fixed at birth and does not vary by cell or tissue type, or in response to stimuli: in this sense it reveals the causal biology of psoriasis. In this review, we describe how genetic studies have helped to disentangle pathogenic mechanisms of psoriasis and informed the selection of therapeutic targets. We also highlight the potential of genetic biomarkers as a stratification tool for the effective clinical management of psoriasis.

GENETICS OF PLAQUE PSORIASIS

Early genetic findings

It has long been observed that the incidence of psoriasis is significantly higher among first- and second-degree relatives of sufferers than the general population (6, 7), and it is more concordant among monozygotic than dizygotic twins (8–10).

Linkage studies identified at least 9 genomic regions (loci) that co-segregated with psoriasis (*PSORS1-9*) in multiplex pedigrees. However, most of these findings could not be replicated, which underscores the limitations of linkage approaches for the analysis of multifactorial conditions (11). A notable exception is the *PSORS1* region, which maps to the class I interval of the major histocompatibility complex (MHC) that primarily encodes genes involved in antigen presentation (12–14). The region also contains the candidate gene corneodesmosin (*CDSN*), which encodes a desmosomal protein involved in keratinocyte cohesion and desquamation (15). *PSORS1* has the largest effect size and accounts for 35–50% of disease heritability explained by known

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Box 1 – Genetic terminology

Alleles: Alternative variants of a gene (or other segment of DNA).

Single nucleotide polymorphism (SNP): A DNA sequence change affecting a single genomic position.

Linkage disequilibrium (LD): Genetic variants are in LD if they are in close proximity on the same chromosome and therefore less likely to be separated by recombination during meiosis, tending to be inherited together and being correlated in the population.

Susceptibility loci: Genomic regions that contain variants showing statistically significant association in a disease susceptibility GWAS (usually more than one variant due to LD).

Imputation: The statistical ascertainment of an individual's probable genotype at known genetic variants that exist in between markers genotyped on a GWAS chip. This requires large panels of reference genomes in which genotypes are available for the "missing" variants.

Polygenic risk score (PRS): A composite measure of genetic risk for a disease. Once a susceptibility GWAS has been completed, the polygenic risk for any genotyped individual (who may not have been included in the original GWAS) can be calculated by summing the number of risk alleles they carry at each susceptibility locus (usually weighted by the effect size observed at that locus).

Genetic correlation: The degree to which the genetic influences on two different traits are similar.

Mendelian randomisation: An approach to assess how far one trait (typically representing a modifiable exposure) is causal of another trait (typically a health outcome) by estimating the effects of genetic variants associated with the first trait on the second.

Next-generation sequencing: High-throughput and highly parallelised DNA sequencing, typically of the whole genome or exome (the protein coding portion of the genome).

loci. Despite the complex correlation structure across the MHC due to extensive linkage disequilibrium (Box 1) (16), *HLA-C*06:02* is now confidently considered the most likely causal susceptibility allele, since single nucleotide polymorphisms (SNPs; Box 1) that tag this allele have generated the most significant association signals in subsequent case-control studies (17, 18). Fine mapping studies have suggested the presence of additional association signals within *PSORS1*, some of which are population-specific (19–22).

The only other successfully validated linkage results are the *PSORS2* and *PSORS4* loci on chromosomes 17q25 and 1q21, respectively. The most likely susceptibility gene in *PSORS2* is *CARD14*, which encodes a nuclear factor-κB (NF-κB) activator and harbours variants associated with rare and common forms of psoriasis (23–25). *PSORS4* contains the late cornified envelope (LCE) genes, which encode stratum corneum proteins involved in terminal epidermal differentiation. This locus has been implicated in psoriasis susceptibility in genome wide association studies of both European and Chinese populations (26, 27).

Psoriasis in the GWAS and post-GWAS era

Genome-wide association studies (GWAS) use highly optimised microarrays that can efficiently and robustly genotype several million genetic markers across the genome. With sufficiently large sample numbers, GWAS allows even small differences in allele frequencies between disease cases and unaffected controls to be detected, making it a much more powerful approach than linkage analysis. As such, GWAS have fundamentally changed the genetic dissection of common complex diseases such as psoriasis. By 2010, initial GWAS efforts in psoriasis had identified 21 susceptibility loci in Europeans (17, 18, 28, 29).

One inherent limitation of GWAS, however, is that it only uncovers statistical relationships. The genetic variants identified by GWAS may actually, by virtue of linkage disequilibrium, be tagging a separate 'causal' variant that exerts a biological effect and modifies disease risk. To refine GWAS signals and thus identify potential causal susceptibility alleles, genotyping arrays with dense coverage in regions of interest have been employed. The immunochip included 200,000 SNPs focused in known susceptibility loci for a range of immune-mediated diseases (30). In psoriasis, meta-analysis of immunochip data almost doubled the number of known susceptibility loci and uncovered candidate causal variants at 10 loci including in the innate immunity genes *DDX58* and *CARD14* (31).

More recently the exome chip aimed to comprehensively genotype protein-altering variants, including rare variants. Exome chip meta-analysis of 12,000 psoriasis cases and 29,000 controls highlighted potential functional SNPs within 11 known psoriasis susceptibility loci. This study provided novel insights into the complex role in psoriasis susceptibility of rare variants in the type I interferon signalling genes *IFIH1* and *TYK2* (32).

Rather than physically genotyping additional SNPs that are not included in GWAS arrays, however, it is becoming standard practice to perform genome-wide imputation (Box 1) using freely-available computational resources (33, 34). Imputation has been critical in facilitating the larger psoriasis meta-analyses, which combine data generated by different GWAS platforms (35–37). Indeed, an improved imputation strategy revealed a novel psoriasis susceptibility locus at *DLEU1*, linked to apoptosis, in previously analysed GWAS data (37).

Finally, combining datasets from international collaborations in meta-analyses of genome wide association studies has been essential to enhance statistical power and uncover novel disease susceptibility loci (18, 28, 29, 31, 38). A recent meta-analysis of psoriasis GWAS with a combined effective sample size of > 39,000 individuals identified 16 novel disease-associated regions (36).

PATHOGENIC INSIGHTS FROM GENETIC DISCOVERIES

As a result of GWAS, targeted association and meta-analysis efforts, the number of independent genomic loci contributing to susceptibility to common plaque psoriasis in populations of European ancestry now stands at 65 (32, 36, 37). More than 30 loci have been implicated in Han Chinese individuals (39). Although these susceptibility loci can span many genes, many of the lead SNPs lie in proximity to genes involved in specific adaptive and innate immune pathways. These include genes involved

in antigen presentation (*HLA-C, ERAP1*), T17 cell activation (*IL23R, IL23A, IL12B, TRAF3IP2*), innate antiviral immunity/type I interferon signalling (*RNF114, IFIH1*) and skin barrier function (*LCE3B/3D*) (**Fig. 1**) (17, 18, 26, 28, 40–42). The coding variants in genes such as *IL23R, TYK2* and *TNFSF15* uncovered by targeted association analyses further underscore the involvement of the interleukin (IL)-23/T17 axis in disease pathogenesis (31, 32, 38, 39, 43).

Genetic studies have thus provided important mechanistic insights into the aetiology of psoriasis, and support a pathogenic interplay between immune activation and disruption of skin barrier function (44). There is also evidence of gene-gene interactions (epistasis) contributing to disease heritability, since variants in *ERAP1* (encoding an enzyme that trims peptide antigens for loading onto MHC class I molecules) only confer disease susceptibility in individuals also harbouring the *HLA-C* risk allele (18). Once GWAS association summary statistics are in hand, there are several additional *in silico* approaches that can help to pinpoint relevant causal genes and variants before costly hypothesis-driven functional experiments are undertaken.

Statistical fine-mapping jointly considers correlated groups of associated variants to estimate the likely causality of each (45). This has been undertaken for several psoriasis susceptibility loci, revealing multiple independent association signals (46).

Pathway analysis methods look for known biological pathways for which gene annotations are enriched across multiple susceptibility loci. NF-κB and type I interferon signalling pathways have thus been implicated in psoriasis pathogenesis (36).

If GWAS summary results are available from other studies that have assessed the genetic basis of relevant molecular traits, colocalisation with the disease association signal can be assessed (47, 48). In particular, expression quantitative trait loci (eQTLs) are SNPs associated with the level of expression of a gene in a specific tissue. Colocalisation of a psoriasis susceptibility signal and a skin- or immune-based eQTL would thus provide strong evidence that the variant directly modifies psoriasis risk and suggest a probable mechanism of action. This powerful approach has been successfully employed in GWAS studies of acne (49) and atopic dermatitis (50) but has yet to be employed systematically in a large psoriasis dataset, with only suggestive colocalisations being reported in cross-disease studies (51, 52).

It is worth remarking that all of these approaches rely to a greater or lesser extent on *open science*: the continuing efforts of research groups around the world that are committed to making reference data, summary results, annotations, tools and computational resources publicly available in the interests of collaborative science.

TRANSLATION OF GENETIC DISCOVERIES INTO NOVEL THERAPEUTICS

The genetic insights gained from large-scale association analyses have paved the way for transformative novel therapeutics in psoriasis. Indeed, it has been shown in general that pipeline drugs whose mechanisms are supported by direct genetic evidence are more likely to reach the clinic (53, 54). Based on the mechanistic insights that have emerged from genetic studies in psoriasis, the IL-23/T17 axis has been a particular focus for drug development. Biologic agents such as ustekinumab (targeting the common p40 subunit of IL-12 and IL-23), secukinumab and ixekizumab (targeting IL-17A), and newer monoclonal antibodies targeting the p19 subunit specific to IL-23 (including guselkumab and tildrakizumab), have shown progressively increasing efficacy rates in clinical trials (55). These agents are now licensed for use in the USA and Europe and have impressive effectiveness and tolerability in real world practice (55, 56).

In addition to informing the targets of biologic medications, genetic studies have opened new avenues for small molecule therapeutics. Following genetic association data highlighting *TYK2* as a causal allele (31, 32), an oral, selective

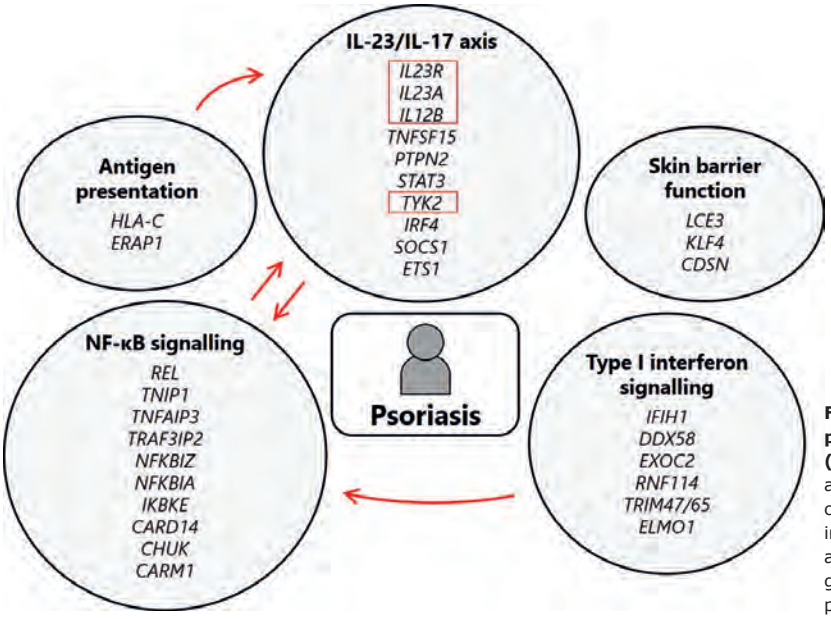

Fig. 1. Biological pathways implicated in psoriasis pathogenesis via genome wide association studies (GWAS). Candidate causal genes from selected disease-associated loci identified by GWAS. *Arrows* signify the crosstalk between the immune pathways shown (e.g. interleukin (IL)-17 and type I interferon signalling both activate nuclear factor-κB (NF-κB) pathways). *Red boxes:* genes involved in mechanisms currently targeted by psoriasis treatments.

inhibitor was developed, which has shown promising efficacy in phase II trials (57).

Missing heritability

Despite the recent progress in psoriasis genetics, less than a quarter of heritability is thought to be explained by the susceptibility loci identified to date (32). There are several reasons for this missing heritability.

A substantial fraction of heritability may arise from rare variants that are not genotyped or well tagged by GWAS arrays. A recent analysis of 22,000 whole genomes makes a compelling case for this, since the heritability of both height and body mass index could be almost fully explained when very rare variants are accounted for (suggesting also that pedigree-based estimates of heritability are not overestimated) (58). The same may be true for psoriasis susceptibility, although sequencing efforts will need to surpass those performed to date (59) to confirm this.

More generally, the estimated heritability explained at a GWAS-identified locus may be underestimated where the lead GWAS SNP is a poor tag for the true causal variant, or where there are multiple true causal variants (60).

Another explanation could be high polygenicity, where many common SNPs across the genome may modify psoriasis risk, but with effect sizes too small to have been identified with current GWAS sample sizes. Although increasingly large case-control study populations will help to address this (61), sufficient numbers to fully elucidate the role in psoriasis pathogenesis of every individual common SNP are impractical. One approach to overcoming this limitation is to consider genetic variation aggregated according to known biological function. For example, functional network-based analyses have been applied to suggest novel mechanisms involved in psoriasis (36, 62).

It could also be the case that psoriasis risk attributable to individual genetic variants does not accumulate additively and independently, so that simple GWAS association tests mask more complex causal biology. Alternative models of genetic architecture have been explored (63), including genetic interactions genome-wide (64, 65) (recall the *HLA-C/ERAP1* interaction described previously).

Missing heritability in genetic studies could be due in part to epigenetic variation: DNA modifications that can cause differences in gene expression even when no differences are present in DNA sequence. Numerous studies have begun to explore the role of epigenetics in psoriasis, although the types of modification and study designs have varied widely, making it difficult to assess their overall contribution to heritability (66).

The complex genetic nature of psoriasis and the unresolved missing heritability have implications for the growing industry of direct-to-consumer genetic testing. While genetic risk profiles can offer additional information beyond family-history based risk estimates (67), this information will likely be insufficiently precise or consistent to offer substantial clinical utility (68, 69) and it is vulnerable to misunderstanding by the public (70, 71).

As we shall describe, however, the genetic risk profiles of larger cohorts still hold great potential to refine our understanding of the biology and to inform effective clinical management of psoriasis.

BEYOND DISEASE SUSCEPTIBILITY

The possibilities of GWAS-based analysis have now moved beyond the study of simple susceptibility and towards disease stratification. With large collections of genotyped and deeply phenotyped individuals, the genetic basis of many other aspects of psoriasis natural history and treatment response can be characterised (**Fig. 2**). These collections could comprise psoriasis patients (e.g. PSORT (72)) or be derived from the general population with phenotype data from linked electronic medical records (e.g. UK Biobank (73)).

These "post-susceptibility" genetic studies still currently utilise much smaller samples than the susceptibility GWAS meta-analyses described above. However, they can benefit from numerous methods that incorporate or compare genetic information (typically GWAS summary statistics) from related traits to make novel inferences. Relevant methods include polygenic risk scores (PRS) (74) and genetic correlation (75, 76) to assess shared genetic associations, Mendelian randomisation (77) to assess causality, and methods for deconvoluting genome-wide association signals into functionally relevant constituents (78) (Box 1). While findings from psoriasis susceptibility studies offer a natural starting point (and efforts to accurately document and annotate these associations are ongoing (79, 80)), the utility of these methods are greatly enhanced by the availability of GWAS summary results for thousands of other traits, including physiological, disease-based and molecular traits (81).

We offer here a brief overview of recent progress in psoriasis genetics beyond susceptibility.

Onset

It remains unclear how genetic susceptibility variants interact with environmental risk factors such as infection, ultraviolet exposure, smoking, alcohol, and psychological stress to trigger psoriasis onset (82). Initial findings suggest that the risk attributable to *HLA-C*06:02* may be modified by smoking and stress (83). The pathogenic contribution of smoking may also be mediated via variants in *CYP1A1*, a key gene in the aryl hydrocarbon receptor signalling pathway (84). The availability of large datasets with environmental exposure and GWAS

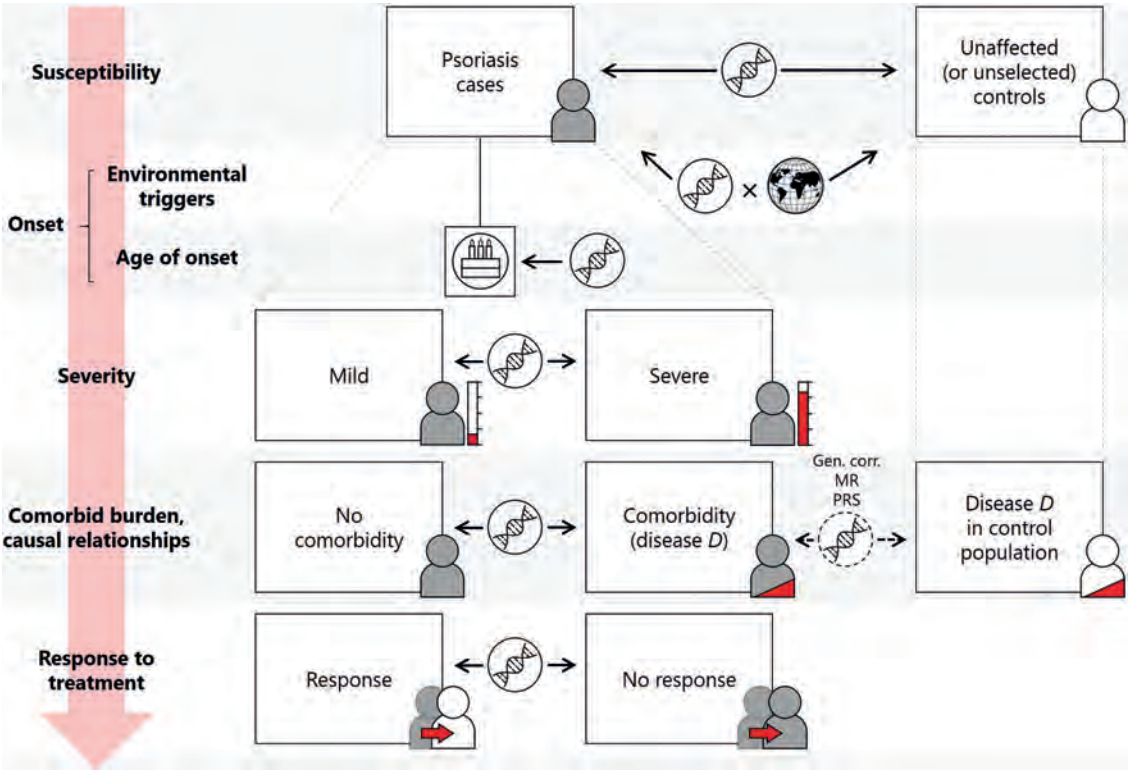

Fig. 2. Psoriasis genetics beyond susceptibility. Various strategies are employed to study the genetic factors that influence the conceptual trajectory from risk of psoriasis through disease onset and prognosis to patient outcomes (red arrow). *Susceptibility*: allele frequencies are compared between psoriasis cases and controls to reveal genetic variants contributing to psoriasis risk. *Onset:* gene × environment studies may integrate genetic data with environmental exposures (indicated by globe symbol) to identify relationships between genes and environmental triggers; age-of-onset is also influenced by genetic factors and this can be investigated where age-of-onset data (denoted by birthday cake symbol) are available for psoriasis cases. *Severity:* genetic profiles can be compared between psoriasis patients with mild and severe disease; severity may also be studied as a continuous outcome. *Comorbidities*: genetic profiles are compared between psoriasis patients with and without comorbid disease *D*; more sophisticated methods will also consider the genetic basis of disease *D* in the wider population. *Response to treatment*: genetic profiles can be compared between psoriasis patients responding and not responding to a treatment; response may also be studied as a continuous outcome. Gen. corr.: genetic correlation; MR: Mendelian randomisation; PRS: polygenic risk score.

data (such as UK Biobank (73)) now offers the opportunity to study gene-environment interactions in a more systematic manner, and this is an active area of research in the field. Age of onset is better studied. It is well established that the *HLA-C*06:02* susceptibility allele is associated with earlier disease onset (85, 86). Tsoi et al. used PRS analysis to show that a greater burden of psoriasis susceptibility variants is associated with earlier disease onset, even when only non-HLA susceptibility loci are considered (36).

Comorbidities

Psoriatic arthritis (PsA), with prevalence estimates ranging from 6–41% among individuals with psoriasis (87), has been the subject of large genetic studies as a disease in its own right (88–90). Particularly revealing, however, are studies comparing individuals with psoriasis and PsA against cutaneous-only psoriasis cases, (either directly or with reference to unaffected controls). Several studies focusing on the HLA region suggest that certain *HLA-B* alleles, including *HLA-B*27*, are associated with increased PsA risk in the presence of psoriasis (21, 91, 92), while *HLA-C*06:02* is not (91). Genome-wide analysis has identified additional associations of interest, including independent alleles in known psoriasis susceptibility loci (including at *IL23R* and *TNFAIP3*) (93). The translational potential of these approaches was recently explored using a "risk score" of 200 genetic markers that proved predictive of PsA development (area under the receiver operator curve = 0.82) (37). While this finding requires replication and may benefit from phenotype refinement (there are at least five recognised subtypes of PsA (94)), it offers a first step towards prognostic genetic risk profiling.

Obesity and related cardiometabolic traits have also been studied. While a large GWAS-based investigation found the genetic architectures of psoriasis and cardiometabolic traits to be largely distinct (95), an epidemiological association with obesity is well established (96, 97) and twin studies suggest a genetic correlation (98). Based on psoriasis and body mass index (BMI) GWAS data, Mendelian randomisation reveals a causal relationship: higher BMI increases the risk of psoriasis, whereas psoriasis does not have a causal effect on BMI (99, 100). Given the relatively large effect that *HLA-C*06:02* exerts

on psoriasis risk, it may be interesting to examine the causal role of BMI separately in patients positive and negative for this allele.

In principle, the shared genetic aetiology between psoriasis and any other associated condition can be readily explored at scale via GWAS data. A recent example looked at psoriasis alongside 4 other inflammatory diseases (ankylosing spondylitis, Crohn's disease, primary sclerosing cholangitis and ulcerative colitis), finding genetic overlap between the conditions that may drive co-occurrence, but with the qualification that patients affected by multiple conditions are likely to be genetically distinct from those with a single disease (101). Shared genetic factors have been found to extend beyond inflammatory disease, such as the positive genetic correlation observed between psoriasis and schizophrenia (102).

Stratified medicine

Genomic information has an exciting role in potential future personalised models of disease prevention and treatment (103). Although highly discriminative genetic prediction for complex diseases such as psoriasis (which are influenced by many genetic factors of modest effect) is unlikely (74), there remains ample opportunity to "stratify" individuals into broader groups according to distinct risk and response profiles, thus leading to more effective and economical care.

Effective deployment of expensive biologic therapies is an area of promise in psoriasis. Patients positive for the *HLA-C*06:02* psoriasis susceptibility allele demonstrate better response to ustekinumab than *HLA-C*06:02*-negative patients, particularly during the initial months of treatment (104, 105). Numerous candidate gene studies (and one small GWAS (106)), have tested for genetic associations with response to TNF antagonists such as etanercept, adalimumab and infliximab, often pooling observations for multiple drugs. Robust associations have until recently been scarce, but we are beginning to see better-powered investigations; a recent Danish study found significant associations with anti-TNF response in several immune genes (107). We recently showed via a comparative approach that *HLA-C*06:02* status could inform choice of treatment between adalimumab and ustekinumab, particularly when used in combination with clinical factors. Specifically, we found that *HLA-C*06:02*-negative patients with psoriatic arthritis were significantly more likely to respond to adalimumab than ustekinumab after 6 months (odds ratio, 5.98; $p = 6.89 \times 10^{-5}$), with no such difference observed in *HLA-C*06:02*-positive patients (108). This has promising clinical utility.

PRS may also help to define strata relevant to the management of psoriasis. Several studies have explored the predictive ability of PRS in psoriasis susceptibility (36, 109, 110) but the true translational benefits of this approach may lie in identifying and characterising groups of patients with very high or very low PRS scores (74). More research in this area in psoriasis is therefore warranted.

PUSTULAR PSORIASIS

Pustular psoriasis is a rare subtype characterised clinically by the presence of sterile pustules on variably erythematous skin, and histologically by diffuse dermal neutrophilic infiltration (111). It can be classified as either acute generalised (generalised pustular psoriasis (GPP)) or chronic localised disease (palmoplantar pustulosis (PPP) and acrodermatitis continua of Hallopeau (ACH)) (112). Pustular psoriasis has a distinct genetic architecture to plaque psoriasis, underscored by a lack of association with the *PSORS1* locus (113). The severity and rarity of the clinical phenotype indicate that pustular psoriasis could be associated with rare alleles of moderate to large effect, which has been supported by the identification of three disease genes (*IL36RN, AP1S3* and *CARD14*) using next-generation sequencing technologies (Box 1).

Linkage studies of consanguineous pedigrees and exome sequencing of unrelated GPP patients identified autosomal recessive loss of function mutations in *IL36RN* (114, 115). *IL36RN* encodes the IL-36 receptor antagonist (IL-36Ra), which modulates the activity of the IL-1 family cytokines IL-36α, -β and -γ. The screening of expanded patient resources subsequently identified a spectrum of *IL36RN* mutations that are distributed throughout the length of the protein and are associated with pustular psoriasis in a variety of populations (116, 117).

Genotype-phenotype analyses indicate that *IL36RN* disease alleles are less common in individuals with PPP (frequency 0.03) than GPP (0.19) and ACH (0.16) (116). Although recessive *IL36RN* alleles are typically observed in patients presenting with a severe clinical phenotype (early-onset GPP characterised by a high risk of systemic involvement) (118), deleterious *IL36RN* variants have also been associated with localised pustular disease (119). Individuals harbouring a single *IL36RN* mutation are occasionally affected, and they classically present with disease at a later age, indicating a dose-dependent effect (116, 118). Thus, genotype-phenotype analyses provide evidence for variable penetrance of disease alleles and a potential role for genetic modifiers and environmental factors.

Since *IL36RN* mutations are only found in a minority (~25%) of pustular psoriasis cases (118), exome sequencing was undertaken to gain a better understanding of the genetic basis of the disease. This uncovered two recurring founder mutations in the *AP1S3* gene (120). While these defects were found to account for 12% of pustular psoriasis cases of European descent, no AP1S3 mutations were found in Asian patients. AP1S3 encodes the σ1 subunit of AP-1, an evolutionarily conserved

hetero-tetramer that has been implicated in the formation of autophagosomes (specialised vesicles that mediate autophagy). Autophagy is an intracellular degradation pathway for misfolded proteins and damaged organelles (121) and has been shown to regulate cutaneous immune responses (122, 123). *AP1S3* mutations may lead to defective autophagy, causing accumulation of p62 (an adaptor protein that mediates NF-kB activation) and upregulation of IL-36 mediated cutaneous inflammation (124). Therefore, mutations in different disease genes converge on the de-regulation of IL-36 signalling in pustular psoriasis, highlighting IL-36 blockade as a promising therapeutic strategy regardless of the specific gene affected.

CARD14 was subsequently confirmed as a third disease gene for GPP (25). *CARD14* is highly expressed in keratinocytes and encodes a scaffold protein that, upon oligomerisation, mediates TRAF-2 dependent activation of NF-κB signalling. A deleterious gain-of-function substitution in *CARD14* has been associated with GPP in an extended case series and shown to cause spontaneous CARD14 oligomerisation *in vitro* (25). The same variant was also found in two patients with PPP (125), which provides further evidence for an overlap in the genetic basis of generalised and localised forms of pustular psoriasis. Indeed, gain-of-function *CARD14* mutations have been detected in cases of familial plaque psoriasis (23, 24), indicating shared aetiological mechanisms in plaque and pustular subtypes of disease.

There is a substantial unmet need for effective treatments for pustular psoriasis (111). The conventional systemic agents used for the treatment of plaque psoriasis are often ineffective in pustular phenotypes and there is a paucity of robust clinical trial data, such that current guidelines are mostly based on isolated case reports (111). However, recent exciting progress in this area shows a clear throughline from genetic discovery to treatment advances. IL-1 blockers are being investigated as potential treatments for pustular psoriasis and a multicentre double-blind randomised controlled trial of anakinra in PPP is currently underway (http://apricot-trial.com/). In GPP, anakinra has been shown to cause initial rapid clinical improvements in case reports (126, 127), although full disease remission was seldom achieved. This incomplete response supports the notion that IL-1 itself is not the dominant disease driver but participates in positive regulatory feedback loops driven by IL-36 (128).

In vivo and *ex vivo* research has validated IL-36 signalling as a powerful therapeutic target in psoriasis, and indicates that IL-36 blockade would not substantially compromise host defences (129). A recent phase I proof of concept study of 7 patients demonstrated that blockade of the IL-36 receptor (using a single intravenous dose of a monoclonal antibody) reduced the severity of GPP over a 20-week period (130). The agent was efficacious irrespective of the presence of known causal genetic variants and larger scale clinical trials of IL-36 antagonists in pustular psoriasis are currently underway.

FINAL THOUGHTS

Non-European ethnicities

We have shown that genetics will be instrumental in moving healthcare provision towards stratified, and even personalised, models. However, such progress is dependent on robust genetic associations with disease susceptibility, clinical outcomes and other related traits. The majority of genotyping efforts to date have focused on populations of European, and to a lesser extent Han Chinese, origin, meaning the translational potential of GWAS is largely limited to these groups at present.

A trans-ethnic GWAS meta-analysis of psoriasis susceptibility demonstrated heterogeneous genetic associations between European and Han Chinese populations (20). Other ethnic groups in which smaller GWAS and candidate gene studies have been undertaken include Indian (131), Japanese (132) and Omani Arab (133) populations. We are unaware of genetic studies of psoriasis in people of African descent. While lower prevalence might make psoriasis a smaller population burden among predominantly non-white populations (134) the disease burden for individual psoriasis patients is high, and large-scale genetic studies across ethnic groups are warranted.

Such endeavours will benefit from recent community efforts to generate the necessary supporting resources, including statistical tools for trans-ethnic meta-analysis (135), reference panels for genome-wide (136) and HLA allele (137) imputation, and GWAS summary results for common traits (138).

The future of genetics in psoriasis

As with other complex diseases, we believe that genetics will be at the heart of future success in translational psoriasis research. Increasingly large GWAS studies will improve power to detect genetic variants with small effects on psoriasis risk, refining our understanding of the genetic basis of the disease. This increased resolution should allow more accurate deconvolution of susceptibility associations into functional mechanisms of disease, aided by a growing catalogue of systematically derived and publicly available GWAS datasets for intermediate molecular traits. There is also an increasing awareness in the investigative dermatology community of the importance of precise phenotyping. When combined with genetic data, larger and more detailed clinical datasets will help reveal genetic differences between patients that differ in phenotypic presentation or outcome and therefore inform the development and deployment of effective therapies. Finally, as patients become more likely to undergo GWAS profiling or whole-genome sequencing as part of standard healthcare provision, there will almost

certainly be benefits to be derived from PRS or related genome-wide measures. These benefits are unlikely to come from very precise diagnostic or prognostic predictions but rather from prioritising individuals for early screening or closer monitoring, thus making optimal use of clinical resources and reducing the significant disease burden of psoriasis at the population level.

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REVIEW ARTICLE

The Woronoff Ring in Psoriasis and the Mechanisms of Post-inflammatory Hypopigmentation

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The Woronoff ring is a ring-like hypopigmentation zone around regressing psoriasis lesions. Although it was first described more than 100 years ago, its aetiology has remained a mystery. Recent insights into the pathogenesis of psoriasis can now explain the origin of the Woronoff ring. Psoriasis involves an HLA-class I-restricted autoimmune response of CD8⁺ T cells against melanocytes in the epidermis. The pathogenic CD8⁺ T cells are not cytotoxic, but are characterized by the production of interleukin-17, interleukin-22 and tumour necrosis factor-α. Interleukin-17 and tumour necrosis factor-α act synergistically on melanocytes by increasing proliferation while inhibiting melanogenesis. This reduces the cellular melanin content despite an increased number of melanocytes in psoriatic lesions. As a consequence, during healing the prior influence of interleukin-17 and tumour necrosis factor-α, despite the increased density of melanocytes, leaves a hypopigmented zone at the edge of regressing psoriasis lesions, which becomes visible as the Woronoff ring. This mechanism can explain a long-discussed puzzling phenomenon in dermatology.

Key words: psoriasis; Woronoff ring; melanocytes.

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SIGNIFICANCE

The Woronoff ring is a depigmented zone arising around healing psoriasis plaques. Analysed in detail for the first time approximately 100 years ago, our current understanding of the pathogenesis of psoriasis enables its explanation due to the cytokine pattern of the T-cell-mediated pathogenic melanocyte-specific psoriatic autoimmune response. The production of interleukin-17 and tumour necrosis factor-α causes suppression of melanin synthesis with a simultaneous increase in melanocyte proliferation. This results in an inflammation-induced hypopigmentation surrounding the healing psoriasis lesions. The emergence of the phenomenon is thus coherently integrated into our immunological understanding of psoriatic pathogenesis.

Nearly 100 years ago, Dr D. L. Woronoff, a dermatologist at the clinic for skin diseases of Moscow University in Russia, published his investigations on a pale annular zone that was known to appear around healing psoriasis lesions, and was thought to be caused by a spastic vessel contraction or hypopigmentation (1). He described the clinical appearance of these rings as a "pseudoatrophic" annular zone surrounding acanthotic psoriatic plaques, and gave a precise histological description (**Fig. 1**). He reported that this zone was histologically distinct from both the psoriasis plaque and the surrounding normal skin due to the absence of parakeratosis in the stratum granulosum, a broadened stratum Malpighii due to more layers of living cells leading to increased epidermal thickness, and irregularly shaped papillae without dilation of the capillaries. He concluded

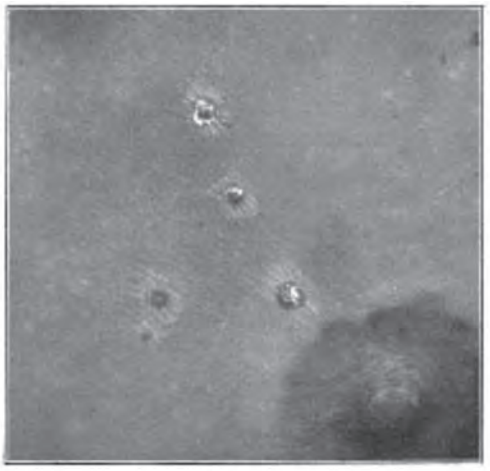

Abb. 1.

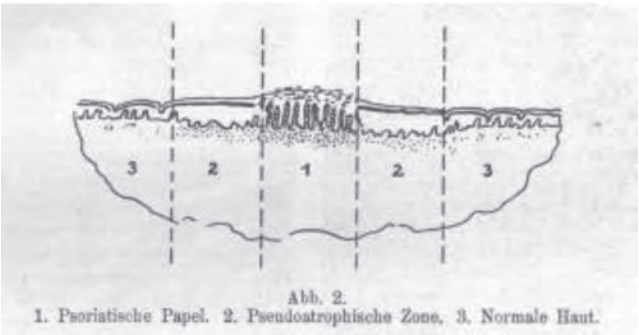

Abb. 2.
1. Psoriatische Papel. 2. Pseudoatrophische Zone. 3. Normale Haut.

Fig. 1. The pseudoatrophic zone around the psoriatic papules and scheme of histological changes. From the original publication by Woronoff, 1926 (1).

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that this zone, which he sketched in a scheme (Fig. 1), inhibited further development of the psoriatic plaque and was more likely to result in regression of psoriasis lesions. Since he also observed these changes around corymbiform syphilitic skin lesions, he concluded that these "achromatic phenomena" were to the utmost extent better able to withstand all possible pathological stimuli than normal skin.

Based on these studies, the annular zones of hypopigmentation developing around psoriatic skin lesions are referred to as the Woronoff ring. The Woronoff ring is a phenomenon that is mentioned in major dermatological textbooks as a morphologically distinct alteration of healing psoriasis plaques, and it has been occasionally addressed in the medical literature with only a few photographic illustrations. The width is usually between 2 and 6 mm, with regional fluctuations, and increases with the size of the central psoriatic plaque (2). The Woronoff ring has been observed after ultraviolet (UV) phototherapy or photochemotherapy (3), topical treatment, such as anthralin (4) or glucocorticosteroids, or systemic treatments including fumaric acid esters (5) or the tumour necrosis factor (TNF)-α antagonist adalimumab (6), but it may also occur spontaneously. The nature of the Woronoff ring is still not fully explained. This article discusses the aetiology of the Woronoff ring in terms of new insights into the pathogenesis of psoriasis, using the example of a patient who developed Woronoff rings around regressing psoriasis plaques under UV (311 nm) radiation therapy.

CASE REPORT

A 71-year-old female patient with a long history of psoriasis had undergone phototherapy with UVB (311 nm) radiation. The resolving psoriasis plaques developed annular zones of hypopigmentation and, further outward, circumferential hyperpigmentation (**Fig. 2**A). A biopsy from a whitish Woronoff ring showed a dense population of c-Kit$^+$ melanocytes in the basal epidermal layer (Fig. 2B) compared to lesional and normal skin (Fig. 2 C, D).

DISCUSSION

Different approaches have tried to explain the aetiology of the Woronoff ring. Disturbed vascularization, as discussed in early descriptions, appeared unlikely as a cause, since injections of prostaglandin E_2 (7), histamine phosphate and metacholine chloride (8) produced wheals with surrounding red flare involving both the Woronoff ring and adjacent normal skin. Furthermore, by measuring the cutaneous and subcutaneous blood flow in the Woronoff ring by the ^{133}Xe washout method, cutaneous vasoconstriction corresponding to a white dermographism could be excluded as the cause of the white discoloration (9). A major cause of the Woronoff ring was suspected in alterations in prostaglandin metabolism. Observation of a decreased level of prostaglandins in the tissue corresponding to the Wornoff ring has led to the hypothesis that UV therapy induces an inhibitor of prostaglandin synthesis, which causes the white ring by reducing inflammation (10). In addition, diminished inflammation in the Woronoff ring was attributed to a decreased level of endoglin, a scavenger of transforming growth factor beta (11). A histological study using the Masson-Fontana stain observed a marked decrease in the amount of basal-zone epidermal melanin in both the halo and the psoriatic lesions (8).

Recent insights into the psoriatic pathogenesis now provide another explanation for the aetiology of the Woronoff ring. The HLA-class I allele, HLA-C*06:02, is the main psoriasis risk gene (12). Psoriasis develops upon epidermal recruitment, activation and clonal expansion of CD8$^+$ T cells (13, 14). CD8$^+$ T cells recognize peptides that are presented by HLA-Class I molecules. Because the peptide antigens are derived from intracel-

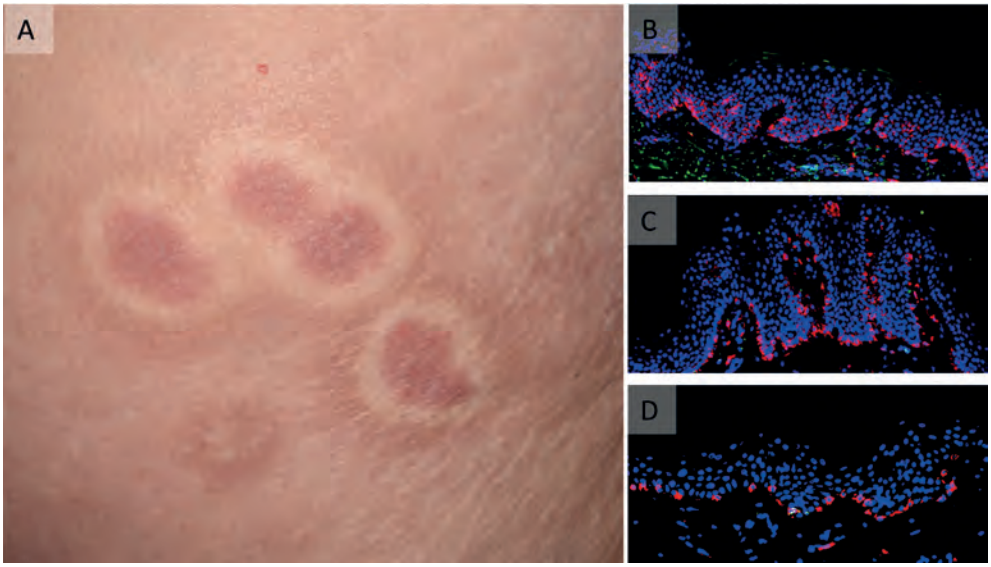

Fig. 2. Clinical features and immunostaining of melanocytes in patient and control skin. (A) Woronoff rings developing as depigmented zones around regressing psoriasis lesions during UVB (311 nm) radiation on the buttocks. Note the slight hyperpigmentation surrounding the depigmented halo. (B) Dense population of c-Kit+ melanocytes (red) in the basal epidermal layer of a biopsy from a Woronoff ring compared with (C) a psoriasis lesion or (D) normal skin; blue: DAPI. Staining by Akiko Arakawa, MD, PhD. Original magnification ×25.

lular proteins, an HLA-class I-restricted pathogenic $CD8^+$ T-cell response is primarily directed against a particular target cell type expressing the parent protein of said antigenic peptides (15, 16). In fact, in psoriasis HLA-C*06:02 mediates an autoimmune response against melanocytes through autoantigen presentation as the underlying pathomechanisms of T-cell mediated chronic inflammation (17).

The pathogenic psoriatic $CD8^+$ T cells represent a particular subtype of epidermal $CD8^+$ tissue-resident memory cells. Such $CD8^+$ T cells are characterized by the expression of CD103 and by CD69, and develop in the skin from epithelium-infiltrating precursor cells (18). CD103 is the α subunit of the $\alpha_E\beta_7$ integrin receptor, binds E-cadherin, which is highly expressed on epithelial cells and thus promotes lodging of $CD8^+$ T cells in the epidermis (19), while CD69 inhibits egress of T cells from tissue via the sphingosine 1-phosphate receptor 1 (20, 21). The epidermal psoriatic $CD8^+$ T cells belong to the $T_{helper/cytotoxic}17$ ($T_{h/c}17$) T cell type and produce the cytokines interleukin (IL)-17, TNF-α and IL-22 (22), which are the signature cytokines of the lesional psoriatic immune response (23). These cytokines promote the epithelial hyperplasia, accumulation of neutrophilic granulocytes and production of antimicrobial peptides, and thus convey the clinical manifestation of psoriasis with the scaly erythematous squamous plaques.

In addition to these clinically apparent cytokine effects, IL-17 and TNF-α have another impact: they alter the functional state of melanocytes. IL-17 and TNF-α synergize in reducing skin pigmentation while promoting melanocyte proliferation (24). They inhibit pigmentation-related signalling and melanogenesis by suppressing pigmentation-related genes and lowering cellular tyrosinase levels in melanocytes, thereby reducing cellular melanin content. IL-17 and TNF-α further jointly induce the expression of melanocyte mitogens, including CXCL1 and IL-8, thus enhancing melanocyte proliferation. In psoriasis, epidermal melanocytes are under the constant influence of IL-17 and TNF-α. Accordingly, the combined effect of these 2 cytokines causes hypopigmentation and, at the same time, increases the number of melanocytes by stimulating melanocyte proliferation, which is reflected by the expression of the proliferation marker Ki67 on melanocytes in psoriatic lesions (24). This impact on melanocytes can now explain the emergence of the Woronoff ring. During healing, the effect of IL-17 and TNF-α leaves a hypopigmented zone on the edge of regressing lesions, where melanogenesis is still suppressed despite the increased density of melanocytes along the basement membrane (Fig. 2B) seen in lesional psoriatic skin (24, 25). The progressive recovery of pigmentation genes, along with the numerically increased melanocytes can then lead to an abundant melanin production and thus cause post-inflammatory hyperpigmentation. This is already evident here as a hyperpigmented zone around the Woronoff ring (Fig. 2A) and can eventually induce hyperpigmentation of the entire lesion. Accordingly, selective therapeutic blockade of TNF-α or IL-17 caused rapid recovery of pigmentation and post-inflammatory hyperpigmentation in healing psoriatic lesions of treatment responders (24). The ring may develop if the psoriasis plaques regress centripetally and not evenly over the entire lesion, so that a remaining central plaque is surrounded by a healed skin zone. If the psoriasis lesions evenly heal, either a uniform post-inflammatory hyperpigmentation or hypopigmentation may remain at the site of the former psoriasis plaque, as is often observed.

At the same time, these findings raise the question of how psoriasis differs from vitiligo. Both diseases are based on an autoimmune response against melanocytes. The pathogenic T cells of vitiligo correspond with the expression of $CD8^+CD103^+CD49a^+$ to a tissue-resident memory phenotype, which is characterized by a high production of interferon (IFN)-γ and has the protective purpose to mediate local immunity to viruses (26). CD49a is the α-subunit of the $\alpha_1\beta_1$ integrin receptor and is expressed on ~15% of all skin-derived T cells (27). The $CD8^+CD103^+CD49a^+$ T cells express cytotoxic granules including granzymes and perforins and have a high cytotoxic potential, which can be further amplified by IL-15 (26). When activated in an autoimmune response against melanocytes they promote T-cell-mediated killing of melanocytes and induce the persistent depigmentation of vitiligo through the permanent elimination of melanocytes. The melanocyte-specific $CD8^+CD103^+$ psoriatic T cells are clearly distinguished from the melanocyte-specific $CD8^+$ T cells of vitiligo by the absence of the expression of CD49a, by a different cytokine transcription profile and a lack of cytotoxicity, which even IL-15 cannot overcome (26). Instead, they belong to the $T_{h/c}17$ phenotype, whose actual role is the antimicrobial immune defence against extracellular bacteria and fungi (23, 28). When activated against melanocytes in psoriasis, they are not cytotoxic, but induce an antimicrobial, yet sterile, immune reaction. Psoriasis can therefore be considered as a T-cell-mediated antibacterial defence reaction against melanocytes (29, 30). The reason for the different functional outcomes of the melanocyte-specific autoimmune response in psoriasis and vitiligo may lie in the different genetic predisposition of the 2 diseases. According to genome-wide association studies, psoriasis and vitiligo arise on a different genetic background and HLA-association, which may then decide the respective functional differentiation of the pathogenic immune response (31, 32).

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REVIEW ARTICLE

Psoriasis and Treatment: Past, Present and Future Aspects

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The management of psoriasis has evolved considerably over the past 100 years. This has occurred in parallel with our understanding of the pathogenesis of this common, complex and enigmatic disease. It should be celebrated as an outstanding example of successful translational research. With precise targeting of immune pathways for the treatment of psoriasis with new biologics and small molecules has come the realisation that the most effective approach to patient management is a holistic one which encompasses the biopsychosocial nature of the disease. This involves a stratified medicine approach to identifying the best drug for an individual allied to patient education, screening for comorbidity, and regular review as both the clinical presentation and the patient's needs will change over time. Although there is not yet a cure for psoriasis – the whole person, systems approach to patient management, that is in part dependent on early intervention, should help to ensure an optimal outcome.

Key words: psoriasis; treatment.

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SIGNIFICANCE

Psoriasis is a common and disfiguring chronic skin condition. Over the past 100 years, our understanding of the disease has improved and as a direct result, more effective therapies have been developed. In addition to the cutaneous manifestations, it is associated with an increased risk of psoriatic arthritis, depression and cardiovascular disease. The best approach to care is an individualised one which focuses on improving the physical symptoms of the rash while proactively screening for and treating any associated comorbidities to minimise the impact of the disease and empower patients to live well.

This psoriasis-themed edition of *Acta Dermato-Venereologica* provides an opportunity to reflect on the progress which has been made in the treatment of psoriasis over the intervening 100 years since the journal was established in 1920.

The first volume of the journal featured a patient with 'psoriasis universalis' (1); a case which would fit with our current definition of erythrodermic psoriasis. The patient was treated with, a to us unusual, combination of bran baths, borvaseline emollient and injections of sterilised milk. An improvement was noted after the third cycle of injections, at which point cignolin (dithranol) was introduced to treat the remaining plaques. Fortunately, therapies for patients have evolved greatly in terms of efficacy, safety and tolerability. Over the past century therapies for psoriasis were more commonly discovered by chance and recommendations were largely based on anecdote. Today, serendipity still plays a role in the advancement and discovery of therapies but the reductionist approach to targeted therapies and evidence-based guidelines hold sway (2).

One hundred years ago, psoriasis was recognised as a relapsing and remitting skin condition for which temporary remission, but not cure, was possible with treatment (3). Although a cure remains elusive, treating psoriasis as an isolated skin disease is widely viewed as an outdated approach. The condition is now accepted as a systemic immune-mediated inflammatory disease associated with several comorbidities including psoriatic arthritis, mood disorders and cardiovascular disease. When selecting therapy, several factors should be considered in addition to the extent and clinical severity of the cutaneous involvement. These include psoriasis phenotype and previous treatment history, clinical severity and psychosocial impact, presence of psoriatic arthritis and other comorbidities, concomitant medications, conception plans and of course individual preferences and treatment goals. An effective approach to treatment is holistic, recognising the multi-faceted nature of the disease, and should be flexible as this chronic disease evolves and patient needs change over time.

The evolution of psoriasis treatment over the past century is an excellent example of successful translational research whereby an enhanced understanding of the pathogenesis of the disease has facilitated the development of increasingly precise targeting of therapies. Biologics are the proof of concept in this modern approach to drug design and development and the management of patients with moderate-severe disease has been transformed by these therapies. Complete skin clearance or psoriasis area and severity index (PASI) 100 has become a realistic treatment goal with use of the recently available anti-interleukin (IL) 23p19 therapies. Despite this progress, patients face many challenges including timely access

to appropriate care; the cost of under-treatment to the individual and to society remains and is considerable (4).

This review outlines the major treatments used for psoriasis over the past 100 years, focusing on important milestones through the decades. It illustrates a shift in approach from serendipity to science, as modern-day drug development is based on targeting key effector molecules in psoriasis, and a shift to a whole patient approach to care.

1920's

Even a hundred years ago, a variety of treatments, both systemic and topical, were available for psoriasis and salicylic acid, coal tar and dithranol preparations were all in use. **Fig. 1** illustrates those therapies which were available 100 years ago and tracks major therapeutic developments to the present day.

In the 19th century, arsenic became established as a popular treatment for psoriasis. It was trialled for a variety of dermatological conditions but appeared to be most effective for psoriasis. It was taken orally or applied topically – and even added to spa water. A narrow therapeutic range meant it was usually ineffective at low doses, and high doses were associated with clinically significant ocular and gastrointestinal tract disturbances (5). With more widespread prescription, the adverse effects associated with chronic use became more apparent. Cutaneous adverse effects including hyperpigmentation, keratotic and cancerous growths were noted towards the end of the 19th century but it continued to be used to treat psoriasis during the first half of the 20th century. Today, arsenic toxicity is well recognised (6).

Balmanno Squire first described the use of Goa powder (chrysarobin), a forerunner of dithranol (anthralin), for the treatment of psoriasis and published this in the British Medical Journal in 1876 (7). Produced from the araroba tree in Brazil, Goa powder had been used for centuries to treat fungal infections. Squire used Goa powder for a patient with psoriasis who he thought had tinea corporis; the psoriasis cleared prompting his accidental discovery of it as an effective treatment for the condition. Importing this product from Brazil to Europe became difficult during World War 1; in 1916, a synthetic version known as cignolin or dithranol was synthesized which seemed more efficacious than the natural variant. There is a correlation between efficacy and side-effects of irritancy and discoloration of skin, nails, clothes. In 1953, Ingram suggested using dithranol as a photosensitiser with ultraviolet-B radiation (UV-B) (8). The use of short contact dithranol became popular in the early 1980's. This involves applying a concentrated version of dithranol which is washed off after a few minutes and so is more practical and acceptable for patients. Nowadays dithranol is used only rarely by outpatients with most cases being treated in day treatment centers or as inpatients.

X-rays were first used to treat psoriasis at the beginning of the 20th century. Carcinogenic and other side effects became apparant over time, and so this method was phased out by the 1950's (3, 9). The beneficial effects of heliotherapy in treating psoriasis were first reported in 1923 (10), although patients had been aware, for centuries, that sunlight improves their psoriasis. In 1925, Goeckerman used a high-pressure mercury lamp to produce artificial broadband UV-B and demonstrated that the effect of UV-B was enhanced with prior application of crude coaltar as a photosensitiser (11).

1950's

Corticosteroids were discovered in 1950 and two years later, the first report of a topical steroid (17 hydroxycorticosterone-21-acetate) used to treat two patients with psoriasis was published; it did not have any noticeable

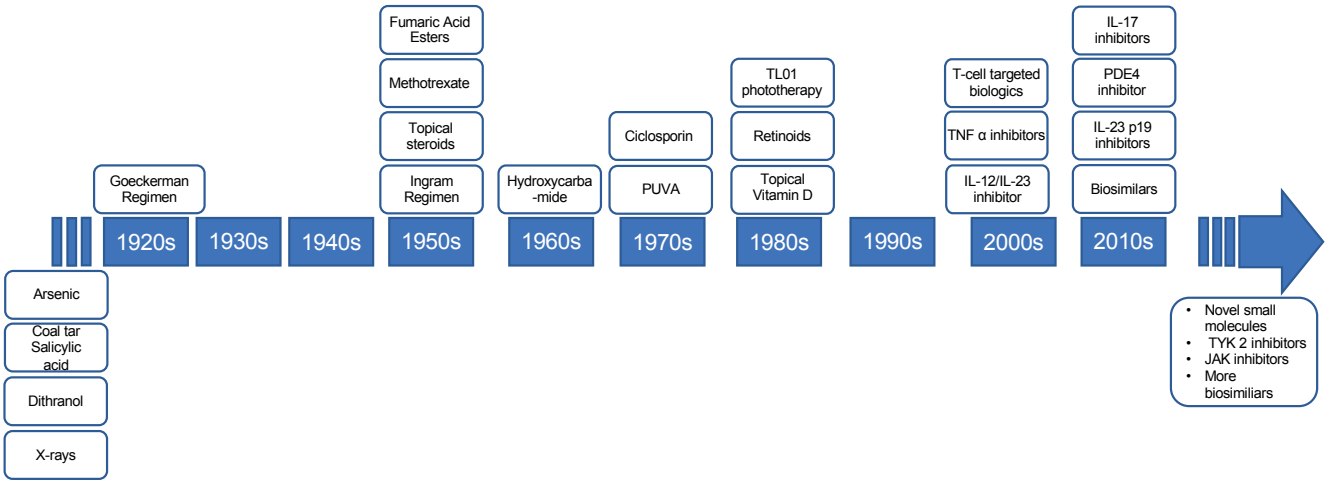

Fig. 1. Advances in the treatment of psoriasis over the past century. This timeline illustrates the major pharmacological advances which have occurred in the management of psoriasis over the past 100 years. It also speculates as to what may be the important therapies in the near future. IL: interleukin; PDE: phosphodiesterase; PUVA: psoralen plus ultraviolet (UV)-A; TNFα: tumour necrosis factor alpha; TYK: tyrosine kinase; JAK: janus kinase.

effect which may have been due to its low potency (12). Following this report, the structure of the compound was modified and by the end of the 1950's, a variety of local and systemic corticosteroid drugs had been developed. Prednisolone and triamcinolone were both shown to be moderately effective when taken orally (3). In the early 1960's, potent topical steroid preparations were developed including betamethasone 17-valerate (Betnovate®) and fluocinolone acetonide (Synalar®) marking a significant breakthrough in the treatment of dermatoses in general. We now know that potent steroids only suppress psoriasis temporarily. Today, topical corticosteroids are the acknowledged first line recommended topical therapy either alone or in combination with vitamin D analogues (2). They are known to have an anti-inflammatory, immunosuppressive, and anti-proliferative mechanism of action in psoriasis. Steroid-related side effects are well recognised however, and include skin atrophy with increasing potency, tachyphylaxis, suppression of the pituitary-adrenal axis, and a rebound phenomenon which may lead to psoriasis becoming unstable or pustular.

Methotrexate was first used to treat psoriasis in the early 1950's. Aminopterine, a folic acid inhibitor which had been used to treat leukaemia, was shown to suppress arthritis experimentally. Thus, it was trialled in a group of patients with rheumatoid arthritis. One of these patients had psoriasis which improved markedly. The authors of this original report proposed that aminopterine probably worked via direct effect on epithelial cells; at the time, psoriasis was thought to be a disorder of keratinocytes (13). Ametopterine (methotrexate) a next generation folic acid antagonist was subsequently developed; it was shown to be as effective as aminopterine but less toxic (14). Methotrexate is currently recommended as first line therapy for most people with psoriasis who are eligible for systemics. It is also effective for psoriatic arthritis (15). It is typically given as a once weekly oral dose. Folic acid supplementation is recommended when prescribing methotrexate. Folic acid use may also decrease the gastrointestinal and mucosal side-effects of methotrexate (16) whilst having a protective effect against hepatotoxicity (17). Its specific mechanism of action remains uncertain. It is thought to exert an anti-inflammatory effect via adenosine pathways. Some of the immunomodulatory effects are mediated through the inhibition of nucleic acid synthesis in keratinocytes and activated T cells (18). A recent meta-analysis showed that 45% of patients achieve PASI75 at primary endpoint (12 or 16 weeks, respectively) (19). The side effect profile of methotrexate is well characterised, in particular the hepatotoxicity risk. Appropriate patient selection to minimise this risk is important (2) and subcutaneous administration may reduce gastrointestinal side-effects and enhance efficacy (20).

The German chemist Schweckendiek was the first to use fumaric acid esters FAE to treat psoriasis in the late 1950's. He postulated that psoriasis occurred due to a deficiency in fumaric acid levels leading to defects in the Krebs citric acid cycle, and that oral supplementation of fumaric acid might neutralize these defects. He suffered from psoriasis, and used esters of fumaric acid in self-experimentation (21). The drug was subsequently modified to produce Fumaderm®, which comprises dimethyl fumarate (DMF), and calcium, magnesium, and zinc salts of monoethyl hydrogen fumarate; licensed for oral use in Germany since 1994. A second oral product, Skilarence® (dimethyl fumarate as a single acid ester), was introduced to the European market for the treatment of psoriasis in 2017. The mechanism of action of (FAE) remains unclear, but evidence suggests that it has nothing to do with the Krebs cycle. Recent systematic reviews have investigated the efficacy and safety of FAE using data from randomised controlled trials (22, 23). Meta-analysis showed that a PASI50 response rate at 12–16 weeks was achieved by 64% receiving FAE compared to 14% in the control group – it was not possible to calculate PASI75. It is ineffective for treating psoriatic arthritis. Of note, 8–39% of patients discontinue FAE treatment owing to adverse events, mostly relating to intolerable gastrointestinal or flushing complaints (23).

1970's

Psoralens, photosensitisers extracted from plants, have been used for centuries to manage skin conditions such as vitiligo. The beneficial effect of topical and the subsequent use of oral psoralens combined with UVA (PUVA) in treating psoriasis was first reported in 1973 (24) and subsequently became widespread (25). It was the most effective systemic therapy in use for psoriasis in the 1980s. PUVA can increase the lifetime risk of cutaneous squamous cell carcinoma, and this limits its use (26); indeed the use of PUVA has diminished markedly in recent years as it has been usurped by narrow band UVB, which was introduced in the 1980s after it was found to be more effective than broadband UVB in the treatment of psoriasis (27, 28).

In 1979 it was reported, serendipitously, that ciclosporin improved psoriasis in patients with psoriatic arthritis (29). By this time, it was already known to be an immunosuppressant drug which exerted its effect through inhibition of T-cell proliferation and had transformed outcomes in solid organ transplant recipients (30), but the mechanism of action in treating psoriasis remained unclear. A few years later, the active selective recruitment of T-helper cells into psoriasis plaques was demonstrated (31, 32). The authors proposed that psoriasis should be considered a T-cell-mediated disease and this hypothesis was subsequently proven by the remarkable efficacy of ciclosporin in its treatment (33). Today, there is substantial evidence for efficacy of ciclosporin in psoriasis vulgaris (34) but its use is limited by a relatively narrow therapeutic index. Nephrotoxicity and hypertension

are the most significant common risks of ciclosporin. Nephrooxicity risk is directly related to the dose and duration of ciclosporin (35). Thus, single or intermittent short courses of up to 16 weeks are recommended to limit nephrotoxicity (34, 35). Ciclosporin is particularly effective for patients who need rapid or short-term disease control (for example a psoriasis flare), have palmoplantar pustulosis or are considering conception and systemic therapy cannot be avoided (2).

The discovery of ciclosporin marked a turning point in the history of psoriasis treatment and the direction of future translational research as it became clear that a deeper understanding and subsequent modulation of the immune system would lead to more effective disease control.

1980's

Vitamin D analogues were investigated in the 1980s for a range of dermatoses. Calcipotriol, a vitamin D3 analogue, was shown to be effective in reducing proliferation and inducing differentiation of epidermal keratinocytes, indicating potential efficacy when used topically for psoriasis (36, 37). This therapy was subsequently shown to be significantly more effective than either dithranol (38) or tar (39). Although not as effective as potent topical steroids, calcipotriol has the advantage of not being subject to the same side-effects. Calcipotriol may protect against corticosteroid-induced dermal atrophy (40). Local irritation at the site of application affects up to 20% of patients (41) and this may lead to discontinuation. The vitamin D analogue calcitriol tends to be less irritating than calcipotriol and so may be better tolerated on face and flexural sites (42). Combination of calciptriol and betamethasone valerate as either ointment, cream, gel, or, more recently, a foam spray preparation, has proven to be a highly effective topical preparation for psoriasis (43).

The importance of vitamin A in maintaining healthy skin was recognised over 100 years ago. Synthetic vitamin A drugs, retinoids, were subsequently developed and trialled for a variety of skin diseases. Etretinate was found to improve psoriasis but this was replaced by acitretin, its pharmacologically active metabolite, in the late 1980s, because of a more favourable and less lipophilic pharmacokinetic profile. The precise mechanism of action is not understood. It is believed to interfere with epidermal growth factor receptor gene expression which reduces epidermal cell proliferation and differentiation to a normal rate (44). Additional anti-inflammatory effects may be mediated through nitric oxide (45).There is considerable variability in reported effectiveness of acitretin and anecdotally, it tends to work best for the less common pustular and erythrodermic variants of psoriasis (46). Acitretin may be combined with PUVA which is more effective than PUVA alone, reducing the number of PUVA treatments needed and hence UVA exposure (47). The use of acitretin is limited by its safety profile. It is highly teratogenic and pregnancy should be avoided for at least 2 years after the last dose. These days, acitretin is recommended for adults and in exceptional cases for children and young people if other conventional systemics (ciclosporin and methotrexate) are not appropriate or have failed, and for cases of pustular psoriasis (2).

With the development of an increasing number of systemic therapies, it became apparent that a valid and objective approach to the assessment of cutaneous disease severity and response to treatment was needed for psoriasis. Fredriksson & Pettersson created the PASI in 1978 as an objective means to evaluate the clinical efficacy of retinoids for psoriasis (48). Today, it is the most widely recognised outcome measure in psoriasis management. The Dermatology Life Quality Index (DLQI), the first dermatology-specific health-related quality of life questionnaire, was published several years later in 1994 (49). Although not specific for psoriasis, it is widely used to assess the subjective effectiveness of psoriasis treatments and their effect on quality of life.

BIOLOGICS ERA

Biologics are a subgroup of drugs comprised of large complex protein molecules including monoclonal antibodies and receptor fusion proteins. Unlike the traditional systemics which are taken orally, these are administered parenterally – as they would otherwise be degraded by the gastrointestinal tract. These target specific components of the immune system that are involved in psoriasis pathogenesis.

Biologics are indicated for moderate-severe psoriasis which has not responded to conventional systemic therapies. This licensing reinforces the current stepwise approach to psoriasis treatment. Patients with mild or limited extent disease are typically prescribed topical therapy in the first instance. If this is not sufficient, they are deemed to have moderate-severe disease and phototherapy or conventional systemic therapies (methorexate, ciclosporin, acitretin) are used next. If these fail, small molecule therapies (FAEs, apremilast) or biologics are indicated. Unfortunately, we do not yet have the tools in clinical practice to predict which patient will respond favourably to a given drug. As a result, between 11 and 35% of patients do not respond sufficiently to their first biologic drug during the first year of treatment, either because the drug is not effective or adverse effects develop (50).

2000's

T-cell targeted biologics

Research on the mechanism of action of ciclosporin in treating psoriasis (29) affirmed it being a T-cell-mediated

disease, and subsequent mouse models added further evidence to the theory that immune cells are the primary effector cells in driving the disease (51). Ensuing from this, the first biologics to be developed for the treatment of psoriasis targeted T cells.

The first was alafacept, which was approved for psoriasis use in 2003. This is a human lymphocyte function-associated antigen (LFA)-3/immunoglobulin (Ig) 1 fusion protein. It binds to CD2 molecules on the surface of activated T cells, blocking their co-stimulation by antigen presenting cells. It selectively targets memory-effector T cells, blocking their activation and migration (52, 53). Despite high hopes resulting from the known mechanism of action of this drug and what was known about the pathogenesis of psoriasis at that time, the results of phase III studies indicated a modest overall efficacy (54, 55). The overall PASI75 response rate was 33%. Median duration of remission (time to retreatment or maintenance of PASI50) was 7 to 10 months in phase II and III studies (56). In 2011, alefacept was withdrawn from the market as it had become clear that more efficacious and cost-effective options had become available.

Efalizumab was the first biologic to be approved in the UK for the management of psoriasis in 2003. This drug is a humanized monoclonal IgG1 antibody, directed against CD11a, the α-subunit of LFA-1. This inhibits T-cell trafficking into the skin. In phase III studies, the PASI75 response rate was approximately 30% when compared to placebo (57, 58). Increasing the duration of treatment from 12 to 24 weeks resulted in a PASI75 of 44% (58). Post-marketing drug surveillance revealed an association between long-term treatment with efalizumab and progressive multifocal leukoencephalopathy (PML) which is a rare but life-threatening infection of the central nervous system (59). As a result, efalizumab was withdrawn from the market in 2009. This reminds us of the importance of monitoring drug safety in the post-marketing phase.

Tumour necrosis factor-α inhibitors

Tumour necrosis factor (TNF)-α is recognised as a key effector cytokine in chronic immune-mediated inflammatory diseases, including psoriasis.

Etanercept was the first TNF-α inhibitor approved for treatment of psoriasis in 2004. It is a recombinant human TNF-receptor fusion protein. Each molecule can bind two TNF-α molecules. Phase III studies show that 100 mg weekly results in PASI75 at week 12 in 47–49% compared with placebo (60–62). Infliximab, a chimeric IgG1 monoclonal antibody which can bind to and neutralise soluble and membrane-bound TNF-α was approved for the treatment of severe psoriasis in 2006. This derived from the observation that it cleared the concomitant psoriasis of a patient in whom it had been administered for the management of Crohn's disease. Two phase III studies reported that intravenous infliximab 5 mg/kg at week 0, 2, and 6 resulted in PASI75 responses at week 10 of 75.5% and 80% compared with placebo (63, 64). This level of efficacy had not previously been recorded with any treatment for psoriasis. Adalimumab was approved for the treatment of psoriasis in 2005. Similar to infliximab, adalimumab is a fully human monoclonal antibody of the IgG1 isotype. PASI75 response rates of around 70% have been reported in clinical trials (65).

A meta-analysis has confirmed that infliximab is the most efficacious drug in this class in terms of PASI, followed by adalimumab (66). However, infliximab is associated with an increased risk of serious infection (67) and infusion reactions can occur.

Targeting TNF-α is particularly effective for treating psoriatic arthritis. Therefore, adalimumab is currently the recommended first line biologic for psoriasis with psoriatic arthritis (68). There are rare but potentially severe adverse events associated with this drug class including multiple sclerosis, congestive heart failure, opportunistic infection such as tuberculosis, and lupus. The risk of developing neutralizing anti-drug antibodies is well described for this class which in turn is associated with reduced clinical response to infliximab and adalimumab treatment (69).

Anti-TNFα therapies continue to develop and evolve. Certolizumab pegol (CZP) was licensed for psoriasis in 2018, and psoriatic arthritis in 2013. It is the only biologic agent with clinical trial data in its label supporting potential use in both pregnancy and breastfeeding. Prospective studies showed a lack of placental transfer of CZP from mothers to infants (70), and no to minimal transfer from plasma to breastmilk (71). The adalimumab and etanercept labels have recently been updated to allow potential use during pregnancy while acknowledging that they may cross the placenta (72). These recent developments have brought the issue of managing psoriasis in women of childbearing age into sharper focus, highlighting the specific challenges faced by this large group.

Ustekinumab anti-IL12/IL-23

Psoriasis was the first inflammatory disease for which ustekinumab was licensed by the US Food and Drug Administration (FDA) in 2009. It is a human IgG1 monoclonal antibody that targets the shared protein subunit p40 of IL-12 and IL-23. PASI75 response at week 12 in phase III studies was 66% (73) and 76% (74) and this was maintained at week 28. For patients with a bodyweight ≤100 kg the dose of ustekinumab is 45 mg and with a body weight of >100 kg the dose is 90 mg.

Registry data show that ustekinumab has a longer drug survival compared to the anti-TNF-α therapies (50, 75). Due to its effectiveness, weight-based dosing and safety record – it is recommended as first line biologic

for patients with psoriasis without psoriatic arthritis in the UK (68).

2010's

The current consensus is that psoriasis is a disease driven by the IL-23/TH17 cell pathway. For this reason, current therapeutic strategies are now focused on the development of novel agents that disrupt IL-23 or IL-17 cytokine signalling.

Three IL-17 pathway antagonists have been approved for the treatment of psoriasis: secukinumab was the first approved in 2015, and since then ixekizumab and brodalumab have come to market. Ixekizumab and secukinumab target IL-17A, while brodalumab targets the receptor subunit IL-17RA. Both secukinumab and ixekizumab have been approved for psoriatic arthritis. Phase III studies have demonstrated favourable efficacy and safety profiles. For the first time, significant numbers of patients are achieving PASI90 or PASI100 with treatment. In the CLEAR trial nearly 80% of patients treated with secukinumab achieved a PASI90 response at week 16 compared with only 58% in a comparison cohort treated with ustekinumab (76). The available safety information is overall reassuring, but there are specific adverse events associated with IL-17 inhibition, including increased risk of mucocutaneous candida and a slightly increased risk of developing inflammatory bowel disease (77). Four suicides were reported in clinical trials for brodalumab which raised some concern, but no causal relationship was demonstrated when these cases were reviewed (78). Bimekizumab is a novel drug in this class as it inhibits both IL-17A and IL-17F. The result of phase 3 comparative studies in patients with psoriasis and psoriatic arthritis are pending.

The latest biologic group to be licensed for the management of psoriasis are those which specifically target the p19 subunit of IL23. Three drugs have been licensed: guselkumab, rizankizumab and tildrakizumab. Guselkumab, the first of the 3 to be approved by the FDA in 2017, was compared to adalimumab in the VOYAGE 1 and 2 clinical trials. The PASI90 response at week 16 was 73% versus 50% (VOYAGE 1) and 70% versus 47% (VOYAGE 2), confirming the superior efficacy of guselkumab (79, 80). Guselkumab also showed superior long-term efficacy based on PASI90 at week 48 when compared with secukinumab (81).

In phase 3 studies comparing rizankizumab to ustekinumab at week 16, PASI90 was achieved by approximately 75% of patients receiving risankizumab versus 45% receiving ustekinumab and 4% receiving placebo (82). Pivotal trials for tildrakizumab selected PASI 75 at week 12 as the co-primary outcome measure. In one study, 64% of those who received the study drug achieved PASI75 compared to 9% of those who received placebo. In a subsequent clinical trial, 61% who received tildrakizumab and 48% who received etanercept achieved PASI75 (83).

The selective IL-23 p19 inhibitors have proved to be highly efficacious in clinical trials and no specific safety concerns have been raised to date (84). Although the depletion of IL17 by the anti-IL 17 biologics class has been associated with an elevated risk of opportunistic infections, mucocutaneous candida infections, and triggering or worsening of inflammatory bowel disease; the IL-23 p19 inhibitors have not been associated with these side effects (84). This is thought to be because residual IL17 is produced by non TH17 cells such as innate lymphoid cells and mast cells, so function is not clinically impaired. In addition, no increase in rates of malignancy, major adverse cardiovascular events, demyelinating disorders, active tuberculosis or reactivation of latent tuberculosis infection have been reported, although these have been associated with other biologic drug classes (84).

The real test will be how IL23p19 inhibitors perform in the real-world clinical setting. In addition to PASI90 and PASI100, other novel outcomes have been assessed for these drugs. For example, the efficacy of withdrawal and retreatment with guselkumab was assessed in VOYAGE 2 and it was shown that few patients required retreatment by week 48 (80). Amongst patients treated with guselkumab, efficacy was maintained at 2 years with continuous therapy while efficacy improved amongst those who switched from adalimumab to guselkumab at week 52. Reassuringly, there was no significant increase in adverse event rate compared with rates through week 48 (85).

The recent increase in published head to head comparator studies amongst biologic therapies is a welcome addition to the literature as this provides more meaningful results than placebo comparator alone.

Apremilast is a small molecule therapy which was licensed in 2014 to treat moderate–severe psoriasis and active psoriatic arthritis. It inhibits phosphodiesterase (PDE) 4 and thus reduces expression of proinflammatory mediators such as TNF-α and IL-23 (86). The PASI75 response to apremilast 30 mg twice/day ranges from 29–41% at week 16 in clinical trials (87). It is moderately effective for both psoriasis and psoriatic arthritis, with an efficacy level comparable to methotrexate. Advantages include its oral administration and it is anti-inflammatory rather than immunosuppressant. It also has a favourable safety profile, laboratory monitoring is not required and a potentially advantageous weight loss effect (88). Gastrointestinal intolerance is the most common adverse effect reported in clinical trials – diarrhoea (18%) and nausea (17%) (89) and rates appear higher in real world clinical practice (90). Apremilast has potentially been associated with an increased risk of depression, although the incidence is low – caution and close monitoring is advised in patients with a history of depression.

RECENT THERAPEUTIC DEVELOPMENTS

A variety of small molecule oral and topical drugs are in development for psoriasis. Indeed, the majority of drugs in the clinical trial pipeline for psoriasis are small molecules. The drugs listed below interfere with the IL-23/TH17 cell pathway that is key in driving the disease.

Tofacitinib is an oral Janus kinase (JAK) inhibitor targeting JAK1 and JAK3, thus regulating immune response via interruption of intracellular signalling pathways involved in the pathogenesis of psoriasis. A recent meta-analysis showed that approximately one third of those receiving tofacitinib 5 mg twice/day and half of those receiving tofacitinib 10 mg twice/day achieve PASI75 at week 12–16. Results to date indicate that it is generally well tolerated in treating psoriasis (91, 92). Although of modest efficacy, the favourable safety profile is appealing. JAK show efficacy in the topical treatment of psoriasis as well as atopic dermatitis and may have utility in facial and flexural disease as they are without corticosteroid side-effects (91). Research into this new topical therapy is welcomed as there has not been very much development in this area in recent decades.

Tyrosine Kinase 2 (TYK 2) signalling pathways are implicated in psoriasis pathogenesis and recent Genome Wide Association Studies have identified TYK 2 as a "druggable target". This molecule is an intracellular signalling enzyme which can activate functional responses of interleukin-12, interleukin-23, and interferon receptors – key cytokine pathways in psoriasis pathogenesis. A recent phase 2 study of TYK 2 inhibitor therapy for moderate to severe psoriasis has shown promising results (93). Several different doses were trialled and the primary outcome measure was PASI75 at week 12. This was achieved by 75% of patients on the maximal dose of 12 mg daily. Trials of longer duration and with a larger population are required to determine the longer-term safety and effectiveness of this agent.

MANAGEMENT OF PSORIASIS AS A COMPLEX CHRONIC DISEASE

The management of a patient with psoriasis involves much more than selecting and prescribing the recommended drug. Effective chronic disease management demands a holistic and proactive approach. Management incorporates patient education, screening for comorbidity and adjusting therapy depending on changes in clinical presentation.

Patient education improves patients' understanding of psoriasis and imbues a sense of control (94) in addition to improving adherence and coping. Screening for comorbidity is included in some national guidelines for managing psoriasis (2). This is particularly important for psoriatic arthritis because early diagnosis and commencement of appropriate treatment goes some way to prevent irreversible joint damage (95). It is also important for the detection of risk factors for cardiovascular disease and mood disorders, both of which are highly prevalent amongst this group and contribute to the multi-morbidity complexity of psoriasis (96, 97).

Alcohol excess, smoking and obesity are more prevalent amongst patients with psoriasis and are predictors of poor outcome to systemic therapies. Pharmacovigilance registry data demonstrate that being either a current or ex-smoker, and high body mass index are associated with a reduced odds of achieving PASI90 at 6 months when treated with biologic therapy. This underscores the need for lifestyle management as such factors are modifiable (98). A recent systematic review indicated that weight loss can improve pre-existing psoriasis and psoriatic arthritis, and prevent the onset of psoriasis in obese individuals, highlighting the importance of this intervention as an adjunct in psoriasis management (99). Further investigation into the role of lifestyle management has been identified as a key research priority by the Psoriasis Association in their recently published priority setting exercise (100). Management of these lifestyle factors using motivational interviewing techniques as espoused by the Psoriasis Wellbeing (PsoWell™) (94, 101) programme is likely to play an increasingly prominent role as part of a more integrated approach to psoriasis management going forward.

Never before have there been so many treatment options for psoriasis. However, clinicians are often faced with a challenge when selecting which systemic or biologic drug to commence for their patient as it is not possible to predict which patient will respond to a given therapy. The resultant primary and secondary treatment failures are costly from a patient and socio-economic point of view. With this in mind, the PSORT (Psoriasis Stratification to Optimise Relevant Therapy) (102) consortium was established to develop predictors of clinical response to biologic therapies. This involves analysis of genomic and other biological data in well-phenotyped patients who are commencing a new biologic therapy. It is now clear that due to the complex and multifactorial nature of the disease, multi-omic data is the key to effectively stratify patients and guide systemic therapy accordingly. Another limiting factor is the great expense associated with biologics. Biosimilar drugs which are similar but not identical to established biologics, have now become available as the originator drugs have come off patent. These should reduce the cost of therapy, and so hopefully make biologics more accessible for more patients. It is important to consider the true burden of psoriasis in any health economic evaluation. Direct costs such as medications and hospital appointments are well characterised. Indirect costs such as lost productivity can be more difficult to assess accurately. It has been estimated that indirect costs account for 43% of the mean annual cost of psoriasis amongst those with moderate-

severe disease (103). Some of this could be offset by timely and effective treatment. Psoriasis patients with comorbidities use more healthcare resources and generate higher costs compared to those without comorbidities (104). Screening for and more aggressive treatment of such comorbidities may lead to better patient outcomes. Further research is needed in this field to establish the true burden of disease and relative cost effectiveness of therapy.

Although there are lots of therapies licensed for psoriasis, access to appropriate care remains a problem for many patients around the world. These inequalities are highlighted by the World Health Organisation in their Global Report on Psoriasis (105). The Global Psoriasis Atlas (GPA) aims to address this problem, firstly by establishing the true incidence and prevalence of disease, and then investigating the true burden of disease internationally. This in turn will enable any person with psoriasis, wherever they live in the world, access to the best available care locally.

The biologic revolution has transformed the standard of care for patients with severe disease. However, the majority of patients with psoriasis have mild–moderate disease in terms of cutaneous extent. Unfortunately, there have not been many new therapeutic developments for this group. Topical therapies remain the most commonly prescribed class of drug for psoriasis (106). It is hoped that small molecule therapies which are currently in the pipeline may be accessible for patients with moderate disease.

Randomised controlled trials (RCTs) are the gold standard when it comes to investigating the efficacy and safety of new therapies and most of the evidence which informs clinical guidelines is based on this principle. However, rigorous inclusion and exclusion criteria often mean that the study population is not representative of the usual, real world clinic population. For instance patients with psoriasis identified as being ineligible for RCTs of biologics are at least twice as likely as eligible patients to suffer serious adverse events (107, 108) and reduced efficacy. Prospective longitudinal data collection through pharmacovigilance registers such as The British Association of Dermatologists Biologics and Immunomodulators Register (BADBIR) provides an invaluable service to patients and clinicians by providing real-world safety and efficacy data.

Patients with psoriasis accumulate excess physical, psychological and socioeconomic morbidity throughout their lives (4). The reason for this is multifactorial and due to a combination of genetic, behavioural and environmental factors which are unique to an individual. Unfortunately, it is not yet possible to predict which newly diagnosed patient with psoriasis will go on to develop severe disease and associated co-morbidity. The natural history of psoriasis remains poorly understood. It has been suggested that systemic inflammation in psoriasis, perhaps emanating from adipose tissue, contributes to the increased risk of comorbidity (109) and provides further rationale for managing psoriasis with systemic therapies. It has been hypothesised that early intervention with systemic therapy could modify the course of disease and, as a result, reduce the potential for this cumulative impairment which can severely limit a patient from reaching their full potential. Identifying patients at an early stage in their disease course would also provide an opportunity to proactively screen for comorbidities and unhealthy lifestyle behaviours associated with psoriasis, providing an integrated systems approach to management. The collection of multi-omic (genomic, biochemical, demographic, phenotypical, clinical) data from patients with recent-onset disease could provide novel insight into subclinical predictors of disease progression and multi-morbidity (110). Ideally, longitudinal follow up could help determine the characteristics of patients who develop specific disease and comorbidity patterns. Stratification using algrithms based on these multi-omic data is likely to play a key role in guiding the management of immune mediated inflammatory diseases, such as psoriasis, in the future.

CONCLUSION

The evolution of psoriasis treatment over the past 100 years is a celebration of the advances which have been made in understanding and improving care for patients with this disease. A collaborative approach between clinicians, patients, academics and the pharmaceutical industry has been instrumental in enabling this progress. Whilst a cure for psoriasis is unlikely anytime soon, complete clearance of the disease with the newest biologic therapies is now a realistic goal for some. A whole person approach to disease management that embraces the P4 medicine principles of prediction, prevention, personalised therapy and patient participation is the logical extension of our realisation that psoriasis is a "systemic disease" with important physical and psychosocial consequences. Although systemic therapy of psoriasis has advanced considerably there is still an unmet need for more effective topical therapies and a more widespread use of biosimilars.

Future research will focus on the use of integrated multi-omic data to stratify patients and guide therapy. Improved access to care and early intervention with systemic therapy are concepts which are being discussed increasingly. This is where service development overlaps with research, calling for innovative approaches and research methodologies to achieve this goal.

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REVIEW ARTICLE

Psoriasis and Co-morbidity

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Psoriasis is associated with multiple co-morbid medical conditions. The purpose of this study is to evaluate the relationships between psoriasis and cardiovascular disease, psoriatic arthritis, mental health conditions, and immune-mediated diseases, respectively. A literature search was performed during the study period January 1, 2015 to December 18, 2018. Of 2,499 records identified, 28 met our criteria selection and were included in this review. The relationships between psoriasis and these multiple comorbid disease conditions are discussed and are important to consider when developing the treatment plan and overall management of patients with psoriasis. Early recognition and treatment of comorbid disease conditions is important to help improve the quality of life for these patients.

Key words: psoriasis; cardiovascular disease; psoriatic arthritis; mental health conditions; depression; immune-mediated disease.

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SIGNIFICANCE

Psoriasis is associated with many different medical conditions. In this study, the relationships between psoriasis and cardiovascular disease, psoriatic arthritis, mental health conditions, and immune-mediated diseases, respectively, are assessed. A literature search was performed during the study period January 1, 2015 to December 18, 2018. Based on these findings, the relationships between psoriasis and these multiple comorbid disease conditions are identified and discussed. The treatment of comorbid conditions can promote an enhanced quality of life for patients with psoriasis. Therefore, the recognition and treatment of co-morbid medical conditions is important to consider when taking care of patients with psoriasis.

Psoriasis is a chronic inflammatory skin disease that affects approximately 125 million individuals worldwide (1). Cardiovascular disease, psoriatic arthritis, and mood disorders are common comorbid disease conditions associated with psoriasis (2). Moreover, autoimmune diseases have been reported to be associated with psoriasis, which may suggest that the pathogenesis of psoriasis may involve autoimmune mechanisms. In this review, the relationships between psoriasis and cardiovascular disease, psoriatic arthritis, mental health conditions, and autoimmune diseases, respectively, will be evaluated.

METHODS

A literature search was performed to identify comorbid disease conditions associated with psoriasis. Psoriasis and the associated comorbid conditions of cardiovascular disease, psoriatic arthritis, psychiatric conditions, and autoimmune disorders were examined. Articles from the past 3 years, specifically January 1, 2015 to December 18, 2018, were searched via PubMed and Google Scholar with the following keywords: psoriasis, comorbid, cardiovascular, psoriatic arthritis, psychiatric disease, and autoimmune disorders. The available abstracts and literature that investigated the relationship between psoriasis and cardiovascular disease, major adverse cardiovascular events (MACE), psoriatic arthritis, depression, suicidal ideation, suicidal attempts, and immune-mediated disorders, respectively, were evaluated. We restricted the search results to English-only records. Case reports, case series, and studies including pediatric patients were excluded. A manual inspection of reference lists and relevant studies was also performed to identify any additional relevant studies.

RESULTS

A total of 2,499 records were identified. After application of criteria selection and removal of duplicates, 28 of these records were included in the review (**Fig. 1**). Citations within identified articles and relevant studies were also reviewed, and 18 articles that were not originally detected in database searches were also included.

PSORIASIS AND CARDIOVASCULAR DISEASE

Psoriasis and cardiovascular risk factors

Patients with psoriasis have a higher prevalence of traditional cardiovascular risk factors, including diabetes mellitus type 2, hypertension, dyslipidemia, and obesity (1, 3). Studies have shown that obesity is an independent risk factor for psoriasis (2). Specifically, studies have demonstrated a dose-dependent relationship between psoriasis severity and obesity (2). Moreover, independent of traditional risk factors, psoriasis is associated with a greater risk of diabetes. Recent evidence has suggested

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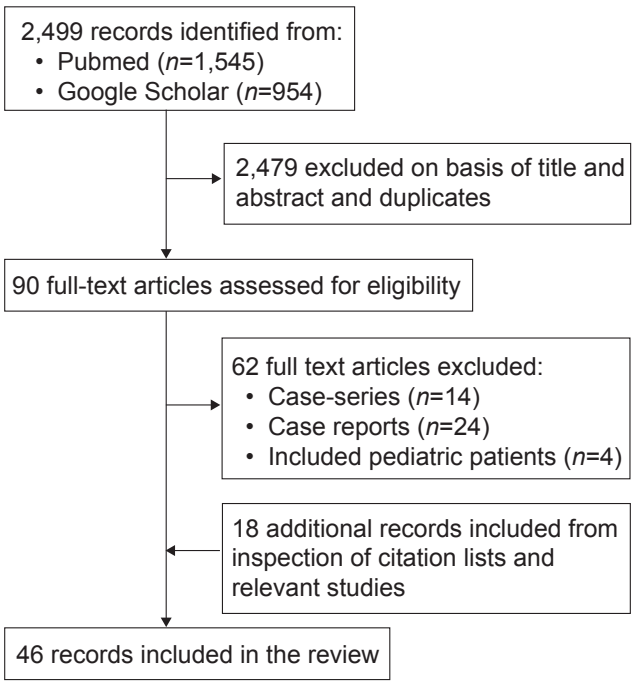

Fig. 1. Study-flow diagram of the included studies.

that the risk of diabetes, likelihood of insulin resistance, and diabetic complications increases with greater psoriasis severity, as defined by treatment patterns or BSA body surface area involved, independent of traditional risk factors (2). Studies have also demonstrated a higher prevalence of metabolic syndrome among patients with psoriasis compared to patients without psoriasis in adult and pediatric populations (2). The underlying mechanism of the association with psoriasis and these cardiovascular risk factors is not yet known, yet common inflammatory pathways, cellular mediators, and genetic susceptibility may contribute to these findings.

Association of psoriasis with vascular inflammation and cardiovascular events

Fluorodeoxyglucose F-18 positron emission tomography computed tomography (FDG PET/CT) is commonly used to measure aortic vascular inflammation and has been used as an indicator of cardiovascular risk and vascular disease (4, 5). Joshi et al. (5) demonstrated that patients with psoriasis with increased aortic vascular inflammation as evidenced by FDG PET/CT had significantly increased coronary artery disease indices, including total plaque burden, luminal stenosis, and high-risk plaques (5). Additionally, after adjustment for traditional cardiovascular risk factors, patients with severe psoriasis were at a higher risk for MACE compared to the general population (6). A prospective cohort study evaluated 115 psoriasis patients to determine the association between psoriasis disease severity and vascular inflammation as measured by FDG-PET/CT (7). At baseline, psoriasis severity was significantly associated with vascular inflammation. At one-year follow-up, improvement in psoriasis severity was associated with improvement in vascular inflammation, which maintained significance after accounting for traditional cardiovascular risk factors. Moreover, a significant 11% reduction in aortic vascular inflammation was observed for patients with greater than 75% reduction in psoriasis severity (7).

A study by Egeberg et al. (8) evaluated the impact of psoriasis duration on vascular disease and cardiovascular events. Among young patients with low cardiovascular risk by traditional risk scores and a high prevalence of cardiometabolic diseases, vascular inflammation as measured by FDG-PET/CT was significantly associated with disease duration. Moreover, duration of psoriasis demonstrated a strong association with MACE risk. Therefore, cumulative exposure to chronic inflammation among psoriasis patients may facilitate the development of vascular disease and MACE (8).

Patients with non-severe psoriasis also appear to be at an increased risk of developing cardiovascular events. Vascular indices of early arterial atherosclerosis (carotid intima-media thickness), endothelial dysfunction (flow-mediated dilation), and bioserum markers of oxidative stress (serum levels of advanced oxidation protein products) were assessed among patients with non-severe psoriasis. Compared to controls, patients with mild-to-moderate psoriasis without a history of any cardiovascular disease had significantly increased carotid intimamedia thickness, impaired flow-mediated dilation, and increased serum levels of advanced oxidation protein products (9). Additionally, a study utilizing echocardiographic evaluation demonstrated that left ventricular diastolic dysfunction was present among a greater number of young healthy patients with psoriasis compared with controls (10). Thus, patients with psoriasis appear to be at an increased risk of cardiovascular disease irrespective of underlying cardiovascular risk factors.

Impact of family history of cardiovascular disease

Family history of cardiovascular disease may help explain the increased risk of cardiovascular disease and MACE among patients with psoriasis. Egeberg et al. (11) compared the risk of incident MACE among patients with psoriasis with or without a family history of cardiovascular disease. Among patients with psoriasis and a family history of cardiovascular disease, the incidence ratios of MACE were 1.28 for mild and 1.62 for severe disease. No increased risk of MACE was detected among patients with psoriasis without a family history of cardiovascular disease (11). Therefore, it is important to determine the presence or absence of a family history of cardiovascular disease, as family history is likely a main contributor to MACE risk among patients with psoriasis.

Tumor necrosis factor inhibitor therapy and biomarkers of inflammation

C-reactive protein (CRP) is a biomarker of inflammation that has been utilized as a marker of cardiovascular risk (12). A retrospective cohort study evaluated the relationship between tumor necrosis factor (TNF) inhibitor therapy and changes in CRP among patients with psoriasis, psoriatic arthritis, or rheumatoid arthritis (13). At time of follow-up, mean change in CRP was lower among patients exposed to both a TNF inhibitor and methotrexate compared to methotrexate alone. For patients exposed to both a TNF inhibitor and methotrexate, the difference in mean CRP change was significantly lower compared to the methotrexate group after accounting for baseline CRP. Given that inflammation is a key contributing factor to the pathogenesis of cardiovascular disease (13), the results from this study suggest that exposure to TNF inhibitor therapy potentially reduces the risk of MACE among patients with chronic inflammatory conditions, including psoriasis. Thus, the results from this study further support the notion that TNF inhibitor therapy may offer a protective effect against developing MACE for patients with psoriasis.

Impact of biologic therapy on cardiovascular risk for patients with psoriasis

Treatment of psoriasis with TNF inhibitor therapy has been linked with a reduced risk of MACE among patients with psoriasis (14–16). A retrospective cohort study assessed MACE risk among patients with psoriasis receiving TNF inhibitor therapy compared to oral/phototherapy and topical therapy, respectively (14). After adjustment for cardiovascular risk factors, patients with psoriasis on TNF inhibitor therapy experienced significantly lower MACE hazard rate (HR) compared with patients on topical therapy. The MACE HR for patients in the oral/phototherapy group was similar to the topical group (14). The results from this study suggest that TNF inhibitor therapy may offer a protective effect against risk of MACE for patients with psoriasis.

Cumulative exposure to TNF inhibitor therapy may help further lower the risk of MACE for patients with psoriasis. A retrospective study by Wu et al. (15) compared the risk of MACE among patients with psoriasis receiving methotrexate versus TNF inhibitor therapy. After 12 months, patients receiving TNF inhibitor therapy developed fewer MACE and had lower cardiovascular event hazards compared to patients receiving methotrexate. Specifically, the MACE HR was 45% lower for patients receiving TNF inhibitor therapy compared to methotrexate. By 24 months median follow-up, every additional 6 months of TNF inhibitor exposure was associated with an 11% reduction in MACE risk (15). Therefore, cumulative exposure to TNF inhibitor therapy may help lower the risk of MACE for patients with psoriasis (15). A retrospective cohort study assessed the risk of MACE in patients with psoriasis receiving a TNF inhibitor versus phototherapy (16). Compared to patients receiving phototherapy, patients on TNF inhibitor therapy had a reduced MACE HR. Furthermore, every 6-month incremental cumulative exposure to TNF inhibitors was associated with a statistically significant reduction in MACE risk over a median observation period of 15.4 months. Furthermore, the risk reduction in MACE with 6 months of cumulative exposure was 11.2% greater among patients receiving TNF inhibitor therapy compared to phototherapy (16). Thus, the results of this study further suggests that cumulative TNF inhibitor exposure may lower the risk of MACE for patients with psoriasis (16).

Biologic therapy may attenuate coronary artery disease progression in patients with severe psoriasis. A prospective, controlled clinical study by Hjuler et al. (17) evaluated the association of biologic therapy with changes in coronary artery disease progression. The study evaluated coronary CT angiography among patients with severe psoriasis without symptomatic coronary artery disease at baseline and after 13 months of receiving biologic therapy (adalimumab, etanercept, infliximab, and ustekinumab) compared to control group. Among the control group, the severity of luminal narrowing in diseased segments was increased at 13-month follow-up, yet in the intervention group this was unchanged. In addition, the non-contrast coronary artery calcium scores were stable in the intervention group and progressed in the control group. A likely explanation for this finding is that biologic therapy helps decrease systemic inflammation, thus preventing cardiovascular disease progression. A limitation of this study is the small sample size of 28, which should be taken into consideration when interpreting these results. Nevertheless, biologic therapy appears to be associated with reduced coronary artery disease progression in patients with severe psoriasis.

On the other hand, there are also studies with opposing data regarding the effect of TNF inhibitory therapy on vascular inflammation. A randomized multicenter study by Bissonnette et al. (18) evaluated the impact of the TNF inhibitor adalimumab on vascular inflammation in patients with psoriasis. Utilizing PET/CT, no difference in vascular inflammation was appreciated over 16 weeks in the adalimumab group compared to placebo. Moreover, a randomized clinical trial by Mehta et al. (19) compared vascular inflammation and levels of cardiovascular biomarkers among patients with moderate-to-severe psoriasis treated with adalimumab, phototherapy, or placebo. At week 12, there was no difference in change in vascular inflammation as measured by FDG PET/CT among the adalimumab group (change compared with placebo, 0.64%) or the phototherapy group (−1.60%). Biomarkers of inflammation, serum CRP and IL-6, were decreased in both the adalimumab and phototherapy

groups. Therefore, while studies have demonstrated that TNF inhibitor therapy may lower the risk of vascular inflammation for patients with psoriasis, studies have also revealed evidence that is contradictory to these findings. For this reason, the exact impact of TNF inhibitor therapy on cardiovascular risk is currently still debated.

PSORIASIS AND PSORIATIC ARTHRITIS

Psoriatic arthritis is an inflammatory disease that involves the peripheral and axial joints, skin, nails, and entheses (20, 21). Psoriatic arthritis has been reported to affect approximately 6–42% of patients with psoriasis (2). The prevalence of psoriatic arthritis appears to increase with greater severity of skin disease and duration of psoriasis (2). Clinically, patients experience joint pain and swelling secondary to chronic joint inflammation that, if left untreated, can lead to long-term irreversible joint damage and disability (20, 21). Cutaneous lesions tend to precede joint involvement, which can develop years after being diagnosed with psoriasis (18, 19). Yet, according to a meta-analysis by Villani et al. (22), the prevalence of undiagnosed psoriatic arthritis in patients with psoriasis at time of seeking medical care is approximately 15.5%. Thus, all patients with psoriasis should be screened for psoriatic arthritis at every stage of their disease.

Screening for psoriatic arthritis

Multiple questionnaires are available to help diagnose psoriatic arthritis (21). These questionnaires include the Toronto Psoriatic Arthritis Screening Questionnaire (TOPAS), Psoriasis Epidemiology Screening Tool (PEST), Psoriatic Arthritis Screening and Evaluation (PASE), and the Psoriasis and Arthritis Screening Questionnaire (PASQ) (21). Despite the development of these screening questionnaires, the ability to differentiate psoriatic arthritis from other forms of arthritis remains difficult and the diagnosis of psoriatic arthritis is often delayed (23). In a large population-based survey (24), 37.6% of dermatologists indicated that their greatest challenge in managing patients with psoriatic arthritis is discerning psoriatic arthritis from other arthritic diseases, while 25% of rheumatologists indicated that delayed referral is one of their greatest challenges. Moreover, joint pain was reported among 51.8% of psoriasis patients without a diagnosis of psoriatic arthritis, however only 18.6% of dermatologists reported that their patients had joint pain (24). Based on these results, there could be a discrepancy in the interpretations of joint involvement between physicians and patients with psoriasis. Additionally, there may be a need for enhanced communication between dermatologists and rheumatologists as well as within rheumatologists using enhanced tools to differentiate psoriatic arthritis from other arthritic diseases (25, 26). Cohen et al. (23) offered a simple, concise screening tool that encompasses key characteristics of psoriatic arthritis. The tool consists of the mnemonic "PSA," for which P stands for pain (joint pain), S stands for both stiffness (>30 min after a period if inactivity) and sausage digit (dactylitis), and A stands for axial (axial joint involvement/back pain referring to stiffness that improves with activity) (23). Moreover, the Classification Criteria for Psoriatic Arthritis (CASPAR) incorporates clinical findings specific to psoriatic arthritis, including presence of psoriatic nail dystrophy, a negative rheumatoid factor test, dactylitis, and radiographic evidence of juxta-articular bone formation) (21). CASPAR is highly specific (99.1%), however has lower sensitivity for detecting early psoriatic arthritis (87.4%) (21). Thus, it serves better as a confirmatory test rather than a screening tool, and can help physicians differentiate psoriatic arthritis from other forms of arthritis.

Symptom and complications of psoriatic arthritis

Patients with psoriasis and comorbid psoriatic arthritis tend to experience more symptoms and complications with respect to physical functioning compared to psoriasis patients without psoriatic arthritis (27). Compared to psoriasis patients without psoriatic arthritis, patients with psoriasis and psoriatic arthritis appear to have significantly more comorbid conditions, including hypertension, diabetes mellitus, and hyperlipidemia (20). In addition, patients with psoriasis and psoriatic arthritis have higher health care utilization and costs compared to patients without comorbid psoriatic arthritis (20). A study by Edson-Heredia et al. (28) demonstrated that patients with moderate-to-severe psoriasis and comorbid psoriatic arthritis experienced a greater impact on quality of life and symptoms of itching, physical irritation, and pain compared to patients with moderate-to-severe psoriasis alone. Among psoriasis patients with comorbid psoriatic arthritis, a greater frequency of comorbid diseases was reported, including type 2 diabetes mellitus and hypertension, compared to patients with psoriasis alone (28). Thus, psoriasis patients with psoriatic arthritis may experience greater psoriasis-related disease burden compared to patients with psoriasis alone.

Importance of recognition and treatment of psoriatic arthritis

Early recognition of psoriatic arthritis is imperative because improved control of inflammation can prevent joint destruction and improve quality of life. According to a study by Haroon et al. (29), a diagnostic delay of over 6 months from time of symptom onset to visit with a rheumatologist contributed to the development of joint erosions and worse functional disability as evidenced by Health Assessment Questionnaire (HAQ) scores for patients with psoriatic arthritis. Yet, a delayed diagnosis of psoriatic arthritis over one year was not associated with a significant difference in HAQ scores. A large

United Kingdom multicenter study that evaluated factors contributing to work disability among patients with psoriatic arthritis found that worse physical function was associated with unemployment (30). Moreover, among participants that were employed, greater disease activity and worse physical function were associated with higher levels of productivity loss. Thus, among patients with psoriatic arthritis, productivity loss could potentially be prevented with treatment of psoriatic arthritis (30). Rahman et al. (31) also found that treatment of psoriatic arthritis was associated with improvements in physical function and health-related quality of life. Additionally, a study by Kirkham et al. (32) demonstrated that treatment of psoriatic arthritis was associated with improved quality of life as measured by patient reported outcomes (specifically, EuroQol-5D scores). Moreover, patients with shorter disease duration exhibited significantly greater improvements in disease activity and patient reported outcomes of joint pain and quality of life (32). The results from this study suggest that early intervention may have a more prominent impact on patient-reported outcomes of disease activity and quality of life. Moreover, treatment options for psoriasis, including biologics, can have different efficacy on cutaneous disease versus joint disease, which is important to consider when choosing appropriate therapy, which should ultimately be tailored for the individual patient (33).

PSORIASIS AND MENTAL HEALTH CONDITIONS

Patients with psoriasis are at an increased risk of depression compared to the general population (34). Moreover, depression in psoriasis patients may increase risk of other comorbidities. In a prospective cohort study, patients with psoriasis and major depressive disorder (MDD) were found to be at a significantly increased risk of developing psoriatic arthritis compared to psoriasis patients without MDD (35). Additionally, a study by Egeberg et al. (36) found patients with psoriasis and comorbid depression to be at an increased risk of myocardial infarction, stroke, and cardiovascular death. Moreover, Aberra et al. (37) demonstrated that vascular inflammation (as measured by FDG PET/CT) and total and non-calcified coronary plaque burden (as measured by coronary CT angiography) were significantly higher among patients with psoriasis and self-reported depression versus patients with psoriasis alone. After adjustment for traditional cardiovascular disease risk factors, vascular inflammation, total plaque burden, and non-calcified burden were significantly associated with self-reported depression (37). The reported findings may be due to the chronic inflammation present in psoriasis, which potentially increases the risk of developing cardiovascular events. Furthermore, psoriasis patients with comorbid depression are at an even greater cardiovascular risk due to the reported association between depression and cardiovascular events, subclinical atherosclerosis, and all-cause mortality, independent of traditional cardiovascular risk factors (37). Therefore, patients with psoriasis and comorbid depression appear to be at a greater risk of inflammation-induced atherosclerotic plaque development, ultimately increasing the risk of developing cardiovascular events. Comorbid depression could also result in reduced adherence to treatment for psoriasis as well as utilization of healthcare resources, consequently interfering with cardiovascular risk factor management (36, 38). Thus, detecting and treating comorbid depression may prevent the development of complications for these patients.

Psoriasis is also associated with anxiety and suicidal ideation (39, 40). A systematic review and meta-analysis by Singh et al. (41) assessed the relationship between psoriasis and suicidality. Compared to patients without psoriasis, patients with psoriasis were twice as likely to exhibit suicidal ideation and suicidal behaviors (combined attempted and completed suicides; pooled (41). Thus, it is important to detect and treat comorbid mental health conditions in patients with psoriasis.

PSORIASIS AND IMMUNE-MEDIATED DISORDERS

An increased frequency of immune-mediated disorders has been reported among patients with psoriasis (42–46). However, a definite relationship between psoriasis and immune-mediated diseases remains unclear (42). Compared to patients with mild psoriasis, patients with severe psoriasis demonstrated significantly higher diagnosis rates of rheumatoid arthritis, lupus, and Crohn's disease (43). In addition, the presence of autoreactive T cells have been demonstrated in the pathogenesis of psoriasis, which suggests that psoriasis may be autoimmune in nature (44–46). A nationwide, population-based, cross-sectional study evaluated the association between psoriasis and various immune-mediated rheumatic diseases among 267,230 patients with psoriasis and 267,230 controls without psoriasis (47). Psoriasis was significantly associated with ankylosing spondylitis (AS), rheumatoid arthritis (RA), Behçet disease, systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and dermatomyositis/polymyositis (DM/PM). Moreover, male patients with psoriasis exhibited higher associations with AS, RA, SLE, SSc, and DM/PM compared to female patients with psoriasis (47).

A case-control study evaluating 287 patients with bullous pemphigoid (BP) and 1,373 matched controls found that the prevalence rate of psoriasis was greater among patients with BP versus controls (48). Moreover, psoriasis preceded the diagnosis of BP, by a mean duration of 25.2 years (48). Additionally, a cross-sectional study detected a significant association between psoriasis and Hashimoto's thyroiditis that sustained after adjusting for confounding variables, including sex, age, psoriatic

arthritis, and use of systemic anti-psoriasis agents (odds ratio 2.49) (49). Chronic inflammation and subsequent damage to the basement membrane has been suggested as a possible mechanism for the reported association between BP and psoriasis (42). Another theory is that treatment for psoriasis may worsen subclinical bullous pemphigoid (42). Further studies would help determine the exact association between psoriasis and BP, as well as other autoimmune disorders.

CONCLUSION

The association between psoriasis and comorbid disease conditions is important to consider when developing the treatment plan and overall management of patients with psoriasis. Psoriasis is associated with cardiovascular disease, and chronic inflammation likely plays a major role in this relationship. Treatment of psoriasis improves underlying inflammation and TNF inhibitor therapy may provide a protective effect against risk of MACE for patients with psoriasis, which would ultimately promote better health outcomes for these patients. Moreover, psoriatic arthritis is a common comorbid condition associated with psoriasis that can lead to permanent disability. Early treatment is imperative to help prevent complications of psoriatic arthritis and improve quality of life for these patients. Furthermore, it is important to address and treat comorbid psychiatric conditions among patients with psoriasis, including depression, suicidal behavior, and suicidal ideation. Future clinical trials would help better assess the role of biologic therapy on improving health outcomes, wellness, and quality-of-life for patients with psoriasis. Certain immune-mediated disorders have been reported to be associated with psoriasis. Further research will help better assess these associations as well as the autoimmune aspects of the underlying pathogenesis of psoriasis.

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REVIEW ARTICLE

Pustular Psoriasis: The Dawn of a New Era

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Pustular psoriasis is a clinically heterogeneous entity of different, orphan disease subtypes, among which the most clearly defined are generalized pustular psoriasis, palmoplantar psoriasis, and acrodermatitis continua of Hallopeau. Although phenotypically and genetically distinct from psoriasis vulgaris, these subtypes may be associated with plaque psoriasis lesions, establishing the rationale for their inclusion in the psoriasis spectrum. Unlike psoriasis, however, their genetic background is thought to be mainly monogenic, as shown by the recent identification of mutations in 3 different genes of the skin innate immune system; *IL36RN*, *CARD14* and *AP1S3*. These major advances in the understanding of the disease pathogenesis have led to the design and ongoing development of tailored therapeutic approaches, which are highly necessary given the refractory nature of pustular psoriasis in response to most available antipsoriatic drugs.

Key words: pustular psoriasis; pustulosis; generalized pustular psoriasis; palmoplantar pustulosis; interleukin-36.

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SIGNIFICANCE

Pustular psoriasis defines a heterogeneous group of skin inflammatory diseases, which have in common the presence of aseptic pustules. Genetically distinct from psoriasis vulgaris, they have been shown to be related to mutations in any of 3 genes of the skin immune system, respectively called *IL36RN*, *CARD14* and *AP1S3*. These recent advances have initiated the design of biological drugs specifically targeting key actors of inflammation in pustular psoriasis, with interleukin-36 inhibitors as the most advanced example of therapeutic development.

Psoriasis is a chronic inflammatory disease entity that includes different clinical phenotypes, of which the so-called psoriasis vulgaris (PV) or plaque psoriasis variant is by far the most prevalent, representing approximately 80% of cases, and resulting from the combination of a multigenic, complex genetic background with environmental triggers (1, 2). Aside from this most frequent clinical form of psoriasis, much rarer clinical phenotypes, all characterized by the presence of neutrophilic skin inflammation with macroscopically visible, non-infectious or aseptic pustules, have been termed pustular psoriasis and, in some cases, psoriasis-related subphenotypes (2). Long-neglected, these phenotypes have attracted a lot of interest over the last 10 years due to major advances in the understanding of their pathogenic mechanisms, involving a major deregulation of skin innate immune responses, reflected at the histological level by an intense afflux of neutrophils and monocytes in the lesional dermis and epidermis (2). The current review integrates long-established clinical features with the more recently re-defined disease subtypes classification and, more importantly, advances in physiopathological scenarios, which have already driven therapeutic innovations.

Pustular psoriasis consists of several clinical entities, of which the best defined are: (*i*) localized pustular psoriasis dominated by palmoplantar pustular psoriasis (PPPP), also called palmoplantar pustulosis (PPP); (*ii*) acrodermatitis continua of Hallopeau (ACH), which predominantly involves acral areas of the hands and/or feet; and (*iii*) generalized pustular psoriasis (GPP), a disseminated, severe and potentially life-threatening form of psoriasis. The question of whether these pustular skin disorders belong to the psoriasis spectrum has been debated for a long time. Their intersection and overlap with PV is reflected by their frequent coexistence in a given patient, and by some commonalities in their respective mechanistic models. In this sense, studies of pustular psoriasis have also been insightful regarding mechanisms of skin inflammation, both in physiology and for other skin-inflammatory diseases.

GENERALIZED PUSTULAR PSORIASIS

Clinical characteristics and diagnostic procedures

GPP, the most severe of all the psoriatic disease variants, is an orphan skin and multisystemic inflammatory disease characterized, in its typical forms, by intermittent flares or attacks with partial or complete remission in between (**Fig. 1**). Estimated prevalences of GPP are 0.0002% and 0.0007%, in France and Japan, respectively (3, 4). Each flare consists of the acute onset of a rapidly disseminating cutaneous eruption an extensive skin rash covered with aseptic pustules at an early stage, combined at some point

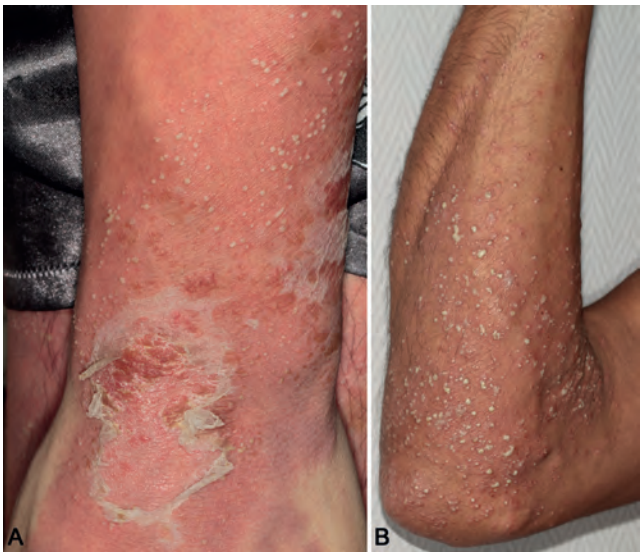

Fig. 1. Skin lesions in patients with generalized pustular psoriasis. (A) A diffuse erythema is covered with confluent pustules, leading to formation of pustular lake, with superficial scaling at a later stage, in a patient free of *IL36RN* or *CARD14* mutation. (B) Disseminated, separated pustules on an erythematous basis of the forearm of an adult patient with identified deficiency of IL36 receptor antagonist.

with systemic symptoms, such as a variable degree of fever up to 40°C, and general malaise with fatigue. Other extracutaneous manifestations, such as polymyalgia and polyarthralgia, are common, and arthritis may occur (5, 6). Several subtypes of GPP have been defined depending both on disease course and clinical presentation: (*i*) the acute von Zumbusch type; (*ii*) GPP in pregnancy, previously named impetigo herpetiformis; (*iii*) the annular GPP clinical subphenotype; and (*iv*) GPP associated with PV. GPP is an unpredictable disease; the spectrum of severity of attacks varies widely between patients and in any given patient, ranging from the absence of any systemic symptoms, which does not rule out diagnosis, to the presence of high fever or even life-threatening complications requiring admission to the intensive care unit (6). Biological test abnormalities typically consist of raised serum concentrations of inflammatory proteins, mainly C-reactive protein (CRP), peripheral blood hyperleukocytosis with neutrophilia, and a high prevalence of liver test abnormalities, sometimes delayed with respect to the onset of ongoing GPP flare (7, 8). Extracutaneous manifestations of GPP, such as osteoarthritis, uveitis, acute respiratory distress syndrome, and cardiovascular aseptic shock (the last related to the massive release of inflammatory cytokines), may occur at any stage during the disease course (7, 9–11). The reported high prevalence of liver test abnormalities during GPP attacks, mainly mild to moderate cholestasis and/or cytolysis, raised the hypothesis of aspecific liver/biliary involvement by the inflammatory process. This hypothesis was reinforced by results from magnetic resonance imaging (MRI) of the liver and biliary ducts, showing, in some patients, strictures alternating with dilatations of the principal and/or accessory biliary ducts, and was definitively ascertained by histopathological analysis of liver biopsies showing innate immune cells, mainly neutrophils, infiltrating the epithelium of biliary ducts and the portal and periportal spaces (6). This last entity, which shares features of other types of inflammatory cholangitis, was further termed neutrophilic cholangitis, and seems to have a benign short- and medium-term evolutive profile, although additional follow-up studies are needed to investigate its long-term prognosis (8). More recently, cases of neutrophilic cholangitis have been reported in patients with localized pustular psoriasis, and in those with PV with or without psoriatic arthritis, raising the hypothesis that deregulation of the extracutaneous innate immune system is not exclusive to GPP, but may also be observed in more frequent psoriatic variants (12).

One very specific evolutive feature of GPP is the spontaneously self-remitting pattern of disease flares, at least in classical intermittent forms. Typically, this spontaneous remission of attacks happens in a matter of weeks following the onset of attacks, but there are cases in which chronic skin lesions persist in between attacks of GPP (9). Whatever the genetic background, the intermittent, acutely flaring course of GPP allowed triggering factors to be identified, the best known being infections, stress, corticosteroid treatment withdrawal, and pregnancy (5, 6, 13). Cases of GPP with onset during pregnancy, usually early during the third trimester, have been also termed impetigo herpetiformis, but they are now acknowledged as part of the GPP entity. Their prognosis may be severe both for the mother and the foetus, potentially leading to intrauterine growth restriction, miscarriage, or foetal death (14, 15). Therefore, GPP in pregnancy requires close monitoring of foetal viability. GPP in pregnancy should be considered as a serious, potentially life-threatening situation for both mother and foetus.

Physiopathology and prognosis

Infectious respiratory viral triggers have been identified recently by multiplex PCR-based analysis of nasopharyngeal swabs in a small cohort of patients with different subtypes of psoriasis, including GPP (16). Interestingly, viral nucleic acids are potent agonists of the innate immune system, and can stimulate the release of inflammatory cytokines operating in psoriasis pathogenesis, including interleukin-36 (IL-36) (16). The role of these viral triggers has been raised in several studies, and a striking observation is the short time interval between the infection and onset of GPP flare, in keeping with a potent stimulation of the innate immune system (9, 13, 16). The role of these infectious triggers raises the challenge of immune intervention with immunosuppressants during GPP attacks with simultaneous infection, emphasizing the need for effective therapeutic strategies with appealing infectious safety profiles. The deciphering of

the pathogenesis due to identification of causal genetic abnormalities, mainly mutations of the *IL36RN* gene encoding a regulator of the IL-36 inflammatory pathway in a subset of patients with GPP, established a strong rationale for the development of targeted therapies, a crucial breakthrough which is addressed below (13).

Mortality rates for recent cohorts of GPP are not available, but its life-threatening potential is acknowledged. Likewise, some skin and systemic signs and symptoms-based attempts to score the severity of GPP flares have been launched in Japan, paving the way for more specific, reproducible scoring tools, in a disease where PV-specific Psoriasis Area Severity Index (PASI) is not suitable, notably due to the absence of any induration in GPP lesions (7).

Recent advances in the assessment of the severity of pustular psoriatic diseases are addressed below.

PALMOPLANTAR PUSTULAR PSORIASIS

PPPP, also called palmoplantar pustulosis (PPP), is the most common of pustular psoriasis variants, and the most common localized pustular variant (**Fig. 2**) (17). Prevalence estimates of 0.01% have been reported, and the disease predominates in women, with a strong link with smoking (18, 19). The disease usually presents as aseptic pustular lesions following a chronic course, going through different stages in the form of yellow scales or crusts, and at a later stage brown macular residual lesions. The onset of PPP usually occurs in adulthood, severely impairs patients' quality of life in severe cases, and may associate with extracutaneous involvement, such as nail disease, arthritis, and, rarely, with neutrophilic cholangitis (10). Occasionally PPPP may associate with autoimmune conditions, such as thyroiditis, although it is not known if the prevalence of clinical autoimmunity is higher than in the general population (20). One particular axial spondyloarthritis feature observed in patients with PPPP led to the definition of a syndrome called SAPHO (synovitis, acne, pustulosis, hyperosteosis osteomyelitis) (16). This is characterized by painful swelling of sternocostal and manubrial areas, and its diagnosis is usually established by bone scintigraphy (21).

While GPP typically follows a relapsing, intermittent course with disease-free intervals that may last for months or sometimes years, the evolutive pattern of PPPP is usually chronic and, like other pustular psoriatic subtypes, may associate with PV. It may also combine in some patients with ACH or, rarely, with GPP (13, 16).

ACRODERMATITIS CONTINUA OF HALLOPEAU

The ACH subphenotype is defined by pustular lesions involving extremities of the hands and feet, with progressive destruction of the nail apparatus, with or without underlying bone erosions (Fig. 2C) (22). Like PPPP, this rare, debilitating form has been reported in between GPP flares in patients with deficiency of IL-36 receptor antagonist (DITRA) (13). The threat of definitive nail and/or bone damage warrants early treatment of patients with ACH.

DIFFERENTIAL DIAGNOSES

Diagnosis of pustular psoriasis relies mainly on clinical features and is usually easy. Characteristic histopathological findings include the formation of intraepidermal neutrophilic abscesses, with marked dermal infiltrate composed of neutrophils, monocytes and T-lymphocytes (1, 2). One major differential diagnosis of GPP is acute

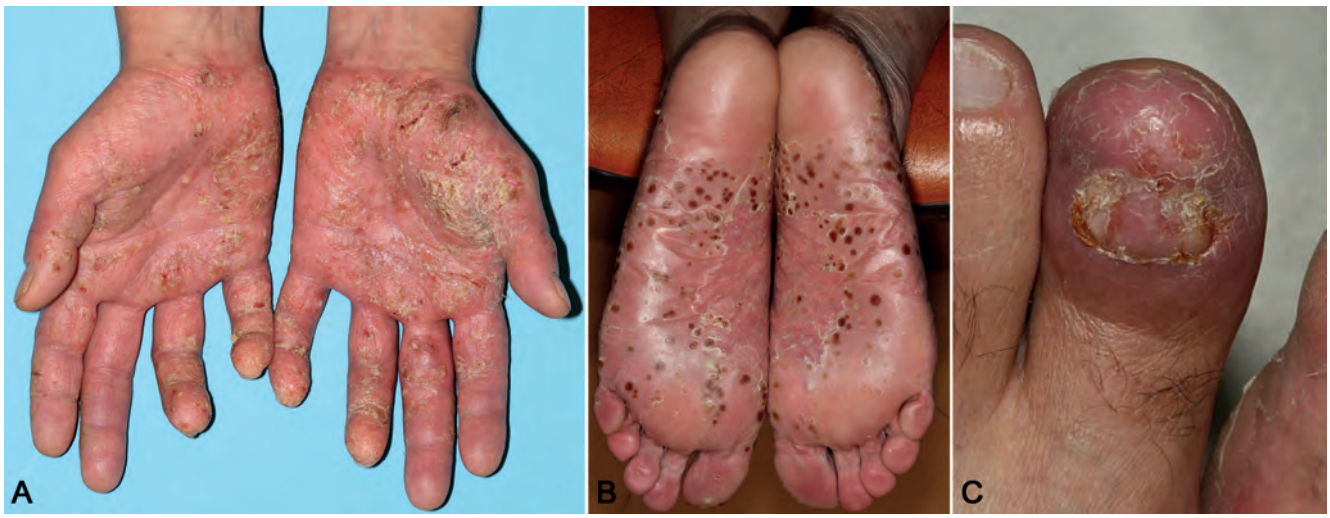

Fig. 2. Typical lesions of palmoplantar pustular psoriasis in 3 different patients free of any mutation in *IL36RN*, *CARD14* and *AP1S3* genes. (A) Pustular lesions involving palmar areas of hands, with some degree of acropustular damage, reflecting the possible association between palmoplantar pustular psoriasis and acrodermatitis continua of Hallopeau (ACH). (B) Disseminated pustules of the soles leave dark-brown macular lesions, coexisting with fresh evolutive pustules and erythemato-squamous lesions. (C) Typical lesions of ACH involving the toes, leading to destruction of the nail apparatus.

exanthematous generalized pustular eruption (AGEP), the clinical signs and symptoms of which may be impossible to differentiate from GPP, but which is caused by drugs, notably by anti-infectious chemotherapy, such as pristinamycin and amoxicillin, but also other classes, such as non-steroidal anti-inflammatory drugs, among others (23, 24). The recent detection in patients with AGEP of mutations in *IL36RN*, sometimes identical to the ones identified in patients with GPP/DITRA, challenges the current view of AGEP and GPP being separate entities (25).

GENETICS AND IMMUNOPATHOGENIC MECHANISMS

The extreme severity of these inflammatory pustular skin disorders, especially GPP, and the existence of Mendelian familial cases, raised the hypothesis of a monogenic model, unlike most cases of PV. This monogenic model has been robustly established by the identification of homozygous or composite heterozygous, loss-of-function mutations of the *IL36RN* gene, which encodes a negative regulator of the IL-36 pathway, which is involved in the limitation of the intensity of skin and systemic innate immune responses. Indeed, *IL36RN* mutations have been found in sporadic or familial cases of GPP in patients from different geographical territories worldwide (13, 26–32). These *IL36RN* mutations are more prevalent in patients with GPP without plaque psoriasis, and influence the age of disease onset (32). Mutations of *IL36RN* lead to major structural and functional impairments of its encoded protein, the IL36 receptor antagonist (IL36Ra), leading to increased inflammatory responses resulting from unrepressed interactions of the IL36 pathway agonists IL36α, IL36β and IL36γ with their receptor, and from subsequent uncontrolled activation of the transcription factor NFκB (13). This results in the massive release by keratinocytes, macrophages and dendritic cells, of several inflammatory mediators including CXCL8, TNFα, IL1 and IL23 (33). Dysregulated activation of the IL-36 pathway has also been shown to trigger the expansion and activation of TH17 cells in GPP (34). So far, different scale studies of cohorts from various geographical territories have reported various prevalences of *IL36RN* mutations, ranging from approximately 5% to 70%, while much lower prevalences have been observed in patients with PPPP, and no causal *IL36RN* mutation has been detected in patients with PV without pustular psoriasis (29–32, 34). An interesting finding has been the identification of identical *IL36RN* mutations across the different subtypes of pustular psoriasis (35). However, mutations leading to the absence of IL-36Ra protein expression are preferentially associated with the most severe entities of GPP and AGEP, while hypomorphous mutations seem to be more prevalent in PPPP and ACH (35). The major breakthrough in the identification of causal mutations of the *IL36RN* gene has been instrumental in establishing without ambiguity the autoinflammatory nature of GPP, and led to the definition of a new entity called DITRA, which differs from the previously described deficiency of IL-1 receptor antagonist (DIRA) by the presence of striking lesions of joints and bones (13, 37, 38). Finally, although causal mutations of *IL36RN* have not been found in patients with PV, several studies have shown deregulation of the IL-36 pathway in PV lesions (39).

The 2 other genes associated with pustular psoriasis so far are *CARD14* and *AP1S3*. Likewise, heterozygous gain-of-function mutations of *CARD14* (caspase activating recruitment domain, member 14), a gene expressed in keratinocytes the protein of which interacts with Bcl 10, a positive regulator of NFκB activation, has been shown to be primarily involved in autosomal dominant forms of PV and in some patients with pityriasis rubra pilaris (40–43). The Adaptor Related Protein Complex 1 subunit sigma 3 (AP1S3) gene has been also found to be heterozygously mutated in patients with different subtypes of pustular psoriasis, mainly GPP and ACH, leading to structural and functional alterations of the protein, a member of the Adaptor Protein 1 (AP1) family, contributing to deregulation of skin innate immune responses (42, 44, 45). Likewise, it is notable that some patients have "digenic" features, e.g. a pattern characterized by mutations reported to be damaging in 2 of the 3 genes identified so far (32). Further identification of other genes, especially in GPP, will undoubtedly complement the current genetic map of pustular psoriasis, and is likely to greatly contribute to personalized therapeutic approaches.

THERAPEUTICS: TOWARDS PRECISION MEDICINE

The low prevalence of pustular psoriasis and the capricious course of the disease with unpredictable flaring frequency in many cases of GPP, explain the low level of scientific evidence regarding treatment efficacy. Indeed, although topical steroids and/or vitamin D derivatives, used as single agents or combined, or phototherapies are still used in mild forms of pustular skin disease with limited involved body surface area, pustular psoriasis often requires systemic therapy. In PPPP, cyclosporine has the highest level of evidence for efficacy, while there is weak or very weak evidence, respectively, for acitretin and methotrexate (46–48). More recently, randomized, placebo-controlled phase 3 clinical trials have been conducted in PPPP with secukinumab and guselkumab, targeted inhibitors of IL17A and IL23p19, respectively (49, 50). However, neither drug showed clinically relevant superiority over placebo at the population level, suggesting that the IL23/IL17 pathway is not the major

pathogenic axis in pustular disease (49, 50). Randomized clinical trials are currently being conducted in PPPP with inhibitors of cytokines of the IL-1 family, the most advanced programme investigating the efficacy and safety of anakinra, the recombinant form of the IL-1 receptor antagonist, based on encouraging responses in isolated cases, including with ACH (51, 52).

There is even less available evidence in GPP, due to the previously exposed challenges, but also to the spontaneously self-remitting evolutive pattern of acute GPP flare. Thus positive responses reported with conventional or biological drug interventions in the setting of retrospective, or open-labelled prospective trials, should be considered with caution. Therefore, although high-dose steroids, cyclosporine, acitretin and apheresis have been promoted for severe acute flares, and although some biologics, such as IL17 inhibitors, have been approved for GPP in Japan, these interventions lack randomized controlled studies to assess the magnitude of their efficacy effect (53, 54). Furthermore, the efficacy of anakinra, the recombinant form of the IL-1 receptor antagonist, has been reported only in a case series of GPP with or without DITRA, reporting most often transient and partial responses (55, 56). These cases should be confronted with the outstanding efficacy of IL-1 inhibitors in patients with DIRA, emphasizing the fine specificity of pathogenic pathways across different monogenic autoinflammatory syndromes of the skin (36). Therefore, the emerging development of specific inhibitors of the IL-36 pathway in GPP and PPPP is not surprising. The most advanced development investigates an anti-IL-36 receptor monoclonal antibody, which, administered as a single intravenous dose, proof-of-concept study in acute GPP, showed very encouraging results in 7 patients, only 3 of whom were carrying *IL36RN* mutations (57). Ongoing phase 2 and 3 studies will provide a more accurate picture of the efficacy and safety of this new targeted strategy.

CONCLUSION

Pustular psoriasis is a very challenging spectrum of autoinflammatory skin diseases, with both clinical and genetic heterogeneity. However, the increasing collaboration between medical experts and scientists is encouraging in enabling the better nosological classification of subentities, as well as the development of specific therapeutic strategies, approximately 100 years after the pioneering description of GPP by von Zumbusch (58).

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BLISTERING SKIN DISORDERS

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REVIEW ARTICLE

Skin Fragility: Perspectives on Evidence-based Therapies

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The term skin fragility disorders describes a group of conditions in which the structural integrity of the skin is compromised and its resistance to external shear forces diminished. Skin fragility can have different causes, ranging from genetic variations to inflammatory or physical phenomena. The genetic skin fragility disorders, collectively called epidermolysis bullosa, serve as a paradigm for the study of causes and mechanisms of skin fragility. Recent biomedical research has revealed substantial genetic heterogeneity of the epidermolysis bullosa group, delivered ample new knowledge on its pathophysiology, and facilitated the design of evidence-based therapeutic strategies. The therapy development process extends from *in vitro* testing to preclinical validation in animal models, and clinical trials. This article reviews different approaches to curative and symptom-relief therapies, and appraises their status and perspectives for clinical implementation.

Key words: skin blistering; genodermatosis; molecular therapy; symptom-relief.

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SIGNIFICANCE

The term skin fragility describes skin that blisters and breaks easily upon mild friction or trauma. Skin fragility can have many causes, ranging from genetic variants to a compromised immune system, infections or adverse drug reactions. Studies of genetic skin fragility disorders, such as epidermolysis bullosa, have provided better understanding of their causes and mechanisms. At least 20 genes may be involved in epidermolysis bullosa, and secondary phenomena, such as inflammation or fibrosis, can worsen the disease. No cure is yet available, but international research is developing novel approaches to cure the disease and alleviate its symptoms. This article reviews these new developments and appraises their clinical implementation.

The term skin fragility refers to pathologically altered skin that blisters and breaks easily upon mild friction, pressure or trauma. The breakage can occur in different skin layers, within the epidermis, along the dermal–epidermal junction, or in the upper dermis. The factors that can cause skin fragility and blistering range from genetic variations to (auto)immune, inflammatory, physical, mechanical, infectious, or drug-induced processes. Correspondingly, many classes of disorders can be described using this term, and the differential diagnosis is broad (1) (**Table I**). As a genetic skin fragility disorder, epidermolysis bullosa (EB) serves as a useful paradigm for these disorders, and research into EB has delivered new information about the pathophysiology of skin fragility that is clinically relevant (2, 3). For example, molecular characterization of autoantigens in acquired blistering diseases has led to the development of molecular diagnostic tests that are in standard use in diagnostics, management and monitoring of autoimmune bullous disorders (4, 5).

Table I. Differential diagnosis of skin fragility

- Genetic skin fragility disorders
- Autoimmune blistering disorders
- Skin fragility induced by infections
- Skin fragility induced by acute inflammation
- Metabolic conditions with blisters
- Bullous drug reactions
- Mechanically induced skin blisters
- Physically induced skin fragility

EPIDERMOLYSIS BULLOSA AS A PARADIGMATIC SKIN FRAGILITY DISORDER

EB has been studied intensively, and the genetic causes and disease mechanisms of the different EB types are rather well understood (1–3). The initial simple assumption that a single pathogenic gene variant/mutation explains all symptoms still holds true in principle. However, the complexity of cellular and molecular processes unleashed by mechanical stress on EB skin is far greater than anticipated; a fact that has major consequences for the design and development of therapies.

As background for the discussion and appraisal of therapy developments, a short introduction to EB, its current diagnostics and management follows.

Epidermolysis bullosa classification

The EB group encompasses 4 main types: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler syndrome (6) (**Table II**). The division into types is based on the morphological level of separation

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Table II. Major types of epidermolysis bullosa (EB)

EB types (abbreviation)	Level of blistering
Epidermolysis bullosa simplex (EBS)	Intra-epidermal
Junctional epidermolysis bullosa (JEB)	Along the basement membrane
Dystrophic epidermolysis bullosa (DEB)	In the upper dermis
Kindler syndrome (KS)	Mixed*

*The blistering can occur at any or all of the above levels.

within the dermal–epidermal junction zone. In EBS, the blisters form within the epidermis, in JEB within the basement membrane, and in DEB just below the basement membrane. In Kindler syndrome, blisters can form at all levels. A common hallmark for all EB types is trauma-induced skin blistering and fragility, but each of them contains a number of subtypes, in which the extent of skin lesions and the associated organ manifestations can vary to a great extent (**Fig. 1**). In April 2019, an international EB consensus classification meeting took place in London. Experts from all over the world updated and revised the consensus classification; the new classification paper is in preparation (6). The main changes are related to the EBS group that has expanded significantly in the past 5 years. Some of the very severe forms in this group, but also mild disorders with minimal skin fragility, were clearly regarded as skin fragility disorders, but not as EB. The new classification includes for the first time syndromal EB subtypes with multi-organ involvement.

Modern diagnostics of epidermolysis bullosa

A well-defined diagnosis, with as much molecular precision as possible, is recommended for all patients with EB. A clear diagnosis facilitates disease management, including prognostication and genetic counselling (7, 8). Furthermore, as novel therapies emerge, molecular diagnosis is often a prerequisite for inclusion in clinical trials; it will also be needed for application of future personalized therapies (8). The recommended diagnostic procedure involves immunofluorescence mapping of a skin biopsy as a first step; this enables identification of the blistering level and definition of candidate gene(s) for subsequent genetic analysis. In cases with inconclusive clinical presentation, genetic diagnostics using next generation sequencing (NGS) technologies, such as EB gene panel-based diagnostics or clinical exome analysis, are recommended (7).

Current disease management

Since there is currently no cure for EB, a combination of symptomatic treatment modalities is used, depending on needs. Protection from trauma, cleaning, disinfecting, and moisturizing the skin belong to daily basic measures. Different wound management modalities are defined in guidelines (http://www.debra-international.org/clinical-guidelines). Furthermore, since involvement of other organs is common in more severe EB, and since chronic skin fragility and painful wounds diminish the quality of life of the affected individuals and their families, interdisciplinary and multi-professional management, including psychosocial care, are highly recommended (www.debra-international.org/clinical-guidelines/complete-eb-guidelines.html).

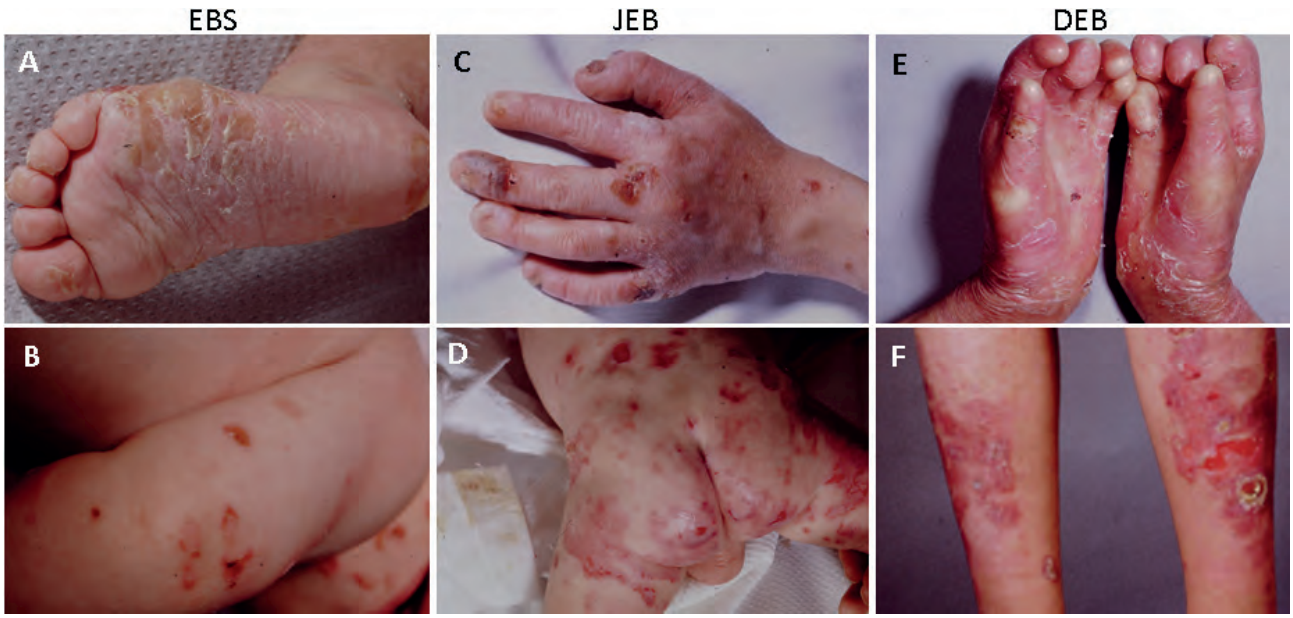

Fig. 1. Typical clinical presentations in different types of epidermolysis bullosa (EB). (A, B) EB simplex (EBS). (A) Blisters, erosions and scaling in the foot of a 2-year-old child. (B) Disseminated blisters on the trunk and extremities of a newborn. (C, D) Junctional EB (JEB). (C) Blisters, erosions and loss of nails in the hand of a 7-year-old girl with moderate JEB. (D) Typical extensive skin fragility in the buttocks area and back of a newborn with severe generalized JEB. (E, F) Dystrophic EB (DEB). (E) Strong scarring and fusion of digits in the hand of an 8-year-old girl with severe generalized DEB. (F) Trauma-induced blistering, inflammation and scarring on the shins of a 12-year-old girl with moderate DEB.

Expert centres and European Reference Networks

Numerous expert centres for EB exist worldwide. Most of these are members of the EB-Clinical Network "EB-Clinet" (www.EB-Clinet.org), which works together with the patient groups (www.debra-international.org). The centres provide information and advice to patients and caregivers, as well as services ranging from diagnostics to genetic counselling and interdisciplinary management plans. In 2017, the European Commission launched European Reference Networks (ERNs) for rare diseases for high-quality diagnostics, management, and research. The goal is to tackle complex or rare diseases with a concentration of knowledge and resources (https://ec.europa.eu/health/ern). The ERNs provide a dedicated IT platform, telemedicine tools and a virtual advisory board of specialists from different disciplines to evaluate the diagnosis of a patient and plan the treatment. An important principle is that the medical knowledge and expertise "travel", and not the patients, who should have the comfort of staying at home in their supportive environment. ERN-Skin encompasses 56 healthcare providers from 18 countries who are endorsed by their national authorities and committed to pool their knowledge and expertise within the framework of the ERN-Skin (https://ern-skin.eu/). Two approaches are taken: (*i*) a disease approach with 8 sub-thematic groups on high-level patient management and research; (*ii*) a transversal approach focusing on teaching and training, E-health, registries and research, deep phenotyping and clinical outcomes. One of the 8 sub-thematic groups deals with EB.

EMERGING NOVEL THERAPY APPROACHES

Despite all the structural developments in the field of rare skin diseases, the unmet medical need remains high, and novel evidence-based therapies are urgently needed. Development of new treatments is strongly promoted by patient advocacy groups, which are very active in setting priorities and funding patient-oriented research (www.debra-international.org; www.ebresearch.org/, www.cure-EB.org).

As the therapeutic era for skin fragility disorders progresses it becomes clear that therapy strategies with "intention to cure" are far more complex and difficult than expected. Gene therapy development faces technological challenges with vectors, targeting skin stem cells, achieving long-term therapeutic effects, etc. Therefore, a variety of methodologies relating to gene replacement, gene editing, and modifying transcription and translation are being tested. Because patients demand more rapid development of treatments that bring relief, the focus has turned to so-called symptom-relief and regenerative therapies that, although they do not bring cure, will alleviate symptoms, offer relief and improve quality of life. The therapies that have reached a clinical

Table III. Currently recruiting clinical therapy trials for epidermolysis bullosa (EB) (as of June 2019)

Therapy type	Investigational drug	EB type	Trial identification number
Therapies with curative aim			
Gene therapy	Transplantation surgery of genetically corrected cultured epidermal autograft	JEB with COL17A1 mutations	ClinicalTrials.gov ID: NCT03490331
	Genetically corrected cultured epidermal autograft	RDEB*	ClinicalTrials.gov ID: NCT02984085
	FCX-007, Genetically modified autologous human dermal fibroblasts	RDEB*	ClinicalTrials.gov ID: NCT02810951
	KB103, topically applied non-integrating, replication-incompetent herpes simplex virus vector expressing human collagen VII protein.	DEB	ClinicalTrials.gov ID: NCT03536143
Antisense oligonucleotides	QR-313, topically applied antisense oligonucleotide	DEB with mutations in exon 73 of COL7A1	ClinicalTrials.gov ID: NCT03605069
PTC read-through	Gentamicin, intravenous	RDEB*	ClinicalTrials.gov ID: NCT03392909
	Gentamicin, topical	JEB	ClinicalTrials.gov ID: NCT03526159
Protein therapy	PTR-01, recombinant human collagen VII	RDEB*	ClinicalTrials.gov ID: NCT03752905
Regenerative cell-based therapies			
Cell therapy	Serial mesenchymal stem cell (MSC) infusions from a related donor	All EB types	ClinicalTrials.gov ID: NCT02582775
	Allogeneic stem cell transplantation and "off-the-shelf" mesenchymal stem cells	All EB types	ClinicalTrials.gov ID: NCT01033552
	Allogeneic ABCB5-positive stem cells	RDEB*	ClinicalTrials.gov ID: NCT03529877
	Epidermal grafts generated using the Cellutome System	EB after hematopoietic cell transplantation	ClinicalTrials.gov ID: NCT02670837
Symptom-relief therapies			
Anti-fibrotic	Losartan, systemic	RDEB	EudraCT No.: 2015-003670-32
Anti-inflammatory	Pharmacokinetics, safety of diacerein after maximum use	EBS	ClinicalTrials.gov ID: NCT03472287
	Oleogel-S-10, topical	All EB types	ClinicalTrials.gov ID: NCT03068780
	BPM31510 3.0% cream, topical	All EB types	ClinicalTrials.gov ID: NCT02793960
	Sirolimus, topical	EBS	ClinicalTrials.gov ID: NCT03016715
Accelerator of wound healing	RGN-137, a thymosin beta-4 gel, topical	JEB, DEB	ClinicalTrials.gov ID: NCT03578029
	Amniotic membrane	RDEB	ClinicalTrials.gov ID: NCT02286427
Analgesic	Ropivacaine, topical	All EB types	ClinicalTrials.gov ID: NCT03730584
Anti-pruritic	Neurokinin-1 receptor Antagonist, oral	All EB types	ClinicalTrials.gov ID: NCT03836001
Anti-hidrotic	Botulinum toxin	EBS	ClinicalTrials.gov ID: NCT03453632

EBS: EB simplex; JEB: junctional EB; DEB: dystrophic EB; RDEB; recessive DEB.

trial stage and are recruiting trial participants are summarized in **Table III**.

Gene therapies

Retrovirus-mediated gene correction in keratinocytes and subsequent grafting of gene-corrected epidermal sheets was developed many years ago as a principally valid method to treat JEB or DEB skin (9, 10 and references therein). Recently, this method was used to replace approximately 80% of the skin surface in a very severely ill child with JEB (9, 10). A similar approach is being tested in DEB for maintenance of wound healing (11). So far, 7 patients with RDEB have been treated with *COL7A1*-gene corrected keratinocyte grafts, many of them have durable wound-healing (www.abeonatherapeutics. com). However, the classical gene therapy approaches still deal with technological issues relating to vector safety and to optimal transfection/transduction efficiency of stem cells. Gene editing using the CrispR/Cas technology has shown promise in correcting *COL7A1* mutations in RDEB keratinocytes (12) and RDEB fibroblasts (13) *in vitro* and at a preclinical level. Further research strategies encompass approaches with gene-corrected iPS cells (14–17). A newly introduced technology employs a non-integrating, replication-incompetent herpes simplex virus 1 (HSV-1) vector expressing human collagen VII (www.krystalbio.com). The vector preferably targets keratinocytes/epidermis, and a pilot trial using topical treatment of DEB addresses wound-healing as a primary outcome marker (Table III).

Natural gene therapy

The term "natural gene therapy" describes revertant mosaicism, i.e. the spontaneous conversion of a somatic cell with a mutation and pathological phenotype into a cell that has acquired a second, compensating mutation and gained a normal phenotype (18). Revertant mosaicism is relatively common in genetic disorders, and in most classic EB types revertant mosaic skin patches can be found by a well-trained expert. Approximately 5 years ago, the first "natural gene therapy"-based treatment of EB was reported, JEB skin was transplanted with small split-thickness revertant grafts (19). More recently, cultured epidermal autografts generated from clinically revertant skin were applied to treat DEB wounds in 3 patients. The take was 55–87%, and the clinical effects remained for at least 76 weeks of follow-up (20).

RNA-based therapies

Different approaches can be used to skip or replace exons at the RNA level. In an ex vivo RNA trans-splicing-based approach 7 exons were replaced, including the one with a *KRT14* mutation, to correct the cellular phenotype in EBS keratinocytes. The corrected keratinocytes formed a stable epidermis in a xenograft model, indicating that trans-splicing-mediated RNA therapy could have potential for clinical implementation (21). Another option is to employ antisense oligonucleotides to skip the mutated exon in the transcription process. Subsequently, a polypeptide that lacks the amino acid sequence encoded by the skipped exon is synthesized; this is usually at least partly functional. Collagenopathies are particularly suitable for this approach, since exons of collagen genes are typically in-frame and small. Their deletion is not likely to cause major structural changes in the affected protein. Of the EB genes, the collagen VII gene is interesting, since exon 73 harbours a high number of mutations. *In vitro* experiments showed that antisense oligonucleotide-induced skipping of exon 73 leads to a partially functional collagen VII that could potentially improve DEB skin functions (22, 23). A phase 1/2 multicentre clinical trial plans to test this approach in DEB patients carrying specific mutations (www.wings-tx.com).

Premature termination codons read-through

The idea of read-through of premature termination codons (PTC) arose from the knowledge that nonsense-mediated mRNA decay is often caused by PTC (24). Overriding the mutation during transcription would presumably generate a full-length translation product, i.e. a polypeptide with a minor modification that is likely to be adequately functional. Aminoglycoside antibiotics induce PTC read-through. However, the neighbouring nucleotides of the mutations influence the efficiency of the read-through and, therefore, not all PTC are suitable for aminoglycoside treatment. Gentamicins suppressed *COL7A1* and *LAMB3* mutations with some efficacy in vitro and *in vivo* (25, 26). Human therapy trials assess the suitability and tolerability of intravenous gentamicin in RDEB and topical gentamicin in JEB (Table III). A challenge with this category of drugs is the spectrum of adverse effects, such as renal and ototoxicity, or potency to induce contact sensitization. Gentamicin B1, a minor gentamicin constituent, has been suggested to be superior in this context due to its high potency to suppress PTC and its low toxicity (27). Amlexanox, an anti-inflammatory drug, can also induce PTC read-through. *In vitro*, in collagen VII-negative DEB cells with PTC mutations, it induced collagen VII protein production (28).

Protein therapy

Protein therapies, in particular enzyme replacements, have been designed and tested for several inborn errors of metabolism (29). In case of EB, the challenge is that many of the proteins that are mutated and/or missing (collagens, laminins, keratins) are large and, by the nature of their physiological functions, have a tendency to

form aggregates. These characteristics do not facilitate intravenous administration and homing of the protein to the required site of action. With this background it seems surprising that intravenous and intradermal injections of recombinant collagen VII in DEB model mice resulted in homing of some collagen into the skin and the dermal–epidermal junction, without major adverse effects (30). A clinical trial is currently testing the safety of recombinant collagen VII in RDEB (Table III; http://phoenixtissuerepair.com).

DISEASE-MODIFYING APPROACHES

With increasing experience in preclinical and clinical development of therapies for EB, the complexity of treatment-related issues has surprised most scientists (8, 31). We realize that curative therapies will need many years to enter the clinics and, at the same time, the pressure from patients for treatments increases. The scientific community has reacted by searching for possibilities to modify disease activity and to alleviate symptoms. The rationale for such symptom-relief approaches comes from basic research on disease mechanisms in EB. Many *in vitro* and preclinical studies have laid the foundation for using cells or targeting, for example, cytokines or growth factors that drive EB phenotypes (8). The goal of these treatments is to improve functions of the skin and make the patients feel better. Three groups of symptom relief therapies are delineated below: (*i*) regenerative cell-based therapies; (*ii*) topical pharmacological therapies; and (*iii*) systemic therapies with biomolecules and repurposed drugs.

Regenerative cell-based therapies

From many different angles, cell therapies for EB have turned out to be more challenging than initially expected. They are very unlikely to bring cure, and have recently been re-grouped into the category of disease-modifying treatments. Currently, both local and systemic applications are being tested for disease-modifying capacity.

Intradermal cell injections

Early investigations with intradermal injections of fibroblasts or human bone marrow-derived mesenchymal stem cells into RDEB mice demonstrated that the cells produced collagen VII that homed into the dermal–epidermal junction and ameliorated its stability (32–34). However, in humans the tolerability and efficacy of this therapeutic approach were poorer than expected. The injections were very painful and improvement of the skin very limited (35). One study observed a comparable improvement of wound healing in DEB, regardless of whether fibroblasts or vehicle was injected (36). Recently, the approach has been modified with the use of gene-corrected fibroblasts that produce large amounts of collagen VII. Preliminary information indicates that the injections bring some *de novo* collagen VII into the treated areas, but the full potential of this approach remains to be seen (37; www.fibrocell.com).

Systemic stem cell therapies

Bone marrow transplantation has been tested as treatment for different genetic diseases, including severe DEB (38). Disappointingly, the therapeutic effect and duration were not as positive as hoped for and, as is well known, the complications of bone marrow transplantation can be life-threatening (39). Subsequently, different conditioning regimens have been tested, most recently a regimen that combines reduced-intensity conditioning, post-transplant cyclophosphamide and infusions of immunomodulatory allogeneic mesenchymal stromal cells (40). Treatment of children with RDEB with intravenously administered human allogeneic mesenchymal stem cells made them feel better, but brought no collagen VII into the skin (41). The efficacy of an ABCB5-positive subpopulation of mesenchymal stem cells for symptom-relief in adults with RDEB is assessed in a current trial (*www.rheacell.com*). In addition, cord-blood derived stem cells have shown some potential as systemic anti-fibrotic treatment in a preclinical setting (42).

Topical pharmacological therapies

Diacerein from rhubarb root extracts has been implicated as possible treatment for EBS skin (43, 44). The rationale involves the capacity of diacerein to dampen the inflammatory response caused by epidermal cell rupture in EBS (43). The cell disruption is a consequence of keratin 5 and 14 mutations that cause intermediate filament aggregation and loss of stabilization by the cytoskeleton. *In vitro* data demonstrated both anti-inflammatory properties of diacerein and its potential for stabilizing EBS cells, then a pilot clinical trial demonstrated fewer blisters in diacerein cream-treated skin in part of the study population (44).

Wound-healing in EB can be supported by another plant-derived compound with anti-inflammatory properties, namely betulin-based oleogel isolated from birch bark. Betulin was shown to support keratinocyte differentiation (45), enhance re-epithelialization and facilitate wound healing *in vitro* and *in vivo* (46, 47). An ongoing placebo-controlled phase 3 study assesses the efficacy of oleogel in patients with EB, regardless of subtype (48).

Systemic disease modifying therapies

Anti-inflammatory approaches. Recent basic research, followed by preclinical and clinical validation, has revealed an unanticipated role for inflammatory cascades in EB. In EBS, keratin mutations and keratinocyte fragility induce expression of specific cytokines and T-cell-

mediated inflammatory responses, which manifest with itch as a bothersome symptom (49, 50). A vicious circle is generated by itch, scratching and subsequent skin blistering, which leads to a stronger inflammatory response. Although non-specific anti-inflammatory therapies with NSAIDs are not beneficial, first pilot studies with specific systemic treatments show promise. For example, anti-IL17 interval therapy with apremilast worked well in 3 individuals with of EBS (50).

Antifibrotic therapy approaches. Based on an ample body of scientific literature, severe DEB can be regarded as a systemic disease, since systemic inflammation is prominent and the secondary progressive fibrosis affects many organs (51). Therefore, drugs that inhibit inflammation and fibrosis could potentially relieve symptoms in DEB, such as inflammation-caused itch or formation of strictures and contractures, including fusion of digits.

A repurposed drug, losartan, has shown such benefits in DEB on the preclinical level (52). This drug for treatment of high blood pressure also has anti-fibrotic potential in some disease constellations. The mechanism is based on its ability to inhibit TGFβ signalling via AT-1 receptor antagonism (52). Since inflammation and hyper-active TGFβ signalling contribute to DEB-associated fibrosis in a major manner (8, 53, 54), losartan appeared suitable as treatment. The expectations were met in losartan-treated RDEB model mice, inflammation and TGFβ activity were reduced, progression of fibrosis inhibited and fusion of digits delayed (53). As a logical next step, a clinical trial currently assesses safety and tolerability of losartan in children with moderate-to-severe DEB. The study is also likely to generate preliminary information on the ability of losartan to alleviate symptoms in human DEB (Table III).

Another modulator of TGFβ signalling is the small leucine-rich proteoglycan decorin. Endogenous decorin levels are known to correlate with clinical severity in RDEB (55). In a preclinical study, systemic administration of lentivirally overexpressed human decorin reduced TGFβ levels and fibrotic traits, and enhanced survival of the RDEB mice (56). These observations indicate that extracellular matrix biomolecules modulating TGFβ signalling may have potential for systemic anti-fibrotic therapy for DEB.

In addition to the above small (bio)molecules, a high mobility group box 1 (HMGB1)-derived peptide may improve systemic fibrosis in DEB. HMGB1 has variable functions and has been implicated in both physiological and pathological processes (57). In the context of EB, its relevance lies in its ability to release a specific anti-inflammatory population of mesenchymal stem cell from the bone marrow into the circulation and from there into damaged skin (58). First treatments of RDEB mice with a HMGB1-derived peptide resulted in improvement of skin fibrosis and gastrointestinal strictures (K. Tamai, personal communication).

APPRAISAL AND PERSPECTIVES FOR CLINICAL IMPLEMENTATION

The multitude of approaches to EB treatments and the rapid developments of research methodologies raise our hopes that first evidence-based therapies for EB will enter clinics in the foreseeable future. To date, biologically valid treatment modalities for most severe EB types have advanced to preclinical and clinical testing, but all strategies still face substantial challenges, including technical issues, safety considerations, or issues related to practical clinical implementation and the duration of the clinical effects. Many of the pilot studies have made us realize that much work is still needed for better understanding of the disease mechanisms and skin stem cell properties. These must be further elucidated, and new therapeutic targets identified. Based on all we know today, the prediction is that future treatments for EB will represent individualized medicine based on the patient's mutation constellation, phenotypic characteristics and prominent disease mechanisms. They are likely to encompass combinations of different therapeutic principles: curative and symptom-relief therapies. It is easy to imagine therapeutic regimens using alternating gene, cell and drug therapies to win the best clinical outcomes and to reduce adverse effects. Once therapies are available for wide clinical implementation, the next big challenges will have to be tackled, such as cost and worldwide access to therapy.

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REVIEW ARTICLE

Collagen XVII Processing and Blistering Skin Diseases

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Collagen XVII (COL17) is a hemidesmosomal transmembrane protein in the skin, which, in several autoimmune blistering skin diseases, may be targeted by autoantibodies. In addition, loss-of-function mutations in the *COL17A1* gene induce a subtype of junctional epidermolysis bullosa. The extracellular domain of COL17 can be physiologically cleaved from the cell surface by ADAM family proteins in a process known as ectodomain shedding. COL17 ectodomain shedding is thought to be associated with the migration and proliferation of keratinocytes. Furthermore, the C-terminal cleavage of COL17 may be associated with basement membrane formation. COL17 can be targeted by various proteases, including MMP9, neutrophil elastase, plasmin and granzyme B, which may be associated with blister formation in pemphigoid diseases. Interestingly, cleavage of COL17 may induce neoepitopes on the proteolysed fragments, and such induction is associated with dynamic structural changes. This review summarizes the current understanding of cleavage of COL17, and how such cleavage relates to blistering skin diseases.

Key words: ectodomain shedding; BP180; bullous pemphigoid; linear IgA bullous disease; epidermolysis bullosa.

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SIGNIFICANCE

Collagen XVII (COL17, also known as BP180) is an important molecule, which maintains stable adhesion between the dermis and epidermis. Genetic and acquired dysfunctions of COL17 lead to blistering skin diseases. However, the expression of COL17 is tightly regulated, depending on various settings, including wound-healing, proliferation and differentiation. Dysregulation of COL17 processing may be associated with the development of blistering skin diseases; thus, it is important to understand the mechanism by which COL17 is processed and the diseases associated with such processing.

Type XVII collagen (COL17), also known as BP180/BPAG2, is a type-II-oriented transmembrane collagen composed of 3 identical 180-kDa α-chains (1). COL17 is one of the hemidesmosomal components of basal keratinocytes. It links keratin intermediate filaments to the underlying dermis via plectin, BP230, laminin 332 and type VII collagen (2). Loss-of-function mutations in the *COL17A1* gene result in a subtype of junctional epidermolysis bullosa (JEB), which clinically manifests as blister formation and abnormalities of the hair and teeth (3). Since JEB associated with *COL17A1* gene mutations shows a relatively mild phenotype, the disease was previously called "generalized atrophic benign epidermolysis bullosa (GABEB)".

Autoimmunity to COL17 induces bullous pemphigoid (BP), a major autoimmune blistering skin disease, which commonly develops in elderly people (4, 5). In BP, itchy urticarial erythema and tense blisters develop on the entire body, and the mucous membranes may be involved. Major epitopes for BP autoantibodies cluster tightly within the juxtamembranous extracellular noncollagenous 16th A (NC16A) domain of COL17 (6), and previous studies have revealed the pathogenicity of immunoglobulin G (IgG)-class autoantibodies directing this region (7, 8). COL17 may also be targeted by autoantibodies in other autoimmune blistering skin diseases, including mucous membrane pemphigoid (MMP) and linear IgA bullous disorder (LABD) (4).

The two COL17-associated blistering disorders, JEB (GABEB) and BP, suggest that COL17 is a functionally important structural molecule that maintains stable adhesion between the dermis and the epidermis at the dermal-epidermal junction (DEJ). However, basal keratinocytes are dynamic, and they migrate or differentiate in a context-dependent manner. Therefore, processing of COL17 may be involved in various physiological settings. In addition, dysregulated processing of COL17 may be associated with blistering skin diseases. This review summarizes the current understanding of COL17 processing and the blistering skin diseases associated with such processing.

COL17 PROCESSING IN PHYSIOLOGICAL SETTINGS

COL17 ectodomain is constitutively cleaved within the NC16A domain

In cultured keratinocytes, the 120-kDa extracellular domain of COL17 is constitutively shed from the cell surface and is detectable in soluble form in culture me-

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dium (9, 10). COL17 ectodomain shedding is mediated by ADAM 9, 10 and 17 (11), and mass spectrometry analyses have revealed that the cleavage occurs at different regions within the NC16A domain (**Fig. 1**A) (12, 13). The results are consistent with the nature of ADAM family proteins, which cleave substrate proteins more preferentially, based on the distance from the cell surface rather than on amino acid sequences. The detection of cleavage sites within the NC16A domain of COL17 enables the production of cleavage-site-specific antibodies specifically detecting the cleaved COL17 ectodomains. Unique antibodies have revealed that migrating normal human keratinocytes cleave COL17 ectodomains, which co-localize with laminin 332 (Fig. 1B), (14) and cleaved ectodomain fragments exist in the DEJ of normal human skin (13, 15). Interestingly, the cleavage site(s) of COL17 in pathological settings may differ from that in physiological settings (15). In genetically manipulated mice whose NC16A domain includes amino acid sequences that impair ectodomain shedding, the inhibition of COL17 ectodomain shedding somewhat accelerated re-epithelialization after skin wounding (16). The suppression of re-epithelialization by COL17 ectodomain shedding is associated with dampening of mTOR signalling (17). However, wound healing processes differ greatly between humans and mice, with wounds in mice healing mainly by contraction (18). Therefore, further studies are essential to address the physiological roles of COL17 ectodomain shedding in human skin.

C-terminal cleavage of COL17

The cleaved 120-kDa ectodomain of COL17, also called as linear IgA dermatosis antigen (LAD-1) , may be further processed at the C-terminal region around the NC4 domain, which migrates around 97 kDa (19, 20). The 97-kDa processed COL17 polypeptide is called linear IgA bullous disorder (LABD)-97 (**Fig. 2**A). Although it remains uncertain whether LABD-97 is present in normal human skin, C-terminal processing is expected to be physiologically associated with correct basement membrane formation in skin, as described later in this article.

Cleavage in unfolded COL17

Within the NC16A domain, COL17 has a distinct furin consensus sequence: ‘’RIRR’’. Early studies have suggested that ectodomain shedding of COL17 may be induced by this distinct motif; however, the furin consensus motif is not used under physiological settings (10). What is the physiological role of the furin consensus motif in COL17? As illustrated in Fig. 1A, there are potential coiled-coil sequences just before the furin consensus motif, and these sequences initiate the folding of COL17

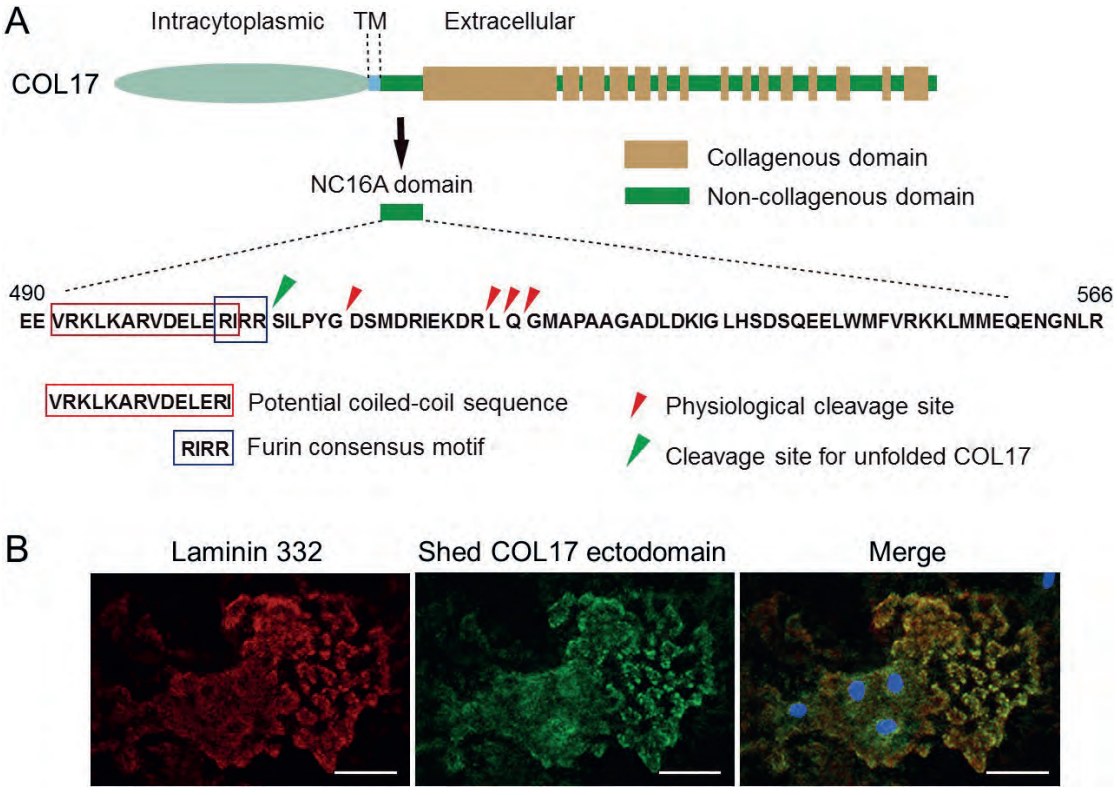

Fig. 1. Collagen XVII (COL17) processing in physiological settings. (A) Schematics of COL17 and sequences of the NC16A domain. (Copyright 2010: The American Association of Immunologists, Inc.). (B) The shed COL17 ectodomain (green: antibody HK139) and laminin 332 (red: antibody 6F12) co-localize in the extracellular matrix of normal human skin. TM: transmembrane. Blue: DAPI. Scale bar: 40 μm. The figures have been partially modified from previous studies (13, 14). Permission given by publisher.

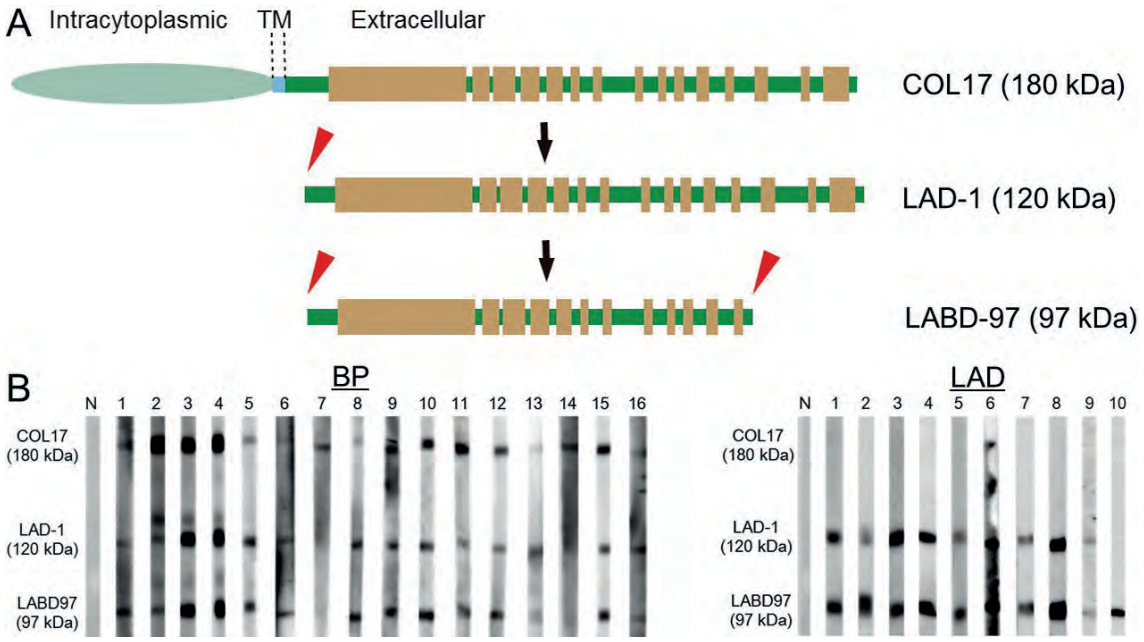

Fig. 2. Neoepitope development in the cleaved collagen XVII (COL17) ectodomains. (A) Schematics of linear IgA dermatosis type 1 (LAD-1) and linear IgA bullous disorder (LABD)-97 polypeptides. (B) LAD IgA-class autoantibodies show more intense reactivity to the cleaved COL17 ectodomains LAD-1 and LABD-97 than to full-length COL17. Note that LAD sera numbers 2, 5 and 10 react more strongly to LABD-97. N: normal control. The immunoblotting data have been partially modified from a previous study (23). BP: bullous pemphigoid. Permission given by publisher.

as a collagen triple helix in the N to C direction (21). When coiled-coil disruptive mutations are introduced, COL17 folding is impaired and unfolded COL17 accumulates in cells. The unfolded COL17 is cleaved by furin at the ''RIRR'' furin consensus motif in the Golgi apparatus before being incorporating into the cell membrane. Finally, cleaved 120-kDa ectodomains derived from unfolded COL17 are expelled from the cells (21). Thus, cleavage at the furin consensus motif within the NC16A domain of COL17 is important for maintaining the quality of the molecule.

COL17 CLEAVAGE AND BLISTERING SKIN DISEASES

Cleavage within the NC16A domain induces neoepitopes in processed COL17 ectodomains

As described, cleavage of COL17 within the NC16A domain yields a 120-kDa ectodomain polypeptide, known as LAD-1 (Fig. 2A). LAD-1 partially contains sequences of the NC16A domain, with which BP autoantibodies preferentially react (12, 13). Similarly, MMP autoantibodies targeting the C-terminal regions of COL17 may react with LAD-1. It is notable that, in some cases of BP and in many cases of LABD, autoantibodies show more preferential reactivity to LAD-1 than to full-length COL17 (Fig. 2B) (19, 22), indicating that cleavage within the NC16A domain induces neoepitopes on the cleaved LAD-1. *In silico* predictions based on detected cleavage sites reveal that the antigenicity of the remnant NC16A sequences in the cleaved COL17 ectodomains increase despite the different cleavage sites (13). Furthermore, monoclonal antibodies target the 15th collagenous (COL15) domain with preferential reactivity to LAD-1, suggesting that cleavage within the NC16A domain induces dynamic structural changes in COL17 (23).

C-terminal cleavage of COL17 induces neoepitopes on the LABD-97 fragment

Since LABD autoantibodies react more preferentially with LAD-1 than with full-length COL17, they usually have strong reactivity to LABD-97 (Fig. 2B) (24). Interestingly, LABD autoantibodies may have greater reactivity to LABD-97 than to LAD-1 (Fig. 2B), suggesting that C-terminal cleavage has additional effects on neoepitope development (23). A previous study reported that epitopes on the 15th collagenous domain appear after C-terminal cleavage (23), which is consistent with an epitope mapping study of LABD autoantibodies (25).

COL17 cleaving enzymes in bullous pemphigoid

In BP lesional skin and blister fluid, several proteolytic enzymes are known to exist, including plasmin, neutrophil elastase and MMP-9 (4, 5). *In vitro* studies have revealed that neutrophil elastase (26), MMP-9 (27) and plasmin (19) are able to cleave COL17. Of these, plasmin is known to cleave within the NC16A and NC4 domains of COL17 ectodomains, yielding 120-kDa LAD-1 and 97-kDa LABD-97 fragments (19, 20, 28). In addition, a recent study has reported that granzyme B, a serine protease secreted by immune cells, is highly expressed

in infiltrated cells in BP lesional skin and that not only does this enzyme cleave COL17, but it also cleaves other molecules present at the DEJ, including α6/β4 integrins and collagen VII (29).

Impaired C-terminal cleavage of COL17 may induce disorganized basement membrane formation

When homozygous R1303Q mutations occur in the *COL17A1* gene, a mild and localized form of JEB develops that is clinically characterized by mechanical blisters, tooth and nail abnormalities, and sclerotic fingers associated with a loss of fingerprints (**Fig. 3**) (30, 31). Histopathologically, duplication of the basement membrane is characteristic of JEB patients with R1303Q mutations (Fig. 3B). Since R1303Q mutations impair the C-terminal processing of COL17, such processing is thought to be essential for normal basement membrane formation in skin (28).

Impaired cleavage of COL17 may induce the breaking of tolerance to bullous pemphigoid autoantigens

BP is induced by autoantibodies targeting the hemidesmosomal components COL17 and/or BP230. Although the pathomechanism of autoantibody-dependent blister formation has been studied extensively, there has been no full elucidation of why tolerance to these autoantigens may be broken in certain individuals. Immune tolerance to molecules may be broken by various triggering events, including thermal burns, ultraviolet (UV) irradiation and surgery (32). In addition, recent studies have reported that anti-type II diabetes mellitus drug dipeptidyl peptidase IV inhibitors (DPP4i) are a risk factor for the onset of BP (33, 34). Furthermore, impaired Treg function may break the tolerance to COL17 and BP230 (35, 36). However, it remains unclear whether the impaired expression of pemphigoid autoantigens may induce the breaking of tolerance. In 2015, Hurskainen et al. (37) produced a genetically manipulated mouse lacking the immunodominant NC14A domain of Col17, a domain that corresponds to the human NC16A domain of COL17. Since NC14A is essential for the ectodomain shedding of mouse Col17, this is another shedding-deficient model. It is notable that the mice are prone to scratching themselves and spontaneously developed anti-Col17 autoantibodies, although no blistering was observed. Whether impairments of BP autoantigens induce the breaking of tolerance had not been elucidated, therefore this study brought important

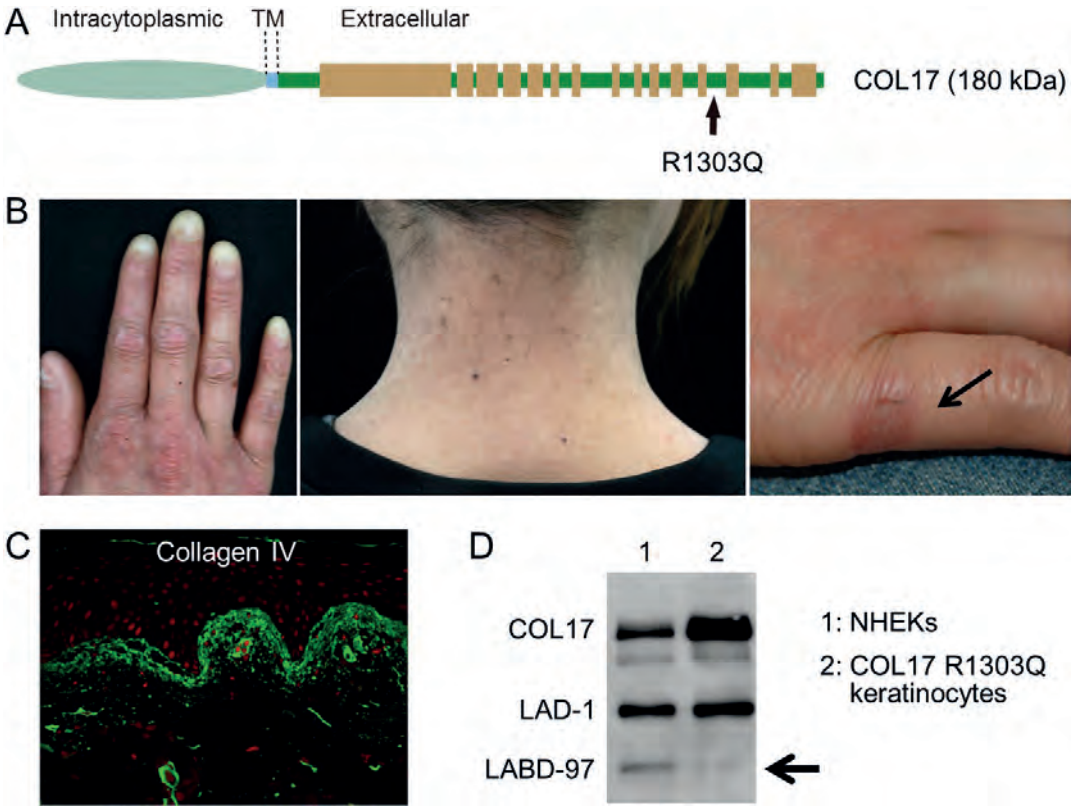

Fig. 3. Collagen XVII (COL17) R1303Q mutation induces blistering disease associated with disorganized basement membrane formation. (A) The R1303Q mutation is located within the NC4 domain. (B) A previously reported 32-year-old COL17 R1303Q$^{+/+}$ patient (28). The *arrow* indicates a mechanical blister. (C) A disorganized and duplicated basement membrane is a characteristic histopathological feature, which can be detected by anti-type IV collagen antibodies (PHM-12+CIV22). (D) Western blotting using anti-COL17 NC16A antibodies (NC16A-3) on extracellular matrix proteins derived from mal human epidermal keratinocytes (NHEKs) and keratinocytes from a R1303Q$^{+/+}$ junctional epidermolysis bullosa patient. The *arrow* indicates that linear IgA bullous disorder (LABD)-97 is absent in R1303Q$^{+/+}$ keratinocytes, suggesting that the C-terminal cleavage of COL17 is impaired. The figures have been partially modified from previous studies (28). LAD-1: linear IgA dermatosis type 1. Permission given by publisher.

information on the pathomechanism behind the breaking of tolerance to COL17.

Cleaved fragments on immune cells in bullous pemphigoid lesional skin

The roles of IgG-class anti-COL17 autoantibodies in the development of blisters have been studied extensively; in contrast, the pathomechanism for urticarial erythema has not been fully elucidated. Previous studies have reported that both IgG- and IgE-class anti-COL17 NC16A autoantibodies are present in BP sera (38, 39). Although *in vivo* IgE deposition at the DEJ may be observed in BP, the positivity rate is not high (40). Notably, Freire et al. recently reported that IgE is rarely observed at the DEJ, but that it is prominent on mast cells and eosinophils in the dermis, in which COL17 ectodomain fragments co-localized with IgE (39). This observation is consistent with the fact that the shed COL17 ectodomain is soluble after being cleaved from the cell surface, as described previously.

CONCLUSION

JEB and pemphigoid diseases have proven that COL17 is a vital player in the stable adhesion between the dermis and epidermis at the DEJ in the skin. However, this adhesion needs to be tightly regulated in a context-dependent manner, for basal keratinocytes to migrate, differentiate and proliferate. Undoubtedly, the processing of COL17 is involved in various normal physiological, as well as pathological, settings, and will be the focus of future study.

The author has no conflicts of interest to declare.

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REVIEW ARTICLE

Drug Development in Pemphigoid Diseases

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Pemphigoid diseases are organ-specific autoimmune diseases of the skin and/or mucous membranes. They are caused by autoantibodies targeting adhesion molecules located at the dermal–epidermal junction. While the diagnostics of pemphigoid diseases and insights into their pathogenesis have improved significantly, the development of novel treatments that are effective and safe remains an unmet medical need. However, numerous pre-clinical studies and early clinical trials have recently been launched. This review summarizes some pathways leading to drug development in pemphigoid diseases, namely: (*i*) hypothesis-driven drug development; (*ii*) omics-based drug development; (*iii*) drug repurposing; (*iv*) screening-based drug development; and (*v*) drug development based on careful clinical observations. Ultimately, it is hoped that this will lead to personalized and curative treatments.

Key words: bullous pemphigoid; epidermolysis bullosa acquisita; translational medical research; disease models; animal autoantibodies.

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SIGNIFICANCE

Despite detailed insights into the pathogenesis of pemphigoid diseases, their treatment still relies on unspecific immunosuppression. Since such treatment contributes significantly to the high patient morbidity and increased mortality, we propose pathways that may facilitate drug development for pemphigoid diseases. With this we aim to foster translational research to develop new treatment strategies for patients with pemphigoid diseases.

(Muco)-cutaneous blistering is the clinical hallmark of pemphigoid diseases (PD). They are characterized and caused by autoantibodies targeting adhesion molecules located at the dermal–epidermal junction. Depending on clinical presentation, the specificity and isotype of the autoantibodies in the following PD can be distinguished (1):

- *Bullous pemphigoid* (BP) is the most prevalent PD and predominantly affects elderly people. BP is caused by autoantibodies targeting BP180 and/or BP230 (2).
- *Mucous membrane pemphigoid* (MMP) is defined as a PD with autoantibodies against components of the dermal–epidermal junction (i.e. BP180 or laminin 332) and predominant mucosal involvement (3, 4).
- *Pemphigoid gestationis* (PG) is a pregnancy-associated immunobullous disease with autoantibodies against BP180 (5).
- *Linear IgA disease* (LAD) is characterized by the linear binding of IgA autoantibodies at the dermal–epidermal junction. LAD is the most common PD in children and clinically presents with urticarial plaques, erosions, and blisters, frequently in a ring-shaped pattern with blistering along the edge of lesions, forming the so-called string-of-pearls sign (6).
- *Epidermolysis bullosa acquisita* (EBA) is a rare and clinically very heterogeneous PD, but due to the availability of pre-clinical model systems it is well-studied (7, 8).
- *Anti-p200/anti-laminin γ1 pemphigoid* (p200) clinically mimics BP, but patients are younger and p200 usually responds well to treatment (9).
- *Lichen planus pemphigoides* (LPP) is, like BP, caused by anti-BP180 antibodies, but in LPP these occur together with lichen planus. Patients with LPP are also younger than those with BP (10).

UNMET MEDICAL NEED IN PEMPHIGOID DISEASES

Treatment of all PD centres on unspecific, systemic immunosuppression, whereby corticosteroids are usually the first line of treatment. Among PD, PG, LAD and p200 usually respond well to treatment and long-term remissions are common. Likewise, BP also responds well to either systemic or topical corticosteroids. However, after withdrawal of treatment, BP relapses in almost 50% of patients within 6 months, requiring long-term corticosteroid treatment, which contributes to patient morbidity and mortality. Both, MMP and EBA are notoriously difficult to treat, and often remission is achieved only after months of immunosuppressive therapy, usually a combination of several drugs (1, 11–16).

This "need for better treatment options" has been identified recently by patients and physicians in a survey to identify the medical need in PD (17). In addition to the current limitations regarding treatment options, the increasing incidence of PD, especially in ageing socie-

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ties (18, 19), further contributes to the medical need to develop novel treatment strategies for PD that are both effective and safe. This increasing medical need has also prompted a significant number of translational studies and clinical trials in PD (20, 21). Unfortunately, however, these clinical trials will not fully address the medical need in PD. Thus, ongoing translational research is required to continuously improve the treatment options, ultimately aiming for personalized and curative treatment.

PATHWAYS TO NEW DRUGS FOR THE TREATMENT OF PEMPHIGOID DISEASES

There are many pathways that may contribute to drug development in PD (**Table I, Fig. 1**). While it may be simplistic, it could be useful to categorize these pathways to new drugs, as follows: (*i*) hypothesis-driven drug development; (*ii*) omics-based drug development; (*iii*) drug repurposing; (*iv*) screening-based drug development; and (*v*) drug development based on careful clinical observations. Examples of each of these pathways to novel treatments for PD are given and discussed in more detail below. The aim of this review is to promote drug development for patients with PD by providing these examples. Another important aim of this article is to initiate a discussion on how this goal is best achieved. Hence, the authors are looking forward to comments from the community, which it is hoped will lead to a fruitful discussion.

Hypothesis-driven drug development: anti-C1s antibodies in bullous pemphigoid

Complement deposition at the dermal–epidermal junction is one of the diagnostic pillars of PD (22). The functional contribution of complement to the pathogenesis of PD has been well documented in pre-clinical model systems (23, 24). Recent data, however, suggests that complement has a more complex role in pemphigoid, whereby some complement receptors confer protection from development of clinical disease (25), or where PD develops independent of complement activation (26). Nonetheless, the complement component C5a has to be considered as one of the main drivers of autoantibody-induced tissue damage in PD (27, 28).

Based on these considerations, function-blocking antibodies to C1s, which initiate the classical complement activation cascade, were developed (29). These anti-C1s antibodies, dose-dependently inhibited the immune complex-induced complement fixation on human skin cryosections (30). More recently, a phase I clinical trial in patients with BP was successfully completed, in which the anti-C1s antibody TNT009/BIVV009 was found to be safe and tolerable in this elderly population, with only mild to moderate adverse events (31). Furthermore, a phase II clinical trial using the dual C5/LTB4 inhibitor coversin is currently being conducted in BP, with promising initial data (32). What is perhaps most striking about the clinical development of these 2 complement inhibitors is the long time needed to translate the clinical and experimental findings on the importance of the complement system into clinical trials. The presence of complement deposits in BP was discovered in the late 60th of the last century (33), and the central role of the complement system in disease pathogenesis was described over 20 years ago (34).

Interestingly, complement activation in PD seems to be restricted to the skin, where C3 deposits are regularly observed, both in patients and animal models of the diseases. More specifically, plasma concentrations of C3a, C4a and C5a in patients with BP were identical to those observed in age- and sex-matched controls. In the same cohort of patients, concentrations of these complement compounds did not change after clearance of skin lesions. In contrast, all of the patients had C3 deposits in the skin at the time of diagnosis (30). Recently, targeted complement therapeutics have been developed, which preferentially bind to sites where complement is activated (35, 36). These targeted complement therapeutics are expected to be both more effective and have fewer adverse events compared with non-targeted complement inhibitors.

Omics-based drug development: validation of spleen tyrosine kinase as a target for treatment of pemphigoid disease

With the availability of novel technologies; for example, genetics, proteomics and RNA sequencing, an unbiased exploitation of novel therapeutic targets can

Table I. Examples of drugs evolving from the outlined pathways to drug development in pemphigoid diseases

Pathway to drug development	Target (compound)	Evidence	Development state
Hypothesis-driven	C1s (Sutimlimab)	Pre-clinical, *in vitro* (30) Phase I trial in patients with BP (31)	Phase I clinical trial completed
	C5/LTB4 (Coversin)	Pre-clinical, *in vivo* (67)	Ongoing Phase IIa in BP (68)
Omics-based	SYK (BAY61-3606)	Pre-clinical, *in vivo* (37, 38)	Target validated in PD mouse model
Drug repurposing	Doxycycline	Case report(s) (series) (15)	Phase III clinical trial successfully completed (43)
	DMF (Skilarence)	Pre-clinical, *in vivo* (47)	Phase II clinical trial in preparation (20)
Drug screening	Not disclosed	Pre-clinical, *in vitro* (69)	Pre-clinical
Clinical observations	Autoantibodies	Case report series (59) Pre-clinical, *in vitro* (60)	Pre-clinical (60)

BP: bullous pemphigoid; DMF: dimethyl fumarate; LTB4: leukotriene B4; PD: pemphigoid disease; SYK: spleen tyrosine kinase.

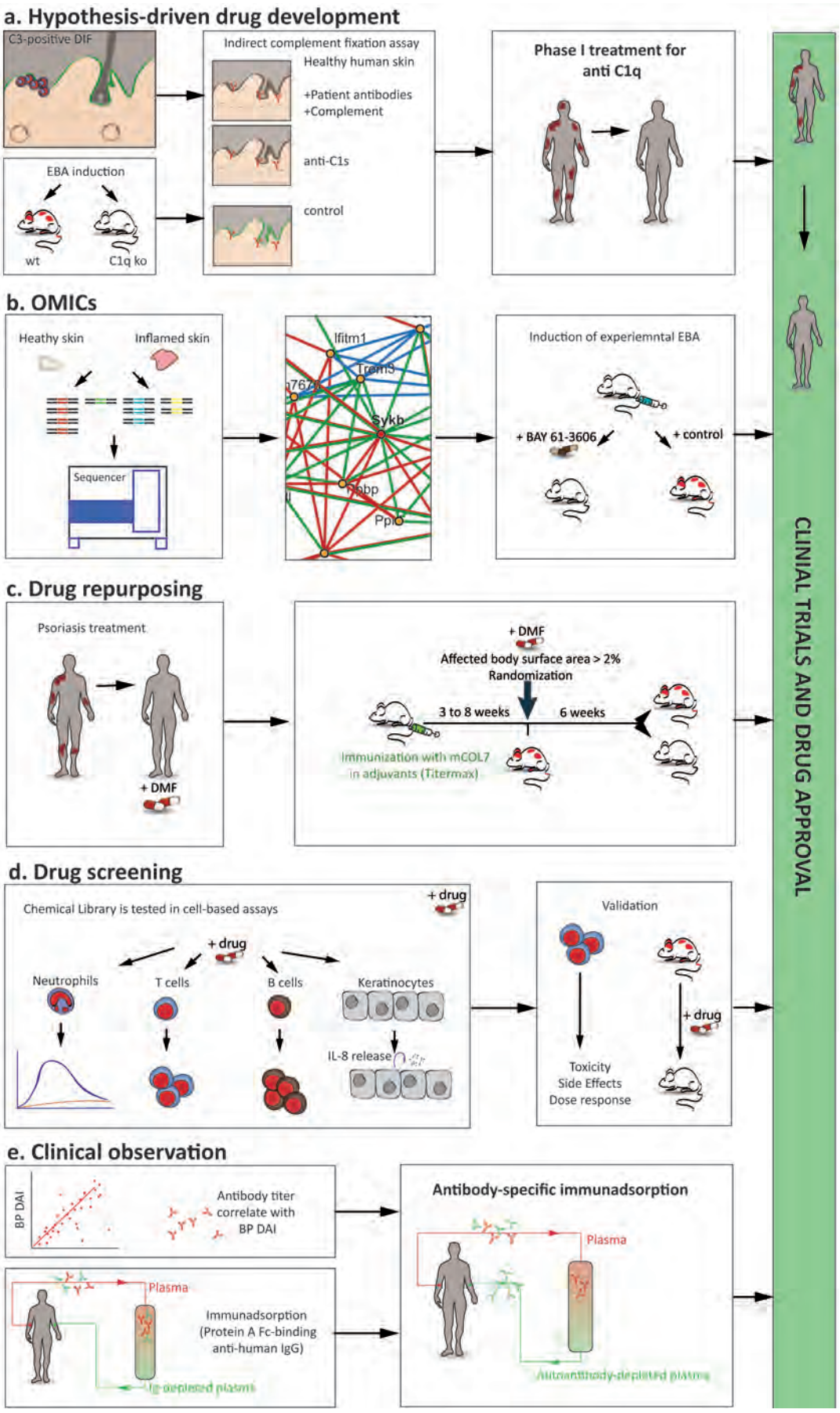

Fig. 1. Pathways to new drugs for the treatment of pemphigoid diseases. (a) Development of new pemphigoid treatments based on hypothesis-driven research. As an example, the development of new complement inhibitors, such as anti-C1s antibodies and coversin are depicted. Based on the clinical observation that complement deposits (in *green*) are highly prevalent in the skin of patients with pemphigoid disease (PD) (1) and the observation that mice deficient (ko) in specific complement proteins are protected from the induction of experimental PD (34), targeting complement activation was assumed to have disease-modifying effects in PD. Both (*in vitro* assays, *middle panel*) and a phase I clinical trial demonstrated that anti-C1s impairs/reduced complement deposition along the dermal-epidermal junction. (b) Development of new pemphigoid treatments based on complex data sets and omics. Here biological specimen, i.e. affected vs. non-affected skin from patients or pre-clinical model systems (*left-hand image*), are subjected to unbiased measurement, for example RNA-sequencing or proteomics. In the example provided, RNA expression in the skin was contrasted between healthy mice and mice with EBA. Subsequently (*middle image*), data analysis is performed, leading to the identification of potential pharmacological targets, such as *Sykb*. For functional validation, in vitro systems or pre-clinical model systems (*right-hand image*) may be used. (Example from Samavedam et al. (37)). (c) Development of new pemphigoid treatments based on drug repurposing. Already licensed drugs, for indications than other pemphigoid, can be repurposed for PD. The rationale for drug repurposing in pemphigoid can either be based on clinical observations, i.e. case report series that a given drug is also effective in pemphigoid, such as doxycycline, or be hypothesis-driven, as shown for dimethyl fumarate, which has a long history as an anti-psoriatic agent (*left-hand panel*), which also ameliorates experimental PD (*right-hand panel*). (d) Development of new pemphigoid treatments based on drug screening. If putative defined drug targets are not known, drug screening can be performed in in vitro model systems, which are up-scalable and highly reproducible. In PD, examples for these assay systems are immune complex-induced release of reactive oxygen species from neutrophils, anti-CD3/CD28-induced T cell proliferation, IL-21/antiCD40L-induced B cell proliferation and anti-BP180 IgG-induced cytokine release from keratinocytes (51). Drug libraries, for example the Prestwick Chemical Library (66) or the FDA-approved Drug Library from Selleckchem, can be obtained commercially. After the initial screening the identified potential drugs need to be validated *in vitro* and *in vivo* (left-hand panel). (e) Development of new pemphigoid treatments based on clinical observations. After the identification of the pathogenic relevance of autoantibodies in PD and the clinical observation of a correlation of the levels of the circulating autoantibody titres with disease severity, immunoadsorption/plasmapheresis were introduced to the management of PD. However, immunoadsorption is limited because all antibodies are removed. Hence, the procedure has to be paused, and does not elute all autoantibodies from the patients. Using the insights from detection of specific autoantibodies in PD, first attempts were made to develop antigen-specific immunoadsorption.

be performed. Regarding PD, such approaches have, however, been sparsely used, and have been limited to mouse models (7). In detail, contrasting cutaneous RNA expression from mice with and without experimental EBA, several potentially disease-promoting genes were identified, i.e. *Sykb*, the gene encoding for the spleen tyrosine kinase (SYK). To evaluate the functional role of differential *Sykb* expression in EBA, experimental EBA was induced in mice that were treated with selective SYK inhibitors, or EBA was induced in SYK-deficient mice. In both experiments, complete protection from induction of experimental EBA was observed if SYK was blocked (37). In parallel, hypothesis-driven research, made similar observations (38). Thus, SYK has been independently identified and validated as a potential therapeutic target for PD.

Unfortunately, however, omics datasets are quite sparse for PD. To the best of our knowledge, only one GWAS has been published so far, reporting an association of MMP with *HLA-DQB1*03:01* and rs17203398, in which the intronic region of *GALC* is located (39). Therefore, in the future, a joint community effort is required to collect well-defined patient samples using standardized procedures for sample acquisition and storage. Alternatively, or in parallel, multi-omics data from model systems (as reported for SYK) may be used for target identification, as well as functional validation. For translation into clinical use, expression of the identified targets may be performed in corresponding patient samples. The advantage of such an approach is that fewer patient samples would be required.

Drug repurposing: doxycycline and dimethyl fumarate for bullous pemphigoid treatment

In dermatology, the use of the anti-CD20 antibody rituximab, initially developed for the treatment of B cell malignancies (40), for the treatment of pemphigus (41) is a good example of drug repurposing. In contrast to "conventional" drug development, already licensed compounds are evaluated for efficacy in other indications. The already known safety profile of the licensed drugs, the decreased time and costs of drug approval are the main advantages of drug repurposing (42).

Regarding PD, the antibiotic doxycycline has recently been demonstrated to be effective in the treatment of BP (43). In a comparative clinical trial, 200 mg of doxycycline, achieved clinical remission in 74% of patients within 6 weeks; while prednisolone (initial dose 0.5 mg/kg) induced remission in 91% of patients. Regarding adverse events, 18% of doxycycline-treated patients experienced a grade 3 or greater adverse event. This was significantly lower, compared with prednisolone, where the number of adverse events was 2-fold higher. Another compound that is currently evaluated for repurposing in BP is dimethyl fumarate (DMF). In Germany, the compound has a long-standing history as an anti-psoriatic agent (44), and more recently has also been licensed for treatment of multiple sclerosis (45). DMF has a multitude of biological effects, including a shift in cytokine expression, a suppression of leukocyte extravasation, anti-oxidant properties, and many others (46). Based on these properties, we hypothesized that DMF may also be beneficial for the treatment of PD. Indeed, treatment of mice with already established clinical EBA manifestations led to a significant improvement in disease activity, while clinical disease severity increased in solvent-treated mice (47). On a molecular level, the beneficial effects of DMF in EBA are mediated through the hydroxycarboxylic acid receptor 2 (48). Based on these findings, the DPem consortium was established to evaluate the safety and efficacy of adjuvant DMF in BP patients responsive to corticosteroid treatment. Centres in France, Poland, Turkey and Germany will recruit 210 patients with BP and allocate these to DMF or placebo.

To the authors' knowledge there are additional drugs soon to be published that have the potential for repurposing in PD. We expect that this pathway to novel drugs for PD will lead to the approval of several new treatment options for pemphigoid patients, using "old" drugs from other indications.

Drug screening

The use of chemical libraries to identify inhibitors of specific molecules, or the use of complex, but up-scalable, model systems is well established for drug development (49, 50). While the use of specific (enzymatic) assays is very well suited to identify new compounds for known pharmacological targets, the use of complex, up-scalable systems in chemical screens offers advantages in instances where molecular defined targets are not known. Despite the fact that up-scalable complex *in vitro* models of PD are already established (51), these have, so far, not been used for drug development in pemphigoid. Examples of these up-scalable model systems are immune complex-induced release of reactive oxygen species (ROS) from neutrophils, or autoantibody-induced cytokine release from keratinocytes, as well as stimulation of T cells using anti-CD3/CD28 and B cell stimulation with IL-21 and anti-CD40L (51, 52).

An envisioned work-flow of such an approach would be to screen compounds of a chemical library to inhibit activation of immune cells or autoantibody-induced cytokine release from keratinocytes with a relatively small sample size. Candidate compounds would be selected based on pre-defined cut-off criteria. Subsequently *in vitro* and *in vivo* validation (using appropriate animal pre-clinical model systems (53), would be employed before clinical trials.

It is hoped that these models, as well as computational approaches to drug development, such as the Connectivity Map (54), will lead to the identification of novel compounds suited for the treatment of PD.

Clinical observations: immunoadsorption for bullous pemphigoid

The detection of IgG deposits along the dermal–epidermal junction in PD (55) and the identification and cloning of the corresponding autoantigens (56) led to the development of serological test systems for the diagnosis of PD (1). This, by itself, is a good example, of how clinical observations and basic research can improve diagnosis. In addition, insights into the pathogenetic role of these autoantibodies (24) prompted the use of immunoadsorption/plasmapheresis in PD (57). More recently, 2 case series have been published, reporting the outcome of immunoadsorption in 26 patients with BP. Interestingly, and in contrast to other autoimmune skin blistering diseases, such as pemphigus, long-lasting remissions were observed in the majority of patients (58, 59). This data, however, should be interpreted within the limitations of case series, as well as the use of concomitant treatments.

Currently, removal of autoantibodies by immunoadsorption is, however, limited because all antibodies are removed, rather than selective removal of autoantibodies. Hence, vigorous and prolonged removal cannot be performed using unspecific immunoadsorption. In mice, at least, this limitation has been overcome: by using insights on the autoantigens in pemphigus and PD, which are currently exclusively used for diagnosis (22), columns specifically removing autoantibodies targeting the NC16A domain and Dsg3 were developed, and (in part) successfully employed in animal models (60, 61). If these insights from pre-clinical model systems can be translated into clinical use, immunoadsorption will most likely become a more widely used treatment modality for PD. Another, potentially very selective and antigen-based, treatment is the use of chimeric autoantigen receptor (CAAR) T cells, which have been shown to selectively deplete specific autoreactive B cells in mouse models of pemphigus (62).

FUTURE DIRECTION OF TRANSLATIONAL RESEARCH IN PEMPHIGOID DISEASES

With the increasing number of clinical trials in PD (21), approval of several new treatments for PD can be expected within the next 3–5 years. However, these trials only recruit patients with BP. For all other PD, to the best of our knowledge, there are currently no ongoing clinical trials, despite the high medical need in MMP and EBA. Therefore, specific, or maybe basket, trials that also include these patients would be highly warranted. Regarding curative treatments, the above-mentioned approaches towards the development of antigen-specific immunoadsorption for BP, or the CAAR-T-cell approach could be tailored to each patients' autoantibodies. In particular, removing the autoreactive B/plasma cell population could induce long-lasting remission, or even a cure, for PD. While translating these interesting findings from pre-clinical model systems into clinical use will take considerable time, a personalized treatment for PD could be implemented relatively quickly using established diagnostic and therapeutic procedures: in single-centre and retrospective studies, several biomarkers have been identified that indicate relapse in BP; for example, the presence of anti-type VII collagen autoantibodies, variations of the glucocorticoid receptor β, or CXCL10-induced matrix metalloproteinase 9 secretion (63–65). Given, that (some of) these are validated in prospective multicentre diagnostic clinical trials, tapering of immunosuppression could be adjusted to the expression of these biomarkers.

Collectively, the high medical need to develop new treatments for PD has prompted a very exciting new area

of translational research in this field, which is expected to improve the treatment of patients with PD in the future. New drug approvals, more clinical trials, and personalized and curative treatments are expected.

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Centenary theme section: BLISTERING SKIN DISORDERS

REVIEW ARTICLE

Current Concepts of Dermatitis Herpetiformis

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Dermatitis herpetiformis (DH) is an autoimmune skin disease that causes itchy, blistering rash, typically on the elbows, knees and buttocks. DH and coeliac disease share the same genetic background, gluten-dependent enteropathy and antibody response against tissue transglutaminase. DH is currently considered a cutaneous manifestation of coeliac disease, and the prevailing hypothesis is that DH develops as a late manifestation of subclinical coeliac disease. The incidence of DH is decreasing contemporarily with the increasing incidence of coeliac disease. The IgA immune response in DH skin is directed against epidermal transglutaminase, while the autoantigen in the gut is tissue transglutaminase. Granular IgA deposition in the papillary dermis is pathognomonic for DH, and is a finding used to confirm the diagnosis. The treatment of choice for DH is a life-long gluten-free diet, which resolves the rash and enteropathy, increases quality of life, and offers a good long-term prognosis.

Key words: dermatitis herpetiformis; coeliac disease; gluten-free diet; transglutaminase; immunoglobulin A; villous atrophy.

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SIGNIFICANCE

Dermatitis herpetiformis is an itchy, blistering rash, which occurs on the elbows, knees and buttocks. Dermatitis herpetiformis is considered a cutaneous manifestation of coeliac disease. Even though obvious gastrointestinal symptoms are rare in dermatitis herpetiformis, intestinal coeliac-type villous atrophy or inflammation is present at diagnosis. The diagnosis is confirmed by skin biopsy revealing typical IgA deposits, and the majority of patients also have coeliac autoantibodies in the serum. The treatment of choice for dermatitis herpetiformis is a life-long gluten-free diet, which resolves the rash and enteropathy, increases quality of life, and offers a good long-term prognosis.

Dermatitis herpetiformis (DH) is an intensively itching skin disease, which causes papulovesicular eruption, predominantly on the elbows, knees and buttocks. DH is considered an autoimmune-based disease, since pathognomonic granular immunoglobulin A (IgA) response in the dermis, directed against epidermal transglutaminase (TG3), and circulating autoantibodies against tissue transglutaminase (TG2) and TG3 exist in DH (1, 2). Moreover, the predisposing genetic background, more specifically HLA DQ2 or DQ8 haplotypes, is a necessity for development of the disease (3). DH is considered a specific variant of coeliac disease, manifesting primarily in the skin, but coeliac-type enteropathy also exists in DH, albeit more subtle than in coeliac disease (4). Currently approximately 13% of patients with coeliac disease have DH (5, 6) and the highest reported prevalence of DH to date has been 75 per 100,000 from Finland (5). The prevalence is lower in some areas of the globe and in specific populations, for example in Asia and in African-Americans (7, 8) and, overall, the geographical differences in the prevalence of DH and, likewise, coeliac disease, have been explained mainly by HLA genetics and wheat consumption habits (9). Also the incidence figures of DH have ranged from 0.4 to 3.5/100 000/year, even in different studies performed in Europe or North America (5, 10). DH is typically diagnosed during adulthood, and the incidence of DH is highest in females and males aged 50–69 years (5, 6). Interestingly, the diagnostic age of DH has increased (5) and, although the reasons for this increase remain largely obscure, a possible explanation could be changes in dietary habits. Nonetheless, even though childhood diagnosis is rare in northern Europe (5, 6, 11) it seems to be more common in Italy and Hungary (12, 13).

The focus of this review is to describe the current, clinically relevant, concepts of DH diagnostics, treatment and prognosis. In addition, the close link between DH and coeliac disease is elaborated, and unique features of DH, the cutaneous manifestation of coeliac disease, are presented.

SKIN MANIFESTATION OF COELIAC DISEASE

The clinical manifestations of DH were first described as early as 1884 by Louis Duhring (14) and, 4 years later, the classical abdominal and malabsorptive symptoms of coeliac disease were described by Samuel Gee (15). The link between DH and coeliac disease was found when Marks et al. (16) detected that coeliac-type enteropathy was also a common finding in DH, and importantly, when gluten-free diet (GFD), the treatment of choice in coeliac disease, was shown to heal small bowel mucosal

changes in DH, and to alleviate DH rash (17, 18). Subsequent family and genetic studies have coupled DH and coeliac disease even more convincingly together: DH and coeliac disease have been shown to occur often in the same families and even in monozygotic twins, and furthermore, predominantly HLA DQ2 and, more rarely, DQ8 haplotypes have been shown to be the predisposing haplotypes in both (19–21). Moreover, it has been shown that the phenotype of coeliac disease is not invariably constant, since it can convert from classical disease into DH, especially when dietary compliance is poor (22).

A major breakthrough occurred in coeliac disease research in the 1990s when TG2 enzyme was identified as the autoantigen of the disease (23). Subsequently an enzyme-linked immunosorbent assay (ELISA)-based method for detecting TG2 antibodies was developed and found to be accurate in coeliac disease (24) and, furthermore, a similar TG2 antibody reaction was shown to occur in the serum of patients with DH (2). Moreover, TG2-targeted autoimmune response has been detected in the small bowel mucosa of untreated coeliac disease and DH patients (25, 26).

DH, however, has some distinct features compared with coeliac disease in general. DH is more rarely diagnosed during childhood compared with coeliac disease (11, 27). Furthermore, DH is slightly more common among males than females (5), which contradicts the female predominance known to exist in coeliac disease (6, 28). Moreover, the incidence of DH has decreased, but in coeliac disease a marked increase in the incidence figures has been detected (5, 6, 28). One prevailing hypothesis is that DH develops as a late manifestation of coeliac disease, affecting individuals with subclinical or neglected coeliac disease. It has, moreover, been suggested that the TG3 immune response typical for DH develops as an epitope spreading phenomenon from an autoimmune response initially targeting TG2 (29). Coeliac-type dental enamel defects detected in adults diagnosed with DH indicate that these individuals were already sensitive to gluten in early childhood (30). Moreover, the rarity of childhood DH and the changing phenotype of coeliac disease during poor dietary adherence support this hypothesis, and furthermore, the divergent trend of incidences of DH and coeliac disease also fits well with this hypothesis: better diagnostics of coeliac disease due to increased awareness, availability of accurate serum autoantibody tests, and screening of risk groups has resulted in a smaller pool of patients with undiagnosed coeliac disease and, consequently, fewer individuals with potential for development of DH.

DIAGNOSING DERMATITIS HERPETIFORMIS

The suspicion of DH typically arises from the characteristic skin symptoms, which are an intensely pruritic rash with small blisters and papules affecting most commonly the extensor surfaces of the elbows, knees and buttocks (**Fig. 1**a, b and **Table** I). Occasionally other sites, such as the scalp, face, upper back and neck, are also affected. There is individual variation in the severity of the rash and pruritus, but commonly due to the intense itch and scratching, the blisters are broken and only erosions, crusts and post-inflammatory hyperpigmentation are consequently present. Acral purpura is one, albeit quite rare, finding in DH and can be found either as a sole presentation or concomitantly with the typical DH rash (31–33). Despite the gluten-sensitive enteropathy, obvious gastrointestinal symptoms and signs of malabsorption are rare in DH, but some kind of abdominal symptoms have, however, been reported in up to one-third of patients (34, 35). Interestingly, although the clinical picture of coeliac disease has been shown to become milder and more heterogenic with increasingly common non-classical symptoms (36–38), it seems that the clinical picture and the severity of DH rash have remained quite unchanged during recent decades (39).

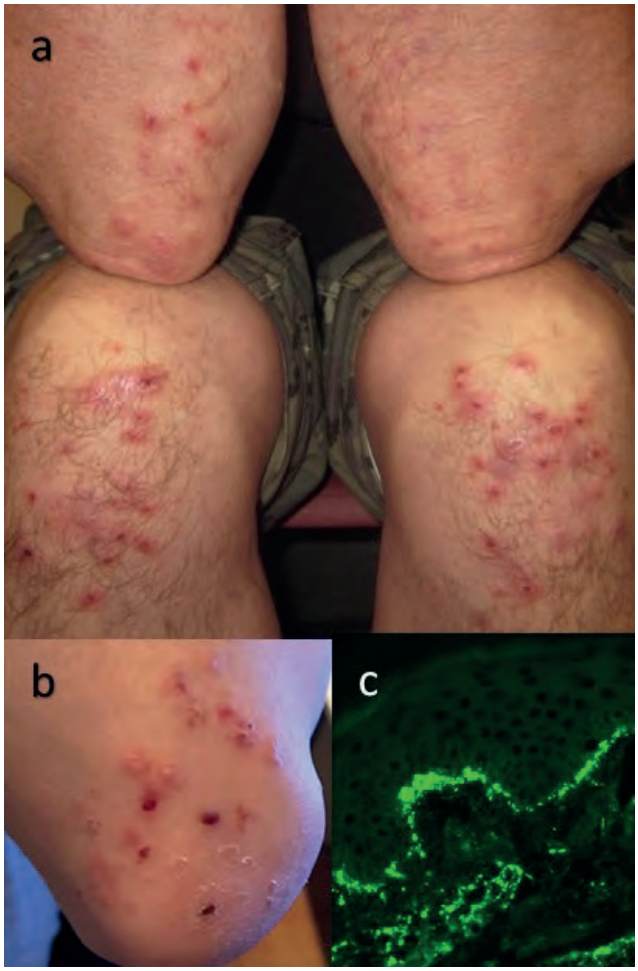

Fig. 1. Clinical characteristics of dermatitits herpetiformis (DH). (a) A typical clinical picture of dermatitis herpetiformis (DH) with excoriated blisters and papules on the elbows and knees. (b) Intact and excoriated blisters, papules and crusts on the elbow. (c) Direct immunofluorescence (×40) finding in DH; granular IgA deposits in the basal membrane zone and in the dermis.

Table I. Diagnostic procedures in dermatitis herpetiformis (DH) and recommendations regarding when they should be applied

Procedure	Recommendation
Patient history and physical examination	
Duration, severity and type of skin symptoms	Always
Presence of gastrointestinal and malabsorptive symptoms and signs	Always
Family history of coeliac disease and DH	Always
Presence of associated autoimmune diseases	Always
Diagnostic procedures	
Direct immunofluorescence examination of perilesional skin biopsy	Always
Histopathological analysis of lesional skin biopsy	In obscure cases
Serum tissue transglutaminase or endomysial antibodies	Always
Small bowel biopsy examination	Only if gastrointestinal symptoms not compatible with coeliac disease exist
HLA DQ2 and DQ8 typing	Only in obscure cases

The differential diagnosis of DH includes other subepidermal blistering diseases, especially linear IgA disease and bullous pemphigoid. In addition, other itchy skin diseases, such as atopic and nummular dermatitis, lichen planus, urticaria and scabies may sometimes be difficult to differentiate from scratched DH rash, although the typical predilection sites of these diseases differ from those of DH (40).

The gold-standard method to verify DH diagnosis is direct immunofluorescence (IF) examination, which shows the pathognomonic granular IgA deposits in the papillary dermis and/or at the dermoepidermal junction (Fig. 1c). IgA deposits are widespread, but not totally uniformly distributed in the skin of patients with DH, and therefore the ideal site for the diagnostic skin biopsy is uninvolved perilesional skin, where the deposits are found in greater amounts (41). The immune response in DH skin is directed against TG3, an enzyme closely related, but not identical, to TG2 (1). It has recently been demonstrated that TG3 disappears from the dermis of patients with DH on a GFD, in parallel with IgA, but the disappearance is prolonged, often taking years even on a strict diet (42). There are a few rather interesting studies reporting that granular IgA also exists in the skin of coeliac disease patients with healthy skin or with inflammatory skin diseases other than DH (43, 44). However, the number of the patients in these studies has been small, and further research evidence is needed before conclusions can be drawn about the existence of granular IgA in non-DH skin. For the time being, at least, this finding can be considered DH-specific.

In addition to the characteristic granular deposition of IgA, mostly sporadic cases of fibrillary IgA deposits in DH have been presented (45–47). The fibrillar pattern of IgA appears to be more common in Japan, where it has been reported to occur in approximately one-third of patients with DH. However, Japanese patients with DH also show other distinct features that differ from Caucasian patients; the Japanese patients with DH do not carry the predisposing HLA-DQ2 and HLA-DQ8 haplotypes, the occurrence of gluten-sensitive enteropathy is rare, and coeliac-disease-specific autoantibodies are seen only in low proportion of patients. These findings suggest that the pathogenesis of Japanese DH differs from that of Caucasian DH, and may not be dependent on gluten (48, 49).

Histopathological examination of lesional skin biopsy is not required for diagnosis of DH, but, in obscure cases, compatible findings with DH support the diagnosis (40). Ideal areas for histopathological biopsy specimen are an intact vesicle or erythematous skin, and the typical findings include non-specific subepidermal blister and papillary microabscesses, together with neutrophil and a few eosinophil infiltrates (50). However, the above-mentioned findings alone do not allow the differentiation of DH from other autoimmune bullous disorders.

A recent study from Finland demonstrates that diagnosis of DH is not always easy. The study investigating the diagnostic delay of DH during the last 45 years detected that the duration of skin symptoms before the diagnosis was 2 years or more in one-third of patients with DH. Female sex, villous atrophy at diagnosis, and a DH diagnosis prior to the year 2000 were significantly associated with long diagnostic delay. Fortunately, the same study established that the diagnostic delay has shortened during recent decades from 12 to 8 months (39). Correspondingly, the diagnostic delay in coeliac disease has become shorter (51).

SEROLOGICAL AND SMALL BOWEL MUCOSAL FINDINGS IN DERMATITIS HERPETIFORMIS

In DH, there are often circulating IgA-class autoantibodies against both transglutaminase isoenzymes, TG2 and TG3. TG2 is also the target for endomysial antibodies (EmA) (52), and ELISA-based TG2- and indirect IF-based EmA tests can equally be utilized in clinical practice (Table I). However, the evaluation of EmA is subjective and requires skilful laboratory personnel. TG2 antibodies have proven to be highly accurate in coeliac disease, but in DH these antibodies are mostly confined to those patients with small bowel mucosal villous atrophy, and hence a negative result does not exclude DH (53). However, together with a compatible clinical picture, TG2 antibodies are suggestive of DH, and further, indicative of small bowel mucosal damage. If elevated, TG2 antibody measurement can further be utilized in the follow-up of GFD adherence after the diagnosis. Circulating TG3

antibodies have been suggested to be DH-specific, but surprisingly, these antibodies occasionally also occur in the serum of coeliac disease patients without any detectable skin lesions (1, 54, 55). It has been shown, however, that in coeliac disease the affinity of the antibodies to TG3 is lower than in DH (1) and that TG3 reactivity increases with age in coeliac disease (55). Therefore, it can be speculated that skin symptom-free coeliac disease patients with TG3 reactivity are susceptible to future development of DH, especially if not compliant with a strict GFD. However, since the exact role and value of TG3 antibodies in DH and coeliac disease is, thus far, to some extent obscure, these antibodies are currently mostly used in research settings.

Small bowel mucosal biopsies obtained during upper gastrointestinal endoscopy are not necessary for DH diagnosis. It is widely recognized that the majority of the untreated DH patients have coeliac-type small bowel mucosal villous atrophy, but at least one-quarter of the patients evince normal villous architecture (53). However, virtually all subjects without evident small bowel mucosal damage evince intestinal coeliac-type inflammation and/or immune response. Characteristic for both DH and coeliac disease is increased densities of γδ+ intraepithelial lymphocytes in the small bowel mucosa (56), but even more specific finding is the presence of intestinal TG2-targeted autoantibody deposits (25, 26). However, both of these investigations require frozen small bowel mucosal samples, which are not available in every diagnostic centre. Importantly, even though small bowel mucosal changes vary from inflammatory changes to severe villous atrophy in DH, recent evidence has shown that the severity of mucosal damage at diagnosis does not have any effects on the long-term prognosis of DH (57, 58), which naturally strengthens the rationale behind the current policy of not obtaining routine small bowel biopsies when DH is diagnosed.

GLUTEN-FREE DIET AND DAPSONE TREATMENT IN DERMATITIS HERPETIFORMIS

The essential treatment for DH is a strict, life-long GFD. When adhering to a GFD, wheat, rye, barley and foods otherwise containing gluten are permanently excluded from the daily diet, but gluten-free oats (i.e. oats not contaminated by other cereals) are currently allowed in most countries and tolerated by the majority of patients with DH (59). Adherence to a GFD leads to healing of the small bowel mucosa and alleviation of the clinical symptoms, but total clearance of the DH rash may take several months or even a couple of years (17, 60). Therefore, at the beginning of GFD treatment the individuals with widespread, active rash need additional treatment with dapsone.

Dapsone is a sulfone drug with potent antimicrobial and anti-inflammatory properties, which relieves the DH rash and itch effectively, but has no effect on the enteropathy. The starting dose of dapsone should be 25–50 mg/day. If needed, the dose can be increased gradually up to 100 mg/day, and then, once the rash has disappeared, the dose should be slowly tapered and finally discontinued as the GFD alone controls the rash (60). Dapsone is usually well tolerated when recommended doses are used, but side-effects are possible, of which dose-dependent haemolysis is the most common and, for example, methaemoglobinaemia, agranulocytosis and hepatitis less frequent. Hence, clinical and laboratory monitoring during treatment is necessary. In Finland approximately 70% of patients with DH require dapsone treatment after being diagnosed, and when initiated, it is usually needed for 2–3 years (57, 60). In rare cases of DH, the rash continues despite long-lasting, strict, adherence to a GFD. Recently this condition, named refractory DH, was found to occur in less than 2% of patients with DH (61). The patients with refractory DH in that study had followed a strict GFD for a mean of 16 years, but dapsone was still essential due to the active DH rash. Interestingly, despite the ongoing clinical symptoms, the small bowel mucosa had recovered in all subjects, and none had developed lymphoma, which suggests that refractory DH probably diverges from refractory coeliac disease, in which the small bowel mucosa does not heal on a GFD and the risk of lymphoma is increased (62). However, since refractory DH seems to be very rare, in cases of non-responsive DH, intentional or accidental dietary lapses are a more common reason and have to be excluded by dietary consultation.

Current recommendations are that treatment with a GFD should be life-long in DH, as in coeliac disease. However, there are some reports suggesting that a proportion of patients with DH following a GFD could later re-introduce gluten to their diet without developing symptoms or signs of DH (60, 63, 64). Three gluten-challenge studies have also investigated the possible redevelopment of gluten tolerance in DH. The first gluten-challenge study by Leonard et al. reported 11 out of 12 (92%) patients with DH relapsed with rash and 7 (64%) of these also with villous atrophy (65). However, when Bardella et al. later challenged 38 GFD-treated DH patients with gluten, they reported 7 (18%) who did not manifest any type of relapse in the skin or small bowel during the prolonged gluten challenge (66). Very recently, a 12-month gluten-challenge study was performed in 19 long-term GFD-treated DH patients in Finland (67). In this study, 18 (95%) of the patients relapsed in a mean of 6 months; 15 (79%) developed DH rash, 12 of whom also showed small bowel villous atrophy, and 3 patients showed progression of small bowel mucosal villous atrophy without skin symptoms or cutaneous IgA deposits. One patient, however, did not show any skin symptoms or IgA deposits, nor did he develop intestinal villous atrophy or inflammation. However, a long follow-up is needed

before it can be concluded that gluten is truly tolerated by this patient, and at present, it seems that development of gluten tolerance in DH is rare or even non-existent, and life-long strict adherence to a GFD is still justified in all patients with DH.

Long-term prognosis on a gluten-free diet

Coeliac disease is known for increased all-cause and lymphoma mortality risk (68). Therefore it is interesting that, in a recent Finnish DH study, the all-cause mortality rate in DH was, in contrast, significantly decreased (standardized mortality rate 0.70), and the lymphoma mortality was increased during the first 5 years after diagnosis, but not thereafter (58). Similarly, a previous DH study from the UK found a slightly, but non-significantly, reduced mortality rate (hazard ratio 0.93) (69). In the Finnish study, 98% of patients with DH adhered to a GFD, which may explain their excellent prognosis, whereas in the study from the UK, data about dietary adherence was absent for one-third of patients (58, 69). Evidence clearly confirms that adherence to a GFD reduces the risk of lymphoma in DH, the risk of which has been shown to be similarly increased in DH and coeliac disease (70–72). In DH, the risk of gastrointestinal carcinomas has not been reported to be increased, which is in contrast to coeliac disease (69, 71, 72). Also, the increased bone fracture risk associated with coeliac disease seems not to be a complication of similar extent in DH, although bone complications have been very rarely studied in DH (69, 73).

Quality of life (QoL) aspects in coeliac disease have been widely studied, but only limited evidence of DH and QoL exist. However, according to current knowledge, the QoL of patients with DH seems to be reduced, but importantly, already after adherence to a GFD for 1 year, the QoL increases to the level of controls (35). The positive impact of GFD on DH patients' QoL is also supported by another study, in which the Qol of long-term GFD-treated DH patients was equal to that of controls, and slightly better than that of long-term treated coeliac patients (74).

Similar to coeliac disease, DH has been associated with other autoimmune diseases, and the associations have mostly been explained by common genetic factors. In DH, the frequency of autoimmune thyroid disease has been reported to be as high as 4% and that of type 1 diabetes 1–2% (75–78). In addition, Sjögren's syndrome, vitiligo and alopecia areata have been reported to associate with DH, although these associations are not well documented. Most of the associated autoimmune diseases have been reported to develop prior to the diagnosis of DH, but subsequent development is also a possibility. A recent Finnish register study demonstrated a rather interesting association of DH with bullous pemphigoid (79). In that study, patients with previously diagnosed DH had a 22-fold risk for the later development of bullous pemphigoid, with a mean of 3 years from diagnosis of DH to diagnosis of bullous pemphigoid. The authors speculated that a possible mechanism of this evolvement could be an epitope spreading phenomenon.

CONCLUSION

DH is a chronic, bullous skin disease, which is a skin manifestation of coeliac disease. It is suggested that long-lasting and undetected coeliac disease with TG2-directed immune response serves as a prerequisite for the development of DH and TG3 antibody response and, furthermore, that more accurate and active coeliac disease diagnostics has resulted in a declining incidence of DH (5, 6). The cutaneous symptoms of DH are troublesome and decrease the QoL of patients (35). It is therefore fortunate that the diagnostic delay has become shorter during recent decades (39). However, variable prevalence figures for DH in different countries, and delayed diagnosis in one-third of patients with DH in a high prevalence area (39) indicate that there is still a necessity for further improvement of DH diagnostics. Recognizing the cutaneous signs indicative of DH and IF examination of perilesional skin biopsy remain the cornerstones of DH diagnosis (Table I). Investigation of small bowel mucosal histology has no further value in routine diagnostics, and TG2 antibody testing has a supportive, but not exclusive, role in DH diagnosis. Future studies will presumably reveal whether measurement of TG3 antibody has additional value in DH diagnostics or in the identification of subjects at risk of development of DH. One future prospect is that TG3 antibody-based diagnosis of DH could be a possibility in the long run, which would facilitate the diagnosis of DH and enable diagnostics in centres without the possibility of IF examination. In coeliac disease, serologically-based diagnosis has been recommended in children since the year 2012 (80), and is also utilized in adults in some countries, such as Finland.

According to current knowledge, strict life-long adherence to GFD is justified in all patients with DH. The prognosis seems to be excellent in those individuals with DH who follow the diet rigorously, but other than adherence to a GFD, little is known about the factors that influence the development of complications or associated diseases of DH and mortality. Instead, it has been shown that the degree of villous atrophy has no effect on the above-mentioned outcomes of DH (57, 58). Factual non-responsiveness to GFD is rare in DH, but, in general, refractory DH seems to have better prognosis compared with refractory coeliac disease (61). However, current knowledge of refractory DH is scarce and more research evidence is needed in order to elaborate this entity more thoroughly. In addition, the differing mortality trends currently existing among DH and coeliac disease patients adhering to the same diet is an interesting topic for future studies.

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REVIEW ARTICLE

Bullous Drug Reactions

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Bullous drug eruptions are infrequent, but because they pose a challenge both to affected patients and to treating physicians they are considered to be the most severe cutaneous adverse reactions (SCAR). It is important to recognize these conditions and to differentiate them from other clinical entities involving blister formation. There may be early signs and symptoms that indicate a severe bullous drug eruption even before blisters and erosions of the skin and mucous membranes become obvious. Once the diagnosis is suspected, appropriate diagnostic procedures and adequate management must be initiated. The latter includes identification of the potentially inducing drug, although it should be taken into account that not all cases of bullous eruptions are drug-induced. In cases with drug causality the potentially culprit agent must be withdrawn, while in cases with other aetiology the underlying condition, e.g. an infection, must be treated appropriately. In addition to best supportive care, immunomodulating therapy may be considered.

Key words: severe cutaneous adverse reaction; Stevens-Johnson syndrome; toxic epidermal necrolysis; generalized bullous fixed drug eruption.

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SIGNIFICANCE

Drug reactions with blisters (known as bullous drug reactions) are challenging for patients and physicians. Often there are early signs and symptoms that may lead to the suspicion of a bullous drug reaction before blisters and erosions of the skin and mucosa appear. Once the diagnosis is suspected, appropriate diagnostic and therapeutic procedures must be initiated. A detailed history, including clinical symptoms, drug use and infections, is crucial. In cases with drug causality, the potentially culprit agent must be withdrawn, while in cases with other aetiology, the underlying condition, e.g. infection, must be treated. In addition to best supportive care, immunomodulating therapy may be considered.

Bullous drug reactions generally occur as a result of medication use, but there are also other possible causes. One of the major challenges is to identify at a very early stage whether the reaction will be severe and life-threatening. Once blisters are present, differentiation between types of reaction, such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), generalized bullous fixed drug eruption (GBFDE) and, sometimes, bullous autoimmune disease, is also challenging, since, for example, conditions such as GBFDE or IgA-linear dermatosis can mimic SJS/TEN. Differentiation is important as prognosis and treatment modalities differ substantially.

HISTORICAL CONSIDERATIONS

In 1922, the American paediatricians Stevens & Johnson (1) reported 2 cases of a disseminated cutaneous eruption associated with erosive stomatitis and severe ocular involvement. In 1956, the Scottish dermatologist Lyell (2) described patients with epidermal loss secondary to necrosis, and introduced the term "toxic epidermal necrolysis". However, Lyell did not refer to the findings of Stevens & Johnson at that time, but in a later reappraisal evaluated the original 4 cases in his publication as SJS/TEN, staphylococcal scalded skin syndrome (SSSS) and generalized bullous fixed drug eruption (GBFDE) (3). The histopathological difference between an intraepidermal subcorneal separation in one case had already been described in his first publication, but it took until 1971 to identify a staphylococcal exotoxin as the cause of this reaction and to name it accordingly (4). Around the same time Kauppinen (5) from Finland separated a multilocular or GBFDE from SJS and TEN through clinical features and behaviour in allergological testing.

Over the years, due to similarities in clinical and histopathological features, SJS and TEN have been included in the spectrum of erythema multiforme (EM), which was first described by von Hebra in 1860 (6). However, several attempts have been made to disentangle, regroup and rename the reactions. Ruiz-Maldonado (7), for example, proposed the term "acute disseminated epidermal necrosis" for SJS, TEN and "transmission of forms", but did not separate EM, whereas Lyell (8) suggested the name "exanthematic necrolysis" for SJS/TEN. Based on the original descriptions and the observation that SJS may progress into TEN, an international group of dermatologists developed a consensus definition that separates these conditions from EM. Because SJS and TEN share a clinical pattern, histopathological findings, aetiology, risk factors, and mechanisms, they are considered as severity variants of a single disease entity

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that differs only in the extent of skin detachment related to the body surface area (BSA) (9). Therefore, it seems more appropriate to use the term "epidermal necrolysis" or "epithelial necrolysis" (referring to skin and mucosa) for both (10).

EPIDEMIOLOGY

Epidermal necrolysis (EN) is a rare condition with an overall incidence of 1–2 cases per million persons, estimated using strictly validated cases of a prospective population-based registry (11, 12). However, incidences as high as 5–6 cases per million per year derive from medical databases not primarily designed for epidemiological analysis of rare diseases (13). EN can occur at any age, but the risk increases with age and the highest incidence is seen in elderly persons over 65 years of age (14). The mean age of patients was 53.4 years (range 1–94 years) in a cohort of more than 2,200 patients (15). Women are more frequently affected, with a sex ratio of 0.6. Patients infected with human immunodeficiency virus (HIV) and, to a lesser degree, patients with collagen vascular disease (also called connective tissue disease, including rheumatoid arthritis, systemic lupus erythematosus, Sjögren syndrome, dermatomyositis, polymyositis, scleroderma, mixed connective tissue disease and some types of vasculitis) and cancer are at increased risk (11, 16). The overall mortality associated with EN is 22–25%, varying from approximately 10% for SJS to almost 50% for TEN (17–19). Several factors contribute to poor prognosis, such as larger extent of skin detachment, older age, and underlying comorbidity.

In contrast, the mortality for erythema (exsudativum) multiforme majus (E(E)MM; i.e. EM with mucosal involvement) is very low, affecting few individuals of older age and underlying conditions. The majority of patients are young (80% are younger than 40 years, 45% are under 18 years) and male (approximately 75%) (9, 20). The incidence of cases of severe EMM leading to hospitalization is of approximately the same order of magnitude as that of EN (SJS-TEN), with milder cases (EM minus with only skin involvement or cases with only mucosal involvement) occurring more frequently (15, 20).

To date, estimates of the incidence of GBFDE are lacking, since there are currently no population-based data. As with most types of cutaneous adverse reactions, GBFDE more frequently affects women. Of the affected patients 70% are older than 70 years and approximately 22% of patients die due to advanced age and disease severity (21).

CLINICAL FEATURES AND CLASSIFICATION

EN is characterized by erythematous skin, epidermal detachment and erosions of mucous membranes. The erythematous exanthema consists of atypical flat target lesions (these lack the typical 3-zone, target-like constellation of so-called typical target lesions seen in EM) and/or macules that frequently tend to become confluent and spread from cranial to caudal. Blisters develop on the erythema and coalesce. Usually, at least one mucous membrane is affected by erosion in addition to the skin. Fever and malaise are very common (10). The condition is classified according to the consensus definition: skin detachment of less than 10% of the BSA refers to SJS, and more than 30% of the BSA to TEN. Skin detachment between these values is defined as SJS/TEN-overlap (**Table I**, **Fig. 1**) (22). In approximately 95% of cases, haemorrhagic erosions of mucous membranes, including eyes, lips, mouth, vulva, glans penis, and sometimes also trachea, bronchi, urethra and anus, are present (**Fig. 2**). Due to the fact that the skin detachment progresses, turning a case initially thought of as SJS into TEN, and due to the fact that SJS and TEN share the same aetiology and pathogenesis, they are considered as a single disease entity of different severity (9).

Table I. Consensus definition of epidermal necrolysis (EN) (22)

Criteria	EM majus	SJS	SJS/TEN overlap	TEN with maculae	TEN on large erythema (without spots)
Skin detachment, %	< 10	< 10	10–30	> 30	> 10
Typical target lesions	+	–	–	–	–
Atypical target lesions	Raised	Flat	Flat	Flat	–
Maculae	–	+	+	+	–
Distribution	Mainly limbs	Wide-spread	Wide-spread	Wide-spread	Widespread

EM: erythema multiforme; SJS: Stevens-Johnson syndrome; TEN: toxic epidermal necrolysis.

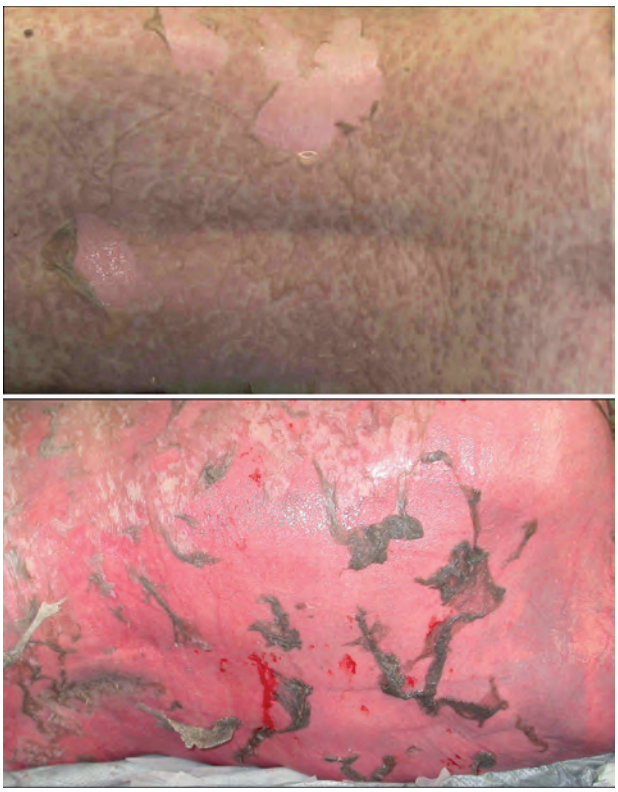

Fig. 1. Confluent macules with confluent blisters, leading to large areas of skin detachment in epidermal necrolysis (patient's back).

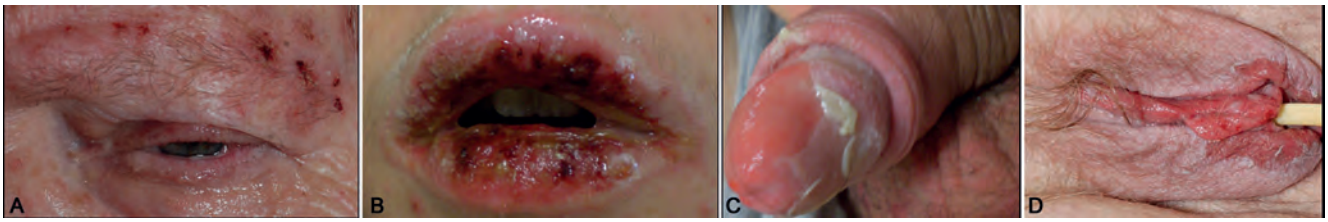

Fig. 2. Haemorrhagic erosions of mucous membranes in epidermal necrolysis or erythema multiforme majus: (a) blepharitis, (b) erosions of lips and oral mucosa, genital erosions in (c) a male and (d) a female patient.

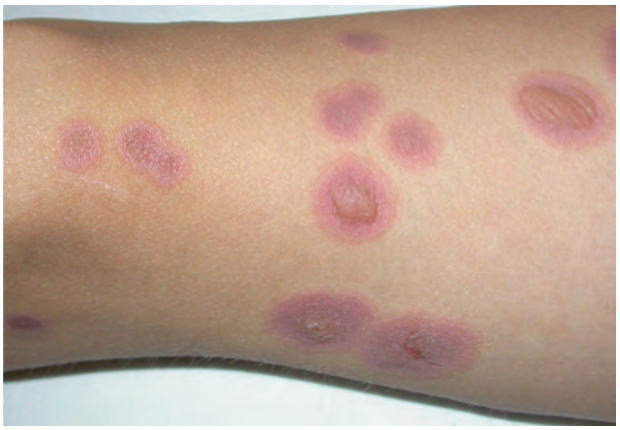

Fig. 3. Typical target lesions with central blisters in erythema multiforme majus (on the leg).

Due to the same type of mucosal involvement, EM with mucosal involvement (erythema multiforme majus; EMM) was assumed to be a less severe form of SJS. However, this incorrect classification may lead to false assessment of causal factors, which in SJS/TEN are predominantly medications and in EMM almost exclusively infections (9, 10, 20). Furthermore, younger patients with EMM may be severely ill with high fever and overall poor general state of health (20).

EMM and SJS can generally be well differentiated based on the consensus definition (in more than 90% of cases), especially when typical targets on the limbs are present (**Fig. 3**). However, differentiation may be challenging in the case of atypical EMM involving atypical "giant targets". This also accounts for the mainly truncal and generalized distribution of typical target lesions, especially in children and adolescents, since these lesions sometimes coalesce. The description of a typical and atypical type of EMM helps to better classify the various patterns of EM and their distinction from SJS (23). Moreover, due to their demarcation towards intact skin, older "giant targets" may resemble resolving patches in GBFDE (18).

Besides EMM, GBFDE is an important differential diagnosis of EN. This reaction is typically characterized by well-defined round or oval, egg-sized patches of dusky violaceous or brownish colour. Blisters may develop on these patches, but the skin remains intact between the areas of blistering and, in most cases, skin detachment does not affect more than 10% of the BSA (**Fig. 4**). However, the reaction may also present with diffuse erythema and blisters, which will show demarcation during the course. There is a debate among experts as to whether the rare cases of TEN on large erythema are potentially severe forms of GBFDE (10, 24).

Patients with GBFDE usually do not develop fever and malaise, but there may be mild mucous membrane involvement, with the genital and/or oral mucosa affected, but not the ocular surface. Milder eruptions are frequent in the patient's medical history (18, 21, 24).

To supplement the consensus definition for EN described above (22), the RegiSCAR-group developed a score for the diagnostic differentiation of GBFDE, which is currently in the validation phase and has not yet been published. There are no specific laboratory parameters to differentiate between the various types of blistering reactions.

HISTOPATHOLOGY

The histology of EN reveals necrotic (dyskeratotic or apoptotic) keratinocytes, either in a disseminated distribution or as complete epidermal necrosis with subepidermal blister formation. Localization and timing of sample collection are important: if the biopsy is taken from the central blister of an EMM target, complete epidermal necrosis may also be visible, as well as a sparse superficial lymphocytic infiltrate in the dermis, often in a perivascular location (25, 26). Therefore, histopathology can only confirm the clinical condition within the spectrum of disease but is unable to proof the specific clinical form. The same accounts for the histology of GBFDE, in which a distinction is sometimes possible in the course of the disease. If a biopsy is taken at a later stage, a deep perivascular infiltrate containing

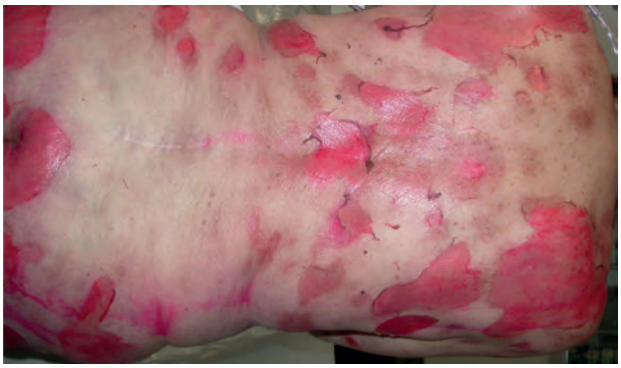

Fig. 4. Well-demarcated erythematous patches with blisters in generalized bullous fixed drug eruption (on the back).

neutrophils and eosinophils may be seen, and potentially also pigment deposits (26, 27).

FURTHER DIFFERENTIAL DIAGNOSES

EN and GBFDE must be differentiated from staphylococcal scalded skin syndrome (SSSS), which histologically shows intraepidermal, subcorneal separation (4). Bullous autoimmune dermatoses, such as bullous pemphigoid, linear IgA dermatosis, pemphigus vulgaris, and paraneoplastic pemphigus, should be included in the differential diagnosis (10). Therefore, if one of these diseases is suspected, a direct immunofluorescence test as well as serological autoimmune parameters (e.g. anti-BP 180-, 230-, desmoglein antibodies) should be performed (10). Linear IgA bullous dermatosis (LABD) can imitate SJS/TEN, as has been described in several case reports and case series (28, 29). Some authors reported a more severe pattern with larger areas of skin detachment in cases that were drug-induced, with vancomycin being a frequent cause (29). Other disorders that should be considered in the differential diagnosis include widespread drug eruptions, erythroderma, exfoliative dermatitis, and subacute cutaneous lupus erythematosus (10, 18). Acute generalized exanthematous pustulosis (AGEP) may mimic EN, when confluence of pustules appears to reveal a positive Nikolsky's sign. However, AGEP does not turn into EN, since there is no primary epidermal necrosis, but there is rapid healing of the subcorneal lesions. Bullae may occur in body areas with oedema, leading to widespread intraepidermal blister formation and secondary necrosis of the blister roof (30). If tension blisters appear due to oedema in drug reaction with eosinophilia and systemic symptoms (DRESS), EN might be suspected, but early histopathology will show that there is no full-thickness necrosis leading to epidermal detachment and that the subepidermal separation occurs first followed by secondary necrosis of epidermal cells (18). In addition, atypical target lesions on the limbs and erosions of the lips may raise the suspicion of EN, although features such as facial oedema and erythema with inflammatory infiltration of the skin point to DRESS. Therefore, it is important to monitor specific laboratory values relevant for a diagnosis of DRESS, e.g. eosinophilia, liver enzymes, kidney parameters, etc. Liver involvement, indicated by at least a 2-fold increase in transaminases, on 2 different days may occur when eosinophilia has already turned to normal values. When the skin eruption heals, widespread post-inflammatory desquamation is frequently observed and sometimes mistaken for skin detachment in EN (18, 31).

Other differential diagnoses vary with the clinical pattern and during the course of the reaction. In the early stage of the disease, maculo-papular, multiforme- or target-like drug eruptions, which can also present with oral lesions and conjunctivitis, must be considered, especially in elderly patients (**Table II**) (32). Varicella and other viral exanthems are important differential diagnoses when the first signs and symptoms occur in children (10, 18, 33).

Table II. Differential diagnoses of epidermal necrolysis (EN) (10)

Most likely
- Limited EN (SJS)
  - Erythema multiforme majus
  - Varicella
- Widespread EN (SJS/TEN overlap and TEN)
  - Acute generalized exanthematous pustulosis
  - Generalized bullous fixed drug eruption
  - Drug reaction with eosinophilia and systemic symptoms

Consider
- Paraneoplastic pemphigus
- Linear IgA bullous dermatosis
- Pressure blisters after coma
- Tension blisters due to oedema
- Phototoxic reaction
- Graft-versus-host disease
- Staphylococcal scalded skin syndrome
- Thermal burns
- Skin necrosis from disseminated intravascular coagulation or
- Chemical toxicity (e.g. colchicine intoxication, methotrexate overdose)

CLINICAL COURSE OF EPIDERMAL NECROLYSIS

EN typically begins with unspecific prodromal symptoms, such as sore throat, runny nose, cough, headache, fever, and malaise, preceding mucocutaneous lesions by 1–3 days. These symptoms are followed by the appearance of erythematous macules and atypical targets of the skin that may be confluent and on which blisters occur. Burning or stinging of the eyes, and pain when swallowing or urinating, develop progressively, heralding mucous membrane involvement. Most reactions start with non-specific symptoms, followed either by cutaneous or mucosal involvement, but some may begin directly with specific lesions of the skin and mucous membranes. The rapid progression of such symptoms, the addition of new signs, severe pain, and rapid decline in the patient's general state of health should prompt the suspicion of a severe disease (10, 33).

In the majority of EN-cases the eruption initially shows a symmetrical distribution on the face, the upper trunk, and the proximal parts of the limbs. The distal parts of the arms and legs are often spared, but the eruption may extend rapidly to the entire body within a few days or even within a couple of hours. The initial skin lesions are characterized by erythematous, dusky-red, irregularly-shaped, purpuric macules, which coalesce progressively (Fig. 1). Atypical target lesions with dark centres are often observed. Confluence of necrotic lesions leads to extensive erythema, and Nikolsky's sign (dislodgement of the epidermis by lateral pressure) is positive on erythematous areas. Flaccid blisters that burst easily are present at this stage, and the necrotic epidermis is easily detached at pressure points or by frictional trauma, revealing large areas of exposed, red, sometimes oozing dermis, whereas the epidermis may remain in other areas (10, 15, 18, 33).

In terms of severity, cases are classified according to the consensus definition (Table I) based on the total area in which the epidermis is detached or detachable (positive Nikolsky's sign). Correct evaluation of the extent of detachment is difficult, especially in areas with spotty lesions and small blisters. Therefore, it may be helpful to remember that the surface area that can be covered by one hand (the

patient's hand in children) represents approximately 1% of the patient's BSA (10, 15).

Mucous membrane involvement (in most cases on at least 2 sites) is observed in approximately 90% of patients (Fig. 2). It typically begins with erythema, followed by painful erosions of the oral, ocular, genital, nasal, anal and, sometimes, tracheal or bronchial mucosa. These symptoms usually lead to impaired alimentation, photophobia, conjunctivitis and painful urination. The oral cavity is almost invariably affected and reveals painful haemorrhagic erosions, often with greyish white pseudomembranes. The lips are covered with haemorrhagic crusts. Approximately 80% of patients have conjunctival lesions accompanied by pain, photophobia, lacrimation, redness and discharge. Severe forms may lead to epithelial defect and corneal ulceration, anterior uveitis, and purulent conjunctivitis and blepharitis. Synechiae often occur between eyelids and conjunctiva, and eyelashes may be shed. Genital erosions are frequent in men and women, but may be more easily overlooked in females, especially in young girls.

To detect such distinct features requires a thorough clinical examination of the patient's entire body, involving further specialists in the examination of eyes, deep throat and genital mucosa in women. Ophthalmological consultation, in particular, is an urgent requirement to prevent complications and long-lasting sequelae (10, 15, 18, 33).

AETIOLOGY AND MEDICATION RISK

Although more than 100 different drugs have been reported in the literature as inducers of EN, less than a dozen have been identified to carry a high risk, and these account for more than half of the cases occurring in Europe according to 2 multinational case-control studies (16, 34). These high-risk drugs are allopurinol, antibacterial sulphonamides, certain antiepileptic drugs, such as carbamazepine, lamotrigine, phenobarbital and phenytoin, non-steroidal anti-inflammatory drugs (NSAIDs) of the oxicam-type, and nevirapine. The risk appears to be confined to the first 8 weeks of treatment and most reported EN cases started after the first continuous use of the medication between 4 and 28 days (16, 34, 35, 36). For lamotrigine and the anti-HIV-drug nevirapine, it was thought that a slow titration of the dosage could prevent such severe adverse reactions, since slow dose escalation had been shown to decrease the rate of mild eruptions. However, there is no evidence for a decreasing risk of EN (37–39). Oxcarbazepine, a 10-keto derivative of carbamazepine, which was considered to have a far lower risk, seems to cross-react with carbamazepine, revealing a lower, but substantial, risk of causing EN. Allopurinol, an old drug used to treat hyperuricaemia and gout, is widely believed to be a very safe medication; however, it was identified as the major cause of EN in Europe and Israel more than a decade ago and remains as such to date (40, 41).

Often the entire group of NSAIDs is suspected to induce EN, but there is a huge difference in risk among the various groups: oxicam derivatives carry the highest risk, acetic acid derivatives (e.g. diclofenac) moderate risk, and propionic acid derivatives (e.g. ibuprofen) no increased risk (**Table III**) (34, 36).

Among anti-infective agents, a significant, but much lower, risk than for antibacterial sulphonamides has been shown for different groups of antibiotics, such as cephalosporins, quinolones, tetracyclines and aminopenicillins. For other medications, such as corticosteroids, proton pump inhibitors or tramadol, the calculated risk was

Table III. Drugs and recommendations in epidermal necrolysis (EN) (34)

A. Drugs with a high risk of inducing EN

Use of these drugs should be evaluated carefully and they should be suspected promptly.

- Allopurinol
- Carbamazepine
- Co-trimoxazole (and other anti-infective sulphonamides and sulfasalazine)
- Lamotrigine
- Nevirapine
- NSAIDs (oxicam type, e.g. meloxicam)
- Phenobarbital
- Phenytoin

An interval of 4–28 days between start of drug use and onset of adverse reaction is most suggestive of an association between the medication and SJS/TEN.

When patients are exposed to several medications with high expected benefits, the timing of administration is important to determine which one(s) must be stopped and if some may be continued or re-introduced.

The risks of various antibiotics to induce EN are within the same order of magnitude, but substantially lower than the risk of anti-infective sulphonamides.

B. Drugs with a moderate (significant but substantially lower) risk of EN

- Cephalosporins
- Macrolides
- Quinolones
- Tetracyclines
- NSAIDs (acetic acid type, e.g. diclofenac)

C. Drugs with no increased risk of EN

- Beta-blockers
- ACE inhibitors
- Calcium channel blockers
- Thiazide diuretics (with sulphonamide structure)
- Sulfonylurea anti-diabetics (with sulphonamide structure)
- Insulin
- NSAIDs (propionic acid type, e.g. ibuprofen)
- Valproic acid

NSAIDs: non-steroidal anti-inflammatory drugs; ACE inhibitors: angiotensin-converting-enzyme inhibitors.

strongly affected by confounding (16, 34). In comparison with the results of 2 case-control studies, recent analysis of systematically ascertained registry data on EN using ALDEN (algorithm for causality assessment in EN) (42) demonstrated that the proportion of validated cases that could be explained by medications with a significant (high and moderate) risk was stable (65–68%) over a period of more than 2 decades (16, 34, 42). ALDEN provides structured help for identifying the most likely culprit drug and is based on the following criteria: time latency between start of drug use and index-day (i.e. onset of the adverse reaction), drug present in the body before index-day (taking into account the drug's half-life as well as the patient's liver and kidney function), information on prechallenge/rechallenge and dechallenge (if available), type of drug/notoriety (based on drug lists that require a regular update) and alternative causes. Numerical score values lead to a causality assessment for each individual drug a patient has taken or was administered, ranging from "very unlikely", "unlikely", "possible", "probable" to "very probable (43). For approximately one-third of cases of EN, no patent drug cause could be identified by using 2 completely different epidemiological methods. Even if new drugs or combinations of old drugs are taken into account as triggers of EN, at least 25% of all cases remain without a plausible drug cause, whereas this proportion reaches 50% among children and adolescents with EN. In these cases other eliciting factors must be saught:

An important non-drug risk factor is infections within one month before reaction onset. Most often these infections are diagnosed by clinical means, but positive serology related to certain well-known infectious agents, such as Epstein-Barr virus, cytomegalovirus, adenovirus or *Mycoplasma pneumoniae*, are rare. In some cases a preceding infection cannot be distinguished from the prodromal symptoms of EN; in others the reaction occurs suddenly with no prior signs or symptoms and must be labelled as "idiopathic" (10, 24).

EN has also been reported in the context of bone marrow transplantation, some eruptions of which may be induced by medication use, others are rather a maximal variant of acute graft-versus-host disease (GVHD). However, clinical and histological findings in EN and extensive acute GVHD are often indistinguishable, but depending on reaction onset after transplantation and the presence of non-cutaneous symptoms of GVHD, this diagnosis seems to be more likely (44). Lupus erythematosus (systemic LE or subacute cutaneous LE) is associated with an increased risk of EN. Often drug causality is doubtful in such cases and keratinocyte necrosis and subsequent skin detachment may be an extreme phenotype of cutaneous LE that must be considered as a differential diagnosis of EN (45).

For drug analysis in epidemiological studies, as well as for causality assessment in an individual case of EN, the correct determination of the day of reaction onset (so-called index-day) is of major importance (10, 15, 33, 34). All medications taken within a month preceding the index-day should be listed with their first and last day of use. Furthermore, information on prior use is very important, since it is rather unlikely for a medication to be the cause of EN if it was taken and tolerated in the past. A drug inducing EN is typically taken as the first continuous use, most often for 1–4 weeks, but sometimes for up to 8 weeks, without prior exposure (34). Thus, the mechanism differs from the classical sensitization in allergic conditions (10, 15, 37, 38, 39).

Frequently, and especially when no obvious drug cause is identifiable, medications taken to treat the prodromal symptoms are suspected of having induced the reaction. This mainly concerns antipyretics, analgesics, and secretolytics, sometimes summarized as "cough and cold medicines." When looking more closely at the use of these medications, they have usually been taken and tolerated previously and/or were started after the onset of prodromal symptoms of EN ("protopathic bias"). Neither of these patterns is typical for drug exposure causing EN (15, 33, 46). In contrast, medications causing EN have not been used previously and their exposure represents the first continuous use that started 4 weeks to at least 4 days before reaction onset. Furthermore, these substances do not belong to the drug groups for which an increased risk was estimated in epidemiological studies (34, 35). Differentiation between infection and drugs as the triggering agent can be challenging in the case of antibiotics used to treat infections ("confounding by indication"), but it helps to consider the type of infection, since classic bacterial infections alone do not seem to have an increased risk of causing EN (33).

For GBFDE, there are numerous case reports in the literature providing information on possible drug triggers (47–50); however, no analyses have been conducted on large patient numbers. The range of triggers include antimicrobial sulphonamides (especially cotrimoxazole), analgesics (especially metamizole, but also paracetamol), and, less frequently, antibiotics, allopurinol, and antiepileptic drugs (especially carbamazepine) (47–50). The latency between the start of drug use and reaction onset ranges from a few hours to a few days. In contrast to EN, the triggering agent has often been used and tolerated in the past (18). Sensitization happens over time, meaning that a reaction consistent with a fixed drug eruption occurs rapidly upon renewed use of the drug. Thus, GBFDE is a classic allergic reaction that must be differentiated from EN.

RISK OF RECURRENCE

The risk of recurrence in EN appears to be rather low, as Kirsti Kauppinen had already observed in 1972 (5). In the multinational RegiSCAR study, few individual patients experienced a second event of EN after accidental exposure to the same drug that had induced the first event. The time latency between the start of drug use and reaction onset was very similar and not necessarily shorter, as reported repeatedly in the literature.

In contrast, fixed drug eruption, including GBFDE, has a high risk of recurrence, which may be explained by memory T cells remaining in the affected skin (51). In many cases there has been a previous, often less severe, event, but cases

with extensive skin detachment may also occur *de novo* and re-occur with the same amount of involvement (49).

EMM appears to be almost exclusively triggered by infections, especially *M. pneumoniae* in children and adolescents, and herpes simplex virus in adults. Recurrence has been observed, in up to 10% of cases, and in some patients even several times, before the reaction resolves (20). Interestingly, infection-induced EN cases do not seem to recur, and it may be assumed that the viral triggers change so rapidly that they are not recognized again as an antigen (52).

PATHOGENESIS AND GENETICS

A T-cell reaction comparable to GVHD is believed to be the pathogenetic mechanism in EN, since immunohistochemical investigations identified primarily $CD4^+$ cells in the dermis and $CD8^+$ cells in the epidermis (53, 54). In contrast to what was postulated in earlier years, these cytotoxic T cells are usually specifically directed against the native form of the drug rather than against reactive metabolites (55). The acute necrosis of keratinocytes in EN is attributed to an extensive process of apoptosis (54, 56). Cytotoxic T-cells are able to initiate apoptosis, enhanced by the release of perforin and cytokines, such as TNF-α or granzyme B (57, 58). It is also assumed that proteins such as Fas antigen (CD 95) and the P55 TNF-α receptor enhance apoptosis in keratinocytes (59). However, it was demonstrated that Fas and Fas ligand are not the most important cytokines in the acute phase of EN, but rather the cationic protein granulysin (60). It showed the strongest cytotoxicity in the blister fluids of patients with EN compared with other blistering diseases, with its concentration correlating with the severity of the clinical reaction (60). Therefore, it was concluded that granulysin is a severity marker in EN and provides a target for possible immunomodulating treatments. It has also been shown that IL-15 is associated with the severity of the reaction as well as the risk of mortality (61).

It has been known for many years that there is a genetic predisposition to develop EN. As early as 1987, different human leukocyte antigen (HLA) loci were found for TEN associated with sulphonamides or with oxicam-NSAIDs (62). Almost 20 years later, a strong association between HLA-B*1502 and carbamazepine was observed in patients with EN who were of Han Chinese descent (63). This association could not be detected in European patients, where HLA-B*5701 was identified to confer genetic susceptibility to carbamazepine-induced SJS/TEN (64). Interestingly, HLA-B*5701 had previously been demonstrated to be associated with abacavir hypersensitivity, which is characterized by fever, rash and constitutional, gastrointestinal, and/or pulmonary symptoms different from SJS/TEN and DRESS (52). A second strong association with HLA-B*5801 was observed in Han Chinese patients with allopurinol-induced disease, not only for EN, but also for DRESS (65). For this allele an association of 55% was found in allopurinol-induced EN cases of European descent (66). Clearly, genetic predisposition is not the only important factor for developing a certain type of severe cutaneous adverse reaction due to a specific drug, but also the patient's ethnicity, as was shown for patients of southeast Asian, European and African descent (52).

To date, there have been no systematic investigations into the genetic pattern of infection-induced EN cases. However, some reports on specific HLA alleles in cases thought to be triggered by antipyretics and secretolytics appear to be ultimately associated with infection-induced reactions (46). Although a large genome-wide association study in European patients with EN demonstrated that the relevant alleles/genetic variants are all located in the HLA locus on chromosome 6, the variability in the European population appears to be too large to deploy a medication-specific predictive test to prevent EN (67). In contrast, this has been successfully demonstrated in Southeast Asian subjects, at least in the case of carbamazepine, for which the predictive test has led to a marked reduction in carbamazepine-induced EN cases (68).

Although no systematic investigations into the pathogenesis of GBFDE have yet been undertaken, there are analyses on the T-cell population in fixed drug eruption. T cells play an important role here, since they remain in the affected areas of skin as "memory cells", which explains why a reaction re-occurs at the same site. The term "fixed drug eruption" takes this fact into account, although the reaction may expand if it recurs (51). Furthermore, several cytokines, such as FAS/FAS-L, perforin and granzyme B, are equally expressed in GBFDE and EN, whereas the concentration of granulysin is much lower in GBFDE compared with EN (27).

THERAPY

Taking a detailed and thorough medication history is crucial. Assuming a medication rather than an infection triggered the reaction, the most likely culprit drug should be identified and discontinued. Thus, it is essential to know the time latency between the start of drug use and onset of the reaction, as well as the drugs that have a high-to-moderate risk of the type of reaction in question. It may be helpful to create a timeline diagram, into which the chronological sequence of clinical symptoms is entered on the x-axis and the medications taken or applied are entered on the y-axis (**Fig. 5**). Based on the diagram and the information on duration of use (start and end of use), it is possible to narrow down or even identify the inducing agent. It then becomes obvious that not all drugs, some of which may be vital for life, need to be withdrawn. Medications that were administered to treat prodromal symptoms and that are often suspected as the cause of EN, can also be excluded as triggers. If an infection is thought to have induced the reaction, patients should receive adequate antibiotic or antimicrobial treatment; reluctance to provide medication

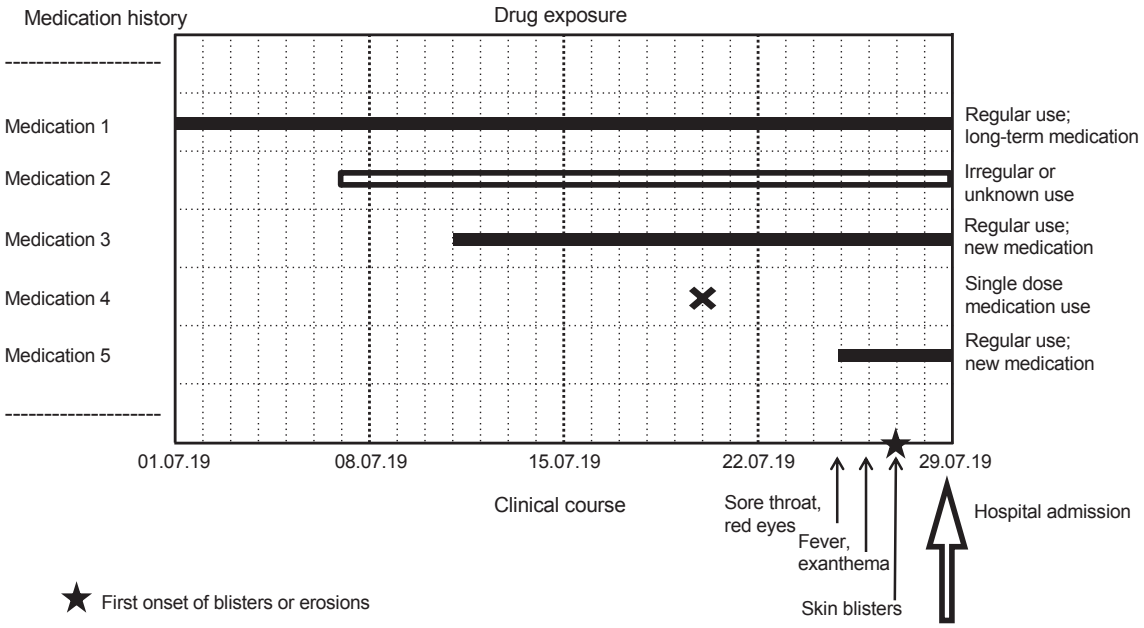

Fig. 5. Timeline diagram with chronologic sequence of clinical symptoms (x-axis) and medication use (y-axis).

in a medical condition frequently caused by drugs may be detrimental (15, 33). The following supportive care and topical treatment is recommended:

In order to assess a patient's prognosis and to decide on the appropriate therapeutic options, the SCORTEN (severity-of-illness score for EN) has been developed (69). Seven independent, but equally significant, factors are used for the calculation of score points: (*i*) age (≥40 years), (*ii*) heart rate (≥120/min), (*iii*) malignancy, (*iv*) percentage of detachment relative to BSA on day 1 (≥10 %), (*v*) serum urea (>10 mmol/l), (*vi*) serum bicarbonate (<20 mmol/l), and (*vii*) serum glucose (>14 mmol/l) (69). The positive score points are added and the higher the value, the higher is the risk of death and the lower the chance of survival (69–71) (**Table IV**).

Only patients with limited skin involvement, and a SCORTEN value of 0 or 1, and a disease that is not rapidly progressing can be treated in non-specialized wards. Depending on the local or national facilities, patients who do not need intensive care may remain in dermatology units or hospitals (in many European countries), others should be transferred to intensive care facilities or burn units (72, 73). Supportive care is still the cornerstone of treatment and includes maintaining haemodynamic equilibrium and preventing life-threatening complications. Due to significant fluid loss in patients with large amount of skin detachment, hypovolaemia and electrolyte imbalance must be adjusted on a daily basis. Infusion volumes are usually lower than for burns of a similar extent of skin detachment (approximately 1/3–/4 of the infusion volume in burns) because interstitial oedema is absent. In order to select the correct amount of fluid replacement, correct estimation of the denuded BSA is important (74). Peripheral venous lines should be used, if possible, since the sites of insertion of central lines are far more prone to infection. Increasing the environmental temperature to 25–30°C is important to compensate for loss of thermoregulation in patients with extensive skin detachment (72). Air-fluidized beds may help to increase the patient's comfort. To reduce the risk of infection, aseptic and careful handling is required. Skin, blood, and urine specimens should be cultured for bacteria and fungi at frequent intervals. Prophylactic use of antibiotics should be avoided, and instead patients with EN should receive antibiotics when an infection is suspected based on clinical features and laboratory results. Prophylactic anticoagulation is needed and early nutritional support should be provided through nasogastric tubes in order to promote healing and decrease the risk of bacterial translocation from the gastrointestinal tract (10, 18, 72, 73). For adequate enteral nutrition, intensive care guidelines (e.g. ESPEN guidelines) should be followed (75).

Table IV. SCORTEN (69) (severity-of-illness score for epidermal necrolysis) to assess a patient's prognosis

Factor	Score	Weight/score value
Age, years	≥40	1
Malignancy	Yes	1
Body surface area detached (day 1), %	≥10	1
Tachycardia, /min	≥120	1
Serum urea, mmol/l	≥10	1
Serum glucose, mmol/l	≥14	1
Serum bicarbonate, mmol/l	<20	1
Possible score		0–7

Topical treatment plays a special role in bullous reactions. Antiseptic solutions or gels, as well as non-medicated and non-adhesive gauze dressings are used. There is no standard policy concerning the use of antiseptics and wound dressings, which remains a matter of experience in each centre. Careful handling and skilful wound care, performed by experiences nurses, in addition to adequate pain management, are essential (10, 72, 73).

Some experts recommend leaving the blister roof in place as a natural cover to protect the dermis, while others recommend complete removal of detached skin and the consecutive use of biosynthetic dressings in order to protect against infection. Although this remains a topic of debate, it was recently suggested that aggressive debridement is neither necessary in superficial burns nor in EN, because superficial necrosis is not an obstacle to re-epithelialization and might even accelerate the proliferation of stem cells due to inflammatory cytokines (76).

In the case of erosive mucous membrane involvement, local antiseptic treatment is recommended and the appropriate medical specialist should be consulted. In terms of eye involvement, an experienced ophthalmologist should examine the patient immediately after admission. Preservative-free emollients, antibiotic or antiseptic eye drops, often alternating with anti-inflammatory (e.g. corticosteroid) eye drops are recommen-

ded every 2 h in the acute phase. In case of early synechiae, mechanical disruption is indicated and graft of cryopreserved amniotic membrane has been proposed to decrease the rate of severe ocular sequelae. In any case, severe ocular involvement requires daily consultation with an ophthalmologist (73, 77).

Disinfectant mouthwash can be used for treatment of oral erosions, whereas erosions of the lips should be treated with bland ointment, e.g. dexpanthenol. Genital erosions in male and female patients may lead to adhesions or strictures. To avoid such complications wet dressings or a sitz bath are helpful. If deeper vaginal involvement is suspected in young girls, a gynaecological examination should also be performed, since early adhesions must be carefully disrupted. To avoid these, dilators covered with ointment can be applied (78).

Since GBFDE is considered to be a self-limiting disease that ceases to progress shortly after discontinuation of the triggering drug, supportive care alone is adequate. However, complications requiring intensive care can occur, especially in older patients and patients with extensive skin detachment. Topical treatment is the same as in EN. Since the mucous membranes are most often unaffected, interdisciplinary consultations are not mandatory, but can be helpful in some cases (24, 48).

Immunomodulating treatment. Because of the immunological mechanisms with involvement of cytotoxic T-cells and release of cytokines, several immunosuppressive and anti-inflammatory treatments have been tried to halt the progression of the disease. Data on therapeutic approaches largely derive from uncontrolled case series and case reports. Due to the rarity of SJS/TEN and the resulting low patient numbers, as well as the unexpected onset and rapid progression of the reaction, it remains a huge challenge to conduct a controlled randomized study on treatment efficacy. Therefore, existing data on treatment of EN must be evaluated with care:

- Glucocorticosteroids are the most frequently used immunomodulating treatment in patients with EN (18), but their use is controversial, since they may increase the risk of infection and septicaemia and delay wound healing (79). However, a recently published meta-analysis on the treatment of EN that investigated publications in the period 1990–2012 demonstrated that the administration of systemic glucocorticosteroids conferred a survival benefit compared with supportive care alone (odds ratio (OR) 0.54; 95% confidence interval (95% CI) 0.29–1.01) (80). A number of smaller case series on the administration of glucocorticosteroid pulse therapy with methylprednisolone or dexamethasone (100 mg/day for 3 days) demonstrated a benefit when comparing the expected number of deaths by SCORTEN with the actually observed death rate (81, 82). A case series of 5 patients reported on the positive effect of methylprednisolone pulse therapy (500 mg/day for 3 days) in massive eye involvement on the development of ocular sequelae; this effect could not be confirmed in larger observational studies (82, 83). Thus, individual case reports and small case series should be viewed with caution. Nevertheless, if administered short-term at a medium dose (50–250 mg) for only a few days, glucocorticosteroids are a treatment option with a positive effect on swollen and painful mucous membranes, but little impact on the progression of skin detachment (80, 84).
- Intravenous immunoglobulins (IVIG) have been suggested as therapy option based on the assumption that Fas-induced keratinocyte apoptosis is blocked by antibodies present in human IVIG (85). Their use remains a subject of controversy, given that some reports described a positive effect (85, 86), whereas others were unable to show any benefit (80, 84, 87, 88). However, a number of methodological weaknesses and problems were found in the studies showing a positive effect for IVIG (89). Furthermore, the effect of IVIG dose is often the focus of the discussion. In studies that showed a disadvantage for IVIG, the dose was mostly ≤2 g/kg BW, whereas it was at least 2.8 g/kg BW in positive studies (88). Nevertheless, using SCORTEN for comparison, a more recent retrospective study of 64 patients revealed that the administration of IVIG did not have a positive effect on survival, not even at a higher dose (90). Two extensive meta-analyses also found no survival benefit for patients with EN who received treatment with IVIG compared with supportive therapy (80, 91).
- Cyclosporine A has strong immunomodulating capacity and thus has been used in the treatment of EN. Its mechanism may, on the one hand, be activation of T-helper cells and cytokines, and, on the other hand, inhibition of CD8+ cytotoxic mechanisms followed by an anti-apoptotic effect of several cytokines. The first larger retrospective case series, in which 11 patients were treated with 2×3 mg/kg BW/day, was published as early as 2000 (92). The progression in skin detachment stopped and wound healing was faster in the patient group receiving cyclosporine A compared with the control group, which received cyclophosphamide and glucocorticosteroids (92). In the following years, individual case reports and case series were published, all showing a survival benefit in patients treated with cyclosporine A compared with SCORTEN values and/or other systemic therapies (93–95). A recent larger study was conducted in Madrid and used 3 different approaches to assess the effect of cyclosporine A. Again, re-epithelialization began earlier than in the comparison group (IVIG, glucocorticosteroids, supportive care only), and the observed mortality was lower than expected by application of SCORTEN, whereas in the comparison group more patients than estimated died (96). Children and adolescents were not included in many of these studies, but cyclosporine A has been used successfully in children with EN in smaller case series (97). The 2 meta-analyses mentioned above concluded that cyclosporine A is a very promising treatment, because first, re-epithelialization begins earlier and, second the observed mortality is lower than expected (80, 91). The recommended dose is 3–5 mg/kg BW/day for a total of 10 days, but adjustment of the dose may be needed in patients with impaired renal function (98). Therefore, it is necessary to monitor creatinine levels during treatment. Close surveillance of creatinine levels is advisable in the case of higher doses and renal insufficiency, but not necessarily mandatory in other cases. Strict contraindications to short-term treatment with cyclosporine A at the suggested doses are rare, but there are only a few reports on the treatment of elderly patients (>70 years) with EN (98).
- TNF-α inhibitors have also been tried for treatment of EN, since elevated TNF-α levels were found in blister fluids, serum, and skin samples of patients with EN, and the level correlated with the severity of the reaction (99, 100). Therefore, the use of TNF-α inhibitors appeared as a potential treatment approach in EN. In 1998, a randomized double-blind placebo-controlled treatment study using thalidomide in patients with EN was terminated early, because significantly more patients in the thalidomide group died than in the placebo group (100). Paradoxical high levels of TNF-α were detected in the serum of patients in the treatment arm of the study. However, later studies used other TNF-α inhibitors, e.g. infliximab and etanercept, for the treatment of EN, but only scant reports of treatment success have been published (101, 102). In a randomized treatment study that was published recently a lower mortality in patients with EN treated with etanercept compared with the achieved SCORTEN values was observed. Wound healing started earlier and the inhibitor reduced the levels of

TNF-α and granulysin in serum and blister fluids compared with the glucocorticosteroid-treated control group (103). The prospective randomized study design can be regarded positively, since treatment studies of that kind are lacking in the area of severe skin reactions. However, most results are not significant and this study also had a number of methodological problems. The delayed re-epithelialization in the control group could be due to the prolonged use of corticosteroids.

- *Other immunomodulatory treatment options*. Other therapies have been used to treat EN, but the reliability of the findings is very low due to the small number of patients treated. In some cases, these options are no longer, or only rarely, used, as in the case of cyclophosphamide (80). Other treatments, such as plasmapheresis, which is based on the removal of cytokines involved in apoptosis, are still used, although they were not able to demonstrate verifiable positive results (104, 105).

To date, there are no data from clinical trials on the benefit of systemic immunomodulating therapy in the treatment of GBFDE. Systemic glucocorticosteroids are also used in some patients, but it appears that their short-term use does no harm and does not result in faster healing (18, 49).

COMPLICATIONS AND SEQUELAE

During the acute stage of the disease, EN may be accompanied by hepatitis, tubular nephritis, or tracheobronchial mucosal involvement, which usually resolve rather quickly (10, 73). The most common complications include nosocomial infection and septicaemia, frequently caused by central venous catheters. Therefore, peripheral catheters should be preferred wherever possible and specific hygiene measures are advised, e.g. reverse isolation, etc. (72, 73).

The majority of EN survivors experience long-term sequelae of varying severity, affecting primarily the skin and mucous membranes (106, 107). Whereas skin lesions generally heal without scarring, hyper- and hypo-pigmentation of the skin as a result of the inflammatory reaction often persist for months to years. Reversible loss of hair and nails, as well as nail growth disorders are frequently observed. Mucosal adhesions that may cause strictures in, for example, the urethra or oesophagus, represent a greater problem. By far the most hazardous and, for the patient, most dramatic, sequelae affect the eyes by symblepharon formation with entropium and trichiasis, which can even cause blindness (10, 15, 77, 106, 107).

Many patients still experience somatic as well as psychological sequelae years after their reaction. These sequelae may range from symptoms of post-traumatic stress, sleep disorders, and nightmares, to fear of using any medications. A large survey, performed 5 years after EN, revealed that many patients and their relatives are inadequately informed about their reaction, its sequelae, and how to deal with these in the long term (107).

ALLERGY WORK-UP

EN is not an allergic reaction in the strict sense, since there is no classic sensitization as in other delayed hypersensitivity reactions. In the latter, initial use of the substance is well tolerated, with a reaction developing only upon renewed exposure. EN differs in that it typically occurs during the first course of treatment with a drug (34).

GBFDE, on the other hand, is a true allergic reaction, since previous exposure to the triggering drug has usually occurred, and repeated use often causes localized fixed drug eruptions. While renewed administration of a triggering drug in patients with GBFDE can be expected to cause a rapid onset, and possibly even more extensive, repeated reaction, EN was rarely observed following similar re-exposure (5).

Skin tests, such as the patch test, are generally safe, but most often are not helpful for confirming the suspected trigger in EN. The success of testing depends, to a great extent, on the type of reaction and the T-cell populations involved, as well as on the drug to be tested. In a study performed a few years ago in France, for example, the triggering agent was confirmed by patch testing in less than 25% of patients with EN (108). One should also bear in mind that allopurinol, a very common trigger of EN, is not suitable for skin testing due to the lack of lipophilicity and skin penetration (108, 109).

In vitro tests were the most suitable instrument to identify the inducing agent in bullous drug reactions; however, their use is yet not part of routine diagnostics and remains rather experimental. This may, in part, be due to the fact that the specificity of the various tests, e.g. the lymphocyte proliferation test, the lymphocyte stimulation test, and cytokine assays, is high, while their sensitivity is much lower (109).

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GENODERMATOSES

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REVIEW ARTICLE

An Early Description of a "Human Mosaic" Involving the Skin: A Story from 1945

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In 1945, the *Journal of Heredity* published an impressive article entitled "A human mosaic: bilaterally asymmetrical noevus pigmentosus pilosus et molluscіformis unilateralis." The author was M. Zlotnikoff, a Russian physician working in Ivanovo, a city located approximately 250 km northeast of Moscow. Zlotnikoff described a 24-year-old woman with a congenital linear epidermal naevus in a systematized and strictly unilateral arrangement. For the first time, the author explained this disorder as a mosaic resulting from a somatic mutation that occurred at an early stage of embryonic development. However, because this article was published immediately after the war, it fell into oblivion, despite the fact that it was of utmost importance in clinical dermatology. Zlotnikoff's work is all the more remarkable as the author had never heard of the lines of Blaschko.

Key words: epidermal naevus; unilateral involvement; mosaicism; postzygotic mutation; lines of Blaschko.

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SIGNIFICANCE

In 1945, M. Zlotnikoff from Ivanovo, former Soviet Union, documented a unilateral systematized epidermal naevus in an adult woman. Without knowing about the lines of Blaschko, Zlotnikoff precisely described a Blaschko-linear cutaneous pattern. He explained this epidermal naevus as a biological mosaic resulting from an early postzygotic new mutation. However, because Zlotnikoff's manuscript was published immediately after the war, it remained unnoticed. During the second half of the past century, Blaschko's lines were "rediscovered" in dermatology. Today, it should be known that Zlotnikoff was an important forerunner in research on mosaicism and Blaschko's lines in human skin.

In 1945, an impressive article by a Russian author appeared in an American journal, the *Journal of Heredity* (1). It was presumably during the early 1940s that Zlotnikoff had submitted his manuscript to the journal. At the beginning of the report, an Editor's note states: "*This remarkable contribution came to hand some months before the war virtually suspended communications with the Soviet Union. Some suggestions for modifying the discussion of possible causes of the mosaic were addressed to the author and the manuscript was 'put on ice' to await his reply. In press of other matters it remained there much longer than originally intended. It may still be many months before we will hear from the author, so we are proceeding with the publication of the article essentially as submitted.*" Apparently, the Editor never heard from the author again.

The text of the Russian physician begins with the words: "The author has not been able to find a case of mosaic mutation in man in the available literature and therefore, he considers the present case to be worthy of publication. A careful study of the genealogy of this case showed that we may possibly deal with a case of a newly formed mosaic mutation."

ZLOTNIKOFF'S CASE REPORT

A 24-year-old woman, employed as an assistant veterinarian, presented to the Surgical Department of the 1st Medical Institute with a request to have "a pigmented patch" on the left side of her face and neck removed by surgery. Physical examination revealed several somewhat elevated patches forming a linear pattern beginning on the forehead and running to the cheek and neck. Moreover, the left side of her trunk showed similar lesions "going exactly down the midline of the body, from the forehead to the groin" (**Figs 1 and 2**). Her entire left leg was "of a dark brown colour as if it were covered by a stocking." Her scalp was bald on the left side (**Fig. 3**), and there was a difference in hair colour, "scarce light red on the right and abundant chestnut on the left." Fig. 3, however, clearly shows that the remaining scalp hair on the left side was partly depigmented. There was heterochromia iridum, grey on the left and dark brown on the right side. Tendon reflexes were of higher degree on the left side. X-rays did not reveal any pathological features.

At 158 cm the patient was below average height. She had been born as the seventh child in well-to-do peasant family. The skin lesions were present at birth. She began to speak rather late. When she was 2 or 3 years old, her parents noticed that her scalp hair was not uniform, being lighter and poorer on the left side.

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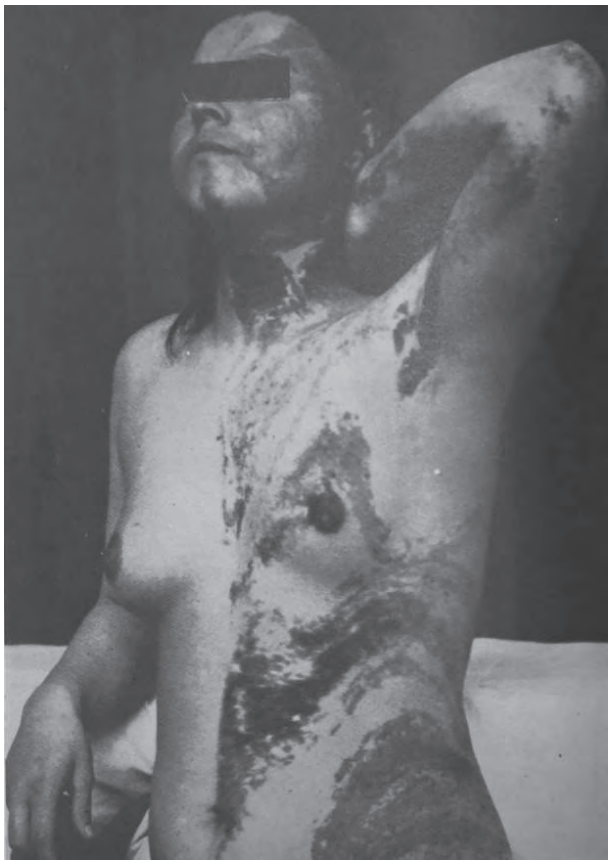

Fig. 1. A 24-year-old woman with systematized linear epidermal naevus, described by Zlotnikoff as "naevus pigmentosus pilosus et mollusciformis unilateralis", with a sharp separation down the midline (1). (Reproduced with permission from the American Genetic Association, USA, and Oxford University Press, UK).

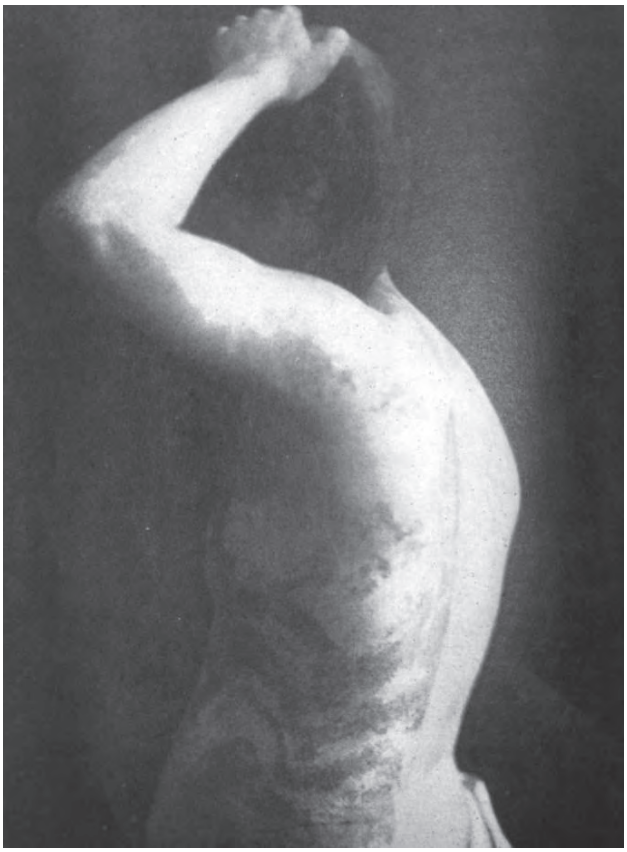

Fig. 2. The systematized linear lesions on the patient's back, likewise show an exact midline separation. (Reproduced with permission from the American Genetic Association, USA, and Oxford University Press, UK).

At 6 years of age, a large bald patch was strictly limited to the left side. Subsequently this hairless area extended to the neck and the frontal hairline.

The right half of her body was entirely normal. "The skin on the left side is partly of intense dark brown colour, partly crimson, and partly 'café-au-lait'."

On this side, "the pigmented regions form stripes like military 'shoulder-knots' from the shoulder to the spine." Ipsilaterally, "the upper abdomen is covered with a granular swelling of soft consistency, dark-red colour, slightly elevated in palpation. The colouration of the skin in this region reminds one of an oil-painting, where the paint has been applied in heavy 'dabs'. These have a semi-circular appearance, the rounded part directed upwards". There was no hair in the left axilla, whereas the right axilla showed abundant hair.

She reported that, since early childhood, whenever she made the slightest exertion, the left side of her body showed pronounced perspiration. The sweat stains were of dark-brown colour, being difficult to remove from the linen. On the right side sweating was normal.

As a child she was sometimes mocked as a "devil". During adolescence, the patient became morose and sullen and preferred solitude: "less mockery, less tears".

ZLOTNIKOFF'S EXPLANATION OF THE CONDITION

"If we assume that at the stage of two blastomeres a somatic mutation had taken place, i.e., one of these blastomeres underwent some mutation, then the development of these blastomeres would proceed in accordance with this mutation, i.e., the difference between the 'normal' and the mutated blastomere would exist in all stages of development of the organism. If we assume that in our case one of the blastomeres (at the two-blastomere stage) namely, the left underwent a mutation then we can easily understand from what has already been said that the left side of the organism would reflect all the features resulting from the mutation, that took place at the stage of the blastomeres, and the pathology of the organism would be strictly asymmetrical…. Assuming that this explanation is the most probable one, we are inclined to apply it in the present case, as it is impossible to give any other explanation to this one-sided asymmetry of mosaic mutation in our patient,…"

ZLOTNIKOFF'S FINISHING NOTES

At the end, the author makes the following touching remark: "The patient considers herself if not a blasto-

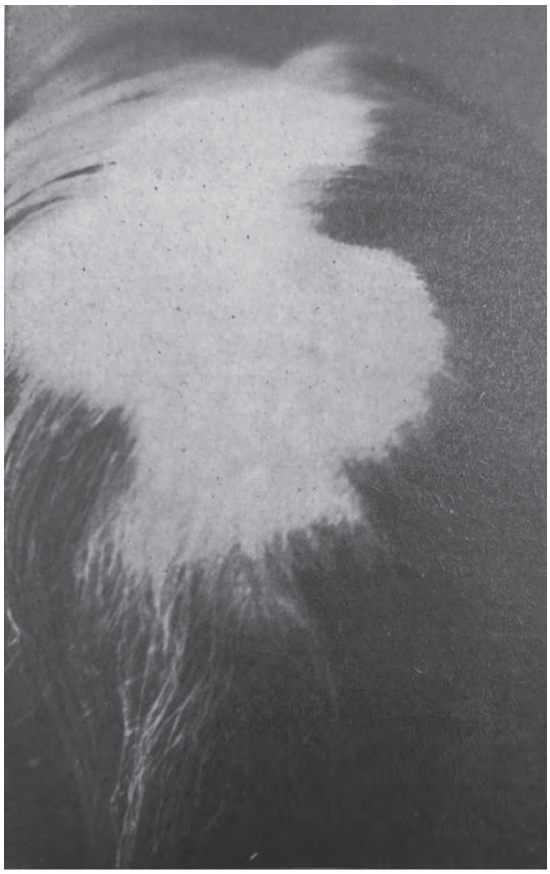

Fig. 3. Hair is lacking to the left of the midline, though part of the left side of the skull is unaffected. There is also a bald area above and back of the ear. (Reproduced with permission from the American Genetic Association, USA, and Oxford University Press, UK).

matous variation then a new species obtained as a result of a somatic mutation at the stage of two blastomeres."

In a last paragraph, Zlotnikoff mentions that "the patient was demonstrated at the Genetic Conference at the Institute of Medical Biology (director prof. Levit) in 1931 in Moscow."

HOW CAN WE CATEGORIZE THE EPIDERMAL NAEVUS IN THIS PATIENT?

The diagnosis is rather difficult. A sebaceous naevus is unlikely because of the presence of pronounced hyperhidrosis/chromhidrosis. Moreover, the bald area of the scalp is not suggestive of Schimmelpenning syndrome (2). Admittedly, lesional hyperhidrosis is a feature of phacomatosis pigmentokeratotica (PPK) that can today be taken as a particular variant of Schimmelpenning syndrome (3), but in the present historical case there was no papular naevus spilus that is known to be associated with hyperhidrosis (4). In oculoectodermal syndrome, an epidermal naevus can be associated with bald areas of the scalp and depigmented hair (5), but other features of Zlotnikoff's patient are not compatible with this diagnosis. In the present author's view, the phenotype described can best be categorized, at present, as an unclassifiable type of systematized epidermal naevus. However, this by no means interferes with the innovative significance of Zlotnikoff's report.

COMPARISON OF ZLOTNIKOFF'S EXPLANATION OF THE CASE WITH PRESENT KNOWLEDGE

At that time Zlotnikoff could not know that all human mosaics represent a mixture of normal and mutant cells (6, 7). The involved left half of his patient contained normal cells within the segmental areas of uninvolved skin as well as within the systematized epidermal nevus. Therefore, the assumption of a mutational event at the two-cell stage of embryonic development is too simplistic and cannot be upheld. In fact, segmental mosaicism tends to develop before the embedding of the fertilized egg into the uterine mucous surface, i.e., during the first week after fertilization (8). Hence, it is elusive to designate a "left" blastomere, as proposed by Zlotnikoff. Such minor historical imperfection, however, does not alter the remarkable fact that the author was on the right track in presenting a genetic theory to explain congenital linear skin lesions as a mosaic phenomenon.

SIGNIFICANCE OF ZLOTNIKOFF'S WORK

When submitting his manuscript, Zlotnikoff did not know about the ground-breaking publications of Alfred Blaschko on his "naevus lines" (9, 10). The intuition of the author from Ivanovo is even more stupendous when we read an additional note on his paper that appeared in the same issue of the *Journal of Heredity* (11). In this comment, the geneticist Bentley Glass from Baltimore, MD, USA, still expounded the then fashionable, but incorrect, theory of dermatomes: "A third interesting feature of the present case is the pattern of the markings, a pattern which strikingly suggests the dermatomes of the neurologists, especially those worked out of Head (12) on the basis of herpetic eruptions."

POLITICAL IMPLICATIONS OF ZLOTNIKOFF'S ARTICLE

In 1937, Stalin had announced, in a well-known speech, that those who still adhered to Mendelian genetics and the chromosome theory of heredity should be considered to be Trotskyist and revisionist enemies of the people. Thus, Stalin supported the charlatan Trofim Lysenko, who wanted to replace the "bourgeois" genetics of "Mendelism-Weismannism-Morganism" by his own absurd doctrine of inheritance of acquired characters (13). As a consequence, many geneticists lost their positions or were even executed. As a prominent example, the renowned Russian plant geneticist Nikolai I. Vavilov was sentenced to death in 1941 because he refused to renounce Mendelian genetics

and the genes as major factors of heredity. In 1943, Vavilov died of starvation in Saratov prison. To date, nothing is known about Zlotnikoff's fate, but we know that, in 1948, Lysenko managed to entirely eradicate scientific genetics in the Soviet Union: "Hail to the progressive Michurinian science! Glory to the great Stalin, the leader of the people and coryphaeus of progressive science!" (14). Hence, the question arises whether Zlotnikoff was aware of the fact that submitting his manuscript to an American journal was a very dangerous step.

CONCLUSION

As far as we know, M. Zlotnikoff was the first to explain the linear arrangement of a congenital human skin disorder by the concept of mosaicism, reflecting the action of a postzygotic mutation that occurred at an early developmental stage. This highly original idea was astounding, because the author had never heard of Alfred Blaschko's "naevus lines" (10). In 1976, Blaschko's work was "rediscovered" simultaneously and independently in Canada (15) and Germany (16). Robert Jackson from London, Ontario, Canada, discussed mosaicism as a possible mechanism, but concluded that "the embryological explanation on Blaschko's lines is not at all clear…I have been unable even to make a guess at what stage of development the changes occur which could provide a mechanism by which the localization of Blaschko's lines is determined. It would be helpful to tie in Blaschko's lines with some other dateable embryological event…" (15). Concurrently, such dateable event was proposed at a meeting in Heidelberg, Germany, in the form of X-inactivation (16, 17). This mechanism is known to occur at approximately day 5 after fertilization, prior to implantation of the blastocyst (18). By 1970, however, Widukind Lenz had already proposed to explain, without mentioning Blaschko's lines, the streaky pattern of incontinentia pigmenti by lyonization, and systematized epidermal naevi by early somatic mutations (18). Today, we can add M. Zlotnikoff's name to the group of authors who developed a genetic concept of how to explain Blaschko's lines. This early description of a human mosaic is all the more admirable because the author from Ivanovo did not know about Blaschko's work.

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REVIEW ARTICLE

Spectrum of Genetic Autoinflammatory Diseases Presenting with Cutaneous Symptoms

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Autoinflammatory diseases comprise a group of chronic disabling entities characterized by inflammation without the presence of infectious agents, auto-antibodies or antigen-specific T-cells. Many autoinflammatory diseases are caused by monogenic defects, which lead to disturbed immune signalling with release of proinflammatory mediators. In addition to interleukin-1β and interleukin-18, interferons play a key role in the pathophysiology of these disorders. Patients with autoinflammatory diseases show a broad variety of clinical symptoms, including skin involvement. Wheals, pustules and ulcerative lesions are the most common cutaneous findings observed. Knowledge of the clinical presentation of autoinflammatory diseases is crucial for establishing the diagnosis and guiding appropriate treatment. This review focuses on the dermatological findings in selected autoinflammatory disorders based on their distinct pathomechanisms.

Key words: autoinflammatory; genetics; interferon; interleukin-1.

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SIGNIFICANCE

Autoinflammatory diseases are rare disabling disorders characterized by excessive inflammation of the skin and inner organs. Many autoinflammatory diseases are caused by genetic defects, which subsequently result in disturbed immune signalling. In the skin, wheals, pustules and ulcerative lesions dominate. As autoinflammatory diseases are associated with a high burden and limited awareness, knowledge of their clinical presentation is crucial for establishing the diagnosis and guiding appropriate treatment.

Autoinflammatory diseases are a group of chronic disabling entities characterized by self-directed inflammation, which is mediated via disturbances in innate immune signalling pathways. The term "autoinflammatory" was established in the late 1990s to classify systemic diseases that lack high-titre autoantibodies and autoreactive T cells as known from autoimmune diseases (1). Within the last 2 decades, the spectrum of autoinflammatory diseases has grown rapidly. In addition to rare monogenic entities, it comprises a variety of multifactorial diseases with variable onset. Even for common disorders, such as gout, cardiovascular, metabolic and neurodegenerative diseases, autoinflammatory disease mechanisms have been claimed (2–5). Furthermore, the coexistence of both autoinflammatory and autoimmune features in several inflammatory disorders demonstrates the close link between the innate and adaptive immune signalling cascades (6).

As a joint disease pathomechanism, excessive cytokine secretion from innate immune cells (e.g. macrophages, monocytes) drives the inflammation in various organs. In particular, the accumulation of interleukin (IL)-1-associated cytokines, including IL-1β and IL-18, plays a crucial role in many diseases. In addition, increased amounts of interferons (IFN) have been recognized as the main inflammatory mediators in other conditions (7).

The clinical presentation of autoinflammatory diseases comprises recurrent fever attacks, musculoskeletal, gastrointestinal and neurological involvement. Also, the skin is affected in many of these disorders. Typical symptoms include urticarial, pustular and ulcerative lesions. This review focuses on the dermatological findings in selected autoinflammatory disorders based on their distinct pathomechanisms.

INTERLEUKIN-1 AND INTERLEUKIN-1-RELATED DISORDERS

In 1984, the nucleotide sequence of IL-1 was identified, and decades of research revealed its importance as a central mediator of innate immunity and inflammation (8). The human IL-1 family consists of a total of 11 members with distinct biological functions (9). Among them, the proinflammatory cytokine IL-1 is the best-characterized member, composed of 2 individual forms, IL-1α and IL-

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1β. IL-1β is the predominant circulating isoform of IL-1 and initiates a cascade of activities in almost every tissue during host defence against pathogens and injuries. IL-1α and IL-1β exert their action through binding to a single ubiquitously expressed membrane-spanning receptor, known as IL-1 receptor type 1 (IL-1R1) (10). The binding of IL-1 to IL-1R1 mediates a conformational change that allows the co-receptor IL-1R accessory protein to bind. Hence, the trimeric complex triggers a signalling cascade, leading to the activation of NFκB. The naturally occurring IL-1 receptor antagonist (IL-1RA) competes with free IL-1, whereby interaction with its receptor is prevented (11).

Inflammasomes are multimeric protein complexes and play a crucial role in the cleavage of pro-IL-1β. Cryopyrin, encoded by the *NLRP3* gene, is a member of the NOD-like receptor family and is expressed by monocytes, granulocytes, T cells, chondrocytes, keratinocytes and mast cells (12). It is a protein that consists of 3 domains: an amino-terminal pyrin domain (PYD), a central nucleotide-binding and oligomerization domain (NACHT) and a C-terminal leucine-rich repeat (LRR) domain. The PYD is crucial for the assembly of the nucleotide-binding domain like receptor protein 3 (NLRP3) inflammasome, an intracellular macromolecular structure responsible for recognition of dangerous signals and important for host immune defence against pathogens (13, 14). In detail, the PYD of the cryopyrin interacts with the PYD of an adapter molecule, known as apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC), and leads to the activation of the precursor protein pro-caspase-1. The activated caspase-1 contains a processing activity, whereby pro-IL-1β is cleaved to the mature active form (IL-1β). The synthesis of biologically inactive pro-IL-1β is mediated by NF-κB binding to the consensus binding site to transcribe the IL-1β gene (15, 16).

Cryopyrin-associated periodic syndrome

Cryopyrin-associated periodic syndrome (CAPS) is the prototype hereditary inflammasomopathy, with over 200 different underlying heterozygous gain-of-function mutations within the *NLRP3* gene (INFEVERS database; https://infevers.umai-montpellier.fr/web/index.php, accessed November 2019).

These *NLRP3* mutations, mainly concentrated in exon 3, constitutively activate cryopyrin, leading to increased conversion of pro-IL-1β into its active form with subsequent IL-1β hypersecretion (**Fig. 1**) (17, 18). CAPS consists of a group of 3 phenotypes: familial cold autoinflammatory syndrome (FCAS) as the mildest subform, Muckle-Wells syndrome (MWS) as the intermediate variant, and neonatal-onset multisystem inflammatory disease (NOMID) as the most severe phenotype (19, 20). Patients with FCAS present with cold-induced skin symptoms and musculoskeletal complaints. Patients with MWS show additional neurosensory hearing loss and may develop amyloidosis, whereas patients with NOMID have bone malformations and can develop severe neurological defects caused by aseptic meningitis (**Table I**). The physical complaints mainly start in early childhood or adolescence, but can also occur later in life due to rare cases of somatic mutations. The symptoms in CAPS are often accompanied by recurrent fever episodes and elevated levels of inflammatory markers, such as C-reactive protein (CRP), leukocytosis, serum amyloid (SAA) and S100 A8/9 or A12 (21). The crucial role of IL-1β in the pathogenesis of CAPS was proven by increased IL-1β secretion from leukocytes of patients with CAPS and highly effective anti-IL-1 treatment (22–26).

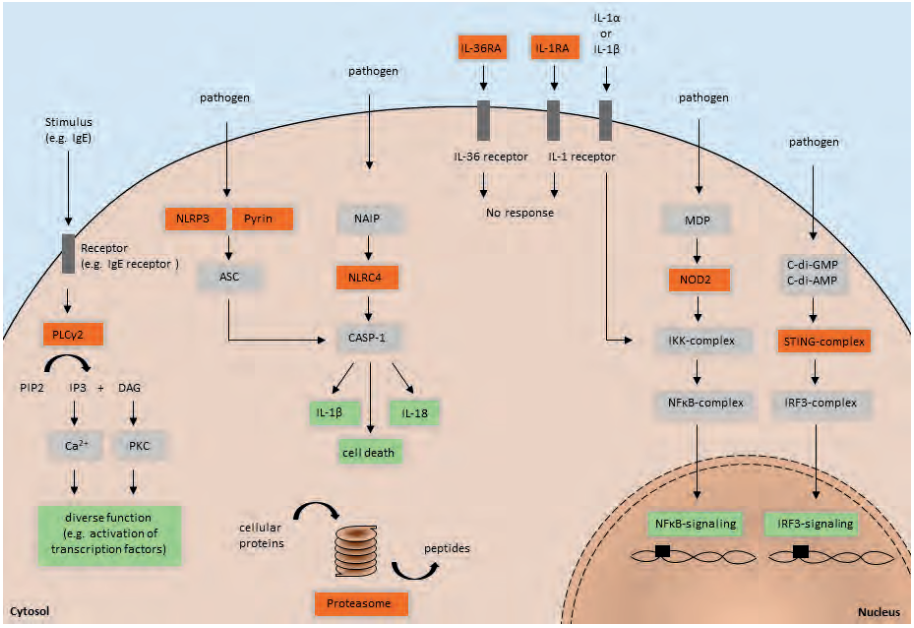

Fig. 1. Schematic representation of innate immune pathways and related pathomechanisms of the described autoinflammatory diseases. *Red squares* highlight the position of mutations associated with PLCG2-associated antibody deficiency and immune dysregulation (PLCγ2), autoinflammation and PLCG2-associated antibody deficiency and immune dysregulation (PLCγ2), cryopyrin-associated periodic syndrome (NLRP3), familial Mediterranean fever (Pyrin), nucleotide oligomerization domain (NOD)-like receptor family CARD domain-containing protein 4-inflammasomopathy (NLRC4), deficiency of interleukin-36 receptor antagonist (IL-36RA), deficiency of interleukin-1 receptor antagonist (IL-1RA), Blau syndrome (NOD2), STING-associated vasculopathy with onset in infancy (STING-complex), and chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature/proteasome-associated autoinflammatory syndrome (Proteasome).

Table I. Overview of affected genes, epidemiology, main clinical findings, skin symptoms and treatment options in selected autoinflammatory diseases grouped by their pathophysiological mechanisms of inflammation

Medicator and disease	Affected gene	Epidemiology	Main systemic symptoms	Skin symptoms	Treatment
Interleukin-1					
Cryopyrin-associated periodic syndrome (CAPS) Disease spectrum: FCAS – mild MWS – moderate NOMID – severe	*NLRP3*	Worldwide distribution; Estimated prevalence of 300,000–1,000,000 (84, p. 348)	Spectrum of: fatigue, fever, lymphadenopathy, splenomegaly, myalgia, arthropathy, sensorineural hearing loss, uveitis, amyloidosis, meningitis, bone deformations	Urticarial/maculo-papular rash, cold-induced, rarely itchy	IL-1 blockade: (23–26) Canakinumab Anakinra Rilonacept
Familial Mediterranean fever (FMF)	*MEFV*	Primarily among ethnic groups of Mediterranean ancestry (120, 121); more than 10,000 affected patients worldwide (120, 121)	Recurrent self-limiting attacks of: fever and serositis, peritonitis, synovitis and pleuritis; arthritis, myalgia	Erysipelas-like skin lesions, purpuric exanthema, urticarial rash, diffuse palmoplantar erythema, Raynaud-like phenomena, erythema nodosum	Colchicine IL-1 blockade: Canakinumab Anakinra (122)
Deficiency of interleukin-1 receptor antagonist (DIRA)	*IL1RN*	Only few cases known from literature (54–59)	Multifocal osteomyelitis, osteolysis and osteopaenia, periostitis	Generalized pustulosis, ichthyosis, nail dystrophy	IL-1 blockade: Anakinra (54, 55, 123) Canakinumab (60)
Deficiency of interleukin-36 receptor antagonist (DITRA)	*IL36RN*	Estimated prevalence of generalized pustulosis 1.76/mio. in Europe (84, p. 692)	Fever	Repeated flares of generalized pustulosis, acrodermatitis continua of Hallopeau	IL-1 blockade: Anakinra IL-36 blockade (68) IL17 blockade: Secukinumab (124, 125), TNF blockade: Infliximab (126), Etanercept (127) IL12/23 blockade: Ustekinumab (128, 129)
Interferon					
STING-associated vasculopathy with onset in infancy (SAVI)	*TMEM173*	Only few cases known from literature (130)	Recurrent fevers, interstitial lung disease, failure to thrive	Acral blistering, pustular rashes, violaceous plaques/nodules, nail dystrophy, distal gangrenes and septum perforations	Janus kinase blockade: Baricitinib (75) Tofacitinib (76)
Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE)/Proteasome-associated autoinflammatory syndromes (PRAAS)	*PSMB3, PSMB4, PSMB8, PSMB9, POMP*	Worldwide less than 100 cases described (84, p. 436–437)	Growth delay, musculoskeletal symptoms, hepatosplenomegaly	Cold-triggered acral erythematous oedematous plaques, annular purpuric plaques with raised borders, violaceous periorbital and perioral oedema	Janus kinase blockade: Baricitinib (75)
NF-KB					
Blau syndrome	*NOD2*	Overall incidence for childhood sarcoidosis 0.29/100,000/year (84, p. 368)	Arthritis, joint deformities, uveitis	Monomorphic micropapular erythematous rash with fine desquamation, progresses to brownish desquamating rash; erythema nodosum; rare: erysipelas-like lesions, urticarial rash	TNF blockade: Infliximab (131, 132) IL-1 blockade: Anakinra (133) Canakinumab (134)
Interleukin-18					
Nucleotide oligomerization domain (NOD)-like receptor family CARD domain-containing protein 4- (NLRC4)-inflammasomopathies	*NLRC4*	various origins (e.g. American, Eastern European, Japanese, Dutch and Italian) Only few cases known from literature (84, p. 526)	Fever, enterocolitis; macrophage activation syndrome: hepatobiliary dysfunction, haemophagocytosis, disseminated intravascular coagulation, acute respiratory distress syndrome; CAPS-like symptoms	Erythematous or urticarial rash	IL-1-blockade: Anakinra (114, 135) IL-18 blockade (113)
PLCG2					
PLCG2-associated antibody deficiency and immune dysregulation (PLAID) and autoinflammation and PLCG2-associated antibody deficiency and immune dysregulation (APLAID)	*PLCG2*	PLAID: identified in nearly 50 members of 3 independent families (115) APLAID: only few cases known from literature (116)	PLAID: susceptibility to recurrent infections, atopic features, autoimmune phenomena APLAID: recurrent sinopulmonary infections, early-onset ocular inflammation, colitis	PLAID: pruritic wheals, erythematous patches cold-induced; neonatal-onset acral ulcerative granulomatous skin lesions (fingers, nose, ears) APLAID: epidermolysis bullosa-like eruptions; recurrent erythematous plaques and vesiculopustular skin lesions	PLAID: Antihistamines with moderate efficacy (136) APLAID: TNF-blockade with limited efficacy (116) IL-1-blockade with limited efficacy (116)

FCAS: Familial cold autoinflammatory syndrome; MWS: Muckle-Wells syndrome; NOMID: Neonatal-onset multisystem disease.

Patients with CAPS present with an urticarial or maculo-papular rash, which is often symmetrically distributed on the trunk and/or extremities (**Fig. 2**). Skin lesions usually occur on a daily basis, last for up to 24 h and aggravate during the course of the day, with a peak in the evening (27). In patients with FCAS and those with MWS, the urticarial rash and systemic symptoms are triggered and exacerbated by cold air or evaporative cooling of the skin. In contrast, direct cold exposure does not induce the skin symptoms (27, 28). The skin lesions are rarely itchy, but can be accompanied by burning sensations and pain (27).

Based on its rarity, there is only limited data on the characteristics of skin inflammation in patients with CAPS. Skin histopathology is characterized by a neutrophil-rich dermal infiltrate (29–31). Accumulation of IL-1β and IL-6 after cold provocation testing was shown in lesional skin of patients with FCAS and dermal mast cells were identified as main producers of IL-1β (23, 32). In addition, IL-17-positive cells were observed in FCAS skin. These are believed to be stimulated by IL-1β, resulting in neutrophil recruitment and further production of IL-17 (33). The urticarial rash is thought to be mediated by NLRP3 inflammasome activation and consecutive IL-1β production of skin mast cells. IL-1β leads to vascular leakage und neutrophil accumulation as the pathological hallmark in neutrophilic urticaria.

Familial Mediterranean fever

Familial Mediterranean fever (FMF) is mostly an autosomal recessive disease caused by mutations within the *MEFV* gene, encoding a 781-amino acid pyrin/marenostrin protein (34, 35). Pyrin has a PYD and an N-terminal homotypic interaction domain, expressed by monocytes, granulocytes, dendritic cells and synovial fibroblasts (36, 37). To date, around 300 different mutations of the *MEFV* gene have been reported (INFEVERS database, accessed November 2019). The inflammation of FMF is mediated by ASC-dependent, NLRP3-independent production of IL-1β due to gain-of-function pyrin mutations (Fig. 1) (38).

The main clinical findings in patients with FMF comprise recurrent self-limiting attacks of fever and serositis as well as peritonitis, synovitis and pleuritis (Table I) (39). There is considerable inter-individual variability in the intensity and frequency of attacks. Between attacks, most patients with FMF are asymptomatic. In general, onset occurs within the first 2 decades and the disease becomes more severe during the course of life (40, 41). In untreated patients, amyloidosis can develop, with subsequent kidney failure (42, 43). Laboratory indicators are elevated acute-phase reactants, similar to those in patients with CAPS (see above) (44).

In up to 40% of patients with FMF, erysipelas-like skin lesions are reported. Those non-infectious lesions mostly affect the lower extremities and present as erythematous, painful infiltrated oedema (40, 45). Erysipelas-like lesions resolve spontaneously within several days and can be accompanied by fever and/or arthralgia (**Fig. 3**) (41). These skin lesions are typical for patients with FMF and do not occur in the context of other autoinflammatory disorders. Histopathologically, erysipelas-like lesions show dermal oedema and sparse perivascular infiltration of lymphocytes and neutrophils. Direct immunofluorescence revealed deposition of C3 in the small vessel wall of the superficial vascular plexus (46). Also, a strong association of FMF with polyarteriitis nodosa and Henoch-Schönlein purpura was reported (47–49). Less frequently, patients with FMF can present with other skin symptoms, such as purpuric exanthema and urticarial rash, diffuse palmoplantar erythema, Raynaud-like phenomena and erythema nodosum (50, 51). As a

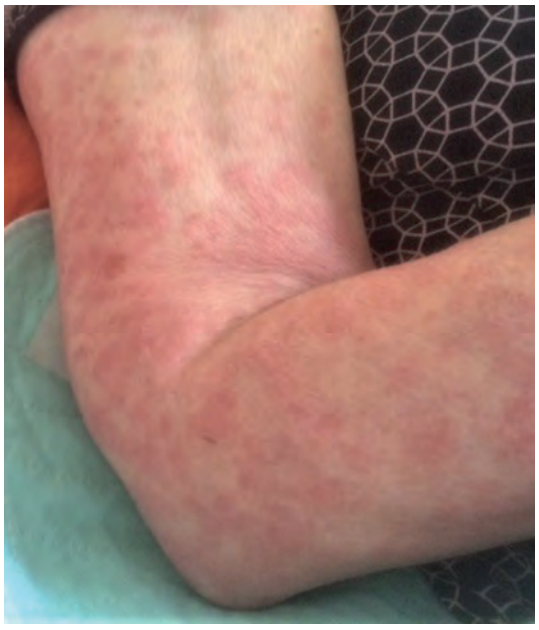

Fig. 2. Urticarial rash on the right arm in a 77-year-old woman with cryopyrin-associated periodic syndrome.

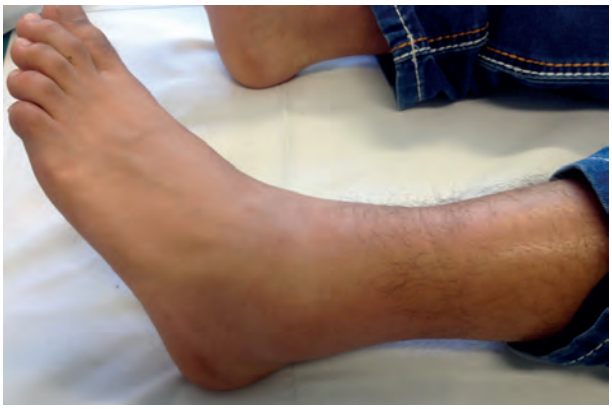

Fig. 3. Patient with familial Mediterranean fever with erysipelas-like lesion of the left lower leg and accompanying arthritis of the left ankle joint.

hypothesis, the co-occurrence of numerous immune-mediated disorders may be linked with inappropriately polarized T-cell responses in FMF, which enhances the occurrence of Th1- and Th17-driven diseases (52, 53).

Deficiency of interleukin-1 receptor antagonist

Deficiency of IL-1 receptor antagonist (DIRA) is an autosomal recessive autoinflammatory disorder caused by a homozygous mutation in *IL1RN*, a gene that encodes IL-1RA, which inhibits the pro-inflammatory cytokines IL-1α and IL-1β (Fig. 1) (54). Several disease-causing mutations have been reported, including missense mutations, nonsense mutations and deletions (54–59). Due to these mutations, IL-1 signalling is increased leading to uncontrolled systemic inflammation.

Onset of symptoms occurs at birth or at the age of few weeks. Patients with DIRA present with multifocal osteomyelitis accompanied by severe bone inflammation and consecutive osteolytic changes and osteopenia, periostitis and pustulosis (Table I) (55). The disease is characterized by premature birth and failure to thrive, as well as respiratory distress. Abnormal laboratory findings include leukocytosis with elevated inflammatory markers and anaemia despite the absence of fever (54, 55).

Cutaneous findings range from the occurrence of disseminated small pustules to severe generalized pustulosis and may be accompanied by ichthyosis. They are mainly located on the trunk and the extremities (54, 55). In most patients with DIRA, the pustular dermatitis is associated with nail dystrophy, such as onychomadesis (60). As the nail matrix is integrated in the enthesis of the extensor tendons, bone inflammation may merge into enthesitis and nail involvement. Histopathologically, DIRA is characterized by epidermal acanthosis and hyperkeratosis (55). The lesional epidermis and dermis is infiltrated by extensive amounts of neutrophils that form subcorneal pustules (54, 55). The exact mechanisms of pustule formation in patients with DIRA is not known. Activation of proinflammatory cytokines including IL-8 may mediate the expansion of IL-17-producing T cells, leading to consequent cutaneous neutrophilic influx and pustule formation. In line with this, IL-17 expression is upregulated in DIRA compared with controls (54). In contrast to urticarial neutrophilic dermatoses, autoinflammatory pustular disorders are characterized by epidermal involvement contributing to skin inflammation. Further investigations are necessary to clarify the role of keratinocytes and antimicrobial peptides, such as LL-37/cathelicidin, to better distinguish disease pathomechanisms.

Deficiency of interleukin-36 receptor antagonist

Analogously to DIRA, deficiency of IL-36 receptor antagonist (DITRA) is caused by a recessive homozygous or compound heterozygous mutation in the *IL-36RA* gene, resulting in deficiency of the IL-36 receptor antagonist (Fig. 1) (61). Consequently, pro-inflammatory cellular signals via IL-36 are enhanced, leading to systemic inflammation and generalized pustulosis. Patients with DITRA present with attacks of fever, elevated inflammatory marker CRP and leukocytosis with neutrophilia. In contrast to DIRA, there is no bone inflammation or involvement of inner organs in patients with DITRA (Table I). This can be explained by the fact that IL-36RA is physiologically mainly expressed in the skin and absent in bones or solid organs. Hallmarks of skin symptoms are flares of generalized pustulosis, as observed in patients with DIRA (61) (**Fig. 4**). Mutations in the *IL36RA* gene can also cause acrodermatitis continua of Hallopeau, a sterile pustular eruption, mainly acrally located with subsequent affection of the nails (62). DITRA is often named as a monogenic form of pustular psoriasis. In addition to *IL36RA*, mutations in *CARD14* and *AP1S3* have been identified in patients with generalized pustular psoriasis. All these mutations lead to enhanced IL-36 signalling with subsequent systemic and skin inflammation (63–65). However, in many patients with pustular psoriasis no underlying mutations are detectable. Furthermore, there is no association of DITRA with the occurrence of plaque psoriasis, and it is important to differentiate

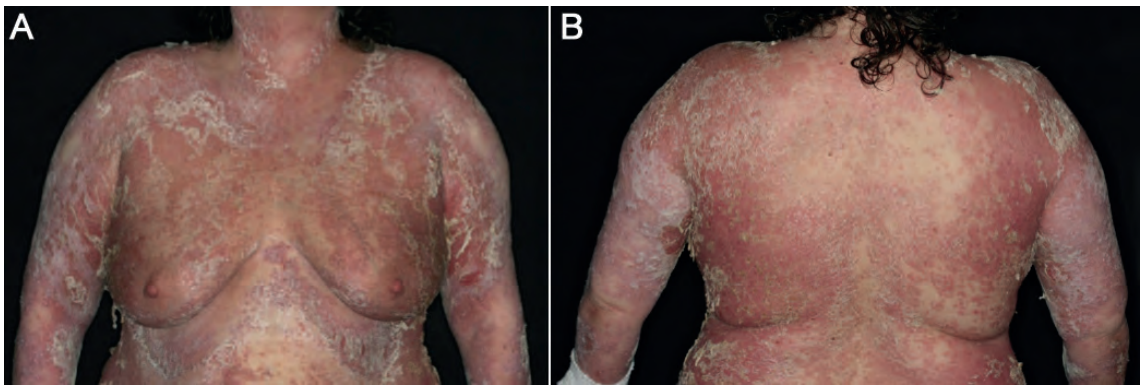

Fig. 4. (A and B) Front and back of a female patient with acute flare of generalized pustular psoriasis presenting with erythroderma, pustules, pustular lakes and erosions.

between DITRA and other types of pustular psoriasis, such as palmoplantar psoriasis. Histopathology revealed massive neutrophilic infiltration of epidermis and dermis (61). Immunohistochemistry showed IL-36γ in epidermal keratinocytes and absence of IL36RA in lesional skin of patients with DITRA (61, 66). Interestingly, biological inhibition of TNF-alpha, IL-12/23 and IL-17 seems to be more effective than anti-IL-1 treatment in patients with DITRA (67). Also, a monoclonal antibody against the IL-36 receptor (Spesolimab, BI 655130) has been shown to be efficacious in patients with generalized pustular psoriasis regardless of their mutation status (68).

INTERFERON AND INTERFERONOPATHIES

Microbial molecules from viruses, bacteria or parasites are recognized by pattern recognition receptors and drive the expression of IFN via activation of downstream signalling (69). IFNs are a family of signal proteins that are released in an autocrine or paracrine manner by host cells to regulate and activate immune response. They are classified into 3 groups, type I IFN, type II IFN and type III IFN, and are differently produced (70). Type I IFN, represented by 13 subtypes of IFN-α and a single IFN-β, is ubiquitously produced, while type II IFN is produced by T cells and type III IFN by epithelial cells. In addition, type I IFN plays a crucial role in antiviral immunity and has been part of the standard treatment of hepatitis C and hepatitis B infections in recent years. Upon releasing, IFNs bind to different kinds of surface receptors, resulting in the activation of the JAK-STAT signalling pathway. Hence, activated STAT complexes act as intracellular transcription factors and regulate the expression of interferon-stimulated genes which are involved in cellular immunity, proliferation, differentiation and apoptosis (71).

Interferonopathies are a group of monogenic disorders defined by impaired interferon-mediated immune responses and upregulated interferon gene expression.

Stimulator of interferon genes-associated vasculopathy with onset in infancy (SAVI)

Stimulator of interferon genes (STING), encoded by the *TMEM173* gene, is an endoplasmatic reticulum transmembrane protein that exists as a homodimer (72). It functions as an adapter that is essential for interferon-β (IFN-β) induction. Binding to its ligands, cyclic dinucleotides, triggers conformational changes leading to phosphorylation of TANK-binding kinase 1 and interferon regulatory factor 3 (IRF-3). Then, phosphorylated IRF-3 translocates into the nucleus and mediates the expression of *IFNB1* (interferon-β) (Fig. 1) (73). Gain-of-function mutations within *TMEM173* causes STING-associated vasculopathy with onset in infancy (SAVI) by constitutive STING activation, resulting in an increase and chronic hypersecretion of IFN-β (74). SAVI presents with recurrent fevers, interstitial lung disease, failure to thrive and systemic inflammation (Table I). However, the main clinical finding is vasculopathy (72).

Regarding the skin, patients with SAVI initially present with teleangiectatic, blistering and/or pustular rashes, mainly distributed on the fingers, toes, soles, cheeks and nose. Cutaneous symptoms start in the first weeks or months after birth, worsen by cold exposition, and can progress to severe ulcerative lesions due to peripheral vascular inflammation. Chronic involvement of the skin can manifest as acral violaceous plaques or nodules, and includes nail dystrophy, distal gangrenes and nasal septum perforations. These symptoms result from further vascular and tissue damage (72). Histological examination of lesional skin samples shows small vessel vasculitis. IgM, C3 and fibrin deposition was observed in lesional skin of single SAVI patients, indicative of an immune-complex-mediated mechanism (72). Given the pathogenic mechanisms in SAVI, inhibition of Janus kinase with blockade of type 1 IFN signalling is a promising treatment option. Both baricitinib and tofacitinib had favourable effects on skin and systemic symptoms in patients with SAVI (75, 76).

Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature/proteasome-associated autoinflammatory syndrome

Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE), also known as proteasome-associated autoinflammatory syndrome (PRAAS), is an autosomal recessive genetic disorder that affects the skin and subcutaneous tissue and presents with systemic inflammation. It is caused by mutations in proteasome or immunoproteasome subunit genes (*PSMB3, PSMB4, PSMB8, PSMB9, POMP*) (77–81).

CANDLE is not a primary interferonopathy, but is characterized by a proteasome – immunoproteasome dysfunction, leading to constitutional hypersecretion of type 1 IFNs (82). The proteasome and immunoproteasome are responsible for the degradation of impaired ubiquitinated cellular proteins by proteolysis (Fig. 1). In patients with CANDLE, proteasome and immunoproteasome dysfunction leads to an intracellular accumulation of ubiquitinated protein. The resulting cellular stress induces type I IFN genes to enhance IFN signalling and IFN synthesis. IFNs modulate the release and production of pro-inflammatory cytokines and cell recruitment, which culminates in further organ inflammation. Infections or cold exposure are potent trigger factors that can aggravate proteasome and immunoproteasome dysfunction.

Systemic symptoms in patients with CANDLE include growth delay, musculoskeletal symptoms and hepatosplenomegaly. Skin symptoms accompanied by fever are often the initial clinical manifestations of CANDLE,

with onset in early infancy (Table I) (83). Mostly, they are located on the fingers, toes, ears and nose, and may be cold-triggered as reported for patients with SAVI. Initially, they present with periodic erythematous to purplish oedematous plaques that resemble perniotic lesions and can be accompanied by localized swelling (**Fig. 5**). The skin symptoms change over the course of the disease. With increasing age, transient annular, purpuric plaques with raised borders become the more common finding. In contrast to SAVI, tissue destruction with ulceration, perforation and development of gangrene is uncommon. Furthermore, a persisting violaceous periorbital and perioral oedema occurs (84, p. 438). Later on, progressive lipodystrophy is a main characteristic of patients with CANDLE. It starts in the face and progresses to involve the trunk and the extremities (77, 83).

Lesional skin biopsies revealed dense mixed infiltrates of mononuclear cells with irregular nuclei, atypical myeloid cells, but also some mature lymphocytes, neutrophils and eosinophils in the dermis. It has been postulated that the atypical myeloid cells are recruited by increased release of IFN from the bone marrow, and that they further infiltrate peripheral organs (85). Immunohistochemistry demonstrated dermal myeloperoxidase- and CD68-positive myeloid cell infiltrates in patients with CANDLE (77, 85, 86). In addition, CD163-positive histiocytes, as well as CD123-positive plasmacytoid dendritic cells, were observed (85).

In line with SAVI, patients with CANDLE benefit from inhibition of Janus kinase. This underlines the pathophysiological role of type 1 IFN signalling (75).

NF-κB AND NF-κB-RELATED DISORDERS

The NOD2 pathway is involved in the innate immune defence against invading pathogens. NOD2 is a member of a family of pattern recognition molecules and is mainly expressed by antigen-presenting cells and intestinal Paneth cells (87, 88). It contains 2 N-terminal CARD domains for downstream signalling through CARD-CARD interaction, a NOD/NACHT domain with ATPase activity and a C-terminal domain comprised of 10 LRR motifs (89). In addition, the LRR domain provides a binding-site for its natural ligand muramyl dipeptide (MDP), a degradation product of ubiquitous peptidoglycan (90). Without a stimulus, NOD2 is silenced via auto-inhibition. The engagement of NOD2 and MDP induces a conformational change and oligomerizes the exposed NOD/NACHT domain. This leads to NOD2 activation and recruitment of the serine/threonine kinase receptor-interacting protein kinase 2 (RIP2) (91). The CARD-CARD interaction between NOD2 and RIP2 promotes the activation of NF-κB and mitogen-activated protein kinase, resulting in production of inflammatory cytokines, chemokines and adhesion molecules (Fig. 1) (92).

Blau syndrome

Blau syndrome is a NOD2-associated granulomatous inflammatory disease with an autosomal dominant inheritance that usually starts between infancy and the age of 5 years (93). Several *NOD2* gain-of-function mutations were described to cause Blau syndrome, most of them were reported in the NOD/NACHT domain (93–96). The main clinical characteristics are arthritis, skin inflammation and uveitis (Table I; **Fig. 6)** (97).

In infancy, patients with Blau syndrome show a monomorphic micropapular erythematous rash with fine desquamation as the initial symptom (84, p. 373–374).

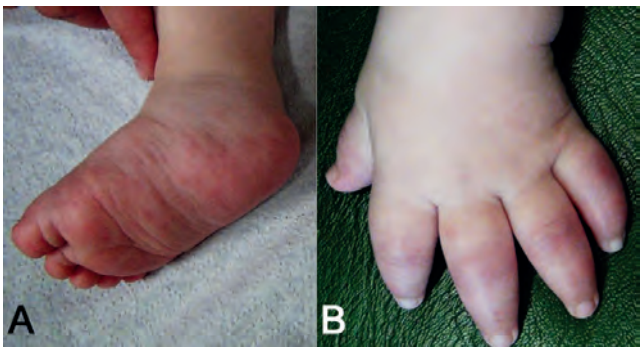

Fig. 5. Skin symptoms in an infant with chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature. (A) Erythematous papules and plaques on the right foot and right lower leg. (B) Purplish oedematous plaques on the fingers that resemble perniotic lesions with accompanying swelling of the hand.

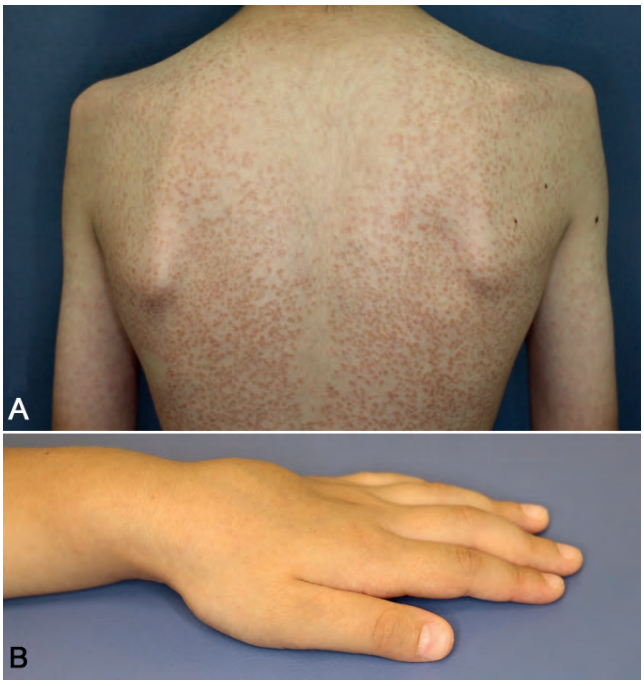

Fig. 6. Blau syndrome. A) A 12-year-old boy with disseminated small scaly solid papules with onset at age 6 months. These asymptomatic eruptions improve spontaneously, but relapse again without specific events. (B) A 4-year-old boy showing joint involvement with cystic swelling of the dorsal sides of the left hand.

The rash often starts on the dorsal trunk and further affects face and extremities. Over time, the initially erythematous rash becomes brownish and scaly. Furthermore, patients can develop subcutaneous nodules on the lower extremities, mimicking erythema nodosum (98). In single cases, skin affection, such as erysipelas-like lesions, urticarial rash, livedoid lesions and vasculitis, have been observed (99–101). Histologically, the skin lesions are characterized by naked sarcoidal granuloma formation (100).

INTERLEUKIN-18

Nucleotide oligomerization domain (NOD)-like receptor family CARD domain-containing protein 4 (NLRC4) and NLCR4 inflammasomopathies

The NLRC4 inflammasome is activated by at least 2 compounds of Gram-negative bacteria, flagellin and type 3 secretion protein (T3SS) (102–104). However, the interaction between ligand and NLRC4 does not occur directly. Instead, the sensor protein NLR family of apoptosis inhibitory protein (NAIP) physically binds flagellin or T3SS and co-assembles with NLRC4, leading to its activation through conformational change (105). Furthermore, studies have shown that phosphorylation by the kinase Pkcδ is required for the complete NLCR4 activation (106). Once activated, NAIP-NLRC4 forms a multimeric complex, known as inflammasome, which recruits and activates caspase-1 (CASP-1) (107). CASP-1 is involved in anti-bacterial responses by triggering pyroptosis, a form of inflammatory cell death (108). In addition, it mediates the processing and release of IL-1β and IL-18 (Fig. 1) (109, 110).

Gain-of-function mutations within the *NLCR4* gene are linked to NLCR4 inflammasomopathies (111, 112). These autosomal dominantly-inherited mutations promote the spontaneous formation of the NLCR4, inflammatory cell death and production of IL-1β and IL-18 (113). The clinical spectrum is manifested by a variety of symptoms, and can range between mild CAPS-like phenotypes with urticarial rash and little inflammation as well as severe conditions of macrophage activation syndrome and enterocolitis with onset in infancy (Table I) (111, 112). Macrophage activation syndrome comprises a life-threatening condition of fever, hyperferritinaemia, hepatobiliary dysfunction and haemophagocytosis. Disseminated intravascular coagulation and acute respiratory distress syndrome may occur (111).

With respect to the skin, patients present with erythematous or urticarial rashes. In contrast to patients with CAPS, the lesional skin of NLRC4 inflammasomopathies patients is characterized by a lymphohistiocytic infiltrate (114). As serum IL-18 levels are markedly increased in NLRC4 inflammasomopathies compared with patients with CAPS, it could be speculated that IL-18 may mediate cutaneous recruitment of lymphocytes and macrophages (112).

MONOGENIC AUTOINFLAMMATORY SKIN DISORDERS OVERLAPPING INNATE AND ADAPTIVE IMMUNE-SIGNALLING PATHWAYS

PLCG2-associated antibody deficiency and immune dysregulation and autoinflammation and PLCG2-associated antibody deficiency and immune dysregulation

Phospholipase C-gamma 2 (PLCγ2)-associated antibody deficiency and immune dysregulation (PLAID) and autoinflammation and PLCγ2-associated antibody deficiency and immune dysregulation (APLAID) are autosomal dominant syndromes, which are based on mutations in *PLCG2* (115, 116). In-frame deletions of exon 19 and exons 20–22 are known for PLAID (115). APLAID is induced by the S707Y mutation in *PLCG2* (116).

PLCγ2 is a transmembrane phospholipase enzyme that catalyses the hydrolysis of phosphatidylinositol bisphosphate (PIP2) to diacylglycerol (DAG) and inositoltriphosphate (IP3). IP3 modulates the calcium release as a second messenger from the endoplasmic reticulum and thereby mediates cellular signal transduction. PLCγ2 regulates various cellular functions, such as protein transport, apoptosis/cell survival, migration and immune responses (Fig. 1). It is expressed mainly in lymphoid and myeloid cells. In patients with PLAID, *PLCG2* deletions alter the carboxyl-terminal Src homology 2 domain (SH2), which is critical for PLCγ2-autoinhibtion. As a result, PLCγ2 is constitutively activated, but with reduced intracellular signalling at physiological temperatures (115). In APLAID, the S707Y substitution within the autoinhibitory SH2 domain leads to hyperactivation of the PLCγ2 enzyme and exhibits exactly the opposite effects, with increased cellular signalling at physiological temperatures (115, 116). Interestingly, it has been shown that this PLCγ2 hyperactivation results in enhanced NLRP3 inflammasome activity via intracellular Ca2+ signalling (117).

PLAID and APLAID comprise a wide spectrum of clinical and laboratory findings. Both present with an increased susceptibility to recurrent infections and variable atopic features and/or autoimmune phenomena.

Regarding the skin, patients with PLAID present with pruritic wheals or erythematous patches since birth. Skin lesions are provoked by cold air or evaporative cooling of the skin and last from minutes to hours (115). In some cases, oesophageal burning sensations after consumption of cold/frozen foods were reported (115, 118). Furthermore, syncopal episodes were described, when completely exposed to cold water (118). The urticarial lesions are based on cold-induced mast cell activation with consecutive degranulation (115). In addition, PLAID can present with neonatal-onset acral ulcerative granu-

lomatous skin lesions (fingers, nose and ears), which may or may not disappear during childhood. Ulcers are often haemorrhagic and can affect the subjacent tissue, such as erosion of the nasal cartilage. The granulomas are characterized by $CD68^+$ histiocytic infiltrates with multinucleated giant cells, a mild CD4/CD8 lymphocytic infiltrate and scattered eosinophils (118).

In contrast to PLAID, patients with APLAID do not show any cold-induced symptoms, but present with epidermolysis bullosa-like eruptions in early childhood. Over time, patients with APLAID develop recurrent erythematous plaques and vesiculopustular skin lesions, which worsen after heat, sun exposure and pressure (116). The underlying pathophysiological mechanisms remain to be elucidated.

CONCLUSION

All autoinflammatory skin diseases have in common that they occur in flares of systemic inflammation with elevated acute phase reactants and characteristic clinical symptoms. Many are caused by gene mutations that impact critical immune responses resulting in autoinflammation, but also autoimmunity and immunodeficiency. The identification of various genetic variants has broadened our understanding of host defence mechanisms and their interactions. Still, the recognition of dermatological phenotypes and clinical presentation of patients with autoinflammatory diseases are crucial for diagnosis and treatment. The occurrence of skin lesions as the only symptom is exceptional and associated complaints and symptoms should always be assessed. In most conditions, inflammatory markers, such as CRP, ESR, SAA and S100 proteins, are elevated. Although these are non-specific findings, they may prompt further investigations towards autoinflammatory disorders, such as genetic analyses in early-onset and/or familial cases.

Targeted inhibition of cytokines is effective in many of these disorders and has significantly improved the health-related quality of life of patients. Based on a better pathomechanistic understanding, novel small molecules (e.g. inflammasome inhibitors) are currently being developed (119) and may enable even more precise therapies. In addition, novel technologies such as CRISPR/Cas may enable targeted gene therapy in autoinflammatory diseases.

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SYSTEMATIC REVIEW

Dental Manifestations of Ehlers-Danlos Syndromes: A Systematic Review

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Ehlers-Danlos syndromes (EDS) are a group of inherited connective tissue disorders characterized by joint hypermobility, skin hyperextensibility, and variable tissue fragility. However, there are limited published data on the dental manifestations of EDS. This review systematically assessed the spectrum of published dental anomalies in various types of EDS. Twenty-four individual case reports/series and 3 longer case-control studies, reporting on a total of 84 individuals with a clinical diagnosis of EDS, were included in the data analysis. The main dental features listed in classical EDS were pulp calcification and localized root hypoplasia. Common dental abnormalities observed in vascular EDS were pulp shape modifications (52.2%), exceeding root length (34.8%), and molar root fusion (47.8%). Dentinogenesis imperfecta is a consistent finding in osteogenesis imperfecta/EDS overlap syndrome. Data on dental manifestations in other types of EDS are both rare and generally inconclusive.

Key words: Ehlers-Danlos syndromes; hypermobility; oral manifestation; dental anomaly.

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SIGNIFICANCE

Ehlers-Danlos syndromes are a group of rare inherited connective tissue diseases. In general, dental problems in Ehlers-Danlos syndromes are minor compared with the serious systemic manifestations, such as complications of generalized joint hypermobility or life-threatening events (e.g. vascular and organ ruptures). Nevertheless, dental problems can severely impact on patients' quality of life. In order to clarify the range of dental manifestations in Ehlers-Danlos syndromes, and create precise disease-specific information for medical and dental practitioners, a systematic search of the medical literature for relevant reports was carried out.

Ehlers-Danlos syndromes (EDS) are a clinically and genetically heterogeneous group of hereditary connective tissue disorders characterized by variable connective tissue fragility, mainly affecting skin, ligaments, blood vessels, and internal organs. The current classification recognizes 13 distinct types of EDS (**Table I**) (1), 12 of which are monogenic, with known genes that allow diagnostic confirmation. Most types of EDS are caused by disease-causing variants (mutations) in collagen-encoding genes or in genes encoding collagen-modifying enzymes (1).

The biology of dental tissues implies that tooth abnormalities might occur in various types of EDS (**Fig. 1**). *Enamel* is an epithelially derived and highly (approximately 96%) mineralized tissue with traces of non-collagenous organic material (2). Recent biochemical studies have found that enamel contains low amounts of collagen I and VII (3, 4). Immunofluorescence studies have localized type VII collagen to the organic matrix near the dentino-enamel junction, suggesting a role of collagen VII in bonding enamel to dentine (4). Animal studies have revealed that proteoglycans control early stages of tooth formation (5). They promote dentine formation and mineralization, but also restrain amelogenin synthesis and consequently enamel formation.

Dentine is a mineralized tissue composed of approximately 70% hydroxyapatite crystals embedded in a 3-dimensional collagenous network. The organic matrix is enriched in type I collagen with traces of type III and V collagen, associated with non-collagenous proteins and proteoglycans. Histological studies have revealed a network of dentinal tubules crossing the mineralized tissue and extending through the entire thickness of the dentine, from the dentino-enamel junction to the pulp (6). These tubules harbour odontoblast cell processes and tissue fluid. Electron microscopic studies have investigated the assembly of the collagenous matrix prior to mineralization, a key step in the formation of dentine (7, 8). During tooth development, odontoblasts secrete collagen fibrils with high concentrations of non-collagenous proteins (9) and proteoglycans (10). These matrix constituents regulate the process of mineral deposition (8).

The *dental pulp* is a loose connective tissue characterized by its specific anatomical location (11). Various types of collagens were isolated by differential salt

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Table I. Present and past clinical classifications of Ehlers-Danlos syndromes (EDS), inheritance pattern and genetic basis

New nomenclature, 2017	Villefranche nomenclature, 1998	Former nomenclature/other names	Gene(s)	Protein	Inheritance pattern
Genetically defined frequent types					
Classical EDS (cEDS)	Classical type	Gravis, EDS I Mitis, EDS II	*COL5A1; COL5A2 (COL1A1)*	Type V collagen (Type I collagen mutation p.Arg312Cys)	AD
Vascular EDS (vEDS)	Vascular type	Arterial-ecchymotic, EDS IV	*COL3A1*	Type III collagen	AD
Genetically defined rare types					
Periodontal EDS (pEDS)	EDS periodontitis	EDS VIII	*C1R; C1S*	C1r; C1s	AD
Arthrochalastic EDS (aEDS)	Arthrochalastic type	Arthrochalasis multiplex congenita, EDS VIIA, EDS VIIB	*COL1A1; COL1A2*	Type I collagen loss of exon 6	AD
Dermatosparactic EDS (dEDS)	Dermatosparactic type	Human dermatosparaxis, EDS VIIC	*ADAMTS2*	ADAMTS-2	AR
Classical-like EDS (clEDS)	–	–	*TNXB*	Tenascin XB	AR
Kyphoscoliotic EDS (kEDS)	Kyphoscoliosis type	EDS VI; EDS VIA	*PLOD1; FKBP14*	Lysyl hydroxylase 1; FKBP22	AR
Musculocontractural (mcEDS)		Adducted thumb clubfoot syndrome EDS Kosho type; D4ST1-deficient EDS	*CHST14* *DSE*	Dermatan-4 sulphotransferase-1 Dermatan sulphate epimerase-1	AR
Myopathic EDS (mEDS)			*COL12A1*	Collagen XII	AD/AR
Spondylodysplastic EDS (spEDS)	EDS progeroid type	EDS progeroid, β3GalT6-deficient EDS, spondylocheiro-dysplastic EDS	*B4GALT7; B3GALT6; SLC39A13*	Galactosyltransferase I/II ZIP13	AR
Brittle cornea syndrome (BCS)		Brittle cornea syndrome	*ZNF469; PRDM5*	ZNF469; PRDM5	AR
Cardiac-valvular EDS (cvEDS)		Cardiac-valvular EDS	*COL1A2*	Type I collagen (complete loss of A2 chain)	AR
Unresolved forms of EDS					
Hypermobile EDS (hEDS)	Hypermobility type	Hypermobile, EDS III	?	?	AD

AD: autosomal dominant; AR: autosomal recessive.

precipitation and extraction. Types I, III and V collagen represented 56%, 41% and 2% of the total collagen, respectively (12).

The *periodontal ligament* belongs to the tooth-supporting tissues and anchors the tooth root to the alveolar bone. It is a highly specialized connective tissue and contains well-defined collagen fibre bundles embedded in ground substance – predominantly collagen types I, III and XII (2). Acellular *root cementum* is a mineralized hard connective tissue that anchors the periodontal ligament fibres to the tooth root. Its main function is tooth attachment. The cellular cementum covers the apical root and adapts to mechanical loading. Cementum consists of approximately 50% inorganic hydroxyapatite. Collagen (mainly type I with traces of type III and XII) and non-collagenous proteins, including several proteoglycans, form the organic matrix.

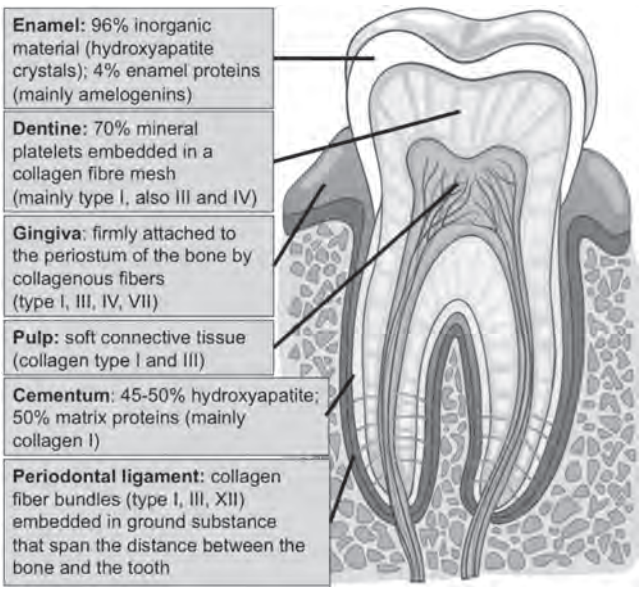

Fig. 1. The tooth and its supporting tissues. The biology of dental tissues implies that tooth abnormalities might occur in various subtypes of Ehlers-Danlos syndrome.

Patients with EDS often have a low oral health-related quality of life due to physical pain, psychological discomfort, and handicap (13). A questionnaire study among a large group of adults with EDS, mostly hypermobile or unspecified types of EDS ($n = 144$), revealed a high prevalence of oral problems, including pain in the masticatory muscles, periodontal disease, spontaneous fractures of teeth and complicated tooth extractions (14). Although EDS-related dental problems may appear less relevant in comparison with severe, sometimes life-threatening, systemic manifestations, they can strongly impact on quality of life. Major issues include pain during oral hygiene procedures, time-consuming dental treatments, and impaired cosmetic appearance. Dental health professionals are often overwhelmed with the medical care of individuals affected by rare diseases; the general dentist may not be familiar with special requirements or disease-specific oral symptoms, and treatment guidelines and precise disease-specific information are currently lacking. The complexities of EDS complications are difficult to handle in general dental practice and vary considerably among individual types of EDS. In 2017 we reviewed periodontal manifestations of EDS (15).

The aim of the present study was to systematically assess manifestations of dental tissues (dentine, enamel, cementum, and pulp) in various types of EDS. This

approach allows the delineation of dental anomalies in specific types of EDS, with clinical implications for practicing clinicians.

MATERIALS AND METHODS

Protocol and registration

A systematic literature search was performed according to Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines (16) and was registered at PROSPERO.

Literature search strategy

Two authors (IK, DS) systematically searched the literature up to 1 April 2019 in the following electronic databases: Medline (PubMed), LIVIVO, and Google Scholar. Medline (PubMed) was searched with the following keywords: ("Ehlers-Danlos syndrome" OR "joint hypermobility" OR JHS OR BJHS) AND ("dental abnormalities" OR "dental abnormality" "dental anomalies" OR "dental anomaly" OR "pulp stones" OR "hypercementosis" OR "tooth colour" OR "tooth color" OR "root deformities" OR microdontia OR transposition OR "supernumerary teeth" OR enamel OR dentine OR dentinogenesis). In addition, grey literature (www.opengrey.eu) was browsed and a "manual search" was performed on the reference lists of the selected articles and identified reviews.

Screening and selection

The inclusion criteria applied during the literature search were: (*i*) population: individuals affected with any type of EDS; (*ii*) outcome: dental anomalies (enamel, dentine, cementum or the pulp); (*iii*) English, German, or Italian language; (*iv*) full text available. There were no restrictions on publication date (available data from 1969 to 2019). Clinical trials, case-control studies, cross-sectional studies, cohort studies, case series, and case reports published in peer-reviewed scientific journals were included. Exclusion criteria were: cell culture laboratory studies, animal studies, and reviews. Titles and abstracts were checked with regard to the listed criteria. Abstracts with unclear methodology were included in full-text assessment to avoid exclusion of potentially relevant articles. Discrepancies detected during the selection process were discussed regularly.

Assessment of heterogeneity

The heterogeneity of the included studies was evaluated based on following factors: (*i*) study design, and (*ii*) subjects' characteristics.

Quality assessment

Quality assessment tools for case series and case-control studies were available from the National Heart, Blood, and Lung Institute (Bethesda, MD, USA) (17). Quality assessment of case reports was performed according to the Joanna Briggs Institute (Adelaide, Australia) (18). Each study was classified into the following groups: low risk of bias if all quality criteria were judged as "present", moderate risk of bias if one or more key domains were "unclear", and high risk of bias if one or more key domains were "absent".

Data extraction

The following main outcome(s) were individually extracted: (*i*) EDS subtype; (*ii*) type of dental manifestation(s). The following secondary outcomes were extracted for each individual as available: (*iii*) clinical characteristics of dental manifestations; (*iv*) clinical and/or genetic diagnosis of EDS; (*v*) EDS-specific features. Types and prevalence of dental manifestations and clinical characteristics available on the subjects level were analysed by standard descriptive measures, such as absolute and relative frequencies of dental manifestations in the present cohort.

RESULTS

The article selection process is documented in **Fig. 2**. Out of a total of 106 studies identified originally, 59 were excluded based on title and abstract. Of these, 45 did not report on either EDS or tooth anomalies, or covered other dental aspects in patients with EDS, such as dental implants, temporomandibular joint disorders, periodontal disease, mucosal alterations and aberrant frenula, or non-specific oral treatments, such as wisdom tooth extraction or orthodontic treatment. Six reviews, 2 animal studies, and 6 studies in other languages (Japanese, French or Dutch) were also excluded.

Out of 47 publications selected for full-text review, 20 were excluded after subsequent evaluation. Two did not report on EDS: one described dental treatment in a child with Keratitis-ichthyosis-deafness syndrome; the other was a cohort study on dentinogenesis imperfecta not including patients with EDS (19, 20). Six studies did not report on EDS dental manifestations (21), but on unspecific dental treatments, such as tooth extraction, caries therapy, etc. (22–25), or on oral manifestations other than tooth anomalies, such as aberrant frenula or craniomandibular disorders (26, 27). One letter to the editor (28) and 2 case reports (29, 30) were excluded

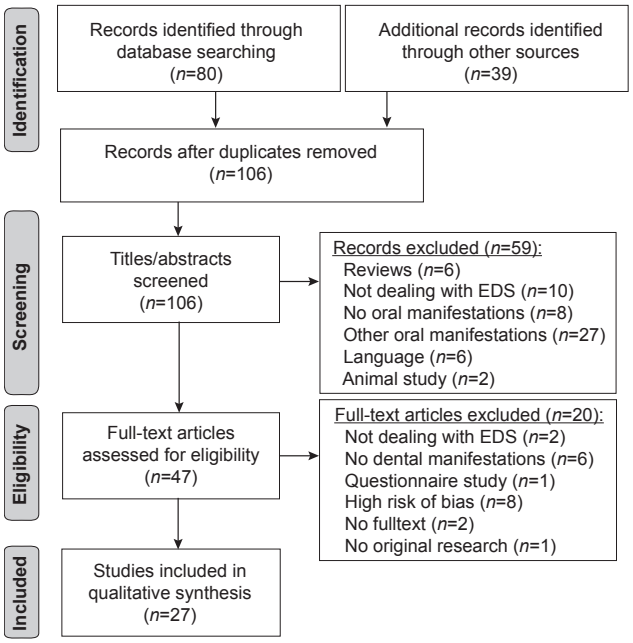

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Fig. 2. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram illustrating the study literature search and selection process.

because no full text was available. One questionnaire study on EDS oral symptoms including 144 individuals was excluded from clinical data analysis because of insufficient data quality, but is mentioned in the introduction (14). Finally, after content classification, 8 papers with high risk of bias due to insufficient data on dental or EDS-specific manifestations were excluded from data analysis (31–38).

The review thus included a total of 27 articles (24 case reports/series with 1–3 individuals each and 3 case-control studies), of which 22 were judged as high quality and 5 as moderate quality due to lack of data or incorrect classification of EDS necessitating reclassification by MP based on clinical descriptions provided in the paper.

Population

Two studies addressed only histological dental anomalies of classical EDS (cEDS) and hypermobile EDS (hEDS) (39, 40). Two subjects were reported twice. In total, 84 individuals with EDS were included in this systematic review: 24 with hEDS, 23 with vascular EDS (vEDS), 17 with cEDS, 10 with spondylodysplastic EDS, 3 with dermatosparactic EDS, 3 with osteogenesis imperfecta/EDS overlap syndrome, 2 with periodontal EDS (pEDS), one with arthrochalastic EDS (aEDS), and one with kyphoscoliotic EDS.

Classical Ehlers-Danlos syndromes

Study characteristics. Seven studies, including 17 affected individuals with classical Ehlers-Danlos syndromes (cEDS), reported various dental manifestations. The included studies were of 2 different designs:

- Case-control studies ($n=1$) (41).
- Case reports and series ($n=6$; with sample sizes of 1–3 affected individuals) (42–47).
- Histological studies on extracted teeth ($n=2$) (39, 40); 1 clinical case series also provided histological analyses (44).

Clinical manifestations (**Table II**). The diagnosis of cEDS was based on the clinical characteristic features of joint hypermobility, skin hyper-elasticity, easy bruising, and atrophic scarring. Two studies reported on genetic testing (41, 43). The age range was 11–40 years; sex distribution was 36% male to 64% female.

Fifteen out of 17 individuals with cEDS showed variable pulp calcification, ranging from single pulp stones to complete pulp obliteration. Other typical dental cEDS changes included localized aplasia or hypoplasia of tooth roots, also described as shortened or sometimes bulbous roots in 9 individuals (44). Technically, severe root aplasia may lead to premature tooth loss mimicking periodontal disease (43, 44).

Two of 17 patients with cEDS showed 2–5 supernumerary teeth (42, 45). None showed crown malformations, tooth discoloration or hypodontia, the latter was validated by inspection of the published orthopantomographs.

Histological analysis. Three papers including 26 teeth reported on histological and ultrastructural features of extracted teeth (39, 40, 44). The dental tissues demonstrated significant structural abnormalities in all investigated samples. Both Pope et al. (44) and De Coster et al. (40) reported on consistently fewer uniform dimensions and cross-sections of the dentinal tubules. A number of dentinal tubules were dysplastic (enlarged), ill-defined and irregularly branched; collagen fibres were short and of irregular size and diameter (40). Klingberg et al. (39) focused on enamel analysis of primary teeth, exhibiting a high frequency of postnatally hypomineralized enamel and postnatally located incremental lines.

Vascular Ehlers-Danlos syndromes

Study characteristics. Two case-control studies investigated dental manifestations in 23 individuals with clinically and genetically diagnosed vEDS (age range 4–61 years) and 95 age- and sex-matched controls with no history of cardiovascular, endocrine, haematological, infectious or connective tissue diseases (41, 48). In both studies, teeth were clinically and radiologically assessed for structural abnormalities and secondary lesions (decay, traumatic injury), as well as root or pulp anomalies. Panoramic radiographs and bitewings were examined for anomalies of tooth number, shape and structures.

Clinical manifestations. Dental abnormalities observed with patients with vEDS affected dentine formation rather than more common dental pathologies, such as

Table II. Clinical studies on dental manifestations of classical Ehlers-Danlos (cEDS) syndrome

Authors	Pulp calcification: pulp stones/obliterations	Root deformities: hypo-, aplasia, bulbous roots	Tooth transposition (code of teeth)	Abnormalities in tooth number
Cho, 2011 (42) ($n=1$)	No	Yes	23, 24	Supernumerary teeth
De Coster et al., 2005 (41) ($n=9$)	Yes ($n=9$)	Yes ($n=2$)	No	No
Hakki et al., 2017 (43) ($n=1$)	Yes	Yes	No	No
Pope et al., 1992 (44) ($n=3$)	Yes ($n=3$)	Yes ($n=3$)	No	No
Premalatha et al., 2010 (45) ($n=1$)	N/a	N/a	N/a	Supernumerary teeth
Selliseth, 1965 (46) ($n=1$)	Yes	Yes	No	No
Sadeghi et al., 1989 (47) ($n=1$)	Yes	Yes	No	No

Six case reports/series and 1 case-control study on a total of 17 individuals with cEDS were published to April 2019. Age range was 11–40 years (with De Coster et al., 2005 (41) excluded). No crown malformations or tooth discoloration were reported. None of the individuals presented with hypodontia or periodontal bone loss, which was validated by inspection of the orthopantomographs provided.
n/a: not available.

Table III. Clinical studies on dental manifestations of vascular Ehlers-Danlos syndrome (vEDS)

Authors	Abnormalities in tooth number *n* (%)	Pulp shape modifications *n* (%)	Root fusion *n* (%)	Exceeding root length *n* (%)	Pulp calcification *n* (%)
De Coster et al., 2005 (41) (*n* = 6)	0	0	0	0	0
Ferre et al., 2012 (48) (*n* = 17)	0	12 (70.6)	8 (47.1)	11 (64.7)	0

Two case-control studies on 23 individuals with clinically and genetically diagnosed vEDS were published up to April 2019. None of the cases reported crown malformations or tooth discolorations. If not stated otherwise, number (*n*) and percentage (%) of affected individuals are given.

caries, pain or periodontal features (48). Pulp shape modifications (e.g. reduction in pulp volume) were reported in 52.2% of patients with vEDS; however, no pulp calcification occurred. Root malformations included exceeding root length, especially in the second mandibular molars in 34.8%, and molar root fusion in 47.8% of patients. No abnormalities were observed in tooth number or crown morphology. De Coster et al. (41) reported on demarcated enamel opacities, possibly due to caries (**Table III**).

Hypermobile Ehlers-Danlos syndromes

Study characteristics. A total of 9 clinical studies, including 24 affected individuals, reported on dental manifestations in hypermobile Ehlers-Danlos syndromes (hEDS). The included studies were of 2 different designs:

- Case-control studies (*n* = 1) (41).
- Case reports and series (*n* = 8; with sample sizes of 1–2 affected individuals) (41, 49–56). Two case reports described the same individual on separate occasions (Norton & Assael (52) and Norton (54)).

Clinical manifestations (**Table IV**). The clinical diagnosis of hEDS was based primarily on obvious joint hypermobility without major features of EDS. Ages ranged from 10 to 15 years, sex distribution was 6 males and 2 females (no absolute frequencies given by De Coster et al. 2005 (41)).

There were no consistent dental features reported. Partial or total pulp obliterations of several teeth, pulp stones, and/or shortened roots were reported in 5 out of 24 individuals (41, 50, 53). In 4 individuals, 1–8 supernumerary teeth were reported (49, 51, 56). Rotation and/or transposition of single teeth were reported in 2 individuals (50, 53). In none of the cases were crown malformations or tooth discoloration reported. None of the individuals had hypodontia, as validated by inspection of the orthopantomographs provided.

Histological analysis. Histological observations of extracted teeth were reported by one paper, including 8 primary teeth of individuals diagnosed with hEDS (39). Four out of 9 teeth exhibited areas of postnatally hypomineralized enamel and postnatally located incremental lines. The dentine was always normal.

Rare subtypes

Study characteristics. Nine clinical case reports/series published up to April 2019 described various dental manifestations in 20 individuals with rare subtypes of EDS. These included aEDS, dermatosparactic EDS, kyphoscoliotic EDS, pEDS, spondylodysplastic EDS, and osteogenesis imperfecta (OI)/EDS overlap syndrome. One individual was separately included in 2 papers (57, 58).

Clinical and histological manifestations (**Table V**). Dental features of dermatosparactic EDS described in 3 separate individuals included agenesis of 4 permanent teeth (*n* = 2), irregular occlusal morphology of deciduous molars (*n* = 2), localized tooth discoloration (*n* = 2), enamel attrition of the deciduous dentition (*n* = 2), and localized tooth pulp obliteration (*n* = 1) (58).

In a case of *kyphoscoliotic EDS* dental changes included irregular occlusal morphology and malocclusion (59). No other abnormalities were reported or evident on available X-rays.

One female diagnosed with *aEDS* had enamel discoloration and microdontia (60). No pulp stones, pulp shape modifications, or root abnormalities of deciduous teeth were present. Microscopic examination of an extracted tooth demonstrated abnormal collagenous patterns in both the dentine and the pulp.

Table IV. Clinical studies on dental manifestations of hypermobile Ehlers-Danlos syndrome (hEDS)

Authors	Pulp calcification: Pulp stones, obliteration	Malocclusion	Rotation, transposition	Abnormalities in tooth number	Other dental findings
Awal et al., 2015 (49) (*n* = 1)	No	Yes	No	Supernumerary teeth	Ectopic tooth
Cohen-Levy & Cohen, 2014 (50) (*n* = 1)	Yes	Yes	Yes	No	None
De Coster et al., 2005 (41) (*n* = 16)	Yes (*n* = 3)	Not available	Not available	No	None
Ferreira et al., 2008 (56) (*n* = 1)	N/a	Not available	Not available	Supernumerary teeth	Odontokeratocyst
Kaurani et al., 2014 (55) (*n* = 1)	No	Not available	Not available	No	None
Melamed et al., 1994 (51) (*n* = 2)	No	No	No	Supernumerary teeth	None
Norton, 1997=1984 (54) (*n* = 1)	No	Yes	No	No	None
Yassin & Rihani, 2006 (53) (*n* = 1)	Yes	No	Yes	Hypodontia	None

Seven case reports /series and one case-control study on 23 individuals with hEDS were published up to April 2019. Age range was 10–15 years of age. In none of the cases, crown malformations or tooth discolorations were reported. None of the individuals presented with periodontal bone loss, which was validated by inspection of the provided orthopantomographs.

Table V. Clinical studies on dental manifestations of rare types of Ehlers-Danlos syndromes (EDS)

Authors	EDS type	Dental abnormalities reported
Ooshima et al., 1990 (60) ($n=1$)	Arthrochalastic EDS	Tooth discoloration; crown malformation
Malfait et al., 2004 (58)=De Coster et al., 2003 (57) ($n=3$)	dDermatosparactic EDS	Agenesis of two teeth; pulp calcifications; shortened roots; tooth discoloration; irregular occlusal morphology
Arun et al., 2006 (59) ($n=1$)	Kyphoscoliotic EDS	Crown malformations; malocclusion
Majorana & Facchetti, 1992 (64) ($n=2$)	Periodontal EDS	One supernumerary tooth; dens in dente; malocclusion
Van Damme et al., 2018 (65) ($n=10$)	Spondylodysplastic EDS	Hypodontia; hypoplastic teeth; dentinogenesis imperfecta
Budsamongkol et al., 2019 (61) ($n=1$)	OI/EDS overlap syndrome	Dentinogenesis imperfecta
Shi et al., 2015 (63) ($n=1$)	OI/EDS overlap syndrome	Dentinogenesis imperfecta
Nicholls et al., 2000 (62) ($n=1$)	OI/EDS overlap syndrome	Dentinogenesis imperfecta

Nine case reports/series on 20 individuals diagnosed with rare subtypes of EDS were published up to April 2019.
n/a: not available; OI: osteogenesis imperfecta.

Three individuals with *OI/EDS overlap syndrome* were diagnosed with dentinogenesis imperfecta (61–63). A severely malformed exfoliated primary incisor was subjected to histological analysis (61). The dentine contained unorganized calcified masses and loss of typical dentinal tubules. The hardness and elasticity of probands' enamel and dentine was significantly lower than those of the controls, which was attributed to the abnormal structure or quality of collagen and not to a decreased level of calcification.

Majorana & Facchetti (64) described dental features other than periodontal destruction in 2 children, aged 7 and 10 years, diagnosed with *pEDS*. Pulp calcifications and supernumerary teeth occurred in one individual, the other individual presented with a dens in dente. No further abnormalities were reported.

DISCUSSION

Various narrative reviews and case reports describe dental anomalies in EDS (66–69), but the true prevalence and relevance is unknown. Previously, the absence of genetic confirmation and problems of separating dental features in EDS from other conditions have confused the understanding of the oral phenotypes of EDS. Lack of evidence has led to various incorrect assumptions. For example, the recent Classification of Periodontal and Peri-Implant Diseases and Conditions claims that individuals affected by cEDS are at higher risk of periodontitis (70). This assumption is based on a case series including 3 individuals describing early loss of teeth due to root aplasia (44). In contrast, our recent systematic evaluation of published data did not reveal any individuals with cEDS and periodontitis (15). We now aimed to provide adequate information on the prevalence of dental hard-tissue abnormalities in specific types of EDS.

To date, the strongest evidence of disease-specific dental abnormalities is available for vEDS. Two case-control studies evaluated oral manifestations in a total of 23 affected individuals and 96 healthy controls. Pulp shape modifications, such as decreased pulp volume and malformed pulp chambers, were reported in 75% of affected individuals, but in only 30% of healthy individuals (48). Root abnormalities, including root fusion (50%) or exceeding root length (69%), especially in mandibular molars, were significantly more prevalent in individuals clinically and genetically diagnosed with vEDS than in controls (20% and 2%, respectively) (48). No pulp calcification, abnormalities in tooth number or other dental findings were reported.

Calcification of the pulp, i.e. pulp stones or obliteration (**Fig. 3**), is a common finding in cEDS and was reported in 15/17 individuals. Since cEDS is caused mainly by heterozygous mutations in *COL5A1* or *COL5A2*, this implies a regulatory function of collagen V in pulp homeostasis. The general opinion is that pulp chamber calcification is a response to chronic irritants, such as carious and/or tooth restorations (70). Pulp calcification has also been reported in individuals with hEDS ($n=5$), dermatosparactic EDS ($n=1$) and pEDS ($n=1$). Howe-

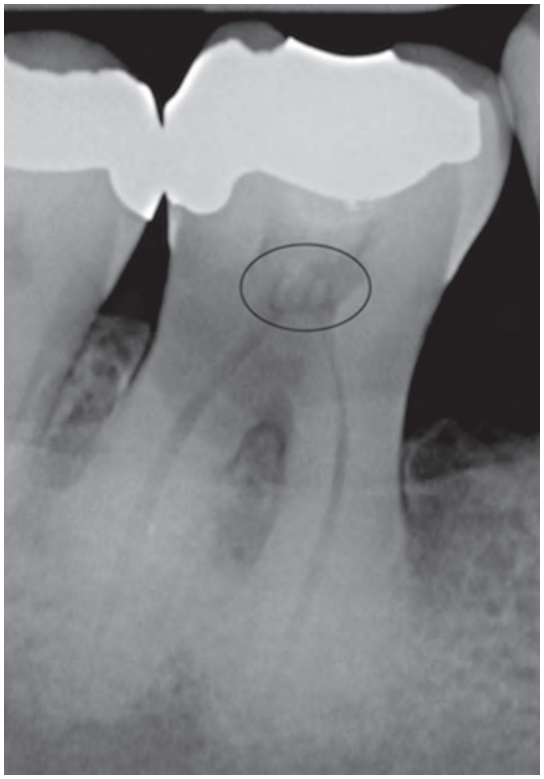

Fig. 3. Pulp stones. Dental radiograph of a 41-year-old woman, clinically and genetically diagnosed with periodontal Ehlers-Danlos syndrome (pEDS), showing pulp stones (black circle) in the mandibular right first molar.

ver, there is a high prevalence of pulp calcification in the general population (71), and the mutual presentation of EDS and pulp calcification in individual cases may be a coincidence rather than a disease manifestation. Since calcification does not usually cause pulp disease or subjective symptoms, it is not clear whether it represents pathology or biological variation (71). However, calcification complicates root canal treatments, and their large size in the pulp chamber may block access to canal orifices and alter the internal anatomy (72). In the absence of any additional signs or symptoms, pulp stones should not be interpreted as a disorder requiring endodontic therapy (72).

Defective dentinogenesis with bulbous enlargement or localized root hypoplasia (shortened roots) is both common and specific to cEDS. Subsequent loosening of the tooth can mimic localized periodontal destruction (44). The diagnostic distinction of these 2 pathologies is essential, as adequate treatment is completely different: mobile teeth with hypoplastic roots should be securely splinted to minimize subsequent bone loss. In contrast, tooth mobility from reduced periodontal attachment in the course of periodontitis requires periodontal treatment. Four patients with cEDS presented with an identical clinical phenotype of severe root aplasia restricted to the lower front teeth (43, 44). There were also 2 further case reports with identical dental phenotype, but without appropriate clinical subtyping of EDS (32, 35). Assuming that these individuals may also have had cEDS, it appears possible that root aplasia is a specific dental manifestation of this type of EDS. Further prospective studies in a cohort with validated cEDS are needed to test this hypothesis.

Dentinogenesis imperfecta is a rare published feature of aEDS, which is characterized by particularly severe joint hypermobility, with bilateral congenital hip dislocation the presenting finding in a high number of patients. aEDS is caused by loss of exon 6 in either *COL1A1* or *COL1A2*, leading to failure to enzymatically remove the N-terminal propeptide of either alpha1(I) or alpha2(I) collagen by procollagen N-propeptidase (OMIM 130060, 120160 and 617821) and, in consequence, altered stability and assembly of triple helical collagen type I. This disorder also overlaps with certain milder forms of osteogenesis imperfecta type I (OI; OMIM 166200), also known as OI/EDS overlap syndrome. In this condition, N-terminal helical collagen *COL1A1* and *COL1A2* mutations typically cause dentinogenesis imperfecta and increased joint hypermobility with variable bone fractures. aEDS is distinguishable from OI/EDS both by DNA analysis (exon 6 splicing or deletion mutations in aEDS vs. mutations between exons 7 and 16) or electron microscopy of dermal cutaneous collagen fibril patterns (which are normal in aEDS). Type I collagen is the major faulty structural protein responsible for many types of OI, as well as aEDS and cardio-valvular EDS.

Previously we systematically reviewed *periodontal manifestations* of various EDS subtypes (15) and identified 30 articles on pEDS and 13 articles on other subtypes of EDS. In pEDS, early severe periodontitis (98.4%) and gingival recession (87.1%), as well as a striking lack of attached gingiva, were the predominant features. Early severe periodontitis was also reported in one individual clinically diagnosed with vEDS (73) and in one with hEDS (74), although from current knowledge these cases more likely represent pEDS. In vEDS, a particular gingival phenotype (generalized thinness and translucency of the gingiva and the mucosa, and decreased stippling with a papyraceous aspect) was observed in 94% of affected patients (48). Reports on periodontal manifestations in other types of EDS were rare. Severe gingival enlargement was described in 3 individuals with dermatosparactic EDS (58). Our systematic review concluded that early severe periodontitis is the hallmark of pEDS, but does not appear to be part of the clinical phenotype of other types of EDS. Just like dental manifestations, stringent analyses of periodontal manifestations in most subtypes of EDS are missing.

The published evidence on dental manifestations of EDS has substantial limitations. Specification of the EDS type based on molecular data was missing in many papers. Many published cases with dental descriptions fail to adequately specify EDS typing, and molecular data supporting a particular diagnosis are missing in the majority of reports. Due to the rarity of the syndromes themselves, mostly isolated cases were reported; therefore, uncommon dental features, including tooth rotation or transposition and abnormalities in tooth number, may be coincidental findings. Cross-sectional and longitudinal studies with systematic dental examination and radiographic analysis should be performed in various subtypes of EDS. In future, only case reports/series and studies with genetically validated EDS diagnosis should be published.

CONCLUSION

Dental phenotypes of the various types of EDS have been poorly studied. Pulp calcification and localized root hypoplasia or aplasia appear to be specific findings in cEDS. vEDS is associated with pulp shape modifications, molar root fusions and exceeding root length. Data on dental manifestations in other subtypes of EDS are inconclusive.

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Centenary theme section: GENODERMATOSES

REVIEW ARTICLE

Legius Syndrome and its Relationship with Neurofibromatosis Type 1

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Neurofibromatosis type 1 (NF1) is the most common disorder characterized by multiple café-au-lait macules. Most individuals with this autosomal dominant disorder also have other features, such as skinfold freckling, iris Lisch nodules and benign or malignant peripheral nerve sheath tumours. Legius syndrome is a less frequent autosomal dominant disorder with similar multiple café-au-lait macules and skinfold freckling. Legius syndrome is not characterized by an increased risk of tumours, and a correct diagnosis is important. In young children with a sporadic form of multiple café-au-lait macules with or without freckling and no other manifestations of NF1 these 2 conditions cannot be differentiated based on clinical examination. Molecular analysis of the *NF1* and *SPRED1* genes is usually needed to differentiate the 2 conditions. Other less frequent conditions with café-au-lait macules are Noonan syndrome with multiple lentigines, constitutional mismatch repair deficiency and McCune-Albright syndrome.

Key words: CAL; NF1; Legius syndrome; SPRED1.

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SIGNIFICANCE

Neurofibromatosis type 1 and Legius syndrome are both autosomal hereditary conditions with the same type of hyperpigmentation macules and skinfold freckles. Patients with neurofibromatosis type 1 usually develop additional signs, such as tumours of the peripheral nerves, and iris Lisch nodules. At a young age these additional signs might not be present, and the correct diagnosis can only be made by genetic testing, because these 2 conditions are caused by mutations in different genes. A correct diagnosis is essential because the medical follow-up is different.

In 2007 an "NF1-like syndrome" was reported resulting from heterozygous mutations in the *SPRED1* gene. The phenotype of affected patients in this autosomal dominant condition resembled the phenotype of neurofibromatosis type 1 (NF1) (1). More specifically, these patients show the same multiple café-au-lait macules (CALMs) typically seen in patients with NF1. Freckling in the axillary region and the groin is another feature that is equally present in both syndromes. Unlike the dermatological phenotype, other phenotypic features differ substantially between the 2 syndromes. This NF1-like syndrome is considered to be a milder condition than NF1, since neurofibromas and other typical tumoural manifestations of NF1 are not present. In order to differentiate clearly between both disorders the NF1-like syndrome was named "Legius syndrome" at the 13th European Neurofibromatosis Meeting (2008). Both NF1 and Legius syndrome are caused by mutations in genes related to the rat-sarcoma-mitogen-activated protein kinase (RAS-MAPK) signalling pathway. This review summarizes overlapping and non-overlapping clinical features of these 2 syndromes, as well as their underlying molecular mechanism and relationship with other disorders caused by mutations in the same signalling pathway (**Fig. 1**) (the so-called RASopathies).

NEUROFIBROMATOSIS TYPE 1

The clinical phenotype of NF1 is characterized by multiple CALMs, skin-fold freckling and Lisch nodules in the iris. Patients with NF1 need clinical surveillance during childhood because of the risk of multiple complications, such as optic pathway gliomas, learning difficulties, social and emotional difficulties, skeletal problems, such as scoliosis and tibial pseudarthrosis and disturbances in growth (2, 3). Patients with NF1 have a high risk of development of neurofibromas. Neurofibromas are benign nerve sheath tumours composed of different cell types. The tumoural cells in the nerve sheath tumours are the Schwann cells. Cutaneous neurofibromas are benign and mostly start appearing at puberty and continue to arise in adulthood. Their number and size can increase with age. Plexiform neurofibromas are frequently diagnosed in early infancy and can grow throughout childhood. During adulthood their growth tends to stabilize. They can be asymptomatic, although they can also cause pain and disfigurement. Internal plexiform neurofibromas cannot be discovered by clinical examination alone. Nodular plexiform neurofibromas that continue to grow in adulthood are at risk of malignant degeneration. They might evolve into an atypical neurofibroma and further progress to a high-grade malignant peripheral nerve sheath tumour (MPNST). Adults with NF1 should be

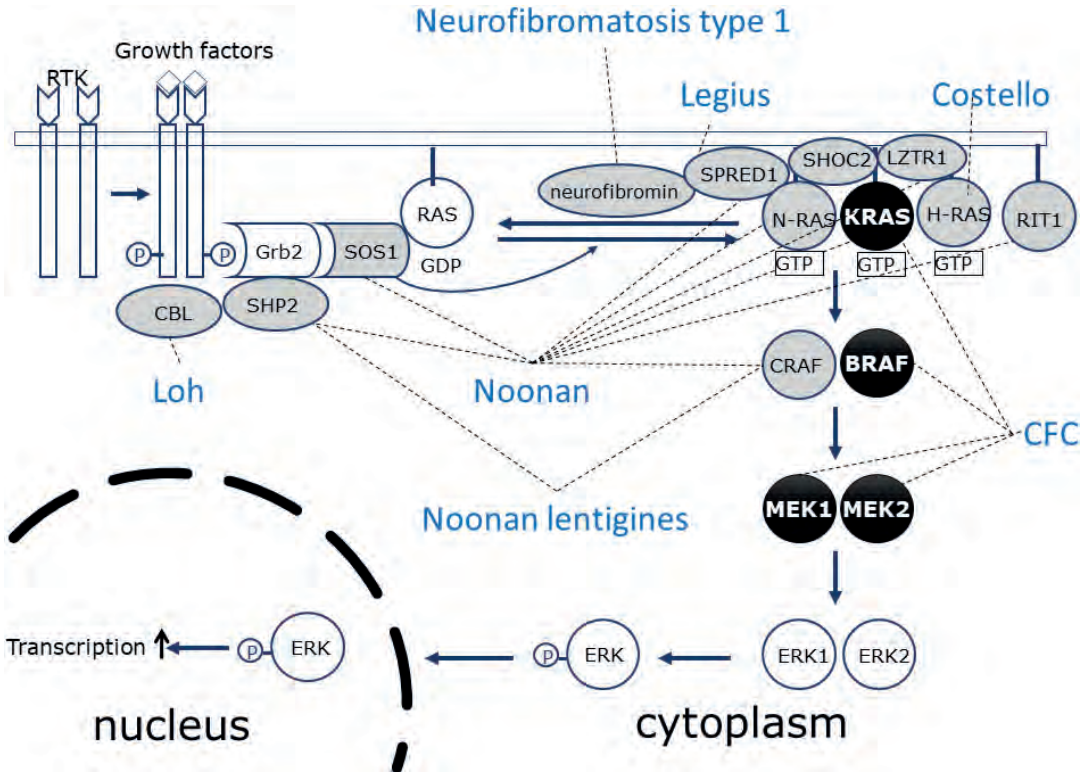

Fig. 1. The RAS-MAPKinase pathway and the proteins involved in the different RASopathies. SOS: son of sevenless; RAS: rat sarcoma; SHOC2: suppressor of clear homolog; LZTR1: leucine zipper like transcription regulator 1; N-RAS: neuroblastoma RAS; KRAS: Kirsten RAS; H-RAS: Harvey RAS; RIT1: RAS like without CAAX 1.

monitored during their lifetime for abnormal growth of plexiform neurofibromas, as well as for the appearance of some other tumours, such as pheochromocytoma, glomus tumours of the digits, gastrointestinal stromal tumours and breast cancer in females (4). The phenotype in patients with *NF1* can be extremely variable, even within families.

Inactivating mutations in the *NF1* gene were identified as the molecular cause for *NF1* in 1990 (5–7). In half of patients a *de novo NF1* mutation is identified, and in the other half the mutation is inherited from one of the parents. Most mutations identified are limited to the *NF1* gene, but approximately 5% of individuals have a microdeletion on chromosome 17q11.2 including the *NF1* region and other genes. These patients have a more severe tumoural phenotype with more neurofibromas at a younger age and a 2-fold increased risk of MPNST. Moreover, these patients sometimes present with an overgrowth phenotype and usually have more learning problems and a lower mean total IQ score compared with individuals with intragenic *NF1* mutations (8, 9). A milder phenotype, consisting of CALM and skinfold freckling, but without neurofibromas, is seen in individuals with a 3-bp in-frame deletion of exon 17 (c.2970-2972 delAAT) (10, 11) and in patients with a missense mutation at codon 1809 (12). NF1 individuals with a missense mutation affecting codons 844 to 848 generally show an important internal neurofibroma load (13).

Diagnostic criteria for NF1 were established at the National Institutes of Health Consensus Development Conference in 1988. However, young children without a family history of NF1 frequently do not fulfil the diagnostic criteria, because they often only show multiple CALMs. The other diagnostic criteria of the disease are frequently seen only later in childhood or adulthood. Moreover, differential diagnosis with other CALM-manifesting disorders is often difficult on clinical grounds. Since molecular techniques for identifying the underlying genetic mutation have become increasingly available, molecular genetic testing is now performed more frequently at initial diagnosis in order to differentiate from other CALM-presenting disorders and to guide clinical follow-up.

The *NF1* gene is a tumour suppressor gene, and NF1-associated tumours show a bi-allelic inactivation of *NF1* (14). A somatic inactivation of the wild-type *NF1* allele is needed in combination with the germline *NF1* mutation in a specific cell to start the oncogenic process. In neurofibromas a second hit is found in the Schwann cells, and they have been identified as the cells driving the growth of the neurofibromas (15, 16).

NF1 codes for the neurofibromin protein, which is highly conserved among species and is composed of different domains. The RAS-GTPase (guanosine triphosphatase) activating protein (GAP)-related domain (NF1-GRD) is the best-studied functional domain of the

NF1 gene and corresponds to a small region located in the central part of the protein. GAP proteins are negative regulators of rat sarcoma (RAS); they stimulate the hydrolysis of RAS-GTP to RAS-GDP, converting RAS from the active to the inactive form. This NF1-GRD interacts with active RAS through an arginine finger of neurofibromin that binds to RAS in a specific groove. This interaction results in a GAP-stimulated hydrolysis of GTP (17). Inactivating mutations in the *NF1* gene result in an increased activation of the RAS-MAPK pathway due to a deficient downregulation of active RAS proteins.

RAS-MAPK PATHWAY AND RASOPATHIES

NF1 and Legius syndrome are part of a group of overlapping disorders previously known as the Neuro-Cardio-Facio-Cutaneous (NCFC) syndromes (18). The phenotype associated with this group of disorders consists of neurological symptoms (e.g. psychomotor delay, learning difficulties, intellectual disability), cardiac abnormalities (most frequently pulmonary valve stenosis and hypertrophic cardiomyopathy), facial features (e.g. hypertelorism, ptosis, low implanted posteriorly rotated ears) and cutaneous findings (e.g. café-au-lait macules). Other frequently encountered features in these conditions were short stature and macrocephaly. Moreover, an increased risk of malignancy has been described in some of these syndromes. This group of disorders consists of NF1, Costello syndrome, cardio-facio-cutaneous syndrome, Noonan syndrome, Noonan syndrome with multiple lentigines, Loh syndrome, and Legius syndrome. These disorders not only share phenotypic features, but they are also caused by mutations in genes coding for proteins of the RAS-MAPK pathway. These disorders are now grouped under the name RAS-MAPK syndromes or RASopathies. A review of RASopathies can be found in (19).

This RAS-MAPK pathway had previously been extensively studied for its role in cancer biology. *RAS* genes are proto-oncogenes controlling pathways that are important regulators of cell growth. Many solid tumours show mutations in one of the RAS genes. The RAS homologues (neuroblastoma RAS (*NRAS),* Kirsten RAS *(KRAS),* Harvey RAS (*HRAS*)) code for proteins that are active in the GTP-bound and inactive in the GDP-bound state. Membrane-bound receptor tyrosine kinases are activated by binding to growth factors, and this leads via different adaptor proteins to activation of RAS-guanosine nucleotide exchange factors (GEFs) such as son of sevenless (SOS). RAS-GEFs activate RAS by stimulating the exchange of GDP to GTP bound to RAS. Active RAS-GTP has different downstream effector molecules. GTP-bound RAS binds to and actives the serine-threonine kinase rapidly accelerated fibrosarcoma (RAF) (MAPKKK= MAPkinasekinasekinase). Activated RAF-kinases phosphorylate and activate the protein kinase MEK (MAPKK= MAPKinasekinase). Active MAPK-ERK kinase (MEK) kinases (MEK1 and MEK2) phosphorylate a threonine and tyrosine on their only known substrate MAPKinase (ERK) (MAPK= MAPkinase). ERK activates transcription factors and signalling proteins. Activation of the RAS-MAPK signalling cascade thus results in stimulation of cell proliferation, promotion of cell survival and control of cell differentiation. Signalling is downregulated when RAS-GTP is hydrolysed to RAS-GDP. RAS proteins have intrinsic GTP-ase activity, which is strongly stimulated by GTP-ase activating proteins (GAPs), such as neurofibromin.

Some rare large families with autosomal dominant Noonan syndrome showed linkage to a locus on chromosome 12q24.1. Later it was shown that activating mutations in tyrosine-protein phosphatase non-receptor type 11 (*PTPN11*), located in this region on chromosome 12, were identified for a large group of Noonan syndrome individuals. *PTPN11* codes for the Src homology region 2 (SH2)-containing protein tyrosine phosphatase (SHP2) protein, which interacts in a stimulating way with the RAS signalling cascade (19). Noonan syndrome is an autosomal dominant syndrome characterized by short stature, a specific facial dysmorphism, macrocephaly, ptosis of the eyelids, epicanthal folds, low implanted and posteriorly rotated ears, low posterior hairline and a broad webbed neck. Widely spaced nipples and pectus abnormalities are also frequently observed, but are less specific. Heart defects, such as pulmonic stenosis and hypertrophic cardiomyopathy, are found in 50–80% of patients. Developmental delay can be present and is rather mild.

Heterozygous mutations in *HRAS* were identified in individuals with Costello syndrome in 2005 by Aoki et al. (20, 21). This was a remarkable finding, because it was the first time that constitutional mutations in one of the RAS genes was identified in a human disorder. Prior to that report it was assumed that germline dominant activating mutations in one of the RAS genes were not compatible with life. Costello syndrome is a sporadic disorder. It usually presents with high birthweight and neonatal feeding problems. Postnatal failure to thrive and growth retardation are observed. Patients with Costello syndrome have redundant subcutaneous tissue with deep palmar and plantar creases. Coarse facial features and cardiac abnormalities are frequent. Relative macrocephaly and intellectual disability are usually present. Many individuals develop papillomata in the peri-oral and peri-anal region. Tumour risk by 20 years of age is estimated at 15% and rhabdomyosarcoma, neuroblastoma and bladder carcinomas are observed.

Knowledge of the genetic mechanisms in this group of disorders has been expanding rapidly over the years. Mutations in several other genes of the RAS-MAPK pathway were identified in Noonan syndrome (*KRAS, NRAS, SOS1, BRAF, RAF1,* suppressor of clear homolog (*SHOC2*), RAS like without CAAX 1 (*RIT1*),

Casitas B-lineage lymphoma (*CBL*) and eucine zipper like rranscription regulator 1 (*LZTR1*)) and in cardio-facio-cutaneous syndrome (CFC) (*BRAF*, *MEK1, MEK2* and *KRAS*). Germline KRAS mutations do not overlap with the mutational hotspots in solid tumours. KRAS is an important protein during embryogenesis. Strongly activating mutations in *KRAS* as seen in cancer tissues are most probably not tolerated in the germline and are probably lethal during development.

LEGIUS SYNDROME

Linkage analysis in 2 families with multiple CALMs and freckling, but without a pathogenic *NF1* mutation was used to map the condition to a region on chromosome 15 where *SPRED1* was localized. Existing literature data at that moment pointed to the SPRED1 protein as a negative regulator of the RAS-MAPK signalling pathway (22). Sequencing of the *SPRED1* gene in affected patients from those families showed inactivating heterozygous germline mutations in the *SPRED1* gene as well as in 3 other families and in 6 unrelated patients with a phenotype of "familial CALM only" (1).

The *Spred1* gene (Sprouty-related, EVH1 domain containing 1) was identified in 2001 and has 7 exons coding for 444 amino acids. The SPRED1 protein has 3 functional domains: an N-terminal EVH1-domain, a central c-KIT-binding domain and a C-terminal SPRY-related domain. The highest expression of human *SPRED1* is seen in lung, brain, spinal cord and spleen. Expression is lower in liver, pancreas, muscle, prostate, heart, thymus, kidney and bone marrow.

The initial report by Brems et al. reported families with a phenotype similar to the phenotype seen in mild cases of NF1, showing multiple CALMs, axillary freckling, macrocephaly and sometimes Noonan-like facial features. Learning difficulties and/or attention deficits were less frequent compared with NF1. Of special note is the observation of multiple lipomas in several adults in 2 unrelated families. Some typical features of NF1 were not observed, such as Lisch nodules, typical bone defects, and NF1-associated tumours (1).

After this first report *SPRED1* mutation analysis in several other cohorts of patients in follow-up in a multidisciplinary outpatient clinic for patients with NF1 were reported. Pasmant et al. (23) identified 5 unrelated individuals with a *SPRED1* mutation in 61 cases. They confirmed the phenotype observed in the first publication with CALMs, freckling and learning disability without neurofibromas or Lisch nodules. Lipomas were seen in only one family.

In another study 6 individuals were identified with *SPRED1* mutations in 85 unrelated patients negative for an NF1mutation. None of the 6 had cutaneous neurofibromas and 5 out of 6 individuals met NF1 diagnostic criteria (24). All individuals had multiple CALMs. Noonan-like facial features, macrocephaly, Lisch nodules or neurofibromas were not reported, and developmental or learning problems were not described.

Messiaen et al. (25) reported a genotype-phenotype study in 22 unrelated individuals carrying a *SPRED1* mutation. These 22 individuals were identified through clinical testing. Fifty percent fulfilled the NIH diagnostic criteria for NF1due to multiple CALMs with or without freckling and/or a positive family history. No increased frequency of lipomas was reported. Other NF1 diagnostic features, such as symptomatic optic pathway gliomas, neurofibromas or osseous lesions, were not present. Relative macrocephaly was observed in 27% and language/speech problems were mentioned in 25% of children. In a separate cross-sectional study *SPRED1* mutation analysis was performed in 1,318 unrelated patients with a NF1 phenotype but without a *NF1* mutation (25). In 33 unrelated individuals 26 different pathogenic *SPRED1* mutations were identified. Seven, probably benign, missense mutations were seen in 9 individuals. In 19% of *NF1* mutation-negative families with an autosomal dominant phenotype of "CALMs only" with or without freckling a pathogenic *SPRED1* mutation was detected. Following this study, it can be estimated that 1–4% of individuals with multiple CALM have Legius syndrome (26, 27).

In a report on individuals from 14 families with Legius syndrome one patient had a vestibular schwannoma and one a desmoid tumour. It is not known whether these tumours are related to the germline *SPRED1* mutation. (28). Learning difficulties were observed in 14/25 individuals. Unilateral postaxial polydactyly was found in 2 patients in this study and in one patient reported by Messiaen et al. (25).

A small study investigated whether Legius syndrome is associated with neurocognitive problems, since learning difficulties (1, 23, 29), hyperactivity (1, 25) and language or speech delay (23, 25) had been reported in Legius syndrome and other RASopathies are also associated with neurocognitive problems (30). In 15 patients with Legius syndrome a mean Full scale intelligence quotient (FSIQ) of 101.57 (SD=17.57; median=107; IQR=23) was reported, which did not differ significantly from the control group (unaffected siblings). The FSIQ was higher than the mean FSIQ in 103 patients with NF1 from the same outpatient clinic. These preliminary data suggest that, in addition to the somatic phenotype (25), the cognitive phenotype is also milder in Legius syndrome than in NF1 and other RASopathies. In Legius syndrome individuals a large variability in mean FSIQ was observed. In comparison with NF1, there were few behavioural problems as assessed by the CBCL.

Another common feature of the RASopathies is the increased malignancy risk. This risk varies between the different RASopathies. It is low in individuals with Noonan syndrome, with an estimated 4% increase in cancer

risk vs. a higher risk in Costello syndrome, estimated at 15% by age 20 years (31). In NF1 benign neurofibromas are seen in the majority of patients at an adult age. In children with NF1 the risk of an optic pathway glioma is estimated at 15%, but more than 2/3 are asymptomatic. The lifetime risk of MPNST is estimated at 10–15%, with a higher risk in patients with NF1 microdeletion. Adult women with NF1 have an increased risk of breast cancer between the ages of 30 and 50 years, and it is recommended to start screening at the age of 30 years for early detection of breast cancer (32, 33).

At present we cannot completely exclude that Legius syndrome is associated with an increased risk of malignancies. Pasmant et al. (34) found one leukaemia in a patient with Legius syndrome with a *SPRED1* loss of heterozygosity in the leukaemic cells in a set of 230 paediatric lymphoblastic and acute myeloblastic leukaemias. Currently there is no documented increased risk of malignancies in Legius syndrome.

The CALMs in patients with NF1 and Legius syndrome are clinically indistinguishable. Naevus anaemicus has been suggested to be a clinical sign useful to differentiate NF1 from other CALM disorders (35). However, naevus anaemicus has been reported in a patient with Legius syndrome, as well as in a patient with Noonan syndrome with multiple lentigines due to a *PTPN11* mutation. Naevus anaemicus is not specific for NF1 and cannot be used as a criterion to differentiate between NF1 and Legius syndrome (36).

Sporadic cases of CALMs without *NF1* mutation are infrequently associated with a *SPRED1* mutation and might represent *NF1* mosaicism or other conditions (37). A specific surveillance for tumoural complications is not recommended in children and adults with Legius syndrome, in contrast to NF1.

MOLECULAR FEATURES

It has been shown that SPRED1 binds to neurofibromin with its EVH1 domain and it recruits neurofibromin to the plasma membrane. SPRED1 is anchored in the plasma membrane by its sprouty-related domain. At the plasma membrane SPRED1, neurofibromin and RAS form a multiprotein complex resulting in down-regulation of RAS-GTP levels (38).

Previously reported mutations and polymorphisms in the *SPRED1* gene can be found in the Leiden Open Variation Database (http://www.lovd.nl/SPRED1). No clear mutational hotspots in the gene have been identified. Most of the pathogenic mutations are predicted to be truncating (nonsense or frameshift mutations). A minority are missense variants. Most of the missense variants are classified as benign polymorphisms. For some missense mutations functional characterization was able to classify them as pathogenic. In Legius syndrome cultured melanocytes from a CALM showed a biallelic mutation in the *SPRED1* gene. The same mechanism (biallelic *NF1* inactivation) was previously reported in melanocytes from CALM in NF1 (1).

DIFFERATION OF OTHER CONDITIONS PRESENTING WITH MULTIPLE CALMS FROM NF1 AND LEGIUS SYNDROME

Constitutive Mismatch Repair Deficiency (CMMRD) is an autosomal recessive inherited condition caused by bi-allelic mutations in the mismatch repair genes *MLH1, MSH2, MSH6* or *PMS2*. The proteins encoded by these genes are responsible for correcting base substitution mismatches or insertion-deletion mismatches generated during DNA replication.

Heterozygous mutations in these genes are responsible for the autosomal dominant Lynch syndrome, a cancer predisposition syndrome characterized by increased risk of adult malignancy, including colorectal cancer, gynaecological cancer (ovarian cancer and endometrial cancer) and uro-endothelial tumours. Tumours in individuals with Lynch syndrome frequently demonstrate microsatellite instability (MSI) and lack of expression of the mutated MMR gene by immunohistochemistry.

Most frequently described malignancies in children with CMMRD are haematological malignancies, brain tumours and gastro-intestinal cancers, but also low-grade gliomas and premalignant gastro-intestinal lesions have been identified. These children present with multiple CALMs that are clinically difficult to distinguish from those in NF1 or Legius syndrome. A study from the international CMMRD consortium (39), showed cutaneous findings resembling NF1 in all children, suggesting that CMMRD should be considered in the differential diagnosis of children presenting with CALMs and other variables associated with CMMRD, such as consanguinity in the parents or a family history of childhood, brain, haematological or gastro-intestinal malignancies. In the review of Wimmer et al. (40), more than 60% (91/146) of the patients with CMMRD were reported to show at least 1 CALM or hyperpigmented skin area and 27/146 presented CALM and other signs of NF1. Interestingly, in up to 75% of families with CMMRD no Lynch-associated malignancies were identified in adult family members carrying the heterozygous MMR gene mutation (37). This is probably related to the fact that, in CMMRD pedigrees, mutations in *PMS2* and *MSH6* are mostly found. These genes are known to be less penetrant than the other Lynch syndrome-associated genes. Diagnostic criteria for CMMRD are given in a review paper by the C4CMMRD consortium (40).

CALMs can also be found in other autosomal dominant conditions, including piebaldism, neurofibromatosis type 2 (NF2), Schwannomatosis, Noonan syndrome with multiple lentigines, and in McCune-Albright syndrome

caused by mosaic mutations in the guanine nucleotide binding protein (G protein), alpha stimulating activity polypeptide 1 (*GNAS*) gene. The cutaneous phenotype in these latter conditions is often distinguishable from NF1 for trained clinicians.

Piebaldism is a rare autosomal dominant condition that is characterized by depigmented areas of the skin and hair. Patients often have a white forelock of hair and depigmented skin patches in a specific pattern. Irregularly shaped depigmented spots can be present on the face, trunk and extremities. Typical CAL spots can be present. The condition is caused by heterozygous mutations in the v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (*KIT*) proto-oncogene or sometimes in the zinc finger transcription factor snail family transcriptional repressor 2 (*SNAI2*).

NF2 is an autosomal dominant condition caused by mutations in the *NF2* gene on chromosome 22. NF2 individuals develop typically bilateral vestibular schwannomas. Schwannomas localized on other nerves are also seen as well as meningiomas and ependymomas. Mononeuropathy occurring in childhood may present as facial nerve palsy or hand/foot drop. Multiple CALMs can be present in children with NF2, although usually there are fewer spots and they are smaller than in NF1. Moreover, they tend to be paler and have more irregular borders than in NF1. Hypopigmented areas can also occur (41).

A related disorder is familial Schwannomatosis, a rare autosomal dominant condition characterized by multiple schwannomas, predominantly occurring in the spine, but also in the peripheral nerves and cranial nerves. Heterozygous germline mutations in /SNF related, matrix associated, actin dependent regulator of chromatin, subfamily B, member 1 (*SMARCB1)* or *LZTR1* have been reported is most individuals with familial Schwannomatosis, both located on chromosome band 22q11. Merker et al. (42) reported that 23% of patients had at least 1 CALM >1.5 cm; none had more than 4 CALMs >1.5 cm. No intertriginous freckling was reported in these patients.

Noonan syndrome with multiple lentigines belongs to the group of RASopathies. This condition presents with a Noonan syndrome phenotype and multiple lentigines. The associated heart defect is frequently a hypertrophic cardiomyopathy or pulmonic stenosis. Sensorineural hearing loss is present in approximately 20% of patients and intellectual disability, usually mild in 30%. The condition can be caused by heterozygous mutations in *BRAF, MEK1, PTPN11* or *RAF1*. A couple of CALMs are observed in a large number of patients and may precede the appearance of the typical lentigines, leading to a suspicion of NF1 or Legius syndrome in young children (43).

In individuals with fibrous dysplasia/McCune Albright syndrome (FD/MAS) large CALMs with irregular borders are seen in combination with polyostotic fibrous dysplasia. The large CALMs do not cross the midline. FD/MAS results from a postzygotic somatic activating mutation of *GNAS*. Characteristic features of CALMs in this condition are the irregular borders resembling the "coast of Maine" (in contrast to the smooth-bordered "coast of California" lesions seen in NF1) and the distribution which reflects the embryonic cell migration of melanocytes. Fibrous dysplasia (FD) can range from a monostotic lesion to severe polyostotic disease. Endocrinological complications can include gonadotropin-independent precocious puberty, thyroid abnormalities and growth hormone excess.

CONCLUSION

Legius syndrome and NF1 share a similar dermatological phenotype, consisting of multiple CALMs and freckling. Legius syndrome is a much milder condition lacking the tumour phenotype seen in NF1. The neurocognitive phenotype also seems milder. Since the number of reported patients is still limited it is uncertain whether some rare malignancies are associated with Legius syndrome, such as certain types of leukaemia. CALMs are the most frequent and easily recognizable manifestation of both conditions. In young children without other manifestations of NF1, differential diagnosis between the 2 conditions can be difficult on clinical grounds. Molecular genetic testing may help in establishing a correct diagnosis and ensure appropriate surveillance for the affected individuals. Another condition to consider in children with multiple CALMs is CMMRD. Although rare, it is important to recognize this syndrome because it is associated with a high risk of childhood malignancies. CMMRD should be considered in children with CALMs from consanguineous parents or with a personal or family history of childhood haematological malignancies, brain tumours, gastro-intestinal malignancies or pilomatricomas (40). A family history compatible with Lynch syndrome may be present, but is often lacking. Other CALM manifesting disorders can usually be distinguished by their disease-specific manifestations and different aspect of the CALMs.

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REVIEW ARTICLE

Diagnosis and Management of Inherited Palmoplantar Keratodermas

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Inherited monogenic palmoplantar keratodermas are a heterogeneous group of conditions characterised by persistent epidermal thickening of the palmoplantar skin. Palmoplantar keratodermas are grouped depending on the morphology of the keratoderma into diffuse, focal/striate or papular/punctate. Some palmoplantar keratodermas just affect the skin of the palms and soles and others have associated syndromic features which include changes in hair, teeth, nails, hearing loss or cardiomyopathy. Next generation sequencing has helped discover genes involved in many of these conditions and has led to reclassification of some palmoplantar keratodermas. In this review, we discuss the diagnostic features of palmoplantar keratodermas and management options.

Key words: keratoderma; palmoplantar; keratin; genetic; inherited.

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SIGNIFICANCE

The palmoplantar keratodermas are a complex group of diseases where the main feature is thickening of the skin of the palms and soles. Genetic testing has given insight into the biology of these conditions and has allowed experts to reclassify them. In this review, we present a summary of the key features of the major types of palmoplantar keratodermas and discuss their management.

The palmoplantar keratodermas (PPK) are a complex group of conditions that are characterised by persistent epidermal thickening (hyperkeratosis) of palmoplantar skin. The PPK are traditionally classified as hereditary (HPPK) or acquired. The main feature distinguishing hereditary from acquired PPK is the presence of a positive family history, early onset of disease, associated syndromic features and relative treatment resistance (1). Sporadic (spontaneous) mutations need to be considered in those without a family history or late onset disease (2).

Next generation sequencing has given us a better understanding of HPPK pathophysiology and has shown that one genotype can have several phenotypes. This has led to reclassification of some PPK thought previously to be distinct entities. Laboratory investigation shows that palmoplantar skin is a site at which multiple molecular pathways converge: gap junctions via connexins, intracellular adhesion through desmosomes and mechanical stability by means of the keratin cytoskeleton amongst others (3).

An initial approach to PPK is to take a history asking about age of onset, palmoplantar pain and/or blistering, sweating and infection and other associated features including hearing loss, abnormal hair, nail or teeth/ mucosal problems, cysts and family history including family history of cancer. Clinical examination can usually differentiate PPK into 3 groups: diffuse, focal or punctate **(Fig. 1**). The clinical features and management will be discussed in this review and are summarized in **Table I**.

DIFFUSE HEREDITARY PALMOPLANTAR KERATODERMAS: NO ASSOCIATED FEATURES

Diffuse epidermolytic PPK (EPPK; MIM# 144200, *KRT9, KRT1*) is the most common diffuse PPK with epidermolytic changes in suprabasal keratinocytes seen on histology (4). It is inherited in an autosomal dominant (AD) fashion due to mutations in *KRT9* and sometimes *KRT1* (5, 6). The *KRT9* gene encodes for the type I keratin, keratin 9, which is mainly expressed in suprabasal palmoplantar skin. Type I keratins form heterodimers with type II keratins, in this instance, possibly keratin 1, found in the epidermis inclu-

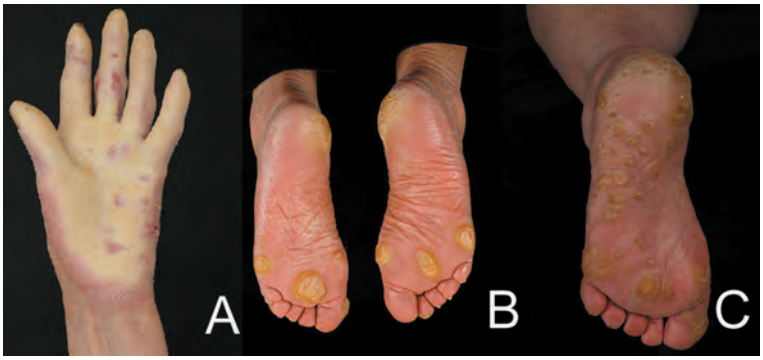

Fig. 1. Patterns of palmoplantar keratodermas. A) diffuse, B) focal and C) punctate.

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Table I. Summary of hereditary palmoplantar keratodermas (HPPK)

Type	MIM(#)	Gene	Inheritance	Features	Trangrediens	Superinfection	Specific Treatment
DIFFUSE HPPK: NO ASSOCIATED FEATURES							
EPPK	144200	*KRT9 (KRT1)*	AD	Brown-yellow, fissuring	Limited in *KRT1*	Very occasional	Keratolytics Retinoids Calcipotriol
NEPPK type Bothnia	600231	*AQP5*	AD	Brown-yellow, smooth, White-spongy with water immersion	No	Bacterial Fungal	Retinoids
NEPPK type Nagashima	615598	*SERPINB7*	AR	Mild hyperkeratosis, Erythema +++, extension to dorsal acral surfaces, spongy with water immersion	No	Bacterial Fungal	Treat superinfection Treat hyperhidrosis
MDM	284300	*ARS*	AR	Thick ivory macerated hyperkeratosis, malodour, lesions on elbows and knees, constrictive bands, contractures	Yes	Bacterial Fungal	Treat superinfection Treat hyperhidrosis Retinoids – hyperkeratosis improves; erythema worsens
DIFFUSE HPPK: NO ASSOCIATED FEATURES							
LK	604117	*LOR*	AD	Collodion +/– generalised scaling, diffuse honeycomb PPK, extensor surface fixed plaques, constrictive bands	Yes – ill-defined	No	Isotretinoin
KLICK	601952	*POMP*	AR	Ichthyosis similar to LK, smooth PPK, flexural linear & starfish keratosis on large joints	No	No	Acitretin
PPK with scleroatrophy (Huriez)	181600	*SMARCAD1*	AR	Scleroatrophy on palms/fingers, mild hyperkeratosis, erythema, palms > soles, 100x risk SCC	No	No	Acitretin – PPK/SCC
Palmoplantar hyperkeratosis with SCC & sex reversal	610644	*RSPO1*	AR	Similar to Huriez syndrome	No	No	Acitretin
OODD	–	*WNT10A*	AR	Mild PPK, diffuse, erythematous, late onset hyperhidrosis, overlap with SPSS – ectodermal abnormalities, late onset hidrocystomas	No	No	Surgery/laser – tumours & cysts
OLS	614594 300918	*TRPV3* *MTBSP2*	AD, AR, Semi-dominant, XLR	Diffuse PPK, digital flexion deformities, constrictive bands, periorificial keratoses	No	Bacterial Fungal	Variable response to acitretin Surgery +/– grafting for PPK ?EGFR inhibitors
PLS HMS	245000 245010	*CTSC*	AR	Thickening/erythema palmoplantar skin, peridonitis, hyperkeratotic plaques on extensors, HMS also has skeletal changes	No	Bacterial Fungal	Retinoids Dental care essential Tetracyclines (>12y/o)
CEDNIK	609528	*SNAP29*	AR	Diffuse keratoderma & ichthyosis, neurological manifestations	No	No	Symptomatic
ARKID	–	*VPS33B*	AR	Progressive hearing loss, diffuse PPK, flexion deformities, constrictive bands	No	No	
PPK, leukonychia, exuberant scalp hair	–	*FAM83G*	AR	Diffuse, verrucous thickening, soles > palms	No	No	
FOCAL PPK: NO ASSOCIATED FEATURES							
PPKS1 PPKS2 PPKS3	148700 612908 607654	*DSG1* *DSP1* *KRT1*	AD AD –	Linear bands of hyperkeratosis on palm. Plantar surface typically focal and precede palms.	No	No	Keratolytics
FOCAL PPK: ASSOCIATED FEATURES							
TOC	148500	*RHBDF2*	AD	PKK by 8 years of age, follicular hyperkeratosis/oral leukokeratosis (cf PC). Oesophageal carcinoma – 95% risk by 65 years of age	No	No	Screening of carcinoma Avoid smoking and alcohol
Tyrosinaemia type II	2766000	*TAT*	AR	Ocular symptoms – photophobia and scarring, hyperkeratosis of dermatoglyphs → focal PPK	No	No	Phenylalanine/tyrosine free diet
PC	Multiple MIM#	*KRT6A,6B, 6C, 16,17*	AD	90% toenail dystrophy, PPK and plantar pain. Nail dystrophy in early life followed by plantar keratoderma when weight bearing	No	Polymicrobial sometimes	Mechanical debridement Acitretin – low dose Botox Rapamycin
HOPP	607658	–	–	Similar to PLS/HMS – CTSC mutation not seen. Progressive hypotrichosis and lingua plicata may be noted	No	No	–
PPK-deafness syndromes	Multiple MIM#	*GJB2 (GJB6)*	AD	PPK with hearing loss. Vohwinkel syndrome – marginal translucent papules, constrictive bands	No	No	Acitretin/surgery – constrictive bands
PPK & Cardiomyopathy	601214 (Naxos) 605676 (Carvajal)	*JUP* *DSP*	AR AR/AD	Woolly hair at birth with diffuse/striate PPK. Naxos – cardiomyopathy in adolescence. Carvajal – cardiomyopathy earlier in life	No	No	Woolly hair and PPK → cardiac investigations for patient and family

Table I. Contd.

Type	MIM(#)	Gene	Inheritance	Features	Trangrediens	Superinfection	Specific Treatment
PAPULAR HPPK: NO ASSOCIATED FEATURES							
Punctate PPK	148600	*AAGAB*	AD	Teenage years, papular lesions that coalesce, worse I manual labourers	No	No	Mechanical debridement Footwear Retinoids
	614936	*COL14A1*	AD				
Marginal papular keratoderma	–	–	(AD)	AKE – crateriform papules on 'Wallace's' lines FAH – knuckles pads & hyperkeratosis extending up Achilles tendon	No	No	–
TAK	–	–	–	White papular palmar eruption after a few minutes exposure to water. Minimal hyperkeratosis on drying	No	No	–
PAPULAR HPPK: NO ASSOCIATED FEATURES							
Cole disease	615522	*ENPP1*	AD	Congenital/early punctate keratoderma. Well-defined hypopigmented macules, calcification	No	No	–
PLACK syndrome	616295	*CAST*	AR	Peeling skin, acral keratoses, leukonychia, knuckle pads	No	No	–

PPK: palmoplantar keratoderma; HPPK: hereditary PPK; AD: autosomal dominant; AR: autosomal recessive; XLR: X-linked recessive; EPPK: epidermolytic PPK; NEPPK: non-epidermolytic PPK; MDM: Mal de Meleda; LK: loricrin keratoderma; KLICK: keratosis linearis with ichthyosis congenita and sclerosing keratoderma; SCC: squamous cell carcinoma; OODD: odonto-onycho-dermal dysplasia spectrum; SPSS: Schöpf-Schulz-Passarge syndrome; OLS: Olmsted syndrome; PLS: Papillon-Lefèvre syndrome; HMS: Haim-Munk syndrome; CEDNIK: cerebral dysgenesis: neuropathy, ichthyosis and PPK syndrome; ARKID: AR keratoderma ichthyosis and deafness; PPKS: Striate PPK; TOC: Tylosis with oesophageal cancer; PC: Pachyonychia congenita; HOPP: Hypotrichosis-osteolysis-periodonitis-PPK syndrome; AKE: acrokeratoelastoidosis; FAH: focal acral hyperkeratosis; TAK: transient aquagenic keratoderma.

ding the palms and soles, to form intermediate filaments which provide strength to the skin (7, 8).

This PPK develops in infancy and in adults the hyperkeratosis is brown-yellow and confluent with fissuring, confined to the palmoplantar surfaces with an erythematous edge. Limited transgradient lesions or flexural hyperkeratosis may indicate *KRT1* mutations (9). There may be a history of blistering and knuckle pads have been reported.

Treatment is mainly by mechanical debridement and use of keratolytics like urea, salicylic acid and lactic acid in emollient, sometimes under occlusion. Oral retinoids can help but pain from increased fragility limits their use (10, 11). Topical calcipotriol has been reported to be of benefit (12). Small inhibitory RNA therapy may be a possibility for the future (13).

DIFFUSE NON-EPIDERMOLYTIC PALMOPLANTAR KERATODERMAS

Non-epidermolytic PPK type Bothnia (MIM# 600231, *AQP5*) was first described in Northern Sweden and is due to heterozygous missense mutations in *AQP5* (14). This gene encodes for the water-channel protein aquaporin-5, which is expressed in exocrine glands but also the plasma membrane of palmar stratum granulosum. Mutations in the gene allow these cells to transport water by forming open water channels at this site.

This PPK usually starts in the first few months of life and is classically a brown-yellow, smooth keratoderma with an erythematous edge. Due to the defect in aquaporins, water immersion leads to a white spongy appearance which lasts for about 30 min. Pitted keratolysis and dermatophyte superinfection is common and can be treated with topical erythromycin or oral anti-fungals (15). Acitretin at low doses can be helpful.

NEPPK type Nagashima (MIM# 615598, *SERPINB7*) is an autosomal recessive (AR) PPK due to mutations in *SERPINB7* described in Japanese and Chinese patients. Mutations in this gene may cause uncontrolled activity of proteases in the stratum corneum leading to increased water permeation (16, 17).

The condition presents in early life and is characterised by mild hyperkeratosis and striking redness extending to the dorsum fingers/feet and anterior wrist (18). A white spongy appearance after water immersion is seen (19) and associated hyperhidrosis and bacterial/fungal superinfection can be present.

Mal de Meleda (MDM; MIM#248300, *ARS*) is an eponymous AR PPK named after the Island of Mljet (née Meleda) (20). Mutations in *ARS* which encodes SLURP-1 cause MDM (21). SLURP-1 stimulates nicotinic acetylcholine receptors which regulate keratinocyte growth. When SLURP-1 is not functioning, it is thought that there is a reduction in keratinocyte apoptosis regulation (22).

MDM is characterised by a diffuse, ivory-yellow macerated hyperkeratosis with a characteristic malodour and

striking erythematous trangradiens that extends to dorsal surfaces. A key feature includes lesions on the elbows and knees (21). Perioral hyperkeratosis and erythema can be present (23). The disease starts in infancy and progresses through life. Flexion contractures can occur and constrictive bands can lead to spontaneous amputation (24). Nail thickening, subungual hyperkeratosis and koilonychia can be present. The diffuse keratoderma of Gamborg-Nielsen also due to *ARS* mutations is likely a mild variant of MDM (25). Interestingly female heterozygotes can also present with a mild phenotype (26).

Treatment of bacterial/fungal superinfection and the hyperhidrosis is helpful, although the mainstay of treatment is oral retinoids which improve the hyperkeratosis although the erythema may worsen (27, 28).

DIFFUSE HEREDITARY PALMOPLANTAR KERATODERMAS: WITH ASSOCIATED FEATURES

Loricrin keratoderma (LK; MIM# 604117, *LOR*) is AD and starts in early childhood. It is due to a mutation in *LOR* which interferes with the regulation of epidermal cornification (29). Some children are born with a collodion membrane and generalised scaling from birth may be noted (30). During childhood the PPK develops with a characteristic diffuse, honeycomb pattern which can extend to wrist/ankles and is associated with non-migratory red plaques on the extensor surfaces of joints (31). Trangradiens is present but the edges of the hyperkeratosis are ill-defined. Constrictive bands can develop. Knuckle pads may be present (32) and hearing is intact.

Isotretinoin has been reported as helpful (33). In the future, there may be a role for treating LK with vascular endothelial growth factor 2 receptor inhibitors (34).

Keratosis linearis with ichthyosis congenita and sclerosing keratoderma (KLICK, MIM#601952, *POMP*) presents in early childhood and similar to LK, starts with generalised erythema and fine scaling with the subsequent diffuse, smooth PPK (35). Inheritance of this PPK is AR caused by mutations in the *POMP* gene, which lead to endoplasmic reticular stress and subsequent dysfunctional profilaggrin processing (36, 37). Flexural linear and starfish keratoses overlying large joints are distinctive (35). Acitretin can be helpful for both the ichthyosis and keratoderma (38).

PPK with scleroatrophy (Huriez syndrome, MIM #181600) is a cancer-related PPK caused by haploinsufficiency in *SMARCAD1* (39). Scleroatrophy is seen across the entire palm and fingers (40) with mild hyperkeratosis of the palms. The affected skin is often red and the palms are usually more severely affected than the soles. Hypoplastic nail changes may be present and 50% experience hypohidrosis. The most important characteristic is the 100-fold increased risk of developing squamous cell carcinoma (SCC) in the affected skin. Acitretin may be helpful for the PPK and prevention of SCC (41).

Palmoplantar hyperkeratosis with squamous cell carcinoma of skin and sex reversal (MIM#610644, *RSPO1*) is similar to Huriez syndrome as it is a mild PPK with sclerodactyly and nail hypoplasia (42). This condition is AR caused by mutations in *RSPO1* (43). This gene is responsible for stabilising β-catenin in the Wnt signaling pathway which antagonises SRY/SOX9 actions for sex determination (44). A characteristic feature is the female to male sex reversal seen in females. The karyotype is 46, XX. Predisposition for cutaneous SCC and also laryngeal SCC is noted (45). Periodontitis with loss of teeth may be present.

Odonto-onycho-dermal dysplasia spectrum (OODD) is an AR condition caused by mutations in *WNT10A* which starts in early life (46). In absence of WNT10A, β-catenin pathway activity and epithelial progenitor proliferation are reduced. In these patients, Wnt-active stem cells are seen in sweat ducts, hair follicles, nails and taste buds and there are differentiation abnormalities in palmoplantar skin (47).

Typically the PPK is mild, diffuse and erythematous with late onset palmoplantar hyperhidrosis (48). There is overlap with Schöpf-Schulz-Passarge syndrome (SSPS) and patients can have hypodontia with abnormal teeth, nail hypoplasia, smooth tongue and hypotrichosis (49). Eyelid hidrocystomas and other benign adnexal tumours can present at a later age (50). Biopsy of palmoplantar skin shows eccrine syringoadenomatosis (46). Tumours/cysts may need treatment with surgery or laser.

Olmsted syndrome (OLS; MIM# 614594 - *TRPV3*, 300918 - *MTBSP2*) typically presents as a severe mutilating transgradient keratoderma. AD, AR, semi-dominant and X-linked recessive (XLR) forms have been described caused by mutations in *TRPV3* (AD, AR) and *MBTPS2* (XLR) (51, 52) The Ca^{2+}-permeable cation channel TRPV3 is expressed abundantly in keratinocytes, associated with TGF-α/EGFR signalling and may play a role in keratinocyte differentiation by elevating Ca^{2+} within these cells (53). MBTPS2 mutations in skin may cause a decrease in responsiveness to sterols subsequent to depletion of proteases (54).

The keratoderma is diffuse and can be associated with digital flexion deformities and constrictive bands. Periorificial/ear/nose/umbilical keratoses can also be present. Dystrophy of teeth, nails and cornea, alopecia, erythromelalgia and joint laxity have also been reported. A milder phenotype can simulate pachyonychia congenita (PC) (55). Melanoma and SCC have been reported in OLS (56).

Treatment in general is difficult with variable response to systemic retinoids. Topical anti-inflammatories can be helpful for hyperkeratosis and itching. Surgery with excision and grafting of the keratoderma can lead to more favourable long-term outcomes (2). Finally, there has been one report of a patient treated with the EGFR inhibitor, erlotinib, which gave a transient improvement (57).

PPK with periodontitis (MIM#245000, allelic disease: Haim-Munk #245010, *CTSC*) encapsulates both Papillon-Lefèvre syndrome (PLS) and Haim-Munk syndrome

(HMS). Both conditions are caused by homozygous mutations in *CTSC*. CTSC is expressed in the palms, soles, alveolar bone and keratinized gingiva; it plays a role in immune cell protease activation and possibly has a role in epidermal differentiation leading to this particular phenotype (58, 59).

Patients have thickening and erythema of the palmoplantar skin, associated with bacterial skin infections and periodontitis (60). The PPK typically starts/worsens with the breakthrough of the deciduous teeth and actually improves after tooth loss/reduction of gingival inflammation (61). Hyperkeratotic plaques on the extensor surfaces are seen. PLS is associated with pyogenic liver abscesses (62). HMS has the same features with arachnodactyly, onychogryphosis and acro-osteolysis, mainly described in Cochin Jews (63).

Retinoids have shown to improve the PPK and oral disease (62). Specialist dental care is essential. For those above the age of 12, low dose tetracycline may be helpful for gingivitis, even at subtherapeutic doses (64).

Cerebral dysgenesis, neuropathy, ichthyosis and PPK syndrome (CEDNIK; MIM#609528, *SNAP29*) is a PPK with neurological manifestations which starts in infancy. This AR condition is caused by mutations in *SNAP29* which lead to abnormal lamellar granule formation with subsequent aberrant epidermal differentiation (65). Around one year of age a diffuse keratoderma and ichthyosis become apparent (65, 66). Histology of CEDNIK demonstrates clear vesicles in the top 3 layers of the epidermis. Treatment is symptomatic.

Autosomal recessive keratoderma ichthyosis and deafness (ARKID) is caused by mutations in *VPS33B*. Mutations in this gene can lead to abnormal lamellar body morphology and function and impaired barrier formation. Patients present with progressive hearing loss (normal at birth) and delayed development. The PPK that develops is diffuse and associated with flexion deformities and autoamputation (67).

PPK, leukonychia and exuberant scalp hair is caused by AR mutations in *FAM83G*. FAM83G may have a role as a suppressor of the Wnt signalling pathway. Diffuse, verrucous thickening of soles and mild palmar involvement is noted. Leukonychia/dystrophy of the toenails and rapid hair growth are also seen (68).

FOCAL HEREDITARY PALMOPLANTAR KERATODERMAS: NO ASSOCIATED FEATURES

Striate PPK (PPKS) can be separated into PPKS1 (MIM# 148700, *DSG1*) (69), PPKS2 (MIM# 612908, *DSP1*) (70), and PPKS3 (MIM# 607654, *KRT1*) (71). The *DSG1* (desmoglein 1) and *DSP1* (desmoplakin) genes encode for desmosomal proteins required for intercellular adhesion of keratinocytes (72). Mutations in the V2 domain of *KRT1* cause PPKS3 and disrupt the intermediate filament network.

Classically, striate PPK presents with linear bands of hyperkeratosis on the palmar surface (73). Diffuse or focal changes may also be present. It is usual for plantar changes to be focal and to present early in life (i.e. first or second year) and the palmar changes follow (71). If patients exhibit woolly/curly hair or abnormal dentition associated cardiomyopathy should be considered. Histology in PPKS can be helpful as it will demonstrate acantholysis of keratinocytes pointing to a desmosomal mutation (74).

FOCAL HEREDITARY PALMOPLANTAR KERATODERMAS: WITH ASSOCIATED FEATURES

Tylosis with oesophageal cancer (TOC; MIM#148500, *RHBDF2*) is rare condition that is AD and caused by gain of function mutations in *RHBDF2* which create a hyperproliferative phenotype through continuous EGFR signalling (75). Patients present with focal keratoderma at sites of pressure usually by 8 years of age. Patients also have follicular hyperkeratosis and oral leukokeratosis similar to PC (76). Most patients with tylosis have a family history of oesophageal carcinoma and carry a risk of oesophageal cancer of 95% by age 65 years (77). Regular screening for oesophageal dysplasia is required and smoking and alcohol should be avoided.

Tyrosinaemia type II (MIM#276600, *TAT*) (78) is a very rare AR condition that initially presents in the first few months of life with ocular symptoms including photophobia and pain and subsequent ocular scarring (79). Hyperkeratosis of the palms that follows the fingerprints develops prior to a focal plantar keratoderma (80). About 50% of patients will have some form of intellectual disability and neurological signs. Increased tyrosine levels found in the bloods/urine, due to abnormal tyrosine aminotransferase function, can aid diagnosis (79) and symptoms may be prevented by a phenylalanine/tyrosine free diet.

Pachyonychia congenita (PC; Multiple MIM#) is a heterogeneous groups of conditions characterised by nail dystrophy and a painful focal keratoderma. The Pachyonychia Project (www.pachyonychia.org) has collected extensive data on PC collated in the International PC Research Registry (IPCRR). The current classification is based on keratin gene mutation: PC-6a, PC-6b, PC-6c, PC-16 and PC-17 (81). These 5 subtypes have replaced the original PC type 1 and 2 classification. Mutations in these genes lead to increased palmoplantar skin fragility due to disruption of keratin filament formation, nail changes and changes in the pilosebaceous unit.

The IPCRR data has shown that 90% of patients > 3 years old will have 3 clinical features: toenail dystrophy, plantar keratoderma and plantar pain (82). The hypertrophic nail dystrophy starts in the first few months of life up to 9 years. *KRT6A* mutations are associated with early onset disease. All nails need not be affected. The focal plantar keratoderma starts when children begin to weight bear with blistering under the calluses (83). Plantar pain

has a neuropathic component and can be severe enough to require ambulatory aids. The palmar lesions are usually less prominent than the plantar lesions, except in the case of PC-KRT16 with striate lesions (82).

Follicular hyperkeratoses are seen in areas of friction. Oral leukokeratosis can resemble oral candidiasis and laryngeal involvement can lead to hoarseness and infantile respiratory obstruction (83). Cysts occur in all subtypes of PC although *KRT17* mutations are usually associated with more steatocystomas/pilosebaceous cysts and natal teeth (less commonly *KRT6A*) (83). *KRT6A* mutations can also be associated with ear pain with feeding difficulties in infants/toddlers. PC-6C has a limited keratoderma and mild nail dystrophy (84).

Current treatment is largely mechanical paring of the calluses, assisted by a podiatrist, if necessary. Low dose acitretin can help in some patients but is associated with increased pain. Botox injections can also help reduce pain (85). The IPCC has had some promising results with siRNA and rapamycin (86, 87). Clinical trials of topical rapamycin are ongoing. As with most PPKs, comfortable foot wear and customised insoles are helpful.

Hypotrichosis-osteolysis-periodontitis-palmoplantar keratoderma syndrome (HOPP; MIM 607658) is a rare syndrome with a phenotype similar to PLS/HMS although CTSC mutations were not found. There is a striking keratoderma with a reticular pitted/punctate pattern (88). Progressive hypotrichosis from 6 years of age is seen sometimes with pili annulati. Lingua plicata can be noted at an early age (89).

PPK-deafness syndromes are mainly caused by *GJB2* and rarely *GJB6* mutations. Numerous gap junctions are present in the skin and inner ear. Mutations in gap junction genes lead to abnormal keratinocyte differentiation/growth and dyfunctional inner ear potassium ion recycling required for hearing (90).

Phenotypically these PPK-deafness syndrome are distinct and still carry their eponymous names. Despite having mutations in the same gene, keratitis-ichthyosis-deafness-like (MIM# 148210), hystrix-like ichthyosis-deafness (MIM#602540), palmoplantar keratoderma-deafness (MIM#148350), Bart-Pumphrey (MIM# 149200) and Vohwinkel (MIM#124500) syndromes have phenotypic differences likely explained by mutations in particular domains of connexin 26 (*GJB2*) (91). Cardinal features are PPK and hearing loss of varying severity. For example, Vohwinkel syndrome has marginal translucent papules which become confluent over time. It also has the 'classic' starfish keratoses on the knuckles and extensor surfaces of joints and pseudoainhum (92).

Oral retinoids are helpful for the constricting bands seen in Vohwinkel syndrome (93) but surgery may be required.

PPK and cardiomyopathy are similar to PPKS1&2 as they are also associated with keratinocyte disadhesion. Naxos syndrome (AR) caused by mutations in *JUP* (MIM# 601214) encoding plakoglobin presents with woolly hair at birth followed by a diffuse/striate keratoderma in the first year of life. Cardiomyopathy presents in adolescence and has 100% penetrance (94). Carvajal-Heurta syndrome (CHS), caused by mutations in *DSP* (MIM# 605676), is like Naxos although the cardiomyopathy presents earlier in the teens and is usually biventricular. Some patients with CHS have short woolly hair and keratoses on the elbows/knees (95, 96). The *DSP* and *JUP* genes encode desmosomal proteins required for formation of cell junctions in hair, skin and cardiac tissue (97). Mutations in *KANK2* can cause woolly hair, hypotrichosis and a PPK without cardiac involvement (98). The *KANK2* gene regulates steroid receptor coactivators. Patients with a striate keratoderma/PPK and woolly hair should have cardiac investigations. Family members should also be screened as these can have AR or AD inheritance.

PAPULAR HEREDITARY PALMOPLANTAR KERATODERMAS: NO ASSOCIATED FEATURES

Punctate PPK occurs in 1 in 100,000 people and has AD inheritance. Mutations in the *AAGAB* gene occur in about 1/3 (99). The *AAGAB* gene is involved in recycling of EGFR proteins and impairment in this function leads to keratinocyte proliferation (100). Also, mutations in *COL14A1*, encoding collagen XIV required for fibrillogenesis, have been found in Chinese families (101). Lesions seem to develop after the teenage years. Lesions are typically papular sometimes coalescing into plaques (102). The lesions are worse in manual labourers. Rarely, there is an association with malignancy (99). Treatment with mechanical debridement is helpful. Comfortable shoes are key. Acitretin and alitretinoin can be helpful for some (103).

Marginal papular keratoderma describes acrokeratoelastoidosis (AKE) and focal acral hyperkeratosis (FAH), thought to be inherited in an AD manner. AKE is characterized by small crateriform papules along 'Wallace's' line on the medial aspect of the foot and the border of the palmar thenar/hypothenar eminences (104). FAH, differentiated by the lack of fragmented dermal elastic fibres on histology, is associated with knuckle pads and hyperkeratosis extending onto the Achilles tendon (105) presenting in the teenage years in individuals of African or Afro-Caribbean ethnicity.

Transient Aquagenic keratoderma (TAK) is an unusual keratoderma that mainly effects the palms and is triggered by contact with water or sweat. Patients are typically young women and after a few minutes of exposure to water a fine white papular eruption is present on the palms (106). The eruption resolves after drying, leaving minimal hyperkeratosis. TAK can be differentiated from hereditary papulotranslucent acrokeratoderma (MIM 101840) as the papules in TAK do not persist. Aquagenic wrinkling of the palms, seen in 50% of patients with cystic fibrosis and 10% of *CFTR* mutation heterozygotes, can also look similar (107).

PAPULAR HEREDITARY PALMOPLANTAR KERATODERMAS: WITH ASSOCIATED FEATURES

Cole disease (MIM# 615522, *ENPP1*) is a very rare genodermatosis characterised by congenital or early-onset punctate keratoderma (108). The condition can be AD and AR and is due to *ENPP1* mutations which impair homodimerization of the ENPP1 protein leading to impaired melanocyte regulation and function (109).

Over time children develop well-defined hypopigmented macules which are most prominent on the extremities. Cases of associated calcinosis cutis or tendon calcification have been reported.

PLACK syndrome (MIM# 616295, *CAST*) is an AR disorder characterised by peeling skin, acral keratoses, leukonychia, cheilitis and knuckle pads causes by mutations in *CAST* which causes dysregulation of keratinocyte adhesion and apoptosis (110).

CONCLUSION

The PPK are a heterogeneous group of conditions with a biologically fascinating diversity of genetic mutations. Modern sequencing techniques have aided our ability to re-classify these conditions. Targeted gene sequencing and keratoderma specific gene panels will aid in confirming diagnoses.

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Centenary theme section: GENODERMATOSES

REVIEW ARTICLE

Molecular Genetics of Keratinization Disorders – What's New About Ichthyosis

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The heritable forms of keratinization disorders, including various forms of ichthyosis and keratodermas, comprise a phenotypically heterogeneous group of diseases which can be divided into syndromic and non-syndromic forms. In the non-syndromic forms, the clinical manifestations are limited to the cutaneous structures while the syndromic ones are associated with a spectrum of extracutaneous manifestations. The inheritance in different families can be autosomal dominant, autosomal recessive or either X-linked dominant or recessive. Currently at least 67 distinct genes have been associated with different forms of ichthyosis. These genes can be grouped on the basis of their physiological involvement, including genes encoding structural components of epidermis, those involved in epidermal lipid metabolism, or those critical for cell-cell adhesion, and keratinocyte differentiation. This overview highlights some of the recent progress made in understanding the molecular genetics of keratinization disorders, and presents selected, recently characterized cases as representative of different forms of heritable ichthyosis.

Key words: autosomal recessive congenital ichthyosis; ichthyosis; non-alcoholic fatty liver disease; non-syndromic ichthyosis; syndromic ichthyosis.

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SIGNIFICANCE

Patients with ichthyosis manifest with dry and scaly skin, with considerable phenotypic variability. The heritable forms of ichthyosis are associated with mutations in over 60 different genes which encode proteins critical for normal physiological function of the skin. This overview highlights some of the new findings in the genetics of heritable forms of ichthyosis and emphasizes the connection of skin findings to extracutaneous manifestations, in some forms of the syndromic ichthyosis. The presentation also emphasizes the importance of determining the specific mutations in the underlying genes, which allows subclassification of the patients into distinct categories, with the capability to prognosticate the severity and the overall outcome of the disease in general terms. The knowledge of mutations in specific genes is also required for application of allele-specific therapies being developed for this group of disorders currently without specific treatments.

The heritable forms of ichthyosis, also known as generalized Mendelian disorders of cornification (MeDOC), comprise a heterogeneous group of diseases caused by mutations in a number of genes that are critical for development and maintenance of physiologic barrier at the outer layer of epidermis (1, 2). Clinically, these disorders manifest with scaling and hyperkeratosis of varying degrees (**Fig. 1**). The pathologic findings in the patients are either limited to the cutaneous structures or are associated with extracutaneous manifestations. Thus, ichthyosis can be divided into two broad categories, the non-syndromic and the syndromic forms. These disorders are present usually at birth or are diagnosed shortly thereafter, but the progression of the disease and the eventual outcome of severity can be highly variable. In the most severe forms, such as the Harlequin ichthyosis (HI), the affected children often die during the early neonatal period while at the other end of the spectrum of severity, such as ichthyosis vulgaris (IV), the manifestations can be relatively mild, the onset of manifestations may occur later in life and the spectrum may represent a continuum with physiologically present dry skin. The longevity of the IV patients is rarely affected by the disease. Also, in some cases the scaling and hyperkeratosis present at birth can be self-healing within a few months' timeframe, as in so-called self-improving collodion ichthyosis (1–6).

Various forms of ichthyosis were initially classified on the basis of predominant clinical manifestations, and early on, different subtypes often carried eponyms of the authors of the original descriptions. More recently, it has been recognized that clinical heterogeneity, coupled with the variable mode of inheritance, i.e., autosomal dominant, autosomal recessive, or X-linked dominant or recessive, largely reflects the genetic heterogeneity, and currently as many as 67 distinct genes have been shown to be associated with different forms of ichthyosis. In addition, there are as many as 28 mutant genes associated with palmoplantar keratodermas. Grouping of the

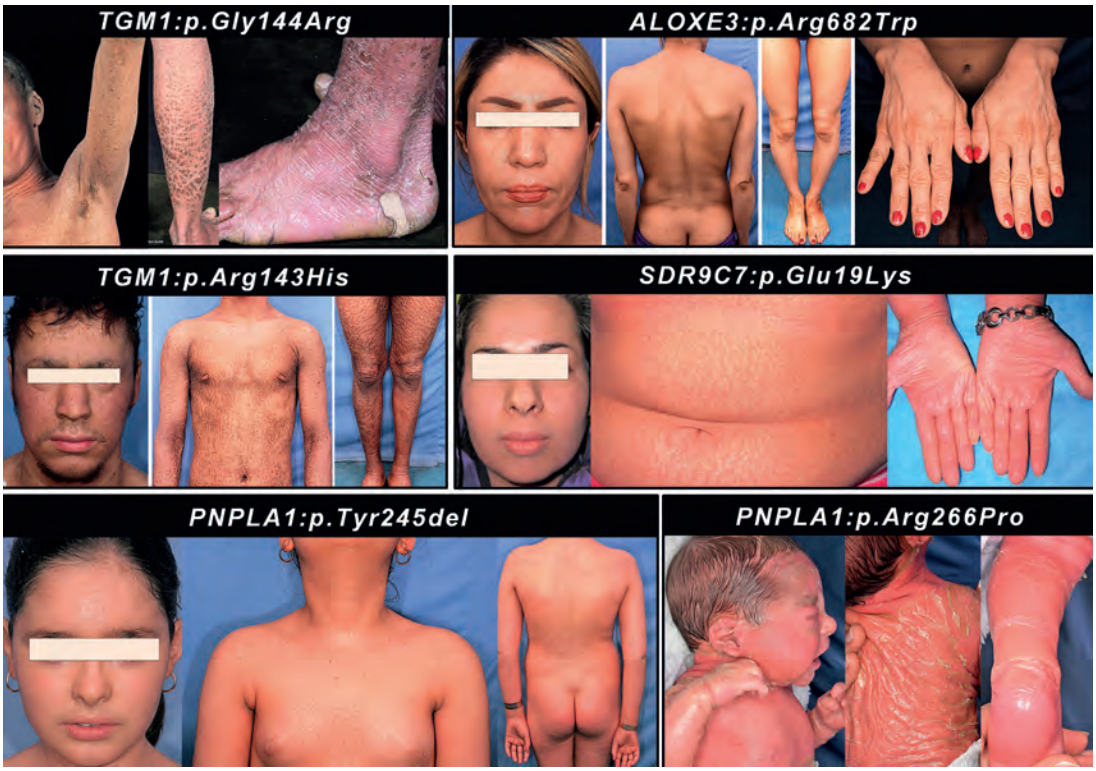

Fig. 1. Phenotypic variability in patients with autosomal recessive congenital ichthyosis associated with defects in different genes. Note the spectrum of severity in association of specific mutations indicated.

genes based on their involvement in distinct biological pathways required for physiological differentiation of keratinocytes and maintenance of the epidermal barrier has resulted in a more granular classification which allows refined diagnostics and nuanced prognostication in general terms (2). In such classification, specific subgroups of syndromic and non-syndromic forms of ichthyosis can be recognized, based on identification of mutations in genes encoding structural components of epidermis, involved in epidermal lipid metabolism or critical for cell-cell adhesion and keratinocyte differentiation as well as homeostasis, essential for formation of functional stratum corneum with uncompromised barrier function.

NEXT-GENERATION SEQUENCING APPROACHES FOR MUTATION DETECTION

As in case of most heritable disorders with extensive genetic heterogeneity the identification of mutations in different forms of ichthyosis was initially based on PCR amplification of exons and flanking intronic sequences in candidate genes identified by clinical observations or by immunofluorescent and ultrastructural examination of epidermis. However, with expanding number of candidate genes associated with keratinization disorders, this approach has proven time-consuming and expensive. The PCR-based approaches are rapidly being replaced by next-generation sequencing (NGS) techniques, including sequencing arrays simultaneously targeting multiple disease-associated genes or the use of whole exome sequencing (WES) and whole genome sequencing (WGS) (7–9). These approaches are assisted by genome-wide tools, including homozygosity mapping (HM) and transcriptome profiling by RNA-seq, which facilitate identification and verification of pathogenic mutations in affected families (10–13).

EXPANDING MUTATION LANDSCAPE OF NON-SYNDROMIC KERATINIZATION DISORDERS: THE PARADIGM OF ARCI

A subgroup of non-syndromic forms of ichthyosis, autosomal recessive congenital ichthyosis (ARCI), is clinically divided into different subcategories: (a) Harlequin ichthyosis (HI), (b) lamellar ichthyosis (LI), and (c) congenital ichthyosiform erythroderma (CIE). HI is a rare and often severe form of ARCI caused by mutations in the *ABCA12*, while LI and CIE, with partially overlapping phenotypes, have been associated with mutations in a total of 14 distinct genes, many of them involved in lipid metabolism and essential for formation of functional stratum corneum. The different genetic subtypes were initially defined by the genomic locations of the corresponding genetic loci (ARCI1-17). The 4th subgroup of ARCI comprises phenotypically variable forms of ichthyosis which can manifest with marked hyperkeratosis at birth, but significant spontaneous improvement during infancy can result in a relatively mild disease in the

adulthood (1–5, 14). This group, known as pleomorphic ichthyosis (14), consists of clinically distinct conditions, such as self-improving collodion ichthyosis, ichthyosis prematurity syndrome and congenital ichthyosis with fine/mild scaling (5, 7, 8). Bathing-suit ichthyosis, also a pleomorphic ichthyosis, is a condition in which arms and legs are not affected by abnormal keratinization (15). In addition, clinically defined forms of ichthyosis include peeling skin syndrome, erythrokeratoderma variabilis (EKV), loricrin keratoderma and congenital reticular ichthyosiform erythroderma. Ichthyosis is often associated with development of palmoplantar keratoderma, a heterogeneous group of disorders which can also present as distinct clinical entities without ichthyosis (see Thomas & O'Toole, 16).

In a recent study by us, the molecular basis of a total of 125 families with diagnostic features of ARCI was probed by a NGS array targeting 38 genes that were at the time known to be associated with different forms of ichthyosis (17). This approach, assisted by homozygosity mapping in this cohort which was characterized by high degree of consanguinity, followed by whole transcriptome analysis by RNA-Seq, identified definitive pathogenic/likely pathogenic mutations in approximately 85% of the families (for examples, see **Fig. 2**). Similar results have been reported in studies examining regional cohorts of families with ARCI, including patients in Czech Republic, England, Israel, Italy, Turkey and the Scandinavian countries (17–22). While some differences in the prevalence of mutations in different genes were observed in different cohorts, mutations in the *TGM1* gene encoding transglutaminase 1, as well as in a number of genes involved in lipid metabolism (*ABCA12, ABHD5, ALOX12B, ALOXE3, CERS3* and *PNPLA1*) were frequently encountered (23, 24). In some populations, *PNPLA1* and *CERS3* mutations are very rare while in others, particularly those with high degree of customary consanguinity, the proportion of these two genes was relatively high (25–29). Interestingly, while *ABCA12* was initially associated with severe, often lethal HI, mutations in this gene can also be encountered in LI and CIE (30). It should be noted that many of the mutations found in different genes are private and population-specific, emphasizing the importance of ethnic-based molecular diagnostics when assessing its impact on the public health in different countries and geographic regions.

The most recent discoveries of ARCI-associated genes include *SDR9C7* and *SULT2B1*. Initially described in 2016 in affected members of 3 consanguineous Lebanese families with congenital ichthyosis, *SDR9C7* was mapped to chromosome 12q13-q14 (ARCI13) (29). This gene encodes short-chain dehydrogenase/reductase family 9C member 7 (SDR9C7) and is highly expressed in the granular and cornified layers of the epidermis. Subsequently, a 1-bp duplication co-segregated in a Turkish family

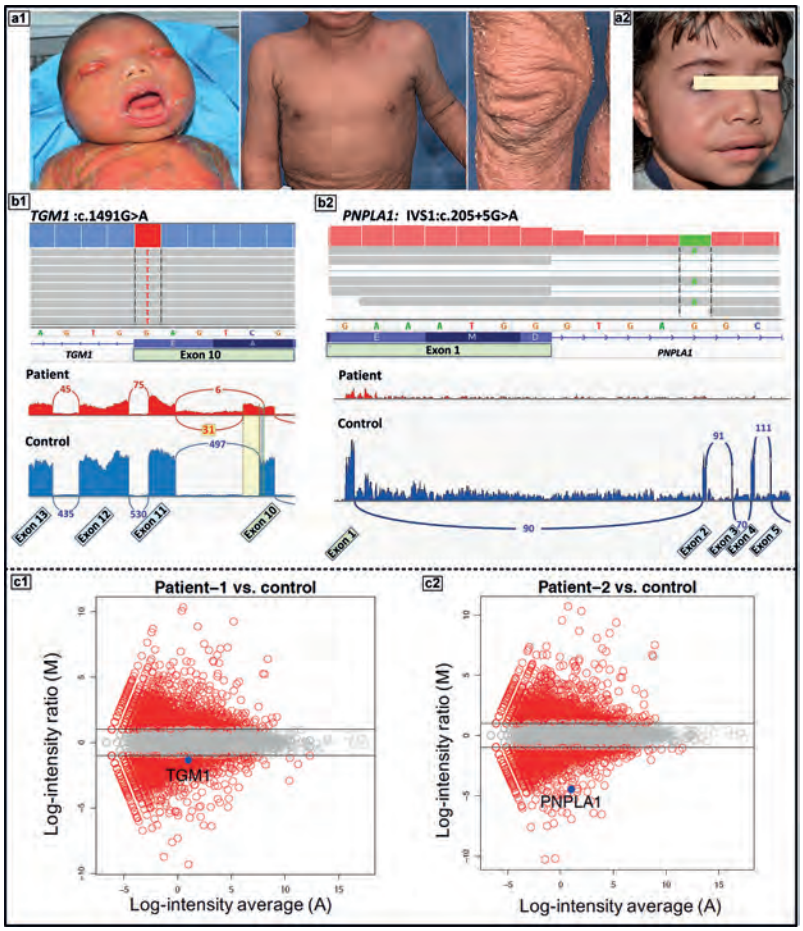

Fig. 2. Utility of RNA-based sequencing in identification and confirmation of a homozygous synonymous/splice-site mutation in *TGM1* (Patient-1) and a non-canonical splicing variant in *PNPLA1* in Patient-2. (a1) The proband, a female at neonate age (left panel) and at 3 years of age (right panel) showing extensive scaling and hyperkeratosis, harboring a mutation in *TGM1*. (a2) A 4-year-old female with generalized fine scaling and hyperlinearity of face harboring a mutation in *PNPLA1*. (b1) Screen shot of the genomic sequence visualized by the Integrative Genomics Viewer demonstrating a homozygous mutation of *TGM1*: c.1491G>A at the border of exon10/intron 10 (upper panel). Sashimi plot of RNA-Seq reveals that this homozygous synonymous mutation results in aberrant splicing and partial intron retention (lower panel, red), as compared to splicing in control RNA (blue). (b2) Screen shot of the genomic sequence visualized by the Integrative Genomics Viewer demonstrating a homozygous intronic mutation of *PNPLA1*: IVS1, c.205+5G>A (upper panel). Sashimi plot of RNA-Seq reveals that the homozygous non-canonical mutation in intron 1 results in the absence of *PNPLA1* transcript (lower panel, red) as a result of possible loss of promoter and/or nonsense-mediated decay, as compared to an age- and sex- matched control (blue). (c1) Mean average-plot showing reduction of *TGM1* gene expression (left panel) in Patient 1 (fold change: −1.4), and (c2) reduction of *PNPLA1* gene expression (right panel) in Patient 2 (fold change: −4.5) in comparison to control. (Modified from ref. 17, with permission).

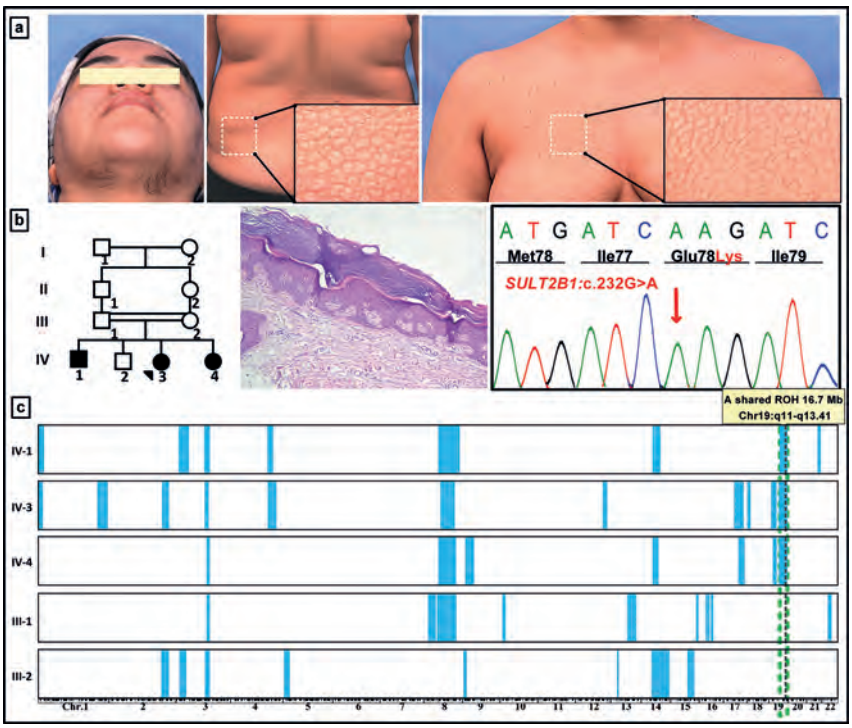

Fig. 3. Utility of homozygosity mapping in gene discovery in a consanguineous family with a *SULT2B1* mutations. (a) The proband, a 32-year-old woman, demonstrated extensive scaling present since birth and hirsutism due to mutations in *SULT2B1*. (b) Pedigree of the three affected individuals with first cousin parents. Histopathology of the proband's skin shows characteristic features of LI. (c) Autosome wide homozygosity mapping identified a region of homozygosity present in all 3 affected individuals (IV-1, 3, 4), but not in their parents (III-1, 2), on chromosome 19. This region harbored the locus for the *SULT2B1* gene, and Sanger sequencing identified a homozygous mutation c.232G>A, p.Glu78Lys, as shown in (b). (Modified from ref. 17, with permission).

was discovered, and as many as 14 mutations have now been reported in populations in Austria, Denmark, France, Germany, Iran, Japan, Sweden, Turkey and the United Kingdom (17, 31–34). Of note, presence of persistent fungal infection was frequently observed in these patients, suggesting that the functional SDR9C7 may physiologically provide protection against such infections.

Mutations in another gene, *SULT2B1*, were initially described in 2017 both in homozygous and compound heterozygous state, mapping to 19q13.3 (ARCI14) in 6 patients from 3 unrelated families (35). Subsequently, two missense mutations in two unrelated families were reported by us (**Fig. 3**) (17). The *SULT2B1* gene encodes a sulfotransferase family cytosolic 2B member 1, expressed in the stratum granulosum-stratum corneum junction in the epidermis.

THE PHENOTYPIC SPECTRUM OF SYNDROMIC FORMS OF ICHTHYOSIS: THE SKIN-LIVER CONNECTION

While the consequences of the mutations in non-syndromic forms of ichthyosis are limited to the skin, a number of cases with cutaneous keratinization disorder in association with extracutaneous manifestations have been reported (1, 2, 36). In such conditions, the pathway disrupted by the mutations in specific genes have consequences not only in the homeostasis of epidermis but also in a number of other tissues. As examples of such conditions serve two syndromes, neonatal ichthyosis associated with sclerosing cholangitis (NISCH) and Chanarin-Dorfman syndrome (CDS), in which in addition to skin, liver can be affected (37, 38). The NISCH syndrome is initially diagnosed with relatively mild ichthyosis at birth, and histology reveals thickening of stratum corneum at the outer layer of the epidermis (**Fig. 4**). Extracutaneous manifestations include hypotrichosis, scarring alopecia, hypodontia and enamel hypoplasia, but a critical element of this syndrome is the involvement of liver with sclerosing cholangitis diagnosed later in life on the basis of hepatomegaly and elevated serum levels of liver enzymes. Mutations in the *CLDN1*, which encodes the tight

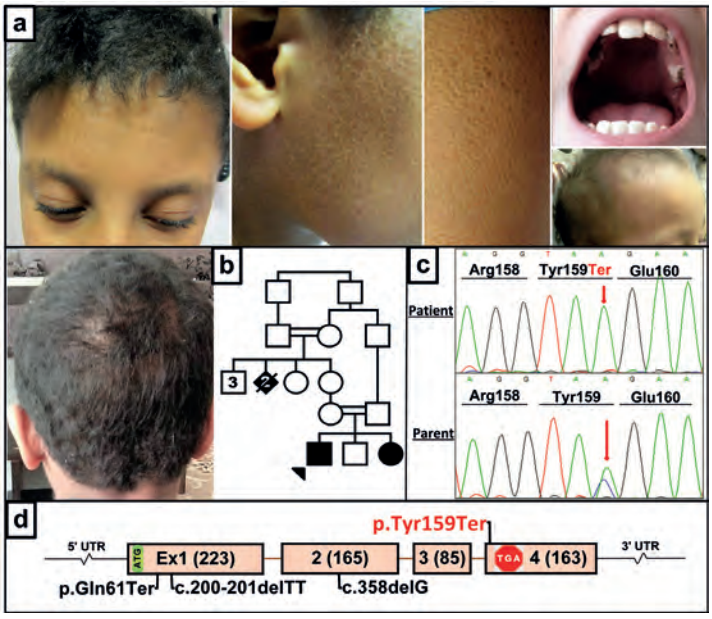

Fig. 4. Clinical features and mutation detection in a consanguineous family with NISCH syndrome with claudin-1 deficiency. (a) The patients with fine scaly ichthyosis and alopecia, oligodontia and enamel hypoplasia. (b) Family pedigree of the affected individuals with consanguineous parents. (c) Gene-targeted next-generation sequencing identified an ultra-rare homozygous p.Tyr159Ter mutation in the patients, verified by Sanger sequencing, the parents being heterozygous carriers (red arrows). (d) Positions of the novel p.Tyr159Ter mutation (red) and those previously reported in the *CLDN1* gene (black). NISCH, Neonatal ichthyosis associated with sclerosing cholangitis. (Modified from ref. 37, with permission).

junction protein claudin-1, has been reported in a limited number of patients with NISCH syndrome (Fig. 4). Thus, congenital presentation of ichthyotic skin lesions together with mutations in *CLDN1* as the molecular confirmation of NISCH syndrome can predict the development of sclerosing cholangitis and liver abnormalities.

In addition to NISCH syndrome, other forms of ichthyosis are associated with liver involvement. An example of such conditions is Chanarin-Dorfman syndrome (CDS) characterized by hepatomegaly and hepatic steatosis, in association with ichthyosis which is readily recognizable during early years of life due to mutations in *ABHD5* (**Fig. 5**). Full-blown CDS with skin and liver findings is inherited in an autosomal recessive fashion due to loss-of-function mutations in *ABHD5* (39, 40). The liver involvement often progresses to hepatic steatosis, liver fibrosis and cirrhosis, and may necessitate liver transplant.

An intriguing genetic constellation was recently recognized in heterozygous carriers of *ABHD5* mutations in CDS families (40). Specifically these individuals had diagnostic features of dyslipidemia and non-alcoholic fatty liver disease (NAFLD), a multifactorial condition and the most common liver disease worldwide, affecting up to one-third of the Western populations (41, 42). The monoallelic loss-of-function mutations, identified in consanguineous families with CDS, resulted in NAFLD in an autosomal dominant inheritance pattern, which was confirmed in a large multi-generation family without consanguinity and with no individuals with biallelic mutations (40). These patients with the heritable form of NAFLD and/or dyslipidemia demonstrated complete clinical expression after the 4th decade of life, and the prevalence of *ABHD5*-associated NAFLD was estimated to be 1:1,137 individuals in general populations (40). Thus, mutations in *ABHD5*, which is involved in neutral lipid

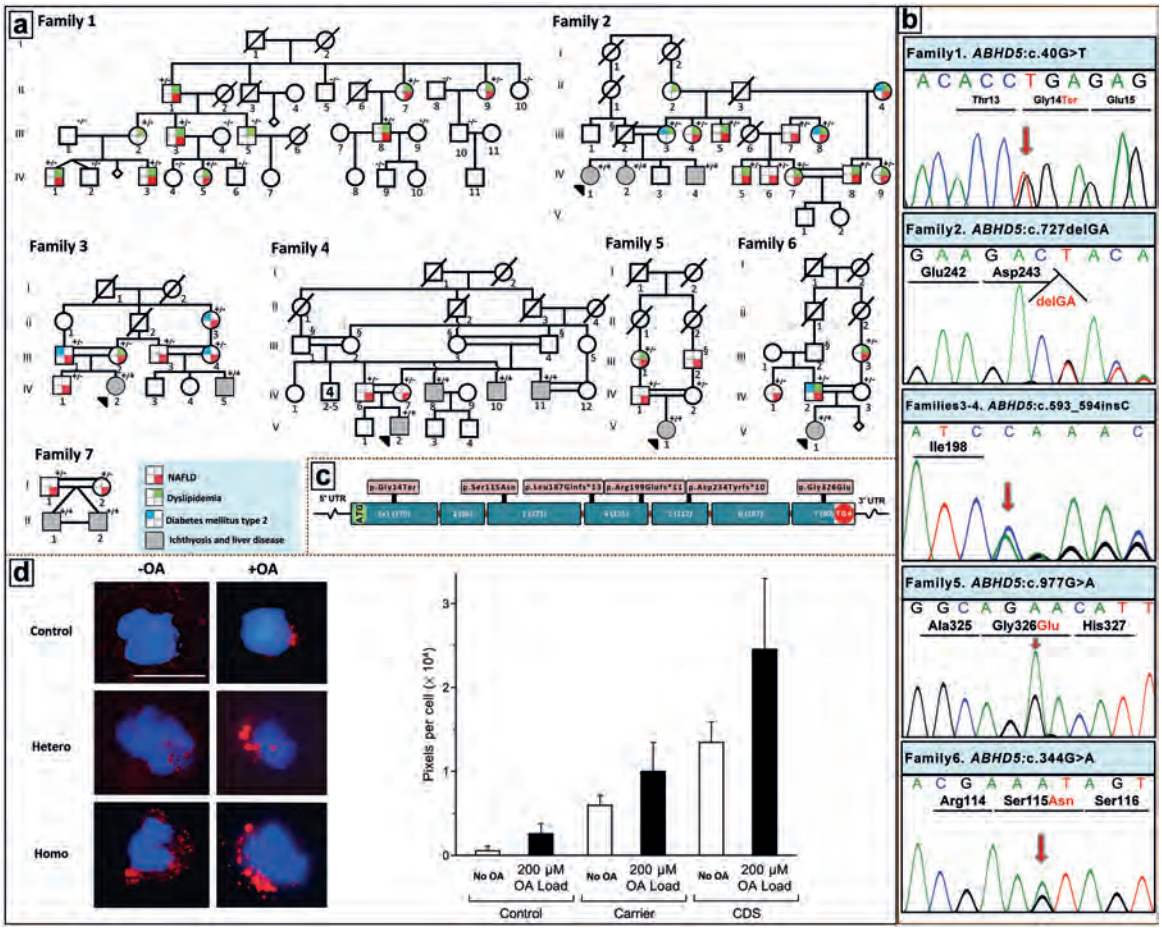

Fig. 5. Pedigree structures and clinical findings in NAFLD families with *ABHD5* mutations. (a) Family 1 is of nonconsanguineous Italian ancestry with a monoallelic mutation in *ABHD5*. Families 2–7 of Iranian ancestry with Chanarin-Dorfman syndrome (CDS) show extensive consanguinity. Heterozygous carriers (+/−) show evidence of NAFLD and/or dyslipidemia and type 2 diabetes mellitus, and patients with biallelic mutations (+/+) manifest with CDS with neonatal ichthyosis and NAFLD. Individuals labeled with § are considered obligatory carriers of the mutation. For the presence of clinical manifestations in individuals tested, see the color code. (b) Sanger sequencing of mutations in Families 1–6. The mutation of Family 7, *ABHD5*:c.560_578 del19 was published previously (61). (c) Positions of the distinct mutations along the *ABHD5* consisting of 7 exons drawn to scale; the introns are not in scale. (d) Presence of lipid droplets (red) in leukocytes from control (upper panels), a heterozygous carrier (middle panel), and a homozygous individual (lower panel) after incubation without (-OA, left) or with 200 μM oleic acid (+OA, right). The lipid content was quantitated by assay of the pixel density of Oil red O and DAPI stained cells (bar graph). The values represent the mean ± SD of 105–125 cells for each sample. CDS, Chanarin-Dorfman syndrome; NAFLD, non-alcoholic fatty liver disease; OA, oleic acid; UTR, untranslated region. (Adapted from ref. 40, with permission).

metabolism, emphasize the pathogenic role of lipid disorders both in NAFLD and in some forms of ichthyosis.

Recent independent studies corroborated the role of CGI-58 (encoded by *ABHD5*) and its partners, such as adiponutrin (encoded by *PNPLA3*) and ATGL (encoded by *PNPLA2*), in the pathogenesis of NAFLD. First, Romeo et al. (43) carried out a genome-wide association study (GWAS) of 9,229 individuals with NAFLD, and they found that a common SNP (rs738409[G]), encoding p.I148M in *PNPLA3*, was strongly associated with increased hepatic fat content (43). These observations have been supported by other studies. For example, Wang et al. showed a direct protein-protein interaction of CGI-58 and adiponutrin (44). In addition, they showed that normal *PNPLA3* overexpression does not enhance lipid accumulation in primary hepatocytes derived from liver-specific *Abhd5*-knockout mice, thus again suggesting that PNPLA3 mediates *ABHD5*-dependent liver steatosis. Finally, Yang et al. showed that *PNPLA3*-I148M allele product in carriers attaches to and sequesters CGI-58 preventing its association with ATGL (Adipose Triglyceride Lipase) in a competitive inhibition fashion (45, 46). ATGL, when associated and activated by CGI-58, is required for the breakdown of triglycerides in the liver and adipose tissue. Thus, in the absence of ATGL available for binding to CGI-58, due to sequestration by PNPLA3-I148M in carriers, triglycerides accumulate in the liver providing a pathomechanistic explanation for hepatic steatosis in NAFLD (47).

GENETIC DEFECTS IN CELL-CELL ADHESION AND COMMUNICATION

In addition to claudin-1, a transmembrane protein in the tight junction complexes which regulates para-cellular permeability in the epidermis (see above), mutations in other genes involved in cell-cell communication have also been associated with aberrant keratinization phenotypes. One of such is *GJB2* encoding connexin 26, previously shown to be mutated in KID syndrome and some forms of palmoplantar keratoderma (see Thomas & O'Toole, 16). Ichthyosis follicularis is a distinct keratinization disorder which has been reported in association with atrichia and photophobia resulting from mutations in the *MBTPS2* gene (48). Recently, however, compound heterozygous mutations in the *GJB2* gene were reported in a novel syndrome of ichthyosis follicularis, bilateral sensorineural hearing loss and punctate palmoplantar keratoderma (10) (**Fig. 6**). One of the mutations (p.Asn176Asp) was demonstrated to significantly reduce the cell-cell gap junction channel activity and to increase the non-junctional hemichannel activity of connexin 26 when tested in Xenopus oocyte expression system (10). This mutation, when associated with a common frameshift mutation in *GJB2* (c.35delG; p.Gly12Valfs*2), frequently documented as a cause of sensorineural hearing loss, resulted in manifestations of this new syndrome, including ichthyosis follicularis phenotype. Collectively, these findings, coupled with previous reports on *GJB2* associations with skin findings, attest to the complexity of clinical consequences of different mutations in *GJB2*.

A number of other genes contributing to cell-cell adhesion and communication have been associated with syndromic forms of keratinization disorders. For example,

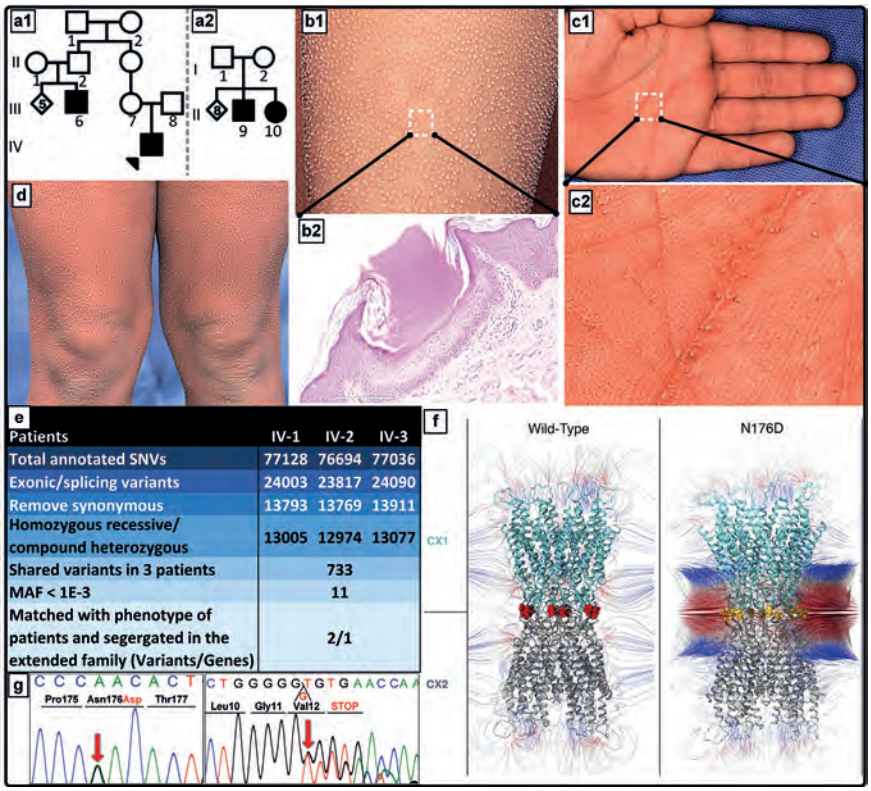

Fig. 6. Example of a novel *GJB2*-associated syndromic form of ichthyosis. Pedigree structure, cutaneous features, histopathology, alteration of the magnetic field and mutations in *GJB2* in families with autosomal recessive follicular hyperkeratosis, PPK, and bilateral sensorineural deafness. (a1, a2) Family pedigrees with autosomal recessive inheritance. (b1, b2) Histopathology of a skin lesion delineated in (b1) revealed a parakeratotic column of hyperkeratotic skin invaginating into epidermis. (c1 and c2). Palm of the proband (IV-1) with hyperkeratosis and accentuated creases which contain punctate pits. (d) Multiple discrete hyperkeratotic projections are centering on hair follicle in widespread distribution, including legs. (e and g). Filtering steps of whole exome sequencing data resulted in identification of compound heterozygous p.Asn176Asp and c.35delG mutations in *GJB2*, followed by Sanger sequencing confirmation. (f) Modeled structure of Cx26 gap junction channels in wild-type (left) and mutant p.Asn176Asp (right). Note the alteration in positive (blue) and negative (red) electrostatic potentials. (Modified from ref. 10, with permission).

the desmosomal proteins *JUP* and *DSP* have both been associated with cardiomyopathy, but cutaneous manifestations, such as palmoplantar keratoderma or hair abnormalities, are highly variable depending on the mutation involved (49, 50). More recently, it was demonstrated that alterations in *PERP*, another component of desmosomes can cause an autosomal dominant Olmsted syndrome or autosomal recessive erythrokeratoderma (51). This observation underscores the genotypic and phenotypic heterogeneity of keratinization disorders: *PERP* mutations confer a spectrum of phenotypes ranging from severe periorificial plaques and palmoplantar keratoderma, as seen in patients with *TRPV3* mutations (52), to varying degrees of erythrokeratoderma observed in patients with mutant *GJB3*, *GJB4*, and *LOR* genes (53–55).

The *PERP* gene consists of 3 exons encoding the p53/p63 tetraspan membrane protein that is expressed primarily in stratified epithelia (56, 57). Although the exact interacting partners of *PERP* are still unknown, its importance in cell-cell adhesion and epithelial integrity was implied by the observation that the majority (95%) of *Perp* knockout (–/–) mice died within 10 days of life due to blistering in the oral mucosa and skin, especially in areas of mechanical trauma, but also showed abnormal thickening of the epidermis (57). The 5% of $Perp^{-/-}$ mice that survived to adulthood had a significantly shorter lifespan compared to $Perp^{+/-}$ and wild-type mice and did not show a predisposition to spontaneous tumorigenesis despite evidence linking p53/p63 to *Perp* expression (56, 58). Nevertheless, with documentation of these cases with *PERP* mutations in humans, clinicians should be aware of this connection and monitor patients accordingly for potential evidence of carcinogenesis.

GENETIC DEFECTS ASSOCIATED WITH KERATODERMAS: THE PARADIGM OF ERYTHROKERATODERMA

Erythrokeratoderma manifests with hyperkeratotic, often transient and migratory erythematous and figurate plaques with sharply demarcated borders typically developing in early childhood (**Fig. 7**). It has been historically divided into two main categories: (a) erythrokeratodermia variabilis et progressiva; and (b) progressive symmetric erythrokeratoderma. However, these two presentations are currently listed on the OMIM catalogue under a single disease entry (OMIM #133200). There are a number of other presentations with erythrokeratoderma (59). Erythrokeratoderma can be inherited either in an autosomal dominant or an autosomal recessive pattern. The autosomal dominant forms have been associated with mutations in the gap junction-related genes (*GJB2*, *GJB3*, *GJB4*, and *GJA1*) as well as in *LOR*, encoding loricrin, a cornified envelope protein (53–55, 59). Autosomal recessive erythrokeratoderma has been associated with mutations in *ABHD5*, *ELOVL4* and *KDSR*. More recently, the genotypic spectrum of erythrokeratoderma has been extended by application of NGS using ichthyosis-associated gene sequencing panels which identified mutations in addition to those previously identified genes, also in *PNPLA1* in

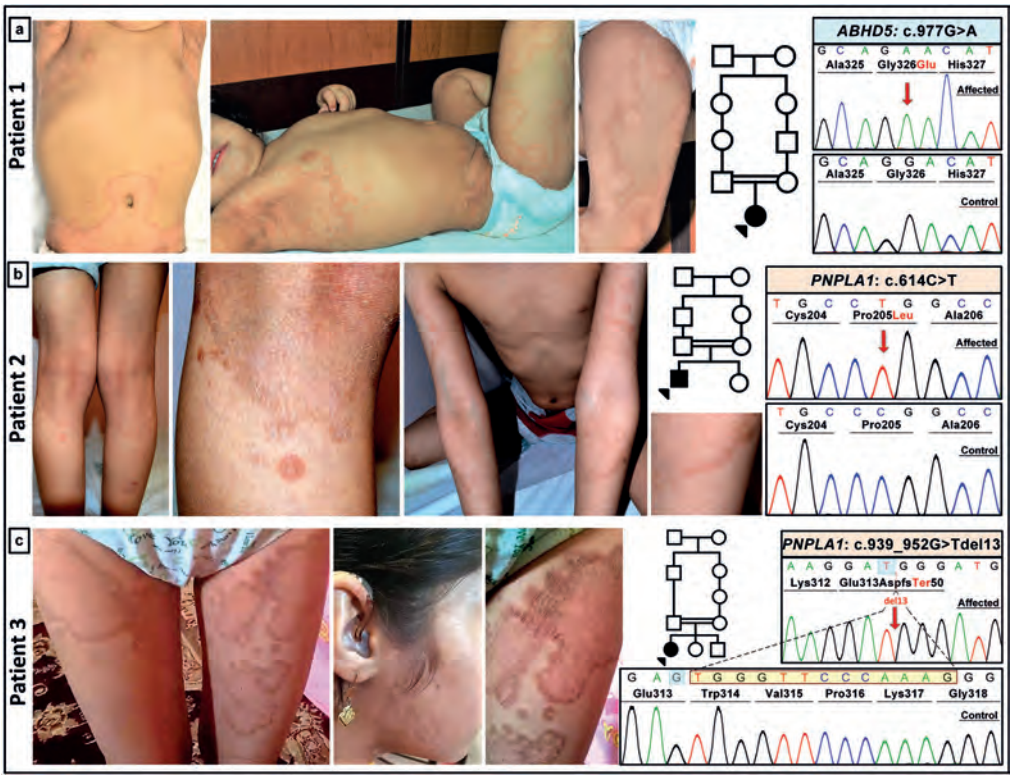

Fig. 7. Patients with ichthyosis and different genetic mutations presenting with erythrokeratoderma. (a) Patient 1, a 2-year-old female patient with erythrokeratoderma and a homozygous missense mutation in the *ABHD5* gene consistent with Chanarin-Dorfman syndrome. (b) Patient 2, a 7-year-old male with erythrokeratoderma and generalized ichthyosis due to a missense mutation in *PNPLA1*. (c) Patient 3, a 12-year-old female with extensive erythrokeratoderma and large brown ichthyotic plaques on the face, consistent with autosomal recessive ichthyosis associated with mutations in *PNPLA1*. Sanger sequencing confirmed the out-of-frame deletion of 13 bp, shown in yellow (lower right panel) (Modified from ref. 57, with permission).

families with the autosomal recessive form of erythrokeratoderma (Fig. 7) (10). These studies provide evidence in support of the notion that erythrokeratoderma can be a manifestation associated with multiple types of ichthyosis with different gene defects (60). Consequently, erythrokeratoderma may not be a distinct genetic entity but rather a manifestation of multiple ichthyosis-related genetic diseases that can occur with or without a more typical ichthyosis presentation.

CONCLUSIONS

This update on recent advances in our understanding the molecular basis of heritable keratinization disorders highlights the tremendous variability, both phenotypic and genotypic, in this group of disorders. The knowledge of the mutant genes and of specific mutations can be used to confirm the diagnosis with subclassification, allows determination of the mode of inheritance, and provides information for prognostication, in general terms, of the severity and overall outcome of the disease. The mutation detection in large consanguineous families also allows identification of heterozygous carriers which can be coupled with genetic counseling for the risk of recurrence in the extended family. The mutations form the basis for prenatal testing and preimplantation genetic diagnosis. Finally, the knowledge of the specific mutations is a prerequisite for allele-specific treatments currently being developed for this group of complex disorders without specific treatment modalities.

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REVIEW ARTICLE

Genetics of Inherited Ichthyoses and Related Diseases

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Inherited ichthyoses are classified as Mendelian disorders of cornification (MEDOC), which are defined on the basis of clinical and genetic features and are mainly divided into non-syndromic and syndromic ichthyoses. Numerous genes, which encode for corresponding proteins, are involved in the normal differentiation of keratinocytes (cornification) and participate in the formation of a functional epidermal barrier. To date, mutations in more than 50 genes are known to result in various types of ichthyoses. Thanks to modern genetic diagnostic methods based on targeted next generation sequencing (NGS), approximately 80–90% of cases can be resolved at present. Further sequencing methods covering the whole exome (WES) or whole genome (WGS) will obviously elucidate another portion of the remaining unknown ichthyoses in the future.

Key words: Mendelian disorders of cornification; ichthyoses; ARCI; genes; mutations; molecular genetic diagnostics.

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SIGNIFICANCE

Knowledge of the molecular genetic causes and mechanisms of hereditary ichthyoses has increased hugely since the 1990s due to the ubiquitous application of modern sequencing technologies. It is important for doctors and scientists that this new knowledge is clinically and genetically correctly classified, in order to make diagnosis and differential diagnosis easier. This article provides an overview of the genetic background and clinical features of ichthyoses and related cornification disorders.

Ichthyoses are genetically determined monogenic (Mendelian) cornification disorders of the epidermis characterized by different degrees of scaling, hyperkeratosis and erythroderma, often associated with palmoplantar keratoderma (PPK) or hyperlinearity. Non-syndromic ichthyoses are limited to skin symptoms and can be subdivided into common and rare forms (**Table I**), whereas syndromic forms are classified according to the additional symptoms (**Table II**).

In congenital ichthyoses, the skin symptoms are present at birth, either as collodion membrane (CM) or as congenital ichthyosiform erythroderma (CIE). Collodion babies (CB) later develop a lamellar ichthyosis (LI) or CIE, or the rarer variants of self-improving collodion ichthyosis (SICI) or bathing suit ichthyosis (BSI) (1).

In common ichthyoses, such as ichthyosis vulgaris (IV) and X-linked recessive ichthyosis (XRI), skin manifestations do not appear until several weeks to months after birth. Occasionally, mild scaling may occur in patients with XRI at birth, which then initially regresses and usually begins again at the age of 4–6 months (1).

In addition to the 2 common forms, non-syndromic ichthyoses also include the much rarer autosomal recessive congenital ichthyosis (ARCI) that clinically manifests as harlequin ichthyosis (HI), LI, or CIE (2).

Ichthyoses caused by keratin mutations, such as epidermolytic ichthyosis (EI), superficial epidermolytic ichthyosis (SEI), and congenital reticular ichthyosiform erythroderma (CRIE), are referred to as keratinopathic ichthyoses. They manifest at birth and often feature episodes of blistering. Most of these types are inherited as autosomal dominant traits, but autosomal recessive forms have also been described on occasion (2).

The family history and pedigree survey can provide important conclusions about the mode of inheritance, and thus contribute to the correct diagnosis. Modern sequencing methods (e.g. next generation sequencing; NGS), including multi-gene-panel sequencing or whole-exome sequencing (WES), help to confirm the suspected diagnosis quickly and reliably.

NON-SYNDROMIC ICHTHYOSES

The 2 most common types of ichthyosis are IV and XRI, whereas ARCI, keratinopathic ichthyosis and a few other non-syndromic forms are much rarer.

Ichthyosis vulgaris

The most common form of ichthyosis is IV, with a prevalence of up to 1:100 (3). It is caused by autosomal semi-dominant inherited loss-of-function mutations in the filaggrin gene (*FLG*). In the majority of patients (approximately 2/3) 2 *FLG* mutations can be detected (4), which are associated with a relatively severe phenotype, whereas patients with

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Table I. Non-syndromic ichthyoses

Name	Abbreviation	OMIM number	Mode of inheritance	Gene mutation	Corresp. figures
Common ichthyoses					
Ichthyosis vulgaris	IV	146750	SD	*FLG*	1 a,b
X-chromosomal recessive ichthyosis	XRI	308100	XR	*STS*	1 c,d
Autosomal recessive congenital ichthyoses	ARCI				
Lamellar ichthyosis	LI				
Congenital ichthyosiform erythroderma	CIE				
	ARCI-1	242300	AR	*TGM1*	1 e-h
	ARCI-2	242100	AR	*ALOX12B*	
	ARCI-3	606545	AR	*ALOXE3*	
	ARCI-4A	601277	AR	*ABCA12*	1 i-k
	ARCI-6	612281	AR	*NIPAL4/Ichthyin*	1 l,m
	ARCI-5	604777	AR	*CYP4F22*	1 n
	ARCI-10	615024	AR	*PNPLA1*	1 o
	ARCI-9	615023	AR	*CERS3*	
	ARCI-14	617571	AR	*SULT2B1*	
	ARCI-13	617574	AR	*SDR9C7*	
Harlequin ichthyosis	HI, ARCI4B	242500	AR	*ABCA12*	
Self healing collodion baby	SHCB		AR	*ALOX12B*	
Self improving collodion baby	SICI			*ALOXE3* *TGM1*	
Bathing suit ichthyosis	BSI		AR	*TGM1*	
Ichthyosis prematurity syndrome*	IPS	608649	AR	*SLC27A4/FATP4*	2 a,b
Keratinopathic ichthyoses	KPI				
Epidermolytic ichthyosis	EI	113800	AD	*KRT1, KRT10*	2 c-e
Superficial epidermolytic ichthyosis	SEI	146800	AD	*KRT2*	
Congenital reticular ichthyosiform erythroderma	CRIE	609165	AD	*KRT10*	2 f
Cyclic I. with epidermolytic hyperkeratosis	AEI	607602	AD	*KRT1, KRT10*	
Ichthyosis hystrix Curth-Macklin	IHCM	146590	AD	*KRT1*	2 g
Other genodermatoses					
Loricrin keratoderma	LK	604117	AD	*LOR*	
Erythrokeratodermia variabilis	EKV	133200	AD	*GJB3, GJB4* *CARD14*	2 h 2 i
Peeling skin syndrome*	PSS	270300	AR	*CDSN*	
Keratosis linearis-I. congenita-keratoderma	KLICK	601952	AR	*POMP*	

*Not a true syndrome. SD: semi-dominant; OMIM: Online Mendelian Inheritance in Man.

only one mutation are significantly more mildly affected. The IV is associated with atopic eczema in approximately half of cases and approximately 40% with allergic rhinitis, conjunctivitis or bronchial asthma, e.g. also overlapping with atopic eczema. Approximately one-third of patients have no atopy (4). Histological analysis reveals an orthohyperkeratosis (thickening of the stratum corneum) with simultaneously reduced or absent stratum granulosum. Electron microscopy shows the defect as reduced, very small (crumbly) keratohyalingranulae. The typical clinical picture of IV is characterized by a fine, pale-grey scaling (**Fig. 1**a, b) with the exception of the large articular flexures and a palmo-plantar hyperlinearity and keratosis pilaris.

X-linked recessive ichthyosis

XRI is the second most common form of ichthyosis, with a prevalence of 1 in 2,000 boys (1). It is caused by steroid sulphatase (STS) deficiency (5) and is often associated with further clinical problems, such as cryptorchidism (~20%) or social communication deficits, such as attention deficit hyperactivity syndrome (40%) or autism (25%) (6). The majority of patients present deletions of a part or the totality of the *STS* gene (isolated non-syndromal XRI); only 10% of cases are due to point mutations. Larger deletions, which also spread to neighbouring genes, lead to much more complex diseases, such as Kallmann syndrome, which is additionally associated with mental retardation, hypogonadism and anosmia. These contiguous gene deletion syndromes are then classified as syndromal ichthyosis. XRI can also be confirmed enzymatically by the determination of sulphatase activity in the blood. The lack of cholesterol hydrolysis leads to the accumulation of cholesterol-3-sulphate in the epidermis. Histological analyses may reveal a normal or rather thickened stratum granulosum (light microscopy), and a lack of degradation of the corneodesmosomes (electron microscopy) as a sign of the retention hyperkeratosis. The predominantly adherent, rhomboid, light-grey to dark-brown scaling is extended over the entire body, with the exception of the hands, feet and the flexor sides of the elbows and knees (Fig. 1c, d). Mothers of affected boys are carriers, who frequently report complications during the birth of their children (weakness of labour) followed by caesarean section or forceps birth.

Autosomal recessive congenital ichthyosis (ARCI)

The generic term ARCI refers to all non-syndromic forms of autosomal recessive congenital ichthyoses that are present at birth and not associated with blistering. This includes HI, which is by far the most severe form of ichthyosis, LI and CIE (2).

Prevalence studies in Germany and Spain show almost identical values of 1.6–1.7: 100,000 (7, 8). Histologically, the different ARCI types show typical signs of epidermal

Table II. Syndromic ichthyoses

Name	Abbreviation	OMIM number	Mode of inheritance	Gene mutation	Corresp. figures
X-chromosomal inherited syndromes					
Syndromic XR ichthyosis[a]	XRI	308100	XR	Xp22-deletion[b]	
IFAP syndrome	IFAP	308205	XR	*MBTPS2*	
Conradi-Hünermann-Happle syndrome	CDPX2	302960	XD	*EBP*	
Autosomal inherited syndromes with:					
Hair anomalies					
Comèl-Netherton syndrome	NS	256500	AR	*SPINK5*	Fig. 2j
Ichthyosis hypotrichosis syndrome	IHS	602400	AR	*ST14*	
IHCS syndrome	IHCS	607626	AR	*CLDN1*	
Trichothiodystrophie (photosensitive)	TTDP	601675	AR	*ERCC2/XPD, ERCC3/XPB, GTF2H5/TTDA*	
Neurological (prominent) symptoms					
Sjögren-Larsson syndrome	SLS	270200	AR	*ALDH3A2*	Fig. 2k
Refsum syndrome	RS	266500	AR	*PHYH, PEX7*	
MEDNIK syndrome	MEDNIK	609313	AR	*AP1S1*	
IKSHD (ELOVL1-deficit)	IKSHD	618527	AD	*ELOVL1*	
Fatal disease progression					
CEDNIK syndrome	CEDNIK	609528	AR	*SNAP29*	
ARC syndrome	ARC	208085	AR	*VPS33B*	
Multiple sulphatase deficiency	MSD	272200	AR	*SUMF1*	
Gaucher syndrome type 2	GS	230900	AR	*GBA*	
Other symptoms					
KID syndrome	KID	148210	AD	*GJB2, GJB4*	
Keratitis-Ichthyosis-Deafness Autosomal Recessive	KIDAR	242150	AR	*AP1B1*	
Chanarin-Dorfman syndrome	NLSDI/CDS	275630	AR	*ABHD5/CGI-58*	Fig. 2l
ARKID syndrome	ARKID	--------	AR	*VPS33B*	
SAM syndrome	SAM	615508	AR/AD	*DSG1, DSP*	
Congenital disorders of glycosylation	CDG				
CDG type 1F	CDG-1F	609180	AR	*MPDU1*	
Dolichol kinase deficiency	CDG-1M	610768	AR	*DOLK*	
Coloboma, ocular, with ichthyosis, brain malformations, and endocrine abnormalities	CDG-1Q	612379	AR	*SRD5A3*	
CHIME syndrome	CHIME	280000	AR	*PIGL*	

[a]Symptoms depending on the size of the deletion. [b]Contiguous gene deletion syndrome: STS and other genes may be deleted. XR: X-linked recessive; XD: X-linked dominant; AR: autosomal recessive; AD: autosomal dominant; IFAP: ichthyosis follicularis-atrichia-photophobia; CDPX2: chondrodysplasia punctate; IHSC: ichthyosis hypotrichosis sclerosing cholangitis; IKSHD: ichthyosis, keratoderma, spasticity, hypomyelination, dysmorphia; MEDNIK: Mental retardation-enteropathy-deafness-neuropathy-ichthyosis-keratoderma; CEDNIK: Cerebral dysgenesis-neuropathy-ichthyosis-palmoplantar keratoderma; ARC: arthrogryposis-renal dysfunction-cholestasis; KID: keratitis-ichthyosis-deafness; NLSDI: Neutral lipid storage disease with ichthyosis; ARKID: Autosomal recessive keratoderma-ichthyosis-deafness; SAM: severe dermatitis, multiple allergies and metabolic wasting; CDG: congenital disorders of glycosylation; CHIME: coloboma, congenital heart disease, ichthyosiform dermatosis, mental retardation, and ear anomalies syndrome.

hyperproliferation with orthohyperkeratosis and thickened stratum granulosum, as well as signs of inflammation with lymphohistiocytic infiltrate of the dermis. Using electron microscopy (EM) it is sometimes possible to detect a change that is typical for the particular defect, e.g. cholesterol clefts in the stratum corneum in patients with *TGM1* and *PNPLA1* mutations, or inflated lamellar bodies in HI.

At present, mutations in 11 different genes are known to cause ARCI (see Table I):

Transglutaminase 1 TGM1 (ARCI1). The most common causes of ARCI are mutations in the *TGM1* gene, first described in 1995 (9, 10) and found in approximately one-third of all cases of ARCI (11). The prevalence in Germany is given as 1:200,000 (7). Patients with *TGM1* mutations are born in 80–90% of cases as a collodion baby and often present severe ectropion. The clinical picture is manifested in approximately 90% as LI and in approximately 10% as CIE (Fig. 1e–h). In general, there are no indications for a genotype-phenotype correlation. However, in some specific phenotypes, such as BSI or self-healing collodion baby, a correlation with specific mutations has been observed (12, 13).

Lipoxygenases ALOX12B (ARCI2) and ALOXE3 (ARCI3). Mutations in 1 of the 2 lipoxygenase genes *ALOX12B* or *ALOXE3* were identified in 2002 using homozygosity mapping in consanguineous ARCI families (14). Overall, 17% of ARCIs are caused by mutations in 1 of the 2 lipoxygenase genes, with 12% *ALOX12B* and 5% *ALOXE3* (11). The 2 enzymes 12R-LOX and eLOX3 catalyse the first 2 steps in the degradation pathway of arachidonic acid (15). Clinically, both LI and CIE occur. Especially in Scandinavian patients with *ALOX12B* mutations, a positive development of the phenotype towards self-improving collodion ichthyosis (SICI) is frequently observed (16, 17).

ATP-binding cassette transporter ABCA12 (ARCI4A and ARCI4B). Defects in the *ABCA12* gene can either lead to LI (ARCI4A) or to a HI (ARCI4B), depending on the nature of the mutation (Fig. 1i–k). In 2003, homozygous missense mutations in *ABCA12* were identified in patients from consanguineous North African families, leading to severe LI, hand and nail deformities, and kyphoscoliosis (18). In 2005, loss of function mutations in the same *ABCA12* gene were identified as the molecular genetic cause of HI (19, 20). The life-threatening HI phenotype is characterized by massively thickened skin with impaired skin barrier function, infection and water loss, requiring intensive care treatment (19, 21). ABCA12 is a transmembrane lipid transporter acting at the lamellar granules (LG) and the cell membrane of keratinocytes. The ABCA12 transporter is important in delivering glucosylceramides (GluCer) to the lipid lamellae through lamellar bodies (LBs) (22).

NIPAL4 (ICHTHYIN) (ARCI6). In 2004, positional cloning was used to identify mutations in *ICHTHYIN*, which was later referred to as *NIPAL4*, according to official nomenclature (23). Approximately 16% of patients with ARCI have mutations in this gene (11), and the recurrent mutation p.Ala176Asp occurs in half of these patients. In some of the patients, a special phenotype is noted with typical reticular lamellar ichthyosis

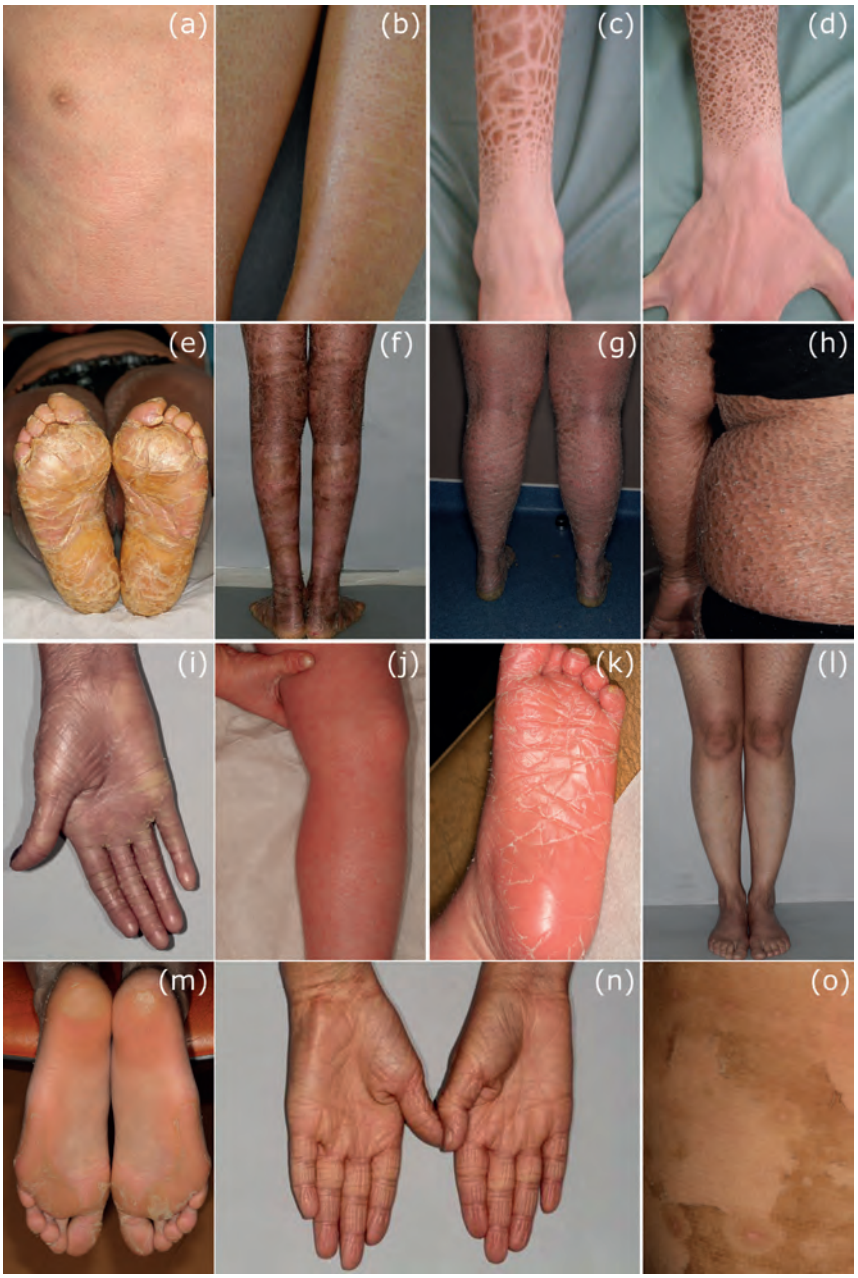

Fig. 1. Examples of skin signs in non-syndromic ichthyoses. (a, b) Fine, pale-grey scales of ichthyosis vulgaris on thorax and legs of a patient with compound heterozygous filaggrin (*FLG*) gene mutations. (c, d) Adherent, rhomboid, dark-brown scaling in X-linked recessive ichthyosis; hands and feet are not affected. (e–h) Severe lamellar ichthyosis and thick palmoplantar keratoderma in a patient with autosomal recessive congenital ichthyosis (ARCI) due to homozygous mutations in *TGM1*. (i–k) Ichthyosiform erythroderma and severe palmoplantar keratoderma in patients with *ABCA12* mutations. (l, m) Lamellar ichthyosis and yellow plantar keratoderma in patients with *NIPAL4* mutations. (n) Typical palmar hyperlinearity in a patient with *CYP4F22* mutations. (o) In patients with *PNPLA1* mutations cyclic superficial scaling can be observed.

and pronounced palmoplantar keratoderma with central cut-outs (Fig.1 l, m). EM classifies these patients as type III with hyperkeratotic stratum corneum and stratum granulosum with vacuoles.

Cytochrome-P450 CYP4F22 (ARCI5). ARCI due to mutations in *CYP4F22* occurs in 8% of cases and results in a relatively mild LI that may be accentuated in the periumbilical region. The patient is usually not born as a collodion baby and, similar to the IV, shows marked palmoplantar hyperlinearity (Fig. 1n) (24, 25).

Patatin-like phospholipase domain-containing protein 1 PNPLA1 (ARCI10). To identify mutations in the gene *PNPLA1*, a spontaneous dog model with golden retrievers with ichthyosis was used (26). Some of the patients with ichthyosis subsequently tested for the human *PNPLA1* showed mutations in this gene. In contrast to the newborn puppies who showed no signs of ichthyosis at birth, all patients were born as collodion babies, and later developed LI. In some patients a phenotype with cyclic skin peeling has been observed (Fig. 1o) (27).

Ceramide synthase 3 CERS3 (ARCI9). In 2013 ceramide synthase 3 (*CERS3*) mutations were identified in patients with ARCI, and this gene encodes the protein responsible for the de novo synthesis of ceramides in the skin (28, 29). Mutations in *CERS3* cause reduced formation of ultra-long-chain epidermis-specific ceramides, which leads to defective epidermal differentiation of

the skin and thus to a disruption of the skin barrier. Clinically, LI dominates with palmoplantar hyperlinearity and hyperkeratosis. Histologically there is an acanthosis with significant thickening of the stratum granulosum in a normal horny layer. Immunofluorescence microscopy localized CERS3 between the stratum corneum and the stratum granulosum.

Sulphotransferase family 2b, member 1 SULT2B1 (ARCI 14). In 2017 Heinz et al. identified mutations in sulphotransferase family 2B member 1 (*SULT2B1*) in ARCI (30). Cytosolic sulphotransferases form a large family of enzymes that are involved in the synthesis and metabolism of several steroids in humans. The absence of cholesterol sulphate, a metabolite of SULT2B1, and an increased level of cholesterol, indicate a disturbed cholesterol metabolism of the skin upon loss-of-function mutation in *SULT2B1*. Mutation in *SULT2B1* leads to an ARCI phenotype via increased proliferation of human keratinocytes, thickening of epithelial layers, and altered epidermal cholesterol metabolism (30).

Short-chain dehydrogenase/reductase family 9C, member 7 SDR9C7 (ARCI13). Mutations in the gene *SDR9C7* were first described in 2016 in patients with congenital ichthyosis; they presented with large erythematous scales over the entire body, with hyperkeratosis of the elbows and knees, mostly associated with palmoplantar hyperkeratosis. The severity of skin lesions decreased with age, and the face and scalp were mostly not affected. Fungal skin infections including onychomycosis were observed frequently. Light microscopic analysis showed mild hypergranulosis and marked hyperkeratosis of the epidermis (31, 32). Hotz et al. reported 7 patients with *SDR9C7* mutations, which also showed a relatively mild ichthyosis with generalized dry and scaly skin and mild or local erythema. With one exception, the patients were not born as collodion babies (33).

Fatty acid transport protein 4 SLC27A4 (IPS). Ichthyosis prematurity syndrome (IPS) due to mutations in *SLC27A4* was initially classified as a syndromic ichthyosis, however the authors and others (34) propose to classify IPS under ARCI. Patients with IPS are typically born well before the calculated date of delivery and often require artificial ventilation due to neonatal asphyxia. The reason for this is the obstruction of the foetal bronchi by massively shed skin scales in the amniotic fluid. At birth an impressive verrucous hyperkeratosis is present, more prominent on the head, forehead and trunk, which heals quickly (35, 36). Subsequent to the critical neonatal phase, mild ichthyosis, atopy, fine hair, and a typical follicular keratosis pilaris are seen (**Fig. 2**a, b). IPS is inherited as an autosomal recessive trait and is caused by mutations in the gene *SLC27A4*, which codes for fatty acid transport protein 4 (FATP4) (37).

Keratinopathic ichthyosis

The term keratinopathic ichthyosis (KPI) summarizes the forms that are caused by mutations in keratin genes (2). Inheritance in this disease group is usually autosomal dominant, although exceptionally, an autosomal recessive pattern of inheritance can occur. Typically, an epidermolytic hyperkeratosis is discovered using light microscopy, while collapsed keratin aggregates can be found by EM. These so-called tonofilaments have clumped/aggregated around the cell nucleus and lost their attachment to the desmosomes.

There are 3 main types of KPI: epidermolytic ichthyosis, superficial epidermolytic ichthyosis and congenital reticular ichthyosiform erythroderma (see Table I):

Epidermolytic ichthyosis. EI has previously been referred to as bullous ichthyosis, bullous CIE type Brocq, epidermolytic hyperkeratosis, or ichthyosis exfoliativa. EI is caused by mutations in the *KRT1* (Fig. 2c, d) or *KRT10* (Fig. 2e) genes. At birth there is usually a non-ichthyosiform erythroderma, which may be associated with blistering, which is why the differential diagnosis is bullous epidermolysis. Following the initial phase of blistering in the first few months of life, hyperkeratosis then occurs (2). Patients with *KRT1* mutations have very severe PPK compared with patients with *KRT10* mutation.

Superficial epidermolytic ichthyosis. SEI was formerly called ichthyosis bullosa Siemens and is caused by mutations in the *KRT2* gene. Clinically it resembles EI, but shows a milder disease course with more localized skin symptoms. Since delineating the phenotype between EI and SEI is not always possible, *KRT2* should be analysed in all patients with KPI in whom no mutations in *KRT1* or *KRT10* have been found (2).

Congenital reticular ichthyosiform erythroderma. CRIE is caused by specific mutations in *KRT10* (38). The clinical picture at birth is dominated by pronounced erythroderma. Palmoplantar blistering and large scaling occurs, similar to peeling skin syndrome. In the later course, lichenification is also observed. In early childhood between the ages of 3 and 10 years, the development of multiple, small white spots begins; these spots can increase in size to 2 cm, which led to the French term "*ichtyose en confettis*" (Fig. 2f). The mechanism is mitotic recombination (2). This phenomenon of revertant mosaicism is also found in other diseases, e.g. epidermolysis bullosa. The same mechanism has been reported in cases with mutations in *KRT1*.

Other keratinopathic ichthyoses. In addition to the 3 main types of KPI (EI, SEI and CIE) there are also rarer types, such as cyclic ichthyosis with annular epidermolytic hyperkeratosis (AEI, OMIM 607602) and ichthyosis hystrix Curth-Macklin type (Fig. 2g) (IHCM, OMIM 146590).

Other non-syndromal ichthyoses

Other genodermatoses among the group of non-syndromal ichthyoses are included as they are phenotypically predominantly characterized by ichthyosis. Examples are the autosomal dominant inherited loricrin keratoderma (OMIM 604117), erythrokeratoderma variabilis (OMIM 133200) (Fig. 2h, i), and the 2 autosomal recessive disorders peeling skin syndrome (OMIM 270300) and KLICK syndrome (keratosis linearis-ichthyosis congenita-keratoderma, OMIM 601952), both inadvertently called "syndrome", even though they are devoid of any extracutaneous involvement (Table I).

SYNDROMIC ICHTHYOSES

The syndromic ichthyoses are generally very rare and are classified based on the mode of inheritance as X-linked or autosomal inherited ichthyosis syndromes and can be further subdivided according to the predominant symptoms (2).

X-linked syndromes

The first group includes the syndromal form of XRI, XR IFAP syndrome and X-linked dominant (XD) chondrodysplasia punctata 2 (see Table II).

Syndromic X-linked ichthyosis. While in patients with mutations or minor deletions of the *STS* gene an isolated, skin-only XRI is present, larger deletions on Xp22.3 often involve multiple

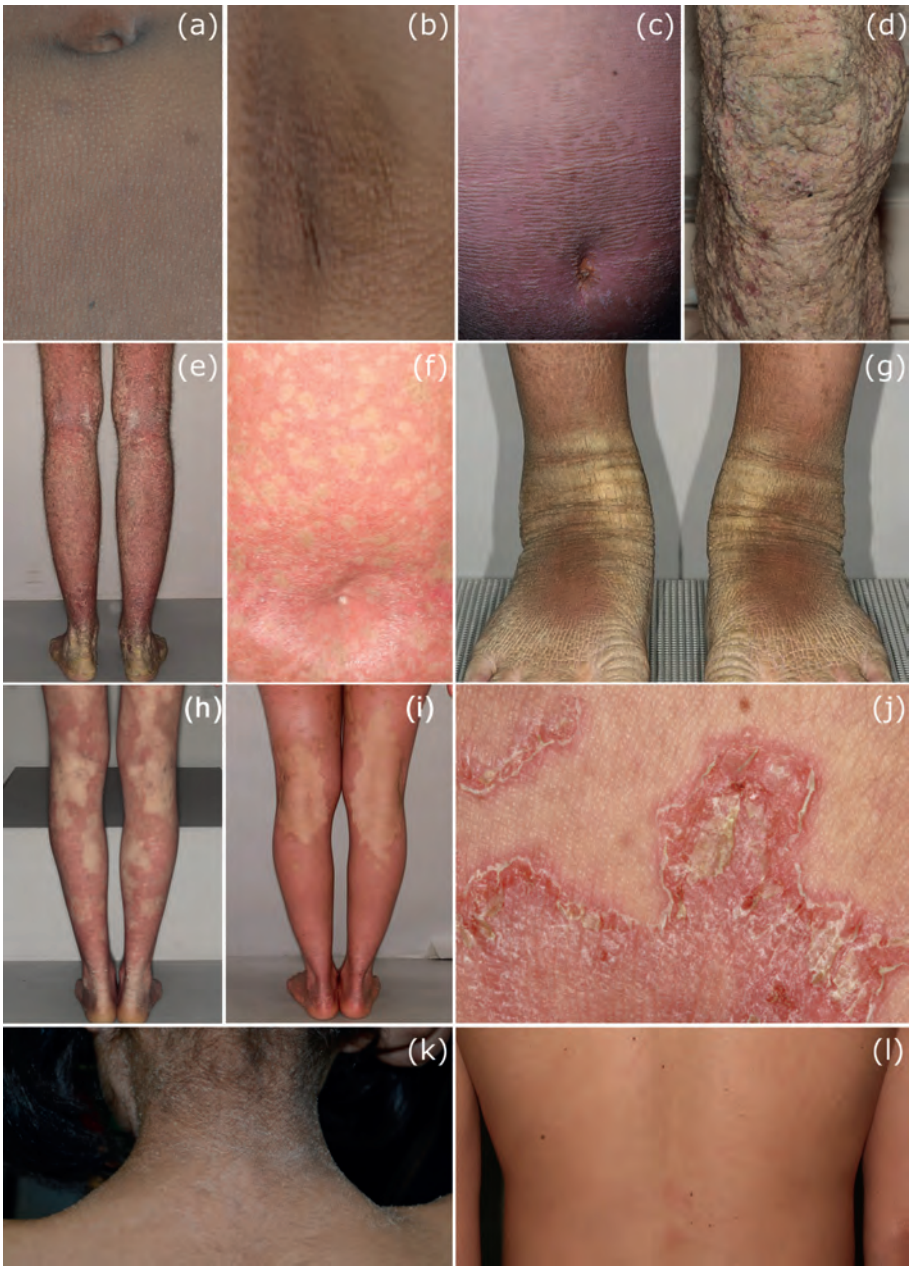

Fig. 2. Examples of skin signs in non-syndromic (continued from Fig. 1) **and syndromic ichthyoses.** (a, b) Follicular hyperkeratosis of the body and affected axilla in adults with ichthyosis prematurity syndrome (IPS). (c, d) Verrucous hyperkeratosis in epidermolytic ichthyosis (EI) of the abdomen and legs caused by heterozygous mutations in *KRT1*. (e) Hyperkeratosis, erythroderma and skin fragility in EI due to a heterozygous mutation in *KTR10*. (f) Congenital reticular ichthyosiform erythroderma (CRIE) with specific heterozygous mutation in *KRT10*; white spots (representing normal skin) appeared since the age of 4 years due to revertant mosaicism. (g) Extensive, spiky hyperkeratosis over the extensor surfaces of the lower extremities in ichthyosis hystrix type Curth-Macklin (*KRT1*). (h) Erythrokeratodermia variabilis (EKV) due to a heterozygous mutation in the *GJB3* gene, also known as *CX31*. (i) EKV due to a heterozygous mutation in *CARD14*. (j) Ichthyosis linearis circumflexa (polycyclic serpiginous migratory plaques with double-edged scales) in Netherton's syndrome caused by biallelic *SPINK5* mutations. (k) Pronounced, dark pigmented ichthyosis on the neck in a patient with Sjögren-Larsson syndrome and mutations in the *ALDH3A2* gene. (l) Mild, ichthyosiform erythroderma in Chanarin-Dorfman syndrome.

adjacent genes, termed "contiguous gene deletion syndrome". As mentioned above, the additional symptoms depend on the extent of the deletion and range from mental retardation, hypogonadotrophic hypogonadism and anosmia in Kallmann syndrome (KAL1, OMIM 308700) to dwarfism (ISS, OMIM 300582, SHOX, OMIM 312865) and ocular albinism (OA1, OMIM 300500) (39, 40).

IFAP syndrome. The IFAP syndrome describes the triad of ichthyosis follicularis, alopecia (atrichia) and photophobia. Less than 100 male patients have been reported in the literature. Female carriers can sometimes present minimal symptoms, such as an asymmetrical distribution of body hair, patchy alopecia or hyperkeratosis along the Blaschko lines. In addition to the absence of scalp hair, eyebrows and eyelashes, the complete atrichia of body hair is part of the full spectrum of IFAP syndrome in male patients. There is often a pronounced ichthyosis follicularis with spine-like outgrowths of the skin follicles. The progressive blindness due to ulceration, scarring and vascularization of the cornea is a known complication. The allelic variant

BRESHEK syndrome has additional symptoms, such as brain abnormalities, mental retardation, ectodermal dysplasia, skeletal deformities, Hirschsprung's disease, ear and eye abnormalities, cleft palate, cryptorchidism and renal dysplasia or kidney hypoplasia. The terminology distinguishes IFAP syndrome with or without BRESHEK syndrome. These 2 phenotypes and also keratosis follicularis spinulosa decalvans (OMIM 308800) (41) are caused by mutations in the *MBTPS2* gene (42).

Conradi-Hünermann-Happle syndrome (CDPX2). Conradi-Hünermann-Happle syndrome is also known as chondrodysplasia punctata 2. It is one of the XD inherited disorders that are lethal in male foetuses. Exceptions are possible in postzygotic mosaics or male chromosome sets with an excess X chromosome, as in Klinefelter syndrome (XXY). The characteristic symptoms of female patients include asymmetrical bone anomalies, sectoral cataracts, and streaky skin changes following the Blaschko lines. The stippling chondrodysplasia punctata is visible as a lime splash in the X-ray picture until approximately the ninth month of life. In the first few weeks of life, there is a very inflammatory phenotype with pronounced feather-like scaling and hyperkeratosis, which subsequently turns into linearly arranged follicular atrophoderma (43). CDPX2 is caused by mutations in the *EBP* gene, which codes for a delta (8) -delta (7) sterol isomerase, also known as emopamil binding protein, involved in cholesterol metabolism (44).

Autosomal inherited syndromes

The second group includes all other syndromic cornification disorders, which follow autosomal recessive or dominant inheritance, and can be further subdivided according to the most characteristic extracutaneous manifestations: Hair anomalies; Neurological symptoms; Fatal disease progression, Other typical symptoms (see Table II).

H1; Netherton syndrome. The AR inherited Netherton syndrome is caused by mutations in the *SPINK5* gene, which codes for the serine protease inhibitor LEKTI. The impaired function of LEKTI leads to inflammatory processes in the epidermis and to a pronounced barrier disorder of the skin. At birth, generalized ichthyosiform erythroderma and severe growth and developmental deficiency are present, in part due to diarrhoea, intestinal malabsorption, hypernatraemic dehydration and recurrent infections. The erythroderma may persist, or develop into an "ichthyosis linearis circumflexa Comèl", which is characterized by polycyclic serpiginous migratory plaques with typical double-edged scales (Fig. 2j). The typical hair anomalies can be detected by light microscopy, but usually only after the newborn phase. Bamboo hair (trichorrhexis invaginata) is considered pathognomonic for NS; trichorrhexis nodosa and pili torti can sometimes be observed. The scalp hairs are brittle and barely grow, eyelashes and eyebrows are also affected. There is a strong tendency to atopy (asthma, allergic rhinitis, atopic dermatitis, food allergies, urticaria and angioedema), increased serum IgE and hypereosinophilia.

H2; Ichthyosis hypotrichosis syndrome. The AR inherited ichthyosis hypotrichosis syndrome (*IHS*) is caused by mutations in the *ST14* gene, which encodes serine protease matriptase (45). It is also listed in OMIM as ARCI11; however it should be classified as syndromic ichthyosis with hair defect. Clinically, in addition to congenital ichthyosis and hypotrichosis, hypohidrosis and follicular atrophoderma are also found. The existing hair appears curly and brittle; sometimes it is pili torti or pili bifurcati. Eyebrows and eyelashes are usually sparse. Photophobia, blepharitis and corneal clouding have also been described in individual patients. Light microscopy shows a pronounced acanthosis and a thickened stratum corneum in the epidermis with orthohyperkeratosis. Using electron microscopy persistent corneodesmosomes and lamellar body-like deposits can be found in the horny layer.

H3; Ichthyosis hypotrichosis sclerosing cholangitis syndrome. IHSC is another inherited AR syndrome with congenital ichthyosis, hypotrichosis and additional sclerosing cholangitis. The synonyms NISCH syndrome (neonatal ichthyosis sclerosing cholangitis) and ILVASC syndrome (ichthyosis leukocyte vacuole alopecia sclerosing cholangitis syndrome) are also common. Liver involvement can provide an important clue to diagnosis, but its severity is highly variable. Individual patients with progressive hepatic insufficiency who needed liver transplantation have been described. Hypotrichosis of the scalp is often associated with scarring alopecia and thinning of eyelashes and eyebrows. Other symptoms, such as oligodontia, hypodontia and enamel hypoplasia, have also been reported (46). Genetic causes are mutations in the *CLDN1* gene, which codes for Claudin-1, a protein of tight junctions.

H4; Trichothiodystrophy. The term trichothiodystrophy (TTD) is based on the characteristic of the disease anomalies with short, brittle hair, longitudinal splitting and reduced content of sulphur-containing amino acids. Typically, a so-called tiger tail pattern with light and dark bands can be detected in polarization light. TTDs are classified as DNA repair or transcription disorders, and subdivided into various forms with or without photosensitivity. The autosomal recessive forms with ichthyosis are caused by mutations in the genes *ERCC2*, *ERCC3* and *GTF2H5*. The hair anomalies are associated with skin manifestations, such as congenital ichthyosis, photosensitivity and nail abnormalities, as well as neurological symptoms, developmental and growth disorders (47).

N1; Sjögren-Larsson syndrome. This AR syndrome was named after the Swedish authors (48) who first described it in 1957. It is clinically characterized by congenital ichthyosis, intellectual deficit with delayed speech development and spastic paresis. At birth there are sometimes only mild hyperkeratoses, which then develop into a pronounced, generalized, often heavily pigmented, dirty-brown ichthyosis with an accentuation in the articular folds, neck, trunk and extremities (Fig. 2k). The SLS is caused by mutations in the *ALDH3A2* gene, which codes for a fatty aldehyde dehydrogenase (FALDH), which oxidizes long-chain aldehydes to fatty acids. Neurological symptoms, such as spastic diplegia or quadriplegia and seizures, may appear later, after early childhood. Many patients never learn to walk and are in long-term care throughout their lives. Life expectancy is reduced. Eye involvement with crystalline retinal inclusions, corneal opacity and macular degeneration, as well as photophobia and myopia, are observed (1).

N2; Refsum syndrome. This AR disorder, named after a Norwegian author, is characterized by increased phytanic acid concentration, which can be detected in plasma or urine. During the course of the disease, specific damage to the retina, brain and peripheral nervous system occur. The symptoms are not present at birth, but usually occur after the age of 15 years. Night blindness (nyctalopia) is a typical first manifestation, followed later by neurological symptoms, such as distal motor polyneuropathy, cerebellar ataxia, mental retardation, deafness and anosmia. Ichthyosis only develops later in the course of the disease. Other symptoms, such as epiphyseal dysplasia, cardiomyopathy and retinitis pigmentosa, have also been reported. Refsum syndrome is an autosomal recessive disorder. In more than 90% of cases, mutations in the *PHYH/PAXH* gene coding for phytanoyl-CoA-hydroxylase can be detected. The function of the peroxisomal enzyme is the degradation of phytanic acid via α-oxidation. Less than 10% of mutations are found in the *PEX7* gene (49).

N3; MEDNIK syndrome. An acronym for the symptoms of *m*ental retardation, *e*nteropathy, *d*eafness, *n*europathy, *i*chthyosis and *k*eratoderma (50). This rare and severe AR multisystem disease is clinically and biochemically related to Menkes syndrome and Wilson disease, in which there is an accumulation of copper in the liver that can be treated with zinc acetate. The causes of MEDNIK syndrome are mutations in the *AP1S1* gene, which codes for the σ1A subunit of the adapter protein complex 1 and controls the intracellular transport of the copper pumps ATP7A and ATP7B (51).

Recently, a new AR inherited syndrome has been described, showing mainly ichthyosis, deafness and photophobia. It is caused by mutations in the *AP1B1* gene, which codes for the 1B-subunit of the adapter protein complex 1. There are some overlapping clinical features with MEDNIK syndrome; however, the new AP1B1-syndrome seems to be less severe: the 5 described patients and our own case do not present neurological symptoms and have no or less -important enteropathy (52, 53).

N4; IKSHD syndrome. Heterozygous mutations in the *ELOVL1* gene have been described in a new phenotype, in which, in addition to symptoms of the epidermis (ichthyosis, keratoderma) and the nervous system (spasticity, hypomyelination), dysmorphism (IKSHD) also occurs (54). So far, only 2 patients have been described in the literature, 1 of which certainly carried a neo mutation (54, 55). Similar to ELOVL4, ELOVL1 has functions in the elongation of fatty acids and is regulated by CERS2, a ceramide synthase, which is important for C24 sphingolipid synthesis (54). A previously described mouse model with Elovl1 deficit clinically showed a skin phenotype, and macroscopically reduced lipid lamellae and defective lamellar bodies in the stratum corneum (56).

F1; CEDNIK syndrome. The acronym CEDNIK syndrome derives from the typical constellation of symptoms, including cerebral dysgenesis, neuropathy, ichthyosis and palmoplantar keratoderma (*ce*rebral *d*ysgenesis, *n*europathy, *i*chthyosis, palmoplantar *k*eratoderma). This rare AR inherited neurocutaneous disease is caused by mutations in the *SNAP29* gene and is characterized by severe developmental disorders of the nervous system. SNAP29 (synaptosomal-associated protein 29) is a t-SNAPE (soluble *NSF* attachment *p*rotein *re*ceptor) that is involved in intracellular transport in various vesicle and membrane fusion processes (Golgi apparatus, focal adhesions) (57).

F2; ARC syndrome. Arthrogryposis-renal dysfunction-cholestasis syndrome is clinically characterized by the association of arthrogryposis, renal dysfunction, cholestasis, and severe failure to thrive. Patients with this AR inherited multisystem disorder also develop severe ichthyosis in addition to a number of symptoms, such as deafness, platelet abnormalities, osteopenia, missing corpus callosum, recurrent infections, and dysmorphism. Most affected children die early. ARC syndrome is caused by mutations in the *VPS33B* (58) or *VIPAS39* (59) genes, after which it is classified into ARCS1 and ARCS2. VPS33B and VIPAS39 play an important role in the biogenesis and function of lamellar bodies in the epidermis (60).

F3; Multiple sulphatase deficiency. Some metabolic diseases present, in addition to variable symptoms of different organ systems, also a more or less pronounced ichthyosis. The AR inherited multiple sulphatase deficiency is caused by mutations in the *SUMF1* gene (sulphatase-modifying factor 1) and is one of the lysosomal storage diseases. The complex clinical picture develops usually only within the first 2 years of life, and in addition to a mild ichthyosis, includes symptoms such as metachromatic leukodystrophy and mucopolysaccharidosis. The diagnosis can be confirmed molecularly or biochemically by detecting increased excretion of mucopolysaccharides and sulphatides (61).

F4; Gaucher syndrome type 2. The presence of ichthyosis or a collodion membrane at birth has only been observed in the rare type 2 Gaucher syndrome (62). The further course of the disease is fatal, due to the occurrence of hepatosplenomegaly and progressive neurological symptoms, such as spasticity, seizures and oculomotor paralysis. Patients with Gaucher syndrome type 2 usually die before their second year of life. Genetic causes are AR inherited mutations in the *GBA* gene, which encodes the lysosomal enzyme beta-glucosidase (or beta-glucocerebrosidase), and plays a role in ceramide metabolism.

O1; KID syndrome. A rare autosomal dominant disease with keratitis, ichthyosis or hyperkeratosis and deafness. At birth, there is a collodion membrane or a non-ichthyosiform erythroderma. Characteristic lesions include progressive erythematous dermatitis with reddened, hyperkeratotic plaques, palmoplantar keratoderma, nail dystrophy, alopecia, and sparse or absent eyebrows and eyelashes. The typical verrucous aspect mainly affects the face, scalp, ears, elbows and knees. As genetic causes, mutations have been described both in the *GJB2* gene (connexin 26) and in the *GJB6* gene (connexin 30). These are mostly neo-mutations. Patients with KID syndrome appear to be at increased risk for squamous cell and tongue cancers. Mutations in the *GJB2* gene may also result in other phenotypes, such as AR inherited deafness or AD inherited palmoplantar keratoderma type Vohwinkel (mutilating), depending on the location of the mutation (63).

O2; Chanarin Dorfman syndrome. Classified as a lipid storage disorder of neutral fats in which the breakdown of triglycerides in the cell is impaired (neutral lipid storage disease with ichthyosis; NLSDI). Due to the defect, lipid droplets accumulate in a wide variety of cell types; in granulocytes this characteristic phenomena is called Jordan's anomalies. By demonstrating Jordan's abnormalities in the blood smear, the diagnosis can be clinically made uncomplicated and cost-effective. The syndrome is inherited in an AR manner and is caused by mutations in the gene *ABDH5* (*CGI-58*) (64). Patients with NLSDI are often born as collodion babies and later develop mild generalized ichthyosiform erythroderma (Fig. 2l) and hepatosplenomegaly. Symptoms such as hearing loss, cataract, nystagmus, mental retardation, and ataxia are less consistent. The accumulation of lipid vacuoles in skeletal muscle cells can lead to muscular complaints (muscle weakness, myopathy) with increasing age. Depending on the extent of liver and muscle involvement, the blood levels of liver and muscle enzymes are raised. In the differential diagnosis, if Jordan abnormalities are detected, another type of lipid storage disorder, similar to NLSDI, but with severe myopathy, and no ichthyosis (NLSDM) (65), should be considered. NLSDM is caused by mutations in *ATGL* (*PNPLA2*) (65).

O3; ARKID syndrome. The acronym ARKID syndrome stands for autosomal recessive keratoderma, ichthyosis and deafness. Some patients exhibit additional symptoms, such as mental retardation, microcephaly, short stature or hip dislocation. Genetic causes of ARKID syndrome are, as in ARC syndrome, mutations in the gene *VPS33B* (66), which is why ARC syndrome and ARKID syndrome are referred to as allelic diseases. All 4 patients described in the literature carried the same mutation at amino acid position 131 (p.Gly131Glu) on at least 1 allele (either homozygous or in combination with a different second mutation). Alter et al. published an 11-year-old patient with liver damage due to copper overload in addition to the well-known ARKID symptoms, as well as exocrine pancreatic insufficiency (67). Copper overload has not previously been reported with ARCID or ARC syndrome, but with MEDNIK syndrome.

O4; SAM syndrome. SAM syndrome is characterized by 3 predominant symptoms: severe dermatitis, multiple allergies

and metabolic wasting. First, patients with biallelic loss-of-function mutations in the desmoglein 1 (*DSG1*) gene with an AR inheritance pattern were described (68, 69). As with Netherton syndrome, these patients have massively elevated levels of IgE. Later, cases of SAM syndrome were also diagnosed with heterozygous desmoplakin (*DSP*) gene mutations (70). Heterozygous mutations in *DSP* and *DSG1* are known in AD transmitted striate palmoplantar keratoderma.

CONGENITAL DISORDERS OF GLYCOSYLATION ASSOCIATED WITH ICHTHYOSIS

Congenital disorders of glycosylation (CDG) are due to deficiencies in the glycoprotein biosynthesis. The spectrum of clinical manifestation comprises multiple organ systems and includes ichthyosis. Four different types of CDG are caused by mutations in the genes *MPDU1* (CDG-If), *DOLK* (CDG-Im), *SRD5A3* (CDG-Iq) and *PIGL*, which is also known as CHIME syndrome or Zunich neuroectodermal syndrome (71, 72).

MPDU1-CDG is a defect in the N-glycan assembly in the endoplasmic reticulum (ER) (73, 74); patients present skin symptoms (ichthyosis, erythroderma), neurological features (psychomotor retardation, seizures), hypotonia, visual impairment, dwarfism and transient growth hormone deficiency.

DOLK-CDG is a defect in dolichol kinase that catalyse the last step of the dolichol phosphate biosynthesis. Patients have dilated cardiomyopathy, ichthyosis, epilepsy, microcephaly, visual impairment, hypoglycaemia and often die within the first 6 months (75, 76).

SRD5A3-CDG (cerebro-cerebello-oculo-cutaneous syndrome) is defined as a defect in polyprenol reductase within the biosynthesis of dolichol. Patients present ichthyosis, erythroderma and dry skin (77, 78).

Patients with PIGL-CDG or CHIME syndrome mainly have *c*olobomas, congenital *h*eart defects, early-onset migratory *i*chthyosiform dermatosis, *m*ental retardation and *e*ar anomalies. The defect is localized in the ER and concerns the second step of GPI-anchor biosynthesis, the de-*N*-acetylation of *N*-acetylglucosaminyl-phosphatidylinositol (79).

CONCLUSION

Inherited ichthyoses comprise a large spectrum of phenotypes that are caused by mutations in more than 50 different genes. Significant progress has been made in understanding the molecular mechanisms of ichthyoses over the past 25 years due to the accelerated development of DNA-based sequencing methods. However, there is still new evidence in genetics and molecular pathology of ichthyoses.

NGS has become a key technology for genetic testing and is applied in routine diagnostics of inherited diseases, since the costs are low, and the outcome is fast and effective. Multi-gene panel sequencing allows analysis of a few to a hundred genes simultaneously and cost-effectively, and guarantees the highest quality. The success rate of this method for the identification of disease-causing mutations in ichthyoses is approximately 80%. Large deletions or duplications cannot be fully detected by this method, although recent technical advances have been made to combine the evaluation of NGS and copy number variation (CNV) in the same analysis. Alternatively, CNVs can be detected by additional methods, such as multiplex ligation-dependent probe amplification (MLPA), quantitative real-time PCR, or aCGH (microarray-based comparative genomic hybridization). If multi-gene-panel analysis fails to detect disease-causing variants, WES can be used as an extended method, in which either only the DNA of the patient or, additionally, the DNA of the parents (trio) is examined. In WES all protein-coding regions (exons) of the approximately 22,000 human genes are analysed. The next possible level is WGS, which looks to the entire genetic information of the human genome, including complex structural variants. Structural variants comprise DNA segments inserted into or removed from the genome, as well as segments that are duplicated and segments whose direction is reversed. They are much more difficult to identify than single nucleotide variants, and it is actually not yet clear how many structural variants exist in a human genome.

The detection rate for disease-causing variants will increase with the help of all these performant technologies. However, the correct interpretation of the identified mutations is still associated with correct description of the phenotype and requires detailed clinical knowledge of diagnoses, differential diagnoses and terminology in dermatology.

The good news is that excellent clinicians will still be in great demand for the next 100 years.

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Centenary theme section: GENODERMATOSES

REVIEW ARTICLE

Ichthyosis: A Road Model for Skin Research[1]

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The understanding of monogenetic disorders of cornification, including the group of diseases called ichthyoses, has expanded greatly in recent years. Studies of the aetiology of more than 50 types of ichthyosis have almost invariably uncovered errors in the biosynthesis of epidermal lipids or structural proteins essential for normal skin barrier function. The barrier abnormality *per se* may elicit epidermal inflammation, hyperproliferation and hyperkeratosis, potentially contributing to the patient's skin symptoms. Despite this and other new knowledge about pathomechanisms, treatment of ichthyosis often remains unsatisfactory. This review highlights a series of approaches used to elucidate the pathobiology and clinical consequences of different types of ichthyosis, and related diseases with the ultimate goal of finding new and better treatments.

Key words: skin pH; ARCI; human epidermis; keratins; ceramides; therapy; epidermolytic; congenital; keratinocytes.

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SIGNIFICANCE

Ichthyosis refers to skin diseases with scaling somewhat reminiscent of fish scales (Greek: *ichthus*=fish). There are more than 50 genetic types of, mostly non-syndromic, ichthyosis, ranging in severity and frequency from mild and common (prevalence < 1%) to severe and rare (< 0.001%). In the latter case, babies are often born with a thick horny layer (collodion), dermal inflammation and impaired skin barrier function, requiring intensive medical care. Nearly all patients with ichthyosis require daily applications of cream, sometimes complemented with retinoid tablets. This review highlights recent progress in the understanding of the causes and consequences of ichthyosis, which may lead to better care and treatments.

Ichthyosis is an umbrella term for more than 50 types of, usually monogenetic, diseases, all characterized by widespread hyperkeratosis, xerosis and scaling of the skin, sometimes also associated with syndromic features. Typically, the skin problems begin at birth or shortly thereafter and usually show lifelong persistence. Depending on the underlying genotype, disease intensity ranges from mild to severe, in the latter case markedly reducing the patients' quality of life (1). Only rarely are there life-threatening consequences; for example, in neonates with harlequin ichthyosis (HI), epidermolytic ichthyosis (EI) and certain types of syndromic ichthyosis (2, 3). Later in life, less severe, but more common, complications occur, such as pruritus, ectropion and anhidrosis (Fig. S1[2]). Careful medical attention is frequently required, including oral retinoid therapy. Yet, the vast majority of patients with ichthyosis have only mild to moderate skin symptoms, which are readily controlled by daily applications of cream (2, 3).

Despite a thick stratum corneum (SC), patients with ichthyosis usually have variably increased transepidermal water loss (TEWL). This is due to various defects in the biosynthesis of proteins and lipids essential for normal barrier formation, specific for each of the 4 main types of non-syndromic ichthyosis (4):

- *Ichthyosis vulgaris* (I. vulgaris; prevalence 1:300) due to semi-dominant *FLG* mutations abolishing filaggrin's compaction of keratin filaments and release of hydrophilic molecules in the corneocytes.
- *X-linked recessive ichthyosis* (XRI; 1:3000 in males) caused by a deficiency of steroid sulphatase, resulting in accumulation of cholesterol sulphate (CSO_4) in the SC.
- *Autosomal recessive congenital ichthyosis* (ARCI; prevalence 1:100,000, including HI) due to mutations in any of >10 genes involved in the biosynthesis of acylceramides (acylCer), lipid lamellae and cornified lipid envelopes (CLE).
- *Keratinopathic ichthyosis* (1:300,000, including EI) caused by dominant negative mutations in keratin 1, 2 or 10, impairing the structural integrity of terminally differentiated keratinocytes.

This overview exemplifies a wide range of approaches used to elucidate the aetiopathogenesis of various types of ichthyosis, research that concurently results in a better understanding of normal human skin biology and yields new ideas about dermatotherapy (**Fig. 1**).

[1]This article is based partly on the Dohi Memorial Lecture presented at the 118th Annual Meeting of the Japanese Dermatological Association in Nagoya, June 2019.

[2]https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-3433

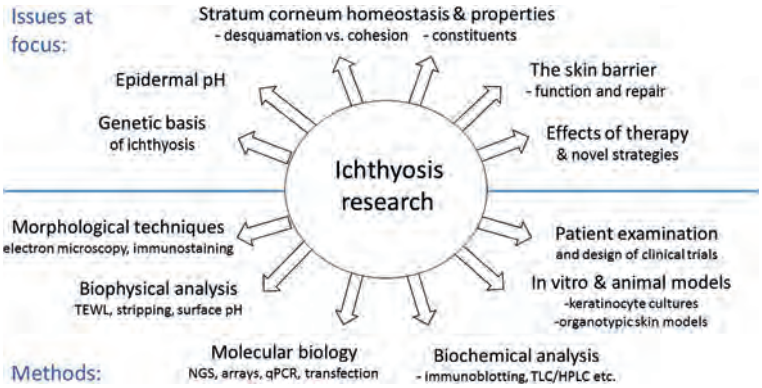

Fig. 1. Examples of scientific issues and methodological approaches in ichthyosis research. NGS: next generation sequencing; LC: liquid chromatography.

MICROSCOPIC EXPLORATION OF ICHTHYOSIS

The ingenious construction of human epidermis, with its many disparate functions and constant renewal of cells, also makes it vulnerable to genetic defects, frequently causing clearcut structural abnormalities. Thus, under light microscopy, I. vulgaris displays an absence of stratum granulosum (below SC) and in EI and ichthyosis with confetti due to various keratin mutations a clumping of intermediate filaments is seen, occasionally leading to cytolysis of suprabasal keratinocytes. However, in most other types of ichthyosis, electron microscopy (EM) is required to disclose the histopathological hallmarks (5–11).

In ARCI, for example, 4 distinctive ultrastructural patterns are identifiable in the granular and corneal layers of the epidermis: EM type 1 (lipid droplets), probably related to epidermal hyperproliferation (5); EM type 2 ("cholesterol clefts"(6)), typically associated with *TGM1* mutations (12); EM type 3 (abnormal lamellar bodies and elongated membranes (7)), often associated with *NIPAL4* mutations (9); and, EM type 4 (aggregated lipid membranes), exclusively associated with *SCL27A4* mutations (13, 14). Furthermore, HI and related conditions due to *ABCA12* mutations often show prominent distortions of the lamellar bodies (11, 15). Finally, and common to most types of ARCI, the lipid bilayers and CLEs are attenuated, best seen after ruthenium staining of the skin specimen (10).

EM analysis is clinically useful for differentiating ARCI from other conditions. For example, in a diagnostic team effort 2 Scandinavian half-brothers, initially believed to have an atypical form of ARCI, showed no signs of EM types 1–4, but the corneodesmosomes were few and abnormally looking (16). Genomic screening revealed novel mutations in the *DSG1* gene, consistent with a mild form of SAM syndrome (severe dermatitis, allergy and metabolic wasting) caused by desmoglein deficiency (17). Another example concerns the rare disorder *k*eratosis *l*inearis, *i*chthyosis *c*ongenita with *k*eratoderma (KLICK), which ultrastructurally exhibits massively enlarged keratohyalin granules (18). KLICK was eventually shown to be caused by recessive mutations in the regulatory elements of the *POMP* gene interfering with the proteasome degradation of numerous epidermal proteins (19).

Although EM is invaluable in many studies of ichthyosis, it is a tedious and costly method only available in certain laboratories. As an alternative, immunofluorescence (IF) analysis can be used, for example, for detecting cytoskeletal abnormalities in patients with ichthyosis with confetti (20) or for experimental studies of cultured cells from patients with EI (21). Regarding the latter, Fig. S2[2] shows IF stainings of keratin 10 in differentiated keratinocytes from a patient with *KRT10* mutation before and after *in vitro* exposure to heat. Clearly, heat stress causes aggregation of keratin filaments to a much higher extent than in healthy control cells (22). However, although the number of cellular aggregates was diminished by pre-treatment with a molecular chaperon designed to stabilize protein polymers (21), any extrapolation to the *in vivo* situation demands circumspection because the efficacy of topical chaperon was disappointing in a recent study of epidermolysis bullosa simplex, another keratinopathic disorder (23).

BIOPHYSICAL PROPERTIES OF STRATUM CORNEUM IN RELATION TO ITS BARRIER FUNCTION

Invasive techniques are not always required for obtaining *in vivo* information about SC. By simply applying an evaporimeter and a flat glass electrode to the intact skin, measurement of TEWL, skin hydration (capacitance) and surface pH is possible. This low-tech approach is useful in both healthy and diseased skin; for instance, when studying the effects of various drugs and noxious agents potentially affecting the skin barrier (24, 25). Another finding from these studies is that TEWL is elevated in untreated ARCI skin and increases further after efficient treatment with topical keratolytics (26). While this might inadvertedly enhance the pathomechanism of ichthyosis, remaining amounts of SC seems nearly always to be sufficient for preventing any harmful losses of water or influx of toxic substances via the skin.

However, when SC is mechanically removed *in toto* down to the glistening layer of epidermis, TEWL will increase dramatically (27). For obvious reasons, a concurrent increase in pH from ~5 on the skin surface to 7.4 in viable epidermis must then also occur, although details of this event for long remained unexplored (28). Fig. S3[2] shows that, soon after a complete removal of SC, pH and TEWL will start to decrease again, reaching normal surface values within 5–7 days, approximately one week before the full restoration of SC (28). Indeed, pH appears to normalize more quickly than TEWL, possibly reflecting its master role during barrier repair.

The importance of pH for SC homeostasis has also been highlighted by the discovery of a sigmoidal pH gradient over human SC, with its steepest slope occurring midway between stratum granulosum and the skin surface (28). This gradient, first demonstrated by repeated monitoring of pH in the course of >100 tape strippings, has since been confirmed using more sophisticated techniques in both human and mouse skin (29).

Interestingly, the pH gradient in SC looks quite different in I. vulgaris and XRI (30); in the former a shift towards less acidic values is observed, whereas the opposite is true for XRI (**Fig. 2**). The proposed explanation for this difference is a paucity of acidic break-down products of filaggrin (e.g. urocanic acid) in I. vulgaris and an accumulation of acidic CSO_4 in XRI (30). Incidentally, CSO_4 is a fascinating molecule, acting both as an inhibitor of SC desquamation (31) and as a signalling molecule during keratinocyte maturation (32, 33). In fact, a deficiency of CSO_4 in epidermis due to recessive *SULT2B1* mutations may also cause ichthyosis (34).

The key components contributing to the pH gradient in normal SC appear to be urocanic acid, free fatty acids and sodium-hydrogen exchanger -1 (NHE-1), all accumulating in acidic microdomains near the skin surface (35). Clearly, a reduction of approximately 2 pH units over a distance of only 10–20 µm (the normal thickness of SC) is biologically huge, and probably affects both lipid organization and protein structure at different depths of SC. Indeed, this makes treatment with pH-adjusting creams an intriguing option for some disorders of cornification (35–37). Examples of two pH-dependent enzymes operating in SC are kallikrein 5 and 7, the principal proteases involved in corneodesmosome degradation and desquamation (38). Besides pH, the activity of these enzymes depends on the amount of endogenous inhibitors, one of which is LEKTI (39). A genetic deficiency of LEKTI, as in Netherton syndrome (NS; *Ichthyosis circumflexa*), accelerates desquamation and reduces SC thickness to almost nil, hence dramatically increasing TEWL (39, 40). Ongoing clinical trials with topical application of synthetic inhibitors may lead to new treatments for NS and possibly atopic dermatitis, which is frequently associated with a secondary deficiency of LEKTI (41). Hypothetically, by modulating desquamation in the opposite direction, e.g. by blocking LEKTI, it might be possible to *increase* desquamation in some hyperkeratotic conditions, such as HI and EI, known to be associated with decreased secretion of proteolytic enzymes from the lamellar bodies (42, 43).

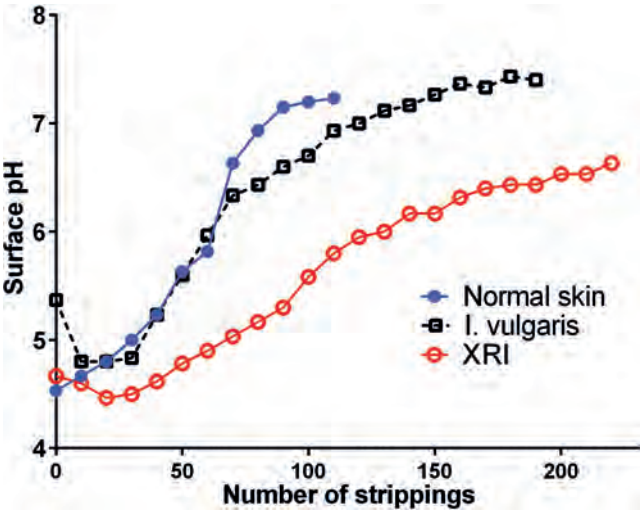

Fig. 2. The pH gradients over stratum corneum in healthy controls and patients with X-linked ichthyosis (XRI) or ichthyosis vulgaris (I. vulgaris). Mean pH values revealed by tape-stripping ($n = 10–11$ males in each group). When adjusting for the higher number of strippings required to remove SC in I. vulgaris (and XRI), the pH gradient will in the former case be shifted to the left compared to normal skin. (modified from refs. 28 and 30 with permission).

BARRIER REPAIR AND GENOMIC RESPONSES

Considering the many different aetiologies of ichthyosis, it is not far-fetched to assume that homeostatic responses in epidermis will differ depending on the genotype and the extent of barrier insufficiency it causes. One way of testing this hypothesis is to study the global mRNA expression in epidermis, using microarray analysis of transcriptomes extracted from tissue biopsies and searching for differently expressed genes (DEGs) in ichthyosis compared with normal skin.

In such a recent study, microarrays consisting of 22,000 genes were applied to pooled skin extracts from healthy controls and untreated patients with either XRI or I. vulgaris due to mono- or bi-allelic *FLG* mutations (44, 45). While patients with XRI showed only 27 DEGs, patients with I. vulgaris showed up to 120 times as many DEGs (Fig. S4[2]). Speculatively, the low number of DEGs in XRI is due to CSO_4-induced hyperkeratosis reducing the need for more active barrier repair (46, 47). In I. vulgaris, since no "silent" generation of hyperkeratosis occurs, a chronic repair process takes place that might explain the abundance of DEGs. This hypothesis gains support from our gene ontology and qPCR analyses, showing activation of numerous genes involved in inflammation, lipid metabolism and hyperproliferation; the response is particularly evident in patients with biallelic *FLG* mutations who are also notoriously prone to develop eczema (24, 44).

When skin samples from patients with ARCI with *TGM1* mutations were similarly investigated, a broad spectrum of 256 DEGs appeared; 25 involved in keratinization and cell mobility, 46 in immune response and 8 in acylCer biosynthesis, the last of which are also known as "ARCI genes" because of their involvement in the ARCI aetiology (48). Speculatively, a marked up-regulation of several ARCI genes reflects a positive feedback loop aimed at generating more omega-O-acylCer for barrier repair. However, in ARCI patients with truncating *TGM1*

mutations such a response is probably useless; no matter how many lipid precursors are available in the granular cells the absence of transglutaminase-1 (TGm-1) will prevent a proper crosslinking of CE and CLE (49).

Further support for a concept of ARCI proteins operating in a feedback regulated pathway comes from our recent studies using IF staining combined with CellProfiler imaging, allowing semi-quantitative comparisons of the protein expression at different depths of epidermis (48). **Fig. 3** shows examples of results obtained in biopsies from 5 patients with *TGM1* mutations and 4 healthy controls. Clearly 2 of the studied proteins, CYP4F22 and CerS3, co-localize in the granular layer of epidermis in both patients and controls, but the protein expression is much higher in the patients, thus corroborating the microarray data. The co-localization of 2 other ARCI proteins, TGm-1 and SDR9C7, was studied in more detail in healthy control skin using *in situ* proximity ligation assay (*is*PLA), which generates a signal when 2 different proteins are at a distance of less than 30 nm from each other (50). While filaggrin did not produce any *is*PLA signals with either of the 2 ARCI proteins, together they produced a strong signal in stratum granulosum consistent with a close interaction between TGm-1 and SDR9C7 in a chain of events leading to a proper formation of CLE (51). TGm-1 has also been found to co-localize with 12R-LOX and eLOX-3 in stratum granulosum of normal epidermis, but not in ARCI epidermis with inactivating mutations in *NIPAL4* (encoding ichthyin) (52). This implies that ichthyin (a tentative transporter of Mg^{2+} (53)) is also essential for acylceramide synthesis, acting in close proximity to other ARCI proteins.

In addition to an increased expression of several wild-type ARCI genes, numerous other genes involved in barrier repair, lipid biosynthesis, inflammation and anti-microbial peptides (AMPs) defence are also heavily upregulated in ARCI epidermis (48, 54–56). Incidentally, increased expression of AMPs might explain why microbial infections are rare in patients with lamellar ichthyosis despite a fissured and scaly skin. Analogously, psoriatic lesions express high levels of AMPs, albeit in this case on a background of much stronger immune and inflammatory reactions (57).

However, not all subtypes of ARCI exhibit a resilience against bacterial infections. For example, patients with HI and IPS often experience neonatal skin infections and septicaemia; in this case possibly related to a defective release of AMPs from the lamellar bodies (43). Furthermore, skin infections are frequent in EI with intrinsic defects in barrier repair, inter-corneocyte lipid deposition and AMP release (43, 58, 59), although in this case skin erosions and blistering are certainly a major contributing factor.

BIOCHEMICAL AND GENETIC STUDIES OF EPIDERMIS

By simply scraping the skin surface with a sharp blade, samples of SC can be collected for analysis of, for example, CSO_4 (46), urocanic acid and natural moisturizing factors (NMFs) (60). Using slightly more invasive techniques, such as superficial shave biopsies, full-thickness samples of epidermis are obtainable without significant risk of scarring. After homogenization and extraction of such samples, sensitive analytical techniques, such as high-performance liquid chromatography (HPLC), allow quantitation of numerous endogenous compounds and drugs, such as vitamin A and retinoids. For example,

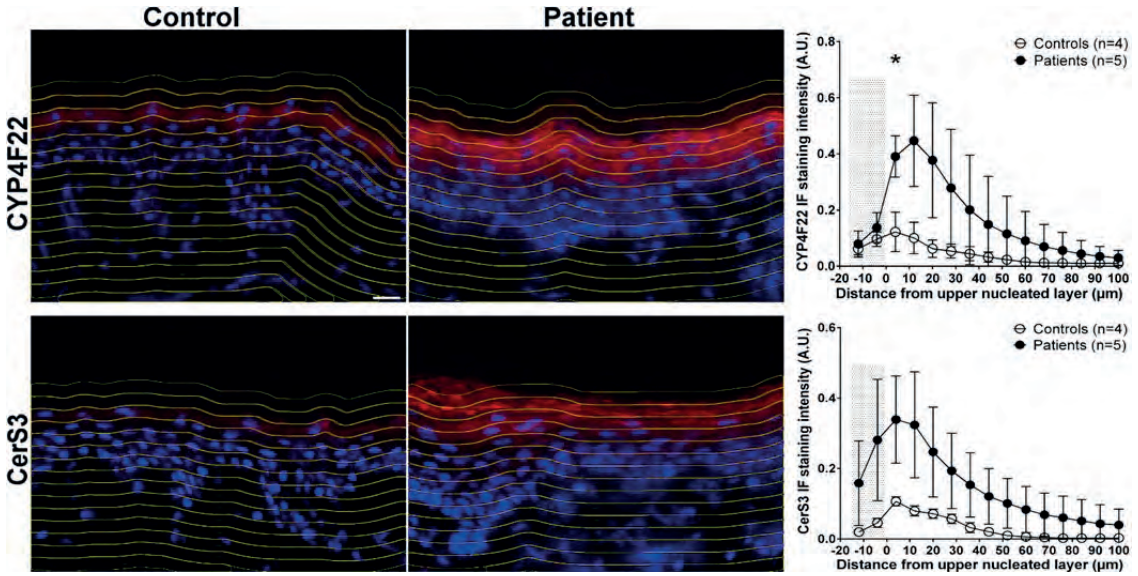

Fig. 3. Examples of immunofluorescence staining (left) and CellProfiler analysis (right) of the CYP4F22 and CERS3 proteins in patients with *TGM1* mutations versus healthy controls. The increased expression in patients' skin extends beyond the granular layer. The shaded area in the diagram correspond to stratum corneum (modified from ref. (48) with permission).

reduced concentrations of retinol (vitamin A_1) were found in I. vulgaris and increased levels of 3,4-didehydroretinol (vitamin A_2) in some types of hyperproliferative keratosis (61, 62), as yet without known significance. Although endogenous concentrations of all-*trans* retinoic acid in epidermis usually fall below the detection limit of the assay, therapeutic levels of isotretinoin and acitretin can be measured in shave biopsies (63, 64).

Using more sophisticated detection methods, such as ultra-performance liquid chromatography and mass-spectrum detection, the skin levels of fatty acids of different chain lengths, squalene and various types of ceramides (Cer) are quantifiable with high sensitivity and specificity (65–67). Indeed, the abnormal levels of various ceramides found in ARCI epidermis gave early clues to the existence of inborn errors of acylCer biosynthesis (68), which was later confirmed via gene hunting. **Fig. 4** summarizes our current understanding of lipid barrier formation in epidermis and the critical positioning of several ARCI proteins (for review see (4, 69, 70)). Subsequent to the enzymatic elongation of fatty acids (FA) by ELOVL4, the ultra-long chains (ULCFA) form amid-linkages with sphingosines, hence constituting Cer. This highly hydrophobic molecule undergoes a series of modifications, including a CYP4F22-mediated ω-hydroxylation of the FA moiety and subsequent transacylation with linoleic acid to form acylCer (71). The latter step, enhanced by PNPLA1, is essential for the formation of the lipid bilayers in SC. A significant fraction of acylCer undergoes further oxidation of the linoleate moiety, catalyzed by 12R-LOX and eLOX-3, and a subsequent covalent binding to CE (72). This final step in CLE formation was previously thought to be catalysed by TGm-1, analogous to the transacylation of involucrin. However, a recent report implicates an alternative pathway involving SDR9C7 (67). SDR9C7 is a dehydrogenase, that converts the oxidated linoleate molecule into a 13-ketone, a reactive moiety known for its non-enzymatic coupling to protein (67). As a corollary, ARCI caused by SDR9C7 deficiency is characterized by absent CLEs on EM examination (67).

Interestingly, Crumrine et al. (70) recently proposed that virtually all the above-mentioned processes take place within the lamellar bodies, subsequently delivering preformed CLE scaffolds and lipid bilayers to the intercellular space via exocytosis. It was also suggested that some previously unexplained ultrastructural features in ARCI are actually caused by toxic levels of free FA accumulating in the keratinocytes owing to a downstream blockade in the acylCer pathway (70). As a possible extension to this "blockage theory", our own findings of an upregulation of several ARCI proteins in the skin of patients with inactivating *TGM1* mutations (48) imply that lipoxygenated acylCer, instead of being converted to CLE by TGm-1, accumulates in the corneocytes as lipid aggregates or membranes. Speculatively, this might explain some of the EM characteristics of *TGM1*-associated ARCI (6). Conversely, more upstream blockages of acylCer biosynthesis, e.g. due to *CYP4F22* or *CERS3* mutations, might instead reduce the acylCer levels and thus impair the formation of both intercellular lipid bilayers and CLE. Although much remains to be clarified about this and the other pathogenic process in ARCI, there are already good arguments for distinguishing aetiologies related to inborn errors of the acylCer metabolism from other causes of ARCI; for example, by using prefixes, such as "lipodysgenic" or "lipid synthetic", for this groups of disorders (48, 70).

Thanks to research mainly from France, Germany, Japan, Scandinavia, UK and the USA, it is now possible to genetically diagnose all forms of common and keratinopathic ichthyosis, and 85–90% of cases with ARCI (for review see (4)). With respect to the latter diagnosis, the leading causes of ARCI in Northern Europe are homozygous or compound heterozygous mutations in *TGM1* (30–35%), *ALOX12B* or *ALOXE3* (combined 15–20%) and *NIPAL4* (12–15%) (4, 73–76). Detailed discussions

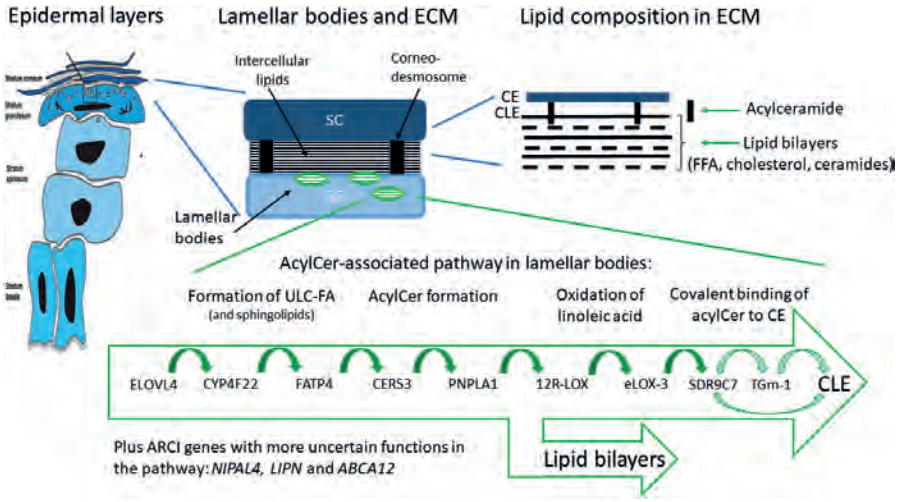

Fig. 4. Crucial components and biosynthetic steps in the formation of an epidermal lipid barrier. *Light green (hatched) arrows* in the box indicate two alternative pathways in cornified lipid envelope (CLE) formation (modified from Am J Clin Derm (4) with permission). SC: stratum corneum, SG: stratum granulosum, ECM: extracellular matrix; CE: cornified envelope, FFA: free fatty acids, ULC-FA: ultra-long chain fatty acid, AcylCer: acyl ceramide, ELOVL4: ELOVL fatty acid elongase 4, CYP4F22: cytochrome P450 family 4 subfamily F member 22, FATP4: fatty acid transport protein 4, CERS3: ceramide synthase 3, PNPLA1: patatin-like phospholipase domain containing 1, 12R-LOX: arachidonate 12-lipoxygenase, 12R-type, eLOX-3: hydroperoxide isomerase ALOXE3, TGm-1: transglutaminase-1, NIPAL4: magnesium transporter NIPA4 (ichthyin), LIPN: lipase member N, *SDR9C7*: short-chain dehydrogenase/reductase family 9C member 7.

about the genetics of ichthyosis are available in 2 other papers (77, 78).

CLINICAL EXAMINATION AND SERENDIPITY AS RESEARCH TOOLS

Patients with ichthyosis frequently exhibit skin signs and symptoms that may be difficult for the examining doctor to describe in a concise way or to score for severity grade, e.g. in relation to a scientific study. Characteristically, skin lesions may be generalized or only occur focally, and the intensity of scaling may range from mild to severe, with scales typically described as lamellar, collodion-like, cobblestone-like, brownish, fine and white, etc. Furthermore, a plethora of other symptoms occurs, such as xerosis, palmoplantar keratoderma, erythema, itch and pain, all with a variable degree of severity. Adding to this complexity, phenotypic fluctuations often occur over time, either spontaneously or as the result of treatment or environmental factors, e.g. work, climate and season of the year. No wonder then a consensus is still lacking about the best severity scoring system to use in clinical trials (for review see (79))

In clinical practice, however, less sophisticated scoring models may still be useful. For example, in a recent study of 132 patients with ARCI, separate scorings (0–4) of ichthyosis (IS) and erythema (ES) severity were made in 10 different body regions, followed by an area-adjusted summation of individual score values (4, 73, 80). When the IS and ES values recorded at age >1 year were plotted against one another in a diagram, the individual ratios roughly distributed into 4 partially overlapping circles seemingly corresponding to the major clinical subtypes identified at first examination, i.e. before the genetic results became known (**Fig. 5**). Unsurprisingly, harlequin ichthyosis (HI), the rarest and most severe subtype of ARCI due to truncating *ABCA12* mutations, shows the highest IS and ES values. Lamellar (LI) and erythrodermic ichthyosis (CIE), with more varied and partially overlapping phenotypes and genotypes, show high values of either IS or ES. The 4th entity, shows low values of both IS and ES, although most of the patients had severe skin symptoms at birth, healing spontaneously over a period of several weeks. This altering pattern is consistent with *pleomorphism*, "a condition in which an individual assumes a number of different forms during its life-cycle". Accordingly, pleomorphic ichthyosis (PI) is a suggested new name for this subgroup of ARCI previously known as "non-LI/non-CIE" (80). It comprises several distinct conditions, such as self-improving collodion ichthyosis (mostly due to mild *ALOX12B* mutations), bathing-suit ichthyosis (due to temperature-sensitive *TGM1* mutations) and IPS (specifically caused by *SLC27A4* mutations) (73, 80, 81). (*Nb*: "syndrome" is probably a misnomer for IPS, because all extra-cutaneous symptoms appear to be secondary to the skin malfunction.)

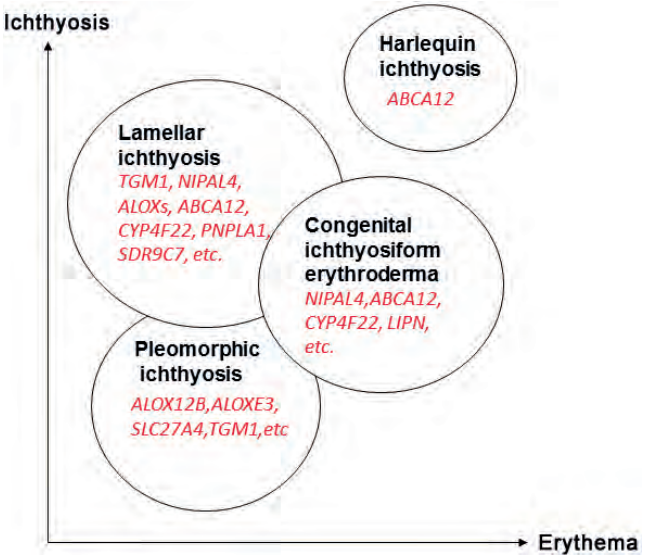

Fig. 5. Tentative correlation between ichthyosis and erythema severity 4 types of autosomal recessive congenital ichthyosis (ARCI) with partially overlapping phenotypes and culprit genes. (modified from refs. (4, 75) with permission).

While a crude classification of ARCI into 4 major subgroups may seem superfluous in an era of exact genetic diagnosing, it is still useful; for instance, when diagnosing ARCI without available genetic expertise or for teaching medical students how to distinguish between the various types of ichthyosis.

Another bonus of a detailed skin examination is the chance of making serendipitous findings. **Fig. 6**A illustrates such a case: a 45-year-old woman, diagnosed in childhood with keratitis, ichthyosis, deafness (KID) syndrome due to a recurrent mutation in *GJB2* (82). She started in her 20s to develop spots of normal-looking skin, which gradually grew in size and number, were histopathologically "non-lesional". A subsequent sequencing of DNA from the healed spots revealed several

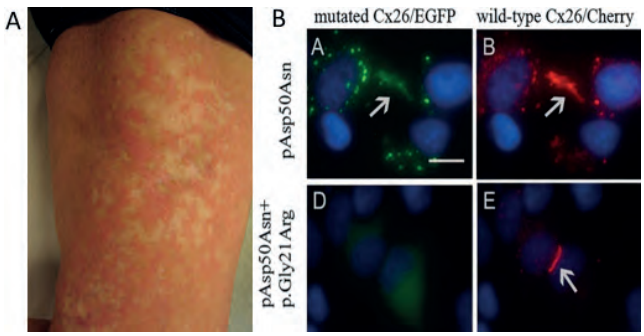

Fig. 6. Patient with keratitis-ichthyosis-deafness (KID) syndrome showing spontaneous revertant *GJB2* mutation restoring the normal phenotype of keratinocytes. (A) Gradually appearing white spots in erythrokeratodermic areas on the thigh, and (B) effects of the patient's somatic (silencing) mutation on an allele with germline *GJB2* mutation (EGFP) when transfected to HeLa cells together with wt-*GJB2* (Cherry). The blurred gap junctions (*top panel*), resembling the situation in lesional skin, are restored by the *de novo* somatic mutation (*bottom panel*) as in healed spots (modified from ref. (83) with permission).

de novo mutations restricted to the disease-causing *GJB2* allele. Co-transfection of germline and *de novo* (somatic) mutations together with wt-*GJB2* in HeLa cells showed that the *de novo* gene product remained intracellular, thus allowing an unopposed incorporation of wild-type connexin 26 into the gap-junctions (Fig. 6B) (83). Similar examples of spontaneous revertants in the skin have been described in epidermolysis bullosa (84), ichthyosis with confetti (85) and loricrin keratoderma (86). This makes drug enhancement of revertance ("natural gene therapy") an interesting possibility for dominant negative genodermatoses (87, 88).

NEW THERAPEUTIC DEVELOPMENTS

Besides emollients and keratolytic creams (2, 3, 26), retinoids remain mainstay therapy for moderate to severe forms of ichthyosis. Acitretin and isotretinoin are the preferred drugs for systemic use, with newcomers, such as alitretinoin, probably having a less favourable risk/benefit ratio (89), and retinoic acid metabolism blocking agents (RAMBAs), such as liarozole, not yet commercially available (90). Broadly speaking, vitamin A agonists have anti-keratinizing and keratolytic effects. However, because many retinoids bind to specific ligand-activated transcription factors and regulate the expression of numerous genes expressed in epidermis, more specific effects on ichthyosis pathogenesis are also to be expected. One example is the different outcome of retinoid treatment in patients with epidermolytic ichthyosis due to *KRT10* or *KRT1* mutations (91). Whereas the former patients respond quite well to low-dose retinoid therapy, consistent with a down-regulation of mutated *KRT10* (92), patients with *KRT1* mutations often get worse and develop more blisters during retinoid therapy (91). A proposed explanation is the ubiquitous down-regulation of *KRT2* by retinoids; this effect is harmless in both normal and *KRT10*-mutated epidermis, but deleterious in patients with *KRT1* mutations who depend on keratin 2 as a replacer of mutated keratin 1 during its dimerization with keratin 10 (93). Conversely, patients with the Siemens type of superficial ichthyosis, caused by keratin 2 mutations that interfere with its heterodimerization to keratin 9, are known to respond most favourably to retinoids (94).

Encouragingly, many new ideas for ichthyosis treatment are in the pipeline, targeting not only the causative mechanisms, but also secondary events, such as inflammation and hyperproliferation. Although gene therapy for skin diseases has not yet proved as successful as initially hoped, topical antisense therapy blocking the translation of mutated mRNA has shown promising results, at least in pachyonychia congenita, a keratinopathic disorder mechanistically similar to epidermolytic ichthyosis (EI) (95). Moreover, disruption of mutated *KRT10* in EI keratinocytes using a transcription activator-like effector nuclease (TALEN) technology reverts the intermediate filament fragility *in vitro* (96). These and other approaches, such as CRISPR/Cas9 gene editing, aimed at correcting the underlying mutation *in situ*, holds promise for a more specific gene therapy for ichthyosis in the future (97, 98).

Substitution and replacement therapy are other interesting approaches. Since various types of ceramides can now be synthetized in large amounts, they are obvious candidates for testing topically in ARCI (99). Another, still preclinical, approach is to enhance the acylCer pathway via ligand stimulation of transcription factors, such as peroxisome proliferator activating receptors (PPARs) expressed in epidermis and known to affect the expression of many ARCI genes *in vitro* (100). Several PPAR agonists are already in use for diabetes and cardiovascular disease. However, for this hypothetical treatment to be effective in ARCI, all genes involved in CLE formation must remain at least marginally intact, implying that lipodysgenic ARCI due to truncating mutations will remain unresponsive. In this context, enzyme replacement therapy (ERT) with topically applied recombinant transglutaminase may become an attractive (but expensive) future option, especially for patients with *TGM1*-associated ARCI (101). Perhaps a combination of ERT and supplementation with synthetic ceramides would prove most versatile, although this approach remains to be studied.

As regards treatment of secondary pathogenic events, it is noteworthy that skin inflammation in ARCI has many similarities to psoriasis, making already approved biological therapies feasible to test in severe cases of ichthyosis (56). In the long term, the search for new therapies in ichthyosis should also focus on alternative ways to restore the skin barrier and to dampen excessive intrinsic responses, which often cause more harm than relief to the patient. Whether this goal is attainable through

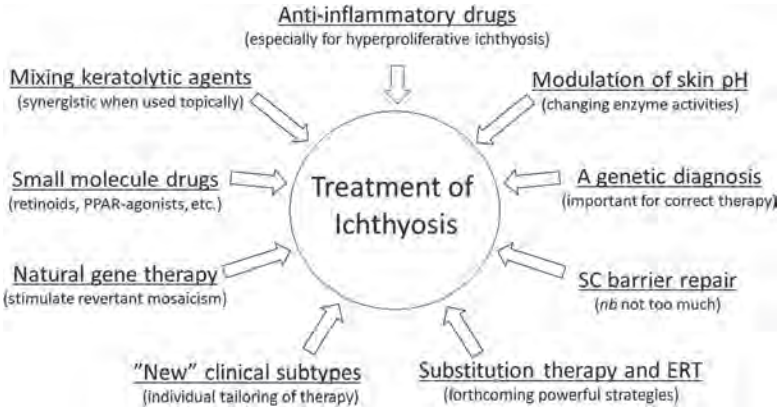

Fig. 7. Summary of ideas about future development of ichthyosis therapy. ERT: enzyme replacement therapy.

gene technology and new biologics, or by specifically tailored molecules and substitution therapies, remains to be determined. **Fig. 7** gives a summary of these and other prospects for ichthyosis treatment.

CONCLUSION

The skin is, both clinically and pre-clinically, a "research-friendly" organ. By combining a wide variety of investigative methods, ranging in complexity from simple *in vivo* measurements of TEWL and surface pH, to high-tech biochemical and genomic analyses of minimally invasive skin biopsies, or *in vitro* cultures of reconstituted skin, much information is attainable about the pathobiology of many skin diseases, not least ichthyosis.

Today, when almost 100 subtypes of ichthyosis have been characterized at both the genomic and ultrastructural level, an exact diagnosis early in life, a definite establishment of mode of inheritance, and an accurate genetic counselling should nearly always be feasible.

Though the treatment options have also evolved over the years, there is still a great need for new developments aimed at improving the patients' quality of life. Through this research, new knowledge may also be gained about many other skin diseases with biological features similar to ichthyosis, such as eczema and psoriasis, which are also characterized by inflammation and a perturbed skin barrier.

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CUTANEOUS AND GENITAL INFECTIONS

Theme Editors:

Roderick J. Hay and Kristian Kofoed

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REVIEW ARTICLE

Skin Infections Caused by *Staphylococcus aureus*

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***Staphylococcus aureus* is the most common pathogen involved in skin infections worldwide, regardless of the patient's age, the climate or geographical area. The main skin clinical manifestations can be linked to a few toxins produced by the bacteria, which give rise to a rich and varied clinical spectrum. Panton Valentine leucocidin, exfoliatins, enterotoxins and toxin shock syndrome toxin 1 are the main toxins involved in most dermatological manifestations associated with *S. aureus*. Other less frequent cutaneous manifestations can occur in endocarditis, bacteraemia. Currently, the most important event is worldwide emergence of community-acquired S. aureus resistant to methicillin (CA-MRSA), mainly causing skin infections.**

Key words: skin infections; *staphylococcus aureus*; bacterial skin infections; cellulitis; furuncle; abscess.

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SIGNIFICANCE

This review describes the characteristics of *Staphylococcus aureus* infections of the skin. Most can be linked to a few toxins produced by the bacteria, which give rise to specific clinical manifestations. Panton Valentine leucocidin, exfoliatins, enterotoxins and toxin shock syndrome toxin 1 are the main toxins involved in most dermatological manifestations associated with *Staphylococcus aureus*. Unfortunately, most reports of *Staphylococcus aureus* skin infections do not consider this complexity. This review should help further research into *Staphylococcus aureus* infections of the skin to consider this rich and varied clinical spectrum.

Staphylococcus aureus is the most common pathogen involved in skin infections worldwide, regardless of the patient's age, the climate or geographical area. The main skin clinical manifestations can be linked to a few toxins produced by the bacteria. Panton Valentine leucocidin (PVL), exfoliatins (ETs), enterotoxins and toxin shock syndrome toxin 1 (TSST-1) are the main toxins involved in most dermatological manifestations associated with *S. aureus*. Other less frequent cutaneous manifestations can occur in the context of bacteraemia. The complex role of *S. aureus* in atopic dermatitis is not considered in this review. Currently, the most important event is the worldwide emergence of community-acquired *S. aureus* resistant to methicillin (CA-MRSA), which is mainly responsible for skin infections.

LOCALIZED *S. AUREUS* SKIN INFECTIONS

Localized *S. aureus* skin infections are either primary or secondary. A primary or "spontaneous" cutaneous infection is an infection occurring without preceding clinically evident lesions or secondary to a minimal skin lesion. These infections include impetigo, folliculitis, furuncles, and primary abscesses. Secondary skin infections are those occurring as a consequence of a pre-existing cutaneous lesion (usually incorrectly called "superinfections"). These include impetiginization, secondary abscesses, lymphangitis, cellulitis and secondary wound infection. This distinction between primary and secondary infection is not strict and may appear somewhat theoretical or artificial, but it allows an understanding of the physiopathology of skin infections.

Impetigo

Impetigo is an epidermal infection caused by *S. aureus*, *Streptococcus pyogenes*, or a combination of both. In northern countries *S. aureus* infections are predominant, representing 90% of the bacterial causes, whereas in developing countries *S. pyogenes* is reported to be predominant (1–5). Impetigo mainly affects children and predominates in underprivileged communities (1–5). It is contagious, with the possibility of self-inoculation and the occurrence of small family or community outbreaks.

The diagnosis of impetigo is clinical. For *S. aureus* impetigo, the primary lesion is a fragile bullae. The bullae quickly becomes inflamed and pustular and ruptures to form an oozing erosion or crust (**Fig. 1**). A frequent and typical localization in children is around the mouth, but any area of the skin can be affected. The grouping of multiple lesions can result in polycyclic erosions with circular contours. The general physical state is preserved. There is no fever; sometimes a satellite lymphadenopathy may be present. Several classical variants have been reported, such as giant impetigo, miliary impetigo, pustules, and dry impetigo. Impetigo neonatorum, previously known as pemphigus neonatorum, is generalized impetigo in neonates. Impetiginization characterizes the secondary

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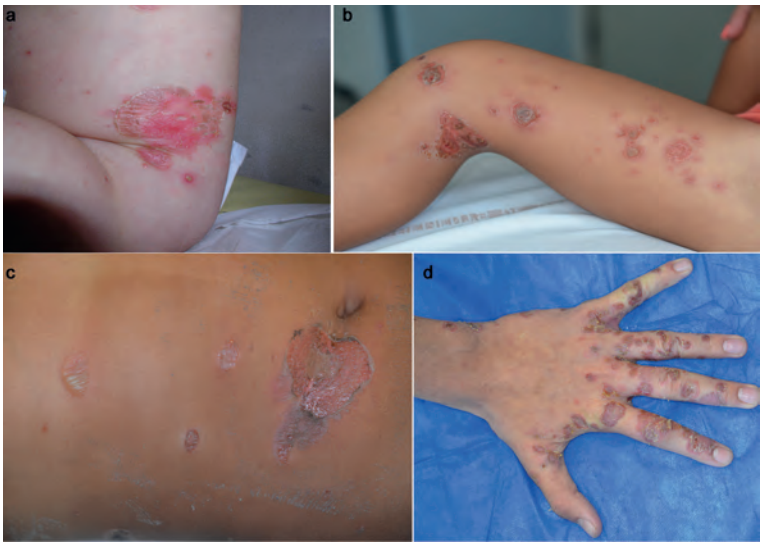

Fig 1. Different clinical presentations of impetigo: a), large dry erosive plaque on adbomen; b) crusted and oozing erosions on the lower limb; c) bullous and oozing erosive lesions on abdomen; d) multiple dry erosions of the hand.

infection by *S. aureus* of a pre-existing dermatosis, usually affecting the epidermis (e.g. eczema, chickenpox, etc.) or secondary to scratching (e.g. pediculosis, scabies, etc.) that results in impetigo or impetigo-like crusted and oozing lesions. A clinical variant of impetigo is ecthyma, in which deep ulceration forms in the dermis (more frequently with *S. pyogenes*). Scabies is a major cause of impetigo in children worldwide and, more specifically, in disadvantaged populations (6–8). Mass or individual treatment of scabies results in a decrease in the prevalence of impetigo in a community (6–8).

The pathophysiology of staphylococcal impetigo is related to the local production of exfoliatin toxins A and B (1, 9–11). The target protein of exfoliatins A and B is desmoglein 1, a desmosomal protein whose role is the cohesion between keratinocytes, and it is mainly located in the most superficial layer of the epidermis (1, 9–11). The main consequence of the action of the toxin on desmoglein 1 is rupture of keratinocyte cohesion and formation of a bullae. Although bullae are not usually reported in impetigo caused by *S. pyogenes*, a similar mechanism could be involved; the streptococcal pyrogenic exotoxin B (SpeB) has been demonstrated to be a proteolytic factor that cleaves the extracellular domains of desmoglein 1 and 3 (12).

According to Koning et al. (13) treatment with topical mupirocin and topical fusidic acid are equally effective to, or more effective than, oral treatment, except in extensive impetigo where research is lacking. Penicillin was not as effective as most other antibiotics (12, 13). Hygiene measures, such as strict attention to handwashing, must be applied to prevent recurrence and cross-transmission.

Regarding the risk of antibiotic resistance in impetigo, rare clones of methicillin-resistant *S. aureus* (MRSA) producing ETA and/or ETB and have been described, mainly from Japan (14–17). In terms of resistance in impetigo, the main concern is with fusidic acid. Resistance to fusidic acid has increased in the early 2000s in some countries of northern Europe, namely Sweden, Norway and the UK. This increase appears to have resulted from the clonal expansion of a strain designated the Epidemic European Fusidic acid resistant Impetigo Clone (EEFIC), which carries the fusidic acid resistance determinant fusB on its chromosome. The high level of use of fusidic acid ointment has been linked to the emergence and spread of fusidic acid resistant *S. aureus* (18–20).

Folliculitis

S. aureus is responsible for the majority of cases of folliculitis (infection of the pilosebaceous follicle).

Superficial folliculitis. In this condition the infection is restricted to the superficial part of the pilosebaceous follicle (follicular ostium). Clinically it manifests as a pustule, centred on a hair associated with a peri-follicular erythema. All parts of the body with high-density hair can be affected: thighs, perineum, arms, back, eyelid (stye). Sycosis barbae (**Fig. 2**), whose spread is favoured by shaving, is a particular clinical form localized on the face, characterized by extensive and chronic lesions. Differential diagnoses include folliculitis caused by other microorganisms, such as dermatophytes in kerions, *Candida albicans* in candida folliculitis, *Malassezia* folliculitis, Gram-negative folliculitis, non-infectious folliculitis (including Behcet's disease) and hidradenitis suppurativa.

Furuncle (boil). Furuncles, or boils, are characterized by a deep and necrotizing form of folliculitis with involvement of the pilosebaceous follicle in its entirety. It presents as a painful inflammatory papule or nodule, centred around a pustule on a hair-bearing area (the hair has usually disappeared due to necrosis) (**Fig. 3**). Within a few days of maturation pus will form, associated with ne-

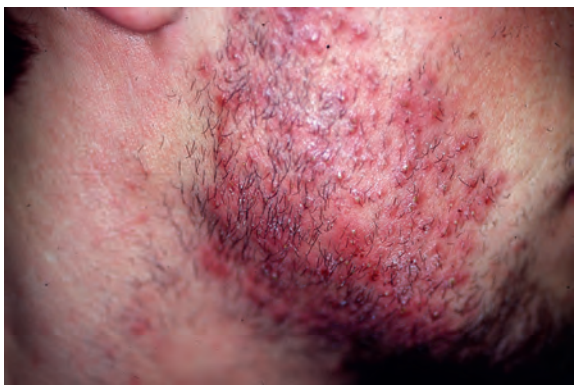

Fig. 2. Sycosis barbae.

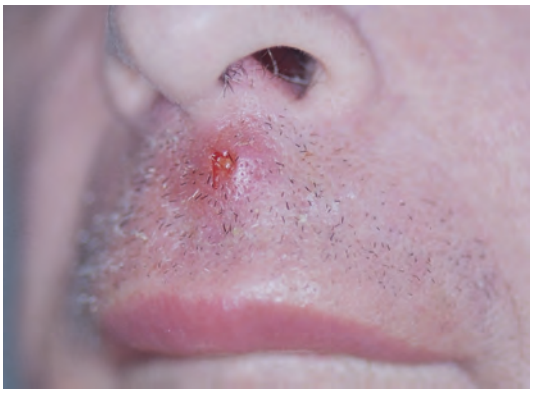

Fig. 3. Furuncle.

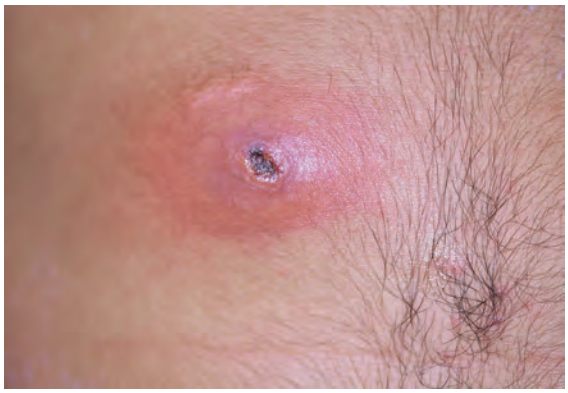

Fig. 4. Primary abscess.

crosis (21). A circular desquamative flange may surround the necrotic centre (22). In recent years it has been found that up to 90% of the *S. aureus*, isolated from furuncles in some areas produce PVL virulence factor (23–26). This leucocidin leads to local destruction of leucocytes with the formation of larger skin lesions, which respond less well to treatment and tend to recur; the organisms can also cause suppurative pneumonia.

The term "furuncle" has sometimes been used in the literature for skin infection caused by other bacteria, such as non-tuberculous mycobacteria (27), but, to avoid confusion, should be reserved for *S. aureus* infection.

A clinical variant of a furuncle is the carbuncle, defined as a cluster of furuncles. Chronic furunculosis is characterized by the repeated formation of furuncles on different parts of the body over several months (21).

Many reports of systemic infection secondary to a furuncle are reported, but this appears to be rare relative to the high frequency of furuncles. Facial malignant staphylococcal infection is a classically described infection, but nowadays it is an exceptionally rare complication of a peri-nasal furuncle leading to a septic facial venous thrombosis that can extend to the cavernous sinus (28).

Abscess

An abscess is a collection of pus. The abscess forms from a tender inflammatory and extremely painful erythematous nodule or plaque. After a few days of evolution, the consistency changes and become soft, indicating the formation of the collection of pus (**Fig. 4**). Abscesses can be primary or secondary. There is no clearly defined size in the literature for an abscess, therefore in primary abscess, the distinction between a large furuncle and a small abscess is difficult or artificial. Fever is rare, cellulitis, lymphangitis, and satellite adenopathies may be associated. The general physical state is preserved. Pus may appear after some days of spontaneous evolution, and if not drained, spontaneous skin necrosis with rupture and drainage of the pus may occur.

S. aureus is by far the main infectious bacteria isolated from abscesses. The majority of primary or spontaneous abscesses are caused by *S. aureus* producing PVL (23, 29–31). Secondary abscesses (accidental direct inoculation, drug addiction, septic injections, etc.) are most often, but not exclusively, due to *S. aureus* (32).

The treatment of suppurative skin infections is based on incision and drainage. The role of antibiotics has been summarized in recent important studies. In the study by Daum et al. (33), 786 participants with a skin abscess 5 cm or less in diameter were treated by incision and drainage and were randomly assigned to receive clindamycin, trimethoprim–sulfamethoxazole (TMP-SMX), or placebo for 10 days; the cure rate among participants in the clindamycin group was similar to that in the TMP-SMX group (221 of 266 participants (83.1%) and 215 of 263 participants (81.7%), respectively; $p=0.73$), and the cure rate in each active treatment group was higher than that in the placebo group (177 of 257 participants (68.9%), $p<0.001$ for both comparisons). Among the participants who were initially cured, new infections at 1-month follow-up were less common in the clindamycin group. Talan et al. (34) compared TMP-SMX with placebo after incision and drainage of abscesses; clinical cure of the abscess occurred in 507 of 630 participants (80.5%) in the TMP-SMX group vs. 454 of 617 participants (73.6%) in the placebo group ($p=0.005$). TMP-SMX was superior to placebo, resulting in lower rates of subsequent surgical drainage procedure, skin infections at new sites, and infections in household members.

Emergence of suppurative skin infection due to community-acquired methicillin-resistant S. aureus. Methicillin has been available since 1961, it was the first semi-synthetic penicillin resistant to penicillinase produced by most of *S. aureus* at that time (35). Its introduction was quickly followed by the appearance of MRSA (35). This resistance is linked to the synthesis of a modified penicillin-binding protein with less affinity to betalactams, PLP2a, leading to resistance to all beta-lactams (except for new cephalosporins ceftaroline and ceftobiprole). The synthesis of this PLP2a is under the control of the *mecA* gene, located on a chromosomal mobile genetic element, called the staphylococcal cassette chromosome mec or SCCmec, bordered at both ends by genes called

chromosome cassette recombinase (ccRA/ccRB or ccRC), which allow horizontal transmission between and within species. Described almost exclusively in hospitals, these hospital-acquired methicillin-resistant (HA-MRSA), clones have spread widely throughout the world. Over time, they have acquired, in addition to the *mecA* gene, other resistance genes against other classes of antibiotics, such as macrolides, fluoroquinolones or aminoglycosides (1). However, these clones are rarely involved in skin infections, except for nosocomial operative site infections.

The epidemiology of MRSA has entered a new era the last 25 years. MRSA with new characteristics have emerged in the community setting, namely outside of healthcare facilities (35–39). First reported in Oceania (Australia and New Zealand), these CA-MRSA are currently present worldwide (35–39). Most strains (80–90%) are isolated from suppurative skin infections (35–39). CA-MRSA infections have specific characteristics that clearly distinguish them from HA-MRSA (35–39); they preferentially affect a young population with no previous medical history (35–39). Unlike HA-MRSA, which are often multi-resistant, CA-MRSA generally remains sensitive to most antibiotics apart from beta-lactams. The genetic origin of CA-MRSA is different, with a few major clonal complexes with relative geographical specificity (35–39), USA 300 being the major clone in the USA. The main SCCmec cassettes for HA-MRSA (SCCmec I, II and III) are significantly longer than those for CA-MRAS (mainly SCCmec IV and V). Almost all of CA-MRSA, including the major clones, produce the PVL toxin, which explains the predominance of suppurative skin infections as clinical presentations of CA-MRSA infections. There are no clinical data to suggest that PVL CA-MRSA skin infections differ from PVL methicillin-sensitive ones (MSSA) and their relative prevalence varies in different countries. As CA-MRSA is isolated mainly from suppurative skin infections, the best way to study its epidemiology is to study those infections. Indeed, some countries, such as the USA, have a high rate of CA-MRSA, at approximately 50% of strains isolated (most USA 300) (40–42) and others have a low rate, at less than 10% (43–45). Outbreaks of CA-MRSA are regularly described mainly in different community settings (military personnel, sports teams, drug users, homosexuals, isolated communities, families, etc.) (46–50).

Acute suppurative paronychia

Acute suppurative paronychia is an acute infection of the eponychial nail folds of the hand or foot. Several bacteria may be implicated, but *S. aureus* is the most common one. The treatment is based on surgical excision; antibiotic treatment plays a minimal role (51).

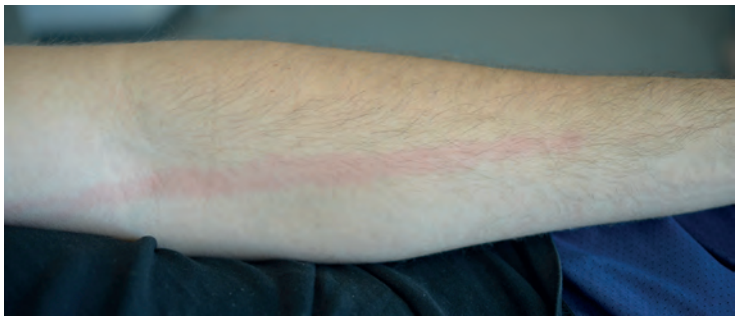

Fig. 5. Lymphangitis.

Lymphangitis

Lymphangitis is caused mainly by *S. aureus* or *S. pyogenes*. It is clinically characterized by an erythematous inflammatory linear band, which usually starts from the origin of the infection towards the draining regional lymph node, namely a local adenopathy (**Fig. 5**). Lymphangitis is sometimes accompanied by fever. Otherwise, general health state is preserved. Treatment is based on systemic antibiotic therapy.

Superficial septic thrombophlebitis

An important feature in the pathophysiology of *S. aureus* infections is its thrombotic capacity. The constitution of a vascular thrombosis allows the infection to develop and cause septic emboli and secondary locations. Staphylococcal skin infection can cause septic thrombophlebitis of the superficial venous network, which can spread to the deep veins. In hospitals, this is most often a complication related to the infection of intravenous catheters. Septic thrombophlebitis is characterized by an inflammatory indurated cord, which begins at the infected site (**Fig. 6**). Treatment is based on antibiotic therapy and treatment of the portal of entry. A particular form of such thrombophlebitis is facial malignant staphylococcal infection (see above).

Cellulitis

Cellulitis may occur associated with an abscess or a thrombophlebitis or complicate an acute or chronic wound as a result of secondary infection. It is more

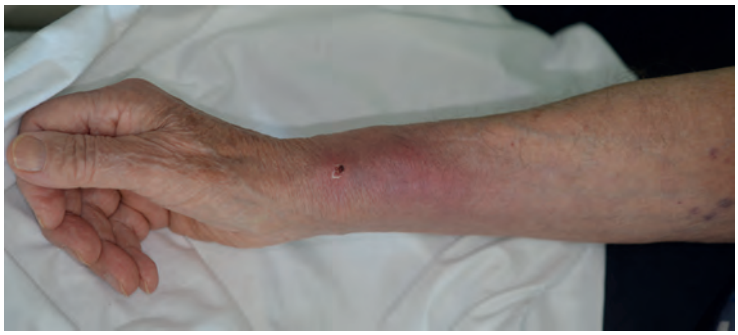

Fig. 6. Thrombophlebitis from catheter site.

common with *S. pyogenes*. The treatment is based on systemic antibiotic therapy.

Necrotizing fasciitis

A few reports of necrotizing fasciitis (NF) associated with *S. aureus* have been published. Miller et al. (52) reported 14 cases in 2005 caused by CA-MRSA. A few other isolated cases have been published since. Given the scarcity of the reports, NF caused by *S. aureus* seems exceptional.

Contiguous infections

These are related to a suppurative focus located near the skin (53). They manifest as an inflammatory mass that simulates an abscess, particularly in the vicinity of septic arthritis, osteomyelitis, bursitis, tenosynovitis or infected false aneurysms or myositis. Sometimes cutaneous fistulization occurs.

Secondary infections of acute or chronic wounds

They are a common situation in practice. Clinically, secondary infections show local inflammatory signs (pain, erythema) or cellulitis, and the possible presence of pus (54). The isolation of *S. aureus* in a wound is not synonymous with local infection, but must be interpreted according to the clinical presentation and, especially, the presence of inflammatory signs. The distinction between secondary infection and colonization may be difficult.

Botryomycosis

S. aureus can cause botryomycosis, a rare, chronic and granulomatous infection characterized by painless slow-growing papulonodules, abscesses and ulcers and, histopathologically, the presence of granules composed of bacterial cocci (55).

SYSTEMIC CUTANEOUS MANIFESTATIONS DUE TO TOXIN-PRODUCING *S. AUREUS*

Toxic shock syndrome

The toxic shock syndrome (TSS) was first described by Todd (1978) in 7 children who had a generalized erythema, fever, hypotension, diarrhoea and multi-organ failure (56). In 1980 many cases were reported in young women who used certain types of tampon (57, 58). The incidence of menstrual TSS in the US peaked in 1980 and has decreased significantly since the removal of these tampons from the market (59).

TSS is due to the production of a toxin by *S. aureus*, mainly TSST-1 and staphylococcal enterotoxins, particularly enterotoxin B and, less commonly, other enterotoxins (56). The 1997 CDC definition (60) includes the following clinical criteria: fever (≥38.9°C) a diffuse macular erythroderma, desquamation (1–2 weeks after

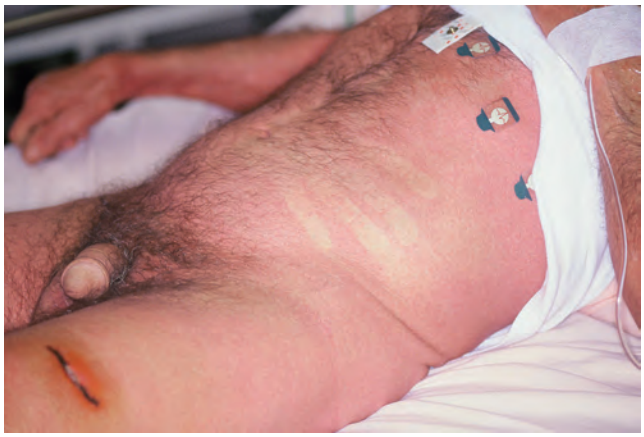

Fig. 7. Erythema of toxic shock syndrome.

onset of illness, particularly on the palms and soles), hypotension, multisystem organ involvement (57). In a study of 130 TSS, Reingold et al. (57) found a skin infection in 30% of cases, a genital focus in 27% (after delivery or abortion), 18% post-surgery focus, and in 13% the source was not identified. The pathogenesis of TSS is linked to the properties of superantigens in *S. aureus* toxins, namely activation of greater numbers of T lymphocytes resulting in the production of high levels of cytokines (33). Skin manifestations of TSS include a generalized erythema (with palm and sole involvement) (**Fig. 7**). Palmar, sole and finger desquamation may occur after recovery (**Fig. 8**). Transient alopecia, nail shedding and increased sweating on the hands and feet have been described (61). Treatment is based on the treatment of the multi-organ failure and the *S. aureus* focus of infection. Some antibiotics acting as protein-synthesis inhibitors with anti-toxaemic properties could provide additional therapeutic benefits (62).

In Japan, Takahashi et al. (63) have reported neonates who developed generalized erythema and thrombocytopaenia in the first week of life associated with MRSA-producing TSST-1. They propose neonatal toxic-shock-syndrome-like exanthematous disease (NTED) as the name for this disease. Similar cases have been reported in Europe (64).

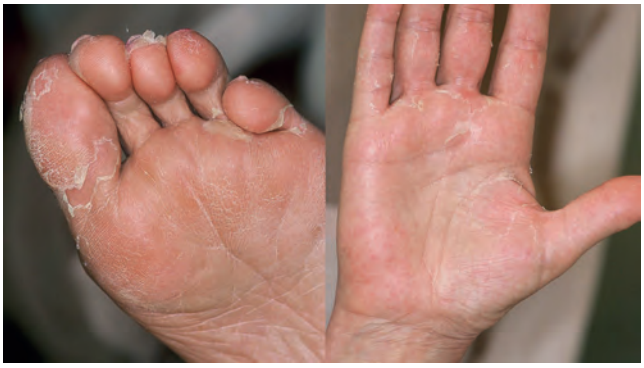

Fig. 8. Distal desquamation after toxic shock syndrome.

"Staphylococcal scarlet fever"

Staphylococcal scarlet fever, also called scarlatiniform erythroderma/rash, was first described in the 1920s. Lina et al. (65) found that 16 out of 17 strains of *S. aureus* isolated from patients with staphylococcal scarlet fever produced TSST-1, enterotoxins, or both. Enterotoxin B was the predominant toxin involved in a study in Taiwan (66). It is possible that most cases of staphylococcal scarlet fever are, in fact, a mild or attenuated clinical manifestation of TSS.

Staphylococcal scalded skin syndrome

When the ETs spread systemically, they can cause SSSS (9–11, 67). It is a generalized blistering disease affecting mainly neonates and young children and, exceptionally, adults with underlying diseases. The disease begins abruptly with fever and generalized erythema, followed by large fragile blisters involving the entire skin surface within the next few hours to days, which rupture rapidly (with a positive Nikolsky sign) (67). Widespread involvement of the entire skin surface can occur, but the mucous membranes are usually spared. Mild forms of SSSS have been described where the SSSS is limited to the body folds associated with a fine generalized desquamation (68). The disease follows localized *S. aureus* infection. Poor renal clearance of the toxins by neonates and by adults with impaired renal function is a major risk factor for developing SSSS. The prognosis of SSSS in children, who are appropriately treated, is good, with a mortality of less than 5%, but it may be fatal in up to 60% of affected adults, usually due to underlying diseases (67). The diagnosis of SSSS is clinically based. Exfoliatins are produced by *S. aureus* at a distant site; the blister fluid in generalized SSSS is usually sterile. The treatment is based on dressings, where there are large blisters, and the eradication of the source of *S. aureus* infection focus.

SKIN MANIFESTATIONS OF *S. AUREUS* BACTERAEMIA

Skin manifestations of S. aureus *endocarditis*

Endocarditis caused by *S. aureus* is classified as acute endocarditis. The description of endocarditis-related skin manifestations is confusing; Janeway lesions and Osler's nodes were described at the beginning of the 20th century (1). Classically reported Janeway lesions are macular, purpuric lesions that occur on hands and feet (**Fig. 9**). Histologically, they show neutrophilic microabscesses in the dermis and vessel thrombosis (69). These lesions are thought to be caused by septic microemboli; results of culture of skin specimens are frequently positive (70–73). Osler's nodes are described as small, painful, nodular lesions on the fingers or toes. Only a few biopsied Osler's nodes gave positive results on culture, and

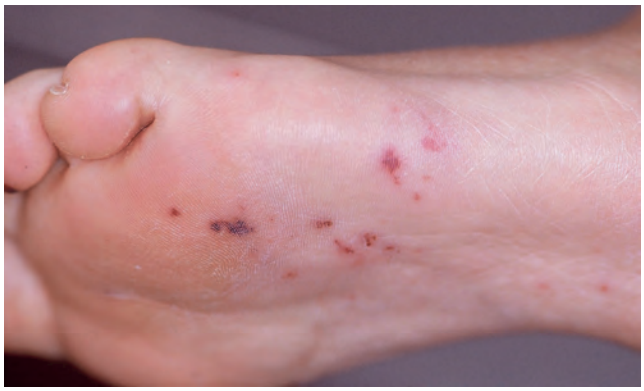

Fig. 9. Purpura during endocarditis.

histological examination showed diverse findings (73). The description of Janeway lesions corresponds better with the skin manifestations of *S. aureus* endocarditis.

Skin manifestations of S. aureus *bacteraemia (without endocarditis)*

Such manifestations related to the frequency of *S. aureus* bacteraemia are extremely rare. Purpuric disseminated eruptions and abscesses are the main clinical manifestations that have been described as a secondary focus of *S. aureus* bacteraemia. Exceptionally, purpura fulminans has also been reported (74).

IMMUNOLOGICAL SKIN MANIFESTATIONS OF *S. AUREUS* INFECTION

Immunological manifestations associated with acute or chronic *S. aureus* infections are rare. A few cases of vasculitis or Henoch-Schönlein purpura have been reported, mainly in the course of *S. aureus* bacteraemia (75–77). Some of these associations may be coincidental.

CONCLUSION

Staphylococcal skin infections are part of a complex group of diseases. Unfortunately, most reports in the literature classify skin infections and *S. aureus* skin infections under the heading "skin and skin structures infections (SSTI)", giving the illusion that all skin manifestations are within the same clinical spectrum. This review shows, on the contrary, how the clinical spectrum of skin manifestations due to *S. aureus* is diverse and related to different physiopathologies. Further reports and studies on skin *S. aureus* infections should take into consideration this rich and varied clinical spectrum of disease.

This review has focused on the clinical and therapeutic aspects of *S. aureus* skin infections, and many other questions are not mentioned, such as the interactions of *S. aureus* with the skin microbiome, the reservoirs of *S. aureus*, the relationships between reservoirs and skin infections, and the decolonization of the reservoirs.

All of these complex topics are currently the subject of intense research.

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REVIEW ARTICLE

A Hundred Years of Diagnosing Superficial Fungal Infections: Where Do We Come From, Where Are We Now and Where Would We Like To Go?

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Superficial fungal infections have been known for hundreds of years. During the 20th century new diagnostic methods were developed and the taxonomy changed several times, which, unfortunately, resulted in many fungi having several names (synonyms). The taxonomy is important, as species-specific identification guides clinicians when choosing the most appropriate antifungal agent, and provides an indication of the source of infection (anthropophilic, zoophilic or geophilic). Traditional diagnostic tests (direct microscopy, culture and histopathology) are still widely used, but molecular-based methods, such as PCR, have many advantages, and increasingly supplement or replace conventional methods. Molecular-based methods provide detection of different genus/species spectra. This paper describes recent changes in dermatophyte taxonomy, and reviews the currently available diagnostics tools, focusing mainly on commercially available PCR test systems.

Key words: diagnostic; microscopy; PCR; dermatophytes; dermatomycoses.

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SIGNIFICANCE

Superficial fungal infections (e.g. ringworm, thrush and fungal nail infections) have been known for hundreds of years. It is crucial to diagnose the fungus correctly, in order to choose the correct anti-fungal medication, and to provide information about the source of infection. Traditionally, diagnosis is based on microscopy, culture and histopathology of the specimen (hair, skin, nails). More recent molecular-based methods have been developed, but there is no standardization as to which fungi they detect. This paper presents an update on fungal taxonomy and describes the diagnostic tools available.

Superficial fungal infections have been known since the 5th century BC, when Hippocrates wrote about thrush in children. It took hundreds of years before the first scientific proof of infection was made by Agostino Bassi in 1835, who showed that the muscardine disease of the silkworm was caused by a fungus (1). In the following years Audouin from France suggested that some human diseases were caused by the same types of plant parasites (fungi). By the end of the 19th century important microbiological methods, such as obtaining pure cultures of the dermatophytes *Trichophyton* and *Achorion schoenleinii*, were introduced. A morphological classification was not established until 1910, when the famous mycologist R. Sabouraud published "*Les teignes*", a monograph based on the standardization of test media and studies on clinical features of skin and hair infections and morphology in cultures (1). At the beginning of the 20th century different nomenclatural systems were suggested, based on clinical presentation and culture characteristics.

The taxonomy of superficial fungal infections has changed several times since then, due to the development of new diagnostic methods. Unfortunately, this has resulted in many fungi having several names (synonyms). An attempt to simplify this, by giving "one fungus one name" has been initiated, and the development of molecular diagnostic methods has contributed to this process. This paper describes the latest changes in dermatophyte taxonomy and reviews currently available diagnostic tools.

TAXONOMY

The taxonomy of dermatophytes changed most recently at the beginning of 2017 (2). The phylogenetic tree in **Fig. 1**, based on molecular data, shows the current valid nomenclature of the family *Arthrodermataceae*. *C. serratus* and *G. ceretanicus* were used as outgroups. Before that, the family of *Arthrodermataceae*, encompassing the dermatophyte fungi, included 3 anamorphic (fungi that have no sexual phase in their life cycle, also called imperfect fungi), *Trichophyton*, *Microsporum*, *Epider-*

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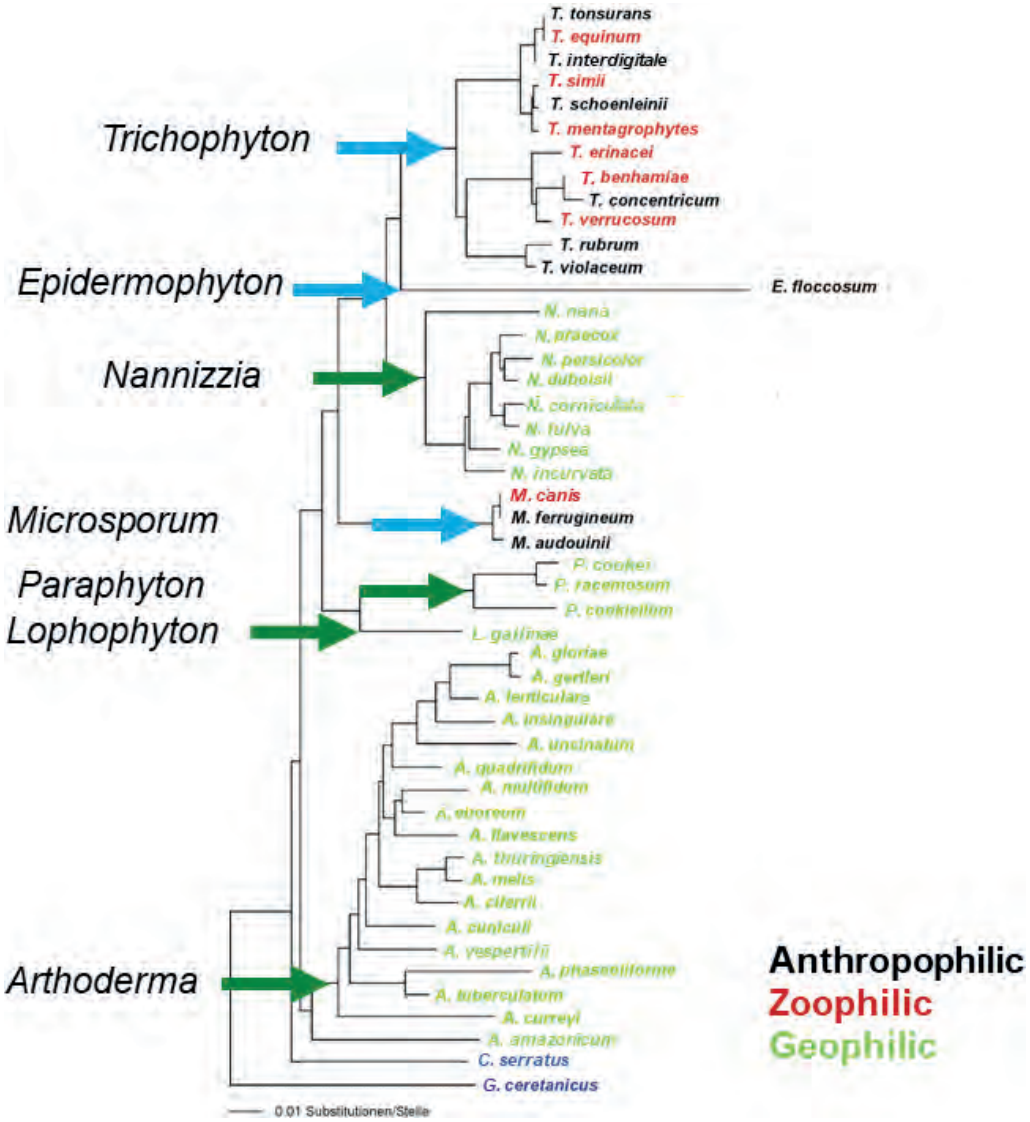

Fig. 1. Phylogenetic tree of the majority of species of the family *Arthrodermataceae*, based on the internal transcribed spacer region of the ribosomal DNA.

mophyton, and one teleomorphic (fungi that have a sexual phase in their life cycle) genus (*Arthroderma*). As early as 2011, this dual nomenclature of fungi was abolished (3), mainly because the basis of taxonomy moved away from using morphological features towards molecular and phylogenetic data. On this basis, the teleomorphic genus in dermatophytes was abolished and 4 additional genera (*Nannizzia*, *Lophophyton*, *Paraphyton* and *Arthroderma*) were introduced to account for the former geophilic *Microsporum* and *Trichophyton* spp. according to the rules of the botanical code. In principle, a separate genus was established at all main clusters (tips of the arrows in Fig. 1) of the phylogenetic multilocus tree. Medical concerns were also addressed, i.e. the anthropophilic and zoophilic species names were retained in the well-known genera *Microsporum*, *Trichophyton* and *Epidermophyton* (2).

At the species level, the nomenclatural changes that affected the medically relevant dermatophytes of the aforementioned genera were minor at this time. Most of these taxonomic changes were proposed at the beginning of the 21st century. For example, the previous 50 anthropophilic and zoophilic *Trichophyton* species were reduced to 19 (4), and in 2017 they were reduced by a further 3 due to the disappearance of the teleomorphic genus. Here, corrections were carried out that mainly affected the classification of the dermatophytes into groups that encompassed their natural sources (anthropophilic, zoophilic, geophilic). For example, the anthropophilic and zoophilic strains of *T. interdigitale*, were separated once again, i.e. the zoophilic strains again received their own species name, *T. mentagrophytes*, whereas the anthropophilic strains were called *T. interdigitale*. Due to this name change, the previous species *T. mentagrophytes*, which was phylogenetically closely related to *T. schoenleinii*, had to be renamed. The name *T. quinckeanum* was used, because the originally described strains of *T.*

mentagrophytes var. quinckeanum clustered here (Fig. 1). The skin fungus, *Trichophyton* sp. of *Arthroderma benhamiae*, which was isolated mainly from guinea pigs, was reduced to *T. benhamiae*. *T. soudanense* was removed from the *T. rubrum* complex and is now again listed as a separate species. New name combinations were also added, which were mostly geophilic species, due to the introduction of new genus names, e.g. *Microsporum gypseum* was renamed *Nannizzia gypsea*. The overall purpose of these changes was to base the new system on genetically robust determinants and to retain well-known dermatophyte names familiar to clinicians (2).

CLINICAL SIGNIFICANCE OF FUNGAL DIAGNOSTICS

Taxonomy may seem remote from everyday clinical practice, but it is important in many ways: first, an accurate diagnosis is important for choosing the correct antifungal treatment (5, 6). Species-specific diagnosis is sometimes also necessary, as different species may have diffierent antifungal susceptibility patterns (7). Secondly, the species name also informs clinicians about the source of the infection. By knowing the source of infection, it is possible to treat the index patient or animal in order to reduce the risk of further spread of disease. Thirdly, sub-species identification (strain typing) is useful in outbreaks as, for example, in India, where a specific *T. mentagrophytes* genotype VIII has been uniformly isolated as a causative agent of a countrywide spread of a chronic, relapsing dermatophyte epidemic (8). By thoroughly studying this sub-species new knowledge about virulence and resistance may become available. Finally, a negative fungal laboratory test is also important as a diagnosis of exclusion, when other dermatological diagnoses have also suspected. Even though identification to genus or species level is important it is not always performed in the clinical setting (9–11). Oral antifungal therapy should not be administered without a confirmed laboratory diagnosis, because up to 40% of the suspected diagnoses are wrong (9), and due to the possible side-effects and drug interaction, particularly in older patients who often have other underlying diseases and take additional medications. A third point is the potentially negative impact that unnecessary treatment may have on the human microbiome, and the increasing threat of drug resistance, which is well recognized with antibacterials, but can be equally applicable to antimycotics.

TRADITIONAL DIAGNOSTICS: DIRECT MICROSCOPY, HISTOLOGY AND CULTURE

Direct microscopy and culture have been used for the purpose of fungal identification over the last 100 years and are still used worldwide. The methods will be described in the following section.

Direct "non-specific" detection of fungal elements in clinical specimens

Direct microscopy is used for the primary identification of fungal elements in specimens after treating with sodium hydroxide or potassium hydroxide (KOH). Conventional light microscopy, without the benefit of any contrast with the background, is difficult to interpret, and stains, such as lactophenol cotton blue, Parker ink, chlorazol black E or Congo red, are therefore often added. Fluorescence microscopy after treatment of the specimen with optical brighteners, such as blankophor or calcoflour, can enhance the detection rate after microscopy (12, 13). *Malassezia* species show characteristic unipolar budding blastoconidia, but with the exception of this genus it is important to note that direct microscopic findings are neither genus- nor species-specific, even though it is possible to distinguish yeasts from hyphae and to detect pigmented fungal cells. Some very experienced technicians may be able to suggest a differentiation between other specific yeasts, dermatophytes and non-dermatophyte moulds, but without absolute certainty (14).

Direct microscopy of hair is important, as the growth pattern of the dermatophyte classifies it as either favus, endothrix (arthroconidia are present within the hair shaft) or ectothrix (where the fungus invades the hair shaft at mid-follicle and the arthrospores then grow out of the hair follicle and surround the surface of the hair shaft). The growth pattern, combined with the conidial size, can be used as a preliminary indication of the genus of the infecting dermatophyte (15–17). Histology is not used routinely in skin and hair infections, but is useful when *Malassezia* folliculitis is suspected, in order to rule out other causes of folliculitis. Some dermatologists use histology routinely for fungal identification in nails, as it may rule out contamination and is able to confirm the growth of the fungus directly in the specimen (11, 18–20). However, the prerequisite for this is an invasive biopsy.

Genus- and species-specific identification

Culture is highly dependent on growth media, e.g. some media are more dermatophyte-specific, while others are better for yeasts and non-dermatophyte moulds. *Malassezia* is lipid-dependent and, as a consequence, is often difficult to culture on normal laboratory media. Culture of nail material is challenging, as up to 30% of microscopy-positive nail specimens are culture-negative (21, 22). This may be due to the presence of non-viable material, either because of insufficient material from the proximal area of infection, or due to previous antifungal treatment.

Combination of the different techniques is usually practiced, as it enhances the chances of fungal detection and provides more clinically useful information. All traditional diagnostic methods are dependent on the skills of the laboratory technicians, whereas molecular

diagnosis does not depend on the acquired skill sets of the laboratory staff, but may have other limitations, as described below.

MOLECULAR-BASED DETECTION OF SUPERFICIAL FUNGAL INFECTIONS

Development of molecular-based methods for detection of dermatomycosis

With the introduction of molecular tools into the taxonomy of dermatophytes approximately 30 years ago, species-specific markers, such as the internal transcribed spacer (ITS) region of ribosomal DNA, were subsequently used for the diagnosis of this fungal group. In the mid-1990s, PCR methods were initially applied to cultured skin material. This included methods such as restriction fragment length polymorphism (RFLP) and random amplification of polymorphic DNA (RAPD) analyses, but also PCR fingerprinting (23–25). Later, so-called in-house PCR methods were developed, which were also able to identify the fungus directly in clinical specimens. These methods are generally based on amplification with a broad range and/or specific primers and, in a second stage, use hybridization with species-specific probes with or without a combination of high-resolution melting curve analysis. A distinction can be made between conventional and real-time PCR techniques. The former are more personnel-intensive because the hybridization step is performed separately and requires additional washing steps (enzyme-linked immunoassay (ELISA), blot or microarray technique) and are more susceptible to contamination because the amplified DNA is further processed manually. On the other hand, the thermal cyclers required are less expensive than real-time devices. However, the advantages of real-time PCR are that both the amplification and hybridization steps are performed in the same closed reaction tube without the risk of contamination. This also eliminates additional bench handling. However, it must be kept in mind that the number of probe hybridizations in conventional techniques is larger (e.g. 78 in the microarray format) than the number of colour labels (4–6), which are used to label different probes in real-time PCR technology. Thus, melting curve analysis is used to extend the spectrum of species to be detected. Nevertheless, these methods are not yet able to differentiate, at the same time, more than 20 clinically relevant dermatophyte species, including the few non-dermatophytes that can play a role in onychomycosis as infectious agents. Such an all-in-one detection test would replace protracted phenotypic diagnostics based on culture, which ultimately requires expert knowledge because morphological features in this fungal group are both polymorphic and partially overlap.

Commercial kits for direct detection of fungal infections on skin, hair and nail samples

Since 2008, commercial systems, that use the above-mentioned detection methods and cover different species spectra, have been available. The Dermatophyte PCR Kit was developed by the Statens Serum Institute (SSI), in Copenhagen Denmark in 2 versions; firstly, as a conventional PCR, and later as a real-time PCR that solely detects *T. rubrum* at species level, as it is the most common pathogen in onychomycosis and tinea pedis

Table I. Species spectra detected by the commercially available test systems

Species/KIT	DPK	FTD	MMD	MMD LF	DG 1.0	DG 2.0	DD	EADM
T. tonsurans	X							V
T. equinum	X							V
T. interdigitale	X							V
T. mentagrophytes	X							V
T. schoenleinii	X						X	V
T. quinckeanum	X						X	V
T. simii	X	nd	nd	nd			nd	V
T. erinacei	X	X	X		X		X	V
T. benhamiae	X	X	X		X		X	V
T. verrucosum	X	X	X		X	V	X	V
T. bullosum	X	nd	nd	nd	X	X	nd	V
T. rubrum	V	V		V	V	V	V	V
T. violaceum	X	V		V	V	V	V	V
E. floccosum	X	V	V	V	V	V	V	V
M. audouinii	X			V	V	V		V
M. ferrugineum	X							V
M. canis	X							V
N. gypsea	X	X	V	V	X	X	V	V
N. fulva	X	X	X	X	X	X	X	V
N. incurvata	X	X	X	X	X	X	X	V
N. persicolor	X	X	X	X	X	X	X	V
pan Dermatophyte	V	X	X	V	X	X	X	V
non Dermatophyte	X	X	V	V	V	V	X	V

Same coloured boxes refer to the detection of species complexes, but not individual species. V: detects, X: do not detect. The identically coloured boxes mark the species in the respective kit, which are detected together (as a complex), i.e. not separated from each other.
Nd: no data; DPK: Dermatophyte PCR Kit; FTD: Fast Track Dermatophytes; MMD: Mentype Mycoderm; MMD LF: Mentype <mycoderm Lateral Flow; DG 1.0: DermaGenius Version 1.0; DG 2.0: DermaGenius Version 2.0; DD: DermaDYN; EADM: Euroarray Dermatomycosis.

(**Table I**). Otherwise, the kit offers the possibility of detecting dermatophytes as a group (pan-dermatophyte), but this will include any non-pathogenic geophilic genera present. The conventional test system is based on a PCR with subsequent size analysis of the amplified DNA fragments in an agarose gel, whereas real-time PCR uses hybridization probes instead. Both test systems can be used for screening for dermatophytes, and this may be followed by subsequent species identification of non-rubrum species via culture or other molecular techniques, such as sequencing (26). In 2011, the FTD Dermatophyte test from Fast Track Diagnostics, was made available in Sliema, Malta. This test system, a 2-tube real-time PCR with probe hybridization, but without melting curve analysis, is able to detect 3 species (*T. rubrum*, *T. violaceum*, *E. floccosum*). The remaining detections are performed at species complex level, i.e. more than 1 species is detected here, but not differentiated from each other. This includes mainly dermatophytes species with different ecological niches: the *T. tonsurans* complex (no differentiation between *T. equinum*, zoophilic and *T. tonsurans*, anthropophilic), the *T. interdigitale* complex (*T. interdigitale*, *T. schoenleinii* antropophilic; *T. mentagrophytes*, *T. quinckeanum* zoophilic) and the *M. canis* complex (*M. canis* zoophilic, *M. audouinii*, *M. ferrugineum* anthropophilic). The kit does not have a detection option for the dermatophytes as a group. Biotype in Dresden, Germany launched the first version of the Mentype MycoDerm kit in 2013. These utilize 2 conventional PCR reactions, which can differentiate 2 species (*E. floccosum* and *N. gypsea*) on the basis of fragment size analyses. There is no differentiation between the *T. tonsurans* and the *T. interdigitale* complexes. *T. rubrum* is identified in a complex together with *T. violaceum*, as is *M. canis* complex. The second version of the test system (Mentype MycoDerm Lateral Flow) was available 2 years later with 3 PCR reactions. Further developments affect, on the one hand, the procedure, because fragment analysis was replaced by probe hybridization on a blot strip. On the other hand, the species spectrum to be detected has been enlarged. Now it is possible to detect *T. rubrum*, *T. violaceum*, *M. audouinii* at species level and *M. canis* in combination with *M. ferrugineum*. The *T. tonsurans* was separated from the *T. interdigitale* complexes. This was also the first kit that could detect *T. benhamiae* in a complex together with *T. erinacei* and *T. verrucosum*. Another concurrent test system, DermaGenius 1.0, from PathoNostics, produced in Maastrict, the Netherlands, based on a single multiplex real-time PCR with melting curve analysis, had the same identification gaps and a similar species spectrum as the FTD Kit. Neither kit included pan-dermatophyte detection. This changed with the 2nd version of the kit (DermaGenius 2.0), which became available in 2018. The species detection is the same as for Mentype MycoDerm Kit Lateral Flow, with 2 exceptions. *N. gypsea* is not included, but *T. verrucosum* is. *T. benhamiae* is clustered together with *T. equinum* (27). At the same time, the DERMADYN kit developed by DYN Diagnostics Ltd in Ha'eshel St., Israel became available. This test system is also based on a 2-tube multiplex PCR with a melting curve analysis and detects a similar spectrum to FTD dermatophytes (Table I). In addition *N. gypsea* is detected, but the *T. simii* complex is not included (28). In 2018, the last of the kits discussed here, Euroarray Dermatomycosis from Euroimmun, was launched in Lübeck, Germany. This is a multiplex PCR reaction with subsequent probe hybridization in the form of a "microarray". This format enables the detection of all relevant (approximately 20) dermatophytes at the species level, including a pan-dermatophyte probe and 6 non-dermatophytes at the species level (*Scopulariopsis brevicaulis*, *Fusarium* and *Candida* spp.). Furthermore, there is species detection for rare pathogens, such as *T. eriothrephon* and *T. bullosum*. Only *T. concentricum*, a pathogen endemic to the Pacific Islands, is not separated from *T. benhamiae*, and *T. soudanense* is not differentiated from *T. rubrum* because the taxonomic change that separated them came after the development of the kit.

Overall, the clinician should be aware that there is a difference between what the commercial tests are able to detect (Table I). Most importantly, the majority of tests do not discriminate between zoophilic and anthropophilic species, which is a necessary step in order to find (and treat) the sources of infection. Another challenge is that many of the non-dermatophytes involved in the pathogenesis of onychomycosis are not detected in many of the kits. The broadest species-specific spectrum is offered by the Euroarray. The other test systems do not detect non-dermatophytes (SSI, FTD), apart from *C. albicans* (DermaGenius), or they provide detection of *Scopulariopsis* and *Candida* to genus level only (Mentype) (Table I).

Non-commercial molecular-based tests

A considerable number of in-house PCR techniques have been developed for the diagnosis of dermatophytes and other skin pathogenic fungi. We do not describe these developments in detail here, because they are not standardized, and in the vast majority of cases are used only by individual, or a few, laboratories involved in routine diagnostics. Of particular interest are the methods based on real-time PCR. Many of these approaches are able to identify up to 6 taxa and dermatophytes in general (29, 30). Ohst and colleagues (31) were able to detect 9 dermatophyte taxa by combining up to 10 PCRs in a sequential algorithm, and Bergmans and colleagues (32) could differentiate 11 species in a single-tube assay with probes and melting-curve analysis. Walser & Bosshard (33) report that using sloppy molecular beacons with species-specific melting temperature signatures allows the identification of 19 dermatophyte species. Until now it has been possible to detect a similar number of

species only by applying post-PCR techniques, such as an oligonucleotide array (34). This may be a promising approach for a commercial test system, if it is possible to increase the sensitivity of the 2nd species-differentiating PCR reaction, which was negative in 76% of cases where PCR1 (pan dermatophytes) was positive.

Another molecular-based method, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), is used to identify micro-organisms based on the characteristic protein spectrum of each species matched with a database. It has been applied to the identification of superficial fungal infection directly on culture material, both yeast, dermatophytes and moulds (35). This technique is fast and reproducible, but until now not applicable directly to clinical hair, skin or nail specimens (36–40). Protein spectra of 7 and 10 dermatophytes species (*T. tonsurans*, *T. rubrum*, *T. interdigitale*, *T. mentagrophytes*, *T. verrucosum*, *T. violaceum*, *M. canis*, *M. audouinii*, *E. floccosum*, *N. gypsea*) are included in the widely used Bruker and Biomerieux reference spectrum databases (41). So far, this method is not able to differentiate the phylogenetically closely related species, e.g. *T. rubrum/soudanense*, *T. interdigitale/mentagrophytes*, *T. tonsurans/equinum* or *T. benhamiae/concentricum* complex (42, 43). Identification can be improved by establishing an in-house database (44). It has also been used to detect antifungal resistance (45).

OTHER DIAGNOSTIC TOOLS

Wood's light, filtered ultraviolet light, is often used as a bedside tool for differentiate tinea capitis caused by a *Microsporum* species (*canis*, *audouinii* and *ferrugineum*) from other dermatophyte infections, as it fluoresces greenish under Wood's light, and endothrix infections are non-fluorescent (17).

Dermoscopy, reflectance confocal microscopy, optical coherence tomography and confocal laser scanning microscopy, all of which are non-invasive methods, can be used as add-on tools to differentiate tinea capitis and/or onychomycosis from other dermatological conditions (17, 20, 46–48).

Dermatophyte screening test media, an agar medium containing a dermatophyte colour indicator can be used for dermatophyte screening. The anti-dermatophyte monoclonal antibody test, an immunochromatographic detection test, is able to confirm a dermatophyte infection, detectable at genus level. Some are known to give a false-positive reaction when non-dermatophytes are grown (22, 49–51).

CURRENT ROUTINE DIAGNOSTIC AND THEIR CHALLENGES

The use of phenotypic methods (microscopy and culture) for the detection of pathogens in tinea is still widespread. They are dependent on the skills of the laboratory technicians and culture is time-consuming. The culture method is still the only diagnostic method that is able to confirm the viability of the fungus, which is important for treatment assessment (11). Microbiological laboratories appreciate the automation possibilities in molecular diagnostics and often have already established similar methods and devices that can also be used for dermatophyte identification. Decisive factors in determining whether to set up a molecular mycology service are the number of samples, the availability of trained personnel for direct microscopy, culturing and cost-effectiveness, which depends strongly on whether and how molecular dermatophyte diagnostics are remunerated. Whether conventional diagnostics will still be used after the wider introduction of the molecular identification method depends primarily on the differentiated pathogen spectrum of the test system used. If not all relevant pathogens are covered, a pan-dermatophyte detection should be used in order not to miss a possible pathogen. However, even then, the detection of potential non-dermatophytes must be considered and, if not included, covered by diagnostics based on culture. It is, therefore, important to be aware of what fungi any locally available molecular test can or cannot detect.

Although molecular diagnostics are up to 30% more sensitive than culture diagnostics, the detection limit is more than one fungal cell (31). Therefore, the clinical specimens must be taken from the correct location and in sufficient quantity. In this respect, there is no difference from culture diagnostics. The detection of pathogenic dermatophytes, whether in culture or PCR, always requires antifungal therapy, because asymptomatic carriers also spread the fungi and can become symptomatic. Disadvantages of the available molecular tests, in general, are that the evolution of fungi can lead to (point) mutations, especially in species-specific sequences used in the primers and probe, so that they can no longer bind, and false-negative results may be generated.

This can be remedied by sequencing with broad-range or only dermatophyte-specific primers, which are more conserved and therefore less susceptible to mutations. Sequencing can then provide accurate species identification. Some laboratories already routinely use these methods for fungal diagnostics. However, the purchase of a sequencing device is expensive and, like an in-house PCR, the method has to be validated. Furthermore, there must be appropriately validated databases to enable correct identification.

CLINICAL AND LABORATORY INTERACTION CAN IMPROVE THE DIAGNOSTIC OUTCOME

It seems logical that there should be coherence between what the clinician suspects and what the mycology laboratory is able to detect. Nevertheless, in our experience

this it often not the case. As described, different fungi have different needs for substrates in order to grow, and some molecular-based tests do not detect all relevant fungi. It is therefore important to inform the mycology laboratory, as a minimum, from which anatomical region (hair, skin or nail) the specimen is obtained, which dermatological disease, and fungal (dermatophyte, *Candida*, *Malassezia* or non-dermatophyte mould) genera is suspected (**Table II**). Furthermore, the attending physician should note on the referral form whether an animal contact is probable and whether, for example, a mycosis with a non-dermatophyte is considered in onychomycosis. The microbiologist needs this information in order to interpret the results of the molecular tests correctly, but also to decide whether a culture should be created in parallel if the kit has gaps in its repertoire.

FUTURE PERSPECTIVES

The advantages of molecular diagnostics for the initial diagnosis of dermatophytosis are beyond question. A few studies have, so far, shown that the method can also be used for therapy monitoring (52, 53). Iwanaga et al., in particular, have demonstrated that the fungal load after 16 weeks of terbinafine therapy is significantly reduced (from 100% to 36%) (53). The patients' culture were already negative at this time, but, microscopically, fungal elements could still be detected in the KOH preparation. The most plausible explanation for this is that resting fungal cells (e.g. in the form of arthroconidia) are still present and may potentially germinate again after discontinuation of therapy. The survival of dormant fungal cells inside the nail is supported by follow-up studies, which after 18 months show a complete cure in only 76% of elderly patients receiving 3-month terbinafine therapy (54). Dormant cells are missed in the culture. Therefore, therapy control with PCR procedures may be suitable in the future, not to mention the short time-span in which such a finding is available, in order to decide whether to continue the therapy. Only very special PCR procedures are able to discriminate between live and dead cells; however, it is not known how long dormant fungal cells survive in the nail, hair or skin of the human body (55).

To date, there has been no significant development of resistance in dermatophytes to the use of antimycotics. This has suddenly changed with the Indian epidemic, which has lasted for approximately 6 years, and goes hand in hand with the use of over-the-counter ointments containing antimycotics (e.g. terbinafine), antibiotics and steroids (e.g. clobetasol). Terbinafine resistance or partial resistance in *T. mentagrophytes* strains with genotype VIII and *T. rubrum* reach rates of more than 65% and

Table II. Helpful information for the clinician to differentiate between suspected fungal pathogens, which is needed for the laboratory for choosing the most appropriate diagnostic methods

Anatomical region	Help for the clinician to differentiate between suspected fungal pathogens			Essential information for the microbiologist	Information helpful for the microbiologist
	Disease	Most common clinical signs	Age	Suspected pathogen	Exposure
Scalp (hair region)	Tinea capitis	Broken hairs Kerion Favus Alopecia Scaling	Children	Dermatophyte	Animal exposure Endemic contacts Woods light results Earlier treatment
	Seborrhoeic dermatitis/ Dandruff	Greasy skin scales on erythematous skin	Newborn Adults	*Malassezia*	
Face	Tinea faciei	Area with raised erythematous border or red patch	All ages, but mostly children	Dermatophytes	Animal exposure Signs of tinea capitis
	Seborrhoeic dermatitis	Greasy skin scales on erythematous skin primary centro-facial and eyebrows	Adults	*Malassezia*	
Upper body	Pityriasis versicolor	Hypo- or hyperpigmented maculae	Young and adults	*Malassezia*	Immunosuppression
	Malassezia folliculitis	Monomorphic pustules mainly located at seborrhoeic areas. No comedones	Young and adults	*Malassezia*	Immunosuppression
	Tinea corporis	Area with raised erythematous border or red patch. Skin scales	All ages	*Dermatophyte*	Animal exposure Other signs of tinea
Hands	Tinea manuum	Area with raised erythematous border or red patches. Skin scales. Hyperkeratosis.	All ages		Other signs of tinea e.g. tinea pedis
Groin & pubic area	Cutaneous candidiasis	Erythematous skin folds with satellite pustules (and skin scales)	All ages	*Candida*	Immunosuppression
	Tinea cruris	Area with raised erythematous border or red patch. Skin scales	Adults	*Dermatophytes*	
	Seborrhoeic dermatitis	Greasy skin scales on erythematous skin	Adults	*Malassezia*	
Feet	Cutaneous candidiasis	Interdigital maceration	All ages	*Candida*	Immunosuppression
	Tinea pedis	Interdigital maceration, skin scales, raised erythematous boarder, 'Moccasin foot', thickening of the soles	All ages	*Dermatophytes*	
	Cutaneous non-D mould infection	Interdigital maceration	Mostly adults	Non-D moulds	Immunosuppression
Nails	*Candida* onychomycosis	Paronychia Nail dystrophy	All ages	*Candida*	Immunosuppression Moist exposure
	Tinea unguium	Hyperkeratosis, superficial white discoloration, yellow streaks.	All ages, but prevalence increases with age	*Dermatophytes*	Concomitant tinea pedis?
	Non-D onychomycosis	Hyperkeratosis, discoloration, paronychia/ inflammation, nail dystrophy	All ages, but prevalence increases with age	Non-D moulds	

Non-D: non-dermatophyte.

17%, respectively, in India and are spread globally (56). This means that *T. mentagrophytes* strains will have to be fine-typed by molecular genetics in order to determine the exact identity, or a susceptibility test has to be performed. The advantage of the latter is that breakpoints can be defined; the disadvantage is that the inoculum, due to the filamentous growth and the often poor conidia formation of the fungi, is challenging and therefore not done routinely. Molecular methods, in particular sequencing with detection of specific genetic mutations leading to antifungal resistance (e.g. squalene epoxidase gene mutation leading to terbinafine resistance), which are independent of the fungal growth could overcome this problem (8, 56–59).

CONCLUSION

The diagnosis of superficial fungal infections has evolved from the first microscopic description more than 100 years ago to current techniques that are able to detect a wide range of clinically relevant fungi using molecular-based techniques. Worldwide traditional diagnostic methods, such as direct microscopy and culture, are still used, as they are cheap and the equipment is already available. The development of molecular-based methods has already improved a lot during the last years, from only being able to detect fungi in cultures to now being able to detect fungi directly in clinical samples. The molecular species-specific fungal detection of clinically relevant fungi is possible, as well as detection of specific mutations causing antifungal resistance. If it were possible to combine all these tests, it would enable the clinician to obtain the correct species identification, the possible source of infection and the susceptibility pattern of the involved pathogen by sending a single sample. The evolution of the diagnosis of superficial fungal infections is not far from this goal.

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Centenary theme section: CUTANEOUS AND GENITAL INFECTIONS

REVIEW ARTICLE

The Management of Scabies in the 21st Century: Past, Advances and Potentials

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Scabies is one of the most common skin diseases worldwide, affecting 150–200 million people yearly. Scabies affects young children in particular, and has the greatest impact in poor overcrowded living conditions. The burden of the disease is now well characterized, including group A *Streptococcus* and *Staphylococcus aureus* bacterial superinfections, with reports of nephritis, acute rheumatic fever, or fatal invasive sepsis secondary to scabies. Management of scabies remains largely suboptimal from diagnosis to treatment, and progress in the development of new therapeutic measures leading to cure is urgently needed. This review gives an overview of the current limitations in the management of scabies, an update on recent advances, and outlines prospects for potential improvements.

Key words: scabies; Sarcoptes scabiei; neglected tropical disease; ivermectin; permethrin; moxidectin; acaricide discovery and development; ovicidal.

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SIGNIFICANCE

Scabies is more than just a disease that provokes a horrendous itch. For more than a century, researchers, clinicians and public health physicians, together with policymakers, have worked to improve the management of scabies. Finally, in 2017, scabies was added to the WHO list of neglected tropical diseases after a long and still ongoing process of documenting the morbidities and burden caused by the disease. This additional and increased research activity, resulting in high-impact publications, has increased our knowledge of the biology, pathology and management of scabies, and has opened doors to new strategies.

Scabies (Latin *scabere* " to scratch") is a common parasitic disease caused by the microscopic mite *Sarcoptes scabiei* var. *hominis* (1). The burrowing mite causes intense itching, associated with typical skin lesions. The disease has been known for over 2,500 years; the Greeks and Romans were the first to write about its contagious nature. The mite was first identified and illustrated in the 17th century (2). Despite marked advances in parasitology in the 19th and 20th centuries, research into scabies has been hampered largely by limited access to the parasite and by low interest in an ectoparasite that mainly affects the poor. The earliest understanding of the mite biology and transmission was provided by Kenneth Mellanby, a British entomologist, in the 1940s during World War II (3, 4). Further advances in the physiopathology and host-parasite interactions have been made in the last 30 years (5, 6), mainly through the development of experimental animal models (7, 8). The therapeutic options for the management of scabies increased considerably in the 1970–80s with the discovery of ivermectin, one of the most important drugs currently used to treat scabies; the researchers were recently awarded, 35 years after its discovery, the 2015 Nobel Prize for Physiology and Medicine (9).

Currently, the recent expansion of multi-omics techniques will enable scientists to design large-scale mite and host molecular and biochemical analyses to develop new diagnostic tools or treatments in the near future (10–13). Scabies was, for a long time, not appropriately considered to be a true health target. In the past 10 years, stupendous efforts, made by a group of experts brought together in the International Alliance for the Control of Scabies (IACS), have given scabies the recognition it deserves (14). Thus, in 2017, the WHO decided to add scabies to the list of neglected tropical diseases and has called for large-scale action to achieve control and eradication (15).

WHY DO WE NEED TO IMPROVE SCABIES TREATMENT?

Scabies is a prevalent disease, which is present in all parts of the world, with greatest prominence in disadvantaged populations living in tropical and subtropical regions, and has a documented significant burden. The latest estimates suggest that 150–200 million people have scabies in the world every year, and that the scabies burden is particularly high in Asia, Oceania, and Latin America (16). Young children in underprivileged populations

living in crowded conditions are more often at risk (17). Transmission of scabies occurs mainly via skin-to-skin contact and, less frequently, via fomites within a patient mite-contaminated environment (generally in the context of severe forms of scabies; see below). As scabies is contagious, persons sharing the same household with patients may frequently be affected. This is especially the case in severe scabies, i.e. profuse or crusted scabies, in which the mite burden per person is dramatically increased, small epidemics around a single case can easily develop, and are fuelled by overcrowded households and transient lifestyles. The risk of transmission is known to depend on the patient's mite load, household size and population concentration, and how individuals interact with each other. Indeed, people living in clustered communities or in crowded housing conditions are at higher risk of scabies and outbreaks. In high-income nations, high endemicity of scabies is often reported in closed communities and institutional settings, such as hospitals, child-care and elderly-care residential facilities (18, 19), prisons, schools, homeless populations, and refugee camps (20–22).

For a long time, the scabies mite has been erroneously perceived as an ectoparasite that just causes itching. However, recent epidemiological studies indicate increasingly substantial morbidity, and even mortality (23), due to scabies infection, mostly caused by bacterial infections appearing with the parasitic infestation (14, 24). It has been hypothesized that scratching of lesions in response to the immense itch is present more often in scabies than in any other pruritic skin affections (25). The discomfort caused by the intense itch can have direct consequences, i.e. depriving patients of sleep (26), interfering with concentration at work or school, leading to a negative impact on attendance, performance (27) and quality of life (28). Scratching scabies lesions themselves leads to breaches in skin barrier that creates an entry point for opportunistic commensal or pathogenic bacteria that can become invasive, such as group A *Streptococcus* (GAS) and *S. aureus* (29). These bacteria lead to secondary infection of the epidermis, also known as pyoderma or impetigo, which can become more severe and cause skin and soft-tissue infections (including necrotizing fasciitis), septicaemia, or more invasive bacterial infections (24). In some cases, immune-mediated diseases can occur following infection, such as glomerulonephritis (30) or acute rheumatic fever (31), both of which can become chronic. This association between scabies parasites and bacterial pathogens is observed mainly in tropical or subtropical areas of the globe and in remote locations (17); with some data suggesting that up to 40% of impetigo lesions can be linked with scabies, especially among young children (32, 33). This particular link was established early in the 1970s, with epidemiological studies showing epidemics of acute glomerulonephritis in Trinidad (34) or Southern Africa (35) contemporaneously with scabies outbreaks, or in interventional studies in the field showing reduction in childhood haematuria following scabies treatment (30), or reduction in impetigo or skin sores prevalence paralleling a reduction in scabies numbers during mass drug administration (MDA) campaigns (36–38). More recent fundamental experimental work has supported and, in part, explained these observations with evidence of direct effects of mite gut proteins (serpins and serine proteases) in downregulating the innate host immunity including complement defence and neutrophil function, thereby modulating the microenvironment around the mite, allowing associated bacteria to flourish (39–43). Beyond the itch, scabies still causes a significant social impact, affecting quality of life and school or job absenteeism amongst infested patients. Its marked social, economic and psychological ramifications are underscored, but are sufficient to justify global improvement in its management.

WHO DO WE NEED TO TREAT?

Typically, the first symptoms of scabies are severe itch that worsens at night (44) and typical skin lesions caused by the penetration and progress of the mite through the epidermis. Most scabies lesions are found in classical sites, such as finger webs, hands, wrists, periumbilical skin, buttocks, genitals, periareolar region in females, or feet (1). In adult patients, the head is usually not affected, although it may be involved in infants, and babies (45). Burrows, vesicles, pustules, nodules, or excoriated pruritic papules are the most common lesions observed, all of which indicate the presence of a mite within the epidermis (**Fig. 1**A). The severe forms of scabies include profuse and crusted scabies. Both presentations are characterized by a very high parasite burden, with hundreds, thousands or even millions of mites per patient, and the development of extensive lesions. In such forms of scabies, hyperkeratotic skin (rather than real crusts) may be restricted to a finger, toe, or the scalp, for example, or diffusely affect multiple skin sites, including the face and palms and soles (1). This condition is seen in the elderly or in patients with underlying immunodeficiency from any cause (transplant recipients, corticosteroid use including topically-applied medication, HIV-, or HTLV-1-infested patients) (46–48).

The diagnosis of scabies is easy if a burrow, the specific lesion of scabies, is observed at a typical site of predilection. However, burrows may not be visible and diagnosis of scabies can be challenging, leading to misdiagnosis and mismanagement. Clinical diagnostic algorithms have been created to assist health-workers in recognizing scabies using a combination of parameters of patient history and clinical arguments, such as, for example, a history of diffuse itch, presence of lesions in typical skin areas, and itching in household members (49, 50). These diagnostic aids, which have proved reliable in endemic regions,

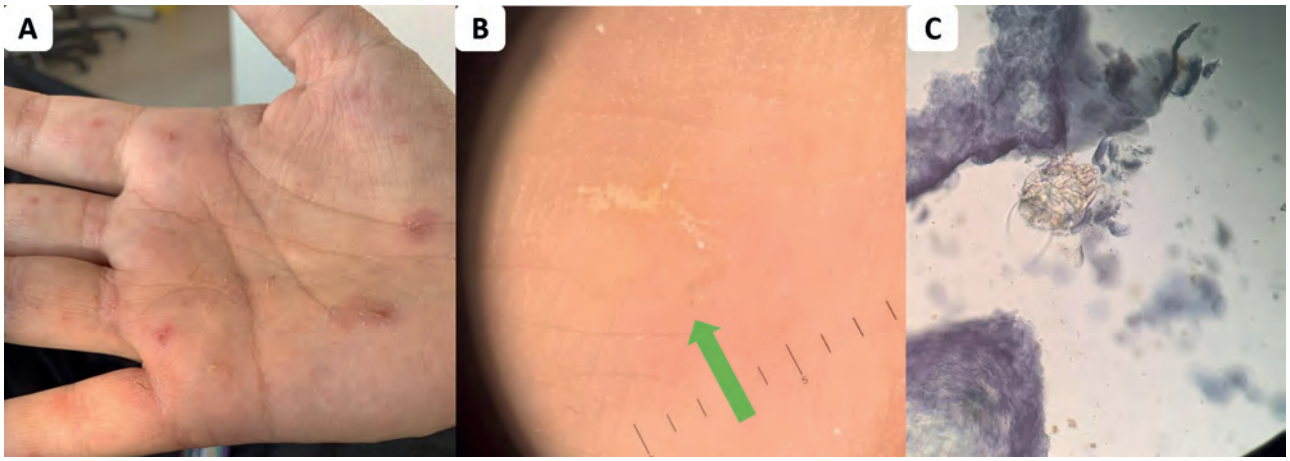

Fig. 1. Typical lesions of ordinary scabies. (A) Scabies lesions on the palm of the right hand with linear burrows, tiny vesicles and papular scabies lesions. (B) A burrow lesion from patient in (A) using dermoscopy showing the "jet-with-contrail" and an image of a brown triangle at the end, the "deltawing jet" (10-fold magnification). (C) Direct examination of skin scraping from patient in (A), showing an adult scabies mite (x10, lactophenol cotton blue staining).

have to be extrapolated and optimized across a larger range of settings, from the dermatologist's daily practice offices to resource-poor field settings, regardless of local prevalence. With this in mind, consensus criteria for the diagnosis of scabies were developed recently using a 4-round Delphi process including 34 international experts under the aegis of IACS (51). The IACS 2019 Criteria includes 3 levels of definition (confirmed scabies, clinical scabies, and suspected scabies case) and 8 subcategories. The accuracy and reproducibility of scabies diagnosis using these criteria have yet to be validated, as well as the ease of using it for GPs, experts in dermatology and other specialties, and non-expert health-workers (52).

As yet, a simple test for scabies based on molecular markers is still not available. Non-invasive methods have been developed for directly identifying the mite in diagnostic scrapings (53). The gold standard remains the visualization of the parasite (adults or immature forms), eggs, eggshell fragments or mite faecal pellets by light microscopic examination of a skin scraping. New technologies have been customized. Their sensitivity and specificity are summarized in **Table I**. Light microscopy examination has an excellent specificity (Fig. 1B), but is highly operator-dependent and is time-consuming, as repeated scrapings may be necessary. Dermoscopy or epiluminescence microscopy are tools used in daily clinical practice by dermatologists for a variety of cutaneous disorders, including parasitic infestations (54). The diagnosis of scabies using dermoscopy is confirmed by the observation of the "jet-with-contrail" pattern in the skin, representing a mite and its burrow, or an image of a black or brown triangle, the "delta-wing jet" sign representing the head of the mite (Fig. 1C) (55). Videodermatoscopy utilizes a dermoscope with a video camera connected to a computer that allows very high magnification and can be used to assess the viability of living mites. These are expensive techniques and therefore some authors have adapted low-cost equipment, used in botanical or entomology investigation, for use in the medical assessment of scabies (56). Reflectance confocal microscopy has been developed more recently for pigmented skin lesions, to differentiate malignant melanoma from benign naevi. The system uses an 830-nm wavelength diode laser and provides high optical resolution to penetrate to a depth of 200–300 µm into the skin. Imaging of the scabies mites and eggs using this device has been described (57). Some authors have tried to develop diagnostic techniques using molecular tools, such as matrix-assisted laser desorption ionization – time of flight (MALDI-TOF), antigen detection system or PCR specifically targeting scabies DNA. With PCR, while most studies have found a very high specificity,

Table I. Comparison of the specificity and sensitivity of the different currently available diagnostic non-invasive methods for scabies. Adapted from Chosidow & Sbidian (62) and Micali et al. (53)

	Clinical diagnosis algorithm	Skin scraping and light microscopy	Burrow ink test	Adhesive tape test	Epiluminescence microscopy (dermoscopy)	Videodermatoscope	Reflectance confocal microscopy	PCR-based method
References	(49)	(55)/(63)	(64)	(63)	(55)/(63)	(65)	(65)	(59)/(66)
Sensitivity, %	96.2	90/46	36.6	68	91/83	95	92	75.7/37.9
Specificity, %	98	100/100	100	100	86/46	97	100	100/100
Positive predictive value, %	87.7	100/100	–	100	88/47	97	100	–/100
Negative predictive value, %	99.4	90/77	–	85	90/85	95	92	–/61.7
Duration of the procedure	15 min for the entire body	30 min	5 min	10 min	5–10 min for the entire body	5–10 min for the entire body	60 s to 10 min for each lesion	Half a day
Prices, USD	–	US$ 500	–	US$ 10	US$ 500–700	US$ 25,000	US$ 150,000	US$ 200

often close to 100%, sensitivity was continually low, ranging from 30% to 60% (58–60), poorer compared with parasite observation either by microscopic or dermoscopic examination (61). No biomarker-based diagnostic kits have been developed for use as a simple and rapid method to identify mite infection without dermatological skills.

Improvement in scabies management is essential and will come from better identification of which patients need to be treated. Thus, the accurate and definitive diagnosis of scabies is crucial. Non-invasive diagnostic tools have been developed, but will have to be improved further.

HOW CAN WE TREAT SCABIES?

Treatment of small clusters (individual and family level)

Treatment must be prescribed for all confirmed cases of scabies, and should be given to all household and family contacts. The options available for treatment of scabies are summarized in **Table II**. Topical medicines were considered first-line treatment until the arrival of oral ivermectin in 1981, which was, at first, reserved for recurrent, difficult-to-treat cases, those with superinfected or eczematous skin, or for patients with crusted scabies (67). Topical agents should be applied to the entire skin surface, from "head-to-toe", avoiding the eyes, nose, and mouth. The application period depends on the specific instructions from the manufacturer. Adverse events are reported with all topical medicines for scabies, but they appear to be limited. Oral ivermectin is given at a standard dose of 0.2 mg/kg and may be associated with lower rates of complete cure if given only once (68–70). This could be explained by the limited ovicidal activity of the drug (71) and the short half-life of ivermectin in the skin, which was shown in 2 experimental trials in a porcine scabies model (72, 73). Giving the drug with a high-fat content meal has been proposed in order to increase its absorption and, accordingly, this might increase its efficacy (74, 75). Most scabicides act by affecting the nerve and muscle function of the parasite, and they are active only against mobile stages (larva, nymph and adults) and not eggs (13). The optimal interval between dosing in the 2-dose regimen still needs to be optimized and should be a short window between larval hatching (occurring at day 2–4 of the mite life-cycle) and the development of the adult stage that can be fertilized (at day 5–8, maximum day 15). Two recent Cochrane systematic reviews of data from respectively 22 (76) and 15 (77) randomized controlled trials (RCTs) placed topical 5% permethrin and ivermectin at the same level of efficacy and safety, and are consequently considered as the reference treatments. Between these 2 systematic reviews, performed in 2010 and 2017, no newer trials were included in the evaluation, but the conclusions were different based on

Table II. Comparison of treatments in use to treat scabies in humans

Drugs	Formulations	Recommended treatments	Cost (Euros)	Efficacies (%)*	Main adverse reactions	Use in children	Use during pregnancy	Use in breastfeeding women
Ivermectin	200 µg/kg Pills	Repeat after 7 days	€19 for 4 tablets at 3 mg=€38 for a complete treatment (weight 70 kg)	70–100	Nausea, rash, dizziness, itching, eosinophilia, abdominal pain, fever, tachycardia	Not approved in children <15 kg or 5 years of age	Only recommended in France	Only recommended in France
	1% Cream/lotion	Overnight (8–12 h) – from head-to-toe – repeat once after 7–14 days	€20 for 15 g cream=€80 for a complete treatment	69–85	Pruritus, burning, stinging, eczema	Not approved in children	Not recommended	Not recommended
Permethrin	5% Cream	Overnight – from head-to-toe – repeat once after 7–14 days	€19 for 30g cream=€38 for a complete treatment	86–100	Pruritus, burning, stinging, eczema	Safe in children ≥2 months of age	Approved	Not recommended
Benzyl benzoate	10–25% Lotion or emulsion	Apply from head-to-toe for 24 h On days 1, 2 and repeat after 7 days	€15 for 125 mL emulsion=€30 for a complete treatment	48–92	Pruritus, burning, stinging, pustules, skin irritation, eczema	Safe in children ≥ 1 month of age	Authorized if necessary	Not recommended
Crotamiton	10% Cream	Overnight on days 1 and 2	€7 for 40g cream	63–88	Pruritus, skin irritation, eczema, erythema, anaphylactic reaction	Safe in children	Not recommended	Not recommended
Precipitated sulphur	6–33% Cream or lotion	Apply from head-to-toe for 3 consecutive nights	–	39–100	Messy application, malodour	Safe in children	Authorized	–
Malathion	0.5% Aqueous lotion	Repeat after 7 days	€12 for 100 ml=€24 for a complete treatment	47–72	Pruritus, burning, stinging, skin irritation, CNS toxicity, dizziness, seizure	Not approved in children <2 years of age	Withdrawn from the European market	Withdrawn from the European market
Lindane	1% Lotion or cream	Overnight Repeat after 7 days	–	64–96	CNS toxicity, dizziness, seizures, renal and hepatic toxicity reported with overdosage	Withdrawn from the European market	Withdrawn from the European market	Withdrawn from the European market

*Efficacies according to Strong & Johnstone (76). Updated from Bernigaud C. et al. (13).
CNS: central nervous system.

the regimen of the drugs used. Strong & Johnstone (76) concluded, in 2010, that topical permethrin 5% was the most efficacious agent for the treatment of scabies. Rosumeck et al. in 2017 (77), reviewing the same trials as in 2010, concluded that topical permethrin was equal to ivermectin when 2 doses were given. Overall, among all the therapeutic trials analysed, a significant heterogeneity in the methods and outcome measurements was found, making the conclusions difficult to evaluate. A French randomized clinical trial, cluster-designed with a robust protocol, is currently recruiting patients with common scabies to establish finally which treatment, 5% topical permethrin or 0.2 mg/kg oral ivermectin, both given twice at 10 days' interval is the most efficacious (SCRATCH, NCT02407782) (78). Treatment also depends on the availability of the drug in the different countries. For example, since it was first approved in France for the treatment of scabies in 2001, oral ivermectin has been licensed only for this indication in 10 nations as first-line treatment, and is mostly used off-label and may not even be accessible in other countries. In those countries, available and cheaper medications are preferred, such as sulphur preparations and benzyl benzoate. To widen access to this key effective medication, in June 2019 the WHO added ivermectin to the 21st WHO Essential Medicines List (79).

Follow-up is necessary after treatment, to evaluate the cure of the patient and to prevent re-infestation. Treatment success should be expected in approximately two weeks. Itching can persist for up to one month after successful treatment. Causes of apparent treatment failure with an effective treatment include incorrect diagnosis, dermatitis secondary to the mite or topical agent, incorrect application of the topical agent, poor penetration of the agent into hyperkeratotic skin or nails, and re-infestation from scabies-infested close contacts (80). Parasite resistance has been reported for both permethrin (81, 82) and ivermectin (83, 84), but its clinical importance remains a matter of debate. Studies are lacking and surveillance for better documentation is warranted. Treatment failure has been observed, mainly because drugs are not 100% effective (74– 93% clearance is observed with permethrin and 68–86% with ivermectin (77)). Some authors recently tried to determine which factors were associated with treatment failure (85, 86). These 2 studies found that incorrect decontamination of furniture or fomites was a key factor in treatment failure. While living mites can be found in samples of environmental dust from floors and furniture of patients with scabies (87), the indirect transmission of mites by fomites is thought to be rare (3), at least in common scabies. Studies are needed to evaluate the impact of environment-decontamination procedures on the success of the treatment (88) in non-profuse cases of scabies and in cases of severe scabies with high mite-load, in order to optimize cure rates. Simplified and generalized algorithms, based on high-throughput experimental data that can be used in a large range of settings, including resource-poor population, were suggested recently (89).

Appropriate treatment should also be given for severe secondary bacterial infection. Topical antibiotic creams (e.g. mupirocin or fusidic acid) are not recommended in cases with profuse lesions. Systemic antibiotics have to target GAS and *S. aureus* (including MRSA in specific areas). Oral trimethoprim-sulphamethoxazole (cotrimoxazole) or intramuscular benzathine penicillin G are used in tropical endemic regions (90), whereas pristinamycin, amoxicillin/clavulanic acid or cephalexin may be used in non-tropical regions. For the itch, there is no specific treatment. Antihistamines can assist, but it is their sedative properties that are effective, rather than an anti-pruritic mechanism (91).

For the treatment of crusted scabies, there is no consensus as, to date, no randomized controlled trials have been performed. Most records come from small studies, and experience in northern Australia, where highly infested patients are seen (47). They suggest a regimen of multiple doses of oral ivermectin with repeated topical permethrin and keratolytic therapy.

For the treatment of children, only topical treatments have been approved. The application time in children can differ from that in adults. Oral ivermectin cannot be used if the patient's body weight is less than 15 kg. Recent reports using ivermectin off-label in infants and young children (aged 1–64 months, body weight 4–14.5 kg) are highly reassuring on the safety and efficacy of this treatment in this age group (92). Recommendations for use of ivermectin in infants may change in the near future. For the treatment of pregnant women, only one country allows the use of ivermectin, as a second-line treatment after 5% topical permethrin, at any trimester of the pregnancy supported by an expert recommendation (93). Most exclusions of women from ivermectin treatment are for basic precaution rather than any anticipated foetal toxicity. In practice, thousands of women have been treated inadvertently before their pregnancy becomes known in onchocerciasis eradication programmes in Africa. Occurrence of miscarriage, stillbirth, or birth defects in the reference population did not differ significantly (94, 95). Continued surveillance is necessary, as well as more fundamental work to enable this target population to be treated adequately.

Treatment of large clusters (collectivity level)

As scabies is a contagious disease spread by skin-to-skin contact, people living in crowded communities are at greater risk. In these collectivity settings, because patients and their close contacts have to be treated all at the same time and because prevalence can be very high in some communities, the opportunity of MDA has emerged to control scabies in endemic spaces (96). The first MDA was performed in a scabies-endemic

region of Panama in the 1970s by Taplin et al. (97). Successively, multiple programmes have evolved in all parts of the world to control scabies by MDA, firstly with lindane, topical permethrin, followed by the use of oral ivermectin (summarized in Table SI[1]). Only one trial, the Skin Health Intervention Fiji Trial (SHIFT) has randomized 3 islands in Fiji to 1 of 2 intervention strategies, i.e. oral ivermectin and topical permethrin, compared with standard care as control (36). At 12 months, mass treatment with ivermectin was found to be the most effective, with a relative reduction of 94% from baseline in the prevalence of scabies and 67% in impetigo. During all these years, many MDA programmes have resulted in successful and significant reduction in the prevalence of scabies in highly endemic or endemic settings. Controversial results have been reported only from Australia (see Table SI[1]), presumably because the adherence of the target population with the treatment regimen was poor. Fewer data exist on the sustainability of success of MDA in the longer term, but this approach seems to be efficient (98, 99), especially in communities with a scabies prevalence higher than 5% (100). Interestingly, the treatment of scabies alone also seems to result in a significant reduction in GAS impetigo (36) and kidney complications, signified by the reduction in haematuria (30). A recent study has indicated that it is not necessary to add antibiotics during MDA for scabies to reduce the prevalence of impetigo (37). Although, as most MDAs target only one disease, some programmes have looked at the potential to integrate scabies MDA in other schemes for neglected tropical diseases eradication programmes (101), such as onchocerciasis, lymphatic filariasis, trachoma, schistosomiasis, yaws, or infection with soil-transmitted helminths. On a smaller scale, combining ivermectin and albendazole administration to treat both lymphatic filariasis and onchocerciasis, or scabies and lymphatic filariasis (102), or scabies and strongyloidiasis (103) were found to be effective and safe. On a larger scale, the co-administration of azithromycin and ivermectin for targeting trachoma and scabies in the Choiseul Province, Solomon Islands, was also found to be effective, feasible and secure in 26,188 enrolled participants (104). Remarkably, MDAs for scabies control have had additional unintended downstream effects, as they have been found to be efficient in controlling head lice (105) and Anopheles farauti, the vector of malaria in the Solomon Islands (106). All these interesting programmes provide positive results and robust evidence to encourage MDA with ivermectin to control scabies in highly endemic populations on a larger scale. Optimization of these programmes will be required in order to understand factors associated with success, defining the appropriate regimen to use, and determining the numbers of rounds of MDA (24).

Similarly, controlling scabies at a larger scale by MDAs has been extrapolated for smaller outbreaks in closed institutions, such as schools (107), prisons (108), age-care facilities (18), and, more recently, in asylum seekers (109).

HOW WILL WE TREAT SCABIES IN THE NEAR FUTURE?

As mentioned above, treatment either with a topical acaricide or oral treatment with ivermectin are the current standards of care for common scabies. Most patients with scabies infection will recover with a suitable medical intervention, but patients often require multiple treatments and/or a combination of topical and systemic drugs. The major limitations of current therapies are poor compliance with repeated treatments, limited activity against eggs, and half-lives too short to cover the whole 14-day life cycle of the mite (13). The recent development of an experimental porcine scabies model provides real potential to conduct translational preclinical and pharmacokinetic studies with new drug candidates.

In order to optimize and improve the therapeutic options for scabies treatment, the concept of translating existing drugs used in the veterinary clinic to humans was investigated (72, 73). Moxidectin is a molecule compound suitable for oral administration. It is a member of the same family as ivermectin, and was recently developed and approved by the US Food and Drug Administration (FDA) for treatment of onchocerciasis. Moxidectin has a very interesting pharmacological profile: rapid absorption, large distribution, and a much longer half-life in plasma and, importantly, in the skin than ivermectin (72), potentially covering the entire life-cycle of the scabies mite. In a pilot trial in the experimental pig model for scabies performed in France, moxidectin used orally, at a single dose of 0.3 mg/kg, was found to be more effective than the conventional 2 doses of ivermectin at 1-week interval (0.2 mg/kg) (72). A multicentre clinical phase II trial in humans is in progress in Australia and France, with the aim of developing moxidectin as a new single-dose treatment for scabies (NCT03905265) (117).

The use of higher doses of ivermectin for treatment of scabies is also an interesting option and currently under investigation (118). The clinical development of ivermectin for scabies and other parasites might have been rushed, and the dose of 0.2 mg/kg was not derived after high-level dose-ranging studies; it was based on a reasoned, but arbitrary, decision. An emerging hypothesis is that the parasite infection may need a higher dose of ivermectin to achieve a cure. This concept was first raised for head lice infestation, as the standard dose of oral ivermectin (0.2 mg per kg body weight) was found to be poorly effective. Further studies found that

[1]https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-3468

treatment with 0.4 mg/kg ivermectin (a double dose) was approximately 95–100% effective (119). Similar results were reported for other parasitic infections (118). Dose-ranging experimental studies in the pig model are ongoing to determine whether higher doses of ivermectin are more effective at controlling scabies infestation. In France, a French Ministry of Health-approved randomized controlled clinical trial is in process, comparing the efficacy of ivermectin given orally as the higher double dose of 0.4 mg/kg with the conventional treatment dose of 0.2 mg/kg, given 3 times 7 days apart (on D0, D7 and D14), supplemented in both arms with daily application of emollient therapy and topical 5% permethrin on D0 and D7 (GALECRUSTED, NCT02841215) (120).

Other novel treatments are also in development, using herbal compounds (121, 122) and even entomopathogenic fungus (123). The use of advanced molecular and biochemical technologies will help to design new therapeutic tools. These next-generation drugs are needed immediately and should be tailored to scabies mites.

CONCLUSION

The worldwide prevalence of scabies remains high, and currently available treatments may not be sufficiently effective to control the disease. During the past 20 years, at the beginning of the 21st century, a lot of important work has been completed concerning the management of scabies, mainly driven by IACS, a global advocacy body formed in 2012. We hope that the next 10 years will provide a significant improvement for patients infested with scabies, and that new drugs and diagnostics will enhance the therapeutic options for the benefit of patients and their families.

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Centenary theme section: CUTANEOUS AND GENITAL INFECTIONS

REVIEW ARTICLE

Skin Disease in the Tropics and the Lessons that can be Learned from Leprosy and Other Neglected Diseases

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Skin disease is a common illness in most tropical regions where the pattern of clinical, presentations is dominated by infections. Along with common diseases such as pyodermas and fungal infections, a group of conditions known collectively as the neglected tropical diseases of the skin or Skin NTDs, which are the targets for worldwide control or elimination are also seen in health care facilities. These diseases range from the common, such a scabies, to those that are less frequent including leprosy and mycetoma. The initiative to use skin presentations of tropical diseases as a route to diagnosis by front line health workers is both logical and welcome. However, this requires training and monitoring and as the work gets under way, it is critically important that time invested in this programme is backed by firm and lasting commitment at regional and national levels.

Key words: skin disease; tropics; NTDs; scabies; tinea; mycetoma; leprosy.

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SIGNIFICANCE

Skin diseases are very common in the tropics. They include illnesses like ringworm, impetigo and scabies. A recent WHO programme has been to take advantage of the fact that many of the serious diseases seen in the tropics, such as leprosy and river blindness, first appear in the skin and that by detecting them, because of their appearances in the skin, their treatment and control becomes much more feasible.

Skin disease is estimated to affect more than 900,000,000 persons globally each year. It is therefore unsurprising that it is one of the common disorders seen at front line health care level in all regions (1). In most countries with a tropical climate between 20–40% of new consultations at primary care level are motivated by skin problems, although this varies, depending on the underlying prevalence of skin disease and the existence of local variations in the normal pattern and distribution of skin disease (1). Generally, in hot climates, this picture is dominated by infections, including bacterial skin infections such as pyodermas and cellulitis, mycoses including dermatophytosis as well as Candida and Malassezia infections. Viral skin infection is generally less common although warts and herpes simplex are seen regularly and the presence of an underlying high level of HIV in the community can be associated with local spikes in the prevalence of molluscum contagiosum and extensive plane warts resembling epidermodysplasia verruciformis (2). Likewise, the prevalence of scabies is variable and changes over time and, while often it affects more than 5% of the population in the tropics, it can reach even higher levels of more than 10%; these prevalence rates have been reported from Ethiopia and the West Pacific and have driven major public health initiatives designed to control the infection (3).

There is less data on the social and economic costs of these infections in poor communities, although they may have a significant impact on overstretched resources. One study in Mexico estimated that, over a 3-month period, households in rural areas were using over 24$ per household to treat scabies – and generally the treatment given was ineffective (4). This used up all cash reserves destined for other needs such as additional food. In Papua New Guinea managing tropical ulcer, or just one skin disease, accounted for more than 30% of the health budget of individual aid posts (primary health centres) in one region (5). Skin infections in the tropics are also associated with significant levels of disability and morbidly which can vary both with region and individual circumstances. For instance, the presence of tinea pedis in patients with lymphoedema, living in areas endemic for lymphatic filariasis, is a major risk factor for the development of recurrent episodes of cellulitis which lead to pain and debility, as well as loss of work (6). Alleviating disability and preventing further damage in patients with tropical ulcerated conditions such as the neuropathic ulcers in leprosy or the massive limb and scrotal swelling in lymphatic filariasis are major challenges to local resources (7). Yet linking their management to that of patients with other conditions with similar needs, such as diabetic foot or podoconiosis, is increasingly used as an effective form of integrated care package, even in remote rural areas (8, 9). Beyond physical incapacity skin disease may also be associated with severe mental

illness particularly depression and in addition it affects household and societal relationships through discrimination and stigma (10). In some countries there has been both community discrimination as well as discriminatory legislation against patients with some skin diseases, including leprosy. This is now changing. In most areas such legislation, which was often introduced many years ago, has been repealed thereby allowing patients to participate in various activities that had previously been prohibited and even simple but important interventions such as ceasing to use the word "leper" or listing leprosy as grounds for divorce are making a difference (11).

While the pattern of skin disease is dominated by infection in tropical countries, it is important to recognise that other skin diseases may have an important impact on health. Although previously sparsely distributed in hot climates eczema, particularly atopic eczema, is now seen more frequently in clinical services and there is evidence that the prevalence of atopic eczema is increasing in some tropical areas (12). There are also specific problems encountered in particular population groups. Oculocutaneous albinism, with its attendant risk of early non-melanoma skin cancer, is more common in tropical regions than the colder north, affecting those living in countries from Central America, to Sub-Saharan Africa and the Pacific Region (13, 14). This poses a strain on resource-limited health systems as, if it is ignored and patients left without sun-protection advice as well as treatment, there is a high risk of fatal squamous cell carcinoma. Certain populations are also susceptible to other specific skin conditions. For instance, some native American groups are genetically predisposed to actinic prurigo which may cause severe itching and persistent debilitating light sensitive dermatoses (15). This is difficult to manage in rural populations whose main occupation is agriculture.

NEGLECTED TROPICAL DISEASES OF THE SKIN

Skin disease or diseases that present in the skin are also important in a different context. Many of the important disabling infections that dominate health care in the tropics, such as onchocerciasis and leishmaniasis, are known collectively as Neglected Tropical Diseases or NTDs. Recently the World Health Organisation, supported by other agencies and scientific journals, has focused on a strategy of integrating preventative, curative and supportive care, as well as research, for NTDs, whose elimination or control forms a core part of global health strategy. A key element of this initiative has been to group these neglected diseases into clusters; in one such cluster diseases such leprosy, lymphatic filariasis, yaws and scabies constitute a group of disorders known as Skin NTDs (16) (see **Table I** for the full list). In order to focus on implementing their control and addressing health inequalities, it has been important to recognise and include the common readily treatable skin conditions, such as impetigo and fungal infections, together with the more serious neglected diseases such as leprosy, as part of an overall skin-centred strategy. By developing integrated schemes for case detection, management and mapping, it will be possible to maximise the advantages of an economy of scale and to rationalise the use of scarce resources (17–19). There is also a further practical benefit to be derived from combining the management of disability caused by these problems, in combatting discrimination and stigma and in sensitising communities and providing health education.

Table I. The neglected tropical diseases of the skin (skin NTDs)

- Buruli ulcer
- Cutaneous leishmaniasis
- Post-kala-azar dermal leishmaniasis
- Leprosy
- Lymphatic filariasis (LF) (lymphoedema and hydrocoele)*
- Mycetoma, chromoblastomycosis and other deep fungal infections
- Onchocerciasis
- Scabies
- Yaws

*Integrated strategy for morbidity management of two tropical endemic diseases with these complications - LF and Podoconiosis.

In furtherance of this initiative a new handbook on the recognition of skin diseases and Skin NTDs has been produced by the WHO and is available in 5 different languages (20). Other strategies to address Skin NTDs such as improving access to training programmes, developing common management pathways and co-distribution of drugs used for mass administration are in development. There have been a number of new initiatives arising from this work and designed to improve case detection, as well as to provide specialist support, that range from the development of diagnostic apps (21) usable in the field on handheld devices, to the provision of diagnostic algorithms and the use of long range expert support through electronic messaging and communication e.g. Telederm and Whats-App technologies (22, 23).

SPECIFIC AND COMMON TROPICAL SKIN INFECTIONS

Fungal disease

The reason for the dominance of skin infections in tropical countries is thought to reflect the prevalence of factors that favour spread and pathogenesis, such as climate and overcrowding, particularly household overcrowding. But there are other changes encountered that are different from those encountered in temperate climates, including the clinical patterns of infection. For instance, with dermatophyte infections, onychomycosis and tinea pedis are frequent presentations in colder climates whereas, in the tropics, tinea capitis and tinea corporis are much commoner. This is also subject to variation in different areas. In sub-Saharan Africa, for instance, tinea capitis is endemic in school children and prevalence rates in schools can

exceed 20% (24–26). Furthermore several studies in recent years have emphasised a changing pattern of infection with *Trichophyton tonsurans* beginning to become more prevalent in both West and East Africa, whereas, previously, *T. violaceum* and *Microsporum audouinii*, although still common, dominated the pattern of disease (24–26). There is little to no surveillance for scalp infection in these countries and effective treatment through oral antifungal therapy is costly and inaccessible to most. Although local communities recognise that, normally, tinea capitis does not persist into teenage years the more inflammatory symptomatic forms of scalp ringworm, including kerion, pose a dilemma as effective management requires control of tinea capitis within the community, a strategy which is currently not achievable. There are other distinctive features of superficial fungal infection in the tropics. In India, for instance, there is a widespread epidemic of recalcitrant dermatophyte infection caused by *Trichophyton rubrum* and increasingly a strain of *T. mentagrophytes* (27, 28). This results in extensive tinea corporis and some strains of *T. mentagrophytes* appears to have mutations in the squalene epoxidase gene, meaning that terbinafine, which targets this enzyme, is less effective. Other factors which may have affected spread of this infection include the ready availability and use of topical potent corticosteroid combinations and low-quality generic antifungals. Over the past few years similar cases have been seen in Europe. The main differences seen in this new epidemic, which is affecting India and some adjacent countries and migrant populations in the Middle East and Europe, are the widespread nature of the infection and its persistence despite adequate treatment or an initial response followed by early relapse. In other tropical countries, particularly in isolated communities, there is persistence of endemic tinea imbricata caused by *T. concentricum* which can result in chronic and very widespread scaling and itching. New endemic areas for tinea imbricata continue to be reported (29) – the latest being in the Solomon Islands and amongst the Batek people of Malaysia (30).

Bacterial infections

Amongst the bacterial infections, there are also differences in the pattern of skin infection seen in the tropics. In colder environments *Staphylococcus aureus* is the main cause of skin infections. But Group A streptococcal infections are commoner in the tropics than in northern climates and the complications of nephritis (31) and rheumatic fever (32) are a potential public health problem in these regions. Streptococcal skin infection is particularly seen in association with scabies (31, 32). The relationship between streptococci and scabies mites is a complex one, as the mites produce different substances, such as Scabies Mite Inactivated Protease Paralogues or SMIPPs (33) which may interfere with complement activation and phagocytosis. As a result, scabies infestation has a direct impact on the development of streptococcal infection. As a result although traditional dermatological teaching often emphasized the need to treat secondary bacterial infection first and then the scabies infestation, current work with the oral drug ivermectin used as mass drug treatment of scabies has shown that this will not only combat the scabies mites, but also bacterial infection declines as well which supports a direct role for scabies mites in predisposing to bacterial infection (34).

NEGLECTED TROPICAL DISEASES

Mycetoma

Mycetoma is a chronic infection, whose first signs are localised swelling and the development of papules and sinus tracts on the skin surface. It is caused either by actinomycetes or filamentous bacteria (actinomycetoma) (**Fig. 1**) or fungi (eumycetoma) in tropical regions from Mexico to Thailand (35). The countries with the highest prevalence are Mexico and Sudan (35). In 2016, mycetoma was formally declared to be a neglected tropical disease by WHO, becoming the first fungal infection to be given this designation. The decision was largely based on the lack of progress in controlling this infection and achieving early diagnosis which reduces the risk of disability caused by the disease that, if unchecked, can proceed to cause severe limb deformities and osteomyelitis (36). There has been a corresponding lack of research on new drug development, new diagnostics and epidemiology. The call for change was taken up in

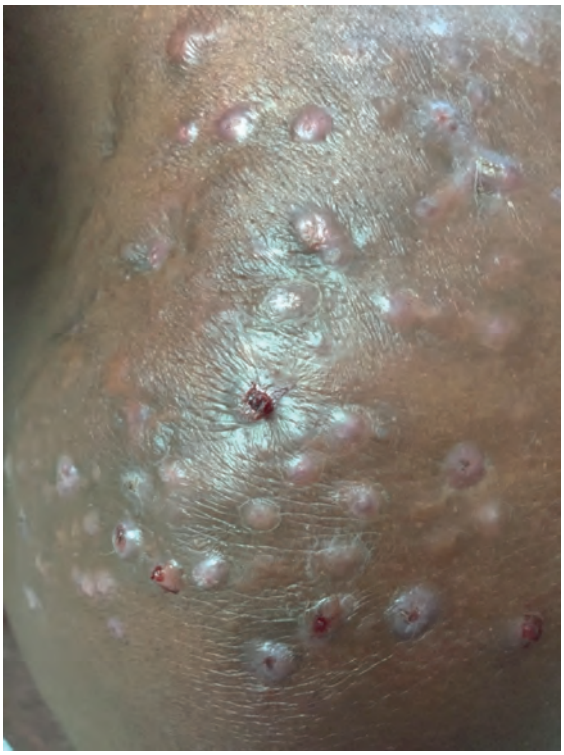

Fig. 1. Actinomycetoma due to *Nocardia brasiliensis*.

February 2019 in a large conference on mycetoma held in Sudan and attended by the Director of the NTD programme for WHO (37).

However, despite slow progress there are several new interesting findings about this infection. In Sudan there is an associated increase of infection in communities where cattle are grazed close to houses and the organism *M. mycetomatis* has been identified close to the thorn hedges around corrals used to pen the livestock (38). The taxonomy of the genera of fungal mycetoma agents has also been changed with several new species identified. For instance, we now recognise *M. mycetomatis*, *M. tropicana*, *M. pseudomycetomatis* and *M. fahalii* (39). This may have implications for accurate laboratory diagnosis and the choice of treatment options. *M. fahalii*, for instance, is not sensitive to itraconazole. There is now a new clinical trial of fosravuconazole for treatment of fungal mycetoma due to *M. mycetomatis*, based on in vitro data supporting the potential effectiveness of this group of antifungals (40). There are also newer approaches to the treatment of actinomycetoma caused by *Nocardia* species which have been found to respond to a wide range of antibiotics including imipenem, moxifloxacin and linezolid, in addition to the more traditional dapsone and cotrimoxazole (41). Yet there remain formidable problems in some of the simplest aspects of disease such as in detection of cases at community level (42) and improving access to laboratory diagnosis, as identification of the causative organisms is a key step in selecting the best treatment for patients (43).

Leprosy

Leprosy has long been a scourge in many tropical countries. While it causes skin lesions, which, if untreated, can be extensive and destructive, nerve damage leads to progressive sensory loss and destructive trophic changes. Changes in immunological responses to the infection, Type 1 and Type 2 leprosy reactions, lead to aggressive local and systemic reactions which are potentially fatal (44). These severe symptomatic reactions are accompanied by tissue swelling, rash, arthritis and eye disease as well as further nerve damage. Most cases of human leprosy are caused by *Mycobacterium leprae*, although a second species, *M. lepromatosis*, is now recognised (45). There is a difference of 9.1% in nucleotides between the two, confirming that they are distinct species rather than clonal variants (46). The latter causes leprosy in certain parts of the world. In Mexico for instance it is probably the commoner of the two as a cause of human disease, and it is responsible for cases of the form of leprosy known as diffuse lepromatous leprosy or Lucio's leprosy where there can be vasculitic ulceration of lesions (46). *M. lepromatosis* has also been detected in Brazil, Myanmar and Singapore (47). In other countries *M. leprae* remains the dominant organism.

Although once endemic in Europe dermatologists in our region now only see imported cases, although it was not so long ago that Gerhard Amauer Hansen carried out his methodically planned studies in Norway, where leprosy was still endemic in the 19th century, to prove the infectious and transmissible nature of the infection. Only one autochthonous case has been reported in Europe, from Italy, in recent years (48). Elsewhere the burden of leprosy in endemic areas has shrunk over the years, although it is still seen in most parts of the tropics. There have been many attempts to identify potential natural sources of *M. leprae* in the environment, yet none of these have yielded results, although two animal species can be infected naturally – the 9-banded armadillo and the red squirrel (49).

A decline in the numbers of cases of leprosy towards the end of the twentieth century followed intensive case detection and the evolution and deployment of a series of drugs with potent antileprosy activity. This also involved the pioneering of suitable effective drug combinations, as well as heightened awareness at national and regional levels (50). A key aspect of leprosy has been the recognition that it presents in a spectrum, with some forms having distinctive changes on the skin and in the nerves accompanied by large numbers of organisms, whereas in others the skin lesions are different and bacteria very sparse or undetectable. These are the multibacillary and paucibacillary forms known as lepromatous and tuberculoid leprosy respectively, although variants intermediate between these two poles of the spectrum such as borderline lepromatous and tuberculoid forms, as well as an indeterminate form, are seen regularly (**Fig. 2**) (51). The other key feature of leprosy is that, as it is not possible to repair the damaged and defunct nerves, once this destructive process has been completed, patients are left with denervated areas resulting in anaesthetic limbs. These are prone to trauma and ulceration as well as trophic change and disfigurement even if the patient is freed from active infection as a result of treatment. Early

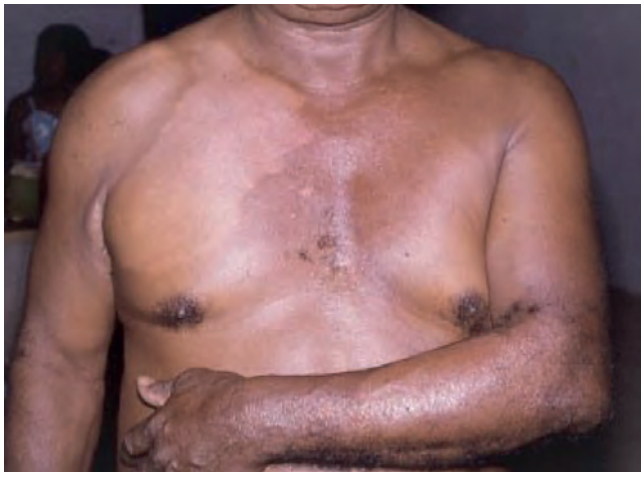

Fig. 2. Leprosy – borderline tuberculoid leprosy on the chest wall.

recognition and treatment of individual cases is the way to prevent this disabling consequence of leprosy. In addition to purely clinical features of the disease, combatting disability and stigma remain other important components of leprosy care, even in the 21st century.

In the course of identifying major health goals to coincide with the millennium, elimination of leprosy was promoted as a suitable target. Ironically, attention was often drawn away from implementation of rigorous programmes for the detection of disease, as case numbers appeared to be on the decline, and, in order to deliver this target, diagnostic criteria were softened. As a result, there was a reduction in planned case detection programmes in some countries, which given the long incubation period of the disease, subsequently allowed the numbers of new cases to stabilise and, in some areas, to increase. Several countries such as India and Brazil are still reporting substantial case numbers and it is likely that the current official figures from other regions are a significant underestimate of the actual picture, as reporting leprosy depends on implementing programmes for the detection of new patients.

The current classification of leprosy used by WHO is based on assessment of the number of skin lesions; patients with less than 5 lesions are classified as paucibacillary (PB) and those with more than 5 as multibacillary (MB) forms of leprosy (52). There are some flaws in this scheme; for instance, acid-fast bacilli (AFB) have been detected in cases classified as PB. Such cases, presumably, provide a risk of onward transmission of the infection. Given the importance of improved case finding there have been a number of different initiatives designed to improve detection rates and simplify the process of making the diagnosis. These include both laboratory-based and clinical actions. The two traditional laboratory-based testing methods the slit skin smear and histopathology of skin biopsies, while useful, have limitations as both are susceptible to variations in observer skills or the availability of staining facilities and depend on an adequately trained work force. So one priority has been to devise a simple laboratory based test deliverable in front line health care environments – a point of care test – using techniques that can be implemented with minimal resource such as a simplified molecular tool or a card antigen detection test (53). Although a great deal of work has been carried out and is still evolving, no single antigen or PCR probe has been identified that will unequivocally identify patients with leprosy of both multibacillary and paucibacillary forms or distinguish between patients and contacts who have no active disease (54). In part this may be due to bacterial load – tuberculoid leprosy, where there is a high level of immunity and very low numbers of organisms, is potentially a difficult target. It is also worth considering the adoption of other options, such as using a combination of different laboratory tests, including some that might be combined with an algorithm based on clinical findings.

The most recent molecular results have focused on *M. leprae*-specific repetitive element RLEP and 16S rRNA which are the most frequent markers used in experimental studies to detect the presence of bacilli with up to 80% sensitivity, although the range of results is wide, starting from as low as 15% (51, 55). Combining molecular methods with a different test system including detection of organisms in smears may be another route for successful diagnosis (56).

Other methods, that have been attempted, have adopted a different strategy, focusing on novel surveillance and training programmes for clinical recognition of leprosy in its many different forms or the identification of the simplest features of the disease that would allow a primary health worker to refer suspected cases to a more expert team – this forms the basic premise of the new WHO training manual (20). Behind most of these programmes lies a realisation that direct clinical observation needs to be supported by at least one or two other signs such as the presence of detectable nerve damage e.g. through loss of sensation or enlargement of peripheral nerves. The detection of both requires training. Attempts to use such approaches in the field as part of broader Skin NTD programmes, aimed at detecting a range of endemic NTDs, are now under way (17, 57).

In order to rationalise the need for extensive programmes for case recognition, a new strategy is being delivered that centres around the treatment of contacts of leprosy patients with single dose rifampicin. It is known as post exposure prophylaxis or PEP (58, 59). While the idea behind this has merits, there are potential problems inherent in the widescale deployment of this strategy, notably that it does not appear to protect contacts of patients with multibacillary leprosy who are the greater sources of spread; also it only protects for up to 2 years post treatment and is only effective in some contacts of leprosy patients (60). There is also a social downside as in drawing attention to the presence of cases of leprosy in small communities, unless there is adequate local patient support, there is a consequent risk of discrimination and social ostracisation. A further practical problem is that it risks diverting attention and resource from the strategy that can deliver control i.e. new case detection, even though this is essential for identifying contacts. Measures are being taken to seek ancillary methods, such as drug combinations or different dosages, to widen the scope and inclusivity of PEP, although this may prove difficult to activate and assess, as the existing PEP scheme has already been adopted as policy in many areas.

Leprosy is a complex disease which has long been feared in many societies and sending mixed messages about elimination has served to confuse as well as divert attention from delivering a potentially achievable goal – a world free of leprosy.

THE WAY FORWARD

The skin remains the key entry point for diagnosis and management of many tropical diseases including NTDs and HIV related skin disease, as well as other common skin diseases such as pyoderma, eczema and fungal infection. Addressing all of these is a difficult task as it means increasing the public profile of skin disease in areas where resources are limited and there is inevitable competition with other calls on financial and clinical support, such as diabetes and cardiovascular disease. But the first step to maximise the potential of using the skin, as the entry point for a radical rethink of health strategies, is to sensitise those responsible for national health care in Ministries of Health and regional health offices about the importance of proper identification and management of diseases that are seen on the skin as a means of health improvement, well beyond the confines of the skin itself. Public support, through advocacy at community level, and through regional or national media services is also key to this mission. But in all this it is important to remember a key message learned from experience with other tropical diseases in the past, that it is important to be unrelenting in reminding and re-reminding all those involved in health care, from Ministry officials to front line workers, not to forget the disease that they aim to control as soon as there appears to be an improvement in its overall burden, whether this is leprosy or scabies. To coin a soccer metaphor, by "taking ones eye off the ball" it is possible to undo years of hard work and surveillance – and for the sake of what?

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REVIEW ARTICLE

The Changing Spectrum of Sexually Transmitted Infections in Europe

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As long as 400 years ago, syphilitic ulcers and gonococcal discharge were observed in connection with sexual intercourse. War, poverty, and lack of efficient therapeutic options led to a high incidence of venereal diseases, many of which had devastating outcomes. This situation continued until the beginning of the 20th century, when the microbial aetiology of venereal diseases was discovered. The infection rate dropped with the availability of antibiotic therapy after the Second World War. However, since the beginning of the 21st century, a steady increase in sexually transmitted infections (STIs) has been recognized worldwide. The number of reported cases of syphilis is increasing in Europe, especially in men having sex with men (MSM). Antibiotic resistance in several genital pathogens, such as *Neisseria gonorrhoeae* and *Mycoplasma genitalium*, causes therapeutic problems. Viral genital infections have become a therapeutic challenge, especially for prevention of STIs. Due to better knowledge of the long-term consequences of STIs and the connection between genital cancer and papillomavirus infections, sexual health services with screening programmes have been established in many European countries. There is general awareness of the importance of human papilloma virus vaccination programmes for young adolescents as a preventive strategy for genital cancer.

Key words: sexually transmitted infection; venereal disease; syphilis; gonococcal resistance.

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SIGNIFICANCE

Sexually transmitted infections (STIs) refer to a broad spectrum of bacterial, fungal, viral and protozoal infections that share a common mode of transmission through sexual contact. While syphilis was recognized as long ago as 1496, it took several centuries to detect *Treponema pallidum* as the agent, and to describe *Neisseria gonorrhoeae* as the cause of gonorrhoea. Discovery of penicillin, and molecular biological diagnostic advances, were enormous steps forward in the control of STIs, leading to a decrease in the reported numbers of infections worldwide. However, the development of antibiotic resistance in gonococci, and the emergence of new viral infections, such as genital herpes, human papilloma virus and HIV/AIDs, have changed the pattern of STIs. Despite efforts to prevent, diagnose, and treat STIs, they remain a major problem.

Although STIs are defined by a common mode of transmission, the diseases vary enormously in their aetiology and clinical manifestations. They may be bacterial, viral, protozoal or ectoparasitic in nature. The number of sexually transmitted or transmissible pathogens is permanently increasing and many of them can be divided into subtypes with differing clinical manifestations, e.g. *Chlamydia trachomatis* (**Table I**) (1).

The so-called venereal diseases (VD) include syphilis, gonorrhoea, chancroid, lymphogranuloma venereum (LGV), and granuloma inguinale, and were recognized as exclusively sexually transmitted when the relevant laws and regulations were written in many countries. Evolution from the term VD to the term sexually transmitted diseases (STDs) or STIs reflects the recognition of the increasing number of symptomatic or asymptomatic infections or conditions due to the high number of sexually transmitted pathogens. While some of them are transmitted predominantly, or even exclusively, by sexual intercourse, others may qualify as sexually transmissible (e.g. hepatitis B virus, HIV, yeasts), and non-sexual routes of transmission may even predominate (2).

In most STIs clinical symptoms first occur at the point of entry of the microorganism and appear as ulcers ("genital ulcer disease" (GUD): syphilis, chancroid, genital herpes), mucosal inflammation or discharge (gonorr-

Table I. Venereal diseases and sexually transmitted infections (STIs)

Venereal diseases	STIs
Syphilis	Chlamydia trachomatis
Gonorrhoea	Mycoplasma genitalium
Chancroid	Mycoplasma hominis
Lymphogranuloma venereum	Ureoplasma urealyticum
Granuloma inguinale	Anaerobic bacteria
	Trichomonas vaginalis (TV)
	Candidiasis
	Pediculosis pubis
	Scabies
	Human immunodeficiency virus (HIV)
	Herpes simplex virus (HSV)
	Human papilloma virus (HPV)
	Hepatitis B virus (HBV)
	Ebola virus
	Zika virus

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hoea, chlamydial and *Mycoplasma genitalium* infection, trichomoniasis), or even neoplasia. In certain STIs the infection may spread to neighbouring (gonococcal salpingitis/epididymitis) and distant organs (gonococcal septicaemia, secondary syphilis, neurosyphilis). While usually benign (genital warts), human papilloma virus (HPV) infections with high-risk genotypes may occasionally bear the risk of malignancy and possibly result in invasive carcinoma of the vulva, cervix, penis or anal region (3). Hepatitis B (HBV), scabies and HIV-disease, mainly affect extragenital sites.

The major complications of STIs include AIDS, cancer, pelvic inflammatory disease (PID) and related sequelae, neurological symptoms, sexually-acquired reactive arthritis (SARA), complications of pregnancy and the puerperium, congenital, perinatal and postnatal infection of the foetus/infant and a variety of other diseases.

HISTORY OF SEXUALLY TRANSMITTED INFECTIONS IN EUROPE

More than 100 years ago, Europe was the leading region in research on STIs. The vision that diseases in the genital tract might be caused by microorganisms was discussed in Paris in 1836, by Donne, who made some important discoveries about *Trichomonas vaginalis* (former name: Trico-monas vaginale), suggesting that it might not only be a harmless commensal, but a sexually transmissible microorganism causing symptoms (4).

As long ago as the 16th century, syphilis had been recognized as sexually transmitted, and was identified as a "venereal disease", stigmatizing the infected individual. This situation continued for 400 years, until the cause of syphilis had been detected and described in Europe. Fritz Schaudinn and Eric Hoffmann, in 1905, recognized pale rotating spiral organisms when using a Zeiss microscope to examine the secretion of an erosive vulval papule from a woman with secondary syphilis (5). They named the microorganism Spirochaeta pallida and published their discovery in the paper "Preliminary report on the presence of Spirochaetes in syphilitic lesions and in Papillomas". This was the beginning of intensive research into syphilis, a little over 100 years ago.

At that time, opinions on the cause of syphilis were diverse, until Karl Landsteiner was able to validate the presence of the microbe in specimens by dark-field microscopy, and created the new genus classification *Treponema pallidum* (6). This observation was followed by an enormous increase in publications on syphilis. A few years later, the "Wassermann reaction" was developed, and the results were presented at a conference in Vienna in 1908 during a congress for internal medicine. This was the first step to a reliable serological diagnosis of syphilis, which, in modified form, is still the recommended diagnostic procedure. Treatment of syphilis ranged from mercury, organic arsenicals, bismuth, and fever cycles, or even their combination. This was often followed by mild to severe side-effects, including death. In the pre-penicillin era, infections with *Treponema pallidum* were a serious cause of disability or even death, and the 10th leading cause of death in the USA in 1923–25 (7). These tortuous treatments ended with the discovery of penicillin by Alexander Fleming in St Mary's Hospital in London.

For a long time gonorrhoea was overshadowed by the severe syphilitic epidemic that threatened the infected population. The recognition of a different genital pathogen was another important scientific advantage in microbiology. Albert Neisser was the leading person in detecting gonococci in smears from men with urethral discharge (8). However, there were doubts, and the cultivation of the microbes was difficult, and they were indistinguishable from other bacteria. In Prague, Wertheim inoculated the urethra of men with pure cultures of *Neisseria gonorrhoeae* and induced the typical clinical picture of gonorrhoea. He also re-cultivated the bacteria from the symptomatic men, finally silencing doubters (9).

By the beginning of the 20th century the aetiology of syphilis, chancroid and gonorrhoea had been discovered, but the identity of urethral infections now known to be caused by *Chlamydia trachomatis* was still not defined. Halberstaetder & Prowazek described inclusion bodies in scrapings of the infected eyes of orangutans in 1907 (10). Lindner postulated a connection between genital (trachoma) and ocular infections; but it took time to discover the organism by cultivation in embryo egg cells and, furthermore, by modern immunological assays and finally by molecular biology (11).

Penile and anal warts were well known for many years, their clinical designation was changed from konylos to condylomata acuminatum, and these were described by different authors, along with an association with gonorrhoea ("gonorrhoeal warts") (12).The hypothesis that anogenital condylomas were caused by a virus identical to skin warts was finally proven by electron microscopy in the late 1960s. Since papillomaviruses could not be cultured either in cell culture-like viruses, or on agar plates, it needed the ability to prepare cloned viral genomes and compare with virus extracted from different anatomical sites to realise that there were differences between papilloma viruses on the mucous lesions in the genital areas and the skin.

During the First World War there were high numbers of different kinds of genital infections with inadequate facilities for treatment. Venereal ablution rooms and washing units were established for infected individuals, especially those with gonorrhoea and syphilis. VD had become a problem for the soldiers and, in particular, for the US army. The importance of medical examinations and Wassermann tests was recognized. Prostitutes were regulated in several countries, and notification of infected individuals became mandatory. After the First World War the International Union against Venereal Diseases and

Treponematoses (IUVDT) was formed in 1923, with the aim of encouraging member nations to collaborate in the prevention and control of VD. There was agreement on the need for a European network in diagnosis, treatment, and post-treatment surveillance of infected individuals and their contacts. While VD clinics were opened in Europe with improvement in health education and clinical and diagnostic development, the interest in STIs had almost disappeared in the USA. It was Thomas Parran (13) who brought the VD problem to national attention. He established programmes for syphilis control, with compulsory blood tests before marriage and during pregnancy, and established contact tracing (14). Clinical services began to improve and VD reporting also became mandatory in parts of the USA.

The Second World War had an enormous impact on the epidemiological situation of VD. Soldiers were looking for sex, not only in Europe, but also in Africa and in the Far and Middle East of Asia, and war poverty added to a high and uncontrolled incidence of STIs. The development of penicillin and other antibiotics after the Second World War changed the pattern of syphilitic and gonococcal infections, with a dramatic decline in the number of early infections throughout the world. The policy of mass testing for syphilis was established in the USA, and this was followed by mass treatment. In many European countries and in the USA the importance of VD diminished. However, despite the availability of effective treatment and better diagnosis, the symptoms of STIs re-emerged, and many men developed epididymitis, became sterile, or even developed uraemia. The resurgence of VD and STIs was due to population movements, oral contraception, the so-called sexual revolution, and the reduced use of condoms to prevent transmission. In addition, resistant microbes became a problem for gonorrhoea in developing, as well as in developed, countries.

SEXUALLY TRANSMITTED INFECTIONS IN EUROPE IN THE 21ST CENTURY

Worldwide, over 357 million new cases of 1 of the 4 major STIs (chlamydia, gonorrhoea, syphilis and trichomoniasis) occur each year in men and women aged 15–49 years, and, according to data from the World Health Organization (WHO), more than 1 million STIs are acquired every day (15, 16). During the last decade, the numbers of genital infections in European countries were collected by the European Centre for Disease Control (ECDC) through annual data collection and online reporting (TESSy).This offers a better insight into the epidemiological situation and STI surveillance in EU/EEA countries, and includes the European gonococcal antimicrobial surveillance programme (Euro-GASP) (17). Reporting, contact tracing, and treatment of STIs is legally regulated and controlled in most, but not all, European countries, and laws have been changed during the last decade. In most of the countries, gonorrhoea, chancroid, and syphilis are mandatory reportable infections, but the procedure differs regarding what and how much on information should be reported. In many European countries, reporting of *Chlamydia trachomatis* infection is recommended, and, in a few countries, even mandatory.

The organization of medical services for STIs varies across Europe. Dermato-venereology is the recognized specialty in Europe, Asia, and Latin America, but not in the anglophone regions. In 1948, in the UK and in Ireland, municipal authorities established a separate specialty, called "venereal diseases" with clinics and services specifically for the management of venereal diseases. In the 1970s, the name changed to genitourinary medicine (GUM) in order to minimize the stigma associated with the term "venereology" in the general population. In contrast to the European Academy of Dermatology and Venereology (EADV), STIs are not part of remit of Dermatology associations in the USA.

In the past 50 years there has been an extraordinary improvement in the quality of science, diagnostic facilities, and the number of services concerned with STIs and HIV, as well as more options for treatment and prevention. The emergence of HIV has influenced the epidemiology and clinical pattern of STIs. Knowledge of the impact of STIs/HIV on reproductive health has increased the recognition of STIs, with public benefit. In Europe the number of reported STIs decreased with the threat of acquiring HIV, but since the beginning of this century, a steady increase has occurred again, when the status of HIV infection was changed to that of a chronic disease. The number and rates of new diagnoses of STIs have increased in the EU/EEA since the 1990s, predominantly due to transmission between men who have sex with men (MSM), except for chlamydia where the increase has been associated with more sensitive diagnostics. Improved molecular biological methods can detect asymptomatic infections in both, men and women (18, 19). Although the surveillance methods vary across Europe, data suggest that HIV-positive men contribute a significant proportion of cases of syphilis, lymphogranuloma venereum (LGV) and gonorrhoea.

SYPHILIS

Despite simple and sensitive diagnostic tests and treatment effectiveness with a single dose of long-acting penicillin, syphilis remains a global public health problem. Since the beginning of this century the infection is re-emerging and affects two-thirds (66%) of MSM, with a high proportion of HIV-infected individuals. (**Fig. 1**) This was especially recognized after, and in connection with, the availability of efficient antiretroviral treatment and with pre- and post- retroviral prophylaxis (PREP and PEP). In 2016, 28 EU/EEA Member States reported

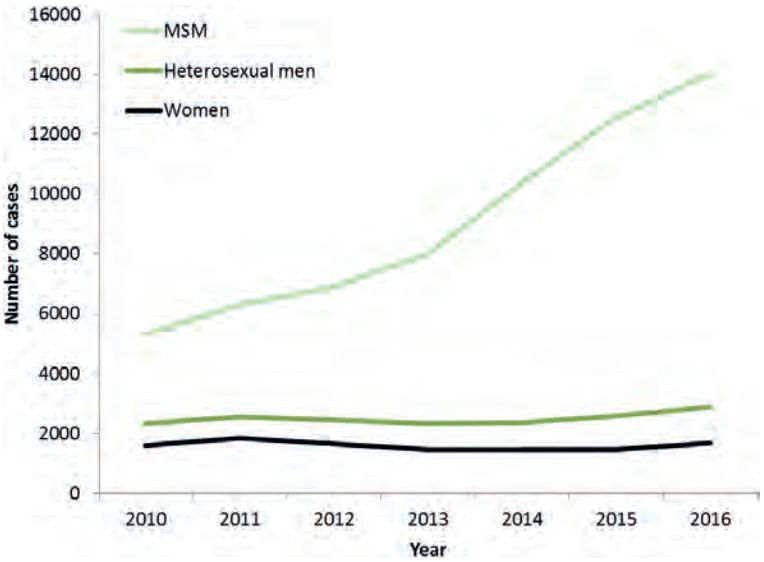

Fig. 1. Number of confirmed syphilis cases by gender, transmission category and year, EU/EEA countries reporting consistently, EU/EEA, 2010–2016. Source: Country reports from the Czech Republic, Denmark, Finland, France, Germany, Hungary, Iceland, Ireland, Latvia, Lithuania, Malta, the Netherlands, Norway, Portugal, Romania, Slovakia, Slovenia, Sweden and the United Kingdom. Permission to publish this figure is given by Gianfranco Spiteri, ECDC. Gianfranco.Spiteri@ecdc.europa.eu.

approximately 30,000 syphilis cases to the ECDC, a rate of 6.1 per 100,000 population, being 8 times higher in men than in women.

The clinical presentation of syphilis has not changed and it can easily be treated, since, so far, no resistance against penicillin has been recognized. However, early forms of neurosyphilis, notably ophthalmic syphilis, often remain under-diagnosed. Syphilis still causes several hundred thousand stillbirths and neonatal deaths every year in developing nations. Therefore, strong advocacy and community involvement are needed to ensure that syphilis is still given a high priority on national health agendas.

NEISSERIA GONORRHOEA

Gonococcal infections are the second most prevalent European and worldwide bacterial STIs. Infection rates vary considerably across Europe, with higher rates reported in northern Europe. In 2016 almost half of the reported cases (46%) were in MSM (ECDC). The number of reported cases increased over the last 15 years in the European region, until 2016, while in the UK the gonococcal cases remained stable in 2015 and 2016 (**Fig. 2**).

In contrast to *Treponema pallidum*, *Neisseria gonorrhoeae* has developed resistance to all antimicrobials previously used as first-line treatments. Most institutions, such as WHO, CDC, ECDC, and IUSTI Europe, currently recommend dual therapy with ceftriaxone (injectable, in variable volumes), together with azithromycin as first-line drugs (20). Alternatively, cefixime (400 mg once orally) has been preferred in many countries, due to its acceptance by patients as an oral single-dose regimen (21). However, the susceptibility to both extended-spectrum cephalosporins (ESCs) is decreasing worldwide (22, 23). The increase in azithromycin MICs or resistant strains, affects the mainstays of dual gonococcal treatment with third-generation cephalosporins and macrolides. The British Association for Sexual Health and HIV (BASHH) has already changed the recommendation from dual therapy to a single-shot therapy with ceftriaxone, at a high dosage of 1 g, influenced by the diagnosis of a gonococcal strain with resistance to both first-line drugs. Progression of resistance of Neisseria gonorrhoea in Europe and a realistic danger of multidrug-resistant gonorrhoea is an ever-present concern (24, 25). In this regard, performance of gonococcal culture is still necessary. In order to obtain better diagnostic information on resistant strains, a real-time PCR-based assay was designed to detect the genomic DNA of strains harbouring mosaic pen A-alleles and to discriminate them from *N. gonorrhoeae* and *Neisseria* spp. strains harbouring other genes (26). A new treatment study with zoliflodacin was performed, with successful results for urogenital and rectal infections, but was less efficacious in the treatment of pharyngeal infections (27).

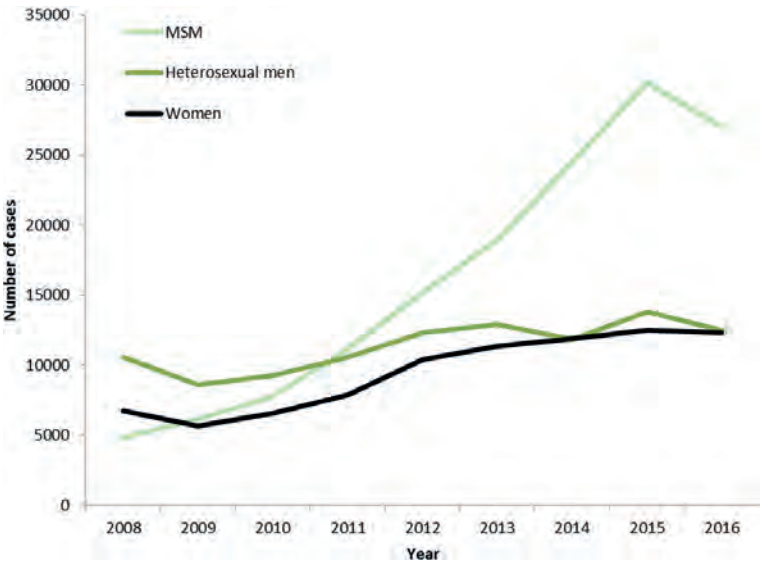

Fig. 2. Number of confirmed gonorrhoea cases by gender, transmission category and year, EU/EEA countries reporting consistently, EU/EEA, 2008–2016. Source: Country reports from the Cyprus, the Czech Republic, Denmark, France, Latvia, Lithuania, Malta, the Netherlands, Norway, Romania, Slovenia, Sweden and the United Kingdom. Permission to publish this figure is given by Gianfranco Spiteri, ECDC. Gianfranco.Spiteri@ecdc.europa.eu.

CHLAMYDIA TRACHOMATIS

Notification rates of chlamydia infections vary considerably across Europe, and continue to be highest among young adult women and heterosexuals. Over recent years, the overall trend appeared stable, both at the European and at the country level, with 150–250 cases per 100,000 population.

A special subgroup of *Chlamydia trachomatis* are the genotypes LGV1-3, causing the Lymphogranuloma venereum (LGV). In 2016, LGV data were reported by 22 European countries, out of which France, the Netherlands, and the UK accounted for 86% of the 2,043 notified cases. Almost all cases were reported among MSM with a 70% HIV-positivity rate (**Fig. 3**) (28). In addition to the classical clinical symptoms, with ulcers and inguinal buboes, rectal ulcerations are caused by these chlamydia strains, especially in infected HIV-positive individuals (29–31). Treatment recommendations are summarized in the guidelines of IUSTI Europe and the EADV (32).

MYCOPLASMA GENITALIUM

M. genitalium has been described recently as an important cause of genital infections in men and women, with a clinical pattern similar to *Chlamydia trachomatis*. It is the aetiological agent in 15–25% of symptomatic men with non-gonococcal urethritis and probably approximately 10% of women having pelvic inflammatory disease (PID) (33). Detection by nucleic acid amplification tests is the only diagnostic method available, and new CE-marked tests are approved for diagnostic use in Europe. Diagnosis and treatment is recommended in symptomatic patients. One of the main concerns is the lack of a universally effective treatment. Doxycycline has a cure rate of only 30%, whereas azithromycin is significantly more effective, with cure rates approaching 90% in macrolide-susceptible infections. However, an increase in macrolide resistance is reported in Europe, and has to be considered in treatment recommendations (34–36).

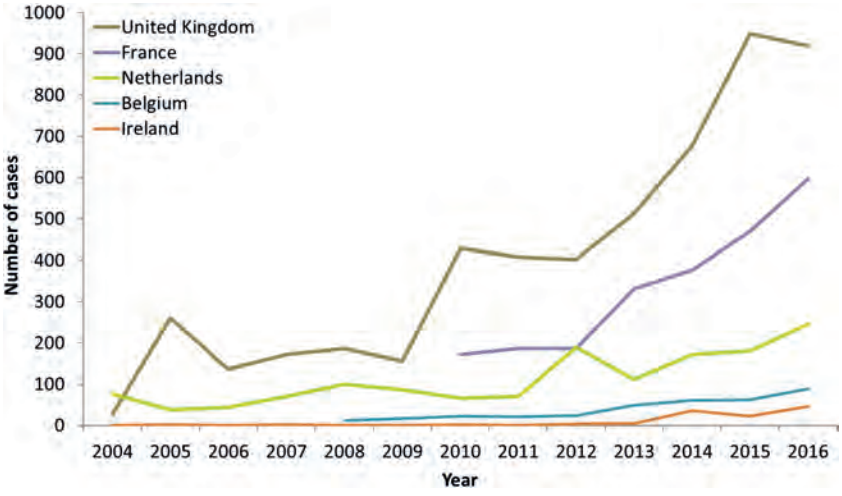

Fig. 3. Confirmed lymphogranuloma venereum cases among the five EU/EEA Member States reporting the largest number of cases in 2016, 2007–2016. Permission to publish this figure is given by Gianfranco Spiteri, ECDC. Gianfranco.Spiteri@ecdc.europa.eu.

VIRAL SEXUALLY TRANSMITTED INFECTIONS

The 21st century, in general, has been characterized by a steep rise in viral STIs, in comparison with bacterial STIs. The major impact of STIs on public health today predominantly derives from viral rather than bacterial infections. This is mainly because the latter are usually amenable to curative treatment, whereas most viral STIs still represent a major therapeutic challenge. Viruses causing STIs belong to various families, such as herpes viruses (e.g. genital herpes), human papilloma viruses (HPV, high- and low-risk groups causing genital warts, intraepithelial neoplasia, genital cancer), hepadnaviruses (hepatitis B), and retroviruses (HIV-disease). Antiviral drugs are effective against HSV infections, and are recommended prophylactically if a high number of genital relapses are observed annually. HSV can cause serious problems, including brain infections, to the newborn at the time of delivery. A close association exists between course and frequency of HIV infection and that of other STIs. Sexually promiscuous patients with GUD and/or gonorrhoea or chlamydial infections are at a particularly high risk of contracting HIV. Once infected, such individuals shed considerably more virus than HIV-infected persons without genital lesions. Conversely, STIs may accelerate the course of HIV-disease; this can be deduced from the observation that different viruses upregulate HIV transcription. These facts imply that the implementation of screening and treatment programmes for STIs will not only reduce the incidence of these infections, but also help to control the spread of HIV infection.

For viral STIs, vaccination against Hepatitis B and HPV has become common, especially in the West and Centre of Europe. The prevalence of high-risk HPV in Europe is 2–16%. Although the majority of acquired genital HPV infections appear to be subclinical or asymptomatic, several high-risk strains of HPV play a central role in the pathogenesis of most squamous cell cancers, and cause the most common cervical carcinoma with potentially life-threatening consequences. High- and low-risk HPV genotypes are also inducing other cancers or genital warts in the genital tract in women and men. Vaccines to protect against high- and low-risk genital HPV infections are available worldwide and modulate the progression of HPV disease (37). Vaccination programmes

are already established in many European countries, and offer pre-adolescent girls and boys HPV prevention free of charge as a public health issue.

In summary, the number and rates of new cases of STIs, such as chlamydia, gonorrhoea and syphilis, have increased in the EU/EEA since the 1990s. STIs are among the most frequently reported infections globally, indicating that national strategies, clinical services, and public health activities are beneficial public health instruments for the prevention and control of STIs and their long-term sequelae.

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Skin Malignancies

Theme Editors:
Veronique Bataille and Nicole Basset Seguin

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REVIEW ARTICLE

Melanoma Epidemiology and Sun Exposure

Sara RAIMONDI[1], Mariano SUPPA[2] and Sara GANDINI[1]
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The worldwide incidence of melanoma has increased rapidly over the last 50 years. Melanoma is the most common cancer found in the young adult population, and its incidence is very high among geriatric populations. The incidence of melanoma varies by sex, and this factor is also associated with differences in the anatomical site melanoma. Adolescent and young adult women have a higher incidence than men. This may be, in part, due to the greater use of sunbeds, as well as intentional sun exposure among girls and, in general, risky behaviours in seeking to suntan, due to socially-determined aesthetic needs. Indeed, the World Health Organization declared that there is sufficient evidence to classify exposure to ultraviolet radiation (sunbed use and sun exposure) as carcinogenic to humans. Although pigmentation characteristics, such as skin colour, hair and eye colour, freckles and number of common and atypical naevi, do influence susceptibility to melanoma, recommendations regarding prevention should be directed to the entire population and should include avoiding sunbed, covering sun-exposed skin, wearing a hat and sunglasses. Sunscreen use should not be used to prolong intentional sun exposure. Primary prevention should be focused mainly on young adult women, while secondary prevention should be focused mainly on elderly men. In fact, after the age of 40 years, incidence rates reverse, and the incidence of melanoma among men is greater than among women. This is probably due to the fact that men are less likely than women to examine their own skin or present to a dermatologist for skin examination.

Key words: sunburn; sunbed; sunscreen; phenotype; melanoma; sun exposure.

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SIGNIFICANCE

Young women present particularly high risky behaviours in terms of melanoma risk, such as tanning, related to social determined aesthetic needs. Indeed, the highest prevalence of sunbed use is found among female adolescents. Prevention recommendations include avoiding sunbed use, covering sun-exposed skin, wearing a hat and sunglasses. Sunscreen should not be used to prolong intentional sun exposure. Primary prevention should focus on young women, and secondary prevention in older men. In fact, at older ages, the incidence of melanoma among men is greater than among women, probably because men are less likely than women to examine their own skin or present to a dermatologist for skin examination.

Melanoma arises through malignant transformation of melanocytes, pigment-containing cells. Melanoma typically occurs in the skin, but may rarely occur in the mouth, intestines, or eye. Cutaneous melanoma (CM) is the most aggressive and lethal form of all skin cancers, which occurs when unrepaired DNA damage to skin cells (most often caused by ultraviolet radiation (UVR)) triggers mutations or genetic defects that lead the skin cells to multiply rapidly and form malignant tumours. CM represents approximately 5% of all skin cancers, but it accounts for approximately three-quarters of all skin cancer deaths (1).

The worldwide incidence of melanoma has risen rapidly over the course of the last 50 years. According to GLOBOCAN 2018 (2), the expected world number of new cases of CM is 287,723 in 2018, with an age-standardized incidence rate of 3.1 per 100,000/year and a mortality rate of 0.63 per 100,000/year. In populations of European origin, incidence and mortality rates were, respectively, 11.2 and 1.7 per 100,000/year in Europe, 12.2 and 1.4 in the USA and 33.6 and 3.4 per 100,000/year in Australia and New Zealand. Worldwide, CM incidence rates vary 100-fold among different populations depending on ethnicity, with the highest rates observed in New Zealand and Australia, intermediate rates in Europe and USA, and the lowest rates in South-Central Asia. In Europe, the highest estimates of CM incidence rates were observed in Sweden and Denmark and the lowest rates in Greece. This variation is mainly attributed to exposure to UVR, and genetically determined phenotypic characteristics. Differences by ethnicity were also observed for CM subtypes and body location. Although the most common melanoma subtype among populations of European origin is superficial spreading melanoma (SSM), melanomas in the African-American population occur more often on non-sun-exposed skin, such as the palms and the soles, and acral lentiginous melanoma

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(ALM) is the most common histopathological type (3). The age range with highest number of CM diagnoses is between 40 and 60 years. The median age at diagnosis and death are, respectively, 57 and 67 years. The incidence rates start to increase from 40 years of age; thus CM is generally considered a tumour affecting young and middle-aged people, almost a decade before most solid tumours (e.g. breast, colon, lung or prostate cancers). A study that examined incidence rates time trends of CM in 39 population-based cancer registries from 1953 to 2008 (4) found that incidence rates of melanoma increased in most European countries (primarily Southern and Eastern Europe). However, indications of a stabilization or decreasing trend were observed in Australia, New Zealand, the USA, Canada and Norway, mainly in the youngest age group (25–44 years). Possible explanations of these results include decreasing sun exposure in children following intensive preventive campaigns in these countries, and changes in the proportion of young individuals at low risk of melanoma due to immigration to these countries over recent decades.

Adjusting for age, adolescent and young adult women have higher melanoma incidence rates than men (5). This may be, in part, due to the greater use of sunbeds by girls, which is associated with increased melanoma risk (6). In general, girls have greater tanning risky behaviours and socially determined aesthetic needs (7). However, after the age of 40 years, rates reverse, and the incidence of melanoma among men is greater than that of women. Men are less likely than women to examine their own skin or seek help from dermatologists for skin examination (8). Considerable sex differences in melanoma awareness and detection practices have been reported in population-based studies (9).

Looking at mortality rates, they were found to increase in the USA and in Europe since 1980s but at much slower rates than incidence. This may be due to overdiagnosis, with diagnosis and removal of very thin, not lethal, melanomas. At all ages, mortality rates are higher in males than in females, with a cumulative mortality at 70 years of 0.37% in men and 0.17% in women in Australia. A pooled analysis of the European Organization for Research and Treatment of Cancer (EORTC) trials showed that, in both localized and advanced disease, women have a significant and independent advantage, across different clinical endpoints concerning disease progression and survival (10). This seems to depend on both biological sex trait and behavioural differences regarding primary (sun exposure, UVR protection) and secondary (skin screening) prevention (11).

We review the literature regarding UV exposure and phenotypical risk factors. A brief summary of risk estimates is presented in **Table I**.

EPIDEMIOLOGICAL RISK FACTORS

Ultraviolet radiation

According to WHO estimates, 65,161 people a year worldwide die from too much sun. Sun exposure is indeed the most significant environmental cause of skin cancer and UVR is the wavelength associated with the occurrence of this disease.

The International Agency for Research on Cancer (IARC) classified the entire spectrum of UVR as "carcinogenic to humans" (Group 1) based on substantial evidence from both basic and epidemiological research. Laboratory data and animal experiments (on DNA mutations and repair, immune function, cell integrity, cell cycle regulation, and other critical biological functions) have documented a role for both UVB and UVA radiation in skin carcinogenesis. Experiments in human volunteers have also shown that exposure to UVA and UVB can weaken the immune system through interacting and overlapping mechanisms, increasing vulnerability to cancer as well as other diseases. Furthermore, evidence

Table I. Summary of epidemiological risk factors for melanoma development

Category of risk factors	Risk factors	Effect estimates	Notes
UV radiation	Sun exposure	High intermittent/intentional vs. low: approximately 60% increased risk High continuous/occupational vs. low: no association	Intermittent: mainly increases risk of SSM Chronic: increases risk of LMM. Decrease risk on occasionally exposed sites
	Sunburns	History of sunburns vs. no history: double increased risk	Increases risk of SSM and LMM, not for NM
	Indoor tanning	Ever exposure vs. never: approximately 20% increased risk	Evidence of dose-response effect; mainly affects young women
	Sunscreen use	Some evidence that high SPF may decrease risk compared with no use	Sunscreen use may increase risk if used to prolong intentional sun exposure
Phenotype	Eye colour	Light colours vs. dark: approximately 50% increased risk	Increased risk of NM and SSM, not for LMM
	Hair colour	Red vs. dark: more than triple risk Blonde vs. dark: almost double risk Light-brown vs. dark: approximately 60% increased risk	
	Freckles	High-density vs. none: more than double risk	
	Skin colour/type	Fair vs. dark: more than double risk Phototype I vs. IV: more than double risk Phototype II vs. IV: approximately 80% increased risk Phototype III vs. IV: approximately 70% increased risk	Dose-response trend of risk according to level of skin type
	Common naevi	>100 vs. <15: almost 7-times higher risk	Total naevus count was associated mainly with intermittently sun-exposed sites (trunk and legs)
	Atypical naevi	≥5 vs. 0: more than 6-times higher risk	

LMM: lentigo maligna melanoma; NM: nodular melanoma; SPF: solar protection factor; SSM: superficial spreading melanoma.

from a large number of observational studies is generally consistent, showing a significant positive association with residing in areas with high ambient UVR through life, in early life, and even for short periods in early adult life (12). Lastly, several meta-analyses showed significant increases in melanoma risk and non-melanoma skin cancer (NMSC) with high sun exposure and indoor UV tanning (6, 13).

A study conducted in Canada estimated the current attributable and future avoidable burden of melanoma related to exposure to UVR and modifiable UVR risk behaviours. They estimated that 62.3% of melanomas in Canada were attributable to exposure to UVR and that 29.7% were attributable to the combination of sunburn (7.4%), sunbathing (17.8%), and indoor tanning (7.0%). They also concluded that a 50% reduction in modifiable UVR behaviour could avoid an estimated 11,980 melanoma cases by 2042 (14).

Recognizing the importance of establishing skin cancer prevention as a national priority, The Surgeon General's Call to Action to Prevent Skin Cancer in 2014 described prevention strategies and called on the community sectors to play a role in protecting Americans from UVR from the sun and artificial sources (15). Strategies that support goals related to lifestyle modifications to reduce the burden of melanoma included reducing the harms from indoor tanning, youth education approaches, and community-wide interventions focused on modifying healthy behaviours, including decreasing UVR exposure (16).

Sun exposure and sunburn

Measurements of individual sun exposure vary between studies, but are commonly classified as "intermittent" (short, intense sun exposure through activities such as sunbathing, outdoor recreation and holidays in sunny locations), "chronic" (continuous exposure, such as occupational sun exposure) and "total" (the sum of intermittent and chronic exposures).

The first systematic review and meta-analysis, summarizing 57 studies on sun exposure and melanoma, found a 60% significant increased risk of melanoma due to recreational sun exposure (summary relative risk (SRR) of CM for intermittent sun exposure of 1.61; 95% confidence intervals (95% CI) 1.31–1.99), while no association was suggested for chronic sun exposure (SRR: 0.95; 95% CI 0.87–1.04).

Sunburn is a biological response to intermittent exposure to the sun in poorly adapted skin and in multiple analyses a stronger predictor than intermittent exposure itself (13). The SRR for sunburns, which is the main indicator of sun exposure, was 2.03 (95% CI 1.73–2.37).

Despite the clear role of sunburn in increasing CM risk, a survey conducted in USA in 2013 (Youth Risk Behavior Survey (17)) highlighted that preventive practices are not regularly followed: most respondents (57%) reported having experienced 1 or more sunburns in the prior year.

Holman et al. (18) first proposed 2 distinct biological pathways by which CM might develop. One by way of intermittent sun exposure, acting primarily as a promoter of melanoma arising on pigmented naevi and mainly of the SSM type, and the other by way of a more continuous pattern of sun exposure, leading principally to lentigo maligna melanoma (LMM). In 1992, Green (19) proposed a theory of site-dependent susceptibility of melanocytes to malignant transformation. According to this hypothesis, people with a low propensity for melanocyte proliferation (small number of common naevi) need a continuous exposure to sunlight in order to drive the clonal expansion of initiated melanocytes. The melanomas arising from this pathway are more likely to be located on chronically sun-exposed body sites, to be of LMM subtype, and to occur in older patients with a history of solar damage and NMSC. On the other hand, people with a high propensity to melanocyte proliferation are more likely to develop melanomas on intermittently sun-exposed body sites, to be of SSM or nodular (NM) histological subtypes and to occur in patients with no history of sun damage or NMSC. Thus, both pathways include early initiation by sun exposure, but later proliferation is driven, in one pathway, by accumulation of sun exposure in non-naevus-prone people and, in the other pathway, by host factors in naevus-prone people (20). In the same study by Green (19), it was found that sun exposure and phenotypic characteristics were positively associated with all the main histological subtypes of melanoma. However, NM was not found to be associated with sunburns, in contrast to LMM and SSM. LMM was not found to be associated with freckling, light eye colour and hair colour, in contrast to NM and SSM, which were significantly associated with all 3.

This 2-pathway hypothesis for melanoma was confirmed and refined by many authors who observed an inverse correlation between number of naevi and clinical signs of sun damage (20–22), and identified a few genes differentially mutated in LMM vs. SSM and NM. Briefly, melanomas characterized by mutations in *BRAF*, *NRAS* and *TERT*, and approximately 80% of melanomas carry UVR signature mutations (C-T or CC-TT), along with other genes coding for downstream components of the tyrosine kinase RAS-BRAF signal transduction pathway (e.g. *CDKN2A* and *CDK4*), were suggested to be more frequent on intermittently exposed skin (23–25). Most of these are considered "passenger" mutations and not "driver" mutations; however, this high prevalence is clearly indicative of a role for UVR in melanomogenesis as is noted also by presence of somatic mutations in normal skin. BRAF mutations, which are present in approximately 40% of CM in people of European origin, are associated with characteristics of the naevus-associated pathway: younger age at diagnosis, occurrence on the

trunk, SSM type and absence of chronic sun damage in the skin (26). *TERT* promoter mutations (associated with UVR exposure) are present in approximately 43% of CM, occur more frequently at sun-exposed sites, and tend to co-occur with *BRAF* alterations (27).

The melanocortin-1 receptor (MC1R), a pigmentation gene associated with melanoma risk (28–30), is involved in the same signalling pathway and has been found to interact positively with *BRAF* and *CDKN2A* in the aetiology of melanoma occurring on usually unexposed skin (31, 32). On the other hand, p53-positive melanomas were usually associated with features of chronical sun exposure (33), supporting the hypothesis that different molecular pathways can lead to melanoma development (34, 35).

Looking at the distribution by body site of different histological types of CM, SSM is the more frequent type on the trunk in men and legs in women, while LMM is more frequent on the face and neck (36). It is likely that melanocytes on different body sites have different characteristic in terms of differentiation: atypical naevi are more commonly found on the trunk, whilst they are very rare on the face. Similarly, intradermal naevi, which are mature melanocytic lesions, are commonly found on the face, but are much rarer on limbs. It is possible that during embryogenesis, melanocytes have different properties according to head and neck, trunk and limb locations, because of migration to different body sites, and this is likely to be influenced by key developmental genes.

The complex interplay between sun exposure, pigmentary characteristics and melanocytic naevi was investigated in a meta-analysis including 24 studies for a total of 16,180 cases of melanoma (37). Considering each measure of sun exposure (intermittent, chronic, sunburns and actinic damage) SRRs for CM risk were 1.31 (95% CI 0.94–1.81) and 1.77 (95% CI 1.30–2.41) respectively for occasionally vs. usually sun-exposed body sites. Chronic sun exposure was weakly, but significantly, negatively associated with CM on occasionally sun-exposed sites. Overall, these results suggest that sun exposure is associated with CM on all body sites (except for mucosal), but in particular with CM on head and neck in older individuals.

The apparently protective effect of chronic sun exposure on CM on occasionally exposed sites and, at most, weakly causal effect on usually exposed sites is puzzling. Enhanced melanin production and melanosome delivery to keratinocytes (38) and increased thickness of the top layers of the epidermis due to continuing sun exposure may be a possible explanation; however, they would not be expected to reduce incidence to a level below that present in the absence of sun exposure. Other possible explanations are the lower melanin content, sunburn, and lower DNA repair capacity of intermittently exposed skin compared with habitually exposed skin. Sunburn can lead to cell proliferation in replacing apoptotic cells, and habitually exposed skin may have somewhat thicker stratum corneum, and thus models protection from tanning, and some upregulation of DNA repair pathways exemplified by fewer thymine dimers after repeated low exposure (39–41). However, it is important to note that the reference category for calculating RRs in epidemiological studies of melanoma and sun exposure is "low sun exposure", not "no sun exposure".

Migrant studies provide convincing evidence that childhood and adolescence are critical periods for the development of melanoma in adulthood. Indeed, it was found that adults were at increased risk of melanoma if they spent their childhood in sunny locations or if they received above average intermittent sun exposure during vacations and/or recreation. In an Australian case-control study published in 1984 (42), earlier age at arrival of immigrants to Australia was a melanoma risk predictor with little residual effect of duration of residence. Specifically, children who migrate from a less sunny country before the age of 10 years had similar incidence rates of native-born Australians, while the estimated incidence in those arriving after age 15 years was approximately a quarter of the native-born rates. Similarly, in a European case-control study (43), age <10 years old at arrival in a sunny location of residence (i.e. the Mediterranean, subtropics, or tropics) conferred a 4-fold increased risk of developing melanoma.

Studies investigating the role of residence in childhood provide further evidence that sun exposure in childhood and adolescence is more closely associated with melanoma risk than adult sun exposure. A case-control study nested in the Nurses' Health Study cohort (44) showed an increased melanoma risk in women whose residence during the ages 15–20 years was more equatorial in latitude, whereas latitude of residence after 30 years of age was not significantly related to melanoma risk. Finally, in another study of 474 cases and 926 controls, those who lived near the coast before the age of 15 years had an increased risk of melanoma compared with those who never lived far away from the coast (odds ratio (OR)=1.6; 95% CI 1.0–2.6) (45).

Sunbeds and indoor tanning

Sunbeds and sunlamps used for tanning purposes represent the major source of deliberate exposure to UVR. Indoor UVR tanning has been widely practiced in Northern Europe and the USA since the 1980s and this trend has gained popularity in sunnier countries, such as Australia. Modern indoor UVR tanning equipment emits mainly in the UVA range, but a fraction (<5%) of this spectrum is in the UVB range, which is needed to induce a deep, long-lasting tan. Both UVA and UVB radiation cause DNA damage and immunosuppression (6, 46–48). Moreover, powerful UVR tanning units may be 10–15 times

stronger than the midday sun in the Mediterranean Sea area, and repeated exposure to large amounts of UVA, delivered to the skin in relatively short periods (typically 10–20 min) constitutes a new experience for human beings. There are several types and denominations of tanning devices (sunbeds, tanning beds/booths/canopies, and solarium): the term "sunbeds" is commonly used to generally define them all.

In 2012, an updated meta-analysis (6) summarized 27 epidemiological studies that quantified risk of CM associated with artificial UVR tanning. The SRR estimate for "ever" vs. "never use" of indoor tanning was 1.20 (95% CI 1.08–1.34) and the risk was independent of skin sensitivity or population and a dose-response effect was evident. When the analysis was restricted to 18 studies with a population-based sampling of cases and controls, the SRR increased to 1.25 (95% CI 1.09–1.43). The analysis restricted to exposure at a young age in 13 studies showed consistent results. For those starting first exposure to sunbeds before the age of 35 years, and increased risk of 1.59 (95% CI 1.36–1.85) was estimated with no significant between-study heterogeneity and no indication of publication bias. Studies on exposure to indoor tanning and NMSC showed a significantly increased risk of basal cell carcinoma (SRR=1.29; 95% CI 1.08–1.53) and of squamous cell carcinoma (SCC) (SRR=1.67; 95% CI 1.29–2.17). Based on the results of a meta-analysis published in 2009, it could be estimated that of 63,942 new CM cases diagnosed each year in Western Europe, 3,438 (5.4%) could be caused by sunbed use. Women represented the majority of this burden, with 2,341 estimated cases (6.9% of all melanoma cases in women) induced by sunbed use; while the figure for men was 1,096 cases annually (3.7% of all cases in men). Taking a melanoma incidence to mortality ratio of 3.7 for European men and 4.7 for European women in EU15 countries, approximately 498 women and 296 men would die each year from a melanoma caused by artificial UVR tanning.

In 2009, Hirst et al. (46) estimated the numbers of potential skin cancers that could be prevented through regulation of solarium and the associated cost-savings to the Federal Government in Australia (for each 100,000 people: 18–31 melanomas, 200–251 SCCs and $AU 256,054 associated costs).

In a paper published the following year, Hery et al. (49) noted a sharp increase in melanoma incidence among young women in Iceland, which began after 1990 with a peak in 2000. At the same time, the prevalence of sunbeds in Iceland rapidly increased, from 1979 to 1988, suggesting a possible link between the 2 observed trends. However, another possible explanation could be the increase in melanoma screening, which occurred all over Europe in the 1990s. Authors also observed a decline in melanoma rates among women after 2001, following a reduction in prevalence of sunbeds. However, it should be taken into account that the lag time between exposure and melanoma onset is quite long and the decline in melanoma incidence is unlikely to be due to the reduced use of sunbeds in the early 2000s.

Some authors hypothesized that indoor tanning could act as a protective factor for melanoma risk, by preventing sunburns. Recently 2 publications expressed scepticism about the carcinogenicity of indoor tanning (50, 51). Some authors have used the lack of randomized clinical trials (which would be unethical) to imply that the relationship between sunbed use and melanoma is not causal. Suppa & Gandini (52) recently showed, however, that the large amount of data coming from observational studies in fact provides enough information to infer that sunbed use does cause melanoma: they were able to demonstrate the applicability of all epidemiological criteria for causality to the relationship between sunbed use and melanoma. They found that recent studies have reinforced previous knowledge about the detrimental effects of first sunbed exposure at young age, especially in women (53, 54). In fact, new insights on sunbed use have emerged, such as its relevance for the development of additional primary melanomas (55), its association with melanoma of the lower limbs (most common in women) (56) and its correlation with other melanoma risk factors, including high naevus count, atypical naevi and sun damage (57).

The large body of evidence prompted both the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) of the European Commission (58) and the WHO (59) to state that there is no safe limit for exposure to UV radiation from sunbeds.

Interestingly, an Italian survey on 4,703 subjects after the ban on sunbed use before 18 years of age estimated the overall prevalence of sunbed use to be as high as 20%, with higher proportion of female, young and highly-educated users (60). Moreover, participants at high risk of melanoma were those who used sunbeds more frequently: subjects with freckles and with red hair had the higher odds of using sunbeds than subjects without freckles and with dark hair (OR were, respectively, 1.89; 95% CI 1.27–2.80 and 3.92; 95% CI 1.91–8.06). Another Italian survey on 3,089 students highlighted the important role of parents on indoor tanning practices of children (61). Indeed, students who attended a targeted educational intervention were more aware that sunbed use cannot prevent sunburns ($p = 0.03$) than those who did not attend; however, sunbed use by parents influenced the desire to use a sunbed more than participation in the educational intervention ($p < 0.0001$).

OTHER EPIDEMIOLOGICAL RISK FACTORS RELATED TO ULTRAVIOLET RADIATION

The association of sun exposure with melanoma risk is influenced by other factors such as phenotype.

Phenotypic characteristics

Pigmentation characteristics, such as skin colour, hair and eye colour, and freckles are well-established host risk factors for melanoma.

A previous meta-analysis found SRR for blue, green and hazel eye colour compared with dark eye colour of 1.47 (95% CI 1.28–1.69), 1.61 (95% CI 1.06–2.45) and 1.52 (95% CI 1.26–1.83), respectively (62). According to hair colour, the highest association with melanoma was found for red-haired individuals, who have a more than tripled risk of melanoma compared with dark-haired subjects (SRR; 95% CI 3.64; 2.56–5.37). Blond-haired and light brown-haired subjects are, as well as increased melanoma risk, compared with dark-haired subjects (SRR; 1.96; 95% CI 1.41–2.74 and 1.62; 95% CI 1.11–2.34, respectively). Looking at skin colour, light-pigmented subjects had a doubled risk of melanoma compared with darker pigmented subjects (SRR 2.06; 95% CI 1.68–2.52). This result was in agreement with the analysis of skin phototype (defined according to the Fitzpatrick classification as indicator of skin sensitivity to sun): indeed, all 3 lighter skin phototypes I, II and III increased melanoma risk compared with skin phototype IV, with a trend in the calculated SRR, that were, respectively, 2.09 (95% CI 1.67–2.58), 1.84 (95% CI 1.43–2.36) and 1.77 (95% CI 1.23–2.56). Finally, high density of freckles was associated with a significantly doubled risk of melanoma: SRR 2.10 (95% CI 1.80–2.45).

In a recently published population-based prospective study including 38,854 subjects, melanoma risk was assessed in association with pigmentation characteristics and other phenotypes, and additive interactions were explored. During a mean follow-up of 3.5 years, 642 (1.5%) participants developed melanoma. Inability to tan was a recognized risk factor (no tan vs. deep tan hazard ratio (HR) 3.11 (95% CI 1.50–6.43)), while propensity to sunburn was not associated with melanoma after tanning inability was adjusted for (63). The highest population attributable fractions (PAFs), helpful in estimating the burden of disease occurring within sub-groups of a population, were observed for skin phototypes I/II (0.27, 95% CI 0.21–0.31), presence of freckles (0.23, 95% CI 0.19–0.26) and blonde hair (0.23, 95% CI 0.20–0.26). For eye colour, the PAF for blue/blue-grey eye colour was higher than for green/grey/hazel eye colour (0.18 vs. 0.13), while the PAF associated with red hair colour was 0.10 (95% CI 0.09–0.11) compared with 0.23 for blonde and 0.15 for light brown hair colour.

Common and atypical naevi

High number of common naevi and the presence of atypical naevi are major risk factors for CM. According to a previous meta-analysis including 10,499 cases and 14,256 controls (64), the presence of more than 100 common naevi was associated with almost 7-times higher risk of melanoma compared with less than 15 common naevi: the SRR was 6.89 (95% CI 4.63–10.25). In the same meta-analysis, the SRR for the presence of at least 5 atypical naevi vs. no atypical naevi was 6.36 (95% CI 3.80–10.33). It was estimated that 42% of melanomas are attributable to having ≥25 common naevi, corresponding to 121,800 patients newly diagnosed with melanoma from an annual worldwide total of 290,000 new cases. Moreover, approximately 25% of melanoma cases are attributable to the presence of one or more atypical naevi, corresponding to an estimated number of 70,000 new cases in 2018. High total body naevus counts (≥50 common naevi) account for approximately 27% of melanoma cases, whereas individuals with few common naevi (0–10) account for only 4% of melanoma cases.

Naevi yield similar relative risks in the UK and Australia, suggesting that genetic factors are important despite different environmental exposure. Multiple naevi might also be an indicator of excessive sun exposure, and thus be associated with an increased risk of CM. A study of Australian children found that increased sun exposure in childhood was significantly associated with an increased number of naevi (65). A separate study of more than 11,000 European children found that sunburns and holidays in the south were significantly associated with high naevus counts and the occurrence of atypical naevi (66). However, it is likely that sun exposure influences smaller naevi on chronically sun-exposed sites and to a lesser extent, larger atypical lesions on intermittently exposed sites, which have more probably a genetic basis (67, 68).

Total naevus count was found to be more strongly associated with CM on intermittently sun-exposed skin (i.e. trunk and legs) than CM on chronically exposed skin (i.e. the head/neck and arms) (37). This may be related to BRAF somatic mutations, which are also more common in CM originating on trunk and legs compared with the head and neck.

A previous prospective cohort study conducted in Australia (64) found that the characteristic most strongly associated with invasive melanoma was self-reported naevus density at age 21 years [many vs. no moles HR 4.91 (95% CI 2.81–8.55)].

Looking at melanoma-related deaths in USA, a recently published prospective study using data from the Nurses' Health Study (n=77,288 women) and Health Professionals Follow-up Study (n=32,455 men) investigated cutaneous naevi and risk of melanoma death (69). During 26 years of follow-up, 2,452 melanoma cases were histologically confirmed and 196 patients died from melanoma. An increased number of naevi was associated with melanoma death: HR for ≥3 naevi compared with no naevi was 2.49 (95% CI 1.50–4.12) for women and 3.97 (95% CI 2.54–6.22) for men. Among melanoma cases, increased number of naevi was associated with melanoma death in men, but not in women. Similarly, the number of naevi was positively associated with

Breslow thickness in men only (p-value for trend 0.01). A possible explanation is that male patients with melanoma and high naevus counts might tend to have their melanomas diagnoses at later stages or may be related to different prevalence of melanoma body sites in men and women. Indeed, melanoma more frequently occurred in men at the head and neck or trunk (sites associated with poorer survival), while it occurred more frequently at the extremities in women (69). The observed differential associations by sex might also reflect other aetiological mechanisms: for instance, the number of naevi had been identified as a phenotypic marker of plasma sex hormone levels, with more naevi associated with higher levels of oestradiol and testosterone (70).

SUNSCREEN USE

Studies have been inconclusive regarding sunscreen use and the development of naevi among children, with a single randomized trial showing evidence of benefit (71), while other studies have shown a positive association between sunscreen use and naevus prevalence (66, 72–74). An Italian large observational study on 1,512 children and adolescents found that sunscreen users were more likely to develop naevi compared with non-users. Moreover, unlike other paediatric analyses (75), a higher frequency of daily application of sunscreen was associated with a higher naevus count, suggesting that this association cannot be due only to residual confounding. On the other hand the use of high sun protection factor (SPF) (>30) sunscreens exclusively, compared with the use of sunscreens with SPF≤30, adequately protected skin during sun exposure and significantly reduced naevus burden. These results were confirmed by subsequent studies (76–78).

The possible explanation of these findings may be interpreted in the light of 2 considerations. First, children who apply more sunscreen are probably fair-skinned subjects with freckles who tend to be burnt by the sun easily and, consequently, lower skin-phototypes have a greater tendency to develop sunburn and naevi. Secondly, the anti-erythematous effect and a false sense of protection against sunburn conferred by frequent application of sunscreen may lead children to spend more time in the sun and to expose themselves in the middle of the day when ultraviolet rays are stronger (79).

Sunscreen use is recommended for sun protection in addition to clothing and shade (80). Sunscreen can decrease the risk of sunburn and SCC (82).

Meta-analyses of observational studies showed no effect of sunscreens on melanoma risk, but the results of the studies are difficult to interpret due to lack of adjustment for potential confounders (82).

The only randomized controlled trial showed a decreased melanoma risk of subjects who used sunscreen daily compared with discretionary sunscreen use (78). However, this trial was conducted among subjects who lived in Australia, a country with very high ambient solar radiation and high awareness of skin cancer.

Recently, the Norwegian Women and Cancer Study (83), a prospective population-based study of 143,844 women and 722 cases of melanoma, showed that sunscreen users reported significantly more sunburns and sunbathing vacations and were more likely to use indoor tanning devices. However, SPF ≥15 sunscreen use was associated with significantly decreased melanoma risk compared with SPF <15 use. The estimated decrease in melanoma (PAF) with general use of SPF ≥15 sunscreens by women age 40–75 years was 18% (95% CI 4–30%).

Primary skin cancer prevention behaviours, focusing on reducing the amount of UVR reaching the skin, include covering sun-exposed skin, wearing a hat and sunglasses, and sunscreen use. There is no high-quality experimental evidence on the efficacy of sunscreen to prevent melanoma; however it is important that patients and consumers do not stop protecting their skin until better-quality evidence emerges. The important message is that sunscreen should not be an excuse to prolong intentional sun exposure.

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Centenary theme section: SKIN MALIGNANCIES

REVIEW ARTICLE

It's Not All Sunshine: Non-sun-related Melanoma Risk-factors

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There is increasing evidence that the behaviour of naevi and melanoma is under significant genetic and/or epigenetic control. Melanoma tumours behaves similarly all over the world. Many genes have now been implicated in melanoma risk and naevi number. Embryogenesis has also been important in the discovery of links between several neurological diseases and melanoma susceptibility. Telomere biology, which regulates cell senescence, is increasingly relevant in melanoma. Melanoma is often found in the context of family cancer syndromes and the identification of these families is important as screening for cancer will save lives. Melanoma is also one of the most immunogenic cancer as the behaviour of naevi and melanoma differ in patients with vitiligo or eczema. The search for non-sun-related melanoma risk factors should continue as it is likely to lead to important discoveries which will, in turn, have an impact on therapeutic targets for this tumour.

Key words: telomere; naevi; vitamin D; family cancer syndromes; body mass index.

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SIGNIFICANCE

Many risk factors for melanoma are non-UV-related and progress in the last 20 years have been instrumental in discovering melanoma genes which are involved in telomere biology, naevi number, pigmentation, body composition, energy expenditure, neural and melanocyte differentiation. Melanoma behaves in a very similar way all over the world in all Caucasian populations and many host factors are under tight genetic control. Research in these areas is important as it sheds new light on genetic and epigenetic factors which are often set early on in life and less likely to be influenced by sun exposure in adulthood. It is also unravelling pathways which could be exploited for future therapies as public health campaigns have, so far, not been very effective. Perhaps, the role of sun exposure in melanoma has been over-estimated in the past as, like all cancers, melanoma is a very complex tumour so addressing environmental exposure cannot be the only focus of our efforts.

In this review, the importance of non-sun-related melanoma risk factors are presented looking at telomere biology, genetics, gender differences, body mass index, body sites, naevi biology, immune-related factors and links to neurological disorders.

TELOMERE AND MELANOMA

Telomeres are strand of non-coding DNA capping the end of chromosomes protecting them from decay. They are having important and complex roles in cell replication and senescence. Protecting against cancer formation is achieved by silencing telomerase which leads to telomere erosion with age. The speed of telomere attrition is under the influence of both genetic and environmental factors. Chronic illnesses and obesity have been associated with shorter telomeres. On the other hand, cancer is usually linked to longer telomeres (1). In 2007, the first report of a link between melanoma susceptibility and telomere biology was suspected with a positive association observed between high number of naevi, the strongest risk factor for melanoma, and circulating white cell telomere length (2). In 2009, case-control studies supported this finding in melanoma case control studies (3). A few years later, a very large melanoma pedigree with no previously known germline mutation, was found to have a germline mutation in the promoter of the *TERT* gene, a telomere gene (4). Mutations in the promoter of the TERT gene were then investigated at the somatic level and were found to be common in melanoma tumours (5). The same year, 11 SNPs in genes predicting white cell telomere length were published (6). Using the same 11 SNPs, genetic scores were created to assess their effects in a large melanoma case control study in the UK. These combined SNPs scores predicting telomere length were confirmed to be predictive of melanoma risk (7).

The associations between TERT promoter mutations, telomerase activity and telomere length are, however, quite complex. It has been shown recently that different SNPs within the TERT promoter have different effects on TERT expression and telomere length despite all being associated with an increased risk of melanoma (8, 9). This implies that the risk of melanoma is not solely explained by elongation of telomeres in some of these families. In rare melanoma families, POT1, another telomere gene, has been identified over the last few years (10). Recent genome wide scan analyses (GWAS) on melanoma and/or naevi number have also identified

further telomere genes (11, 12). There are more telomere genes linked to melanoma susceptibility compared to naevus count highlighting the fact telomere genes do not always drive melanoma risk via an excess of naevi (11). Mutations in telomere genes also raise the risk of many types of cancers so the documentation of all cancers in first- and second-degree relatives of melanoma patients is important. Glioma, neuroblastoma, lung cancer and melanoma are more commonly reported in rare families with telomere mutations but many cancer types can be found (13).

The fact that long telomeres are associated with a susceptibility to melanoma may be behind the observation that individuals within melanoma families with high number of naevi, have reduced cutaneous photoageing. The delayed senescence in melanocytes reflected by the presence of the atypical mole syndrome phenotype is likely to be seen in other cell types such as fibroblasts and keratinocytes (**Fig. 1**). The background for squamous cell carcinoma is, on the contrary, a very photoaged skin. SCC is more likely to be associated with shorter telomeres contrary to melanoma (14). So, by looking at skin phenotypes, short or long telomeres may have opposite effects on signs of cutaneous ageing and, in turn, on specific skin cancer risk (15). This is supported by the negative association between solar keratoses and naevi number both risk factors for melanoma despite adjusting for age (16). This dichotomy has been reported a long time ago via phenotypic studies and is known as the dual pathway to melanoma (17).

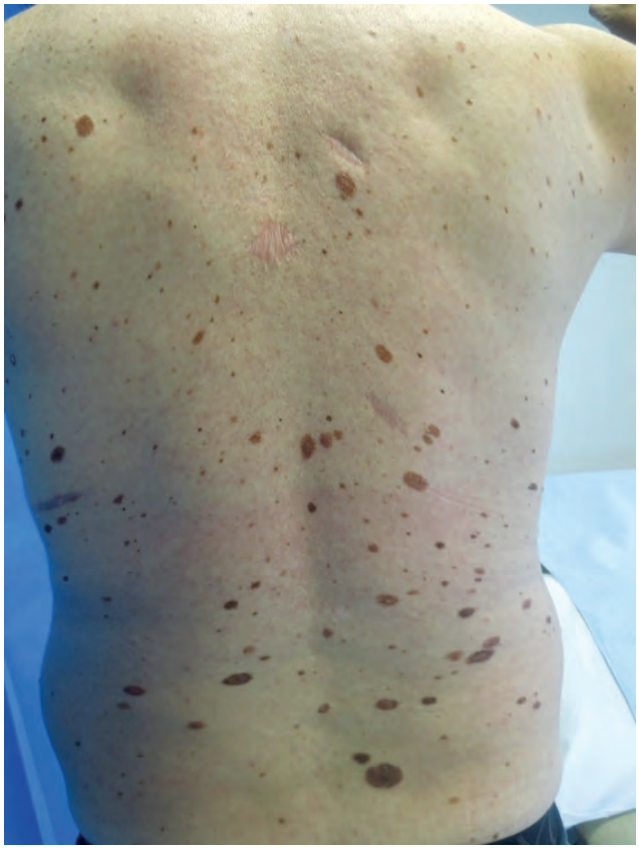

Fig. 1. Male with the atypical mole syndrome phenotype with previous melanoma primaries. Presence of larger atypical naevi towards the lower back.

Melanoma survival is also affected by TERT promoter mutations with worst survival for those carrying different types of mutations. This contrasts with a study published by Ribero et al. (18) showing that large number of naevi (hence predicted longer telomeres) confers a survival advantage in melanoma even in patients with positive sentinel node. However, as mention above, not all telomere gene mutations have the same effect on telomere length so this may explain opposite effects. Telomere biology is also important for potential therapeutic targets: RAS mutated melanomas represent 25% of melanoma tumours and have not had, as yet, effective gene targeted treatments. These RAS mutated melanomas appear to have a dependency on TERT which could be exploited for slowing melanoma growth (19, 20).

The balance between long telomeres leading to an increased risk of cancer versus short telomeres leading to premature ageing with frailty needs to be fine-tuned as the extreme spectrums of long and short telomere syndromes show that belonging to either of these extreme group is not advantageous (1). Most melanoma patients survive their disease and the beneficial impact of longer telomeres is likely to be apparent in old age with reduced senescence in many cell types. It could therefore be speculated that genes associated with melanoma susceptibility may have a survival advantage and have therefore remained common in Caucasian populations.

MELANOMA AND FAMILY CANCER SYNDROMES

Melanoma is more common in cancer prone families as discussed above. However, many other non-telomere genes can be implicated in cancer susceptibility within these families. P16 or CDKN2A was one of the first melanoma gene discovered more than 20 years ago and mutations in this gene lead to an increased risk of melanoma, pancreatic cancer, lung cancer and many other tumours (21). The recruitment of melanoma families for genetic studies over the last 20 years mainly included families with multiple melanomas so family cancer syndromes were excluded. It is, however, well known that some melanoma families may present with many different cancer primaries. These family cancer syndromes are now being studied as well with collaborations from many countries via the GENOMEL consortium (www.genomel.org) with many new genes shared with other cancers being discovered. This is why melanoma germline genetic panel have become more comprehensive. The risk of melanoma in these families is higher in Australia compared to the UK so the penetrance of rare high penetrance genes such as

p16/CDKN2A is affected, in part, by sun exposure. Screening bias is also at play in Australia with many borderline melanomas excised in Australia compared to Europe in view of the active skin surveillance there. Many individuals with p16/CDKN2A mutations have the atypical mole syndrome phenotype usually evident by late teens. However, this phenotype is not always found in mutant individuals so using the naevus phenotype to select family members at risk is not reliable (22). Individuals within these families have reduced senescence in many cell types and not only melanocytes and therefore patients with high number of naevi have reduced photoageing, higher bone mineral density and better cognitive functions with age (23, 24).

Families with BPA1 mutations may present with clinically and histologically recognisable lesions typical of this syndrome called BAPOMAs. These families also have an increased risk of skin and eye melanoma, kidney cancer, mesothelioma and breast cancer (25). BRCA1 and BRCA2 families, apart from the high risk of breast and ovarian cancer, also have an increased but smaller risk of both skin and eye melanoma. However, eye melanoma is a rare tumour and there is no need to offer screening for this as retinal photography is now being offered by many opticians. Melanoma can also occur in rare retinoblastoma families because of the link between the Rb gene and the CDKN2A/CDK4 pathway. Neurofibromatosis families are at risk of melanoma because of the role of NF1 in melanocyte differentiation and growth. This syndrome is part of a group of diseases called Rasopathies where melanoma is more commonly seen such as Noonan syndrome and Leopard syndrome (26). MITF mutations predispose to melanoma and kidney cancer (27, 28). The *MITF* gene is a crucial gene in melanocyte differentiation.

Many cancer genetic clinics now include p16/CDKN2A and CDK4 in their panels as well *MITF*, *BAP1* and several telomere genes. However, it is likely that very soon all melanoma families will undergo much more comprehensive gene panel for mutation screening as they are becoming cheaper. These panels should not be limited to melanoma genes only. This will be beneficial for these families as the identification genes linked to other cancers such as colon, kidney, breast and ovary, for example, can be addressed with specific screening recommendations for the family and will save lives. Genes associated with melanoma in the context of family cancer syndromes are summarised in **Table I**.

Table I. Genes and family cancer syndromes linked to melanoma

Genes	Cancers often clustering in the family with melanoma
CDKN2A/p16 and CDK4	Pancreas, brain and many other tumours
BRAC2	Breast, prostate, pancreas, eye melanoma
POT1	Brain, colon, cardiac angiosarcoma, and other cancers
MITF	Kidney
RB1	Retinoblastoma, osteosarcoma, soft tissue sarcoma
P53	Breast, osteosarcoma, leukaemia and other cancers (Li Fraumeni syndrome)
BAP1	Kidney, mesothelioma, eye melanoma, brain, breast
PTEN	Breast, colon, uterus, hamartomas (Cowden syndrome)
CHEK2	Breast, colon, prostate

BODY MASS INDEX AND MELANOMA

Melanoma risk, like all cancers, is related to body mass index (BMI) (29). Whilst it was thought to be mainly driven by increased weight, the relationship is mainly driven by height. Naevus count is also related to height rather than weight and it is speculated that growth factors are important in melanoma susceptibility. One possible explanation for this observation is telomere biology. Telomere length and cancer risk are also positively associated with height (24, 29). High BMI or obesity is, on the contrary, inversely, correlated with telomere length (30). Another observation is that patient with the atypical mole syndrome phenotype are usually taller than average but not significantly overweight or underweight with strong muscular mass. There is therefore some interesting links between melanoma susceptibility and body composition and growth. Bone mineral density is also correlated with number of naevi and this remains significant despite adjusting for telomere length, so bone senescence is also delayed in these patients as discussed above (24).

Another paradox in melanoma is the lack of cachexia in advanced melanoma. Compared to other cancers, melanoma patients in stage 4 of the disease present with weight loss very late in the evolution of their metastatic disease. There is also some evidence that melanoma patients treated with immunotherapy have different treatment responses according to fat distribution with better responses in patients with higher subcutaneous fat and strong muscle mass but not with high fat mass and low muscle mass (31).

Insulin metabolism and energy expenditure may also have a role in melanoma. However, a recent study showed that levels of IGF1 were not linked to an increased risk (32). The melanocyte-stimulating hormone (MSH) pathway is also relevant as, apart from the *MC1R* gene controlling pigmentation and other immune-related factors, other genes in the MSH pathway such as MC4R gene are also important in energy expenditure. In animal models, weight is related to colour coat pigmentation (33). The *FTO* gene, also linked to obesity, is reported in melanoma GWAS (33). However, there is some evidence that the *FTO* gene may not act via its effects on obesity as SNPs involved in melanoma differ from those reported in high BMI (34, 35). The effects may, in fact, be mediated by pathways shared between the *FTO* gene and telomere genes (36).

VITAMIN D AND MELANOMA

Vitamin D has been found to have a significant role in melanoma survival as low levels of serum vitamin D are a negative prognostic indicator in melanoma (37, 38). On the contrary, patients with high vitamin D levels have thinner melanoma tumours but also have higher number of naevi. The relationship between high number of naevi and higher vitamin D levels is complex but despite adjusting for age and skin type, the association between high number of naevi and high vitamin D remains (39). Further adjustment for telomere length (as telomere length affects vitamin D levels as well), decreases the magnitude of the association but it remains significant. This shows that whilst telomere biology is important in the relationship between melanoma and vitamin D metabolism, other factors are at play. This has implications for public health as patients are advised to avoid sun exposure after a melanoma diagnosis and this may affect their survival. This is supported by a study showing that sun exposure after diagnosis of melanoma was protective in terms of relapse in Italy (40).

MELANOMA AND GENDER

Melanoma behaves differently in women and men both in terms of body sites and survival. It is well established that melanoma in females are more common on the legs compared to males and the reverse is true for males where melanoma is commonest on the trunk. This difference in body sites is observed all over the world and sun exposure levels do not affect it. Furthermore, the distribution of naevi in girls versus boys is already different earlier on in life and mirrors the distribution of melanoma in adults: boys have more naevi on the torso and girls have more naevi on the limbs, especially the legs. There is therefore some sex specific melanocyte migration which does not appear to be related to sun exposure. A recent study showed that genes/loci already known to predict naevi numbers such as IRF4, DOCK8, MTAP, 9q31.2, KITLG and PLA2G6 have different effects on naevi numbers on the torso versus limbs versus head (41). It is likely that epigenetic effects with X inactivation in females explain, in part, some of these sex differences for naevi and melanoma. Females with Turner syndrome with a XO genotype have large number of naevi on the limbs and are also more prone to melanoma and brain tumours (42).

TYPES OF NAEVI AND BODY SITES

It is evident for dermatologists that some type of naevi have a predilection for specific body sites. Intradermal naevi are more common on the face and rarely seen on distal limbs. Atypical naevi are more common on the central body and rarer on distal limbs and extremely rare on the face. This again most probably relates to specific genetic signals for melanocyte migration and growth at different body sites. Unfortunately, not many studies counting naevi have, so far, differentiated between different types of naevi (intradermal versus compound versus junctional). One twin study in Australia, has collected clinical subtypes of naevi. They have shown that SNPs in the *IRF4* gene, which was the strongest signal for their Australian naevi GWAS based on more than 1,800 adolescent twins, was having opposite effects on flat versus raised naevi. Gene may also have divergent effects according to age when comparing adolescent twins to adult twins (mean age 40–50 years) (11). The different gene effect size according to age groups shows that having very large sample size for GWAS with wide age ranges can identify differential gene expression with age. IRF4 is also a gene linked to freckling, fair skin and tanning ability which shows that skin pigmentation is tightly linked to types and number of naevi (43, 44). It is well known that the atypical mole syndrome with many junctional and intradermal naevi is rare in dark skin phototypes so pigmentation genes not only govern naevi colour, but they also have an effect on size, numbers and clinical subtypes of naevi. Visconti et al. (41) have confirmed in a recent study that body site specific genetic effects exist in females for quite a few known naevi genes/loci such as IRF4, DOCK8, MTAP, 9q31.2, KITLG and PLA2G6. In this large collaborative study, based on many cohorts, the analyses of 3,000 UK twins showed that the heritability of naevus number in females (assessed by comparing MZ to DZ female twin pairs) was the highest on the legs (69%) compared to torso (26%). Leg is also the body site where females have more naevi and a predilection for melanoma, so it is interesting to find that this site is under the strongest genetic control for naevi number.

In high risk melanoma families with the atypical mole syndrome phenotype, it is not uncommon to see large atypical naevi in the parietal area of the scalp and rarely at any other sites on the scalp (**Fig. 2**). In embryogenesis, the head development goes through successive phases which may explain the specific behaviour of naevi on specific part of the head and neck. These scalp naevi often are the first ones to appear in children in high-risk families. It is also observed that in patients with the atypical mole syndrome phenotype, atypical naevi increase in size from the upper back to the lower back especially in males which, again, is likely to be governed by genes differentially expressed at different body sites. However, what is puzzling is that many genes involved in melanocyte migration and differentiation in embryogenesis are not found in melanoma/naevi GWAS. It is likely that these early melanocyte genes interact with other gene pathways. One example for this, is the MITF gene, a very important gene early on in embryogenesis for melanoblast/melanocyte migration. Many melanoma

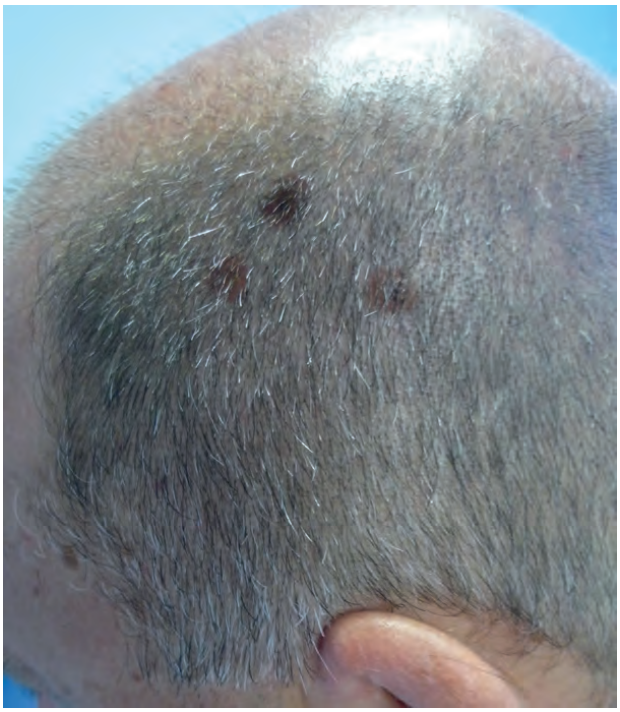

Fig. 2. Large atypical naevi in the parietal scalp. These are usually very stable as scalp melanoma is very rare. They are often found in patients with the atypical mole syndrome and just need monitoring and not prophylactic excision.

genes have MITF binding sites so the discovery of new melanoma genes will need to look at all these gene-gene interactions (44).

NAEVI AND IMMUNOLOGICAL FACTORS

Naevi distribution have an inverse distribution to vitiligo. Vitiligo develops often in folds such as axillae, groins but also on the face around the eyes and mouth as well as on the hands and feet (45). Melanoma and naevi are very rarely found in vitiligo predilection sites. Vitiligo patients also have a reduced risk of melanoma. Melanoma is one of the most immunogenic cancer. It is therefore possible that immunological signals which are inhibitory for melanocyte growth explain this inverse body distribution between vitiligo and naevi/melanoma. Quite of few of the vitiligo genes are shared with melanoma and most of these are related to skin pigmentation. The same SNPs have been reported but have opposite effects in vitiligo versus melanoma which is interesting as it supports the protective effect of vitiligo on melanoma risk. However, how do the same SNPs do offer protection from melanoma in vitiligo patients is unclear (46). Immune-related genes amongst others are likely to affect these divergent associations as CTLA4, a target for the most successful melanoma therapy, is also a vitiligo gene.

Another observation is the lower number of naevi and lower incidence of melanoma in eczema cohorts and this, again, supports the fact that immunological signals in atopic patients may have an inhibitory effect on melanocytes in the skin (47).

Naevi disappear with age, especially junctional and compound naevi and the mechanisms for this process is not fully understood but senescence via genes such as p16, p21 and p53 as well as telomere genes and immune surveillance are likely to all play a role (2, 48). Patients with the atypical mole syndrome phenotype, especially within high risk families, are more likely to present with halo naevi phenomenon than controls. They also show a delayed senescence of naevi with age with large number of naevi persisting well after the age of 50 years. The presence of multiple junctional and atypical naevi after the age of 50 is a reliable sign for dermatologists that an individual is at an increased risk of melanoma.

MELANOMA AND NEUROLOGICAL DISEASES

Naevi originates from the neural crest and it has long been observed that melanoma and Parkinson disease can cluster in some families. Many melanoma genes were later found to be Parkinson genes such as *PLA2G6, BAP1*, *DCC*, *ERBB4*, *KIT, MAPK2*, *MITF*, *PTEN*, and *TP53* (49). Pigmentation may also be important in the link between Parkinson and melanoma as fair skin is more prevalent in Parkinson cohorts and, so far, the *MC1R* gene has been implicated (50).

Charcot Marie Tooth and amyotrophic lateral sclerosis (ALS) are also neurological diseases linked to melanoma (51). The association between these neural diseases and cancer risk is puzzling as Parkinson disease and ALS have, in fact, an overall reduced risk of cancer so the link to melanoma may be because of the neural connection (51).

The NF1 gene is an important prognostic factor for melanoma at the somatic level and patients with neurofibromatosis have an increased risk of melanoma (52). NF1 positive tumours are more likely to be found in the elderly and often have a desmoplastic histology (52). Neurofibromatosis, is part of a group of diseases called Rasopathies such as Noonan syndrome, Leopard syndrome and Leguis syndromes. All these disorders are characterised by the activation of the MAP kinase pathway which is highly relevant in melanoma (26).

SUMMARY

In summary, many risk factors for melanoma are non-UV-related and progress in the last 20 years have been instrumental in discovering melanoma genes which are involved in telomere biology, naevi number, pigmentation, body composition, energy expenditure, neural and melanocyte differentiation. Melanoma behaves in a very similar way all over the world in all Caucasian populations and many host factors are under tight genetic control. Research in these areas is important as it sheds new

light on genetic and epigenetic factors which are often set early on in life and less likely to be influenced by sun exposure in adulthood. It is also unravelling pathways which could be exploited for future therapies. Although excessive sun exposure is associated with melanoma risk, research on non-sun-related risk factors is important to redress the balance. The collection of good phenotypic and familial data as well as tumour and blood DNA is crucial for future genetic-epidemiological studies.

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REVIEW ARTICLE

Melanoma Genomics

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The incidence of cutaneous melanoma continues to increase in pale skinned peoples in Europe and elsewhere. Epidemiological studies identified genetically determined phenotypes such as pale skin, freckles and red hair, and sunburn as risk factors for this cancer. The development of many melanocytic naevi is also genetically determined and a strong melanoma risk phenotype. Not surprisingly then, genome wide association studies have identified pigmentation genes as common risk genes, and to a lesser extent, genes associated with melanocytic naevi. More unexpectedly, genes associated with telomere length have also been identified as risk genes. Higher risk susceptibility genes have been identified, particularly *CDKN2A* as the most common cause, and very rarely genes such as *CDK4*, *POT1*, *TERT* and other genes in coding for proteins in the shelterin complex are found to be mutated. Familial melanoma genes are associated with an increased number of melanocytic naevi but not invariably and the atypical naevus phenotype is therefore an imperfect marker of gene carrier status. At a somatic level, the most common driver mutation is *BRAF*, second most common *NRAS*, third *NF1* and increasing numbers of additional rarer mutations are being identified such as in *TP53*. It is of note that the *BRAF* and *NRAS* mutations are not C>T accepted as characteristic of ultraviolet light induced mutations.

Key words: susceptibility genes; somatic mutations; melanoma.

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SIGNIFICANCE

Melanoma continues to increase in incidence and therefore recognizing individuals at increased risk is especially important. This review discusses the associations between inherited genes which increase risk, and how the presence of those genes is manifest in family history or skin type. Environmental exposures, namely sun exposure leading to sunburn is aetiological in the genetically predisposed.

Melanoma continues to increase in incidence in Europe; figures from the period 1995–2012 recently published showed increases in both *in situ* and invasive melanoma (1). IARC figures generated from data recorded up until 2012 were used to construct **Fig. 1**. It can be seen that the greater proportional rise in incidence in older men in the UK is mirrored in Australia albeit at a considerably higher incidence rate. Australia, however, appears to have achieved a decrease over time in incidence rates in the very young, probably related to the very active and long-standing public health activities in that country.

The common melanoma subtypes, superficial spreading melanomas (SSM), nodular melanomas (NM) and lentigo maligna melanomas (LMM) are essentially diseases of pale skinned individuals, and this observation along with the identification of reported sunburns as a significant risk factor led to the recognition that melanoma is caused by sun exposure. The comparison between rates in England and in Australia is useful as the sub-population of Australians who develop melanoma commonly claim UK ethnicity and previous genome-wide association studies confirmed inherited similarities (2): that is that this comparison in incidence therefore reflects the strong effect of sun exposure (in genetically similar people) on melanoma development.

Fig. 2 shows a principal component analysis (PCA) from a genome-wide association study reported by the GenoMEL consortium (2). PCA analyses of inherited genetic variation effectively examines genome-wide genetic variation across the populations determining the underlying patterns. The first two components explain much of the overall pattern of variation; in this figure, each participant's genome is represented by a "dot" reflecting on a 2 dimension plot the value of that person's first two principal components – each of the principal components consists of many thousands of genetic variants across the genome. The dots in brown, orange, sky blue and dark green represent the genotype of blood samples from the UK, the Netherlands, Sydney and Brisbane respectively. The PCA did not consider the location of residence of the person or the laboratory that recruited them but when the two dimension graph is overlaid on the map of Europe, it is apparent that people recruited from the same location are together on the map and that the pattern of the geographical locations is also retained with the exception of the Australian populations which are superimposed on the map of Western Europe reflecting their ancestry. The map confirms that gene frequencies vary slowly and systematically across Europe reflecting the fact that local migration is the biggest determinant of change. For instance, one of

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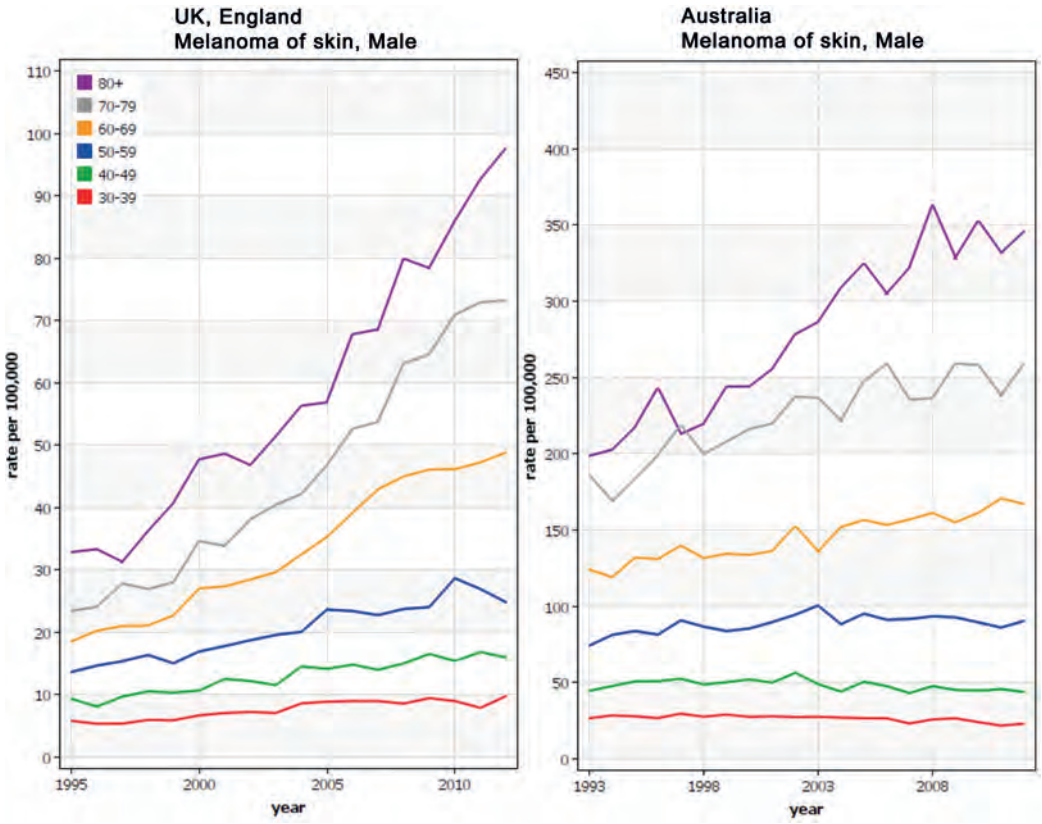

Fig. 1. Incidence rates for melanoma in men in two genetically similar populations in England and in Australia. The figures were generated on line using the Globocan tool (gco.iarc.fr).

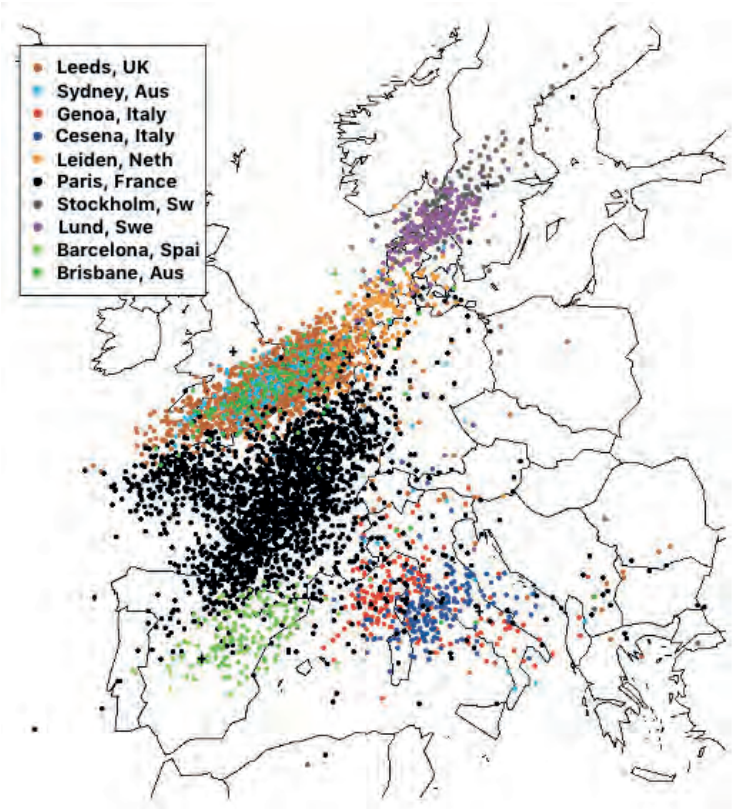

Fig. 2. Principal components analysis (PCA) from a genome-wide association study reported by the GenoMEL consortium (2). The coloured dots represent a measure of the genetic inheritance of participants in a genetic study of melanoma. The superimposed blue, green and terracotta dots over the UK suggests that the participants from two sites in Australia (Sydney and Brisbane) were very similar genetically to those living in the UK. This was expected as many Australian melanoma patients report ethnicity as the UK. Comparing incidence in melanoma then between the UK and Australia is to some degree comparing incidence in two populations similar genetically but with very different sun exposure histories. Figure kindly prepared by Dr Mark Iles of the University of Leeds.

Table I. Incidence rates reported by the North American SEER registry by ethnicity. Modified from Wang et al. (3)

	Non-Hispanic white	Hispanic white	Asian	Black	Total
Superficial spreading melanoma	9.05 (8.96–9.13)	1.12 (1.05–1.19)	0.31 (0.27–0.35)	0.15 (0.12–0.18)	6.18 (6.13–6.24)
Nodular melanoma	1.80 (1.76–1.84)	0.49 (0.44–0.54)	0.14 (0.12–0.17)	0.06 (0.04–0.08)	1.30 (1.28–1.33)
Lentigo maligna melanoma	1.87 (1.83–1.90)	0.23 (0.19–0.27)	0.06 (0.05–0.08)	0.02 (0.01–0.04)	1.37 (1.35–1.40)
Acral lentiginous melanoma	0.21 (0.20–0.22)	0.24 (0.21–0.28)	0.17 (0.14–0.20)	0.19 (0.16–0.23)	0.20 (0.19–0.22)

Age-adjusted incidence rates per 100,000 person-years.

the genes contributing to this pattern is the variation in the lactase gene reflecting the pattern of lactose intolerance across Europe. Thus the melanoma incidence curves in Fig. 1 reflect UK and Australian melanoma patients and this PCA suggests that these are more similar than populations sampled elsewhere in Europe.

INHERITED (GERMLINE) GENOMIC VARIATION AND MELANOMA RISK

Skin colour genes

Although the markedly different incidence rates for genetically similar populations in the UK and Australia reflects the effects of very different patterns of sun exposure, cutaneous melanoma is a strongly genetically determined disease. Melanoma incidence is very strongly related to skin colour being predominantly a cancer of pale skinned individuals. **Table I** indicates that the most common melanoma subtypes, SSM, NM and the less common LMM and desmoplastic melanoma, are very much more common in fair skin, whereas the acral lentiginous melanoma (ALM) variety has approximately the same incidence in most ethnic groups. Table I reports incidence for different melanoma subtypes, SSM, NM, LMM and ALM. The ethnicity terms used are those used in North America: Non-Hispanic white (NHW), Hispanic white (HW), Asian and Black. The data show that the incidence of SSM, NM and LMM is highest in those with ethnicity associated with the palest skins, indeed there is some evidence for a gradation in incidence from typically palest to darkest skins. The data also show that the incidence of ALM does not differ with ethnicity and therefore inherited pigment genes.

Melanocytic naevi genes

The second risk phenotype is the presence of greater numbers of melanocytic naevi (4), both of the "common" or banal type and the presence of larger naevi described clinically as atypical naevi and histologically as dysplastic naevi. Twin studies have reported evidence for high heredity for this phenotype in the order of around 65% (5, 6). Thus the two phenotypes most predictive of melanoma risk (pale skin and the presence of many naevi) are shown to be predominantly genetically determined.

New low-medium penetrance loci

Genome wide association studies have increased steadily in power to identify larger numbers of inherited genetic variation associated with increased risk of the common subtypes of melanoma (7, 8) and indeed with the risk phenotypes as a result of collaboration between multiple research groups. The role of inherited pigmentation genes in melanoma susceptibility is clear but there are also a number of genetic loci associated with increased numbers of melanocytic naevi and with telomere length. Telomeres are nucleotide repeat sequences which protect the ends of chromosomes, from excessive shortening and becoming tangled during cell division. Genes such as that coding for telomerase and additional genes coding for proteins in the so-called shelterin complex play an important role in maintaining telomeres. A number of inherited genetic variants are reported to determine telomere length and a genetic score predicting longer telomeres has been shown to strongly predict melanoma risk (9). In short, common genes associated with paler skin and in particular skin which tends to burn in the sun (predominantly the gene coding for the melanocortin receptor 1, MC1R); others which are associated with having more naevi; and genes associated with longer telomeres are melanoma risk genes, and to a large degree explain variation in melanoma incidence in different populations worldwide. Further genes associated with risk will certainly be found and other pathways may therefore be identified: a recent genome wide association study of naevi reported some evidence of pathways not previously supposed to be associated with naevus pathogenesis (8).

The low to medium penetrance (risk) genes identified in genome wide association studies each increase risk a little and melanoma occurs essentially in individuals who have inherited several risk alleles and who like the sun, in particular intermittent sun exposure. The likelihood is that risk of melanoma increases progressively with higher numbers of the risk alleles.

RARE INHERITED MUTATIONS

Rarer inherited mutations are associated with a high risk of melanoma (high penetrance) so that clustering of cases occurs in families. The definition usually used to define a melanoma family is at least 3 cases in close relatives. The commonest high penetrance susceptibility gene is *CDKN2A* which notably codes for two quite distinct proteins: $p16^{INK4a}$ and $p14^{ARF}$. $P16^{INK4a}$ is a cyclin-dependent kinase inhibitor in the RB1 cell cycle control pathway, and $p14^{ARF}$ binds the p53-stabilizing protein MDM2 in the p53 signalling pathway. The *CDKN2A* gene is therefore involved in the regulation of two critical cell cycle regulatory

pathways. A very small number of melanoma families have causal mutations in the gene which codes for CDK4 to which p16 binds and these families appear to have a very similar phenotype to those with *CDKN2A* mutations (10).

Mutation carriers are more likely to have multiple primaries than those without such mutations (11), a little earlier age of onset and pancreatic cancer occurs in some CDKN2A families reported from mainland Europe and the USA. Studies in specific founder *CDKN2A* mutation families from Sweden (12) and the Netherlands have reported increased rates of cancers associated with smoking (13) but the risks of cancers other than melanoma and pancreatic cancer are not yet sufficiently well established to infer screening requirements, see https://www.ncbi.nlm.nih.gov/books/NBK7030/. That risks remain unclear to some extent reflects bias of ascertainment: in order to identify new high risk inherited cancer genes, researchers typically tested families who had multiple cases of the same cancer. Work is ongoing currently within GenoMEL (www.genomel.org) to address this deficiency.

Familial melanoma has been recognised for many years and between 1994 (14) and 2013, only *CDKN2A* and *CDK4* mutations were recognised as familial melanoma genes. These mutations were identified not least because the majority of affected families are at increased risk of only melanoma, sometimes also with some pancreatic cancer and families were ascertained for investigation on the basis essentially of multiple melanoma cases. There was, in essence, a deliberate bias, in that families with multiple cases of melanoma were selected for invitation to participate in research. This was the usual method for the identification of highly penetrant genes using genetic linkage studies where co-segregation of genetic variants with the cancer was required. Now that whole exomic or genomic sequencing and "panels" of cancer genes are used to identify high risk genes in families, genes are being identified with association with melanoma and an increased number of various other cancer types. As a result, rarer mutations in additional melanoma susceptibility genes have been identified. These have been seen in less than 2% of UK families with 3 or more melanoma cases. They are predominantly genes which are involved in telomere function/maintenance, first the gene named Protection of Telomeres I (*POT1*) which was described simultaneously in two groups in melanoma families (15, 16). Additional mutations were described in other genes in the shelterin telomere protection complex of which POT1 is a subunit (17), and in *TERT* (18, 19). Telomere function is therefore clearly important in melanoma pathogenesis. Finally inherited mutations in the *BAP1* gene, which were originally reported as an inherited cause of uveal melanoma but were quickly then associated additionally with a risk of lung cancer and meningiona (20) are now recognised also to increase the risk of cutaneous melanoma (21). Subsequently the mutations were recognised as also associated with renal cancer and mesotheliomas. Unusual but generally benign "spitzoid" melanocytic lesions of the skin were reported to be part of the syndrome in 2011 (21).

The role of gene testing and screening is therefore in the process of change. As the penetrance of these genes which increase the risk of melanoma and other cancers, becomes clearer then appropriate screening should be possible and gene testing/counselling likely to be increasingly performed.

Families with inherited melanoma susceptibility to melanoma often also have more melanocytic naevi than is usual in that population. This phenotype, called the atypical mole syndrome or the dysplastic naevus syndrome was originally thought to be a key component of the Familial Melanoma "Syndrome" (22). Indeed, there is certainly an association: mutations are more likely to have larger numbers of naevi (23). However, it is recognised now that some families with the same mutation may or may not have many naevi, so that family members with normal naevi may yet be found to carry the susceptibility gene. It has been postulated that the rather variable association between inherited high risk melanoma genes and naevi may be complicated by the variable co-inheritance of common lower risk melanoma susceptibility genes (23). In the dermatology or melanoma clinic, then the factors which should alert the medical team to the possibility of inherited high-risk melanoma susceptibility are, the atypical naevus syndrome, multiple primaries, relatively early onset and the co-occurrence of pancreatic cancer in some populations at least. The single most important factor, however, is family history of cancer. So, only 2% of apparently sporadic melanomas even with 2 primaries have inherited *CDKN2A* mutations (24), but in our own studies > 50% of families with 4 or more melanoma cases have such mutations. In the dermatology or melanoma clinic then, the presence of many naevi or more than one primary should alert the team to the possibility of a higher risk but family history is the strongest evidence for highly penetrant melanoma susceptibility genes. A review published by Sancy Leachman and GenoMEL (25) made recommendations for genetic counselling, but the identification of genes such as *POT1* and *TERT* which increase the risk of cancers other than melanoma means that these recommendations will be revised as more data become available.

Melanoma is an uncommon second malignancy in inherited retinoblastoma (26) and there are reports of a possible small increase of risk in carriers of *BRCA2* mutations (27) and possibly Lynch syndrome susceptibility genes although the evidence for the latter is not at this time convincing.

SOMATIC MELANOCYTIC NAEVUS GENOMICS

Melanocytic naevi are both markers of melanoma risk and precursors of melanoma. They are benign proliferations which arise progressively starting in the first year of life,

but which stop appearing at the age of 40 years or so. The proliferation of melanocytes sufficient to produce detectable naevi results from the development of mutations in genes predominantly in the RAS/RAF/MEK/ERK pathway. The most common mutation is *BRAF*^{V600E} but *NRAS*, and less commonly *KRAS* mutations occur. The prevalence of such mutations differs between naevi of different types, recently reviewed by Roh et al. (28). Roh et al. estimated that *BRAF* mutations drive 78% of common acquired naevi, 60% of dysplastic naevi, 7% of blue and 6% of Spitz naevi. Similarly, they estimated that *NRAS* mutations drive 95% of giant pigmented congenital naevi, 70% of small/medium naevi and 2% blue and Spitz naevi. *GNAQ* mutations occur in 84% of blue naevi.

Neither *BRAF* nor *NRAS* mutations have the classical genetic signature of mutagenesis as a result of ultraviolet (UV) light exposure: C>T mutations (29), but as described above, there is clear epidemiological evidence of a relationship between naevus number and sun exposure. The precise pathogenesis of such mutations remains as yet unclear but these observations suggest a complex relationship between intermittent sun exposure and naevogenesis. It has been queried whether *BRAF* mutations might actually result from DNA damage consequent upon exposure to UVA (30).

Whatever the route, the activation of the RAS/RAF/MEK/ERK pathway appears to drive the proliferation of naevi but the mutations eg in *BRAF* also induce senescence and therefore in the majority of naevus proliferation eventually ceases, resulting in growth cessation and ultimately clinical involution. Where this senescence is overcome as a result of additional mutations, then dysplastic naevi may develop and evolve into superficial spreading melanomas. As reported by Shain et al. (31), as melanoma evolves from benign naevi through to invasive tumours, then the proportion of lesions with loss of the *CDKN2A* gene, increased expression of *TERT*, increased numbers of additional mutations and copy number changes steadily increases resulting in more aggressive tumours. An on-line data source https://www.mycancergenome.org/content/disease/melanoma/ estimates the frequency of the driver mutations in melanoma as *BRAF* in 37–50%, *CTNNB1* (2–4%), *GNA11* (1%), *GNAQ* (1%), *KIT* (2–8%), *MEK1* (6–7%), *NF1* (12%) and *NRAS* (13–25%). The proportion of each in different melanoma subtypes differs, so the same data source reported that in melanomas arising on for example the trunk 50% have *BRAF*, 20% *RAS* compared with melanomas arising in skin with sun damage, whereas *BRAF* is reported to be much lower at 10%, with 10% *NRAS* and 2% *KIT*. Acral melanomas, 15% *BRAF*, 15% *NRAS* and 15% *KIT*. Individual studies have reported additional mutations. As technologies designed to detect mutations and copy number changes become more and more accessible even in formalin fixed tissues, then the knowledge of less common genomic somatic changes in melanoma increases. Hodis et al. (32) for example reported the discovery of 6 novel melanoma genes (*PPP6C*, *RAC1, SNX31, TACC1, STK19* and *ARID2*), 3 of which: *RAC1, PPP6C* and *STK19* were recurrent. Hayward et al. (33) reported in addition significant mutation of *TP 53* in cutaneous melanoma and that the significant mutations were *BRAF*, *NRAS* and *NF1* in acral melanoma and *SF 3B1* in mucosal melanoma.

Large mutation burden in melanomas

Although, the classic driver mutations of naevi do not have C>T mutations, melanomas were shown by the Sanger Institute to have the greatest number of mutations of any cancer and that these mutations were predominantly C>T (29). Mutations are not surprisingly more frequent in tumours which arose on chronically sun exposed skin (31) and the probability is that these mutations are predominantly passenger mutations: that is that they don't play a key role in tumour progression. However, overall mutation rates were reported to be highest in lung cancer and melanoma (29), both of which have good responses to checkpoint blockade and the supposition is that this is at least in part attributable to mutation derived neoantigens capable of stimulation immune responses to the melanoma cells.

Copy number changes

Copy number changes have been elucidated to some extent. Hodis et al. (32) described a low prevalence of amplifications in melanoma overall: 11% *CCND1*, 6% *KIT*, 3% *CDK4*, 13% *TERT*, and 4% *MITF*. The deletions were dominated by those in *CDKN2A* (38%) and *PTEN* (25%). Overall the data support the view that copy number changes are more common in acral lentiginous melanomas than in those in sun-exposed sites. In **Table II** we have summarised some of the recent reports of copy number variation in acral lentiginous melanomas, and by comparison with the proportions reported by Hodis et al. (32) it can be seen that with this albeit limited data, copy number changes are more frequent in acral lentiginous melanoma than in melanomas arising in sun-exposed sites.

In conlusion, cutaneous melanoma is a good example of gene environment interaction, in that it is largely (but

Table II. The recently reported data looking at copy number changes in acral lentiginous melanoma

Copy number alteration	Reference	*n* (%)
Amplification *AURKA*	Yan et al. 2018 (34)	472 (25)
Amplification *GAB2/PAK1*	Chernoff et al. 2009 (35) Yeh et al. 2019 (36)	122 (22)
Amplification *CCND1*	Sauter et al. 2002 (37) Yeh et al. 2019 (36)	122 (21)
Amplification *CDK4*	Curtin et al. 2005 (38) Yeh et al. 2019 (36)	122 (22)
Deletion *NF1*	Liang et al. 2017 (39) Yeh et al. 2019 (36)	34 (12) 122 (15)
Inactivation *NF1 cooperating factor SPRED1*	Yeh et al. 2019 (36)	122 (7)
Deletion *CDKN2A*	Liang et al. 2017 (39)	34 (35)
Amplification or point mutation *TERT* promoter	Liang et al. 2017 (39)	34 (35)

not exclusively) a cancer of genetically determined pale skinned peoples, when they experience sun burn or sun damage. The identification of genes associated with risk from low to high risk has led to the identification of biological processes involved in tumourigenesis. The genetic changes occuring in the tumours adds more to what is known about tumourigenesis but also has lead to the evolution of treatment options for advanced disease.

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REVIEW ARTICLE

Melanoma Risk and Melanocyte Biology

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Cutaneous melanoma arises from melanocytes following genetic, epigenetic and allogenetic (i.e. other than epi/genetic) modifications. An estimated 10% of cutaneous melanoma cases are due to inherited variants or *de novo* mutations in approximately 20 genes, found using linkage, next-generation sequencing and association studies. Based on these studies, 3 classes of predisposing melanoma genes have been defined based on the frequency of the variants in the general population and lifetime risk of developing a melanoma: (*i*) ultra-rare variants with a high risk, (*ii*) rare with a moderate risk, and (*iii*) frequent variants with a low risk. Most of the proteins encoded by these genes have been shown to be involved in melanoma initiation, including proliferation and senescence bypass. This paper reviews the role(s) of these genes in the transformation of melanocytes into melanoma. It also describes their function in the establishment and renewal of melanocytes and the biology of pigment cells, if known.

Key words: melanocyte stem cells; embryonic development; germline mutation; inherited melanoma.

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SIGNIFICANCE

Inherited variants or de novo mutations in approximately 20 genes have been shown to contribute to approximately 10% of cases of cutaneous melanoma. This paper evaluates the function(s) of these proteins in the establishment of the lineage during embryogenesis, melanogenesis, renewal and, of course, during melanomagenesis.

Cutaneous melanoma results from transformation of melanocytes (Mcs). Melanoma accounts for only 10% of skin cancers, but is responsible for approximately 80% of skin cancer-related deaths; the remaining skin cancers are basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and Merkel cell carcinomas. The incidence of melanoma has increased steadily over the last 5 decades. According to Berk-Krauss et al. (1), the overall mortality from 2013-2016 in the US among caucasians was 6%. Melanoma accounts for approximately 2% of all cancers diagnosed annually worldwide (2, 3). Phototype (**Table I**) and geographical location are two key risk factors in the epidemiology of cutaneous melanoma: melanoma incidence is highest in Australia/New Zealand, followed by the USA and Northern Europe, and mostly affects Caucasian populations of phototypes I and II (4). It has been shown that for individuals living in similar environments in the USA, Caucasians have a 25 times higher risk of developing melanoma than African Americans (phototypes IV to VI). The risk of melanoma in red-haired individuals is approximately 3 times that observed in other Caucasians (5). Caucasians were found to have a 4–5 times higher risk of developing and dying from melanoma in Australia than in Europe, showing the effect of the environment. The incidence of melanoma is 56 per 100,000 in Australia and 14 in 100,000 in France, with a similar mortality (approximately 1/100,000).

The frequency of melanoma is lower in dark-skinned individuals than in light-skinned individuals. This may be explained in part by the melanoma inducers present in the environment (ultraviolet radiation). Epidemiological studies have revealed other melanoma inducers, including heavy metals, some pesticides, and alcohol consumption, but none of these were associated with phototypes (6–9). These fundamental data reveal the importance of genetics (mainly senescence, pigmentation and DNA repair genes) and the environment (mainly sun/ultraviolet (UV) exposure) in melanomagenesis.

Melanomagenesis is a multistep process that can be divided into 2 main stages: initiation and progression.

Table I. Phototypes: Fitzpatrick classification

Phototype	Skin colour	Hair colour	Eye colour	Sunburn	Tanning	Freckles
I	Very light	Red/blonde	Light	++++	–	+++
II	Very light	Blonde/brown	Light	+++	+	++*
III	Light	Blonde/brown	–	+	++	+/–
IV	Tan	Brown/dark/black	–	+	+++	–
V	Dark	Black	Dark	+/–	+++	–
VI	Dark	Black	Dark	–	+++	–

The 6 phototypes classification is based on skin, hair, and eye colours, on the ability to tan and sunburn.
*Freckles appear after sun exposure.

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Melanoma initiation requires proliferation of Mcs and bypassing of senescence. After a certain number of divisions, cells enter senescence and, in order to bypass senescence and allow the further growth of the cells, the cell cycle and the length of the telomeres have to be boosted.

Mcs are melanin-producing cells that arise from neural crest cells (NCC) (sometimes called the 4th embryonic layer), a transient population of cells arising from the dorsal part of the neural tube (10). Information concerning the establishment of the Mcs was largely obtained from mouse and chicken studies. The NCC delaminates and migrates away from the neural tube. NCC derivatives exist as single cells throughout development and spread via 2 major migration pathways. Melanoblasts are NCC derivatives migrating in the space between the somites and the non-neural ectoderm (dorsolateral pathway). These cells are the precursors of Mcs and are characterized by an ability to produce the pigment melanin. They are specified in a cell-free area between the dorsal part of the neural tube, the ectoderm and the dorsal part of the somites. This area is known as the migration staging area (MSA), the site at which founder melanoblasts receive proliferation, survival and migratory signals (11, 12). Melanoblasts arising from the dorsolateral pathway travel through the developing dermis. From mid-gestation onwards, the dermal melanoblasts start to cross the basal layer and colonize the epidermis (13). The epidermal melanoblasts then concentrate around the placodes of hair follicles (HF) before entering the forming hair follicles (13, 14). They colonize the future bulge, to generate the melanocyte stem cells (McSC), and the hair bulb, to generate the differentiated Mcs of the first hair cycle ("embryonic" hair), before resting. In addition to colonizing the hair follicle in humans and pigs, melanoblasts also remain in the epidermis in the interfollicular regions. Mcs located in the interfollicular regions are responsible for the tanning response and the protection against UV. Furry animals do not require such protection, since the hair efficiently protects the skin against this type of radiation.

Hair renewal and pigmentation are concomitant processes. Mcs disappear during catagen. In early anagen, McSCs re-enter the cell cycle and divide, for self-renewal and the generation of transit amplifying cells (TAC). These cells migrate and differentiate into Mcs, which participate in the pigmentation of the first "adult" hair. Cutaneous melanoma may arise from the McSCs, TACs and/or from the Mcs (15).

Approximately 20 genes have been found to be constitutively mutated in the germline and associated with a risk of melanoma. Three classes of genes have been defined on the basis of the frequency of the variation and the risk of developing melanoma: ultra-rare variants with a high risk, rare variants with a moderate risk, and frequent variants with a low risk (**Fig. 1**). It has been estimated that ultra-rare variants conferring high risk (~10 genes) account altogether for 2% of the total risk of melanoma. These genes include $p16^{INK4A}$ and $p14^{ARF}$ (located at the same locus, *CDKN2A*), *CDK4, BAP1, RAD51B, POLE, TERT, POT1, ACD*, and *TERF2IP*. Rare variants of *MC1R* and *MITF* confer a moderate risk of melanoma and finally, frequent variants of melanocortin 1 receptor (*MC1R), OCA2, ASIP, TYR, TYRP1, MATP, SCLC45A2, KIT*, and *PARP1* are estimated to account for 12% of the risk of melanoma. It should be noted that the melanoma risk model is very complex, as genetic risk factors interacts together (rare and frequent variants modulate the risk conferred by ultra-rare variants (16)), but also with host phenotypes and environmental factors (17). Functional studies performed *in vitro* and/or *in vivo* has unravelled the role of some of these genes in melanoma. However, no systematic study has yet been performed on all 20 genes in order to evaluate their importance during the natural course of Mcs development and melanomagenesis.

This review focusses first on susceptibility genes for cutaneous melanoma and the melanoma inducers found in the environment. It then discusses the role of these genes, if known, during the various steps of Mcs and melanoma development, including in: (*i*) melanoma initiation and progression, (*ii*) the establishment of the Mcs lineage during embryonic development, (*iii*) the terminal differentiation of Mcs associated with the production and transport of melanin, and (*iv*) the transfer of melanosomes in the keratinocytes of the skin or the hair, (*v*) the renewal of Mcs from McSC, and (*vi*) the maintenance of Mcs function over time.

CUTANEOUS MELANOMA SUSCEPTIBILITY FACTORS

The focus here is primarily on germline/constitutive mutations increasing the risk of melanoma formation and the environmental factors modulating these risks by inducing somatic mutations, epigenetic and allogenetic modifications and/or modifying the micro-environment.

Melanoma susceptibility genes

Variants in melanoma susceptibility genes have been classified according to their frequency and the degree of risk. These variants are generally transmitted from germ cells, but the role of neo-mutations, micro-chimerism and somatic mutations should not be underestimated.

Ultra-rare variants – high risk of developing melanoma. The first genetic studies of familial melanoma mapped markers associated with melanoma risk to chromosome 9, and the 9p21 region in particular (18); subsequently, causal variants at the *CDKN2A* locus were identified (19, 20). The *CDKN2A* (cyclin-dependent kinase inhibitor 2A) gene encodes 2 proteins, p16 and p14, which inhibit cyclin-dependent kinase (CDK), thereby regulating cell

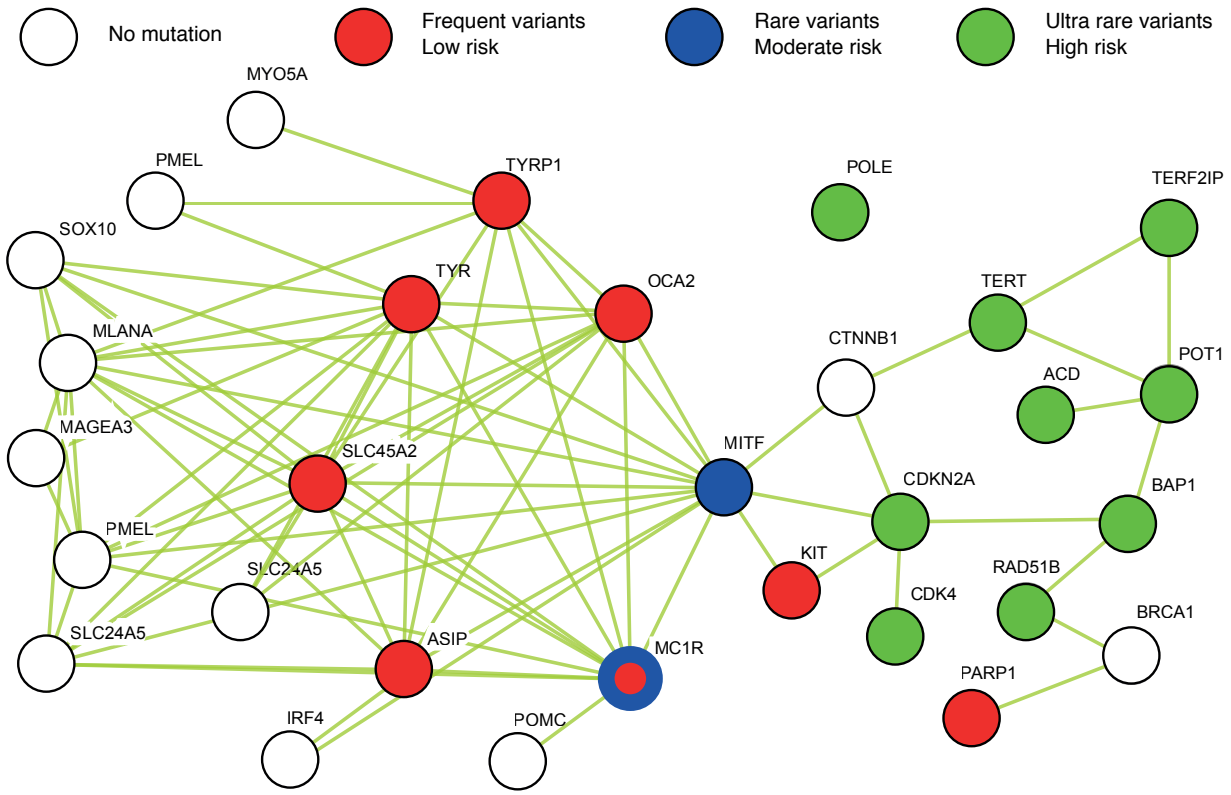

Fig. 1. Interaction network of genes involved in melanocyte biology, and the associated melanoma risk. The list of genes involved either in the establishment and renewal of melanocytes, in the biology of pigment cells, and/or associated with increased melanoma risk, was submitted to the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database for analysis of the gene interaction network (122). Each circle represents 1 gene, and 1 linking line represents a direct (physical) or indirect (functional) association between the proteins encoded by the 2 genes. *Green:* genes with ultra-rare variants associated with a high risk of melanoma; *blue:* genes with rare variants associated with a moderate risk of melanoma; *red:* genes with frequent variants associated with a low risk of melanoma; *white:* genes involved in melanocyte biology with no mutation currently associated with melanoma. Note, the left cluster gathers mainly genes of melanogenesis, and the right cluster gathers genes of the cell cycle, telomere length control, and DNA repair.

cycle checkpoints. *CDKN2A* variants have been found in 10–40% of familial melanoma cases, depending on geographical location, but their prevalence is very low in the general population, either never described or with a minor allele frequency (MAF) <0.001% (21, 22). *CDKN2A* variants confer a high risk of melanoma development with age- and geographical area-dependent variations: in melanoma-prone families ascertained through cancer clinics, the penetrance (a mean age-specific cumulative risk) at age 80 years was 58% in Europe, 76% in the USA and 91% in Australia (23, 24). However, at the same age penetrance was lower (28%) in population-based studies (25). Differences in melanoma risk between *CDKN2A* mutation carriers can be explained partly by the underlying pigment genes these individuals also carry and possibly also sun-exposure variants, host phenotype and sun exposure (17, 26, 27).

Mutations of *CDK4* (cyclin-dependent kinase 4), encoding another cell cycle regulator, have also been linked to melanoma formation in families with phenotypes similar to those of families with *CDKN2A* mutations (28, 29). CDK4 is a kinase that regulates G1 cell cycle progression by phosphorylating Rb proteins. *CDK4* variants are rare and are found in less than 1% of familial cases of melanoma, with a penetrance of 74% at the age of 50 years (29).

More recently, high throughput sequencing studies have added to the list of genes increasing the risk of melanoma, through the identification of rare variants in families. These genes can be divided into 2 groups: *TERT, POT1, ACD* and *TERF2IP*, which encode proteins involved in telomere length control, and *BAP1, RAD51B* and *POLE*, which encode DNA repair proteins. A variant in the *TERT* (telomerase reverse transcriptase) promoter was identified in 2 melanoma-prone families; this c.-57T>G variant is oncogenic through the creation of a new ETS transcription factor binding site leading to increased TERT expression (30, 31). Interestingly, somatic mutations of the *TERT* promoter (1 being identical) have also been detected in 33% of primary melanoma cases and 85% of metastatic cases (30). Mutations of *POT1* (protection of telomeres 1), *ACD* and *TERF2IP* were detected in exome sequencing studies of melanoma-prone families (32, 33). These 3 genes encode proteins of the shelterin complex and explain 9% of melanoma families lacking *CDKN2A* and *CDK4* mutations. Mutations of the *TERT* promoter and the shelterin complex result in longer telomeres, favouring senescence bypass.

BAP1 (BRCA1-associated protein-1) loss of function germline mutations have been found associated to a tumour predisposition syndrome (BAP1-TPDS, OMIM 614327) including both cutaneous (0.52% of families) and uveal melanomas (CM and UM) (28.5% of families) (34–37). In addition to melanomas, the most frequent cancers of this syndrome are renal cell carcinoma, mesothelioma and multiple BCCs but the whole tumour spectrum and lifetime risk are currently unknown (38). *BAP1* is a tumour suppressor gene located at 3p21, encoding a deubiquitylase that participates in multiprotein complexes playing roles in numerous cellular processes, including DNA damage response, cell cycle regulation, cell growth, metabolism, and the regulation of inflammatory responses (38). The *BAP1* loss-of-function mutations are associated with approximately 15% risk of cutaneous melanoma. A germline *POLE* missense mutation located in the exonuclease domain of the protein (p.(Trp347Cys)) was found in a unique melanoma-prone family of 7 confirmed cases of CM and a case with UM, leading to a mutator phenotype in functional assays (39). It should be noted that POLE germline mutations are more frequent in endometrial (7–10%) and colorectal (2%) cancers (22) than in melanoma. Finally, a novel germline *RAD51B* nonsense mutation was identified in a 3-case CM family; a melanoma tissue from a carrier displayed loss of RAD51B staining in most tumoural cells, by immunohistochemistry (40). RAD51B plays an important role in DNA repair through homologous recombination, but up-to-date known germline mutations have been associated with increased risk of ovarian cancer (41).

Approximately 22% of melanoma-prone families are associated with mutations in the 9 high-risk melanoma susceptibility genes (19% for CDKN2A and 3% for the other 8 genes). However, the remaining 78% can be partly accounted for by the rare variants conferring a moderate risk and frequent variants conferring a low risk of melanoma (24).

Rare variants – moderate risk of developing melanoma. A unique missense variant in the microphthalmia-associated transcription factor (MITF), the MITF^{E318K} variant, was linked to melanoma risk in 2 different studies. One of the studies started as a candidate gene hypothesis for melanoma and renal cancer predisposition (42), whereas the other involved whole-genome sequencing of a melanoma-prone family (43). The assumption in the first study was that MITF might be a good candidate for the following reasons: (*i*) it has been proposed to act as a melanoma oncogene (44); (*ii*) it also stimulates the transcription of hypoxia inducible factor (HIF1A), the pathway of which is targeted by kidney cancer susceptibility genes (45); and (*iii*) two members of the MITF-family of proteins, TFE3 and TFEB, have been implicated in renal cell carcinoma through somatic translocations (46). Both studies identified the same rare *MITF^{E318K}* variant (actual Minor Allele Frequency – MAF – in European population of 0.25%, GnomAD database) associated with a 5-fold increased risk of melanoma in genetically-enriched cases and an increased risk of 2.2 in case-controls studies (Yokoyama, 2011 #3370; Bertolotto, 2011 #2771).

The involvement of MC1R variants in melanoma is complex, since it is involved in pigmentation/phototype, naevi, UV/sun exposure (Demenais, 2010 #3357) and more recently, sex-dependence (47–49). The *MC1R* gene (16q24) is a key regulator of the synthesis of melanins (eu- and pheo-melanin) in Mcs, upon UV stimulation. Melanins are transferred to up to 40 surrounding keratinocytes to act as a natural sunscreen to absorb UV irradiation. Eumelanins are prevalent in phototypes IV to VI, while pheomelanins are responsible for red hair and freckles found in phototypes I and II (50). Beyond affecting phototypes, this 7-transmembrane receptor plays a role in DNA repair pathways and antioxidant defences for a complex photoprotective response (51). *MC1R* is highly polymorphic (> 80 variants) in Caucasian populations. Loss-of-function variants, which result in less pigmented phenotypes, are the result of human evolution associated with the classical mutation-selection events. Indeed *Homo sapiens* and *Homo neanderthalensis* migrated, and adapted from Africa to more northern and less sunny regions and subsequently lost pigmentation to allow increased vitamin D production (51). Six loss-of-function *MC1R* variants (p.ins86-87A, p.D84E, p.R142H, p.R151C, p.R160W, and p.D294H) are defined as R-type variants, as they increase the relative risk of melanoma 2–3 times; whereas another 4 variants (p.V60L, p.V92M, I155T, and p.R163Q) are classified as r-type variants as they confer melanoma risk below 2 (48). The effect is additive, as the presence of 2 or more *MC1R* "R" variants is associated with 6-fold increased risk compared to wild-type alleles. Thus, the *MC1R* gene is a moderate-penetrance melanoma susceptibility gene, but it is also a gene that modifies the risk of melanoma in patients with a *CDKN2A* mutation. Recent studies have shown that the risk conferred by *MC1R* variants seems to be independent of sun/UV exposure (52, 53) and independent of phenotype (54–56). Furthermore, *MC1R* polymorphisms seem to influence size and dermoscopic features of naevi (57). And recently, several sex-differences emerged, with *MC1R* variants associated with phototype I and II and higher melanoma risk, but better survival in females than in males (47–49).

Frequent variants – low risk of developing melanoma. Finally, frequent variants of genes associated with pigmentation (*OCA2, ASIP, TYR [OCA1], TYRP1 [OCA3], MATP, SCLC45A2 [OCA4], KIT,* and *PARP1*) are associated with a slight increase in risk of melanoma formation, as shown through genome wide case-control association studies (GWAS) (24, 58–60). These frequent variants have only a small individual effect on melanoma risk,

but combinations of these low-risk variants may account for up to 78% of non-familial melanomas. They are also responsible for the host phototype and are therefore important for determining the interaction between the phenotype and the environment. Further functional studies are required to decipher the associated mechanisms. These frequent pigmentation variants probably play a role as modifiers of melanoma risk in other genetic backgrounds, such as the ultra-rare and rare variants, conferring high and moderate melanoma risk, respectively.

Environmental factors

Sequencing studies on tumours have highlighted the genetic complexity of melanoma in terms of the somatic mutational load in the population and melanoma is the cancer with the highest mean mutation rate (61). The spectrum of driver mutations provided unequivocal genomic evidence for a direct mutagenic role of UV light in melanomagenesis (62). Somatic mutation frequencies also differ considerably between melanoma patients, showing melanoma to be a complex disease with several subtypes and multifactorial origins (63).

The melanoma risk associated with the gene variants, described above, depends heavily on the co-occurrence of other gene variants and environmental (micro- [inside the body] and macro- [outside of the body]) stresses. Protein-protein, protein-microenvironment, and gene/protein-environment interactions undoubtedly account for the complexity and diversity of melanoma. Epidemiological studies have implicated several environmental factors in the induction of cutaneous melanoma, including ultraviolet radiation (UVR), alcohol use, obesity, heavy metals and some pesticides.

UVR from the solar spectrum is the leading external cause of melanoma formation. Epidemiological studies have shown that exposure to UV is correlated with melanoma formation: intermittent rather than chronic exposure, high levels of exposure during childhood and the use of artificial UV lamps are associated with a major risk of melanoma (64, 65). Both UVA (315–400 nm) and UVB (280–315 nm) can promote melanoma formation (66). Most melanomas have many somatic mutations and most of those have a UV signature (61), i.e. ≥60% of mutations are C→T at a dipyrimidine site, with ≥5% as CC→TT changes (67).

As already mentioned, several external factors seem to be associated with melanoma, including alcohol consumption, heavy metals and pesticide exposure (6–9), but the evidence for the associations obtained for alcohol consumption, heavy metals and pesticide exposure is weaker than that obtained for UV. Epidemiological studies on farm workers, a population also exposed to UVR, have highlighted a potential risk of pesticides. The cumulative effects of UV and the pesticide carbaryl are genotoxic in human Mcs (68). Obesity is associated with melanoma initiation and progression, as it increases the risk of melanoma formation and favours melanoma growth, invasion and distant metastasis (69). Indeed, adipose tissue favours the proliferation and aggressiveness of melanoma cells through a direct dialogue, mediated by soluble factors and by exosomes, and through remodelling of the tumour microenvironment. Little is currently known about the molecular mechanisms of melanomagenesis associated with alcohol, heavy metals and pesticides. Extensive *in vitro* and *in vivo* functional studies should be performed to validate the importance of these factors in melanomagenesis.

Melanoma susceptibility genes and environmental factors

One epidemiological study has revealed that *CDKN2A* mutation carriers appear to have the same cumulative risk of melanoma irrespective of the ambient UV irradiation of the region in which they live (27). These results were functionally validated after exposing mice lacking $p16^{INK4A}$ to transient UV irradiations; this did not affect melanoma formation (70). Of course, this does not mean that UV irradiation has no role in melanoma initiation, as there is a strong association between UV irradiation exposure and melanoma risk for the general population. Interestingly, UVB irradiation of mice expressing $CDK4^{R24C}$, the oncogenic form of CDK4, promotes melanoma initiation, with a shorter time lag to tumour formation and faster growth (71). A germline nonsense mutation in *BAP1* (Y646X) and environmental exposure to asbestos and UV irradiation were found to contribute to the high incidence of cutaneous melanoma in a family at high risk of cancer (72). The association of UV and *MC1R* mutations has long been known to promote melanoma, and MC1R has been shown to be a potent regulator of PTEN after UV exposure; interestingly, the major Red Hair Color (RHC) *MC1R* variants R151C, R160W and D294H where shown to bind PTEN less effectively than the wild-type protein (73).

FUNCTION OF SUSCEPTIBILITY GENES IN THE NATURAL COURSE OF MELANOMA

Carcinogenesis is a multistep process. The wild-type cell accumulates genetic, epigenetic and allogenetic (it may affect transiently, for instance, RNA, proteins and lipids; which are modulated by the micro- and macro-environment) modifications, which alter its characteristics and/or environment, leading to self-sufficiency in growth signals, a limitless potential for replication, insensitivity to anti-growth signals, the evasion of apoptosis, sustained angiogenesis and tissue invasion and metastasis (74). A model of the multistep process of melanomagenesis has been described, in which the level of complexity is reduced to provide a schematic view of the process (75).

Melanomagenesis is currently seen as a multistep process with 2 main stages: initiation and progression.

Initiation

Melanoma initiation is characterized by an initial "boost" of cell proliferation and the bypassing of senescence (75). It has been suggested that 25% of melanomas arise from naevi, 1–2-mm wide pigmented spots consisting of Mcs that have hyperproliferated *in situ*, but then stop proliferating and become senescent/quiescent. In the vast majority of naevi, the Mcs remain senescent throughout the life of the individual with a strict control provided by high expression of P14, P15, P16 and/or PTEN. However, in a few cases, melanomas arise due to a subsequent bypass of senescence. The remaining 75% of melanomas do not arise from naevi (76) suggesting that the initial proliferation of these cells is not affected by senescence. These 2 paths may appear different, but the molecular mechanisms are not; molecular mechanisms associated with the bypass of senescence may occur before those associated with proliferation. The RAS/MAPK signalling pathway is activated and involved in the proliferation step of most melanomas (77). Cell cycle proteins, such as CDKN2A/B and CDK4/6, and those of the PI3K/AKT and WNT/β-catenin signalling pathways are involved in senescence bypass/lack of senescence. PTEN loss and β-catenin activation can induce bypass of senescence or lack of senescence (78–81). Melanoma susceptibility genes are clearly involved in melanoma initiation.

Rare variants associated with a high risk of melanoma are involved in melanoma initiation. *CDKN2A* is certainly the best known of these genes. It encodes 2 proteins, one of which is $p16^{INK4A}$, a CDK4/6 inhibitor that represses G1/S cell cycle transition, and is known to promote senescence. Its inactivation induces cell cycle progression and the bypass of senescence. It is therefore considered to be a tumour suppressor. Senescence is controlled by the cell cycle and by telomere length. TERT, POT1, ACD and TERD2IP, which regulate telomere length, may also be tumour suppressors because their inactivation induces checkpoint bypass and promotes uncontrolled cell cycle progression.

Rare variants associated with a moderate risk of melanoma development are also involved in melanoma initiation. Indeed, a rare variant of MITF ($MITF^{E318K}$) has been shown to act through senescence bypass, leading to melanoma formation (42, 82). MITF is the main transcription factor of the Mcs lineage, where it regulates various functions, including melanogenesis/differentiation, proliferation, invasion, and senescence (83). *MC1R* variants modulate the incidence of melanoma, but it remains unclear whether this is linked to the protective effect of eumelanin against UVR or to the intrinsic role of MC1R in melanomagenesis. Studies to resolve this question are underway. One study revealed that *MC1R* mouse mutants in the $BRAF^{V600E}$ background develop more melanomas than control mice, independent of UV exposure. This highlighted the potential role of pheomelanin in inducing oxidative damage (52).

Frequent variants associated with a low risk of melanoma development are also involved in melanoma initiation. Melanoma incidence varies between populations; the higher the phototype the lower is the chance of cutaneous melanoma. Melanin, one of the key parameters for evaluating the level of the phototype, is a natural protector of the skin against external aggression. Variants in genes encoding proteins involved in melanogenesis are responsible for various forms of occulocutaneous albinism (OCA). These genes include *TYR (OCA1), OCA2 (P), TYRP1 (OCA3)*, and *SLC45A2 (OCA4)*. Recently, *OCA5*, *OCA6* and *OCA7* were identified and shown to correspond to *SLC24A5* (*OCA6*), *C10orf11* (*OCA7*), and a locus on chromosome 4q24 for *OCA5*. None of these have yet been associated with melanoma. However, further studies are required to fully evaluate the importance of these OCA genes in melanomagenesis. *OCA1* variants have a general prevalence of 1/40,000, whereas *OCA2* variants are more common in dark-skinned populations (Africa) (1/4,000 – 10,000) than in light-skinned populations (Caucasian) (1/36,000). *OCA1* and *OCA2* variants account for 80% of OCA cases and are the most strongly associated with skin cancer development. Melanomas and BCCs are more frequent in individuals with *OCA1-2* mutations than in the general population. However, SCC is the most frequent type of cancer in patients with *OCA* mutations (84). Melanoma diagnosis is particularly challenging in this population because lesions are often amelanotic. Three other genes (*ASIP, KIT*, and *PARP1*) have frequent variants associated with a low risk of developing melanoma. Mutations of the *KIT* gene affect the tyrosine kinase receptor of the corresponding protein and cause piebaldism, as do mutations of the gene encoding its ligand, Steel (KITLG). *KIT* gene mutations are present in 39% of mucosal melanomas, 36% of acral lentiginous melanomas, and 28% of skin displaying chronic solar damage. Most of the reported mutations are found in exons 9, 11, 13, and 17, and they account for between 5% and 10% of the mutations of diagnosed melanomas (85, 86).

Progression

Tumour progression is characterized by the dissemination of the transformed cells, followed by the formation of metastases. Dissemination involves several fundamental cellular events, including a second boost of proliferation, pseudo-epithelial-to-mesenchymal transition, migration, intravasation of the blood and lymphatic streams, resistance to anoikis, and extravasation to invade new tissues. Cells may form metastases in a suitable niche, in which the cells induce angiogenesis and proliferate.

The function of MITF in melanoma progression is complex and can be explained with a rheostat model

where the level and/or activity determine whether the Mcs or melanoma cells undergo senescence, invasion, proliferation or differentiation (83, 87, 87). MITF amplification is observed in 21% of metastatic melanomas and favours melanoma cell proliferation (44). However, MITF also represses proliferation through the regulation of p21 and p16 (88, 90). The functions of the proteins encoded by the other susceptibility genes in melanoma progression remain unknown.

MELANOCYTE BIOLOGY AND FUNCTIONS OF SUSCEPTIBILITY GENES

We will now focus on the role of melanoma susceptibility genes in the establishment and maintenance of the Mcs lineage and pigmentation. The main function of Mcs, pigmentation, involves the intrinsic synthesis of melanin in specialized organelles called melanosomes, which are transported in Mcs and transferred to differentiating keratinocytes. Normal pigmentation is dependent on the genes involved in melanogenesis, and the transport and transfer of melanosomes and is finely regulated by extrinsic signals and cell-cell interactions.

Melanogenesis

Melanogenesis is a chain of reactions occurring in melanosomes, with tyrosine as an initial substrate, and pheomelanin (yellow, orange) and eumelanin (black, brown) as final products. The first enzyme in this chain, tyrosinase (TYR), hydroxylates tyrosine to generate DOPA, and then DOPAquinone. The second enzyme, dopachrome tautomerase (DCT or TRP2), and the third enzyme, tyrosinase-related protein 1 (TYRP1), catalyse eumelanin production from DOPAquinone. Pheomelanin production from DOPAquinone is dependent on cysteine.

Melanogenesis is regulated through modulation of the level, activity and localization of these enzymes by external signals, including communication between Mcs, keratinocytes and dermal fibroblasts via secreted factors and cell-cell contact. Mcs homeostasis is controlled by a complex network of keratinocyte-derived factors that regulate Mcs proliferation and differentiation. These include melanocyte-stimulating hormone (α-MSH), endothelins (Edn), basic fibroblast growth factor (β-FGF), nerve growth factors (NGF), granulocyte-macrophage colony-stimulating factor (GM-CSF), steel factor (SCF), leukemia inhibitory factor (LIF), hepatocyte growth factor (HGF), transforming growth factor beta (TGFβ), and Jagged1/2 (91, 92). These signals can be regulated by external factors, such as ultraviolet (UV) radiation, chemical compounds, drugs and stress.

The differences in pigmentation depend on the amount and quality of melanin (eumelanin vs. pheomelanin), which are partly controlled by the activity of *MC1R*. The MC1R receptor, which is activated by α-MSH after UV exposure, for example, induces eumelanin synthesis (93). *MC1R* variants are frequent in the Caucasian population, and lead to the expression of a receptor with normal, weak or no activity, associated with a brown, blond or red hair phenotype, respectively (94).

Melanosome transport

Melanosomes are lysosome-related organelles derived from non-pigmented endosomal vesicles (known as pre-melanosome stages I and II). They mature and undergo progressive pigmentation following melanogenesis and go through stages known as stages III and IV (95).

The mature melanosomes are transported from the perinuclear area toward the periphery of the Mcs and the tips of its dendrites. Two types of movement have been observed: rapid microtubule-directed migration over long distances, and short-distance migration along actin fibres at the periphery (96). During their migration from the nucleus toward the periphery of the cell (known as centrifugal movement), melanosomes are transported by a kinesin complex on microtubules. For the reverse migration toward the nucleus (known as centripetal movement), the melanosomes are transported by a dynein complex on microtubules. The motor for peripheral migration is myosin Va (*dilute*) in a complex with melanophilin (*leaden*) and RAB27A (*ashen*), and this migration takes place along actin filaments (97).

Mutations in genes encoding these transporters are associated with abnormal pigmentation, and, in some cases, more severe syndromes, such as Griscelli syndrome type 2 (98). However, they have not, as yet, been linked to an increase in melanoma risk. Conversely, the known melanoma susceptibility genes have not been shown to participate in melanosome transport.

Melanosome transfer

Melanosomes are transferred from Mcs to keratinocytes in order to deliver pigment to all epidermal keratinocytes. Several mechanisms of melanin transfer have been observed and debated: exocytosis-mediated, cytophagocytosis-mediated, tunneling nanotube-mediated and membrane vesicle-mediated transfer (99). The molecular mechanisms associated with the transfer of melanosomes to keratinocytes remain unclear. However, it has been suggested that classical pathways of exo-/endocytosis, membrane blebbing and vesicle biogenesis, filopodium formation and phagocytosis are involved. These processes involve proteins, such as Rab11b, small Rho GTPases, Cdc42 and Par-2 (100–103).

The cell body of the Mcs is located on the basement membrane, but the dendrites of the cell are in contact with 30–40 keratinocytes in the 3 dimensions of the epidermis, and these cells together form an epidermal melanin unit (104). In the basal layer, adjacent Mcs are generally separated by approximately 6 keratinocytes

(105). Melanosomes containing melanin are located in the superior part of the keratinocytes protecting the nucleus against UVR.

Albino individuals, who have Mcs but lack melanin, rarely develop melanomas. However, they develop more carcinomas (BCC and SCC) than individuals with normal pigmentation, confirming the protective effect of melanin in keratinocytes. Several issues should be raised at this point. Albinism is associated with a number of vision defects, including photophobia. These individuals therefore tend to prefer to stay out of the sun, thus leading to few melanomas. This suggests that melanin is protective for keratinocytes, but not necessarily for Mcs. Does this mean that Mcs lacking melanin are intrinsically more resistant to transformation than keratinocytes lacking melanin? If so, what are the molecular differences between these 2 cell types? Melanin, and its intermediates, such dopaquinone, and pheomelanin in particular, may damage Mcs, through oxidative stress, for example.

Melanoma susceptibility genes have not been shown to be involved in melanosome transfer. Melanosomes transfer may play no role in melanomagenesis, but this remains an open question, as the molecular mechanisms of transfer have yet to be fully elucidated.

ESTABLISHMENT AND MAINTENANCE OF THE MELANOCYTE LINEAGE

Embryonic development

In mammals, the establishment of the Mcs lineage during embryonic development involves the production of differentiated Mcs, responsible for the pigmentation of skin, hair and fur at birth, and McSC populations, responsible for maintaining pigmentation in the adult.

Normal development. In mice, normal Mcs development starts at approximately embryonic day 8.5 (E8.5), in mid-gestation, when the neural crest cells delaminate from the dorsal part of the closing neural tube and migrate into the MSA. These neural crest cells include the precursors of Mcs, melanoblasts, which proliferate and migrate between the somites (before they become the dermamyotomes, which subsequently evolve into muscle and dermis) and the ectoderm. At approximately stage E10.5, melanoblasts start to express Dct, which serves as a Mcs marker and can be easily detected by X-gal staining in Dct: LacZ mice (106). Between E11.5 and E15.5, the melanoblasts continue to proliferate and migrate through the forming dermis to cover the whole embryo. Some of these melanoblasts cross the basement membrane to colonize the epidermis, before colonizing the future hair follicles. In mice, the melanoblasts give rise to two Mcs populations at birth: the first differentiated Mcs located in the future bulb of the hair, and McSC pool located in the bulge of the hair. During the last steps of Mcs development in the embryo (from E15.5 until E19.5), the melanoblasts begin to express genes encoding enzymes required for melanin production, including Tyr and Tyrp1, which are produced in Mcs and McSC, for a few days after the birth of the McSC (107).

A second wave of melanoblast development has been described in the skin (108). NCCs give rise to several lineages, including Mcs, neurons, chromaffin cells and Schwann cells. One population of engaged precursor cells, the Schwann cell precursors (SCPs), can differentiate into either Schwann cells or Mcs. After early delamination, the SCPs migrate along the ventral pathway, between neural tube and somites, following the nerve fibres. SCPs retain a Schwann cell fate, while they remain in contact with the nerves. In the absence of signals provided by the nerve, some SCPs acquire a melanocytic fate. This second melanoblast population mostly colonizes the dorsal and lateral body walls, and seems to give rise to most of the limb Mcs.

The patterns of congenital pigmentary disorders in humans, including the congenital giant naevi that frequently display *NRAS* mutations, in particular, helped to identify a third wave of Mcs arising from the ectoderm at the time of gastrulation (109). Temporally, this is actually the first wave, because it occurs before the formation of the neural tube and the NCC formation during embryogenesis. These Mcs are responsible for the non-segmental pattern, through circular, bilateral migration centred on the midline. However, it remains unknown whether these cells contribute to mature Mcs in non-disease states.

Pathological development. Mcs pathology leads to pigmentation disorders of skin and/or hair, and may be associated with deafness and cognitive disorders (110, 111). Waardenburg syndrome (WS) is characterized by pigmentation and hearing disorders, sometimes associated with abnormal development of the face and limbs, and is due to the defective migration and proliferation of embryonic melanoblasts or the abnormal development of other neural crest cells. It is associated with mutations of the *MITF* and *SNAI2* genes (WSII) responsible for the pigmentary and hearing phenotypes; with mutations of the *PAX3* gene (WS I and III) affecting neural crest cell development and leading to morphological defects; and with mutations of the *EDN3, EDNRB*, and *SOX10* genes (WS IV) affecting intestinal neural cells. Piebaldism is characterized by hypopigmented patches of skin and hair and is due to the absence of Mcs in certain areas due to defective embryonic/Mcs development. Mutations of the genes coding the KIT receptor and its ligand, SCF, may cause piebaldism syndrome. Apart from MITF, no other melanoma susceptibility genes have been implicated in the embryonic development of Mcs.

Renewal

Normal renewal. McSCs constitute a reservoir for the replacement of Mcs lost during adulthood. McSC niches

have been identified in the bulge area of hair follicles (92, 112). McSCs are characterized by a specific cell shape and localization in hair follicles. No specific molecular marker of McSCs has been identified, so a combination of markers is used to follow these cells: McSCs are considered to be Dct-positive, Ki-67-negative, BrdU-retaining cells with low or no expression of KIT and MITF. In the pigment disorder vitiligo, repigmentation often begins at the hair follicles, subsequently spreading out to generate continuous colouring of the skin. This observation is consistent with the notion that McSCs from the bulge can migrate from the hair follicles to the basal layer and differentiate into mature epidermal Mcs. Moreover, repigmentation of depigmented regions lacking hair follicles, such as the palms of the hands, is occasionally observed in patients with vitiligo, indicating that McSC niches are also present in other skin structures, such as sweat or sebaceous glands, and the dermis (113, 114).

The maintenance of hair pigmentation has been well studied in mice and humans. Renewal of the hair in the hair cycle is synchronized with a cycling renewal of the differentiated Mcs, resulting in pigmentation of the new hair. After a resting phase and destruction of the previous hair follicle, the McSCs exit quiescence, proliferate and migrate along the hair follicle as transient amplifying cells, eventually reaching the bulb, where they differentiate into pigment-producing Mcs. Bulge cell quiescence is tightly controlled by several different signals. TGFβ represses differentiation and cell cycle progression; SHH, WNT and β-catenin end the quiescence phase, activating anagen; and NOTCH controls the appropriate differentiation of Mcs (115–121).

Failure of renewal. The absence of Mcs renewal by McSCs can lead to unpigmented skin and hair. The McSC population is limited, despite its potential for renewal, and the number of these cells declines during ageing, resulting in physiological greying of the hair in both humans and mice.

Local depigmentation occurs in adult patients with vitiligo. Vitiligo develops as depigmentation of the skin in specific areas, characterized by a disruption of the epidermal melanin unit with the presence of very few, if any, Mcs. Interestingly, the normally pigmented skin of patients with vitiligo also displays an altered Mcs distribution in the basal layer. The number of keratinocytes between 2 adjacent basal Mcs is larger in the pigmented epidermis of individuals with vitiligo than in that of individuals without vitiligo, and the number of suprabasal Mcs in pigmented epidermis from patients with vitiligo is greater than that in a control population. Alterations to E-cadherin levels at the membrane can affect Mcs adhesion to the basal membrane (105). No melanoma susceptibility gene has yet been linked to renewal of the Mcs lineage or its pathology.

CONCLUSION

All 20 melanoma susceptibility genes identified to date have a clear function during melanoma initiation, mainly the bypass of senescence. However, except for MITF they do not have a role in the different aspects of the life of Mcs. Susceptibility genes involved in melanogenesis (*OCA* genes, *ASIP*, and *MC1R*), in control of the cell cycle (*CDKN2A* and *CDK4*), in telomere length control (*TERT*, *POT1*, *ACD*, and *TERF2IP*), and in DNA repair (*BAP1*, *RAD51B* and *POLE*) may not be expressed nor have major function during the establishment of the Mcs lineage. As such, we understand that there is no developmental defect associated with the corresponding defective proteins, but we might expect that they may have a role during Mcs maintenance and renewal. Mutations in MITF and KIT dramatically affect the establishment of the Mcs lineage and both proteins play key roles in Mcs development and function. The importance of these genes during renewal remains unclear. Better understanding of the function of all these genes during normal Mcs renewal is crucial for advancing our understanding of their function during melanoma progression, especially during melanoma phenotype switching (86), which may use some proteins involved in McSC biology.

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REVIEW ARTICLE

Update on the Management of Basal Cell Carcinoma

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Basal cell carcinomas are the most frequent skin cancers in the fair-skinned adult population over 50 years of age. Their incidence is increasing throughout the world. Ultraviolet (UV) exposure is the major carcinogenic factor. Some genodermatosis can predispose to formation of basal cell carcinomas at an earlier age. Basal cell carcinomas are heterogeneous, from superficial or nodular lesions of good prognosis to very extensive difficult-to-treat lesions that must be discussed in multidisciplinary committees. Recent guidelines have updated the management of basal cell carcinoma. The prognosis is linked to the risk of recurrence of basal cell carcinoma or its local destructive capacity. Characteristic molecular events in these tumours are: (*i*) activation of the hedgehog pathway, which has allowed the development of hedgehog inhibitors for difficult-to-treat lesions that are not accessible to surgery or radiotherapy; (*ii*) high mutational burden, which suggests that hedgehog inhibitor refractory tumours could be offered immunotherapy; some trials are ongoing. The standard treatment for most basal cell carcinomas is surgery, as it allows excision margin control and shows a low risk of recurrence. Superficial lesions can be treated by non-surgical methods with significant efficacy.

Key words: basal cell carcinoma; treatment; prognosis; surgery; radiotherapy, hedgehog inhibitors.

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Basal cell carcinoma (BCC) is a slow-growing skin tumour, which is commonly seen in dermatology. BCCs rarely metastasize, but are frequently multiple and recurrent on sun-exposed skin, with some morbidity. BCCs are a heterogeneous group of tumours, with histopathological and clinical characteristics ranging from superficial lesions to very extensive and destructive tumours. The standard treatment for BCC is surgery, but non-surgical options (medical, systemic or physical) have been developed in recent years for each end of the spectrum of these tumours: superficial lesions (sBCC) and advanced BCC (aBCC). Guidelines have been updated to help physicians with these different therapeutic strategies (1).

SIGNIFICANCE

Basal cell carcinoma is the most frequent cancer in fair-skinned adults. The molecular background to these tumours includes activation of a cellular pathway called the "sonic hedgehog pathway". Basal cell carcinomas are induced by ultraviolet light and occur more frequently on areas of skin that are exposed to the sun. Basal cell carcinomas rarely spread to other sites in the body, although there is a risk that they will recur. There are different subtypes of these tumours with different potential to relapse. This paper gives an update of what is known about basal cell carcinomas and their treatment. The standard treatment is surgery. The prognosis for advanced basal cell carcinomas that cannot be operated on has improved with the development of systemic drugs targeting the hedgehog pathway.

EPIDEMIOLOGY OF BASAL CELL CARCINOMA

BCC is the most frequent skin cancer in fair-skinned adult patients (2). The estimated lifetime risk in this population is approximately 30% (3). The worldwide incidence of BCC is increasing continuously, but it cannot be estimated precisely as this tumour is not consistently registered. Marked geographical variations have been reported. The highest incidence is reported in Australia (up to 1,000/100,000 inhabitants per year, followed by the USA (212–407/100,000 female and male inhabitants respectively/year) and Europe (mean range from 76.21 /100,000 person-years in the UK to 157 per 100,000 person-years in 2009 in the Netherlands). This is within the range found in other European countries, such as Germany, France, Italy and Spain (4, 5). The lowest incidence is observed in Africa (<1/100,000 persons years).

BCC is most frequently seen after 50 years of age, with a female/male ratio of 2:1 (6). However, some patients develop BCC at an earlier age (<40 years). Patients with genetic predisposition syndromes, such as xeroderma pigmentosum (XP) or basal cell naevus syndrome (BCNS) can develop BCC earlier, even before 20 years of age (see the section on genetics, below). In the USA the ratio of cases of BCC to that of squamous cell carcinoma (SCC) was estimated at 4:1 and changed to 1:1 in 2012, but this is probably due to earlier SCC lesions being removed, which may have previously been treated non-surgically (7, 8).

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The most significant risk factor for development of BCC is sun exposure, both in childhood and recreationally or occupationally in adult life (9). UVA, and mostly UVB, is implicated. This explains why most tumours are located on sun-exposed skin and are more frequent in fair-skinned people. BCC is the most highly mutated human tumour (65 mutations/megabase) (10). Another risk factor is immunosuppression, with a greater than 10-fold increase in BCC, especially in kidney transplant recipients (11).

HISTOLOGICAL SUBTYPES

BCC develops from follicular and interfollicular keratinocyte stem cells (12, 13). Different clinical and histological types have been described with increasing invasiveness from superficial, nodular, morphoeic and basosquamous tumours **(Fig. 1)**. Nodular lesions represent 60% of all BCCs and appear as nodules or papules with telangiectasia. Superficial lesions are flat, erythematous, and scaly with well-demarcated edges; more frequently found on the trunk of younger adults; and represent 20% of all BCC. Morphoeic lesions are scar-like whitish plaques with indistinct borders. These tumours can also be ulcerated and pigmented.

In a review of 1,039 consecutive cases, Sexton et al. have found that most BCC are mixed (14). In these cases the most aggressive form defines the prognosis of the tumour. Basosquamous tumours are often found in advanced or difficult-to-treat lesions, which have been left without treatment for many years and are seen at an advanced stage. These lesions are classified as difficult-to-treat, in contrast to the former, which fall into the category of common BCC or easy-to-treat tumours unless they have specific management difficulty (1). In fact, these forms of difficult-to-treat BCC are heterogeneous and a classification system has been proposed by the European Association of Dermato-Oncology (EADO) and is under revision. These difficult-to-treat lesions often require imaging, with magnetic resonance imaging (MRI) or tomodensitometry, to determine the tumour extension.

Dermoscopy is useful to help with the diagnosis of BCC, revealing ovoid nests and globules, leaf-like areas, arborizing and superficial telangiectasias, erosions, pigmentation, but absence of pigment network. A recent study has shown that, in a comparison of naked eye examination and dermoscopy, the diagnosis sensitivity and specificity improved from 66.9% to 85% and 97.2% to 98.2%, respectively, with dermoscopy (15). Dermoscopy may also help to recognize the histopathological subtype of BCC (16).

DIAGNOSIS OF BASAL CELL CARCINOMA

The diagnosis of BCC requires a biopsy, unless the lesion is small or clinically and dermoscopically typical, especially in non-high risk locations (trunk). A biopsy is recommended before proceeding to complex surgery or systemic treatment (1). The biopsy can confirm the diagnosis of BCC, but may not be adequate to appreciate the histological subtype in view of the heterogeneous histology.

GENETICS OF BASAL CELL CARCINOMA

Twenty years ago, the candidate gene (germline mutation) for patients with BCNS syndrome (a genodermatose predisposing to multiple BCCs and developmental defects) was reported to be the *PTCH1* gene, leading to activation of the hedgehog pathway (Hh) (**Fig. 2**), which is a crucial event in the pathogenesis of BCC (17). *PTCH1* (located on human chromosome 9q22) encodes a transmembrane protein negatively regulating smoothened (SMO), another transmembrane protein of the pathway. When *PTCH1* binds to an extracellular ligand, such as sonic hedgehog, its negative control on SMO is relieved, allowing SMO to migrate in the cilium and activate Gli transcription factors (18, 19). Since then other germline mutations have been described in Gorlin's syndrome, targeting *PATCH2* and *SUFU* genes (20).

Activation of the Hh pathway has also been demonstrated in sporadic BCC, with 90% of the tumours bearing inactivating mutations of PATCH and 10% activation of SMO (21). These mutations are most often UV-induced: C>T transitions at dipyrimidines sites or even more specific CC>TT tandem mutations.

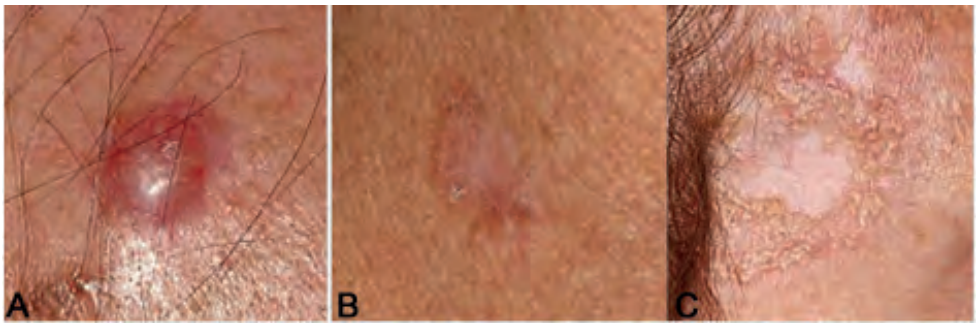

Fig. 1. Various basal cell carcinoma (BCC) clinical subtypes. (A) Nodular BCC. (B) Superficial BCC. (C) Morpheiform BCC.

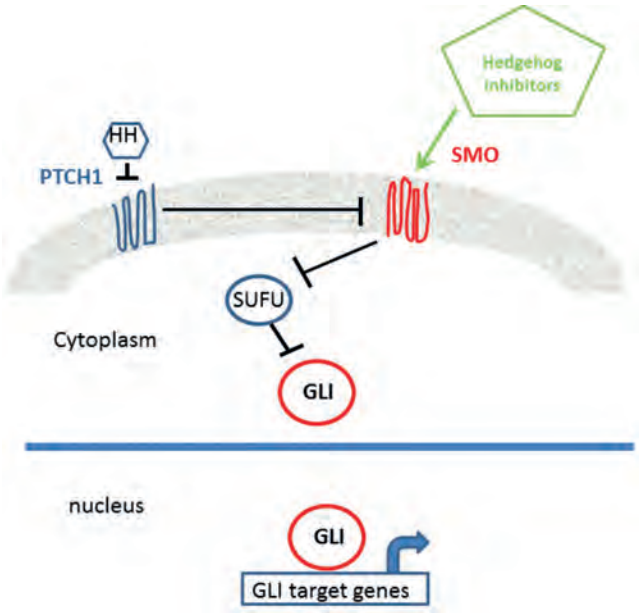

Fig. 2. Schematic view of the hedgehog (HH) pathway. When HH ligand binds to the transmembrane receptor PTCH1 it releases its inhibitory activity toward smoothened (SMO), which inhibits another negative regulator of the pathway *SUFU* leading to activation of *GLI* and *GLI* target genes. Hedgehog inhibitors are anti-SMO molecules. GLI is a transcription factor activated by SMO.

Inactivating the Hh pathway has been a major therapeutic goal for difficult-to-treat lesions and 2 oral-targeted therapies (hedgehog inhibitors or HhI) are currently available: vismodegib and sonidegib (1).

If the occurrence of mutations in the Hh pathway is considered to be the driver event toward formation of BCC, secondary drivers have been found in cancer genes, such as *MYCN, PPPC, SK19, LATS1, ERBB2, PIK23C, N-RAS, K-RAS, H-RAS, PTPN14, RB1*, and *FBX7* (22). Other pathways that increase the transcription factor of GLI include a recently described loss-of-function mutation in *SUFU* in sporadic BCC and a variety of non-canonical hedgehog signalling pathways (the mammalian target of rapamycin (mTOR), insulin-like growth factor (IGF)–PI3K–AKT, epidermal growth factor receptor (EGFR)–MEK–ERK, and Hippo pathways) that are independent of ligand–PTCH1 binding and SMO activation (23).

The impact of these other mutations on the histopathological characteristics and evolution of BCC or their response to systemic treatment is unknown.

Other genetic diseases can predispose patients to the formation of BC: XP due to germline mutations in DNA repair genes (24), which predispose to multiple skin tumours, including BCC, but also melanoma and squamous cell carcinoma (SCC), at an early age, as well as the Bazex-Dupre-Christol syndrome, and dominant X-linked cancer-prone genodermatosis, in which recent studies have reported mutations in the ACTRT1 gene and its enhancer, leading to activation of the Hh pathway in certain families (25).

PROGNOSIS OF BASAL CELL CARCINOMA

BCC very rarely metastasizes; it estimated incidence of metastasis is 0.0028–0.55% (1). A recent review of published cases showed that median survival in case of distant metastases was 24 months, and 36.2% of those had systemic chemotherapies. Regional metastasis were shown to have a median survival of 87 months (26).

The major issues with BCC are local destruction and recurrence. Mortality is low. Risk of recurrence is influenced by the location of the tumour (H zone of the face), the histological subtype, perineural invasion, immunosuppression and prior recurrences. Severe forms of BCC are heterogeneous and rare. A retrospective study from the USA reported that the severe form of BCC accounted for approximately 0.8% of all cases of BCC (27), while another reported 10/100,000 persons (28). No TNM classification is available and a grading method to classify these difficult-to-treat BCC is currently being developed by the EADO group. These advanced tumours are often not measurable by Response Evaluation Criteria of Solid Tumors (RECIST criteria) and can destroy large anatomical surfaces without affecting survival (1).

TREATMENT OF BASAL CELL CARCINOMA

Surgery

Surgery is the standard treatment for the majority of BCC (**Fig. 3**). Standard excision (SE) or micrographic surgery (Mohs) can be used according to the characteristics of the tumour (size, location, previous recurrences, histology) and the skills of the surgeon. Mohs is reserved for high-risk tumours, in recurrent BCC or BCC in critical anatomical sites. A prospective randomized trial comparing SE and Mohs showed a 10-year cumulative probability of recurrence for primary BCC of 12.2% for SE and 4.4% for Mohs and for recurrent BCCs of 13.5 for SE and 3.9% for Mohs (29).

The margins used for SE depend of the BCC recurrence risk profile. Current guidelines suggest a range of peripheral margins between 2 mm and 5 mm in low-risk tumours and between 5 mm and 15 mm in high-risk lesions (1). It has been reported that the size of the BCC also correlates with the risk of subclinical extension with a 4 mm lateral margin sufficient to excise a <2 cm BCC, while a tumour of >2 cm and additional risk characteristics may need a minimal lateral margin of 13 mm for complete removal. Deep margins recommend excision to level of the fat or, in the face, to the level of fascia, perichondrium or periosteum (30).

Clinical and histological margins do not correlate, as tissue shrinkage is observed after fixation. There is no specific recommendation nor evidence-based data to re-excision in case of complete excision with narrow margins (1).

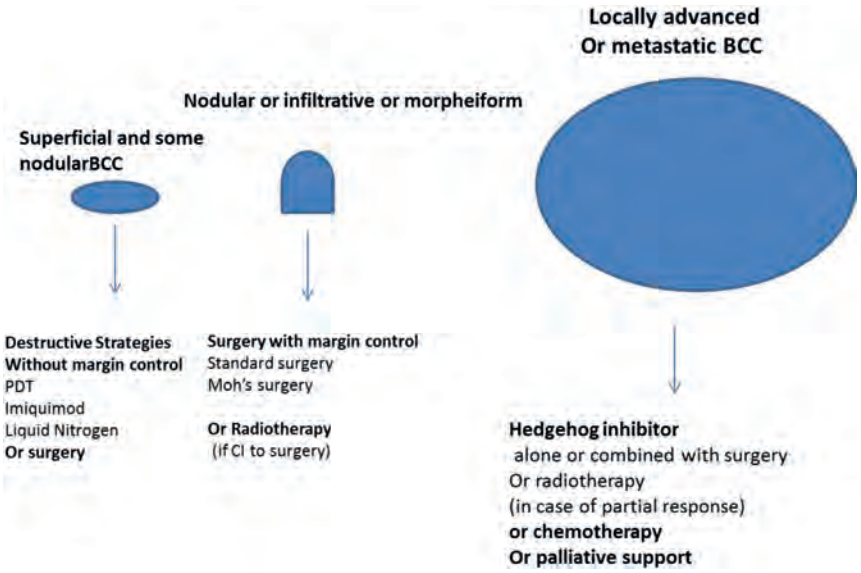

Fig. 3. Schematic landscape of treatment options for basal cell carcinoma (BCC).

What to do in cases of incomplete excision?

Incomplete excision can be reported in 4.7–24% of SE (31), and can lead to recurrence in 26–41% after 2–5 years of follow-up. If incompletely excised lesions recur, it is recommended to re-excise with wider margins, as the risk of multiple recurrence can be as high as 50% once a positive-margin BCC has recurred after surgery (32).

Radiotherapy

Radiotherapy is a good alternative to treat BCC, especially in elderly patients. It is recommended for patients who are not candidates for surgery (due to morbidity, patient's choice, advanced disease, etc.). Radiotherapy can use external beam radiotherapy, or brachytherapy or contact therapy, and this will depend on the size, location of the tumour, the team expertise and resources. It can also be considered, but has never been evaluated, as adjuvant therapy when re-excision of incompletely excised lesions is not possible or when there is perineural evasion.

Recent meta-analysis has reported an estimated recurrence rate of 3.5% with radiotherapy similar to that reported for surgery (1). Radiotherapy is contra-indicated in patients with BCC nevus syndrome (BCCNS), as it may cause further tumours in the field of irradiation

Medical treatments alternative to surgery in superficial lesions

Imiquimod. Imiquimod is an immune-response modifier, which is indicated for the treatment of superficial BCC and small nodular BCC in immunocompetent adults. It must be applied 5 times per week for 6 weeks. The major biological effects of imiquimod are mediated through agonistic activity towards toll-like receptors (TLR) 7 and 8 and consecutively, activation of nuclear factor kappa B (NFkB). The result of this activity is the induction of pro-inflammatory cytokines, chemokines and other mediators, leading to activation of antigen-presenting cells and other components of innate immunity and, finally, the mounting of a profound T-helper (Th1)-weighted anti-tumoural cellular immune response (33). Randomized comparative trials comparing 5% 5-fluorouracil (5-FU) with imiquimod 5% cream and MAL-PDT in patients with sBCC showed a treatment success of 72.8% for MAL-PDT, 83.4% for imiquimod, and 80.1% for 5% 5-FU at 1 year and 62.7%, 80.5% and 70%, respectively, at 5 years (31, 32).

The efficacy of imiquimod was also compared with surgery (S) for low-risk BCC and showed a successful response at 5 years, of 82.5% for imiquimod vs. 97.7% for surgery (34), confirming that imiquimod represents a good alternative to surgery for the treatment of sBCC.

Some local and general reactions can be observed with imiquimod, and patients should be informed of these.

5-Fluorouracil. 5-FU 5% is indicated for the treatment of sBCC (2 applications/day for 2–4 weeks), but very few studies have looked at long-term results. In the trial comparing 5-FU with imiquimod and PDT for the treatment of sBCC, 5-FU was shown to be inferior to imiquimod, but equivalent to MAL-PDT after 3 and 5 years (35).

Physically destructive treatments

Destructive treatments must be reserved for sBCC or small nodular BCC, as they evaluate the complete eradication of the tumour.

Photodynamic therapy. PDT with 5-aminolaevulinic acid (ALA) or its methyl ester (MAL) is indicated for sBCC and small nodular BCC (nBCC) less than 2 mm thickness. MAL-PDT gave clearance rates for sBCC of 92–97% and a recurrence rate at 1 year of 9%, which increased to 22% at 3 years and remained at the same rate at 5 years (36). MAL-PDT was also used and compared with surgery in nBCC and showed 91% clearance at 3 months and a sustained clearance rate of 76% at 5 years, inferior to surgery, but with cosmetic superiority. PDT with ALA nanoemulsion gel was shown to be as efficient as MAL-PDT in low-risk BCC (37).

PDT is a good option for patients with multiple superficial lesions, especially for lesions located on the back, on which application of imiquimod can be difficult.

Cryotherapy. Cryotherapy is indicated for low-risk BCC and has been shown to be as efficient as PDT in clinical trial (35). Its main advantage is the fact that it is an immediate procedure performed during the consultation. Its disadvantages are pain and the cosmetic results, as the treatment often leaves hypopigmented spots, which can last for years. Medical and physical treatments can be combined (i.e. PDT + imiquimod, rituximab and HhI, for example) (1).

Systemic treatments of difficult-to-treat or aBCC

Treatment of aBCC must be discussed in multidisciplinary committee.

Chemotherapy. No clinical trial has evaluated chemotherapy for BCC. Most chemotherapies are platinum-based. The response rate is approximately 20–30%, but the duration of response does not exceed 2–3 months (26).

In addition, in elderly patients, chemotherapy can have life-threatening adverse effects. It is usually proposed as a second- or third-line treatment after failure of HhI.

Hedgehog inhibitors. Major progress has been achieved for the treatment of difficult-to-treat BCC with HhIs (35). Two molecules, with different pharmacokinetics, but targeting the same molecule, SMO, are available: vismodegib and sonidegib. No hed-to-head comparative studies are available. Vismodegib is indicated for laBCC (i.e. not a candidate for surgery or radiotherapy) and symptomatic metastatic BCC (mBCC) at a dose of 150 mg/day, while sonidegib is indicated for laBCC only, at a dose of 200 mg/day.

Vismodegib. Vismodegib was the first approved Hh inhibitor. The ERIVANCE study, an open-labelled non-randomized study, including 104 patients, showed, in the primary analysis, (using independent reviewer assessment) a 43% overall response rate (ORR) for a laBCC cohort, with 20.6% complete response (CR) and 22.2% partial response (PR). The response rate was 30.3% for the metastatic cohort (mBCC) (38). The median duration of response (DOR) was 9.5 (laBCC) and 7.6 months (mBCC). The 30-month update of ERIVANCE showed (using investigator assessment), an ORR of 60.3% for the laBCC (including 33 CR) and 48.5% for mBCC (only PR) and a DOR of 26.2 and 14.8 months, respectively (39). The median survival was 33.4 months for mBCC and was not reached for laBCC.

The STEVIE (SafeTy Events in VIsmodEgib) study, which enrolled the largest amount of patients (1,215, with 1,119 laBCC and 96 mBCC) had a main objective on safety. The secondary objective was efficacy and confirmed results obtained with the ERIVANCE study, with 68.5% of investigator-assessed objective response including 33.4 with CR for laBCC, and a median DOR of 23 months. For mBCC the ORR was observed in 36.9%, mostly PR, and a duration of response of 13.9 months (40). A subgroup analysis showed that BCCNS patients responded better to vismodegib. This was also observed in a clinical trial (41), which objective was to study the efficacy of vismodegib to shrink existing tumours and prevent formation of new BCC, both confirmed. However, long-term follow-up shows that all patients relapse after drug interruption (41).

In a recent report looking at long-term maintenance of CR after drug interruption, it was shown that 60% of patients have relapsed after 3 years of follow-up, with 40% (when BCCNS cases are excluded) having not relapsed at the time. Among relapsing patients, 48% had become eligible for surgery and 50% were vismodegib re-challenged and showed an ORR of 85% (42).

Sonidegib. The second HhI is sonidegib. The pivotal clinical trial Basal Cell Carcinoma Outcomes with LDE225 Treatment (BOLT) was a prospective randomized double-blinded trial comparing a once-daily dose of 200 mg with 800 mg. The 200 mg dose was approved based on the risk/benefit ratio. Evaluation used very stringent modified RECIST criteria showed a response rate of 36% (43). In the 12-month update analysis of the BOLT trial, the response rate for the 200 mg group improved to 57.6% for laBCC and 7.7% for mBCC (44). The Bolt follow-up of 30 months (45) reported a response rate of 56.1% (central review) and 71.2% (investigator review) for laBCC and 7.7% and 23.1% for mBCC. The median duration of responses was 26.1 months (laBCC) and 24.0 months (mBCC). The median survival has not been reached in the 2 groups.

Both vismodegib and sonidegib, which belong to the same class of drug, share common adverse events (most frequent: muscles cramps, dysgeusia, fatigue, hair loss and weight loss). These adverse events are observed in the majority of patients and lead to drug discontinuation in 30% of cases. No treatment-related deaths were reported. Different strategies have been proposed to prevent or manage the side-effects (46). Adverse events with sonidegib seem to be slightly less frequent and less severe, but this has not been evaluated in a comparative study. Some drug holidays have been proposed to overcome these side-effects (1)

The MIKIE trial has reported efficacy results of 2 intermittent regimens of vismodegib, and showed that it did not decrease efficacy (47). The neoadjuvant use of vismodegib has been reported in a small series, and showed that, among patients treated with vismodegib 3–6 months before surgery, only one recurred after 22 months (48). Some clinical trials are ongoing into HhI in the neo-adjuvant setting: Vismoneo (NCT02667574) and NICCI (NCT03035188).

Topical treatment. Earlier attempts with treatment at topical HhI failed, but a study is currently ongoing to evaluate the interest of a topical application of HhI on the face of patients with BCNS (NCT02828111).

FOLLOW-UP

According to the type of BCC observed, the follow-up can vary. Most BCCs are discharged after confirmation of diagnosis and completeness of excision. Some high-risk patients (multiple tumours, high-risk histological subtypes, high-risk anatomical sites, immunosuppression) will need to be followed up at least each year for up to 3–5 years. Difficult-to-treat BCC, which necessitated treatment other than surgery, are followed more carefully at a rhythm decided by the multi-disciplinary board (1).

PERSPECTIVES

BCC, being one of the most highly mutated tumours, could represent a good indication for immunotherapy.

Some isolated reports have shown response to anti-PD1 in treatment-naïve or HhI-refractory patients. In addition, a proof-of-concept study showed that pembrolizumab was efficient in patients with aBCC, but showed no increase efficacy when associated with vismodegib (49).

The efficacy of nivolumab, alone or in combination with ipilimumab, and of cemiplimab (REGN2810) is currently being investigated in patients with laBCC and mBCC in 2 independent phase 2 clinical trials (https://clinicaltrials.gov).

CONCLUSION

BCCs are the most frequent skin cancers, and their management has been thoroughly reviewed in recently published guidelines. Most BCCs have an excellent prognosis and do not need long-term follow-up. For high-risk tumours, the follow-up schedules may need to be adapted to each clinical presentation.

The standard treatment for BCCs is surgery. The understanding of molecular events implicated in their development has allowed the development of new strategies, such as HhI and, more recently, immunotherapy, for difficult-to-treat tumours.

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Centenary theme section: SKIN MALIGNANCIES

REVIEW ARTICLE

Cutaneous Melanoma – A Review of Systemic Therapies

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This decade has brought significantly improved outcomes for patients with advanced melanoma with immunotherapies and targeted treatments offering utility in a variety of settings. In 2020, we can hope for durable long-term responses, and complete remission in a subset of patients with metastatic disease. In the adjuvant setting, approximately 50% improvements in recurrence-free survival are seen both with targeted and immunotherapies. Early data from neoadjuvant immunotherapy clinical trials are very promising. However, responses to treatment are heterogeneous and not always durable; further advances are required, and several emerging strategies are of particular interest. We review the systemic treatment of melanoma, discussing the treatment of unresectable stage III–IV and recurrent disease, outlining curative treatment of cutaneous melanoma in the adjuvant setting and briefly discussing neoadjuvant systemic therapies for advanced melanoma.

Key words: melanoma; systemic therapy; targeted therapy; immunotherapy.

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SIGNIFICANCE

Melanoma is an aggressive and rare skin cancer that can threaten the lives of patients it affects. New treatments have been introduced over the past decade which have dramatically changed the way in which patients with advanced melanoma are managed. Here we review the treatments currently available to patients with advanced melanoma, focusing firstly on patients with stage IV melanoma. We also review treatments available to reduce the risk of a melanoma returning – these treatments can be given either before ("neoadjuvantly") or after ("adjuvantly") a melanoma is surgically removed, but only the latter is currently approved.

Accounting for only 1% of all skin malignancies, melanoma represents the most aggressive and deadly form of skin cancer (1). Melanoma is predominantly a disease of Caucasian populations and affects men and women in equal measure. With a propensity to migrate to draining lymph nodes and any visceral organ, metastatic melanoma carries a poor prognosis.

Prior to 2011, outcomes were poor, with treatment for metastatic disease limited to palliative therapies that offered little or no survival benefit. In 2020, we can hope for durable long-term responses, and complete remission in a subset of patients. The use of immunotherapies and targeted therapies for melanoma in the metastatic, adjuvant and neoadjuvant settings will be reviewed here; the initial management of cutaneous melanoma is discussed separately. This review will cover the systemic treatment of melanoma, starting with a description of therapeutic agents. We will discuss the treatment of unresectable stage III–IV and recurrent disease, outline curative treatment of cutaneous melanoma in the adjuvant setting and briefly discuss neoadjuvant systemic therapies for advanced melanoma.

CLASSES OF THERAPEUTIC AGENTS

Immunotherapy

Immune checkpoint inhibitors. Immune checkpoint inhibitors (CPIs) are a form of immunotherapy designed to target key regulators of the immune system. Immune checkpoints provide stimulatory or inhibitory control of immunity. Tumours can use the inhibitory pathways to protect themselves from being targeted by the immune system. CPIs currently in clinical use act to block these negative pathways enabling T-cells to recognise cancer cells more efficiently. Agonists for stimulatory pathways are also in clinical development. CPIs were the first class of therapy shown to improve the overall survival (OS) for patients with advanced melanoma and provide hope of durable, long-term responses in a subset of patients. The most extensively studied immune checkpoint receptors are cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and programmed cell death protein-1 (PD-1). CTLA-4 and PD-1 induce T-cell suppression through non-overlapping mechanisms and likely impact different populations of T-cells during different phases of the immune response (CTLA-4 during priming and PD-1 during the effector phase), providing a mechanistic rationale for the combination of CTLA-4 and PD-1 blockade.

CTLA-4. Based on promising antitumour activity in preclinical cancer models (2), CTLA-4-blocking antibodies have been developed. Ipilimumab is a fully human monoclonal antibody of the IgG1 isotype that

inhibits CTLA-4 leading to enhanced T-cell activation. For T-cell activation to occur, two sequential signals are required (3–5). Firstly, antigens presented in context with the major histocompatibility complex (MHC) I or II on specialised antigen-presenting cells (APCs) must bind with T-cell receptors (TCRs). Following this, there is a translation of TCR stimulation into T-cell activation which requires a costimulatory signal, occurring when B7 surface molecules on the APC bind with CD28 T-cell-surface receptors. Subsequently, T-cell surface expression of CTLA-4 occurs, competitively inhibiting the binding of B7 to CD28, preventing the costimulatory signal and dampening down T-cell activation and proliferation. Treatment can be associated with mechanism-based, immune-related adverse events more frequently than anti-PD-1 treatment.

A second CTLA-4-blocking antibody, tremelimumab, has been developed. Tremelimumab is a fully human anti-CTLA-4 monoclonal antibody of the IgG2 isotype. However, tremelimumab failed to reach its primary endpoint of improved OS compared to standard-of-care chemotherapy for patients with previously untreated, unresectable stage III or IV melanoma (6). Clinical development of tremelimumab is ongoing in a number of non-melanoma cancers.

PD-1. Like CTLA-4, PD-1 inhibits T-cell activity and is expressed by activated T-cells. However, instead of competitively inhibiting co-stimulation by interfering with CD28/B7 ligand interaction, PD-1 negatively regulates TCR-signalling events. While CTLA-4 inhibits T-cells during the priming phase of immune responses, PD-1 is thought to inhibit activated T-cells at a later stage in peripheral tissues, playing a critical role in the maintenance of peripheral T-cell tolerance.

The first anti-PD-1 blocking antibody developed was nivolumab, a fully human monoclonal antibody of the IgG4 isotype that binds to PD-1, preventing it from interacting with its ligands. Pembrolizumab was the second anti-PD-1 blocking antibody to be used in advanced melanoma; like nivolumab, pembrolizumab is a fully human monoclonal antibody of the IgG4 isotype that binds to human PD-1 preventing ligand interaction. Nivolumab and pembrolizumab are clinically comparable in terms of efficacy and toxicity as monotherapy for inoperable melanoma (despite the absence of any head-to-head comparison), but only nivolumab is licensed for delivery as a combination with ipilimumab. The subtle preclinical and molecular differences between these two agents have been described by Fessas et al. (7). Compared with ipilimumab, anti-PD-1 blockade with pembrolizumab has been shown to have a superior clinical efficacy and improved toxicity profile with fewer SAEs and fewer patients requiring early treatment withdrawal (8).

Oncolytic virus therapy. Oncolytic viruses are a novel class of intratumoural immunotherapies that show promise for treating solid tumours. Talimogene laherparepvec (T-VEC) is a first-in-class, genetically modified, herpes simplex virus type 1-based oncolytic immunotherapy approved for the local treatment of unresectable cutaneous, subcutaneous and nodal lesions in patients with melanoma recurrent after initial surgery. The mechanism of action and clinical applications of T-VEC are described in detail by Raman et al. (9). The key study to note in the context of advanced melanoma is the OPTiM study which randomised 436 participants in a 2:1 ratio to receive intratumoural T-VEC or subcutaneous recombinant granulocyte macrophage colony-stimulating factor (GM-CSF). OPTiM first reported positive findings in late 2015 (10), and recently published final analyses confirmed T-VEC's association with durable complete responses that were associated with prolonged survival (11).

Targeted therapy

The vast majority of cutaneous melanomas harbour mutations in genes of key signalling pathways. Yet, only a small number of these are considered to be driver mutations due to their active role(s) in cancer development and progression; the others are seen as coincidental passenger mutations that are dispensable for cancer cell viability and develop over the course of tumour evolution (12, 13). The mitogen-activated protein kinase (MAPK) pathway is a complex cascade requiring sequential phosphorylation of the different pathway components. In normal cells, when MAPK activation occurs, it leads to cell growth and differentiation. In cells harbouring $BRAF^{V600E}$ mutations, the normal process of negative feedback does not occur and this results in permanent MAPK pathway activation, leading to uncontrolled proliferation. This pathway offers various points at which the protein cascade can be blocked. Mutant BRAF is a "driver oncogene" as mutant BRAF inactivation can induce cancer cell toxicity due to an acquired dependency of cancer cells on oncogenic, mutant forms of BRAF (14). Targeted inactivation of BRAF by pharmacologic inhibitors is an archetypal example of targeted therapy in cancer (14, 15). The recognition of key molecular mutation, $BRAF^{V600E}$ mutation, provided new therapeutic opportunities and facilitated the development of promising small molecule inhibitory compounds later on. Approximately 40% of melanomas harbour a BRAF mutation (16, 17), the most common being $BRAF^{V600E}$, followed by $BRAF^{V600K}$ and rarer genotypes (18).

MEK is the next kinase down from BRAF on the MAPK cascade. BRAF inhibition is the most established form of targeted therapy in melanoma and produces rapid, but often short-lived, tumour regression in the majority of patients. When MEK inhibition is added to BRAF inhibition, increased efficacy and reduced toxicity are seen. Indeed, the combination of BRAF and MEK inhibition offer greater inhibition of MAPK signalling and result in longer durations of response, higher rates

of tumour response, and less cutaneous toxicity often observed from paradoxical MAPK pathway activation with BRAF inhibitor monotherapy (19). The development of acquired resistance to combination BRAF and MEK inhibitor therapy, along with tumour heterogeneity, are formidable obstacles in the treatment of patients with advanced melanoma.

BRAF *inhibitors*. The first BRAF inhibiting tyrosine kinase inhibitor (TKI) approved by the US Food and Drug Administration (FDA) for melanoma treatment was vemurafenib in 2011 (20). The success of vemurafenib in phase I and II settings (21, 22) and then in the BRIM-3 study (23) encouraged intensive investigation of the molecular mechanisms of pathogenesis in melanoma and development of new therapeutic strategies targeting specific molecules in the MAPK pathway. Dabrafenib followed vemurafenib and is another small molecule agent inhibiting $BRAF^{V600}$ mutation-positive melanoma cell growth, demonstrating efficacy as a monotherapy in the BREAK-3 study (24). Encorafenib is a second generation BRAF inhibitor, characterised by a substantially prolonged dissociation half-life (25), and in the phase III COLUMBUS trial demonstrated superior efficacy over vemurafenib monotherapy (26).

MEK inhibitors. Preclinical and early studies demonstrated that the addition of a MEK inhibitor to a BRAF inhibitor decreased tumour growth, delaying the development of resistance and reducing occurrence of skin lesions in metastatic melanoma (27). As a results, there has been considerable interest in various combinations of BRAF and MEK inhibition. Trametinib was the first MEK inhibitor approved for the treatment of BRAF-mutated metastatic melanoma naïve to BRAF-inhibition. Trametinib is approved for use in combination with dabrafenib showing efficacy both as a monotherapy when compared to investigator's choice chemotherapy (28), and when combined with dabrafenib (29, 30). Cobimetinib is another MEK inhibitor which demonstrated efficacy while used in combination with vemurafenib in the CoBRIM study (31), while bimetinib is the most recently-introduced of the MEK inhibitors and has demonstrated efficacy in the COLUMBUS study (26).

Chemotherapy

Prior to recent advances, chemotherapy was the backbone of treatment for metastatic melanoma. Studies reported responses in 10–15% of patients with 5 year survival in only 2–6% of patients (32). Despite the poor survival statistics, agents such as dacarbazine or the combination of a platinum agent and a taxol were the standard of care for many years, due to a paucity of other useful therapeutic options. Currently chemotherapy is used infrequently, and primarily when immunotherapy and targeted therapy options have either failed or cannot be used.

TREATMENT OF UNRESECTABLE STAGE III-IV AND RECURRENT MELANOMA

Systemic therapy is indicated for patients with stage III–IV melanoma in whom surgical metastasectomy is not appropriate. Patients with oligometastatic disease should be evaluated for possible metastasectomy, as complete resection of metastatic disease can achieve cure (33, 34). In such cases, adjuvant therapy would then be recommended following complete resection to reduce recurrence risk (discussed later). This section will focus on systemic therapy for inoperable melanoma.

The primary systemic therapy options for patients with metastatic melanoma are CPIs, and, where a BRAF mutation is the driver mutation, MAPK targeted therapies. The presence or absence of a BRAF mutation is currently the only reliable predictive biomarker that can influence the treatment of advanced melanoma and must promptly and accurately be determined. Many different methods for BRAF testing are currently in use internationally (35–37), but a discussion of these is beyond the scope of this review. Targeted MAPK therapy is not indicated in patients without a characteristic BRAF mutation and may indeed be harmful to this patient group.

Whether patients with $BRAF^{V600}$ mutant melanoma should receive CPIs or MAPK targeted therapy as first line therapy is not always straightforward and prospective head-to-head comparative trials of MAPK inhibitors and CPIs are lacking. A 2019 update of survival in metastatic melanoma reported exploratory analysis of survival data from selected CPI and TKI clinical trials (38). In first line therapy, mean 3-year OS proportions were 41.3% for BRAF plus MEK inhibition, 49.9% for PD-1 inhibition and 58.4% for CTLA-4 plus PD-1 inhibition. Comparison of the mean progression free survival (PFS) and OS curves of kinase inhibition and checkpoint blockade revealed a superiority of combined BRAF plus MEK inhibition within the first 12 months, later changing to a superiority of PD-1 blockers alone or in combination with CTLA-4 blockade. In second-line or higher, BRAF plus MEK inhibition was superior to anti-PD-1 monotherapy throughout the first 3 years; mean 3-year OS proportions were 42.4% for BRAF plus MEK inhibition, and 40.1% for PD-1 inhibition.

Checkpoint inhibitors

Table I outlines key phase III CPI studies in melanoma. Nivolumab (39) and pembrolizumab (40, 41) have been established as preferred monotherapy options for inoperable melanoma given their efficacy over standard of care chemotherapies and acceptable toxicity profiles. Checkmate-067 compared nivolumab and ipilimimab as a combination with nivolumab and ipilimumab monotherapies, recently demonstrating an OS of 52% for the combination group at 5 years. This exceptional survival was associated with 59% of patients receiving the combination suffering

Table I. Landmark checkpoint inhibitor (CPI) trials in metastatic melanoma

Trial	Regimen	Patients *n*	Outcome	G3/4 AEs:
Checkmate 066 (40) Nivo 1st line	Nivo 3 mg/kg q2w vs. DTIC 1,000 mg/m^2 q2w	418	3 years OS: 51.2% vs 21.6% mOS: 37.5 vs 11.2 months	11.7% vs 17.6%
Checkmate 037 (41) Nivo 2nd line	Nivo 3mg/kg q2w vs. ICC	405	ORR: 27% vs 10% mOS: 16 vs 14 mo mPFS: 3.1 vs 3.7 mo	14% vs 34%
Checkmate 067 (42) Ipi + Nivo 1st line	Comparision of 3x 3-weekly regimens: Nivo 1mg/kg + Ipi 3 mg/kg q3w vs. Nivo 3 mg/kg q2w vs. Ipi 3 mg/kg x 4 doses	945	PFS at 60 months: 36%* (Ipi +Nivo) vs 29%* (Nivo) vs 8% (Ipi) OS at 60 months: 52% (Ipi +Nivo) vs 44% (Nivo) vs 26% (Ipi)	59% (Ipi+Nivo) vs 23% (Nivo) vs 28% (Ipi)
Keynote-006 (8) Pembro 1st line	Pembro 10 mg/kg q2w vs. q3w vs. Ipi 3 mg/kg q3w x 4 doses	834	mOS at 60 months: 32.7% vs. 15.9% mPFS at 60 months: 8.4 months vs 3.4 months	17% vs 50%
Keynote-002 (39) Pembro 2nd line (Ipi refractory)	Pembro 2 mg/kg q3w vs. Pembro 10 mg/kg q3w vs. ICC	180	PFS at 28 months: 36% (pembro 2 mg) vs 38% (pembro 10 mg) vs 30% (ICC) mOS at 28 months:13.4 (pembro 2 mg) vs 14.7 (pembro 10 mg) vs 11.0 months	13.5% (pembro 2 mg) vs 16.8% (pembro 10 mg) vs 26.3% (ICC)

mOS: median overall survival; HR: hazard ratio; mPFS: median progression-free survival; PD: progressive disease; G: grade; AE: adverse event; TRAE: treatment-related adverse event; Ipi: Ipilimumab; Nivo: Nivolumab; Pembro: Pembrolizumab; DTIC: Dacarbazine; ICC: investigator's choice chemotherapy.

grade 3 or 4 adverse events (42). As such, combination PD-1 and CTLA-4 blockade is usually considered only for those patients with a very good performance status, with some institutions and oncologists preferring CPI monotherapy for metastatic disease. Untreated brain metastases represent one particular clinical scenario in which combination CPI offers particular advantage and may be preferred in this instance (43).

MAPK pathway inhibition

Overall response rates to vemurafenib, dabrafenib and encorafenib monotherapies are 45%, 51% and 60%, respecively (29, 44, 45). A number of studies have presented clear evidence that the combination of these agents with a MEK inhibitor provide increased efficacy with a reduction in toxicity (**Table II**). In the COLUMBUS study, encorafenib plus bimetinib showed favourable efficacy compared with encorafenib or vemurafenib monotherapy, with the combination associated with an improved tolerability profile compared with either monotherapies (26). The CoBRIM study showed improved survival of vemurafenib and cobimetinib compared with vemurafenib alone, with no significant difference in toxicity (31). Robert et al. recently analysed pooled extended survival data from COMBI-d and COMBI-v trials ($n=563$) which compared dabrafenib and trametinib with dabrafenib and vemurafenib monotherapies, respectively, reporting complete responses in 19% of patients and improved long-term outcomes, with OS rates of 71% and less toxicity seen with the combination of BRAF and MEK inhibition (29).

Checkpoint and MAPK inhibition combinations

Increasing evidence suggests that BRAF and MEK inhibition has an immune-modulating effect, enhancing anti-tumour immunity (47–49). Early evidence from treatment of advanced melanoma with BRAF inhibition demonstrated increased expression of PD-1 and its

Table II. Landmark mitogen-activated protein kinase (MAPK) targeted therapy trials in metastatic melanoma

Trial	Regimen	Patients *n*	Outcome	Toxicity
BRIM-3 (23)	Vemurafenib 960 mg BD vs. DTIC 1,000 mg/m2 q3w	675	mOS: 13.6 vs 9.7 months mPFS: 6.9 vs 1.6 months	Modification/Interruption: 38% vs 16%
BREAK-3 (24)	Dabrafenib vs. DTIC	250	mPFS: 5.1 months vs 2.7 months	G3/4 AEs: 12.8% vs 17.4%
METRIC (28)	Trametinib 2 mg/day vs. ICC	322	mPFS: 4.9 vs 1.5 months 5 year OS: 32% vs 17%	G3/4 AEs: 29% vs 12%
CoBRIM (31)	Vemurafenib + Cobimetinib 60 mg OD vs. Vemurafenib 960 mg BD + placebo	495	mOS: 22.5 months vs 17.4 months mPFS at 5 years: 12.6 vs 7.2 months 5 years OS: 30.8% vs 26.3%	G3/4 AEs: 60% vs 52%
COMBI-d (46)	Dabrafenib 150 mg BD + Trametinib 2 mg OD vs. Dabrafenib 150 mg + placebo	423	3 years OS: 44% vs 32% mPFS: 11.0 vs 8.8 months 5 years pooled resuls with COMBI-d: CR in 19%; OS rates of 71% (29)	G3/4 AEs: 48% vs 50%
COMBI-v (30)	Dabrafenib 150 mg BD + Trametinib 2 mg OD vs. Vemurafenib 960 mg BD	704	3 years OS: 45% vs 32% 3 years PFS: 25% vs 11% 5 years pooled resuls with COMBI-v: CR in 19%; OS rates of 71% (29)	G3/4 AEs: 58% vs 66%
COLUMBUS (26)	Encorafenib 450 mg OD + Bimetinib 45 mg BD (Combo) vs. Encorafenib 300 mg OD vs. Vemurafenib 960 mg BD	577	mOS: 33.6% (combo) vs. 23.5 months (enco) vs 16.9% (vem) mPFS: 14.9 months (combo) vs. 9.6 months (enco) vs. 7.3 months (vem)	G3/4 events ocurred in 68% (combo), 68% (enco) and 66% (vem)

AEs: adverse events; OD: once daily; BD: twice daily; mOS: median overall survival; HR: hazard ratio; mPFS: median progression-free survival; PD: progressive disease; G: grade; AE: adverse event; DTIC: Dacarbazine; ICC: investigator's choice chemotherapy; enco: encorafenib; vem: vemurafenib; combo: combination; CR: complete response.

ligand, PD-L1 (50), suggesting there may be a therapeutic benefit in combing BRAF inhibition with CPI. A phase 1 study showed vemurafenib and ipilimumab to have an unacceptable rate of hepatic toxicity, leading to its discontinuation (51). A preclinical study demonstrated that treatment with BRAF and MEK inhibition, in the presence of the oncogenic $BRAF^{V600}$ mutation, improved CPI anti-cancer effect without any negative impact on immune cell function (47), as had previously been thought may be the case (52). It is believed that MEK inhibition has a protective effect on $CD8^+$ T-cells due to chronic TCR stimulation (53). Such toxicity in the context of BRAF inhibition may be related to the paradoxical activation of MAPK in BRAF wild-type cells and can be ameliorated by the addition of a MEK inhibitor (54).

Preclinical data provide rationale to support testing of a triple combination of BRAF inhibition, MEK inhibition and PD-1 blockade (47, 53). A number of trials have reported relatively initial results with some 1- and 2-year data available, indicating that the combination of CPI and TKI may have a role as standard of care within the next numbers of years (**Table III**).

ADJUVANT THERAPY FOR RESECTED MELANOMA

The role of adjuvant therapy in patients with resected stage III melanoma is a rapidly evolving field. Interferon was the first agent shown to have utility in this space, however, advances in both targeted therapies and immunotherapies have led to a number of practice-changing adjuvant trials in resected stage III and IV disease. By eliminating the micrometastatic disease that remains after surgery, adjuvant systemic therapy aims to reduce disease recurrence and ultimately improve rates of cure following surgical resection of locoregional or stage IV disease. Patients with resected stage III or IV disease have significant differences in predicted survival at 5 years ranging from approximately 80% for stage IIIa disease to less than 20% for resected stage IIId disease (58). Adjuvant treatment with either CPI or MAPK targeted therapy have dramatically changed outcomes for this patient group, with approximately 50% increased recurrence-free survival (RFS) for both treatment approaches (59–62). CPIs and MAPK targeted therapies have not been directly compared in phase III studies and there is currently no clear consensus on choice of approach for patients with a $BRAF^{V600}$ mutation in the adjuvant setting.

For patients with stage I and II primary tumours and a negative sentinel lymph node biopsy, there is presently no indication for adjuvant therapy (63). It is worth noting that patients with high risk (primary tumour >4 mm, or >2 mm with ulceration) but node negative tumours were excluded from the phase III clinical trials that evaluated nivolumab, ipilimumab and the targeted therapy doublet of dabrafenib and trametinib (62, 64, 65). As such, data on adjuvant therapy in this cohort of patients is not available and is currently under investigation.

Adjuvant checkpoint inhibitors

As already discussed, CPI represents an important advance in the treatment of patients with inoperable melanoma. These results led to the evaluation of these agents in the adjuvant setting for patients at high risk of recurrence following initial surgery. Adjuvant treatment with ipilimumab at 10 mg/kg dosing was shown to have a 10% absolute improvement in OS and RFS, but toxicity and high treatment-related death rates limited its widespread use and it was never licensed for this indication in Europe (66). Only 13.4% of patients completed the full planned course of treatment, and nearly 40% of patients discontinued treatment after the first 4 doses due to treatment-related side effects. Adjuvant anti-PD-1 therapy has been tested in two large phase III studies, Checkmate 238 and Keynote 054, which have established nivolumab and pembrolizumab as the CPIs of choice for the adjuvant treatment of resected melanoma (60, 67). **Table IV** summarises the key trials in this setting.

Adjuvant targeted therapy

A key study in this context is COMBI-AD, a study of 870 Stage III BRAF mutant melanoma patients in the adjuvant setting following excision and lymphadenectomy (61, 64). They were randomised to the combination arm of dabrafenib and trametinib, or to matching placebos

Table III. Landmark check-point/tyrosine kinase inhibitor (CPI-TKI) targeted therapy trials in metastatic melanoma

Trial	Regimen	Patients *n*	Outcome	Toxicity
Keynote 022 NCT02130466 (55)	Pembrolizumab 2 mg/kg + Dabrafenib 150 mg BD + Tremetibib 2 mg OD vs. Placebo + Dabrafenib 150 mg BD + Tremetibib 2 mg OD	120	mPFS: 16.0 vs 10.3 mDOR: 18.7 months vs 12.5 mOS: NR vs 23.4	G3-5 AEs: 70% vs 45%
IMspire150 NCT02908672 (56)	Atezolizumab 840 mg D1 and D15 + Vemurafenib 960 mg BD + Cobimetinib 60 mg/D vs. Placebo + Vemurafenib 960 mg BD + Cobimetinib 60 mg/day	514	PFS: 15.1 vs 10.6 months 2 years OS: 60.4% vs 53.1%	G3-5 AEs: 33.5% vs 28.8%
COMBI-i NCT02967692 (57)	Spartalizumab 400mg q4W + Dabrafenib 150 mg BD + Tremetinib 2 mg QDS	36	ORR: 75% (33% CR) 12 months PFS: 65.3% 12 months OS: 85.9%	75% had G3/4 AEs

AEs: adverse events, OD: once daily, BD: twice daily, mOS: median overall survival; HR: hazard ratio; mPFS: median progression-free survival; mDOR: median duration of response; ORR: overall response rate; NR: not reached; PD: progressive disease; G: grade; AE: adverse event; DTIC: Dacarbazine; ICC: investigator's choice chemotherapy.

Table IV. Summary of randomised controlled trials of adjuvant therapy for patients with cutaneous melanoma

Trial	Agents	Patients	Primary Endpoint	12 months RFS	Toxicity
EORTC 18071 (66)	Ipi vs. placebo	Complete resection in Stage III	Median RFS: 26-mo vs 17-months 7-year OS: 60% vs 51.3%	64% vs. 56%	G3/4 AEs: 54% vs. 26% 1% death from Ipi AE
Checkmate 238 (59, 67)	Nivo vs. Ipi	Complete resection in Stage IIIB, IIIC, IV	3-year RFS: 58% vs. 45%	71% vs. 61% Stage III alone: 72% vs. 62%	G3/4 AEs: 14% vs. 46% 0.4% death from ipi SAE
COMBI-AD (61)	D&T vs. placebo	Complete resection in Stage III	RFS 4 years: 54% vs 38% 3 years OS: 86% vs. 77%	88% vs. 56%	SAE: 36% vs. 10% 1 death D&T
Keynote 054 (60)	Pembro vs. placebo	Complete resection in Stage III	12-months RFS: 75% vs. 61%	75% vs. 61%	G 3/4 AE: 15% vs. 3% 1 death pembro
BRIM8 (68)	Vem vs. placebo	Complete resection: Stage IIC-IIIA/B (cohort 1) and IIIC (cohort 2)	Median DFS: Cohort 1: NR vs. 37-months Cohort 2: 23-months vs. 15-months	Cohort 1: 84% vs 66% Cohort 2: 79% vs 58%	G3/4 AE: 57% vs. 15% SAE: 16% vs. 10%

AE: adverse event; DFS: disease-free survival; EORTC: European Organization for Research and Treatment of Cancer; Gr: grade; NR: not reached; OS: overall survival; Pembro, pembrolizumab; Ipi: Ipilimumab; Nivo: Nivolumab; Vem: Vemurafenib; plac: placebo; D&T: Dabrafenib & Trametinib; RFS: recurrence-free survival; SAE: serious adverse event.

for one year. The primary endpoint, RFS, was longer with dabrafenib and trametinib than with placebo (4-year rate: 54% vs 38%; hazard ratio [HR] 0.49, 95% CI 0.40–0.59), with treatment benefits observed irrespective of baseline factors, according to subgroup analysis (61). Vemurafenib was compared to placebo in the adjuvant BRIM8 study demonstrating efficacy but high rates of grade 3/4 toxicity (68).

NEOADJUVANT THERAPY FOR EARLY MELANOMA

Given the success of immunotherapies and targeted therapies for the treatment of advanced melanoma, the natural extension is to identify the role of these therapies in the neoadjuvant setting, with a wealth of clinical trials currently underway. Patients with clinically detectable stage III melanoma represent a high-risk population with poor outcomes when treated with upfront surgery alone and are obvious candidates for investigation of neoadjuvant therapy. However, the clear need to carefully evaluate short-term clinical endpoints such as RFS, and long-term endpoints of neoadjuvant therapy against those of adjuvant therapy remains. Neoadjuvant therapy for melanoma is not presently standard-of-care but represents an active area of research with a large number of completed and recruiting trials with differing designs, endpoints, and methods of analysis under investigation. **Table V** illustrates those neoadjuvant (preoperative therapy) trials which have reported data.

One study of note is OPACIN-NEO study which reported in 2018 (69). OPACIN-NEO examined neoadjuvant combination CPI with 3 different regimens of ipilimumab and nivolumab. A combination of ipilimumab at 1 mg/kg combined with nivolumab at 3 mg/kg given 3-weekly for two cycles was chosen to take forward into later phase studies, as this combination had a response rate of 77%, with responders experiencing excellent outcomes to date. If more mature data confirm these early observations, this schedule will be tested in randomised phase 3 studies versus adjuvant therapies, which are the current standard-of-care systemic therapy for patients with stage III melanoma.

FUTURE DIRECTIONS AND CONCLUSION

The investigation of new immunotherapy and/or targeted therapy combinations, such as anti-PD-1/anti-CTLA-4 CPIs with other immunotherapies (e.g. indoleamine 2,3 dioxygenase inhibitors, antilymphocyte activation 3, histone deacetylase inhibitors, Toll-like receptor 9 agonists, anti-glucocorticoid-induced tumour necrosis factor receptor, pegylated interleukin-2), combination targeted therapies (e.g. MEK and CDK4/6 co-inhibition), and the combined use of immunotherapy and continued research on targeted therapy (e.g. the triplet combination of BRAF/MEK inhibition with anti–PD-1s) are keys for the future of systemic therapy for advanced melanoma. The identification of novel therapeutic targets in the MAPK pathway provides opportunity to improve outcomes by overcoming *de novo* and acquired resistance to BRAF/MEK inhibition. Adoptive cell transfer may have a potential role in patients whose disease has progressed following CPI. Altogether, these new approaches offer potential to build upon past advances and improve long-term survival outcomes for patients with melanoma.

This decade has brought significantly improved outcomes for patients with advanced melanoma with the advent of immunotherapies and targeted treatments that have utility in a variety of settings. However, responses to treatment are heterogeneous and not always durable. Further advances are required, and several emerging strategies are of particular interest.

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Table V. Neoadjuvant trials with available data

Trial	Eligible patients *n*	Regimen	Median follow-up (months)	Results	TRAEs
IMMUNOTHERAPY					
NCT00972933 (70) 2018	Clinical stage IIIB or IIIC and oligometastatic stage IV; *n* = 35	Two neoadjuvant doses of ipi (10 mg/kg), surgery, followed by two adjuvant doses of ipi	18	RFS: 11 months No pPR or pCR reported	G3 AEs: 32%
NCT02437279 (71) 2018	Clinical stage III; 10 per group	Surgery plus 12-week adjuvant ip (3 mg/kg) and nivo (1 mg/kg); 6 weeks of neoadjuvant and 6 weeks of adjuvant ipi (3 mg/kg) and nivo (1 mg/kg)	32	30% pCR, 40% near pCR, 0% pPR	G/43 adverse events: 90% of participants in the surgery group vs 90% of participant in the neoadjuvant therapy group
NCT02519322 (72) 2018	Clinical stage III and oligometastatic stage IV 12 participants in the nivo-only group and 11 in the ipi plus nivo group	4 doses of nivo (3 mg/kg) neoadjuvant therapy, surgery, and 24 weeks of nivo adjuvant therapy; 3 courses of ipi (3 mg/kg) plus nivo (1 mg/kg) neoadjuvant therapy, surgery, and 24 weeks of adjuvant nivo	20	Group A: pCR 45% Group B; pCR 25% RFS: 56% participants in the nivo-only group vs. 81% participants in the ipi-nivo group	Nivolumab-only: 8% participants had G3 AEs; ipi plus nivo: 73% participants had G3 AEs; No G4/5 AEs in any group
NCT02977052 (69) OpACIN-neo 2019	Clinical stage III; 30 in group A; 30 in group B; and 26 in group C	Group A: two courses of ipi (3 mg/kg) plus nivo (1 mg/kg) once every 3 weeks; Group B: two courses of ipi (1 mg/kg) plus nivo (3 mg/kg) once every 3 weeks; Group C: two courses of ipi (3 mg/kg) once every 3 weeks plus two courses of nivo (3 mg/kg) once every 2 weeks	8.3	43% of non-pCRs relapsed; no relapses reported in the other response groups	G3/4 AEs: 40% in group A vs 20% in group B vs 50% in group C
NCT01608594 (73) 2018	Clinically detectable locally and/or regionally advanced melanoma *n* = 28	Ipilimumab 3 or 10 mg/kg high-dose interferon	32	32% pCR	At median follow-up of 32 months, 10/11 patients with either pCR or minimal residual disease remained disease free More grade 3/4 irAEs were seen with ipilimumab 10 mg/kg versus 3 mg/kg ($p = 0.042$)
NCT02339324 (74) 2018	Stage 3 and 4 resected (5 x IIIB, 11 x IIIC and 4 x IV) *n* = 20	Pembrolizumab 200 mg with high-dose interferon	11	35% pCR	90% of patients had to stop early due to G3/4 toxicities
TARGETED THERAPY					
NCT02231775 (75) 2018	Clinical stage IIIB or IIIC and oligometastatic stage IV with BRAFV600E/V600K mutation *n* = 21	Neoadjuvant dabrafenib (150 mg twice a day) plus trametinib (2 mg daily) for 8 weeks followed by surgery and 44 weeks of the same adjuvant treatment versus surgery	18.6	pPR 17% and pCR 58% RFS: 19.7 mo for adjuvant systemic vs 2.9 mo for surgery group	A: G3: 47% of participants in the neoadjuvant systemic therapy group had G3 AEs
NCT01972347 (76) NeoCombi 2019	Clinical stage III with BRAFV600E/V600K mutation; *n* = 35	Dabrafenib (150 mg twice a day) plus trametinib (2 mg daily): 12 weeks neoadjuvant therapy and 40 weeks of adjuvant therapy	27	23 mo of overall RFS (30 mo of pCR, 18 mo of non-pCR)	57% participant had any grade 3 adverse events; 3% had any g G4 AEs and 26% had surgical G3 AEs; 26% had drug-related grade 3 events and 3% drug-related G4 AEs
Sloot et al. (77) 2016	Stage III Of 15, 6 underwent surgery	Vemurafenib 960 mg BID or Dabrafenib 150 mg QD ± Trametinib	25.4	pPR 33% and pCR 33%	Dose reduction or discontinuation because of toxicities occurred in 10/15 patients
Zippel et al. (78) 2017	Stage III *n* = 12	Vemurafenib 960 mg BID or Dabrafenib 150 mg QD ± Trametinib 2 mg QD	20	pPR 62% and pCR 31%	N/a
Eroglu et al. (79) 2017	Stage IIIC and IV *n* = 20	Vemurafenib Dabrafenib + Trametinib Encorafenib + Binimetinib	25	pCR 35%	Not reported

pCR: pathological complete response; mo: months, G: grade; TRAE: treatment related adverse events; AE: adverse event; ipi: ipilimumab; nivo: nivolumab; pPR: pathological partial response; pCR: pathological complete response.

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REVIEW ARTICLE

Biomarkers Predicting for Response and Relapse with Melanoma Systemic Therapy

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Introduction of new systemic therapies in the last 10 years has radically improved outcomes for melanoma patients. Even so, not all patients benefit, so getting the right treatment to the right patient is a priority. These two major drug classes, small molecule targeted kinase inhibitors and immune checkpoint inhibitors, both come at significant cost, with sometimes serious side effects as well as high expense for health services. Almost half of melanomas harbour a *BRAF*^{V600} mutation and virtually all patients receiving BRAF targeted therapy will experience some amount of response. However, duration of response with these agents is uncertain, due to acquired resistance, which means few patients remain in response long term. Most metastatic melanoma patients are potentially eligible for immune checkpoint inhibitors, irrespective of BRAF status. However, only about half of patients will respond to these agents, and only half again will benefit long term. Thus, both primary and acquired resistance limit response. In this era of personalized anti-cancer therapy, biomarkers offer a means to predict for both response and relapse to a particular treatment. To date, the only validated biomarker applied to selecting melanoma systemic therapy is the *BRAF* gene. However, modern technologies are now opening up a wide range of candidate genes, polypeptides and proteins which are being evaluated for their potential clinical application as predictive biomarkers of the future.

Key words: melanoma; biomarkers; immunotherapy; BRAF targeted therapy; response.

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SIGNIFICANCE

Systemic therapy options for melanoma patients are rapidly increasing. They offer life extension for many, but not all patients benefit. These high cost drugs also have complex, life-changing and potentially life-threatening side effects. Modern 'Precision Medicine' aims to personalize therapy for individuals and hence offer the opportunity to selectively treat only those expected to benefit from a particular therapy, while avoiding exposure to ineffective treatment in others. To date, the only validated predictive melanoma biomarker guiding treatment decisions is the BRAF gene mutation, although emerging modern technologies are identifying many more candidates whose clinical application have yet to be ascertained.

In the last decade, treatment of metastatic melanoma has undergone unprecedented transformation, with two new classes of anticancer drugs entering routine clinical practice, tripling overall survival of people whose life expectancy previously was limited to under one year. Both sets of drugs – BRAF targeted therapies and immune checkpoint inhibitors – are now being offered earlier in the disease pathway, to people who have undergone surgery for locoregional melanoma, based on evidence that adjuvant therapy halves the rate of recurrence (1–3). Despite this positive outlook, there are serious limitations yet to be overcome: little more than half of metastatic melanoma patients embarking on systemic therapy will achieve durable response, drug-induced toxicity can be life-threatening and certainly life-changing, while the cost of chronic drug prescribing is crippling many healthcare systems.

This same decade has seen a massive step change in our understanding of cancer biology. We are now in the era of 'Precision medicine', which aims to personalize treatment based on specific biological characteristics of an individual and their cancer. So-called biomarkers should, in theory, enable preferential selection of effective treatment, while avoiding exposure to inactive drugs causing unnecessary side-effects, thus also contributing to more cost-effective healthcare. Primary and acquired resistance to both molecularly targeted agents and immunotherapy limit treatment response. Therefore, biomarkers may be valuable adjuncts to clinical decision-making both prior to initiation of treatment, as well as during treatment, to predict the likelihood of treatment failure and disease relapse (**Fig. 1**). In practice, despite an explosion of research in this field, the role of predictive biomarkers in the clinic currently remains limited. The case of modern melanoma therapeutics well illustrates both the successes and challenges of biomarker discovery and their application.

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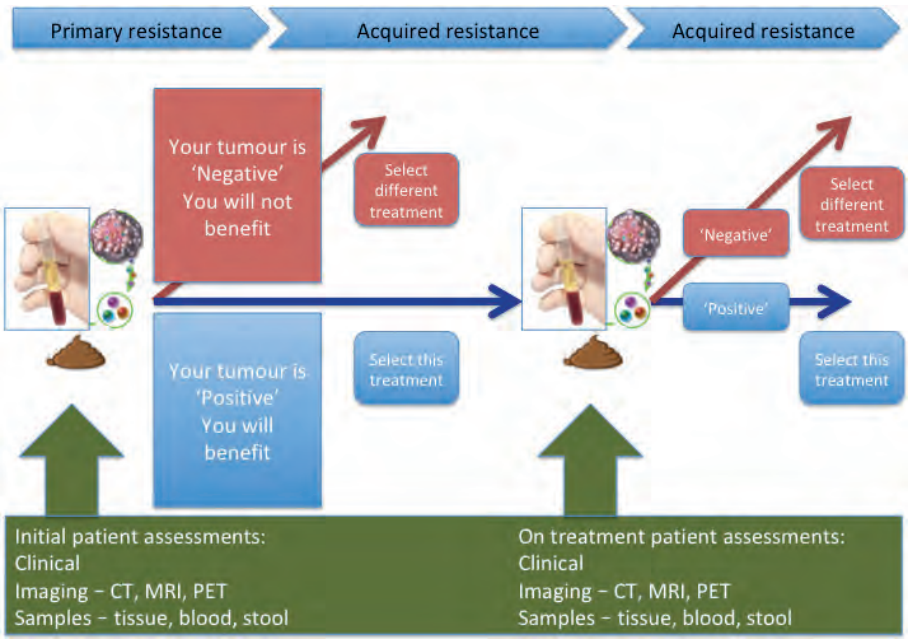

Fig. 1. Integrating biomarkers into routine clinical practice.

BRAF – THE PERFECT BIOMARKER?

In the whole of modern drug development, the mutant *BRAF* gene stands out as a massive success story in biomarker discovery. In 2002, a team at the Wellcome Sanger Institute reported *BRAF* mutations in 66% of melanoma cell lines tested and these findings were subsequently corroborated in melanoma patients (4). Its success as a treatment response biomarker is thanks to a talented biochemist who designed a drug to specifically block the active kinase domain of the mutant BRAF protein. This 'lock and key' approach generated groundbreaking responses in *BRAF* mutant metastatic melanoma patients treated in the phase 1 trial of the first specific BRAF kinase inhibitor, vemurafenib (5). In subsequent large-scale randomised trials, BRAF-targeted kinase inhibitors have generated objective response rates of up to 70% with virtually all treated patients experiencing some degree of response (6). The limitation of BRAF inhibition, however, is duration of response, due to onset of secondary resistance in most cases within a year of starting treatment.

Molecular characterization of tumours biopsied at the time of disease progression showed that reactivation of MEK downstream of BRAF was a consistent feature. Dual blockade with BRAF and MEK inhibitor combination regimens delay onset of secondary resistance, significantly extending duration of response (6). Unequivocal evidence that mutant *BRAF* drives malignancy in some 45% of melanomas led rapidly to adoption of *BRAF* testing of patient's tumour tissue into routine clinical practice worldwide. Progression biopsies identified emergence of new mutations associated with loss of treatment response, some of which might be actionable and offer options for subsequent treatment.

However, accessing tumour is not always practical and is fraught with issues, particularly around tumour heterogeneity. Measuring circulating tumour DNA (ctDNA) in plasma as a 'liquid' biopsy offers an attractive, less-invasive alternative surrogate for disease burden. Preliminary studies support mutant *BRAF* ctDNA as a biomarker predicting for minimal residual disease and recurrence after surgical resection of locoregional melanoma (7) as well as lending value to monitor metastatic melanoma patients on treatment (6, 8), for early signs of both response and disease progression. Although a significant step change in patient management, work is still needed to optimize and standardize liquid biopsy methodologies, while larger scale prospective trials are essential to fully determine the clinical application of ctDNA before being introduced into routine clinical practice.

Other less common driver mutations occurring in melanoma include *NRAS*, *PTEN* loss and *CKIT*. Despite attempts to block signalling from these aberrant pathways, clinical benefits have been modest and no targeted agents have yet been approved for patients with these molecular characteristics. Currently, therefore, their significance as biomarkers is confined to research studies.

CLINICAL BIOMARKERS OF RESPONSE

In contrast to molecular targeted agents, and also to some other cancers for whom they are approved, access to immune checkpoint inhibitors is not limited by any biomarker-determined subgroup of melanoma patients. Since first tested in melanoma patient trials, eligibility has been primarily determined by concerns for patient safety, as well as enrichment for better prognostic groups. Outside of clinical trials, real world experience has widened access and together with increasing understanding of how checkpoint inhibitors work, some clinical features have emerged that may help predict for benefit. This is particularly pertinent for *BRAF* mutant melanoma patients, who must choose which order to access the two drug classes available to them.

Immune checkpoint inhibitors rely on activating cytotoxic ($CD8^+$) T-cell function, which can take a few weeks to kick in after initiating therapy. Evidence suggests that patients with slowly progressing, low disease

burden (reflected in routine clinical and laboratory parameters including good performance status, normal serum lactate dehydrogenase, few organs involved, non-visceral disease) tend to respond to checkpoint inhibitors better than patients with high burden, rapidly progressing disease. These factors are readily identifiable in the clinic, but mainly reflect overall disease prognosis. Similarly, they predict for better outcomes with BRAF-targeted therapy (9) (**Fig. 2**). A recent meta-analysis of advanced melanoma interventional registration trials of systemic targeted therapies and checkpoint inhibitors demonstrated that BRAF-targeted therapies offer superior overall survival in the short term, which may be the priority for those patients with more aggressive disease and poorer prognosis, but checkpoint inhibition offers longer term survival gains for those who respond (10). However, given complex toxicities, high drug cost and limited overall survival benefits, there is a pressing need to utilise modern scientific capability to select the right treatment for the right patient based on their individual disease biology.

CHALLENGES OF CHECKPOINT INHIBITORS

Increasing numbers of melanoma patients are receiving immune checkpoint inhibitors as their first line of treatment both in the adjuvant and advanced setting, striving for long term survival benefits. The dominant agents in clinical use are the anti-PD-1 antibodies, nivolumab and pembrolizumab (6). Both are generally well tolerated in all age groups, so in this modern age, advancing years is not a barrier to access and the numbers of melanoma patients being treated worldwide is rising exponentially, despite relatively modest benefits: response rate in metastatic melanoma is around 40%, while only the minority of those patients receiving adjuvant anti-PD-1 monotherapy are likely to benefit (1,2). Identifying the subgroup of patients expected to respond is a major research priority. Anti-PD-1 agents are licensed to be administered until disease progression, but chronic drug administration is driven by Pharma, not by biology. Can biomarkers also help determine treatment duration for an individual patient?

As a strategy to enhance activity, nivolumab (nivo) was combined with the anti-CTLA-4 antibody, ipilimumab (ipi) and the combination (ipi+nivo) regimen was compared to both monotherapies in the CheckMate 067 international registration trial. Response rates with the combination regimen were higher, reaching 58% for ipi+nivo compared with 45% for nivo and 19% for ipi, but the overall survival gain with ipi+nivo compared with nivo alone was marginal: 4-year overall survival 53% versus 46% (11). On the other hand, ipi+nivo was associated with a three-fold increase (59% versus 22%) in severe or life-threatening adverse events, compared to nivo alone, while 40% and 12% of patients discontinued treatment due to adverse events in these 2 trial arms. There is therefore a pressing need to identify those patients unlikely to benefit from the combination regimen to avoid unnecessary treatment-related toxicity.

In the last 5 years, a huge amount of resource has been invested in better understanding tumour immunology with significant focus on identification of biomarkers to address the questions posed here. As summarised by

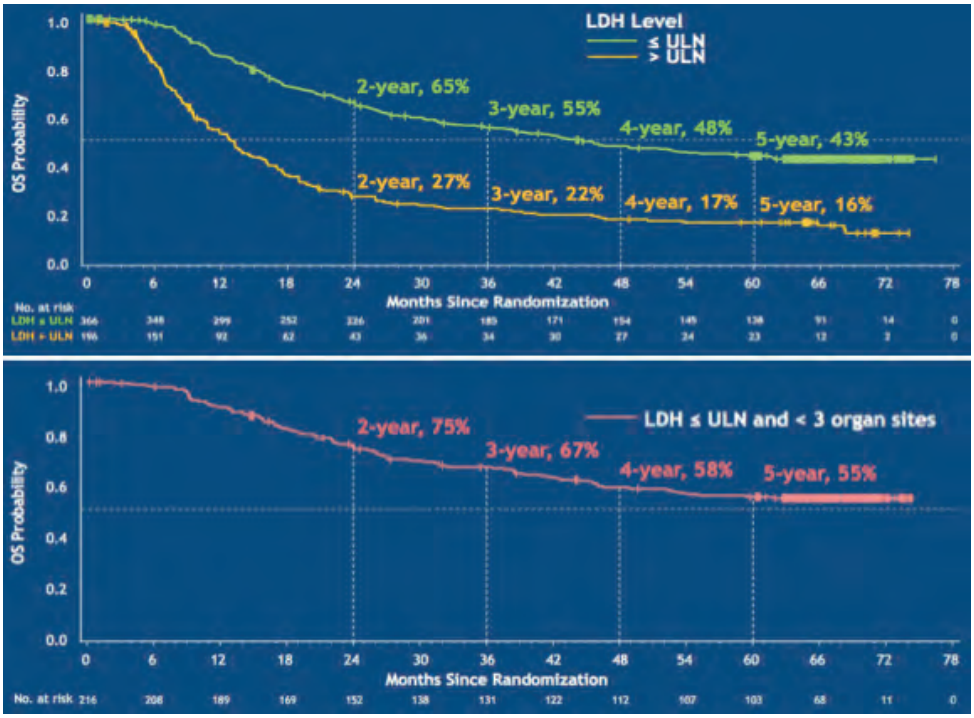

Fig. 2. Impact of tumour burden (as defined by lactate dehydrogenase (LDH) and number of body organ sites affected) on overall survival (OS) following treatment with dabrafenib+trametinib. ULN: upper limit of normal. (Reprinted with permission from The New England Journal of Medicine, Caroline Robert et al., Five-Year Outcomes with Dabrafenib plus Trametinib in Metastatic Melanoma, 381:626-636. Copyright © (2019) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society).

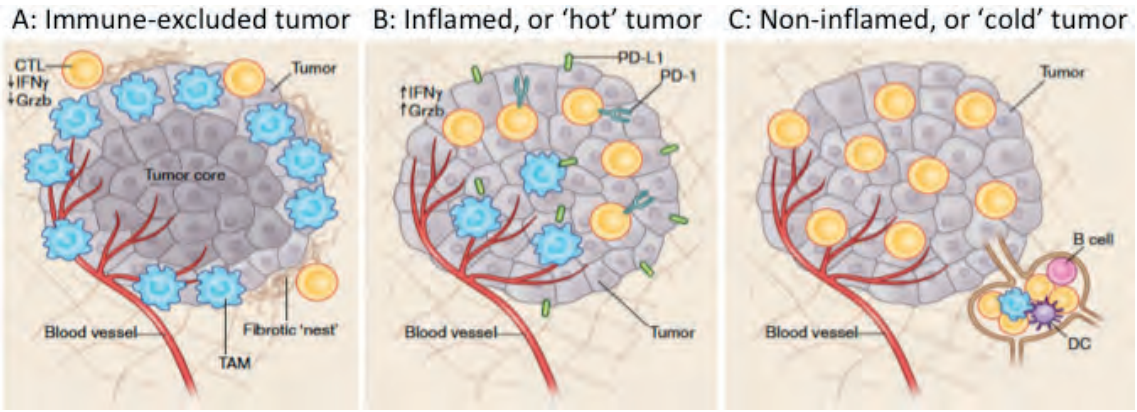

Fig. 3. The tumour immune-microenvironment can be classified as being either (A) immune-excluded, (B) inflamed, or (C) non-inflamed. (Reprinted with permission from Springer Nature: Nature Medicine (Understanding the tumor immune microenvironment (TIME) for effective therapy, Mikhail Binnewies et al. (63), COPYRIGHT (2018)).

Chen & Mellman (12), cancers can be categorized into 3 groups: 1) 'hot' or inflamed tumours, characterized by a high T-cell infiltrate, 2) 'cold' or non-inflamed tumours, devoid of any T-cell infiltrate, and 3) cancers that have T cells and other immune cells present, but only at the periphery or within the stromal tissue and not within the tumour itself (**Fig. 3**). 'Hot' tumours are most likely to respond to checkpoint blockade, and melanomas fall in to this category. However, overall, the minority of melanoma patients respond to checkpoint blockade, demonstrating that the relationship between the tumour, host and microenvironment is hugely complex and no perfect biomarker of response or toxicity is yet available for clinical application. Highlights of expansive research in biomarker identification have been reviewed in various recent publications (for example, see 13–15). While not meant to be an exhaustive list, the role of the most promising biomarkers is summarized here (**Table I**) under these 3 headings.

TUMOUR FACTORS

Programmed death ligand 1 expression

Programmed death ligand 1 (PD-L1) is a protein expressed on cancer cells, tumour infiltrating lymphocytes (TILs) and myeloid cells which, through engagement with its receptor, PD-1, attenuates T-cell responses, thereby helping cancer cells evade immune surveillance. Anti-PD-1 antibodies disrupt PD-1:PD-L1 interactions to reinvigorate T-cell cytotoxicity. PD-L1 expression was therefore the first tumour-associated protein to be explored as a putative biomarker of response to anti-PD-1 antibodies. Initial analysis in the CheckMate 067 trial suggested that patients with high levels of PD-L1 had higher response rates compared with those whose tumours had low, or no expression (16). However, responses still occurred among these patients with low/no expression and the predictive value of PD-L1 expression was not borne out with longer follow-up (11). Since CheckMate 067 was initiated, the limitations of PD-L1 testing have received much attention: which antibody, which cells to count (tumour, immune cells, or both), which cut-off to use (cell count is linear, not binary) and all lack clarity. While in some other cancers PD-L1 expression does appear predictive, currently there is no place for routine testing in melanoma clinical practice.

Tumour mutational burden

Response to immune checkpoint inhibitors is highest among tumour types with a high mutation load and melanomas generally have high levels of mutations (17). This may be attributable, at least in part, to the production of tumour-specific neoantigens. Mutations within a tumour may lead to the formation of peptides unique to tumour cells that have the potential to be antigenic. Therefore, an increase in the tumour mutational burden (TMB) of a tumour could increase the likelihood of production of antigenic tumour-specific peptides, in turn leading to a

Table I. Summary of potential melanoma predictive biomarkers

Tumour	Host	Microenvironment
Tumour mutation burden and neoantigen expression	CD8 T cells	Microbiome
Driver mutations	T-cell receptor	Immunosuppressive stroma/immune cell
Aberrant signaling pathways (including WNT/bcatenin, JAK1/2, VEGF)	Immunoscore	environment including TGFb pathway
MHC	Neutrophil:lymphocyte ratio	PD-L1 expression
B2Microglobulin	Cytokines eg. IL17	
PD-L1 expression	Immune-related gene expression profiles	
Imaging (eg. FDG-PET)	IFN γ signature	
	Inflammatory markers eg. IL6, CRP	

larger pool of tumour-specific T cells. This larger pool of tumour-specific T cells would theoretically produce a greater antitumor response on inhibition of immune checkpoints that may be mediating tumour immune tolerance.

The first confirmatory human data came from whole-exome sequencing of DNA from tumours and matching blood from 25 metastatic melanoma patients treated with ipilimumab (18). There was a significant difference in TMB between patients with a long-term clinical benefit and those with minimal or no benefit, which was then reproduced in a subsequent validation set. High TMB was subsequently shown to correlate with survival following anti-PD1 blockade (19). Even so, as with PD-L1, measuring TMB is not straightforward. Gene sequencing methodology – which platform to use, which cut-off for a non-binary measure – is still evolving. Tumour heterogeneity will influence any measure of TMB in a discrete tumour sample, although some early research suggests this could be overcome by measuring TMB in a blood sample. Therefore, TMB remains an exploratory biomarker for the time being.

Aberrant signaling pathways driven by tumour mutations

Genetic mutations within melanoma cells have downstream effects on signalling pathways, which influence response to immunotherapy. A key pathway implicated in resistance to both anti-PD-1 and anti-CTLA-4 antibodies is the WNT/β-catenin-signalling pathway (20) which induces T-cell exclusion. Studies have demonstrated that loss of *PTEN* correlates with decreased T-cell infiltration at tumour sites, reduced likelihood of successful T-cell expansion from resected tumours, and inferior outcomes with anti-PD-1 antibodies (21). Mutations in several components of the Janus kinase (JAK1/JAK2) pathway have been implicated in both acquired (22) and primary (23) immune resistance in melanoma, by impairing interferon gamma (IFN-γ) signalling. Thus, screening for JAK1/2 mutations has been proposed as a mechanism to identify patients unlikely to respond to immune checkpoint inhibitors.

Recent studies have implicated loss of antigen presentation as a key mechanism of resistance to immune checkpoint inhibitors. β2microglobulin (β2M) is an essential component of MHC class I antigen presentation in which point mutations, deletions or loss of heterozygosity (LOH) have been identified in 30% of melanoma patients with progressing disease (24). In metastatic melanoma patients treated with anti-CTLA-4 and anti-PD-1 agents, β2M LOH was enriched threefold in non-responders compared to responders and was associated with poorer overall survival. Loss of both copies of β2M was found only in non-responders.

A further factor implicated in driving resistance to immune checkpoint inhibitors is transforming growth factor beta (TGF-β) (25). TGF-β is a multi-functional cytokine involved in the regulation of many cellular processes including cell proliferation, differentiation and survival. Melanoma produces increasing amounts of TGF-β with disease progression, inhibiting immune responses and providing an optimal microenvironment for undisturbed tumour growth. Its role as a response biomarker needs further investigation.

HOST IMMUNE-BASED BIOMARKERS

Many immune-based biomarker candidates have been identified to date in retrospective datasets, or preclinical models. The majority of these studies have focused on immune cells, either within the tumour, or circulating in blood.

Tumour-based immune-related biomarkers

The inflamed tumour microenvironment is characterized by the presence of T-cell markers and chemokines that mediate effector T-cell recruitment, with enhanced numbers of $CD8^+$ T cells, macrophages, as well as some B cells and plasma cells. Therefore, it is perhaps not surprising that one of the most reproducible factors predicting response to immunotherapy in melanoma patients has been the presence of tumour-infiltrating lymphocytes (TILs) within tumours: increased numbers of TILs generally correlates with improved response and survival (26). Tumour infiltrating immune cells include T cells, macrophages and various types of immune suppressive cells, all of which contribute to the balance of a pro-immunogenic versus immunosuppressive microenvironment. Thus, low intratumoral CD8:CD4 ratios correlate with lack of response to treatment, while response rates as high as 80% have been reported to be associated with high intratumoral CD8:CD4 in metastatic melanoma patients treated with anti-PD-1 monotherapy (27). Because the nature of the immune microenvironment of a tumour at baseline is associated with efficacy of immune checkpoint inhibition, the assessment of an individual's immune signature to predict treatment outcome is an area of active investigation. This emerging concept, known as immunoprofiling, relies on the 'immunoscore': an assessment of the type, density, and location of immune cells (28). Absolute numbers is a gross oversimplification of a highly complex microenvironment influencing T-cell function. It is likely that multiple markers may need to be combined to fully encompass the heterogeneity of immune cell responses in individual patients receiving specific therapies.

One way of combining multiple factors affecting response to immunotherapy is by gene expression profiling of tumour tissue. A T-cell inflamed tumour microenvironment rich in pro-inflammatory chemokines with an IFN-γ signature has been shown to correlate with the

clinical efficacy of immune checkpoint inhibitors in melanoma patients (29–31). Several multi-gene expression profiles have been proposed as having predictive value, although results are not always consistent across studies. However, evidence from a large cohort of > 300 tumours from multiple cancers including melanoma reported that integrated analysis of an immune gene signature combined with TMB enriches for anti-PD1 responders (32) (**Fig. 4**). This novel approach may provide a precision medicine framework for stratifying patient therapy in the future.

Blood-based biomarkers

Multiple blood-based biomarkers have been identified in retrospective studies and show promise to predict both response, and, potentially, toxicity, and have been extensively reviewed elsewhere (33–35). They include absolute neutrophil count, absolute lymphocyte count, neutrophil:lymphocyte ratio, absolute eosinophil count, relative lymphocyte count (RLC), absolute monocyte count, antibodies against NY-ESO1, T-regulatory cell count, and myeloid-derived suppressor cell (MDSC) count. Recent analysis of patients recruited to the CheckMate 064, 066 and 067 trials identified serum IL6 and CRP as predictors of improved response and survival after checkpoint blockade (36). Even so, most studies have been undertaken on small cohorts using a variety of different evaluation criteria (37) and all require validation in larger prospective trials.

The most extensive analysis of the effects of immune checkpoint inhibitors on peripheral blood was performed in metastatic melanoma patients treated with pembrolizumab (38). The study showed that 1) PD1 inhibition leads to an on-target immunological effect on CD8 T cells and this effect can be detected, longitudinally monitored and mechanistically interrogated in the peripheral blood with the major cell type affected being the $Ki67^+$ CD8 T-cell population, characteristic of exhausted T cells (T_{ex}). 2) Most patients had a single peak of anti-PD-1-induced immune reinvigoration, despite on-going treatment which occurred early during treatment (within 3–6 weeks). 3) Since the T_{ex} cells were the major target of PD-1 blockade in most patients, the authors were able to develop a 'reinvigoration score' by relating changes in circulating T_{ex} cells to tumour burden. 4) Responding T_{ex} cells in the blood contained T-cell receptor clones shared with tumour-infiltrating T cells, and 5) The ratio of T_{ex}-cell reinvigoration to tumour burden distinguished clinical outcomes and predicted for response. The relationship between T_{ex}-cell reinvigoration and tumour

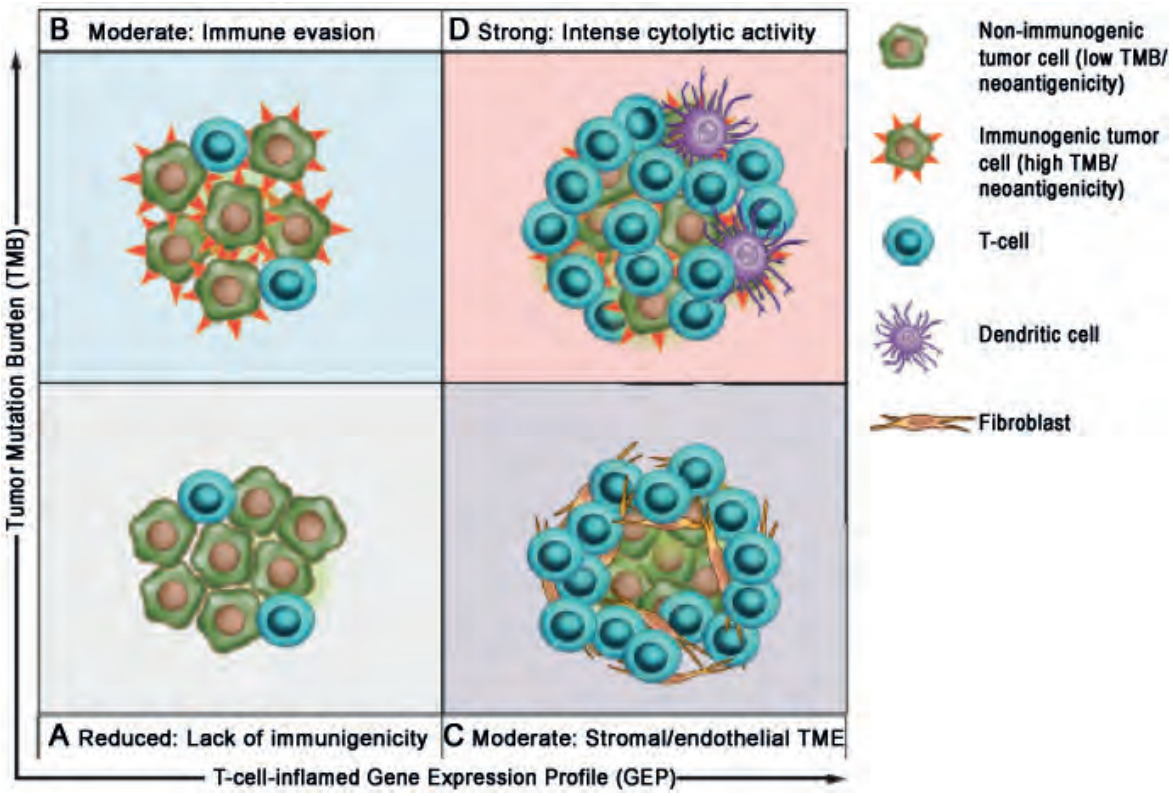

Fig. 4. Biomarker-defined responses to pembrolizumab monotherapy identify targetable resistance biology. (A) Tumours have low TMB and low neoantigenicity and lack a T cell-inflamed TME. (B) Tumours can evade the immune response despite high TMB and high neoantigenicity. (C) Although T cells are present, stromal and/or endothelial factors in the TME, low TMB and low neoantigenicity impede their activity. (D) Tumours have high TMB, high neoantigenicity and a T cell-inflamed TME, typified by activated T cells and other immune cells with cytolytic roles. (From Cristescu R et al., Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. Science. 2018 Oct 12;362(6411). pii: eaar3593. doi: 10.1126/science.aar3593. Reprinted with permission from The American Association for the Advancement of Science).

burden suggests a 'calibration' of immune responses to antigen burden and raises the possibility that even robust reinvigoration by anti-PD-1 therapy may be clinically ineffective if the tumour burden is high. This study provides a clinically accessible potential on-treatment predictor of response to PD-1 blockade which now needs validating prospectively.

There are now several mature technologies available for plasma and serum protein identification and quantification, including mass spectrometry proteome profiling and affinity-based methods (37), which offer the opportunity for larger scale analyses and have identified several potential protein-based biomarkers. They include vascular endothelial growth factor (VEGF). Since an early observation that high serum VEGF were associated with decreased overall survival in metastatic melanoma patients treated with ipi (39), angiogenesis is increasingly appreciated as an immune modulator with therapeutic potential combined with checkpoint blockade. Markers of angiogenesis are now receiving increasing attention for their potential clinical application.

BIOMARKERS PREDICTING FOR IMMUNE CHECKPOINT INHIBITOR TOXICITY

Changes in IL-17, CD8 T-cell clonal expansion, eosinophil counts, and markers of neutrophil activation have been associated with specific immune-related adverse events (irAEs) after treatment induction, but did not predict toxicity development when tested at baseline (40–42). Several other potential baseline risk factors for development of irAEs from ICPIs have been suggested, including a family history of autoimmune diseases (43, 44), but these require further validation. It is intriguing to suggest that similar genetic loci that predispose to autoimmune conditions also contribute towards development of irAES but, to date, no germline factors have been associated with development of irAEs (45). Similarly, preliminary studies suggest the microbiome (discussed in more detail below) may influence risk of irAEs, particularly colitis (46).

A recent study implicated a group of cytokines in predicting immune checkpoint mediated toxicity (47). Eleven cytokines (including pro-inflammatory cytokines such as IL-1a, IL-2 and IFNa2; developed into a score called the 'CYTOX score') measured both pre- and early during treatment were found to be significantly up-regulated in patients with severe immune-related toxicities in 98 melanoma patients treated with PD-1 inhibitors, alone or in combination with anti-CTLA-4. The findings were then validated in an independent validation cohort of 49 patients treated with combination anti-PD-1 and anti-CTLA-4. If validated in larger prospective studies, the CYTOX score could identify toxicity-prone patients to either avoid harmful treatment or consider prophylactic interventions to mitigate side effects.

THE MICROENVIRONMENT

The microbiome

The gut microbiome influences host immunity and has been implicated in multiple diseases including cancer. The presence of certain gut bacteria, including Akkermansia muciniphila and Bifidobacterium, was reported to improve efficacy of PD-1 blockade in animal models. In melanoma patients, significant differences have been reported in the composition and diversity of the gut microbiome between responders and non-responders to anti–PD-1 immunotherapy. However, the reported findings have so far been inconsistent (48–52), which may say more about the limitations of the sequencing technology being used. Even so, the significance of the microbiome is further implicated by preliminary studies suggesting that antibiotic (53, 54), probiotic and prebiotic (ie. dietary fibre) intake all can all influence response to checkpoint inhibition.

IS TOXICITY A BIOMARKER OF RESPONSE?

A key element of drug development is understanding drug-induced toxicity, whether on-target or off-target effects, and whether toxicity has any correlation with predicting efficacy. In the context of BRAF-targeted agents, there is no evidence that the two are connected. With checkpoint inhibitors, the data is far more intriguing, although not at all clear cut. For ipilimumab, immune-related adverse events do not correlate with response, or survival (55, 56). For anti-PD-1 monotherapy, results are conflicting, both in the advanced (57, 58), and most recently in the adjuvant setting (59, 60). The most compelling data comes from the CheckMate 067 trial, when it was observed that 68% of patients receiving combination ipi+nivo who stopped treatment early due to unacceptable toxicity continued to maintain a response over time (15). Thus, at least for metastatic melanoma patients receiving ipi+nivo, it is reasonable to reassure patients experiencing severe, sometimes life-threatening toxicity, that this may predict for good outcome, although the converse is not necessarily true. Understanding the mechanisms that underlie irAEs and their optimal management are key areas requiring active research.

WHEN TO STOP ANTI-PD1 ANTIBODY TREATMENT?

Anti-PD-1 antibodies are licensed to be administered to metastatic melanoma patients for as long as there is evidence of clinical benefit. For those patients who respond, they may be consigned to treatment for many years, risking toxicity, impacting quality of life, and requiring significant healthcare resources. Adjuvant therapy has been approved for a duration of one year. The biological

necessity for long term therapy in either setting is not determined and in fact, there is accumulating evidence arguing against the need. Evidence from following-up advanced melanoma patients stopping treatment due to toxicity suggest that response can be maintained in the absence of drug being administered. Long term follow-up of melanoma patients recruited to the KEYNOTE 006 trial who stopped treatment after 2 years reported durable complete remissions after discontinuation and low incidence of relapse (61). The mechanisms underlying this observation clearly need to be studied, but functional imaging may be a useful adjuvant to clinical decision-making.

Retrospective data from 104 metastatic melanoma patients treated with anti-PD1 antibodies suggests that performing ^{18}F-2-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) at one year accurately predicts long-term outcome: PFS of complete metabolic response (CMR) was 96%, compared with 49% without CMR (HR 0.06, p<0.06) (62). The UK DANTE study is randomizing melanoma patients who are progression-free after one year of anti-PD1 antibody therapy to either stop or continue treatment. A sub-study has been proposed to evaluate prospectively the value of performing PET at one year and will also determine the value of earlier PET scanning performed at or before the first routine 12 week CT response assessment. The rationale for shorter duration of adjuvant therapy also warrants evaluation in randomised trials.

SUMMARY

Now that systemic therapy is established for treatment of both metastatic and high-risk resected melanoma, a key next phase of research is to optimize selection of treatment by identifying biomarkers which can reliably predict both response to and relapse on therapy. This rapidly evolving and expanding personalized approach, offers the opportunity safer, more cost-effective healthcare in years to come.

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Centenary theme section: SKIN MALIGNANCIES

REVIEW ARTICLE

Update on the Management of Cutaneous Squamous Cell Carcinoma

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For all primary cutaneous squamous cell carcinomas (cSCCs), physical examination should include full skin examination, recording of tumour diameter and regional lymph-node–basin status. Surgery is the treatment of choice, with a minimal 5-mm margin. For elderly patients with well-differentiated tumours, other surgical modalities can be explored. Surgery for organ-transplant recipients should not be delayed. The issue with cSCC is identifying high-risk tumours with staging, as this may alter treatment and follow-up schedules. Adjuvant radiation therapy should be considered for incomplete resection, when re-excision is impossible or there are poor-prognosis histological findings. Recommendations are at least biannual dermatological surveillance for 2 years, but in elderly patients with small, well-differentiated tumours long-term follow-up is not always necessary. In case of positive lymph nodes, radical dissection is needed, with regional postoperative adjuvant radiation. Advanced cSCCs are defined as unresectable local, regional or distant disease requiring systemic treatment. Their only approved treatment is the PD-1 inhibitor, cemiplimab. Trials evaluating adjuvant or neo-adjuvant anti-PD-1 are ongoing. Platin-based chemo or anti-epidermal growth-factor–receptor therapies are possible second-line treatments. For transplant patients, minimizing immunosuppression and switching to sirolimus must be considered at first appearance of cSCC.

Key words: cutaneous squamous cell carcinoma; anti-PD-1; adjuvant treatment.

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SIGNIFICANCE

This review updates the management of primary resectable cutaneous and advanced cutaneous squamous cell carcinomas. It is important for physicians treating cutaneous squamous cell carcinoma to know that currently available staging systems can help identify high-risk tumours and should guide work-up and treatment. This article describes risk factors and staging methods, along with an overview of current treatments according to disease stage.

Historically, cutaneous squamous cell carcinoma (cSCC) was the second most common skin cancer after basal cell carcinoma (BCC), but several recent reports on the Australian and US populations have shown a shift in the numbers of cSCCs compared with BCCs. A study of Medicare patients shows a 1:1 ratio of cSCC to BCC (1). The incidence of cSCC has increased markedly over recent decades worldwide, probably because very early cSCC are being resected more often, but also because of increased exposure to the sun (1). cSCC frequency quadrupled for both sexes in Sweden between 1960 and 2004 (2). cSCCs often occur in elderly and male patients. The main risk factors for developing cSCCs are chronic cumulative exposure to ultraviolet (UV), including sunbed use and psoralen and ultraviolet A (UVA), having fair skin or hair, and taking immunosuppressive medication for ≥ 1 month (3–5). Immunocompromised patients, including organ-transplant recipients and human immunodeficiency virus (HIV)-positive patients, are at increased risk of cSCC (6, 7). cSCCs are the most common cancers following organ transplantation, with their risk increasing 100-fold for transplantees (6, 8–10). Oncogenic human papillomavirus, chronic scarring conditions, exposure to arsenic or ionizing radiation, recessive dystrophic epidermolysis bullosa and rare familial syndromes (e.g. xeroderma pigmentosum, albinism and Lynch syndrome) have also been associated with increased risk of cSCCs. Ageing of the population, more organ-transplant recipients, change in attitude toward UV exposure, and increased ascertainment contribute to the increase in incidence of cSCC.

Although initial surgical excision cures 95% of patients, a minority of cSCCs recur locally (3–4%) or metastasize (2–4%), usually to regional lymph nodes or, rarely, to distant locations (11, 12). In addition, 1–4% of cSCCs are fatal (13, 14). cSCC-attributed mortality is increasing in Australia. The mortality rate in the southern and central USA approached that of melanoma, emphasizing that cSCC is a critical public health concern (15).

Awareness of risk factors for cSCC is essential to improve primary prevention with the objective of containing, and hopefully lowering, the increasing incidence of cSCC. Thus, because sunscreens can prevent cSCC (16), its use should be strongly encouraged, and use of sunbeds should be strongly discouraged. Moreover, high-risk patients, i.e. immunocompromised patients, should undergo regular dermatological monitoring and

education about skin self-examination and safe behaviour in the sun.

Application of the currently available staging systems helps to identify patients at high risk of recurrence. The American Joint Committee on Cancer staging 8th edition (AJCC-8) (11) tumour-staging items include tumour diameter, as summarized in **Fig. 1**a. Lymph-node size, number of positive lymph nodes and their location(s) (ipsilateral, contralateral, bilateral) and extranodal extension. However, the AJCC-8 is relevant only for head-and-neck cSCCs, which might limit its usefulness. The Brigham and Women's Hospital (BWH)-staging system (17) is based on the presence of 4 risk factors, summarized in Fig. 1b. BWH stage T3 represents only 5% of tumours, but 70% of nodal metastases and 83% of disease-specific deaths. A recent monocentre retrospective study on 186 head-and-neck cSCCs (18) compared the 2 systems and found an overlapping of poor-prognosis predictions.

Several other poor-prognosis risk factors are not included in these classifications: high-risk locations (lip, ear), histological thickness or Clark level ≥IV, desmoplastic and adenosquamous histological subtypes or immunosuppression. Organ-transplant recipients' cSCCs are often aggressive tumours and in view of the presence of multiple viral warts in these patients, which may be difficult to differentiate from early SCC, it is recommended that dedicated dermatology clinics look after these high-risk patients, if possible.

High-risk cSCCs have a higher recurrence, estimated at 16%. Recurrences occur mainly during the first 2 years post-diagnosis (19). However, a review of the literature showed that, for patients with high-risk cSCCs and clearly documented surgical margins, risks of local recurrence, regional metastasis, distant metastasis and disease-specific death were 5%, 5%, 1% and 1%, respectively (20).

Advanced cSCCs are defined as either locally unresectable, deeply invasive involving muscle, nerve or bone structures, unresectable regional lymph-node disease or multiple distant metastases requiring systemic curative treatment (**Fig. 2**).

A

Tx	**Primary tumor cannot be assessed**
T0	**No evidence of primary tumor**
Tis	**Carcinoma *in situ***
T1	**Tumor diameter ≤2 cm**
T2	**Tumor diameter >2 cm but ≤4 cm**
T3	**Tumor diameter >4 cm, minor bone invasion, perineural invasion# or deep invasion***
T4	**Tumor with gross cortical bone/marrow, skull base and/or its foramen invasion**

#Defined as tumor cells within a nerve sheath lying deeper below the dermis, ≥0.1 mm in caliber, with clinical or radiographic involvement of named nerves without skull base invasion or transgression.
***Defined as that going beyond the subcutaneous fat or >6 mm.**

B

T1	0 risk factor
T2a	1 risk factor
T2b	**2–3 risk factors**
T3	**≥4 risk factors or bone invasion**

High-risk patients

Risk factors

- Tumor diameter ≥2 cm
- Tumor invasion beyond subcutaneous fat (excluding bone invasion, which automatically upgrades tumor to T3)
- Perineural invasion ≥0.1 mm
- Poorly differentiated

Fig. 1. Cutaneous squamous cell carcinoma – staging criteria. (a) American Joint Committee on Cancer 8th edition staging of head-and-neck tumours (adapted from (11). (b) Brigham and Women's Hospital tumour-staging items (adapted from (17)).

MANAGEMENT OF PRIMARY RESECTABLE CUTANEOUS SQUAMOUS CELL CARCINOMAS

Physical examination and biological staging

Staging should systematically include primary tumour diameter, regional lymph-node–basin status, and search for other skin cancers and chronic inflammatory disorders and previous or current immunosuppression. Rare genetic syndromes, such as xeroderma pigmentosum, albinism and Lynch syndrome have to be ruled out in patients who have early onset and/or multiple cSCC without obvious risk factors.

Imaging studies for staging

Because few studies have addressed cSCC imaging, its value for regional and distant staging is uncertain, even for high-risk cSCCs (21). A meta-analysis of head-and neck tumours evaluating the contributions of computed tomography (CT) scans, magnetic resonance imaging (MRI), ultrasonography (US) and US-guided fine-needle aspiration showed that the last accuracy was the best (22). Ultrasound scanning with fine needle aspiration cytology was found superior to CT in assessing primary SCC of the vulva regional disease status (23). Based on a retrospective series of 98 high-risk patients with BWH-stage T2b or T3 cSCCs, with imaging staging (CT, positron-emission tomography (PET–CT scans or MRI) or without, imaging impacted cSCC management for one-third of them; moreover, patients without imaging staging tended to develop nodal metastases more frequently ($p=0.046$) (24). Prospective studies are needed to confirm that an initial imaging work-up can impact management and outcomes, and that imaging should be considered for regional staging in high-risk patients. In 2020, the European Dermatology Forum (EDF), European Association of Dermato-Oncology (EADO) and the European Organization for Research and Treatment of Cancer (EORTC) (EDF–EADO–EORTC) consensus group recommended lymph-node US for high-risk patients (25).

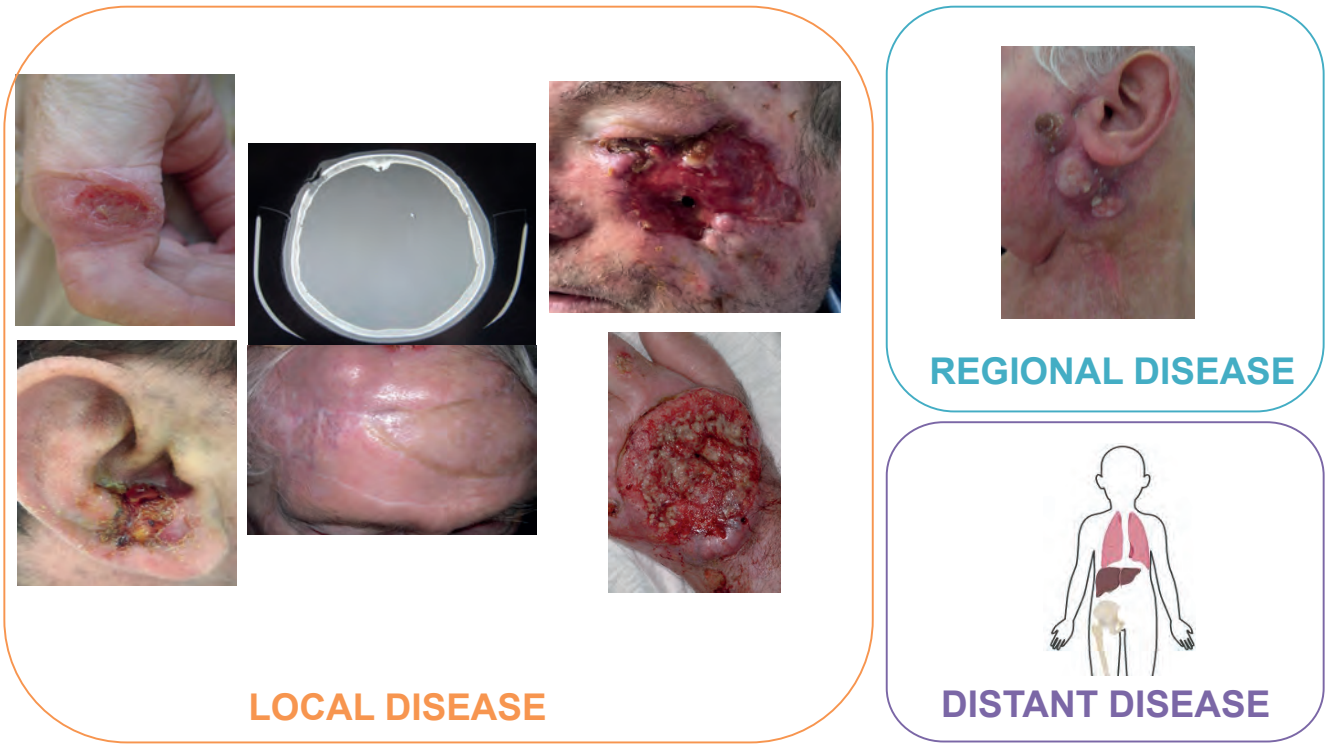

Fig. 2. The different types of advanced cutaneous squamous cell carcinomas. Local disease (*left*): local unresectable disease without regional or distant disease. Regional disease (*top right*): at least regional unresectable disease without distant disease. Distant disease (*bottom right*): at least one unresectable distant metastasis.

Surgery

Biopsy or limited excision of the tumour is usually performed to confirm a clinically suspected cSCC, but if the tumour is small, a single definitive excision is often performed outright with various margins. Surgery is the treatment of choice. Most primary resectable cSCCs are usually cured by conventional excision. Mohs surgery may be needed for high-risk tumours and/or difficult anatomical sites. Randomized controlled trials on resection-margin widths are lacking, therefore excision margins for SCC are controversial.

Excellent cure rates have been reported in several series. Experience suggests that small well-differentiated tumours, which are slow-growing in elderly patients on sun-exposed sites can be removed by experienced physicians with curettage (http://www.bad.org.uk/healthcare-professionals/clinical-standards/clinical-guidelines). Recurrences were rare in a study on 1,174 cSCC patients and did not differ significantly among tumours treated with electrodessication/curettage destruction, excision or Mohs surgery, respectively: 24.3% of 361 vs. 38.3% of 571, or 37.4% of 556 (26).

The EDF–EADO–EORTC consensus group has recommended surgical resection with a minimal 5-mm margin, even for low-risk tumours, which should be extended to 10 mm for high-risk tumours (**Table I**) when additional clinical or histological risk factors are present (25). When technically feasible, 1-step resection and

Table I. Summary of treatment options

Treatment options
Primary resectable cSCCs
Surgical resection (5–10 mm margin)
Alternative: curative radiation therapy
Alternative for low risk small tumours on sun exposed sites: 2 cycles curettage and cautery
*Adjuvant treatment for primary high-risk cSCCs**
Radiation therapy
Ongoing immunotherapy trials
Neoadjuvant treatment
Ongoing immunotherapy trials
cSCCs with regional lymph node involvement
Radical lymph-nodes dissection
Adjuvant radiation therapy
Advanced cSCCs
First line:
• Cemiplimab (350 mg infused intravenously over 30 min every 3 weeks)
Second line:
Cisplatin-based chemotherapies
• or Carboplatin-based chemotherapies (better tolerated in patients with comorbidities)
• or epidermal growth-factor receptor (EGFR)-targeted therapies (cetuximab)
• or hyperthermic isolated-limb perfusion
- or ongoing combined immunotherapy trials
Prevention
Topical treatments
5% 5-FU cream
Alternatives: imiquimod, diclofenac and photodynamic therapy
Oral treatments
Acitretin, nicotinamide
Primary cSCCs in transplant recipients
Minimizing immunosuppression and switching to sirolimus

*Incomplete resection, poor-prognosis histological findings.
cSSC: cutaneous squamous cell carcinoma.

closure is preferred; 2-step resection is recommended when a graft or flap reconstruction is planned. If the resection is incomplete, then surgical re-excision should be performed.

In an earlier prospective, multicentre Australian case series of 1,263 cSCC patients, characterized by an elevated percentage of high-risk tumours treated with Mohs micrographic surgery, 5-year recurrence rates were low: 2.6% in patients with primary cSCCs and 5.9% in patients with locally recurrent cSCC, suggesting that this technique achieves a high cure rate for these high-risk cSCCs (12). However, randomized studies comparing Mohs surgery with conventional surgery are lacking.

The pathologist's report should specify histological differentiation grade, histological subtype, maximum tumour thickness and Clark level, invasion of muscle, cartilage, bone and/or fascia, perineural or lymphatic/vascular invasion, whether or not the resection was complete with minimal lateral and deep margins.

For high-risk cSCCs with negative regional staging on imaging, a sentinel lymph-node biopsy might be considered an option, but is not standard of care, depending on its potential comorbidities. Indeed, sentinel lymph-node biopsies are positive for one-third of the patients with BWH stage-T2b or -T3 cancers (27). However, the authors of a recent prospective German study found that 6% of a series of sentinel lymph-node-negative patients had distant metastases, suggesting the limited prognostic value of the procedure (28).

Curative radiation therapy

Radiotherapy represents an alternative to primary surgical resection for SCC of the lip and when surgery is not appropriate for cSCCs. However, the risk of cSCC recurrence is higher after radiation therapy compared with surgery. For patients with comorbidities that predispose them to radiation-induced cancers, such as basal cell naevus syndrome or xeroderma pigmentosum, radiotherapy must be avoided. Radiation therapy can cause reversible dermatitis or mucositis. Late side-effects include skin atrophy with loss of hair, reduced sweating and sebaceous secretions, discoloration, telangiectasia, hypodermic sclerosis and/or skin carcinomas so should be avoided in younger patients (29).

Adjuvant radiation therapy for primary high-risk cutaneous squamous cell carcinoma

According to a literature review on cSCCs with perineural invasion treated with surgery ($n=30$) or surgery plus adjuvant radiation therapy ($n=44$ cases), outcomes were comparable (20). The role of adjuvant radiation therapy for high-risk cSCCs, including those with perineural invasion, remains controversial. However, authors of a recent retrospective study on adjuvant radiation therapy for cSCCs with perineural invasion found it to be associated with prolonged survival (30), suggesting that such patients might benefit from adding radiation to surgery and decisions have to be made on a case-by-case basis.

Other adjuvant or neoadjuvant strategies for primary high-risk cutaneous squamous cell carcinoma

No significant differences were found for retinoic acid and interferon vs. placebo for the time to recurrence or occurrence of second primary cSCCs in patients with high-risk cSCCs enrolled in a randomized phase-3 trial (31).

O'Bryan et al. prescribed adjuvant cetuximab for 7 patients with high-risk cSCCs (32); only 3 experienced disease recurrence. Neoadjuvant gefitinib therapy in a phase-2 study on 22 patients achieved a 45% response rate, including 3 histological complete responses (CRs) (33). However, disease progressed for 32% and the lack of known biomarkers of response highlights the need for further larger studies, including randomized trials. Jenni et al. (34) more recently reported size reduction after 14 days of lapatinib in 2 out of 8 assessable patients, among 10 with resectable cSCCs.

A recent phase-2 study (35), presented at European Society for Medical Oncology (ESMO) 2019, showed that cemiplimab neoadjuvant therapy given to 20 patients induced histological partial responses (PRs) or CRs in 70% of the patients. Moreover, it was well-tolerated. Ongoing trials are evaluating the potential contribution of anti-programmed cell-death protein-1 (PD-1) agents as adjuvant therapy for high-risk cSCCs.

Monitoring

The majority of all recurrences of cSCC occur within 2 years of the initial diagnosis. In high-risk cSCCs the follow up should be at least 2 years and should include palpation of the primary excision site and of the regional lymph node area every 3 or 6 months depending on the initial stage and medical history. Moreover, the entire skin of all patients should be examined once annually or every 6 months in high-risk cSCCs patients (immunosuppression, multiple primary cSCCs, genetic predisposition) as recommended by the current European guidelines (25). However, in elderly patients with small well-differentiated SCC on sun-exposed sites (excluding high-risk sites, such as lips, ears, digits and mucosa), discharge after 3 months is possible.

MANAGEMENT OF CUTANEOUS SQUAMOUS CELL CARCINOMAS WITH REGIONAL LYMPH-NODE INVOLVEMENT

Histological examination of fine-needle aspirates or resections of any enlarged nodes is mandatory. Available results of studies on lymph-node involvement of head-and-neck cSCCs indicated positive lymph nodes

as a negative factor for survival (36, 37). Extracapsular lymph-node spread is a significant risk factor for recurrence. The most frequently involved lymph-node region is around the parotid. Disease stage should be assessed by imaging studies, including CT or PET–CT scan(s) or MRI. When lymph nodes are histologically positive, they should be subjected to radical dissection. Postoperative adjuvant radiation delivered to the affected lymph-node region is required for head and neck tumours, as it enhances local–regional control and disease-free survival (DFS) and overall survival (OS) of those patients (30).

MANAGEMENT OF ADVANCED CUTANEOUS SQUAMOUS-CELL CARCINOMAS

The PD-1 inhibitor, cemiplimab, is the only approved agent for locally advanced and metastatic cSCCs. Prior conventional treatment for advanced cSCCs, such as cisplatin-based chemotherapies or epidermal growth-factor receptor (EGFR)-targeted therapies, can be used as second-line treatments. Trials evaluating other anti-PD-1 molecules and combinations of anti-PD-1 with other drugs are currently ongoing.

A retrospective study in Europe, completed just before anti-PD-1 became available, described various treatments for patients with advanced cSCCs (38). Among 190 patients (median age 79 years) with locally advanced or metastatic disease, 32% received systemic anti-tumour therapies (excluding anti-PD1), mostly anti-EGFR tyrosine-kinase inhibitors. Half of the patients did not complete systemic therapy as planned. The objective response rate (ORR) was 26% and the mean response duration was 5 months. Among the 152 patients whose survival status was known, 49% had died. The availability of anti-PD-1 agents might allow access to treatment for more patients with cSCC.

Anti-programmed cell-death protein-1

The immune system is important for cSCC, as suggested by the increased risk of cSCCs in transplant recipients (39), the rapid regression of keratoacanthoma, which is characterized by a more active immune response than generally seen in cSCCs (40), and activity of immunotherapy in advanced SCC as combination of interferon and retinoic acid (41). The PD-1 receptor is expressed on T cells, and T cells binding to its ligand (PD-L1) inhibit T-lymphocyte functions. PD-L1 is expressed in 30–50% of cSCCs and its expression was found to correlate with risk of metastases (42). The high mutation rate in cSCCs, as in other UV-induced tumours, is usually a predictor of responsiveness to anti-PD-1 (43).

Cemiplimab (3 mg/kg every 2 weeks) induced a response in approximately half of the 85 patients enrolled in a phase-2 study with locally, regional or distant disease and a phase-1 study with regional or distant disease (44). Those patients were treated, respectively, for up to 48 weeks and up to 96 weeks. Fifty-six to 58% of the patients had received systemic treatment before cemiplimab. Median phase-1 and phase-2 follow-ups were: 11 and 8 months, respectively. Their respective ORRs were 50% and 47%. Median time to response was 2 months for both. In the phase-2 trial, 7% were CRs; median progression-free survival (PFS) and OS had not been reached and median duration of response exceeded 6 months for 16/28 (57%) responders. The most common adverse reactions were fatigue, rash and diarrhoea. Serious adverse events were immune-mediated, such as pneumonitis, hepatitis, colitis, adrenal insufficiency, dysthyroidism, diabetes mellitus and/or nephritis, and, unlike other anti-PD-1 inhibitors, infusion reactions. Treatment was stopped for 7% of patients because of adverse events. Three cemiplimab-related deaths were reported (44). Cemiplimab was approved by the US Food and Drug Administration (FDA) in September 2018 and European Medicines Agency (EMA) in July 2019 for patients with metastatic or locally advanced cSCCs who were not candidates for curative surgery or radiation. The recommended cemiplimab dose and schedule is now 350 mg, infused intravenously over 30 min every 3 weeks. Factors predictive of response are still unknown. Treatment duration needs to be better defined.

Several trials have also assessed pembrolizumab in cSCCs. Interim results of the Keynote 629 study evaluating pembrolizumab (200 mg/3 weeks IV) in advanced cSCC have been presented at the ESMO meeting in 2019 (45). Response rate was 32% in 91 patients receiving pembrolizumab as a second-line treatment and 50% in 14 naïve patients. The median duration of response was not reached. The safety profile was consistent with that of other pembrolizumab monotherapy studies. Interim analysis of the CARSKIN study presented at the ASCO 2019 meeting, showed a response rate of 38.5% in 39 previously untreated patients with advanced cSCC with sustained responses to pembroluzimab (46).

Platin-based chemotherapies

Few prospective trials are available and no treatment regimen has been recommended by health authorities. Because their ORRs are high, platin-based chemotherapies were the first-choice treatment before the anti-PD-1 era, but their administration can be limited by cisplatin toxicity or disease recurrence during treatment. Sadek et al. (47) treated 14 advanced cSCC patients with 1–4 cycles, repeated every 3–4 weeks, of neoadjuvant combination chemotherapy (bolus cisplatin injection, 5-fluorouracil (5-FU) and continuous 5-day bleomycin infusion). The ORR was 78% (4 CRs, 7 PRs). Local control after adjuvant radiation and/or surgery was achieved in 7 (50%) patients. CR lasted >10 months. All patients experienced major toxicities, including grade-3/4

nausea and vomiting; 4 patients had grade-3/4 haematological toxicities and one developed pulmonary fibrosis. In their prospective phase-2 trial, Guthrie et al. treated advanced BCC or locally advanced cSCC patients with cisplatin (75 mg/m^2 and doxorubicin 50 mg/m^2, every 3 weeks) (48). Among the 12 advanced-cSCC patients, 7 responded (4 CRs and 3 PRs). Based on 7 patients with advanced local-regional or metastatic cSCCs, Khansur et al. reported the activity of cisplatin (100 mg/m^2 on day 1) and 5-FU (1 g/m^2/day, days 1–4), given every 3 weeks. Six of 7 patients were responders: 3 PRs and 3 CRs (49). The mean duration for CR was one year. Toxicities included grade-1/2 nausea and vomiting. Carboplatin-combination therapy is better tolerated and can be administered as an alternative to patients with comorbidities. Hyperthermic isolated-limb perfusion can be a second-line limb-saving therapy for patients with unresectable disease located on the extremities (50).

Epidermal growth-factor receptor-targeted therapies

EGFR represents a family of proteins, including EGFR and human epidermal growth factor receptor (HER)-2, 3 and 4. Activation of EGFR tyrosine kinase results in autophosphorylation and activation of RAS serine/threonine kinase, murine sarcoma viral oncogene (RAF), mitogen-activated protein (MAP) kinase and phosphatidylinositol 3-kinase (PI3K), AKT protein kinase and mammalian target of rapamycin (mTOR) pathways leading to tumour growth. EGFR is strongly expressed in metastatic cSCCs and its overexpression in primary cSCCs is associated with poor outcome (18). Anti-EGFR therapy consists of monoclonal antibodies, such as cetuximab or panitumumab, which competitively inhibit EGFR, or small molecules, e.g. gefitinib or erlotinib, targeting the intracellular domain of the receptor. EGFR-targeted therapies have been developed and obtained promising ORRs in several clinical trials and retrospective studies on patients with unresectable cSCCs. So far, phase-3 trial results have not yet confirmed their efficacy against cSCCs. Anti-EGFR tyrosine-kinase inhibitors are not approved to treat advanced cSCCs, but cetuximab is listed in the National Comprehensive Cancer Network (NCCN) compendium as a therapy for recurrent and metastatic CSSCs. No biomarker predictive of a cSCC response has been identified.

Cetuximab was evaluated prospectively as first-line monotherapy in a French phase-3 study on 36 patients with metastatic ($n=3$), regional ($n=16$) or locally advanced ($n=17$) cSCCs. The ORR was 28%, including 2 CRs and 8 PRs, and the overall disease-control rate was 69% (25/36 patients). Median PFS lasted 4 months. The median duration of response was 7 months and the mean OS was 8 months. The more frequent severe adverse events were infections (22%) and tumour bleeding (11%). Cetuximab-related adverse events included 2 grade-4 infusion reactions and 1 grade-3 interstitial pneumopathy (51). Cetuximab can be combined with platin-based chemotherapies and this combination might prolong PFS (9.03 vs. 3.55 months), according to a retrospective series of 14 patients treated with cetuximab monotherapy or cetuximab combined with carboplatin (52). Low-grade specific acne-like rash, pruritus and nail changes have been observed. Severe infusion reactions occurred in 3% of patients.

Panitumumab efficacy (6 mg/kg, repeated every 2 weeks) was of the same order of magnitude for 11 Italian patients with advanced penile SCC (53) and 16 Australian patients with advanced cSCC enrolled in a phase-2 study (54). Median PFS and OS, respectively, were 8 and 11 months for cSCC patients, and 2 and 9 months for those with penile SCC. Severe skin rash, mucositis and diarrhoea occurred.

Efficacy of oral small molecules against advanced cSCCs was variable, with ORR of 10–32%. Based on available phase-2 studies, gefitinib or erlotinib alone obtained only poor ORRs of 15% (6/40 patients) and 7% (3/39 patients), respectively (55, 56). Higher ORRs, of the same order of magnitude as those achieved with monoclonal antibodies, were obtained with second-generation irreversible pan-HER tyrosine-kinase inhibitors, such as dacomitinib: in 28% of cSCCs and 32% (9/28 patients) of penile SCC (57, 58). The tolerance profile of small molecules differed, with more diarrhoea and mucositis than with antibodies.

Concurrent radiotherapy with cetuximab did not significantly prolong PFS and OS compared with concurrent radiotherapy and cisplatin-based chemotherapy in a retrospective series of 23 patients with head-and-neck cSCCs (59).

Further prospective studies are needed to determine the characteristics of patients who would benefit from anti-EGFR and to evaluate combinations of anti-EGFR and other drugs to improve outcomes.

PREVENTION

Available topical agents to treat actinic keratosis and cSCC *in situ* field of cancerization include mainly 5-FU cream, imiquimod, diclofenac and photodynamic therapy. Ingenol metubate (Picato) is now withdrawn because of safety issues. A recent randomized Dutch trial evaluating efficacy of 5% 5-FU cream, 5% imiquimod cream, methyl aminolevulinate photodynamic therapy or 0.015% ingenol mebutate gel in 624 patients with ≥5 actinic keratosis lesions on the head and neck showed that 5% 5-FU cream was the most effective in controlling solar keratoses (60). However, it has not been confirmed that it does, in turn, reduce the risk of SCC.

Oral acitretin can prevent the occurrence of new cSCCs in patients with multiple tumours; for example,

xeroderma pigmentosum patients or transplant recipients. However, cutaneous adverse events often led patients to discontinuation, which, in turn, allowed quick appearance of new cSCCs.

Oral nicotinamide can be prescribed off-label. Indeed, it was evaluated in a randomized study on 386 patients with a history of 2 or more non melanoma skin cancers. Patients received either nicotinamide (500 mg, 2 times per day) or placebo for one year. The nicotinamide group had 30% significantly fewer new cSCCs (61). However, the long-term benefit remains unknown. Liver toxicity can sometimes occur.

TRANSPLANT RECIPIENTS

All transplant recipients are at high risk of developing cSCCs. These cSCCs are more aggressive, with a 5–10-fold higher risk of metastasis (62, 63). Immunosuppression duration and drug types and doses are involved. Surgery must not be delayed in transplant recipients with resectable tumours.

For transplantees, minimizing immunosuppression and switching to sirolimus should be considered as soon as the first cSCC appears. The benefit of switching to sirolimus is maintained for 5 years, with no negative effect on the graft and patient survival (64). However, administration of mTOR inhibitors remains limited because of poor tolerance. Indeed, 25–40% of patients discontinue sirolimus because of adverse events, e.g. hyperlipidaemia, glucose intolerance, interstitial pneumonia and/or lymphoedema. For transplantees with advanced cSCCs, currently available drugs should be used with caution, as anti-PD-1 agents are associated with a high rate of irreversible allograft rejection, while anti-cutaneous T-lymphocyte antigen-4 (CTLA-4) seems to be better tolerated (65). Moreover, the risk of infections with conventional chemotherapy is higher in immunosuppressed patients. Notably, 2 lung-transplant recipients with metastatic cSCCs died 1–3 weeks after their first infusions of cetuximab due to diffuse alveolar damage (66).

CONCLUSION

Due to the increasing incidence of cSCC, it has become a serious public health concern. All tumours should systematically be staged with AJCC-8 or BWH systems, in order to adapt treatment according to the risk of recurrence. Surgery is the treatment of choice whenever the tumour is resectable. Adjuvant radiation therapy must be considered for high-risk cSCCs. PD-1 inhibition is now the standard-of-care for advanced cSCCs. Platin-based chemotherapy or anti-EGFR can be prescribed in the second-line setting. Factors predictive of cSCC response to anti-PD-1 or anti-EGFR remain to be elucidated. Due to the high rate of irreversible allograft rejection associated with anti-PD-1 in organ-transplant recipients, other, less toxic, anti-CTLA-4 or other approaches warrant investigation. Switching from calcineurin inhibitors to sirolimus, or de-escalating immunosuppression, should always be considered. Because most advanced tumours may not respond to various current treatments, the search for new approaches is warranted. Prevention should not be forgotten. SCC incidence is increasing rapidly because of better screening, therefore most cSCC seen in dermatology or plastic surgery clinics are now detected earlier with better prognosis. Only 1–4% of cSCC are fatal; hence patients with cSCC must be accurately staged, to ensure that they are not over-investigated and do not undergo unnecessary surgical procedures or systemic treatments.

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Atopic Dermatitis

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SYSTEMATIC REVIEW

Prevalence and Incidence of Atopic Dermatitis: A Systematic Review

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The primary objective of this study was to systematically review and analyse epidemiological studies of the prevalence and incidence of atopic dermatitis (AD) during childhood and adulthood, focusing on data from the 21st century. A systematic search of PubMed, EMBASE and Google (manual search) was performed in June 2019, followed by data abstraction and study quality assessment (Newcastle–Ottawa Scale). Cross-sectional and longitudinal epidemiological studies of individuals with AD (doctor-diagnosed or standardized definition) were included. Of 7,207 references reviewed, 378 moderate/good-quality studies were included: 352 on prevalence of AD and 26 on incidence of AD. In the 21st century, the 1-year prevalence of doctor-diagnosed AD ranged from 1.2% in Asia to 17.1% in Europe in adults, and 0.96% to 22.6% in children in Asia. The 1-year incidence ranged from 10.2 (95% confidence interval (95% CI) 9.9–10.6) in Italy to 95.6 (95% CI 93.4–97.9) per 1,000 person-years in children in Scotland. There were few recent studies on incidence of AD in the 21st century and no studies on adults only; most studies were conducted in Europe and the USA. Epidemiological studies on childhood and adulthood AD in different continents are still needed, especially on the incidence of AD during adulthood.

Key words: systematic review; atopic dermatitis; prevalence; incidence.

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SIGNIFICANCE

Atopic dermatitis is common, and is often burdensome for the individual. An overview of how often AD occurs is therefore necessary. A systematic review was performed, which included more than 7,000 articles with data from all continents, on children and adults. Each year, up to 17.1% of adults and 22.6% of children were diagnosed with AD; with as many as 9.6% new cases of AD in children. Surprisingly, in adults, studies on new cases were from the 20th century. The results will be useful for patient organizations, physicians, scientists and healthcare planning, especially as new therapies are emerging.

Atopic dermatitis (AD) is a common inflammatory skin disease. AD causes an itchy rash and dry skin and has a substantial impact on quality of life (1, 2). In Europe and the USA, recent data suggests that the prevalence of AD among children is approximately 20% and, among adults, it ranges between 7% and 14%, with substantial variation between countries (1, 3–8).

AD leads to substantial social and financial costs and accounts for the largest global burden of disability owing to skin diseases (9).

The onset of AD occurs during the first years of life in approximately 80% of individuals (10), and that approximately 60% experience remission in adolescence (11). Recent studies indicate evidence of adult-onset AD, but the incidence across different age groups and countries remains unclear (12–14).

Differences in study design and definition of AD contribute to the heterogeneity in reported prevalence and incidence data (15). Differences across studies in factors such as study design, research teams, location, and methods, result in heterogeneity in estimates of the prevalence and incidence of AD, which may underestimate or overestimate the "true" prevalence and incidence of AD in children and adults. Furthermore, AD often features intermittent disease symptoms and signs, which can differ across age groups and skin types.

Knowledge of the prevalence and incidence of AD across different age groups and countries is essential for healthcare planning and patient counselling. Diagnosis based on validated diagnostic criteria, especially physician diagnosis, is often the preferred method. The United Kingdom Working Party diagnostic criteria (UK criteria) are a validated measure for physician assessment of AD and are thus useful (16). Epidemiological data from the 21st century could increase our understanding of the burden of AD.

The primary objective of this study was to systematically review and analyse epidemiological studies of the prevalence and incidence of AD during childhood and adulthood, with a particular focus on publications

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from 2000 through 2019. Secondary outcomes were the prevalence and incidence across age, sex, decade, and country/region.

MATERIALS AND METHODS

A systematic search of PubMed, EMBASE, and Google (manual search) was performed in June 2019. Pre-defined search terms and MeSH (Medical Subject Heading) headings and keywords were developed in collaboration with a medical librarian. The searches are described in **Appendix 1**. Reference lists of included studies and conference abstracts were also screened and Google was searched manually for potential additional studies.

Study selection, data abstraction, and quality assessment

The study included cross-sectional and longitudinal epidemiological studies of individuals with AD, diagnosed by a doctor or using a standardized definition, such as the UK criteria for AD or the International Study of Asthma and Allergies in Children (ISAAC) criteria (17). We primarily searched for studies in English and German. Following a manual search, relevant articles in other languages were also included; specifically, one article in Dutch, 8 in French, and one in Spanish. Exclusion criteria were: intervention studies, clinic-based studies, studies on specific exposed populations (e.g. occupations), and studies of patients with hand eczema only. Title, abstract, and full-text screening was performed independently by two authors in order to assess whether the predefined eligibility criteria were met.

Predefined data extraction sheets and quality assessment sheets were used, which included the Newcastle–Ottawa Scale (NOS) for cohort studies and a modified version of the NOS for cross-sectional studies (18). Screening, data extraction, and quality assessment were performed by two authors (LvK, SB), and discrepancies were resolved by author consensus. Corresponding authors of studies were contacted via e-mail when possible to obtain information about prevalence or incidence by sex.

The primary outcome was prevalence (point prevalence, 1-year (y) prevalence, and/or lifetime prevalence) and incidence of AD. Secondary outcomes included the prevalence and incidence of AD across age, sex, decade, and country/region and quality assessment using the NOS. A particular focus was on publications using data from 2000 through 2019.

This review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (19). When numbers were provided in the original articles but not percentages, then percentages were calculated.

RESULTS

Study selection

The search identified 7,207 abstracts. Of these, 966 articles were selected for full-text review. A manual search and article reference list search identified another 21 studies. Of the articles reviewed, 378 fulfilled the inclusion criteria. In total, 115 of the included studies used data from 2000 onwards. A total of 20 of the studies with data from, and including, 2000 onwards reported 1-year prevalence for doctor-diagnosed AD, and 6 reported incidence for doctor-diagnosed AD. Of the papers included, 337 reported on children and 54 on adults or on both children and adults.

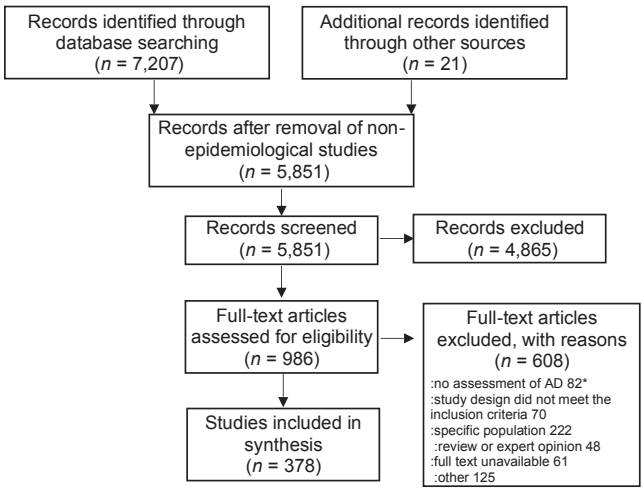

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. Results of search strategy. *No assessment of atopic dermatitis (AD): data on asthma, allergic rhinitis or allergy and not specifically on AD.

The study flow diagram (**Fig. 1**) reports article numbers and reasons for exclusion.

Study characteristics

The studies identified in the search in June 2019 included data from 1958 to 2017. Of these studies, 200 were conducted in Europe, 122 in Asia, 20 in North America, 20 in South America, 23 in Africa, and 14 in Australia; several articles reported on data from several countries. Study samples were between 108 to more than 30 million individuals. Some studies were conducted on several continents and on both children and adults. For study characteristics, see Supplement 1 (http://lup.lub.lu.se/record/e240247d-7664-4263-9918-3b38e704fd06).

There were 342 cross-sectional studies and 36 longitudinal studies. Twenty-eight studies used a doctor diagnosis drawn from study records or patient records and 2 studies relied on a doctor diagnosis based on both physical examination and questionnaire data.The longitudinal studies often used birth cohorts; the earliest of these started in 1958.

The definition of AD varied, and often the ISAAC criteria were used; only 10 studies used the UK criteria and 11 used the Hanifin & Rajka criteria (20), as described in Supplements 1–7 (available from http://lup.lub.lu.se/record/e240247d-7664-4263-9918-3b38e704fd06).

Prevalence of atopic dermatitis

The results for prevalence are presented in **Tables I** and **II** and Supplements 2–7 (available from http://lup.lub.lu.se/record/e240247d-7664-4263-9918-3b38e704fd06).

Studies on children and on both children and adults: all data (1958–2018). The overall point prevalence of AD symptoms in children ranged from 1.7% to 32.8% (21–25). The 1-year prevalence of AD symptoms varied

Table I. Doctor-diagnosed 1-year prevalence of atopic dermatitis (AD) in children assessed in the year 2000 or later by continents

Study	Study type	*n*	Age, years (if not otherwise stated)	One-year prevalence of doctor-diagnosed AD					
				Europe %	Africa %	North America %	South America %	Asia %	Australia %
Aberle et al. 2018 (193)	Cross-sectional study	1,687	10–11	10.1					
Abuabara et al. 2019 (34)	UK primary care cohort study	8,604,333	0–17	12.3					
Civelek et al. 2011 (35)	Cross-sectional study	6,755	10–11					0.94	
Dell et al. 2010 (235)	Cross-sectional study	5,493	5–9			21.4			
Dogruel, et al. 2016 (79)	Birth cohort study	1,377	0–12 months					4.3	
Harangi et al. 2007 (50)	Cross-sectional study	1,454 (2002) 1,454 (2005)	7–14	15.1 16.1					
Horak et al. 2014 (252)	Cross-sectional study	16,019	Mean ± SD age 8.4 ± 1.2	13.9					
Hwang et al. 2010 (255)	Cohort study	277,934	<20					2.0	
Lee et al. 2016 (274)	Cross-sectional study	8,947	1–18					14.3	
Mohn et al. 2018 (378)	Cohort study	373,954	<6	17.0					
Oak et al. 2012 (36)	Cross-sectional study	37,570	Middle-school students					22.6	
Shaw et al. 2011 (306)	National health survey	102,353	Children	10.7					
Simpson et al. 2002 (379)	GP health records	252,538	0–4	9.5					
Wijga et al. 2011 (156)	Survey based on general practitioner records, population surveys and a literature search	79,272	0–9 10–17	5.5 1.8					

SD: standard deviation.

from 0.7% in children and adults in Ethiopia (26), to 2.0% in children in Urumqi (27), and 22.7% in Kuwait (28). The 1-year prevalence in children based on doctor diagnosis of AD ranged from 0.96% to 22.6% (21–25).

The lifetime prevalence of AD varied from 1.2% in Turkey in children aged 7–12 years (132), the same lifetime prevalence of 1.2% was reported in Ethiopia (26) in children and adults with a mean age of approximately 22–23 years, and 36.2% in Beijing (27); the age at assessment of lifetime prevalence was 6–7 years. Lifetime prevalence of doctor-diagnosed AD assessed at age 6–7 years was 1.4% in Lithuania and 36.2% in Beijing (27, 29).

Studies on adults: all data (1958–2018). In adults, the overall point prevalence of AD symptoms ranged from 1.2% to 9.7% (1, 30). The 1-year prevalence of AD symptoms varied from 1.3% in Germany to 22.7% in Kuwait (28, 31), and the 1-year prevalence based on doctor diagnosis ranged from 1.2% to 17.1% (1, 32).

The lifetime prevalence of AD ranged from 1.7% to 17.7% in Kuwait; the age at assessment of lifetime prevalence was 18–26 years. The prevalence of AD in Scandinavia between ages 0–29 years was 34.1%; the lifetime prevalence of doctor-diagnosed AD was 14.6% to 20.2% in Kuwait; the age at assessment of lifetime prevalence was 18–26 years (1, 28, 31, 33).

Studies of 21st century data for children and adults. For children, the point prevalence ranged from 0% in Nigeria to 18.2% in Turkey (39, 40). For adults, it varied from 0.64%–0.9% in Israel to 9.7% in Denmark in 2010 (1, 41). For children, the 1-year symptom prevalence ranged from 4.1% to 22.7% and for adults from 7.3% to 22.7% (28, 42, 43). The 1-year prevalence of doctor-diagnosed AD ranged from 1.2% in Asia to 17.1% in Europe in adults, and from 0.96% to 22.6% in children in Asia (1, 32, 34–36). For children, the lifetime symptom prevalence ranged from 4.4% to 17.7% assessed at age 7–15 years, and for adults ranged from 3.0% to 17.7% (28, 31, 44).

Table II. Doctor-diagnosed 1-year prevalence of atopic dermatitis (AD) in adults assessed in the year 2000 or later by continents

Study	Study type	*n*	Age, years	One-year prevalence of doctor-diagnosed AD					
				Europe (%)	Africa (%)	North America (%)	South America (%)	Asia (%)	Australia (%)
Abuabara et al. 2018 (195)	GP health records	848,435	18–74	5.1					
Abuabara et al. 2019 (34)	Health improvement network	8,604,333	75–99	8.7					
Barbarot et al. 2018 (210)	Multinational cross-sectional survey study	US (*n* = 19,986) Canada (*n* = 10,004) France (*n* = 9,964) Germany (*n* = 9,971) Italy (*n* = 9,897) Spain (*n* = 9,924) UK (*n* = 10,001) Japan (*n* = 10,911)	18–64	Overall 4.9 2.2 for Germany to 8.1 for Italy		US 3.5 Canada 4.4		2.1 Japan	
Hwang et al. 2010 (255)	National health insurance register	997,729	All ages, mean ± SD 33.8 ± 20.70					1.2	
Latvala et al. 2005 (32)	Military services assessment	1.4 million	18–19	1.2 1.2					
Werfel et al. 2018 (374)	Cross-sectional survey	9,971	18–65	2.23					
Zietze et al. 2018 (373)	Health insurance data	3.3 million	18+	1.6–1.9					

SD: standard deviation; GP: general practitioner.

For children, the lifetime prevalence of doctor-diagnosed AD ranged from 4.7% to 20.2% assessed at age 7–15 years and for adults ranged from 17.6% to 20.2% (28, 31, 45).

Trends by continent: 21st century data. In Asia, studies reporting repeated measures indicated higher proportions of AD in the 21st century. For example, Liao et al. (46) assessed the prevalence of parent-reported AD symptoms in 2002 and 2007 in 6–8-year-olds in Taiwan and reported an increase from 5.8% to 7.7%, and an increase in lifetime prevalence of doctor-diagnosed AD from 18.0% to 23.9%. In the 21st century in Europe and North America there was no specific trend and data seemed stable for studies that reported repeated measures (46–53).

Trends by continent: all data (1958–2018). As shown in Supplements 2–4 (available from http://lup.lub.lu.se/record/e240247d-7664-4263-9918-3b38e704fd06), in Africa, prevalence of AD has generally increased; some studies that reported repeated measures of AD across different years confirm this trend (54–56), although one study from Nigeria reported the opposite trend (57). In Asia, some studies suggest an increasing prevalence (46, 58–60), but the results are mixed (61–63) and prevalence of AD was generally lower compared with other regions such as Europe. In the USA, the prevalence reported was somewhat higher in the 21st century compared with the 20th century; however, the few studies reporting repeated measures suggested no clear trend (64–66). In Europe, most studies reported an increasing incidence and prevalence in the 21st century compared with the 20th century and studies reporting repeated measures also suggest an increase in AD (67–74), although other studies found no increase (53, 75). In Australia, most studies suggested a higher prevalence in recent years compared with the 20th century, and this was confirmed in most of the repeated measures studies (76, 77).

Prevalence by sex: all data (1958–2018). Of all studies, 54 reported on the prevalence or incidence of AD by sex. The 1-year prevalence of AD and lifetime prevalence of doctor-diagnosed AD was higher in females (range 0.6–24.3%; 1.0–35.5%, respectively) than in males (range 0.8–17.6%; 1.4–37.3%, respectively) in most studies (Supplements 5–7; http://lup.lub.lu.se/record/e240247d-7664-4263-9918-3b38e704fd06), and this was consistent across different continents, although a higher prevalence in males was also reported (72).

The point prevalence in children assessed in good-quality studies was 24% in females compared with 35% in males at age up to 1 year; in schoolchildren the proportions were 11.1% and 8.1%, respectively (78, 79). One good-quality study that used the NOS assessment in adolescents aged 12–14 years showed a 1-year symptom prevalence for girls of 9.64% and for boys of 17.10% (80). In adults, the point prevalence was 10.2% in females and 5.8% in males (28). The 1-year symptom prevalence in female adults was 13.1% (95% confidence intervals [CI] 12.4–13.8) and in males 10.8% (95% CI 2.4–13.8).

Prevalence by age and continent: all data (1958–2018). The prevalence of AD was stable across age groups and across populations. There were no differences in prevalence across continents; for example, prevalence of AD was high in both Sweden and Africa. However, lower prevalence was observed in China, central Asia, and eastern Europe. There was no clear trend regarding age groups. For example, Burr et al. (82) reported a 1-year prevalence lower than 10% for children, similar to Nissen et al. (83), but higher prevalences were also reported and similar numbers reported for adults by Williams & Strachan (84). However, when considering the range of reported 1-year prevalence in the 21st century, children showed the highest prevalence (22.6%) (28, 36, 81–84).

Study design and assessment methods

There was heterogeneity across study designs and study populations and therefore a meta-analysis was not performed. Studies using signs of AD (ISAAC) reported a higher prevalence of AD than those using physician diagnosis. The number of times AD was measured per study period did not significantly affect the reported prevalence of AD.

Incidence of atopic dermatitis

Atopic dermatitis incidence for 21st century data. The incidence of AD was reported in 17 studies; of these, 6 studies were conducted in the 21st century (**Table III**). The 1-year incidence ranged from 10.2 (95% confidence interval (CI) 9.9–10.6) in Italy to 95.6 (CI 93.4–97.9) per 1,000 person-years in children in Scotland. The incidence of AD in adults was 7.41 (6.27–8.74) per 1,000 person years in 1968 (85).

Atopic dermatitis incidence for all data (1958–2018). In all included studies, the highest incidence of AD occurred during infancy and the incidence was also high in early childhood. For example, Nissen et al. (83) reported the highest incidence of AD during the first 18 months of life, von Kobyletzki et al. (11) reported that approximately 80% of children with AD had disease onset during infancy, and Williams et al. (84) reported that 66% had disease onset by the age of 7 years. Ballardini et al. (81) found that, between age 0–12 years, the proportion of "new" incident cases in the last 12 months in Stockholm, Sweden, was 53% of all prevalent cases. However, a considerable incidence was also reported during adolescence and adulthood. The reported proportion of adult-onset AD was 8.0% in Germany at age 28–30 years (1, 52, 86–90).

Study quality

The study quality ranged from moderate to good, as shown in Supplement 2 (http://lup.lub.lu.se/record/e240247d-7664-4263-9918-3b38e704fd06). One study

Table III. Summary of study results regarding incidence of atopic dermatitis in children and adults from the year 2000 and onwards

Author, year and reference	Study type	Year of enrollment/ study start	Study size (participants, *n*)	Females (%)	Country	Age, (range) at enrollment	Age, years at study end	Follow-up	Definition of eczema	Incidence definition	One-year incidence baseline (if applicable)	One-year incidence (95% CI)	Male incidence	Female incidence
Anandan C et al., 2009 (87)	Cohort	1995	unclear	nr	Scotland	All ages	All ages	8	Doctor diagnose and symptoms	Incidence rate of eczema per 1,000 patients per year	nr	10.2 (9.9–10.6)	8.8 (8.4–9.2)	11.6 (11.1–12.1)
Cantarutti et al., 2015 (37)	Cohort	2006–12	145,233	47.9	Italy	0–13	0–13	6	Doctor diagnose	Incidence per 1,000 person-years	14.1 (13.4–14.7)	16.5 (15.6–17.5)	nr	nr
Halkjaer LB et al., 2006 (344)	Birth cohort	2001	411	50.6	Denmark	At age 1 month	1–2	Scheduled visits every 6 months. Age at last follow-up 3 years	Doctor diagnose and symptoms	Incidence of atopic dermatitis per year	At 1 year 31%	10% from age 1 to age 2 years	nr	nr
Mebrahtu et al. 2016 (38)	Cohort	2012–14	13,734	nr	UK	0	3–7	Median 5.55; range, 0–7.6	Doctor diagnose or treatment-based algorithm	Incidence rate per 1,000 person-years	nr	95.6 (93.4–97.9)	96.5 (93.4–99.7)	94.8 (91.7–98)
Mebrahtu et al., 2016 (38)	Cohort	2012–14	13,734	nr	UK	0	3–7	Median 5.55; range, 0–7.6	Doctor diagnose or treatment-based algorithm	One year incidence, %		52.4 (51.5–53.2)	nr	nr
Mohn et al., 2018 (378)	Cohort	2009–15	357,451 (2009) 373,954 (2014)	nr	Norway	<6	6–12	6	Doctor diagnose or treatment-based algorithm	Incidence rate per patient-years.	2009: 0.028 (0.028–0.029) 2014: 0.073 (0.071–0.075)	2014: 0.034 (0.033–0.035)	nr	nr
Simpson et al. 2008 (52)	Cohort	2001–05	>30 million	nr	UK	All ages	nr	4	Doctor diagnose	Age and sex standardized one-year incidence per 1,000 patient-years	9.6 (9.5–9.7)	13.6 (13.5–13.7)	nr	nr

nr: not reported; SD: standard deviation.

reported on infant-onset AD and this may have excluded prevalent AD diagnosed during later childhood or adulthood.

Some studies, such as the COPSAC study, included high-risk infants in addition to the "general population", thus potentially overestimating the prevalence and incidence of AD (91).

DISCUSSION

This was a systematic review of 378 cross-sectional and birth cohort studies of several million individuals from all continents. The findings indicate a high prevalence of AD across continents.

The studies were heterogeneous, which made it difficult to compare the epidemiology of AD in different settings. Several different diagnostic criteria were used and the study designs differed. Furthermore, the appearance, knowledge of, and definition of AD may differ across continents, cultures, and time periods. This makes comparisons between geographical regions and time periods difficult. The study size also varied considerably. However, with this in mind, the results suggest that there are steady prevalence estimates across different age groups.

There were more studies on children, and doctor-diagnosed 1-year prevalence of AD was seldom assessed in Africa, South America, and Australia. This may be partly explained by differences in healthcare, as the European studies often used general practitioner datasets or insurance data.

The reported prevalence of AD was usually higher during the 21st century than the 20th century, especially in Africa and even in Europe. The data for Asia were more heterogeneous. There was a high prevalence of AD in children and adults. The high prevalence of AD in adults could be explained by high persistence or adult onset of AD. Some studies suggested a higher prevalence of AD for females than for males across all ages; however, there were conflicting results regarding sex differences. A higher prevalence of AD in males may be a result of surveillance bias in some settings (72). Interestingly, the incidence was high in all age groups, and more studies are needed on the definition and associated factors of adult incident AD.

Strengths and limitations

No articles were excluded from the review because of language restrictions, and the search strategy was designed to detect all relevant studies. However, it is possible that some relevant

studies were missed. The definitions of AD may have changed over the decades; however, the trends in data using doctor-diagnosed AD, self-reported AD using ISAAC criteria, and otherwise-reported AD were quite stable.

Some diagnostic criteria included infant onset of disease and thus some cases of AD with onset later than infancy might have been missed (92). As the symptoms and signs of AD may vary across age groups and skin types, using the same diagnostic criteria for different groups of patients may overestimate or underestimate AD in some groups. However, comparison of data using similar diagnostic criteria is very useful, and validated self-report measures to diagnose AD are needed.

Although similar diagnostic criteria were used in some studies, like the ISAAC or adapted ISAAC criteria, differences in study design and slight differences in the questionnaires used made it difficult to summarize the data. In contrast, the study by Williams et al. compared the prevalence of AD symptoms in 56 countries using a similar study design and method (93).

This systematic review included a comprehensive search and a critical assessment of the reviewed studies. The findings report data from representative population-based epidemiological studies, including those with large representative cohorts, data from several decades, and data from all continents. The study thus reports on findings in highly diverse settings and populations.

However, some included studies were designed to assess the prevalence and incidence of AD, whereas others reported on AD as a secondary outcome. The definition of AD is important, as it affects the reported proportions; it is possible that other forms of dermatitis were included. Most epidemiological studies had no information on treatment, which might have influenced disease symptoms and reported prevalence of AD symptoms.

Many studies lacked data on participation rate, and only a few studies reported data on socioeconomic position. It is possible that individuals with AD who had higher socioeconomic status were more likely to participate.

The studies in this review included data from 1958 until 2017. The changes in prevalence and incidence may reflect changes in disease patterns and prevalence of risk factors; however, the fact that studies used different methods of AD assessment should be kept in mind. This review reports point prevalence, 1-year prevalence, and lifetime prevalence. This comprehensive reporting may be useful, as prevalence of AD can show seasonal variations.

Comparison with other studies

The results of this study compare well with results from a systematic review using ISAAC data with a mean 12-month prevalence of 7.9% at age 6–7 years and 7.3% at age 13–14 years. The present data are also in accord with data from ISAAC studies suggesting that there is no clear pattern of prevalence of AD across continents (17). The results are in line with studies suggesting a lower prevalence in the 20th century than in the 21st century (94, 95). In a systematic review by Abuabara et al. (96), a prevalence of AD for adolescents/young adults and children was similar to our findings. A review by Pols et al. (95) reported that the assessed prevalence of AD may vary according to diagnostic methods. More studies are needed using the same validated diagnostic tools and a similar study design. There are more studies on the epidemiology of AD in Europe and the USA; a comprehensive worldwide assessment is needed.

There is also a lack of incidence studies. An understanding of incidence is important for the understanding of disease mechanisms (97). Changes in incidence can even suggest risk factors that need targeting. Most studies use questionnaire data to assess the prevalence of AD, and validated diagnostic criteria are important. The ISAAC criteria and the UK criteria are validated and used worldwide, which permits data comparisons. Further standardization and validation for self-reported assessment of AD may be useful. The results of this study have relevance for healthcare planning and patient counselling.

Below, the 14 references appearing in Tables I–III of this papers are numbered in accordance with the complete list of references also appearing in the supplements shown at: http://lup.lub.lu.se/record/e240247d-7664-4263-9918-3b38e704fd06.

Conclusion

As assessed by both patients and physicians, AD is a common disorder that has increased in most continents and reached a stable plateau in Europe and North America. There are only a few recent studies on the incidence of AD in the 21st century and no studies on adults only; most studies have been conducted in Europe and the USA. More epidemiological studies on childhood and adulthood AD in different continents are needed, especially on the incidence of AD during adulthood. However, assessment of AD must be more standardized across cultures in order to improve future epidemiological studies.

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Appendix 1

Search strategy

Database: Ovid MEDLINE(R) ALL <1946 to June 07, 2019>

Search Strategy:

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1 Dermatitis, Atopic/ or exp Eczema/ or "atopic dermatit*".mp. or eczem*.mp. (42116)

2 Epidemiology/ or exp Epidemiologic Methods/ or epidemiolog*.mp. (6379163)

3 Remission, Spontaneous/ or Remission Induction/ or incidence/ or prevalence/ or remission.mp. or incidence*.mp. or prevalen*.mp. or persisten*.mp. (1891461)

4 1 and 2 and 3 (5085)

5 "population based".mp. (119262)

6 2 or 3 (7138602)

7 1 and 5 and 6 (603)

8 4 or 7 (5321)

9 exp Animals/ not Humans/ (4587438)

10 8 not 9 (5256)

11 limit 10 to (english or german) (4876)

12 remove duplicates from 11 (4869)

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Embase

Session Results Date 10 Jun 2019

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No.	Query Results	Results
#11.	#8 NOT #9 AND ([english]/lim OR [german]/lim)	4,752
#10.	#8 NOT #9	5,274
#9.	'animal'/exp NOT 'human'/exp	5,257,153
#8.	#4 OR #7	5,323
#7.	#1 AND #5 AND #6	867
#6.	#2 OR #3	4,049,189
#5.	'population based'	167,518
#4.	#1 AND #2 AND #3	4,862
#3.	'remission'/exp OR 'incidence'/exp OR 'prevalence'/exp OR remission OR incidence OR prevalen* OR persisten*	2,704,826
#2.	'epidemiology'/de OR epidemiolog*	2,011,417
#1.	'atopic dermatitis'/exp OR 'eczema'/exp OR 'atopic dematit*' OR eczem*	75,678

REVIEW ARTICLE

Counting the Burden: Atopic Dermatitis and Health-related Quality of Life

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Atopic dermatitis is the most prevalent chronic inflammatory skin condition globally. The burden of atopic dermatitis on children and adults is extensive and there is also significant impact on the lives of patient caregivers and family members. It is important to be able to measure this impact to inform clinical decisions and to plan appropriate patient and carer support. The current impact of atopic dermatitis on children and adults can be measured using several different quality of life questionnaires: the most frequently used are the Dermatology Quality of Life (DLQI), Children's Dermatology Quality of Life and Infants Dermatology Quality of Life. The impact on partners and family can be measured using several atopic dermatitis specific questionnaires or the Family DLQI or the generic Family Reported Outcome Measure, FROM-16.

Key words: eczema; atopic dermatitis; quality of life; dermatitis.

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The dry, itchy, eczematous skin of atopic dermatitis (AD) has a profound impact on quality of life (QoL). The pathophysiology of AD is postulated to be a combination of epithelial barrier defects, (1) immune system dysfunction (2) and psycho-neurogenic inflammation (3). The characteristics of AD are heterogenous with varying clinical presentations according to age or anatomical region (4). AD has also been described as a systemic disorder given its wide-ranging associations from malignancies to cardiovascular effects (5). It is the most prevalent chronic inflammatory skin condition globally (6), but there are challenges in collating the extensive epidemiological data. Worldwide, up to 50% of cases labelled as AD are not in fact truly 'atopic' i.e. phenotypic eczema that is associated with circulating allergen-specific IgE. A phase two study of the largest AD sample in the world demonstrated a weak association between flexural eczema and atopy (7, 8) and therefore it cannot be assumed this presentation is always attributable to atopy. Furthermore *ad hoc* prevalence studies are often diverse and based on different diagnostic and sampling methods making true data comparison difficult.

SIGNIFICANCE

Atopic dermatitis is the most common inflammatory skin condition globally that affects both children and adults. The symptoms of atopic dermatitis as well as the demands of treatment often contribute to a significant impact on patient quality of life (QoL). This QoL impairment may also extend to caregivers, partners and close family members of atopic dermatitis sufferers. This review aims to evaluate the impact of atopic dermatitis on the QoL of patients and close relatives. A myriad of tools are available for measuring QoL; a brief description of the most relevant instruments is also presented in this article.

The burden of disease of AD on children is extensive and there is also significant impact on the lives of patient caregivers and family members (9). In affected adults, this effect is multi-dimensional with implications for mental health, work productivity and QoL. This review focusses on the measurement of QoL in AD patients, in particular on the QoL measures recommended by Harmonising Outcome Measures for Eczema (HOME), and the implications of the wider impact that AD has across different ages, social groups and countries.

Health-related quality of life (HRQoL) is a specific aspect of the wider concept of "quality of life". Throughout this manuscript "quality of life" refers to HRQoL.

EPIDEMIOLOGY OF ATOPIC DERMATITIS

The determination of accurate prevalence data for any disease depends on there being clear agreed diagnostic criteria and the ability to gather data from subjects that represent the general population. However, there are several differing diagnostic criteria that may be used in surveys of AD prevalence, contributing to confusion, and the methodology of many surveys leads to selection bias, for example if data from a clinic is measured rather than from a population cross-section. The various prevalence figures quoted in this review relate to the population described in the corresponding reference and may not be generalised to other populations.

Most AD epidemiological data have focussed on the paediatric population (9). The advent of the International Study of Asthma and Allergies in Children (ISAAC) has provided a standardised platform to identify over a

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million children suffering with AD worldwide (10). The prevalence ranged from 0.9% (India) to 22.5% (Ecuador) in a sample of 380,000 children aged 6–7 years from 60 countries (11). For teenagers (ages 13–14, 660,000 subjects) the prevalence values range from 0.2% (China) to 24.6% (Columbia) with generally higher values seen in Latin America and Africa. In the European Union the point prevalence is 4.4% (12).

There have been several studies examining the adult population. The European Community Respiratory Health Survey (ECRHS) study collated data from US and European subjects and identified prevalence rates ranging from 0.3% (Switzerland) to 6.2% (Estonia) (13). Recently, Barbarot et al. (12) conducted an international survey on representative samples of adults (ages 18–64) worldwide using standardised methods and diagnostic criteria. Prevalence values ranged from 2.1% (Japan) to 8.1% (Italy), and there were further variations within countries and regions. Generally, there was a higher prevalence in females, but in the UK and the USA there was no significant difference in prevalence between females and males. Peak prevalence was from age 25 to 45 years, with AD then becoming less prevalent with increasing age ($p < 0.05$). However, a study limitation was that subjects self-diagnosed using modified UK Working Party criteria, with under 10% having a physician diagnosis. Regardless of which measure was used, USA subjects reported having the most severe AD, whereas in southern Europe the prevalence of mild disease was higher than in northern countries such as in the UK (12).

A systematic review of 13 studies conducted in the Netherlands and the UK demonstrated that the prevalence of AD assessed by general practitioners (1.8–9.5%) was lower than when self-reported (11.4–24.2%) (14). This may be because milder cases do not present to general practitioners, or self-reporting may over-diagnose. Kim et al. (15) analysed 110,000 cases and reported that the mean age of AD diagnosis was 1.6 years, with < 5% cases experiencing persistent disease at 20 years follow-up. Disease severity, duration, later onset and female sex were all associated with persistent disease.

As the above studies demonstrate, there is a large burden of disease from AD. It is imperative to measure the impact of this condition in those who are affected by it, because this information is essential to inform the clinician concerning choice of therapy. This data is also useful in the assessment of novel therapies, and in monitoring response to therapy.

PATIENT REPORTED OUTCOME MEASURES

A Patient Reported Outcome (PRO) is any report that comes directly from a patient about a health condition or its treatment, without interpretation by a clinician or anyone else (16). The initial drive for PROs was led by the pharmaceutical industry. In the US during the late 1980s there was an increased awareness of the importance of patient input in assessing treatment. The seminal Rand Health Insurance experiment collected patients' self-report of health status to understand the impact of health insurance plans on health outcomes (17). Following this, Tarlov et al. (18) conducted an observational study to ascertain how outcomes of care were affected by specific components of the health care system. This landmark Medical Outcomes Study concluded that tools should be developed for "monitoring the patient wellbeing in office practice and clinical research." The Food and Drug Administration (FDA) initiated the requirement for QoL assessments in oncology trials (19). However, a report of a PRO measure used as an endpoint in a clinical trial involved anti-hypertensives: when the results were published by the press, although the endpoint measured tolerability rather than efficacy, the stock market value of the pharmaceutical company rose resulting in an economic impact of a health related outcome (20). The term "patient reported outcome" was coined in the year 2000 and the plethora of outcome measures subsequently developed led to the development of a PRO harmonisation group (21).

PROs may include evaluation of symptoms, functional status, or general or HRQoL.

THE IMPACT OF ATOPIC DERMATITIS ON QUALITY OF LIFE

QoL measurement has become an integral aspect of monitoring disease and intervention efficacy across dermatology. Three dimensions in particular have been proposed that are key to QoL evaluation: 'now', 'long-term' and 'family' (22). The 'now' is important for current assessment, but the long-term effects as well as wider implications for family should also influence treatment and health-economic decisions. It is vital to understand the various aspects of QoL impairment across the range of AD sufferers.

The impact of AD on children is comparable to other childhood chronic diseases such as cerebral palsy, epilepsy and cystic fibrosis (23). A review by Olsen et al. (24) identified data from 37 studies on 4082 children with AD and found that AD had, on average, a moderate effect on health-related QoL. However in each study there was a wide range of reported impacts of AD. Children with AD are often affected on a daily basis including problems when feeding, changing clothes and playing, thus depriving them of a 'normal childhood'(25). The chronicity of AD is often not a focus in studies: QoL scores may differ between primary and secondary care settings as the latter are likely to include more severe cases.

There are similar concerns for teens and adolescents. Parents fear that their children may be unable to make friends when older (26). Growing up, they develop a sense of being different due to alienating comments and having to explain several misconceptions (27), eventually

leading to a feeling of isolation and the need to be 'different' (28). Despite the debilitating nature of AD and the wider effect on school-work, AD does not impact academic performance in adolescents (29) and compliance with topical treatment in this group was reported in one study to be as high as 96% (30). Nevertheless, AD may influence career pathways. Advice to adolescents about work where having AD may involve risk is important to help them decide appropriate careers (31). The transition from paediatric to adult clinics is often a challenging period and the Department of Health in England has identified a specialised need in this area (32). A trial of 'young adult' clinics for AD patients with open access psychological support demonstrated significant improvement in QoL with high satisfaction rates.

AD has long been considered mainly a childhood problem, but the prevalence in adults ranges between 3–5% (33). In a review of two cohorts, 38% of adults with AD had symptom onset in childhood. (34). Over half of adult patients report that AD has a moderate to extremely large effect on their QoL. Many describe pain, stinging and embarrassment from their AD impacting their choice of clothing. The burden increases with increasing severity of disease (35): 57% of adults miss at least one day of work in the preceding year and describe problems with intimacy and feelings of guilt due to AD. Over 10% of 1189 people with moderate to severe AD demonstrated depressive symptoms (35). Of those subjects suffering from severe AD, 88% felt their ability to tackle life was at least partly compromised (35).

Whether the patient is a child, teenager or adult, AD impacts on the extended family as well as on caregivers, a concept described as 'The Greater Patient' (36). This effect may be experienced by anyone with a close relationship with the patient (37). This broader impact of disease is increasingly being recognised as another dimension of healthcare, with the advent of several new questionnaires to ascertain this impact. AD, being a common childhood condition, is a particularly relevant field of research given the 'web of relationships' involved from an early stage (38).

Several major life changing decisions, such as choice of education, choice of career, choice of partner or decisions about whether to have children may be influenced by having a chronic skin disease such as AD (39). The impact of the disease on such decisions can therefore alter the life course of people affected, with the impact of the disease echoing through the decades.

A BRIEF HISTORY OF QOL IN ATOPIC DERMATITIS

A plethora of QoL measures have been developed within dermatology, especially in psoriasis and AD. A systematic review by Rehal & Armstrong (40) in 2011 attempted to identify trends in outcome instruments used in AD trials. Of the 382 studies included, only 67 studies incorporated QoL measurements. Eleven instruments were identified for measuring QoL, of which the Children's Dermatology Quality of Life (CDLQI) was the most frequently used followed by Dermatitis Family Index (DFI), Dermatology Life Quality Index (DLQI) and Infant Dermatology Quality of Life questionnaire (IDQoL). Three tools measured the QoL of family members of patients with AD: DFI, Parents Index of Quality of Life in Atopic Dermatitis (PIQol-AD) and Parents of Children with Atopic Dermatitis (PQol-AD). The authors surmised that an overall increase in use of QoL instruments from 1985 to 2010 indicated the emerging importance of QoL measures for patient evaluation and management.

HARMONISING OUTCOME MEASURES FOR ATOPIC DERMATITIS

Noting the myriad of outcome assessments for AD, the first International Conference on HOME was held in 2010 (41) and a decision was made for a core outcome set (COS) to be developed for AD. All scales had to pass the OMERACT filter of truth, discrimination and feasibility (42). The studies assessing the validity of different instruments were required to pass the COSMIN checklist (43). In 2011, 4 outcome domains were agreed on: symptoms, clinical signs, long-term control of flares and QoL (44). At the HOME III meeting Eczema Area and Severity Index (EASI) was recommended as the instrument for the outcome disease severity (45), HOME IV recommended Patient Oriented Eczema Measure (POEM) as the PRO for measuring symptoms (46). Heinl et al. (47) in 2016 conducted a study on QoL instruments used in eczema trials using the Global Resource of Eczema Trials (GREAT) database. In the 303 studies included from 2002–2014, approximately 90% of studies used a PRO, however only 63 used QoL measures. Eighteen named and 4 unnamed QOL instruments were found. Unlike the study by Rehal et al. mentioned above, (40), Heinl et al. (47) did not find evidence of increasing use of QOL measures, however confirming Rehal et al's finding, the DLQI, CDLQI, IDQol and DFI were the most frequently used instruments. Four instruments measured the impact of AD on carers of patients of which two were named (DFI, PIQoL-AD).

Around the same time Hill et al. (48) conducted a systematic review looking at trends in disease severity and QoL instruments for patients with AD. Only 45 of the 135 identified studies measured QoL. Again, the DLQI, CDLQI, IDQoL and DFI were the most commonly used instruments. Hill et al. found 28 QoL measures in contrast to the 22 reported by Heinl and colleagues (47), possibly due to the different databases searched. Hill et al. (48) also found that the number of articles reporting on QoL peaked in 2012. Three instruments (DFI, FDLQI and PIQoL-AD) measured impact of QoL on caregivers.

HOME V concentrated on the definition core outcome for long-term control and its measurement as well as future areas of research for a tool to measure children's QoL (49). It was agreed that a new instrument should be developed for long-term control and that further research on itch intensity was necessary. It was also decided that none of the QoL instruments could be recommended at that point in time due to concerns with validation in certain areas.

However, the sheer number of QoL instruments in the above studies, with some instruments used only in single studies, highlighted the importance of standardised methods for measuring QoL in AD in order to compare various intervention measures. Therefore, at the 2019 HOME VII meeting (50) it was agreed to recommend DLQI and CDLQI to measure the QoL of adults and children and the proxy measure IDQoL to measure the QoL of infants. Two new instruments which had been developed in response to the recommendations from HOME V, Atopic Dermatitis Control Test (ADAPT) and Recap of Atopic Eczema (RECAP) were recommended for measuring long-term control. In addition, the Numerical Rating Scale (NRS-11) (51) to measure the intensity of itch was recommended in addition to POEM as the PRO to measure symptoms. It was also agreed that the COS for AD should be measured at baseline and end of the primary endpoint to ensure comparability in trial results.

QUALITY OF LIFE INSTRUMENTS FOR ATOPIC DERMATITIS CHOSEN BY THE HOME INITIATIVE

Historically, the value of clinical research has been reduced by different outcome measures being used in individual studies, making comparison impossible. The HOME initiative, by identifying a set of core measures provides the potential for improved assessment, comparison and combination of data.

Dermatology Life Quality Index

The DLQI is a dermatology-specific questionnaire developed in 1994 (52). There are over 110 translations, used in over 80 countries (53). The DLQI is quick and easy to perform and score in routine clinical practice. During the initial development, 120 patients answered the open-ended question "list all the ways your skin disease affects you". The questionnaire was developed from the answers.

The DLQI is a 10-item questionnaire with a one week recall period. It is completed, on average, in two minutes. The DLQI assesses the impact of skin disease on symptoms and feelings, daily activities, leisure, work and school, personal relationships and the impact of treatment. The ten question scores (each 0–3) are added to give the DLQI score (maximum 30).

The DLQI has been extensively validated in numerous studies with regards to its psychometric properties as well as its use in clinical research (54–56). The DLQI structure has been examined with respects to dimensionality indicating one to 4 factors across various studies (54). It is responsive to change (57, 58) with high test–retest reliability (59, 60).

The DLQI validated score banding (61) allows meaningful score interpretation. For example, score band 0–1 indicates no effect on a patient's life and 11–20 a large effect. This banding can help inform clinical decisions. The DLQI has been significantly correlated with numerous other measures highlighting its construct validity (54), and used as the standard comparator in the validation of many novel QoL questionnaires. The DLQI has been mapped to the EQ-5D using ordinal logistic regression allowing the prediction of dermatology-specific utility values from generic EQ-5D scores (62). The model allows the capture of disease-specific data that generic measures are often unable to capture, thereby generating more precise health economic data without the need for utilising multiple questionnaires. However, though the model is validated for large groups of data, it requires further testing at an individual subject level. An electronic format has been developed and validated against the paper format demonstrating equivalence (63).

Although the DLQI is the most commonly used measure across dermatology (55, 64), several limitations have been described including concerns regarding under-representation of emotional aspects and its uni-dimensionality (65). Furthermore, there are concerns over score interpretation when "not relevant" options are chosen. In the DLQI, for 8 of the 10 items it is possible for the respondent to choose "Not relevant". If the subject does this for one question, because the life aspect enquired about is not part of the respondent's usual life pattern, then the overall maximum score is reduced. The more questions that are answered "not relevant" the greater the impact on the maximum possible score. Some subjects might therefore not reach a critical level that is used to help inform a clinician concerning the use of some therapy, even though the reason that a question may be "not relevant" may be that the skin disease has severely impacted that aspect of the respondent's life. It has therefore been suggested that the final score should be adjusted depending on the number of "Not relevant" answers given (66).

However, introducing an additional more complicated scoring system may not be appropriate (67) and would be impractical in busy clinics, require a wide range of revalidation studies to be performed and introduce confusion into the interpretation of DLQI scores (68). Whatever method is used to calculate them, DLQI scores should be used to help the clinician take the most appropriate decision for individual patients, and not used to restrict clinical judgement. A simple approach would be for any clinician reviewing a completed DLQI, or indeed any QoL questionnaire, to note whether or not there were any "Not relevant" answers, to enquire further and to take this into account as part of the information informing their clinical decisions.

Although many properties of the DLQI have been extensively validated, the DLQI has been criticised for not having been subject to Rasch analysis (69, 70), a method for the refinement of items and to convert the ordinal scale to a fundamental measure. However, the high face validity of the questions, the simplicity of its use and the easy interpretability of its scores have led to the DLQI being the first QoL measure with which dermatologists worldwide have become familiar (71), contributing to a cultural shift towards patient-centred medicine. Many clinicians have embedded the use of the DLQI in their routine practice because of their experience of its usefulness in routine clinical care, and the DLQI is incorporated in national guidelines or registries in at least 40 countries.

The DLQI has been recommended by the HOME initiative as the core instrument for measuring the impact of AD on the QoL of adult patients with AD (50).

Minimal Clinically Important Difference

The minimal clinically important difference (MCID) is the minimal change in score considered clinically significant by clinicians and patients (72). This provides additional meaning to QoL score changes. The DLQI MCID value is 4 points (73). We have proposed a 'multiple-MCID' concept has (74) to allow a more distinguishing analysis of interventional studies. However, this requires extensive further validation.

Children's Dermatology Life Quality Index

The CDLQI measures the impact of skin conditions on the QoL of children aged 4–16 years (75). A 10-item questionnaire was developed, based on 169 replies from children, asking how their skin condition affected their life. The CDLQI measures impact over the last week on symptoms and feelings, leisure, school or holidays, personal relationships, sleep and treatment. One question has a choice of two options dependent on whether or not within the last week the child was in school or on holiday. Each question has 4 possible answers. A cartoon version appeals to younger children (76). The CDLQI has been validated extensively (77–79). It is completed in mean in 2 min and has score bands to give meaning to the scores (80). There is no published minimal clinically important difference (MCID) for CDLQI described for use across all skin diseases. However, for use in children with AD it has been suggested that the MCID for the CDLQI is between 6–8 points (81).

The CDLQI has been recommended by HOME as the core QoL instrument for measuring the impact of AD on the QoL of children (50).

Infants' Dermatitis Quality of Life index

The IDQoL is a dermatitis specific parent/caregiver proxy measure of the QoL of children under the age of 4 years (82). It is a 10-item questionnaire with a one week recall period. The items measure the perceived impact on QoL of itch and scratch, mood, time to sleep, playing or swimming, family activities, mealtimes, treatment, dressing and undressing, and bath time. An additional question records the severity of dermatitis as perceived by the parent/caregiver. The IDQoL had been translated into several languages and is frequently used in AD trials and validation aspects have been described (83). The IDQoL has been recommended by HOME as the core QoL instrument for measuring the impact of AD on the QoL of infants (50).

The core measures chosen may change in the future if more appropriate measures are developed, but there is huge strength to be gained by always using the same set. The minimal clinically important difference and descriptive score meaning bands have not been described for the IDQoL.

Disability adjusted life years

Whereas Quality Adjusted Life Years (QALYs) are years of healthy life lived, Disability Adjusted Life Years (DALYs) are years of healthy life lost. To calculate the burden of a certain disease, the disability weighting is multiplied by the number of years lived in that health state and is added to the number of years lost due to that disease (84). Using DALYs, the global burden of skin disease survey revealed that eczema causes the highest burden of all skin diseases worldwide (85). Eczema is one of top 50 most common causes of disease, with a global prevalence estimated at 229 million people affected. However, it must be remembered that AD affects the QoL of not only those directly affected but also their close family members.

FAMILY IMPACT OF ATOPIC DERMATITIS

Impact on parents

AD is a chronic disease so the symptoms require constant attention. Treatment for AD includes regular use of emollients along with various topical and systemic measures. The treatment process can have an adverse impact on the QoL of the patient (86) and also the main caregivers, especially when young children are affected. Inevitably parents are affected too. A meta-ethnography study (87) collated parental and childhood/adolescent experiences of AD. It is postulated that parent and child bonding is affected as skin irritation may limit physical interactions (88). Furthermore, the associated behavioural difficulties such as restlessness and hyperactivity may be demanding for parents, resulting in frustration and exhaustion (89). Parents may choose not to have further children because of the current burden on the wider family. Dedicating time for treatment application and extra housework also directly impacts parental work responsibilities and therefore has financial implications (90). The symptoms experienced by children e.g. sleep disturbance, restlessness, psychological strain and embarrassment may all be experienced

second hand by parents and therefore their QoL is a key determinant of the child's well-being (26, 91).

Parents report having to apply creams that children dislike, often resulting in the need for coercion (92). Cultural issues may play an important role in parental attitudes to their affected child. Mothers may feel they did something wrong during pregnancy, or develop a sense of guilt for neglecting other children because of their focus on the child with AD (91). Anxiety may be exacerbated by conflicting advice on management, including the long-term sequelae of topical corticosteroids being inadequately explained by health professionals (93).

Loss of sleep is another familiar theme in parents of children with AD. Angelhoff et al. (94) conducted a study into the perceptions of sleep in such parents. Eleven mothers and one father, with children aged 0–2 years with SCORing Atopic Dermatitis (SCORADs) of >15 were interviewed. All but one parent experienced fragmented sleep. Most parents accepted the sleep loss but expressed a desire for longer uninterrupted sleep. Sleep loss led to fatigue with parents perceiving this had a negative effect on the whole family. The participants felt that the sleep loss was normalised by other family members and ignored by health professionals. The participants also felt that dynamics between parents and other siblings had changed, leading to feelings of guilt and sadness.

Moore et al. (95) reported that parents of children with eczema suffered sleep loss, with the mothers losing a median of 39 min and fathers a median of 45 min of sleep. In contrast, parents of children with asthma lost no sleep. While both parents of children with AD had increased anxiety scores, the mothers had two-fold higher scores of depression than mothers of children with asthma. This was related more to the sleep loss than to a direct effect of the eczema.

In contrast, in an ongoing large prospective, longitudinal, population-based cohort study 11,649 mother–child pairs in the UK were followed up by Ramirez et al. (96) from birth to 10 years. Children were classified as having AD on the basis of the presence of flexural dermatitis on two occasions. After adjusting for confounders, sleep duration and early morning awakening were similar in mothers of children with active AD and mothers with children never having reported AD. However, difficulty in falling asleep, subjectively insufficient sleep and day-time exhaustion were more frequently reported in mothers of children with active AD. The authors also reported larger effects in mothers of children with more severe AD. Adjusting for child sleep disturbances did not change the conclusions, and other factors such as anxiety and stress related to caring for children with AD may have been contributory.

Pustisek et al. (97) studied the QoL of 171 parents (mean age 32 years) of children with AD in Croatia. The mean FDLQI score (range 0–30) was 13.6 ± 6.0. indicating a major effect on the QoL of parents. The most frequently recorded problems were time spent looking after the child, household expenditure and emotional distress, as in a Ukraine study (98). The mean Perceived Stress Scale score was 20.0 ± 5.8, 7 points higher than the average person aged 30–40 years, indicating higher stress levels in parents of children with AD and a correlation with QoL.

The impact of a child's eczema on the QoL of mothers and fathers may vary. Marciniak et al. (99) assessing parents QoL with the FDLQI, found that children's AD had a greater impact on the QoL of mothers than of fathers. Whilst the impact on the social life, spare time and daily expenditure was similar, mothers' relationships with other people were more affected than fathers' relationships with others, however the greatest impact on fathers was on their work or education. This was in contrast to the study by Pustisek et al. (97) where work or education were the lowest scoring items on the FDLQI: this could be because most participants in Pustisek's study were female with over half on maternity leave or unemployed.

Counter-intuitively, there may be positive outcomes resulting from a child suffering with AD. Parents may develop a strengthened bond with their children through the extra time spent treating and supervising them (100). To stop children from scratching, parents spend more time holding children closer, and balanced with the discomfort of physical symptoms, this overall creates a deeper emotional closeness (26). Parents also feel empowered by learning about AD and educating others about this debilitating condition (87).

Impact on siblings

Basra & Finlay proposed the term "Greater Patient' to encompass the interdependence of patients with their close relations (36). In childhood AD this includes the parents, who are generally the caregivers, however, in childhood siblings usually live together and their lives may also be affected. Whilst there are many studies on the QoL of siblings of children affected with other medical conditions, notably cancers (101–106), there is a lack of information on the impact of QoL on siblings of children affected with skin conditions, including AD. It is difficult to compare from the literature the effect on the QoL of siblings of skin disease compared to other diseases, due to the wide variety of instruments that have been used. Siblings of children with chronic conditions may have the same QoL as their peers (107), but it has also been suggested that siblings may have increased levels of distress (102). The parent child relationship and the sibling bond may also be affected when a child in the family has a chronic condition (108).

These negative interactions with family members (94, 99) coupled with sleep deprivation can leave patients, and their carers, feeling exhausted, stressed and depressed (96, 97). There may therefore be repercussions on siblings of patients affected with AD: this area needs further investigation.

The above findings illustrate the importance of assessing the QoL of family members. Several dermatology specific and AD specific validated instruments exist for measuring the impact of QoL on family members of patients with AD. The HOME initiative has not yet addressed this. However, the TREatment of ATopic eczema (TREAT) Registry Taskforce has recommended that for research registries for paediatric and adult patients with AD, if family impact is measured, the Family Dermatology Life Quality Index (FDLQI) should be used (109).

QUALITY OF LIFE INSTRUMENTS FOR FAMILY MEMBERS

Family Dermatology Life Quality Index

The FDLQI is a 10-item questionnaire, with a recall period of one month, assessing the impact on the QoL of adult family members of people of any age with any skin condition (110). The questionnaire includes the domains of emotional and physical wellbeing, relationships, leisure activities, social life, burden of care, impact on job/study, housework and expenditure. Each question is scored on a 4-point scale (0–3). The FDLQI has been translated into several languages (111) and has been used in various studies involving AD and other dermatological conditions (97, 99, 112–116).

Dermatitis Family Index

The DFI, the first family QoL questionnaire in dermatology measures the impact of having a child with AD on the QoL on their adult family members (117). This 10-item dermatitis specific questionnaire measures the impact over the last week on QoL in the domains of housework, food preparation and feeding, sleep of others in the family, family leisure activities, time spent on shopping, expenditure, tiredness, emotional distress, relationships and impact of child's treatment. Each question is scored from 0–3 points. There are no validated banding descriptors for the DFI, but some studies have used non-validated scoring descriptors (118, 119). The DFI has the advantage of being eczema specific and its measurement properties have been reviewed (120). The DFI, along with DLQI, CDLQI and IDQoL is one of the most frequently used instruments for measuring QoL in eczema studies (40, 47, 121).

Parents Index of QoL in Atopic Dermatitis

The PIQoL-AD is another AD specific measure to assess the impact of the child's AD on the QoL of parents (122). Developed on the basis of multinational qualitative interviews with parents of children up to age 8 years with AD, this is a 28-item unidimensional questionnaire (123). The lower the score, the better the QoL, a change of 2–3 PIQoL-AD points over time is considered meaningful.

Childhood Atopic Dermatitis Impact Scale (CADIS)

CADIS is a QoL measure for parents of children with AD combined with a proxy measure for children under the age of 6 years (124). It measures the impact on QoL of the domains of symptoms, activity limitations and behaviour, family and social function, parent sleep and parent emotion. This 45-item questionnaire uses 5-point Likert Scales giving a maximum score of 180. The recall period is the last 4 weeks and the questionnaire can be completed in approximately 6 min (125). Whilst it does not have score band descriptors, the MCID is considered to be a 12% change from the total score or a 12% change from any of the individual domains (126).

Family Reported Outcome Measure

Speciality and condition specific questionnaires cannot compare the impact on QoL of family members between different specialities. Golics et al. (127) developed the Family Reported Outcome Measure (FROM-16), based on relatives of patients from 26 medical specialties.

FROM-16 has 16 questions and can be used to assess the QoL of any adult member of the family of a patient of any age with any disease. The average completion time is 2 min. FROM-16 consists of the Emotional domain with 6 questions and the Personal and Social Life domain with 10 questions. Each question has three possible answers: 'Not at all', 'A little' and 'A lot' scoring 0, 1 and 2, respectively. Validation studies have been completed in Germany and Thailand and further validation characteristics are being studied. FROM-16 can be used to compare the QoL of family members across different disciplines in medicine, thus making it easier to make meaningful comparisons in QoL trials involving different medical conditions.

The Impact on Family Scale (IOF) (128, 129) has been validated to measure the impact of QoL on the adult family members of children suffering with chronic illness or disability. However, unlike the FROM-16, which can be used in the family members of patients of any age, the IOF should only be used for family members of affected children

DISCUSSION

In any scientific endeavour, it is essential to be able to measure some characteristic of what is being studied. Without measurement, it may be possible to describe, but impossible to make meaningful comparisons or detect change. It could almost be said that if you can't measure something, it doesn't really exist, at least to a scientist. The same applies in medicine, a field of science that coexists as an 'art'. Advances have followed the ability to measure: measuring blood pressure has enabled identification and control of hypertension, measuring visual fields has allowed diagnosis of ophthalmic and neurolo-

gical conditions and measuring frequency of micturition is used as an alert to diabetes and prostatic hypertrophy. Perhaps because of the visual nature of dermatology, a focus on measurement came late to our subject. But this delayed focus has coincided with a realisation that, as part of delivering the highest quality of care, we need to better understand what our patients are experiencing (130). In addition, qualitative studies should be used more often in combination with quantitative studies to gain more insight into the real burden of diseases such as AD.

This review has focussed on questionnaires specifically designed to measure the impact on QoL of skin diseases in general or of AD in particular. However, there are also a wide range of questionnaires that are designed to be used across all diseases. Examples of such measures include the Short-Form 36, the WHOQOL and EuroQoL (EQ-5D). Utility information giving QALY information is typically calculated from EQ-5D data, and this is sometimes used by national or international drug regulation agencies to inform decisions concerning resource allocation. However, use of QoL data in this way may overlook critical aspects of the reality of the impacts of skin diseases, such as the psychological impact that understanding the risk of mortality, say of a malignant melanoma, may have. And having a basal cell carcinoma that is treated appropriately may have a low impact on QoL, but if untreated the long-term consequences can be extremely serious. Therefore, when QoL measures are used to inform resource allocation, wider aspects of the conditions must also be considered.

This review has described some of the many ways in which the lives of people with AD are affected by their condition. Large multicentre studies in Europe and the USA determined that patients with psoriasis felt that their dermatologists were not aggressive enough with therapy: it is likely that the same applies at least to adult AD. By having insight into the individual patient's experience, more appropriate therapeutic decisions may be made, especially over the coming decade with the advent of many novel powerful systemic therapies for AD.

The Greater Patient, the close family members, may all experience impact on their QoL through having a family member with AD. But the "Greater Patient" also acts as the "Greater Therapist", as family members support the patient with practical therapeutic help, such as application of topical emollients and drugs, and giving encouragement to persist with therapy. The role of the family in promoting adherence to agreed treatment plans should not be underestimated. Therefore, understanding the experiences of family members, and identifying their needs may make a crucial contribution to the success of therapy.

Being able to measure the QoL impact of AD provides stark challenges to the health care team. Of course, the over-riding aim must be to effectively suppress the disease. Having identified the QoL problems we can no longer ignore them and we are obliged to creatively develop methods to address these issues. We now have the tools to assess prospectively the impact of AD on QoL.

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REVIEW ARTICLE

Disease Mechanisms in Atopic Dermatitis: A Review of Aetiological Factors

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Atopic dermatitis is a prevalent inflammatory skin condition characterized by itch and dry skin, which affects 15–20% of children and 3–5% of adults. This article reviews epidemiological, clinical and experimental data to provide an overview of the most important disease mechanisms in atopic dermatitis. Genetic predisposition, environmental insults, atopic triggers, complex host immune response and skin barrier changes, and altered skin microbiota are discussed. Whilst our understanding of atopic dermatitis has improved dramatically in recent years, many basic aspects are still not understood. Further research is needed to fully understand this complex skin disease.

Key words: atopic dermatitis; aetiology; pathophysiology; pathomechanism; risk.

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SIGNIFICANCE

The aetiology of atopic dermatitis is poorly understood, but studies have provided insight into the pathomechanism, which may improve the prediction of onset of atopic dermatitis and its prophylaxis. This review provides an overview of the pathogenesis and pathomechanism of atopic dermatitis.

Atopic dermatitis (AD) is a prevalent inflammatory skin condition characterized by itch and dry skin, which affects 15–20% of children and 3–5% of adults. In large proportions of affected patients AD is chronic or remitting, as shown by epidemiological studies (1).

The pathogenesis of AD is complex and poorly understood. However, in recent years, there has been major advancements in our understanding of the disease mechanism of AD, e.g. through the discovery of common filaggrin gene (*FLG*) mutations as a strong risk factor for AD, as well as the significant clinical effects of antagonistic therapy against interleukins (IL) 4, 13, 22 and 31.

This review provides a holistic overview of the most important disease mechanisms in AD.

INCIDENCE OF ATOPIC DERMATITIS PEAKS IN EARLY CHILDHOOD

AD predominately begins in early childhood, as indicated by a recent prospective Danish study, which showed that nearly all cases of AD are diagnosed before the age of 7 years (2). It is currently unclear to what degree "late-onset AD" is important in absolute numbers, as studies have shown that patients who present with AD in adulthood may have forgotten about their childhood AD, and that the disease may therefore represent re-activation of previous disease. This notion is strongly emphasized by the finding that approximately 29% of Swedish adults aged 31–42 years with a school health record of AD in childhood did not recall this when asked as adults (3). In asthma, patients with adult onset seem to have different disease mechanisms, and it is possible that this may also be the case for AD. Moreover, the epidemiology of AD may change over time, in concert with new causative exposures. As an example, use of cosmetic products in adolescence has been associated with new onset of AD or recurrence of previous disease (4). Nonetheless, AD normally begins in early childhood; a time where the skin barrier is vulnerable to stress (5–7). This will lead to a decrease in the threshold level against common triggers. As discussed in this review, the skin barrier defect is central to the risk of developing AD.

GENETIC PREDISPOSITION

AD is a clinical syndrome, as indicated by the Hanifin & Rajka criteria for AD (8). These criteria dictate that a certain number of major and minor criteria need to be fulfilled in order to make a diagnosis of AD, including a list of phenotypic and heritable characteristics, such as xerosis, palmar hyperlinearity, keratosis pilaris (all associated with *FLG* mutations), infra-orbital folds or darkening, as well as facial pallor. Importantly, family predisposition to atopic disease is a major criterion of the Hanifin & Rajka criteria, and twin studies have shown that the heritability of AD is very high (9). The Hanifin & Rajka criteria were unintentionally developed for use in patients with predominately European ancestry, and it is clear that the phenotypic characteristics observed in other ethnic groups are under-represented, and that the

criteria may fail when used in these populations (10). An example is the recent observation that pigmentation on the lips is associated with AD in Asian subjects (11).

FLG mutations lead to dry skin, characterized by elevated pH, increased colonization with staphylococci, enhanced penetration and reactivity to chemicals and allergens, and therefore, expectedly, a strongly increased risk of AD (12). Nearly all carriers of *FLG* mutations with AD develop their skin disease within their first 2 years of life (13), whereas children with later onset do not have these mutations (14). The discovery of *FLG* mutations provided a new, and much needed, basis for the study of paediatric AD, and led to a strong re-emphasis on primary skin barrier impairment as a crucial factor for the development of AD. Since then, it has been shown that dry skin at birth and at 2 months of age, independent of *FLG* mutations, can predict AD at 12 months of age, and that daily application of emollients in high-risk infants may reduce the risk of AD (15). Importantly, the normal skin barrier in the 2 first years of infancy is very different from that of adult skin; for example, the levels of natural moisturizing factors (NMF), a degradation product of filaggrin, are much reduced (16). The tendency for AD to begin on the cheeks is also explained by a local, very pronounced, reduction in NMF, which may last until 3 years of age (6). The down-regulation of filaggrin on exposed skin areas, as well as the increased prevalence of *FLG* mutations in populations that have migrated far from the Equator, is probably explained by evolutionary benefits due to increased synthesis of vitamin D following facilitated penetration of ultraviolet (UV) (17). Importantly, a deficiency of filaggrin, whether primary or secondary, results in increased penetration of allergens and risk of sensitization, which, in turn, may explain the increased risk of allergic asthma, rhinitis and food allergy in carriers of FLG mutations who have AD (18).

ENVIRONMENTAL EXPOSURE

The crucial role of environmental exposure and skin stressors cannot be overemphasized when explaining the aetiology of the AD epidemic. Modern society has resulted in dramatic changes in human exposure, with increased use of, or exposure to, household products, cosmetics, tobacco, processed food, and air pollution, but at the same time reduced exposure to microorganisms and solar irradiation, as a result of increased hygiene, fewer people living together in the same household, and less time spent outside. Epigenetic changes due to environmental changes or insults could explain a large part of the endemic proportions of AD. In support of this theory, large genome-wide association studies have identified only a small proportion of genetic factors associated with AD (19). However, how the environmental changes have influenced the risk of AD at a mechanistic level is largely unknown.

Being born in the autumn or winter in the Northern hemisphere, or being exposed to a dry and cold climate, has been strongly associated with AD (20, 21). This is probably explained by skin exposure to low temperatures, as well as low ambient humidity due to indoor heating, which can negatively affect the skin barrier and result in dermatitis (22). Similarly, bathing infants in hard water may increase the risk of AD, possibly due to increased pH, which, among other aspects, results in premature cleavage of cornedesmosomes (20). Exposure to air pollution and being born in a newly built home have also been associated with AD (23, 24), perhaps because chemicals negatively affect the epidermal barrier. For example, short-term exposure to airborne formaldehyde results in increased water loss from the skin surface (25) in patients with AD, and toluene, a common air pollutant, can directly down-regulate synthesis of filaggrin (26). Interestingly, exposure to solar irradiation, which is normally avoided in infancy, to reduce the risk of skin malignancy, seems to protect against AD (27, 28). This could be explained by the positive effects of sub-erythemogenic doses of UVB irradiation on the skin barrier, which, among other aspects, reduces *Staphylococcus aureus* colonization, itch, and T-cell invasion.

EARLY ALTERATIONS IN THE IMMUNE SYSTEM

The crucial role of early-age alterations in immune activity on the development of AD is emphasized by the significantly reduced risk of AD in premature infants (29). Moreover, thymectomy in infancy reduces the risk of AD by 20%, suggesting that removal of the thymus decreases the number of circulating T cells that can act to develop AD (30). In indirect support of this assumption, a study found significantly larger thymus sizes in children with AD compared with controls, although this may also be a consequence of the increased demand for T cells in patients with AD (31). The farm theory suggests that microbial exposure may reduce the risk of diseases mediated by T-helper (Th) cell 2, including AD (32), but it is probably more important for allergic diseases than for AD per se. The finding that neonate exposure to dogs can strongly reduce the risk of AD could be confounded, but it is also possible that changes in the host gut microbiome can affect the tolerance-reactivity balance (33). It is unclear how nutrients and alcohol use in mothers can affect the risk of AD, but is has been suggested that the Th2 skew induced by alcohol intake may lead to a higher prevalence of AD in infants (34). Similarly, nutrients may affect the child's immune response, but this area is complex, and little evidence exists. Collectively, AD occurs mainly in genetically predisposed individuals who have significant skin barrier impairment and who are exposed to AD triggers (or who are overly protected against the crucial microorganisms that could prevent excessive Th2 skew in childhood) (**Fig. 1**).

VICIOUS CYCLE IN ATOPIC DERMATITIS

AD is a skin condition in which primary (or secondary) skin barrier impairment leads to (further) skin inflammation, and in which *S. aureus* colonization may increase, and in turn may drive both eczema severity and the relentless sensation of itch (35). This leads to scratching and additional barrier impairment, thus creating a vicious cycle. Clinicians attempt to stop this cycle by restoring the skin barrier with emollients, reducing inflammation and itch with use of topical/oral immune suppressants or immune modulating drugs, as well light therapy, and, finally, decreasing the burden of *S. aureus* by use of disinfectants and antibiotics. Evidence supporting the benefits of emollient use to treat AD is the strongly increased time to subsequent flares in emollient users, and the reduced need for topical corticosteroids (36). However, barrier restoration without simultaneous control of inflammation seems to be inadequate in the treatment of AD (37). Prophylactic use of topical anti-inflammatory agents, e.g. with application twice weekly, works to reduce the risk of new flares (38).

PATHOGENIC ROLE OF *STAPHYLOCOCCUS AUREUS*

While the exact role of bacteria in the pathogenesis of AD is unclear, colonization with *S. aureus* is very common in lesional and non-lesional AD skin. Antimicrobial peptides, which work as broad-spectrum antibiotics to kill Gram-negative and Gram-positive bacteria, are reduced in patients with AD, which, in turn, allows bacteria to colonize the skin (39). *S. aureus* can induce serine protease activity, which will destroy corneodesmosomes, and allow invasion (40). Moreover, the expression of Th2 cytokines is activated by proteases released by *S. aureus* (41), and *S. aureus* toxin increases the allergic response by activating mast cells (42), and induces up-regulation of T cells via a superantigen-mediated mechanism (43). *S. aureus* also release α-toxins, which forms pores in keratinocyte membranes leading to cellular damage (44). Individuals with AD and *FLG* mutations have a 7-fold higher risk of *S. aureus* skin infections, in part due to increased pH, but also due to the lack of the direct growth inhibition of the filaggrin proteins (45, 46). The levels of filaggrin degradation products, i.e. NMF, seem to regulate the strength of *S. aureus* corneocyte adhesion, the first step in skin colonization (47).

SKIN MICROBIOME AND DISEASE CONTROL

While the skin hosts the most diverse commensal community of humans, with over 1,000 different bacterial species, the role of the skin microbiome in AD is poorly understood (48, 49). An animal study showed that

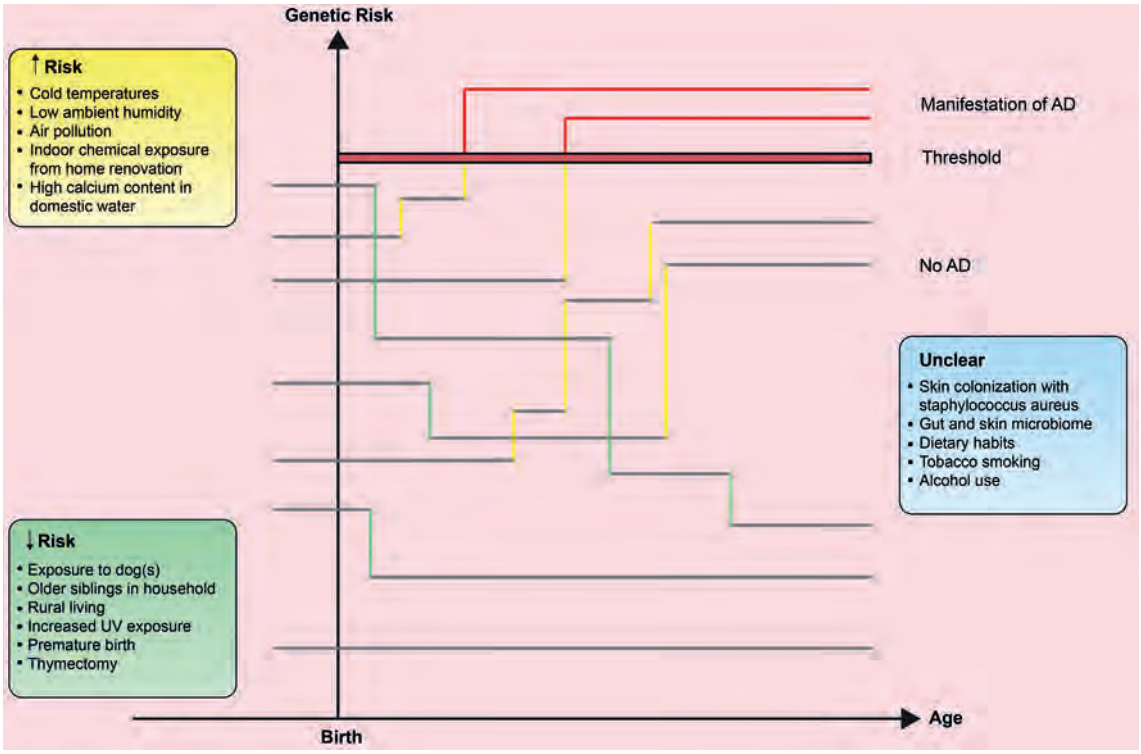

Fig. 1. Theoretical outline of how genetic risk genes and environmental risk exposures interact and may impact the risk of atopic dermatitis (AD). If a child reaches the threshold bar for AD, the disease will manifest. Factors that increase the risk of AD are represented by *yellow vertical lines*, whereas factors that decrease the risk are represented by *green vertical lines*. Once AD has manifested, the lines are shown in *red*.

filaggrin deficiency and microbial dysbiosis triggered intracellular IL-1α secretion and drove chronic inflammation, hence indicating an important pathogenic role (50). Moreover, following successful treatment of AD, *Streptococcus*, *Propionibacterium*, and *Corynebacterium* species increase in numbers along with microbial diversity (51).

DYSFUNCTIONAL LESIONAL AND NON-LESIONAL SKIN

It is important to understand that non-lesional AD skin is also different from the skin of normal controls (**Fig. 2**). It shows decreased or altered synthesis of important epidermal proteins, e.g. filaggrin, filaggrin 2, involucrin, loricrin, hornerin, and tight junctions, but also decreased synthesis of antimicrobial peptides and lipids, (52–58) as well as increased expression of high-affinity IgE receptor on dendritic CD1a, along with increased numbers of T cells and their cytokines. Children with AD and food allergy have stratum corneum abnormalities in non-lesional skin that are not found in children with AD and controls without food allergy. Thus, filaggrin and ω-hydroxy fatty acid sphingosine are reduced, and there are important changes in the epidermal lamellar bilayer architecture (59). Thus, skin measurements in non-lesional AD skin show elevated pH, increased water loss from the skin surface, and increased penetration of chemicals (60). Moreover, AD skin displays a reduced reactivity threshold to exogenous stressors, such as skin irritants, allergens and *S. aureus*, in part due to the creation of resident T-cell populations (61–63). The changes in non-lesional skin are largely determined by disease extent and severity (53), probably reinforcing the impression of AD as a generalized skin disease.

HETEROGENEOUS INFLAMMATORY RESPONSE, DEFICIENT SKIN BARRIER AND EXOGENOUS STRESSORS

Type 2 immunity-associated cytokines, such as IL-4 and IL-13, as well as other cytokines, including, but not limited to, IL-1, IL-17, IL-22, IL-31 IL-33, and thymic stromal lymphopoietin (TSLP) have important roles in AD. It is presently unclear whether significant differences exist between AD skin of children and adults, as well as between different ethnic groups, and to what degree this should affect treatment strategy (64, 65). While certain endotypes of AD are suspected to exist, the heterogeneous cytokine landscape could also, in part, be explained by the crucial pathogenic role of the sustained skin barrier impairment in lesional and non-lesional AD skin. Thus, the continuous bombardment and penetration of microorganisms, chemicals, irritants and allergens into the primary and sustained skin barrier impairment in AD could lead to secretion of various cytokines, and as discussed below, activate the Th1 and Th17 axis in addition to the Th2 axis. The exact immune response would be expected to depend on genetics, age, sites of skin exposure, possible co-infection, climatic effects, and type of elicitor. Interestingly, use of monoclonal antibodies against the IL-4 and IL-13 receptors seems to be slightly less effective in facial skin; an anatomical area which is exposed to environmental pollutants and climatic factors (66).

ATOPIC TRIGGERS

To date, there has been little research into the reactivity to various stressors. A survey in children with AD showed that sweating from exercise was a common exacerbator

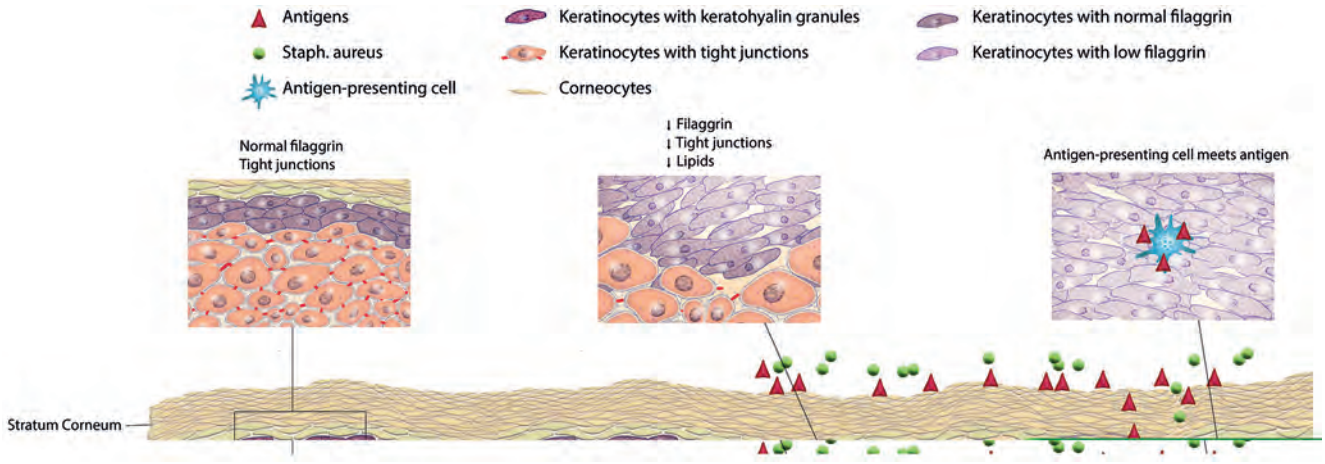

Fig. 2. Important skin barrier changes in atopic dermatitis (AD). Innate and acquired inflammation in AD leads to downregulation and degradation of filaggrin and tight junction proteins, in turn leading to a dry and leaky skin barrier with elevated pH, which allows bacteria to colonize and allergens, irritants and microorganisms to invade. Tight junction reduction further allows antigen presenting cells to move upwards and meet the antigens. Lipid synthesis is compromised at several levels, which acts in concert with protein dysfunction to allow increased loss of water from the skin surface. In an attempt to restore the skin barrier and prevent excessive water loss, acanthosis occurs, often in conjunction with mild spongiosis.

of AD (67). While the exact mechanisms is unknown (68) and, at least in part, could be explained by the direct effects of heating (69), leaking of sweat into the epidermis due to dysfunctional tight junction function could be relevant (70), as well as obstruction of sweat ducts due to filaggrin deficiency (71). Other well-established triggers for AD include exposure to wool, hot weather, psychological stress and sleep deprivation. Induction of stress leads to scratching behaviour in patients with AD, but not in controls (72). The dysfunctional and partly unresponsive peripheral hypothalamic-pituitary-adrenal axis in AD skin could also be important (73). Moreover, psychological stress reduces the recovery time of the stratum corneum, decreases lipid synthesis, and increases the risk of skin infections (74). Exposure to grass allergens may cause worsening of AD in grass-allergic AD individuals through IL-4 release (75). Contact allergens, e.g. fragrances and certain rubber chemicals, have been shown to elicit Th2 immune activity in patch test reactions, as opposed to many other allergens that elicit Th1 immune response (76, 77). Furthermore, exposure to experimental and environmental contact allergens in patients with AD causes Th2 immune response activity, but Th1 immune response in non-atopic skin (78). How this translates into clinical relevance is currently unclear. A recent study examined the skin immune response to various atopic triggers in individuals with normal skin and found that exposure to hard water is associated with IL-4 secretion in the epidermis (79).

CYTOKINE ANTAGONISM AND THE IMMUNE RESPONSE

The most important knowledge about the immune response in AD has been derived from clinical trials using antagonists against specific cytokines. To date, mainly IL-4, but also IL-13, antagonisms have proven to reduce the severity of AD, whereas IL-22 inhibition mostly worked in patients with severe AD (80). While IL-31 inhibition significantly reduced itch in patients with AD, the effects on AD have not been appropriately examined (81). Clinical studies into the development of antibodies against TSLP, IL-33 and IL-17C are ongoing. These published data clearly indicate the relative importance of the above-mentioned cytokines, but other chemokines and cytokines will be targeted in the future.

COMPLEX IMMUNE RESPONSE

It is beyond the scope of this review to describe the immunopathophysiology of AD in detail. Briefly, predominately Th2 (IL-4, IL-5, IL-13, IL-31) and Th22 (IL-22) deviation is observed in acute and chronic AD lesions, which, in turn, down-regulate expression of important skin barrier proteins, such as filaggrin. Innate lymphoid cells also release Th2 cytokines, now increasingly referred to as type 2 immunity. In chronic AD lesions, a parallel activation of the Th1 axis is observed, and in both acute and chronic AD, IL-17 activation can be found (82). Yet, even in healthy skin from patients with AD, there is increased expression of inflammatory cytokines and chemokines, as well as of their receptors, and an increased number of lymphocytes compared with healthy controls, suggesting increased immuno-surveillance in the skin and risk of acute inflammation (53).

Apart from the negative influence on the skin barrier, Th2 inflammation inhibits antimicrobial peptide synthesis and increases *S. aureus* colonization. The Th2 cells may, in many patients, lead to antibody isotype switching to IgE and recruit mast cells, eosinophils, basophils and dendritic cells. Elevated levels of IgE correlate with AD and atopic co-morbidities, including asthma and food allergies (83). Previously, this has been used to subtype AD into extrinsic AD, where allergic sensitization has taken place, and intrinsic AD, in which patients have normal levels of IgE. However, patients with normal IgE levels may also be sensitized and vice versa. It has even been suggested to use the terms intrinsic factors to describe inborn factors e.g. *FLG* mutations, Th2 skewing, etc., which affect the skin barrier function or the immune response in terms of AD and extrinsic factors to describe exogenous factors, e.g. *S. aureus*, detergents, allergens, etc. (82). Interestingly, IgE may target keratinocytes in up to 25% of patients with AD, indicating that IgE may play an important role in impairment of the skin barrier (84).

Regulatory T cells can suppress the Th2 response, and the balance between these 2 cell types is central to development of tolerance. It is not known whether a primary immune-deficiency/imbalance might be the prime cause of AD. Single nucleotide polymorphisms (SNPs) in, for example, ST2 (a member of the interleukin 1 family), IL-13, IL-12, have been reported to be associated with AD, and a huge work in developing a taxonomy for AD subtypes based on serum levels of cytokines has been undertaken (85). A recent work was able to distinguish at least 3 different subtypes of AD, based on analysis of 147 different soluble factors, yet this does not, in itself, show that the immune response is the prime cause of the disease (86). Rather, it indicates that patients with AD have different propensity to react to exogenous stimuli and that, even within the group of patients with AD, this differs slightly and gives rise to different subtypes. The result of this may be the development of personalized medicine for patients with AD (87).

ROLE OF SYSTEMIC INFLAMMATION

Adult patients with AD have significantly elevated levels of circulating cytokines and chemokines (87). While it is intriguing to consider that the systemic inflammation in AD can negatively affect the function of other organs, such as the central nervous system and vascular system,

there is currently no convincing evidence to support this. Nonetheless, AD has been associated with anxiety, depression, autism and attention deficit disorders, and it is possible that cytokines may cause a leaky blood–brain barrier and become absorbed into the cerebrospinal compartments and negatively affect cognitive development, by affecting the glia cells and neurogenesis. Decreased sleep quality due to itch is, however, also a major risk factor for ADD and depressive symptoms. The link between asthma and AD is not fully understood, but the shared type 2 immunity and effect of dupilumab on severity of both AD and asthma support that systemic inflammation could play an important role. While some patients with AD experience worsening of their AD during or after asthma attacks, it is unclear whether this is explained by psychological stress or by cytokines reaching the skin.

CONCLUSION

This review highlights some important disease mechanisms of AD. While understanding of AD has improved in recent years, many basic aspects are still not understood. For example, why do AD lesions outside the flexural areas tend to clear once flexural eczema is controlled? Why is AD a flexural disease? What triggers an AD flare? What explains the resolution of AD in the majority of children? What is the role of foods as triggers for AD? Why do AD children have fewer naevi than controls? These are just some of many unanswered questions. In conclusion, more research is needed into this complex skin disease.

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Centenary theme section: ATOPIC DERMATITIS

REVIEW ARTICLE

Genetics in Atopic Dermatitis: Historical Perspective and Future Prospects

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Atopic dermatitis (AD) is a common, complex trait, arising from the interplay of multiple genetic and environmental factors. This review provides an overview of developments in the field of AD genetics. AD shows high heritability; strategies to investigate genetic risk include linkage, candidate gene studies, genome-wide association and animal modelling. Loss-of-function mutations in *FLG*, encoding the skin barrier protein filaggrin, remain the strongest genetic risk factor identified for AD, but variants influencing skin and systemic immune function are also important. AD is at the forefront of genetic research, from large-scale population studies to *in vitro* models and detailed molecular analyses. An understanding of genetic risk factors has considerably improved knowledge of mechanisms leading to atopic skin inflammation. Together this work has identified avenues for therapeutic intervention, but further research is needed to fully realise the opportunities of personalised medicine for this complex disease, to optimise patient benefit.

Key words: atopic dermatitis; eczema; filaggrin; genetic; genome-wide; risk; phenotype; transcriptome.

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SIGNIFICANCE

Atopic dermatitis (also called eczema) often runs in families, showing that this disease occurs partly because of inherited genetic risk. Research to understand the genetic variation that contributes to an individual's risk of atopic dermatitis has improved our understanding of mechanisms in the skin that can lead to a leaky barrier and inflammation. Already this knowledge has been applied to treatment and eventually it is hoped that these insights will lead to personalised medicine, in which treatment is tailored to a patient's genetic make-up and their individual type of atopic dermatitis.

Atopic dermatitis (AD), synonymous with atopic eczema, is a common chronic inflammatory skin disorder with a lifetime prevalence of 10–20% in developed countries (1, 2). AD is considered to be a genetically "complex disease", with interactions of multiple genetic, biological and environmental factors leading to skin barrier dysfunction and altered immunological response. Having AD has a severely negative impact on health-related quality of life, including self-confidence and sleep; it also implies a socioeconomic burden (3).

AD has been known from ancient times. According to the Roman biographer Suetonius, the Emperor Augustus suffered from symptoms and signs of atopic diseases "…noting a number of hard, dry patches suggesting ringworm, caused by an itching of his skin" as well as "seasonal disorders," noticing that he experienced in the early spring "a tightness of the diaphragm; and when the sirocco blew, catarrh" (4).

This review aims to provide readers with a historical perspective on the progression of genetic studies in AD over recent decades, the rapid escalation of molecular techniques and a view to future opportunities in the field.

WHAT HAVE WE LEARNED ABOUT ATOPIC DERMATITIS GENETICS OVER THE PAST 100 YEARS?

Heritability of AD: family and twin studies

It can clearly be observed that atopic diseases show a familial aggregation, with clustering of affected individuals within families, demonstrating the importance of genetic heritability. The term 'heritability' refers to the proportion of variation within a clinical feature that is attributable to genetic factors (5). A family history of atopic diseases, in particular AD, is the strongest of all risk factors. The presence of any atopic disease in one parent is estimated to increase a child's risk of developing AD 1.5-fold, whereas the risk is increased ~3-fold and ~5-fold, respectively, if one or both parents have AD (6, 7). Familial aggregation can be due to shared environment and/or shared genes and a way to address the genetic component is to study twins. These studies have shown a concordance rate of 72–86% in monozygotic twins and 21–23% in dizygotic twins (8, 9). These data demonstrate that the genetic contribution to the development of AD is substantial and this heri-

tability has been estimated at 70–80% (10, 11) – a high heritability for a complex trait (12). For comparison, psoriasis heritability is approximately 68% (13) whilst other inflammatory barrier diseases show heritability of 7–38% for periodontitis (14) and approximately 67% in ulcerative colitis (15).

Strategies for the investigation of genetic risk

Various different strategies have been used to study genetic components in complex diseases such as AD. In broad genomic analyses (genome-wide linkage, genome-wide association studies) a pre-existing knowledge of the function of genes is not required, nor the biology of the trait in question; it is a 'hypothesis-free' approach. In contrast, directed genetic analysis such as a candidate gene approach is a strategy in which certain loci or genes considered to be of interest for the phenotype are selected for study. The selection can be based on earlier studies, "educated guesses" or knowledge of the pathogenesis and function of previously identified genes or loci; this is a 'hypothesis-driven' approach. Each of these strategies has been used to provide insight into AD.

Linkage studies

Genetic linkage is a method for mapping genes. It exploits the fact that a marker (often a microsatellite marker such as repeated DNA sequences, mostly di-, tri-, and tetra-nucleotide repeats) show variation between individuals. Informative markers have many alleles and are distributed at known locations throughout the genome. The first genome-wide study in AD identified a major susceptibility locus on chromosome 3q21 (16). During the following years, additional genome-wide studies in AD were performed and several more loci were identified including 1q21,3p,17q, 18q,11.13q. However, these loci were often too wide and they required labour intensive fine-mapping. Genome-wide association studies (GWAS) have subsequently replaced genome-wide linkage (17). The technique of GWAS is described in more detail below.

Candidate genes

Filaggrin (FLG). Using a candidate gene approach, and the link between ichthyosis vulgaris and AD, the *FLG* gene was identified as a susceptibility gene for AD in 2006 (18). This was a major breakthrough and also established the impaired skin barrier function as having a key role in the development of AD. Filaggrin is involved in the development of keratinocytes to maintain epidermal integrity and it is an important marker of keratinocyte differentiation. During keratinocyte differentiation, profilaggrin is dephosphorylated and degraded into monomers, which condense in the cytoskeleton of keratin to form an intensive protein-lipid matrix. Consequently, these filaggrin monomers are degraded into amino acids, which contribute to the natural moisturising factors, maintaining skin hydration, a low pH and other aspect of the barrier function of the stratum corneum (**Fig. 1**).

Loss-of-function mutations in *FLG* are present in up to 10% in the Northern European population. They cause the common monogenetic dry skin disorder ichthyosis vulgaris. The most common loss-of-function mutations in Europe are R501X, 2282del4, R2447X and S3247X. Together these 4 null mutations account for >90% of null mutations in the population (21). Among European patients with moderate to severe AD up to 40% of the patients carry a *FLG* null mutation. In meta-analysis the risk of getting AD in a mutation carrier is increased 3-fold

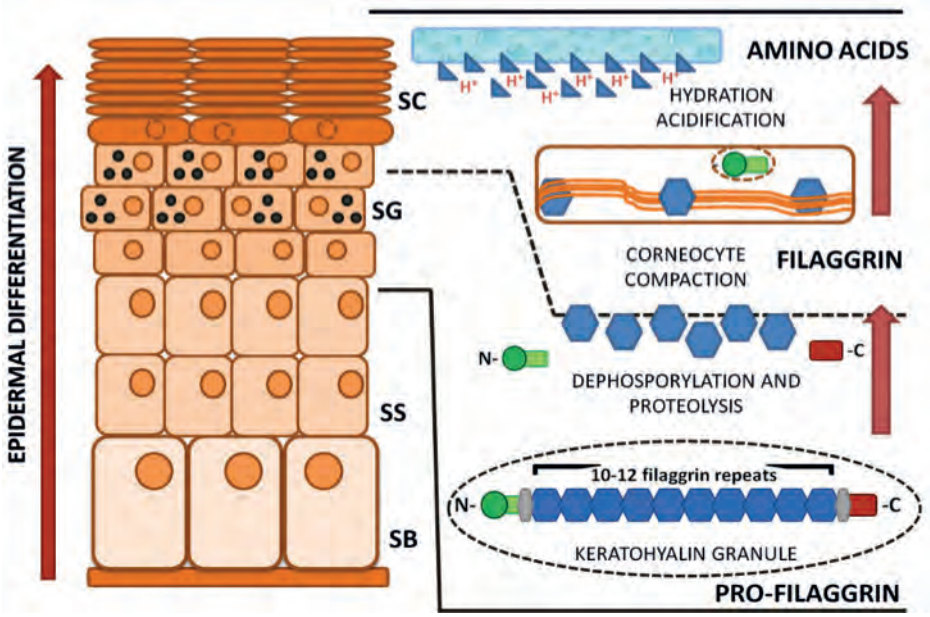

Fig. 1. Filaggrin expression and processing in the epidermis. The pro-protein profilaggrin is cleaved in a stepwise process into filaggrin monomers which are then degraded to release amino acids, contributing to 'natural moisturising factors' in the stratum corneum (19, 20). Filaggrin is an important marker of keratinocyte differentiation. SC: stratum corneum; SG: stratum granulosum; SS: stratum spinosum; SB: stratum basale.

(odds ratio 3.12) (22, 23). However, among Europeans only ~20% of patients with mild-to-moderate AD carry *FLG* null mutations and >50% of individuals carrying *FLG* mutations do not develop any atopic disease and this indicates that *FLG* mutations are neither necessary nor sufficient to cause AD (24).

The frequency of *FLG* null mutations diverges in different populations and >50 have been characterized worldwide (25, 26). In Asian countries, the prevalence of mutation varies from 3% to over 50%, and many mutations are family-specific (25, 27–30). Research in people of African ancestry has been relatively limited to date and the prevalence of *FLG* null mutations appears to be less than 1% (31–33). Studies on African Americans have shown a slightly higher frequency of *FLG* mutations and *FLG2* has also been identified as a possible susceptibility gene (34, 35).

To address the question of why *FLG* mutations are so prevalent in the white European population, it has been hypothesized that this is due to an evolutionary advantage. The increased skin barrier permeability in filaggrin-deficient skin may enhance immunity to infections, conferring 'natural vaccination' to individuals with *FLG* mutations during European pandemics (36). Additionally, filaggrin deficiency may confer an evolutionary advantage in higher latitudes (i.e. Northern Europe) through its role in increasing vitamin D biosynthesis. Vitamin D3 levels are 10% higher in German and Danish individuals with *FLG* null mutations, which may be due to a reduction in filaggrin's role as an endogenous UVB filter in the skin (37).

Besides mutations there are intragenic repetitive gene sequences or 'copy number variations' in *FLG* that determine the amount of filaggrin monomer expressed in the skin. Having more repeats (12 compared to 10 on each allele) is associated with reduced risk of AD (38) by a dose-dependent effect within this repetitive gene sequence. The effects of cytokines, such as IL-4, IL-13, IL-17A, IL- 22, IL-25, IL-31, and TNF-α have also been shown to suppress filaggrin expression in the skin, resulting in additional barrier impairment (39, 40).

Even though *FLG* mutations and the filaggrin protein are extremely important in AD pathogenesis, there must be yet unknown, additional factors/genes or functions of gene involved in AD development that still are to be found.

Some other candidate genes in atopic dermatitis. Other genes that has been detected through a candidate gene approach, supported by knowledge of AD biology, and replicated by GWAS are genes involved in the Th2 immune response, for instance IL-4 located on chromosome 5q31.1, the IL-4 receptor located on chromosome 16p12.1-p11.2 and IL-13 on chromosome 5q31.1 (24, 41).

More candidate genes have been detected through the study of monogenic diseases that have features that resemble AD. Netherton syndrome (OMIM #256500) is a rare monogenic disease with AD-like lesions in the skin and increased IgE levels. The gene mutation underlying Netherton is in the Serine Protease Inhibitor Kazal-Type 5 gene (*SPINK5*) located on chromosome 5q32. *SPINK5* encodes a 15-domain protease inhibitor Lymphoepithelial Kazal-Type-Related Inhibitor (LEKTI) which is expressed in epithelial and mucosal surfaces and in the thymus. In several studies, there has been an association between *SPINK5* variants and AD, also in different populations (42–45). Other candidate genes will be studied as a result of new approaches to assessing monogenic disorders and extreme phenotypes, as discussed below.

Animal models

Animal models have the advantages that one can more easily control the environment and create genetic homogeneity. Apart from humans, dogs have spontaneous AD that has been studied and documented (46).

There are also several AD mouse models that have been described and generated over the years, each focusing on one or more aspects of human AD. The mouse models can be divided into 3 main categories: (*i*) Inbred strains of mice that develop AD-like phenotypes. The most well-known of these are the flaky tail mouse and the NC/Nga mouse (47, 48). The Flaky tail (ft) recessive mouse mutation arose spontaneously on the background of a recessive matted (*ma*) trait (49). The *ft* mutation has been identified as a 1-bp deletion in the *Flg* gene resulting in a premature stop codon (50), analogous to the human *FLG* mutations. More recently the *ma* trait has been separated from the flaky tail mouse and identified as a nonsense mutation in the novel gene *Matt* encoding the protein mattrin which is also postulated to have a role in skin barrier biology (51). (*ii*) Genetically engineered models, in which genes can be silenced or be overexpressed, for example the claudin-1 (52) and *Flg* knockout mice (53). (*iii*) Models that can be induced by exogenous agents with for example the allergens ovalbumin and house dust mite (as recently reviewed (54)).

ATOPIC DERMATITIS IS AT THE FOREFRONT OF CURRENT GENETIC TECHNOLOGY AND ITS APPLICATION

The prevalence of AD and the accessibility of disease-relevant tissues – both skin and blood – has allowed AD research to be at the forefront of applying new technologies. This has been powerfully facilitated by the active collaboration of large consortia across Europe and throughout the world. The advance of genetic and genomic analysis techniques has occurred at a rapid pace over recent decades. Large-scale and more focused molecular analysis techniques provide complimentary information; an overview of these approaches is given in **Fig. 2** and each is described below.

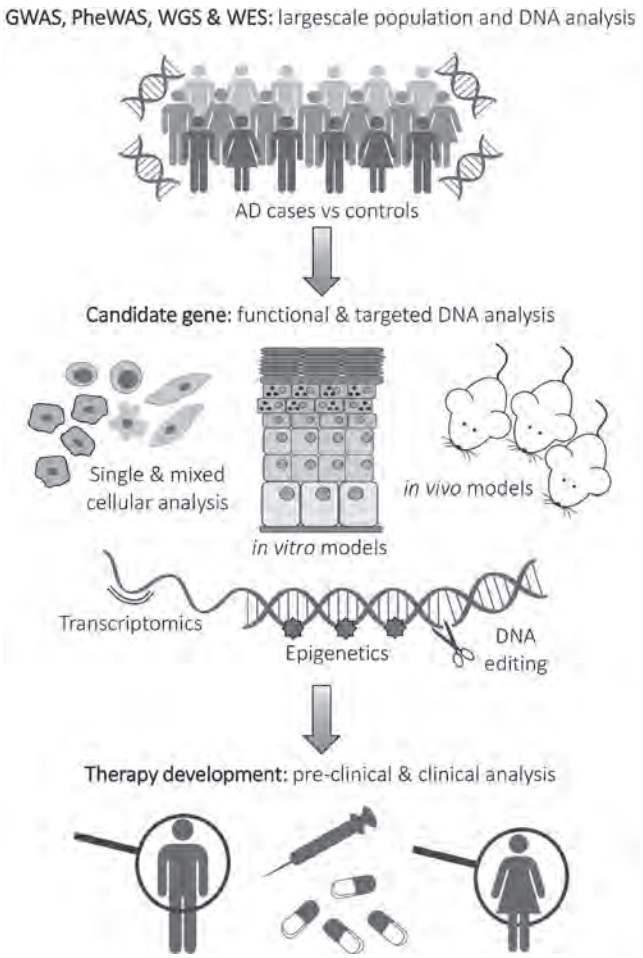

Fig. 2. Complimentary strategies for genetic analysis leading to therapy development. GWAS, genome-wide association study; PheWAS, phenome-wide association study; WGS, whole genome sequencing; WES, whole exome sequencing; AD: atopic dermatitis.

Genome-wide association studies

GWAS is a technique in which very large numbers of single nucleotide polymorphisms across the genome are compared between large numbers of cases and controls, to identify differences that are associated with disease status. GWAS have been conducted in several different populations worldwide, and a recent meta-analysis has synthesized these studies (55). Over 30 loci (regions of DNA) have been identified as showing association with AD risk. Some loci include well established genetic effects, such as the epidermal differentiation complex on chromosome 1q21.3 (which includes *FLG*) and the cytokine cluster on chromosome 5. Many of the other regions are between genes, meaning that their functions require detailed follow-up work to ascertain a functional mechanism. One example is the region on chromosome 11q13.5 which interacts with a gene, *EMSY*, >30 kilobases away; *EMSY* has recently been shown to have an effect on skin barrier formation and function of relevance to AD (56). Another gene, *LRRC32*, >60 kilobases away from the same locus on chromosome 11q13.5, may also play a role in AD pathogenesis (57), demonstrating the pleiotropic effects that arise from genetic variation.

Further, larger, meta-GWAS studies are on-going, because larger sample sizes allow the detection of additional risk loci, although their effect sizes are likely to be smaller.

Phenome-wide association studies

Phenome-wide association (PheWAS) is a technique in which large numbers of phenotypic traits are tested for association with single genetic variants. For example, a loss of function variant in *FLG* shows strong association with atopic phenotypes including AD, asthma, allergic rhinitis and food allergy in a PheWAS study, as expected (58). Unexpected or previously unknown associations with genotypes may be revealed using PheWAS and the technique may also be applied to drug repositioning (59).

Whole exome sequencing and whole genome sequencing

Whole exome sequencing (WES) is a technique that studies the genetic sequence of the DNA in exons that code for proteins, and also exonic regions in non-coding RNAs. WES focuses on exons because they are most likely to have a direct functional effect; however, each variant requires careful assessment to define which may lead to loss-of-function or other functional effect.

WES in 22 Ethiopian people with AD and ichthyosis vulgaris has revealed rare variants in *FLG* and several other genes within the epidermal differentiation complex, as well as nonsense and missense mutations in previously unreported candidate genes including *GTF2H5, ADAM33, EVPL* and *NLRP1* (60). Some of these findings indicate population-specific variation rather than disease-associated variants. There was no evidence of recurrently-mutated causal genes in this population and AD appears to show considerable heterogeneity in genetic susceptibility (60).

Whole genome sequencing (WGS) sequences intergenic regions as well as exons, because many of the regulatory mechanisms are situated in intergenic DNA. WGS generates more data and is potentially more powerful than WES, but the interpretation of non-coding variants on a large scale remains very challenging as their functional effects are not well defined. The cost of WGS is also a limiting factor to sample size and to date no large WGS have been reported in AD.

Epigenetic studies

'Epigenetic' refers to heritable changes in gene expression that occur without alteration to the DNA sequence. In the context of AD, there are multiple environmental and pathophysiological effects which could impact on skin cells via epigenetic mechanisms, ranging from ma-

ternal factors *in utero*, to early life exposures, irritant and allergic effects. Two important epigenetic mechanisms are histone modifications and DNA methylation. These regulate chromatin structure and DNA accessibility to transcription factors and polymerases (61). Specific histone modifications can be used to predict and delineate regulatory features such as promotors and enhancers in the genome. Epigenetic mechanisms are central to the precise control of skin development and homeostasis (reviewed (62)). A number of studies have linked abnormal epigenetic control of the immune system and skin barrier to AD pathogenesis (63). Key differences in DNA methylation are observed between lesional and non-lesional AD epidermis and these correlate with changes in the expression of skin barrier and innate immune genes (64). Non-coding RNA including micro RNAs (miRNAs) confer an additional level of epigenetic control by regulating mRNA translation or degradation. Differential expression of number of miRNAs has been reported in lesional AD skin (63). Considerable further work is needed to fully understand epigenetic control in AD.

Three-dimensional DNA analyses

DNA may be represented diagrammatically as if it were a straight linear molecule, but in vivo it is extensively folded and wrapped around protein structures in three-dimensional space. Due to this folding, genomic regions that are far from each other in the linear DNA are brought in close proximity in the 3D genome (65). This complex and dynamic process facilitates long range control of gene expression by bringing distant promotor and enhancer elements together (66). Recent technological advances including chromosomal conformation capture (5C) and Hi-C or Hi-Cap, have allowed these interacting regions to be delineated. The techniques crosslink DNA with formaldehyde prior to digestion and sequencing so that interacting regions are sequenced together (65, 67). HiCap uses probes to capture promoters across the genome and regions important in gene regulation such as enhancers. Then, selected promoter–enhancer interactions can be sequenced. This analysis is performed in different cell lines and at different timepoints to reveal the dynamic process and identify candidate genes (68). Importantly, since the 3D interactions are cell-type as well as cell-state-specific, Hi-C analysis has been applied to differentiating keratinocytes, to characterise spacial control of promotor-enhancer interactions likely to be of relevance to AD (56, 67).

Transcriptome analysis

Transcriptomic analysis describes the study of RNA molecules that are present in a cell or tissue, having recently been transcribed from DNA. These molecules include protein-coding messenger RNA (mRNA), ribosomal RNA, transfer RNA, long non-coding RNA (lncRNA), micro RNA (miRNA) and others; their half-lives range from seconds to minutes. The transcriptome is a highly dynamic system and it is cell-type and cell state-dependent; differentiated cells show different gene expression compared to undifferentiated cells. Transcriptome analysis performed on skin itself is most relevant for dermatological conditions, but transcriptomics of serum or blood may also provide valuable insight into skin-related inflammatory conditions, including AD. Transcriptomic analyses are very sensitive; skin biopsy samples from so-called 'non-lesional' (clinically uninflamed) skin from an AD patient show profound abnormalities in the transcriptome, including barrier impairment, dysregulation of lipid metabolism and an activated stress response (69). The AD lesional skin transcriptome shows a disease signature (70) that improves after treatment (71).

Single cell analysis

Most of the molecular analyses on skin to date have been carried out using whole skin biopsies, or epidermal samples. However single cell analysis is now feasible, for DNA and RNA sequencing, as well as protein analysis (72). These techniques offer the prospect to study individual cells, define new cell types and gain insight into the functional and structural heterogeneity of skin as a complex organ. The Human Cell Atlas is an international collaboration to make single cell analytical data available to researchers (73) and the skin component of this atlas is eagerly awaited. Several research laboratories have already released published data and tools to allow the interrogation of skin transcriptome analysis, for example murine data from the Kasper lab (74).

CRISPR-cas9 gene editing

CRISPR (clustered regularly interspaced palindromic repeat) sequences are found in bacterial DNA and form part of their immune response to phage infection. Cas9 (CRISPR-associated protein 9) is an enzyme that cleaves DNA selectively at sequences containing the CRISPR motif. In 2012 it was reported that this mechanism can be exploited for genetic engineering; guide-RNAs are used to direct the cas9 enzyme to cleave DNA in precisely-targeted editing. Application of CRISPR-cas9 allows the effects of genetic variation to be tested directly and the technique has revolutionised molecular biology. This cost-effective and relatively easy-to-use technology has allowed researchers to precisely and efficiently target, edit, modify and mark genomic loci in a wide range of cells and organisms (75). Within dermatology, CRISPR-cas9 editing has been used to correct the genetic defects in several forms of epidermolysis bullosa and of relevance to AD, the technique can be used to investigate candidate genes *in vitro* (see below).

Functional analyses in vivo

Clinical observation followed-up with genetic analysis has increased our understanding of severe phenotypes which include features of AD. Following on from Netherton syndrome, these 'human knock-out' models include *CARD11* mutations (causing systemic atopic inflammation), *DSG1* and *DSP* mutations (causing severe dermatitis, multiple allergies and metabolic wasting) and various immunodeficiency syndromes with AD-like skin inflammation (such as Wiskott-Aldrich, caused by mutations in *WAS*) (76).

Functional analysis of the skin of AD patients *in vivo* also offers opportunities to gain understanding of the pathophysiology. Transepidermal water loss (TEWL) (77) measures the 'inside-to-outside' barrier function and *in vivo* it is proportional to skin inflammation; capacitance or conductance of the stratum corneum give a quantitative measure of water content; and tape-stripping can be used as a relatively non-invasive methods for sampling the skin transcriptome, proteome and lipids of relevance to AD (78).

Organotypic models of atopic dermatitis

Three-dimensional organotypic models of human skin bridge the gap between cultured cells in monolayer and animal models. Multi-layered organotypic models recapitulate many features of human epidermis including: morphology, spatiotemporal expression of terminal differentiation/proliferative markers and an appropriate complement of epidermal lipids (79, 80). Several organotypic models of AD have been described which generally use one of two basic approaches: the first involves the treatment of organotypic models derived from normal healthy cells with AD-relevant cytokines and the second models *FLG* deficient AD through gene silencing or the use of *FLG*-mutant keratinocytes (81). Th2 cytokines (IL-4 and IL-13) stimulate a spongiotic epidermal morphology, similar to that observed in AD (82). Organotypic models deficient in filaggrin expression broadly recapitulate many of the structural, molecular and functional defects observed in AD skin. These include a lack of keratohyalin granules, increased paracellular permeability (83, 84) and protein expression signatures consistent with AD skin (85, 86). Filaggrin deficient organotypic skin, therefore, mirrors many changes observed in the AD skin and thus represents a useful model for the study of AD disease mechanisms and therapeutic options.

Organotypic models allow the investigation of tissue-specific genetic effects and the opportunity for testing other AD candidate genes, by knockdown, over-expression, or CRISPR-cas9 editing of genes of interest.

Functional analyses in vitro

Organotypic skin models grown at the air liquid interface develop a competent bidirectional epidermal barrier with similar biophysical properties to human skin. They offer the advantage over monolayer cell cultures, that they are tractable for physiologically relevant functional analysis (87). The outside-in barrier can be quantified in organotypic models using topically applied hydrophilic dye such as Lucifer yellow. This is naturally excluded from the epidermis by the lipid-rich stratum corneum but can permeate into the deeper epidermal and dermal layers if the skin barrier is immature or impaired (83). Analogous to the *in vivo* situation described above, the inside-outside barrier of organotypic cultures can also be determined by measuring the rate of TEWL (56). These techniques have been used successfully to investigate both the effect of previously uncharacterized genes and the *FLG* deficiency on skin barrier function (56, 83, 85).

OUTSTANDING QUESTIONS AND FUTURE WORK

The rapid progress made in recent years still leaves a large amount of work to fully capitalize on novel understanding for the benefit of patients.

More detailed genetic studies

The majority of heritability in AD remains unexplained. Improvements in technology have allowed more and more detailed interrogation of the coding and non-coding regions of the genome which are likely to hold important mechanistic information. Outstanding questions involve tissue-specific effects in skin; the relative accessibility of this tissue allows dermatological studies to take advantage of direct sampling for epigenetic studies and more detailed transcriptome analyses. Copy number variation within *FLG* has a dose-dependent effect on AD (38) and more detailed analyses are required to assess CNV in other risk loci. On a genome-wide level, even larger numbers of cases and controls will be required to achieve the statistical power to detect gene-gene interactions and gene-environment interactions of relevance to AD. These studies remain challenging in their financial cost and computational requirements.

More inclusive genetic research

As described above, the majority of genetic research to date in AD has been conducted in people of white European ancestry. However, the clinical phenotype of AD is different in different ethnicities and studies of genetic risk in African (35) and Asian (88) populations have provided valuable complimentary insight (89). There has been a call in the field to prioritise diversity in human genomics research because this will increase the accuracy, utility and acceptability of using genomic information for clinical care (90). The International Symposium on Atopic Dermatitis (ISAD) has recently

published a position statement calling for more research on AD in Africa (91).

Integration of -omics for personalised medicine

Genetic studies have given important information for understanding AD mechanisms, particularly the initial or 'root cause' of atopic skin inflammation. However, the combination and integration of information provided by the full complement of techniques described above will be required to increase our understanding of AD pathophysiology sufficiently to allow translation for clinical impact. Furthermore, given the complexity and diversity of this trait, further developments in machine learning and more powerful *in silico* analyses (76) are likely to be required to gain full benefit from the wealth of molecular data.

Application of genetic discoveries to drug development

The quest for understanding genetic mechanisms in AD is not merely an academic exercise. Genetic studies can provide a causative link between a sequence variant and a phenotype and drugs developed to target a pathway informed by human genetic studies have above-average chances of clinical success (92). Filaggrin deficiency remains a challenging therapeutic target, even though the genetic discovery was made more than a decade ago, but genetic studies continue to identify causal pathways for AD in increasingly precise and personalised detail. The era of 'personalised medicine' is expected to bring a new relationship between genomics and drug development, testing the physiological and molecular bases for disease, but success in this endeavour would ultimately transform drug development and clinical use (93).

CONCLUSION: THE FUTURE LOOKS BRIGHT

In 1952, Rosalind Franklin was the first to crystallise DNA fibres to study their structure using X-ray diffraction; in 1953 James Watson and Francis Crick reported the double helix structure of DNA; in 1990 the Human Genome Project began and in 2003 the Human Genome Project was completed, providing a sequence of the entire human genome – approximately 3 billion base pairs in length.

Since this time, we have progressed a long way in understanding more of the detail of how DNA sequence variation contributes to human health and disease. There has been a particularly rapid explosion of knowledge in the last 20 years, brought about by increased technical capacity for sequencing DNA and RNA. Whilst it is unlikely that another single gene exists with the impact of *FLG* upon AD risk, the future appears bright for AD patients: New techniques will refine understanding of genetic risk, with a multi-ethnic perspective, providing powerful insight to drive the development of new pharmacological interventions. These will increasingly be targeted to specific disease mechanisms for each individual patient with AD. The next 100 years is likely to see a step-change in the management of this challenging disease.

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REVIEW ARTICLE

Skin Microbiome in Atopic Dermatitis

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Atopic dermatitis is a common inflammatory skin disease with a complex pathogenesis that includes imbalanced immune system signalling, impaired skin barrier and enhanced *Staphylococcus aureus* skin colonization. The skin bacterial communities are characterized by increasing abundance of *S. aureus*, leading to reduced diversity compared with the bacterial communities on healthy skin, and increasing disease severity. In contrast, fungal communities are richer and more diverse on the skin of patients with atopic dermatitis, although distribution of the most common species is similar in patients and controls. Filaggrin deficiency in atopic dermatitis skin might be related to the enhanced skin colonization by *S. aureus*. In addition, *S. aureus* expressing variant virulence factors have been shown to elicit atopic dermatitis-like phenotypes in mice, indicating that specific *S. aureus* strains can induce flare-ups. This review aims to provide an overview of the recent literature on the skin microbiome in atopic dermatitis.

Key words: atopic dermatitis; skin microbiome; *Staphylococcus aureus*; filaggrin.

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SIGNIFICANCE

Atopic dermatitis is a common skin disease characterized by dry and itchy skin with eczema flares. The disease is associated with changes in the skin microbiota, which constitutes all microorganisms present on the skin surface. The greatest difference is due to increased abundance of *Staphylococcus aureus*, a bacterium that can cause skin infections and probably contributes to aggravation of the disease. This review aims to provide an overview of recently published literature regarding changes in the skin microflora in atopic dermatitis and its association with disease severity and exacerbation.

Atopic dermatitis (AD) is a common inflammatory skin disease that affects 10–20% of children and 2–10% of adults in developed countries (1, 2). The pathogenesis of the disease is complex and includes impaired skin barrier function and an imbalanced immune system with enhanced Th2, Th17, and Th22 signalling (3). Furthermore, patients with AD have an increased burden of *Staphylococcus aureus* skin colonization, which is associated with disease severity and exacerbation (4–8). Within recent years, where it has become possible to examine complete microbial communities using advanced DNA sequencing technologies, it is evident that cutaneous *S. aureus* is associated with decreased bacterial diversity on AD skin (8–13). The aim of this review is to provide an overview of the recent literature on the skin microbiome as well as microbe-host interactions in AD.

There is no single established accepted definition of the term "microbiome" in the scientific community. Often, this term is defined as the composition of all microbial genes in a community (14), but it has been argued that this definition rather describes the "metagenome" and that the word "microbiome" should be defined as all microorganisms in a habitat (the "microbiota"), their genomes, and the surrounding environmental conditions (15). In this review, the latter definition is used.

Skin microorganisms can be identified using culture-based assays, and complete microbial communities can be examined by DNA sequencing (**Box 1**) followed by diversity and taxonomy analysis (**Box 2**). Recently, Grogan et al. (16) have summarized the techniques used for studying the skin microbiome, and the methods are therefore not described in detail in this review.

HEALTHY SKIN MICROBIOME

The skin is an important first-line defence against pathogenic microbial invasion. Tight connections between corneocytes in the stratum corneum form a physical barrier, and antimicrobial peptides and lipids secreted from keratinocytes and glands provide a chemical barrier (17). In addition, commensal skin microorganisms can impede growth of pathogens, either directly by secreting antimicrobial molecules, or indirectly by occupying space and competing for nutritional resources (18, 19).

The skin microbiota consists of diverse organisms, including bacteria and fungi. In adults, the microbial community composition is rather stable over time, despite of the constant exposure to external microorganisms from other humans and the surrounding environment (20). However, the composition of the microbiota changes during puberty, with children having a more diverse microbiota compared with adults (21–23).

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Box 1. Assays for examination of microbial organisms and communities

- Culture assays: Plating and culturing of samples in order to detect and isolate specific viable microorganisms of interest. Advantages of this method are the possibility to use isolated strains for additional analysis, e.g. testing for antimicrobial resistance and examine the gene content using molecular methods. Also, it is known that the detected microbes are alive and viable. Disadvantages are that it is difficult to detect microorganisms that do not grow easily under standard laboratory conditions and that it is not possible to examine the microbial community as a whole.
- Whole genome sequencing: Sequencing genomes of specific microorganism of interest allows to examine the genetic content and thus properties of the single strain. This method is especially useful for comparing strains and their relatedness, e.g. to examine the similarity of strains isolated from distinct skin sites within individuals. A disadvantage of this method is that only selected strains are examined.
- Targeted amplicon sequencing: Sequencing of selected variable regions of the 16S rRNA gene can be used to identify most bacteria in a sample, making it possible to examine the composition of the bacterial community. The transcriptional spacer regions ITS1/2 and variable regions of the 18S rRNA gene can be used to study eukaryotic microbial communities, such as fungal communities. An advantage of targeted amplicon sequencing is thus the ability to examine whole microbial communities, though this method has its limitations as it often is impossible to differentiate between related species. Another disadvantage of this method is that all available target DNA is sequenced, including DNA from microbial contaminants and human skin cells, which especially constitutes a problem for low biomass samples, such as skin samples.
- Metagenomic shot-gun sequencing: Shot-gun sequencing is used to examine the complete genomes of the microbiota. This allows to detect all species constituting the microbiota at the strain level as well as examining specific properties of the community, e.g. metabolic pathway genes. A disadvantage of shot-gun sequencing is that deep-sequencing is required in order to obtain a high resolution making the method very expensive. As a consequence, most metagenomic studies are based on minor sample sets. Another disadvantage, which also applies to the amplicon DNA sequencing method, is the lack of discrimination between live and dead organisms.

Bacteria constitute the greatest proportion of the microbiota, representing more than 70% of species in most skin areas (24). 16S rRNA gene sequence analysis has shown that *Corynebacterium, Cutibacterium* (formerly *Propionibacterium* (25)), *Micrococcus, Staphylococcus, Streptococcus*, Betaproteobacteria, and Gammaproteobacteria are common skin-colonizing bacteria (9, 21, 23, 24, 26). Microbial richness and Shannon-diversity are influenced by the microenvironmental conditions on the skin, including pH, moisture, sebum content, and topography (24, 26, 27). Sebaceous skin sites (e.g. facial areas and the upper part of the chest and back) are dominated by *Cutibacterium acnes* and are less diverse and rich compared with moist skin (e.g. nares, axillary vault, antecubital fossa, and popliteal fossa) and dry skin (e.g. volar forearm) (24, 26). *C. acnes* is also the most abundant species in dry skin, whereas no single bacterial species is over-represented in moist skin, although *Corynebacterium* spp. and *Staphylococcus* spp. relative abundances are greatest (24). Fungi constitute 1–5% of the skin microbiota (24), with *Malassezia* being the most common and abundant habitant (27, 28).

Box 2. Diversity and taxonomy analysis

- Alpha-diversity: Diversity within samples.
  - Richness: the total number of species or unique sequences in a sample.
  - Shannon Index: a diversity measure that takes into account both species richness and evenness.
- Beta-diversity: Diversity between samples.
  - Pairwise comparison of community structures can be measured using distance-based methods, e.g.:
    - Weighted UniFrac: Dissimilarity measure based on species presence/absence data. Takes into account the phylogenetic relatedness of species.
    - Unweighted UniFrac: Dissimilarity measure based on the relative abundances of species. Takes into account the phylogenetic relatedness of species.
    - Jaccard: Dissimilarity measure based on species presence/absence data. Does not take into account the phylogenetic relatedness of species.
    - Bray-Curtis: Dissimilarity measure based on the relative abundances of species. Does not take into account the phylogenetic relatedness of species.
  - Ordination plots: Visualization of beta-diversity based on the pairwise distance measures. Samples with similar community structures are clustered together. The axes determine the degree of variance between samples.
  - Hierarchical clustering: Samples are clustered in a dendrogram based on the pairwise distance measures.
- Taxonomy analysis: Analysis of species distributions in a community/sample.

SKIN MICROBIOME IN ATOPIC DERMATITIS

AD is clinically characterized by red, dry, and itchy skin, with eczema flares and disease exacerbation. Interestingly, the clinical presentation of AD changes with age (29). Infants (< 1 year) are primarily affected by acute lesions of the cheeks, scalp, neck, trunk, and extensor parts of the extremities. Children (2–12 years of age) are mostly affected by eczema at the antecubital and popliteal fossa, and adolescents and adults by chronic lesions comprising the head, neck, hands, and flexural areas (and sometimes widespread disease). Consequently, published studies on the skin microbiome in AD have focussed on distinct skin areas depending on the age group investigated. A major genetic risk factor of AD in Asian and Caucasian populations is loss-of-function mutations in the *FLG* gene encoding the skin protein filaggrin (30). Filaggrin is essential for the alignment of keratin in the corneocytes, and filaggrin breakdown products act as natural moisturizing factors (NMFs) important for proper skin hydration. Thus, filaggrin is important for maintaining a functional skin-barrier. Th2 and Th22 cytokines can down-regulate *FLG* expression, and thus lead to filaggrin deficiency in AD independently of loss-of-function mutations in *FLG* (3). Filaggrin deficiency and reduced levels of NMFs and free fatty acids, followed by an increase in skin pH, lead to an altered skin ecology in AD (31–34). Also, microbial communities are altered on AD skin compared with normal healthy skin, as described below.

Bacterial community on atopic dermatitis skin during infancy

As in healthy control skin, the skin microbial composition in AD differs between age groups, with distinct bacteria being over-represented at different ages (10, 21). Two case-control studies have compared the bacterial community composition on skin from infants with and without AD (35, 36). Zheng et al. (35) examined the bacterial community composition in perioral skin in infants with clinical signs of AD at the sample site and in age-matched healthy controls. The microbial diversity was lower on AD skin compared with healthy control skin, with the largest difference observed between patients with severe AD and healthy controls. *Streptococcus* was the most common bacterial genus at the perioral skin, with mean relative abundances exceeding 40% in both healthy

control skin and lesional skin from patients with mild/moderate AD. However, in the severe AD patient group, relative abundances of *Streptococcus* spp. were significantly reduced and replaced with *Staphylococcus* spp, primarily *S. aureus*. In contrast, bacterial communities on skin from the cheeks, nose tip, antecubital fossa, and popliteal fossa were generally similar in infants with or without AD (36). Importantly, the AD group consisted of infants who not necessarily had developed AD or had active disease at the sampling time-points, which very well can influence the results. *S. aureus* was not identified in any of the skin samples (36), despite the fact that *S. aureus* is frequently detected on antecubital and popliteal fossa skin regions in older children and adults with AD (8, 10). Although this could indicate that *S. aureus* colonization at the antecubital and popliteal fossa is not an essential marker for AD during disease development in the first year of life, culture-based analysis has indicated the opposite (37). Thus, Meylan et al. (37) found that frequencies of *S. aureus* colonization at axillary and antecubital fossa skin were significantly higher at the time of diagnosis among infants and toddlers (0–2 years of age) developing AD compared with non-AD age-matched controls. However, frequencies of *S. aureus* colonization were less than 15% and thus remarkably lower compared with the prevalence in older children and adults with AD (6). In addition, *S. aureus* colonization of the anterior nares, which is a major habitat for *S. aureus* in both healthy and AD individuals (6, 38), was not considered to be a risk factor for AD development among infants with familial predisposition (39). Thus, a possible role of cutaneous *S. aureus* colonization during development of AD still needs further investigation.

Bacterial community on atopic dermatitis skin during childhood and adulthood

Several studies have shown that bacterial communities on skin of children and adults with established AD are less diverse, and are dominated by increased proportions of *S. aureus* compared with communities on healthy skin (8–13, 21, 35). Quantification of cutaneous *S. aureus* abundances has shown that the proportional increase of *S. aureus* is due to a significant greater absolute abundance of *S. aureus* on AD lesional and non-lesional skin compared with healthy control skin (40–42). No difference in absolute abundances of the 3 common skin bacteria *Corynebacterium*, *Cutibacterium*, and *Streptococcus* were observed between AD and healthy control skin in children (40). Thus, the increased relative abundance of *S. aureus* on AD skin is probably not due to decreased colonization with these bacteria, but mainly a result of an enhanced burden of *S. aureus* on the skin. This could also explain that the total bacterial load is significantly greater on AD skin compared with healthy control skin (**Fig. 1**) (40, 43).

Kong and colleagues (8) were the first to examine the temporal bacterial variation on antecubital and popliteal fossa skin in children during and after an AD flare episode (8, 12). Bacterial diversity was significantly reduced during flares at both skin sites, compared with baseline and post-flare samples. The decreased diversity during AD flares was associated with increased relative abundances of *S. aureus*, which exceeded 40% in many of the samples. The increase in relative abundances of *S. aureus* was accompanied by a decrease in the relative abundance of *Streptococcus salivarius* (8, 44), a commensal bacteria of the oral cavity, intestines and skin that has been shown to possess anti-inflammatory potentials *in vitro* (45). In addition, higher proportions of *S. salivarius* contributed to greater bacterial diversity on the skin of the cheek, volar- and dorsal forearm in healthy infants with a family history of atopic diseases and thus at higher risk of developing AD (44). The proportional abundances of *S. aureus* decreased significantly at the post-flare sample time-point, but were still slightly higher compared with *S. aureus* proportional abundances in the healthy control samples (8, 12). Yet, no significant difference in alpha-diversity was observed between baseline, post-flare, and healthy control skin, which could indicate that skin bacterial diversity is only reduced during flare-ups. Several studies have compared alpha-diversity on lesional and non-lesional AD skin, but with different conclusions. Three studies found that the bacterial diversity was lower on lesional skin compared with non-lesional skin (13, 21, 46), whereas 2 other studies found that the diversity was equally reduced on affected and un-affected AD skin compared with healthy control skin (9, 10). Neither age nor sampling sites can explain the

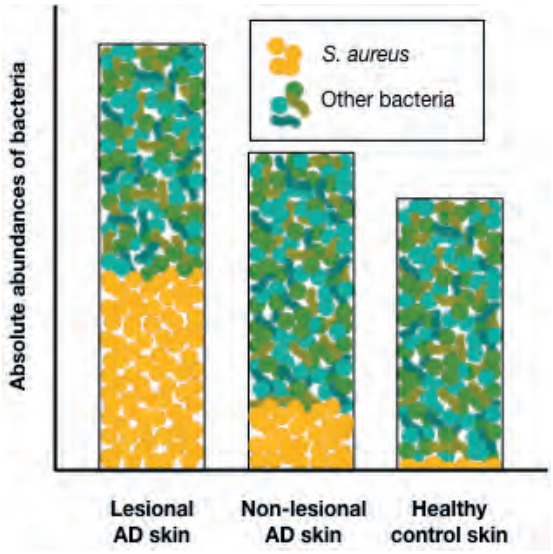

Fig. 1. Absolute abundances of bacteria in atopic dermatitis (AD) skin and healthy skin. Bacterial densities are significantly greater on AD lesional skin compared with healthy control skin, which mainly is due to significantly increased abundances of *S. aureus* in AD lesional skin. *S. aureus* absolute abundances are also increased in AD non-lesional skin, but not as much as in lesional skin.

conflicting results. These studies might indicate that it is not the eczema itself that drives the changes in diversity, but other AD-related factors in or on the skin that not only are associated with lesional skin areas (e.g. *S. aureus* colonization, lipid composition or pH).

Clausen et al. (9) discovered that the bacterial community composition (beta-diversity) varied significantly between lesional and non-lesional skin areas in adult AD patients, with the greatest variance being due to a different distribution of Staphylococcal species. One-third of the lesional skin samples were dominated by *S. aureus* (relative abundances greater than 50%), whereas only a few non-lesional skin samples were characterized by high proportions of *S. aureus*. Instead, coagulase-negative staphylococcal species (CoNS), such as *S. epidermidis* and *S. hominis*, dominated the bacterial community on non-lesional skin in a majority of patients. In accordance, Baurecht et al. have shown that relative abundances of CoNS are reduced and *S. aureus* abundances increased in acute and chronic lesional skin compared with non-lesional AD skin (46). Though the identified CoNS are common colonizers of moist skin, their abundances were lower on healthy control skin compared with non-lesional AD skin at the antecubital fossa (9, 46). It could thus be hypothesized that the skin ecology in AD supports enhanced staphylococcal growth on both lesional and non-lesional skin, and that changes in the distribution among the staphylococcal species towards greater abundances of *S. aureus* can contribute to the development of eczema locally on the skin. However, no changes in the proportion of either *S. epidermidis*, *S. hominis* or *S. capitis* at the antecubital- and popliteal fossa during or after a flare-up episode was identified among paediatric AD patients (12), suggesting that a potential role for the CoNS spp. in AD still needs to be clarified. Furthermore, species level analysis might not be sufficient, as distinct strains within a species can have distinct phenotypes. For example, Nakatsuji et al. have shown that CoNS strains isolated from the skin of healthy individuals more often are capable of killing *S. aureus* compared with CoNS strains isolated from AD skin (42). Also, colonization of specific subspecies of *S. aureus* seems to be favoured in AD, as *S. aureus* clonal complex 1 (CC1) strains are more often detected on skin and in nares from AD patients compared with healthy controls (47). The increased prevalence of CC1 *S. aureus* colonization might be due to intrinsic factors in AD, e.g. CC1 *S. aureus* colonization have been associated with carriage of loss-of-function mutations in *FLG* (7), or due to extrinsic factors, such as treatment practice leading to selection of antibiotic resistance (48, 49).

Eukaryotic microbial community on atopic dermatitis skin

Few studies have examined the eukaryotic microbial community on AD skin, and only in adults and Asian populations (50–52). Focus has been on fungal communities, which was found to be richer and more diverse on AD lesional skin compared with healthy control skin (50, 51). *Malassezia*, especially *M. globosa* and *M. restricta*, was the dominant fungus in both AD lesional skin and healthy control skin (50, 51). An increase in the proportional abundance of *M. dermatitis* and *M. sympodialis* was identified on the skin of individuals with a history of AD (no active disease) compared with individuals without AD (52). However, no differences in the proportions of these 2 species were found between lesional skin of AD patients with active disease and healthy control skin (51, 53). Another fungal species, *Candida albicans*, was found to be over-represented on AD lesional skin on the cheeks (presence in 100% of samples), compared with healthy control skin from the same area (presence in 10% of samples) (51). The literature regarding skin eukaryotic microbial communities in AD is limited, and thus, additional studies with more attendees are needed in order to validate the presented results.

Atopic dermatitis disease severity is associated with changes in skin microbial communities

Significant differences in alpha- and beta-diversity across AD severity scores have been identified in both lesional and non-lesional skin sites, with patients with more severe disease having the lowest bacterial diversity on the skin (9, 10, 13, 54). Brandwein et al. (10) found that the bacterial community composition in antecubital- and popliteal fossa in patients with mild/moderate AD was more similar to the community composition of healthy control skin than to skin areas in patients with severe AD, regardless of whether samples were collected from lesional or non-lesional skin. This finding supports the hypothesis that the AD phenotype, such as an overall impaired skin barrier and skin inflammation, has a widespread effect on the skin microbial community and not only on lesional skin areas.

S. aureus skin and nasal colonization is significantly more prevalent among patients with more severe disease (6, 7, 55, 56), and increased relative abundances of *S. aureus*, at least at the antecubital fossa, have been associated with increasing AD severity scores (10–13). However, conflicting results regarding total *S. aureus* densities on skin in relation to AD severity have been published. Thus, *S. aureus* absolute abundances have been associated with increasing severity scores among adult patients (13, 57), whereas no association was detected in a paediatric AD population (54).

Studies investigating eukaryotic microbial communities on AD skin are sparse, but one study implies that there is an association between AD severity scores and the fungal community on skin, as beta-diversity analysis showed distinct community compositions in samples from patients with severe AD compared with samples from those with mild/moderate AD (51).

MICROBE-HOST INTERACTIONS IN ATOPIC DERMATITIS

Microbiome studies have made it evident that microbial communities on AD skin differ from those of healthy skin, and that the greatest difference is due to an over-representation and greater abundance of *S. aureus* on AD skin. What are the mechanisms behind these differences? Functional analysis studies suggest that the AD phenotype, including impaired skin barrier function, increased pH, and skin inflammation, can promote changes in the skin microbial communities (43, 58, 59). Moreover, *S. aureus* can induce skin inflammation and aggravate AD (12, 60–62). Thus, a vicious circle might exist, with filaggrin deficiency in skin leading to enhanced colonization of *S. aureus*, which through the expression of virulence factors then can induce skin inflammation and contribute to further skin barrier impairment, and, in turn, can facilitate the maintenance of an imbalanced skin microbial community (**Fig. 2**). The mechanism behind these connections is elaborated below.

Atopic dermatitis pathogenesis facilitates changes in skin microbial communities

In AD, loss-of-function mutations in the *FLG* gene have been associated with changes in the overall bacterial community composition on non-lesional AD skin (9, 46), as well as with an increased risk of *S. aureus* colonization on lesional skin and in anterior nares (7). These studies indicate that filaggrin can influence bacterial growth and colonization on the skin. In accordance, presence of the filaggrin breakdown products urocanic acid (UCA) and pyrrolidone carboxylic acid (PCA), which contribute to skin acidification, have been shown to reduce *S. aureus* growth *in vitro* (58). More neutral pH, reflecting the skin pH in AD, has been associated with increased expression of *S. aureus* genes involved in colonization, including the gene encoding clumping factor B, which mediates adherence to keratinocytes (58, 59). Thus, increased *S. aureus* colonization among AD patients with *FLG* loss-of-function mutations (7) might be due to changes in skin pH caused by UCA and PCA deficiency. In addition to increased *S. aureus* adherence in epidermis, filaggrin deficiency is also associated with enhanced migration of *S. aureus* into the dermis skin layer. Nakatsuji et al. (43) showed that skin barrier impairment in mice, induced by genetic predisposition (*FLG* loss-of-function mutations) and physical skin disruptions (tape stripping), led to enhanced penetration of *S. aureus* into the dermis where it could activate the host immune system. In humans, the absolute abundance of *S. aureus* was significantly greater in dermis of AD lesional skin compared with healthy control skin, indicating that *S. aureus* can migrate more easily into the deeper skin layers of patients with AD with a disrupted skin barrier (43). Disrupted AD skin is also more permeable to allergens, which can trigger type I allergic responses in sensitized individuals. To corroborate this, patients with AD are also more often hypersensitive to a wide range of microbial allergens, including allergens from *S. aureus* and the skin colonizing fungal species *Malassezia furfur* and *Candida albicans*, compared with the general population (63–65).

AD skin might not only be more susceptible to *S. aureus* colonization, but also more vulnerable to *S. aureus* virulence. Alpha-haemolysin (also known as alpha-toxin), a virulence factor secreted by *S. aureus*, has thus been shown to adhere more easily to keratinocytes in AD skin compared with keratinocytes in healthy skin (66, 67). Alpha-haemolysin adheres to sphingomyelin lipids in the membranes of keratinocytes, leading to cell lysis and contribution to skin barrier disruptions (68). The density of sphingomyelin lipids, and thus the amount of free adherence sites for alpha-haemolysin, is regulated by the enzyme acid sphingomyelinase. Filaggrin deficiency as well as Th2 cytokines promote down-regulation of acid sphingomyelinase, thus enhancing alpha-haemolysin binding efficiency (66, 67). Thus, filaggrin deficiency in AD probably both favours *S. aureus* colonization and enhanced *S. aureus* mediated cytotoxicity and immune activation (**Table I**).

S. aureus *as an inducer of clinical atopic dermatitis*

Byrd et al. (12) have shown that *S. aureus* isolated from AD skin, but not *S. aureus* from normal healthy skin, was able to induce skin inflammation in wild-type mice with no genetic predisposition. Skin inflammation, assessed by epidermal thickening and cutaneous infiltration of immune cells, including Th2 and Th17 cells, was more pronounced in mice inoculated with *S. aureus* from patients with more severe AD. This study highly suggests

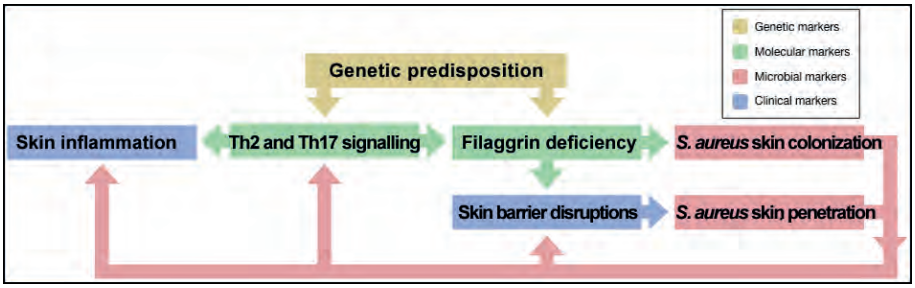

Fig. 2. Proposed connections between human factors involved in AD pathogenesis and *S. aureus* colonization and virulence.

Table I. The effect of filaggrin deficiency on *S. aureus* skin colonization and virulence

Effect of filaggrin deficiency		
Primary outcomes	Secondary outcomes	Refs
Increased skin pH	Enhanced *S. aureus* growth and colonization	(58, 59)
Impaired skin barrier	Enhanced *S. aureus* migration through the epidermal barrier and into dermis	(43)
Increased density of sphingomyelin lipids in keratinocyte membranes	Enhanced binding of alpha-haemolysin (cytotoxic *S. aureus* virulence factor)	(67)

that certain strains of *S. aureus* are able to elicit lesions similar to those observed in AD. The detected effect of *S. aureus* is probably mediated by the production of virulence factors, such as phenol-soluble modulins (PSM) and enterotoxins.

Several studies indicate that *S. aureus* induced skin inflammation and barrier disruption in mice are dependent on secretion of PSM-alpha, which promotes interleukin (IL)-17A mediated pro-inflammatory responses *in vitro* (human keratinocytes) and *in vivo* (mice) (60, 69, 70). Another PSM, known as delta-toxin, was also able to mediate *S. aureus* induced skin inflammation in mice, an effect that probably is mediated by delta-toxin induced mast cell degranulation, IgE production and enhanced IL-4 expression (62, 71). Interestingly, PSM-alpha transcripts are significantly more abundant in *S. aureus* isolated from AD skin compared with those from *S. aureus* isolated from healthy control skin (60), and delta-toxin production has been found to be considerably higher among *S. aureus* from lesional skin compared with non-lesional skin on patients with AD (62). These findings might explain why *S. aureus* strains isolated from AD lesional skin were better at eliciting skin inflammation compared with *S. aureus* from healthy skin (12).

S. aureus enterotoxins have also been proposed to be important mediators of *S. aureus* induced skin inflammation. Thus, topical application of staphylococcal enterotoxin B (SEB) to skin have been shown to cause erythema and epidermal thickening in both healthy volunteers and patients with AD (72), an effect which likely is mediated by enhanced T-cell signalling (72, 73). Studies indicates that *S. aureus* from AD skin more often carry genes encoding enterotoxins (*sea*, *seb*, *sec*, and *sed*) and more often produce these toxins compared with *S. aureus* isolated from non-AD individuals (73, 74). Furthermore, carriage of enterotoxin producing *S. aureus* has been associated with increased AD severity (assessed by SCORAD) (73, 75).

In one study, alpha-haemolysin was also found to be produced more frequently by AD *S. aureus* (91% of isolates) compared with production rates among *S. aureus* from healthy volunteers (33% of isolates) (61), which in combination with AD genetic predisposition for enhanced binding efficiency of the toxin (66, 67) could make alpha-haemolysin a potent inducer of skin barrier disruptions in AD (61). However, two other studies found lower proportions of *S. aureus* producing alpha-haemolysin on AD skin (30–63% of isolates) (76, 77) and a third study reported an alpha-haemolysin gene (*hla*) expression frequency of 59% among *S. aureus* nasal isolates from healthy carriers (78), highlighting that population-based differences and use of distinct assays can influence the results. Thus, future studies need to elucidate whether alpha-haemolysin, and other *S. aureus* toxins, is upregulated in *S. aureus* colonizing AD skin.

The above-mentioned studies support the hypothesis that *S. aureus* virulence is a major driver of AD disease exacerbation and might even be a direct cause of flare-ups. In order to cause disease, *S. aureus* must first colonize the skin. *S. aureus* isolated from AD skin has an enhanced binding activity of clumping factor B, leading to increased adhering to corneocytes, compared with *S. aureus* from healthy skin (79). In addition, CC1 *S. aureus*, which is a dominant clone in AD (7, 79, 80), had a slightly higher binding affinity compared with other *S. aureus* lineages (79). Thus, the increased prevalence of *S. aureus* skin colonization in AD might both be due to host factors and *S. aureus* factors (58, 59, 79). A summary of the described *S. aureus* virulence factors shown to be involved in AD is given in **Table II**.

EFFECT OF ATOPIC DERMATITIS TREATMENTS ON SKIN MICROBIAL COMMUNITIES

Topical application of corticosteroid (glucocorticoids) based creams is a common treatment of AD lesions. Prospective studies examining the effect of topical corticosteroid treatment on skin microbial communities in AD, have shown that 4–6 weeks of treatment led to significant increases in bacterial Shannon-diversity and richness (40, 81), whereas 7–10 days of treatment had no influence on alpha-diversity, though an clinical improvement was observed (35). Thus, a possible effect of topical corticosteroid on skin microbial communities is dependent of several weeks of continuous treatment. Comparative studies also imply that topical corticosteroid

Table II. Virulence factors upregulated in *S. aureus* isolated from atopic dermatitis skin compared with *S. aureus* from healthy control skin

Virulence factors	Clinical outcomes	Mediators	Refs
PSM-alpha	Skin inflammation Skin barrier disruptions	IL-17A signalling Protease activity	(60, 70)
Delta-toxin	Skin inflammation	IL-4 signalling Mast cell degranulation IgE release	(62, 71)
Alpha-haemolysin	Skin barrier disruptions	Keratinocyte lysis	(61)
Enterotoxin B	Skin inflammation	T-cell signalling	(72)
Clumping factor B	*S. aureus* colonization	Cell adherence	(79)

PSM: phenol-soluble modulin; IL: interleukin.

treatments have an effect on the skin microbial community, as AD patients undergoing topical corticosteroid treatments prior to sample collections often have a more diverse bacterial population with lower relative abundances of *S. aureus* compared with non-treated patients (8, 9, 81). This effect might be due to direct inhibition of *S. aureus* as well as to a general improvement on skin conditions due to the anti-inflammatory properties of corticosteroids (82).

A keystone treatment practice in AD is application of emollients and moisturizers, which restore skin barrier integrity and prevents flare-ups. Despite extensive use, little is known about what effect this treatment approach has on skin microbial communities, but one study indicates that emollient application leads to decreased proportions of *Staphylococcus* spp. on AD lesional skin (83). Although it indeed would be interesting to examine the long-term effect of emollient usage on the skin microbiome, it might be challenging and ethically unjustifiable to set up such study, as it would include an AD patient group that will be denied treatment with emollients and moisturizers for a longer period.

One study has examined the effect of dupilumab treatment, an anti-inflammatory systemic therapy offered to adults with severe and chronic AD, on the skin bacterial community (13). Sixteen weeks of treatment led to increased alpha-diversity and a decrease in relative and absolute *S. aureus* abundances on lesional as well as non-lesional AD skin. However, this effect was lost 18 weeks after treatment termination. Dupilumab inhibits IL-4/IL-13 signalling, and the study thus shows that reduction of Th2-mediated signalling may influence *S. aureus* skin colonization.

Another common treatment practice, at least in some countries, is topical application of fusidic acid, which is a narrow-spectrum antibiotic used against *S. aureus*. Unfortunately, bacterial growth of other common bacterial species on skin, including CoNS, are also inhibited by fusidic acid (84), and recent studies have shown a high prevalence of fusidic acid resistant *S. aureus* on AD skin and nares (48, 49), signifying that alternative treatment regimens are needed for the control of *S. aureus* colonization. Future treatment approaches could include *S. aureus* anti-virulence therapy (71) or application of commensal skin bacteria with anti-*S. aureus* properties (42). Oral administered antibiotics might also impact the cutaneous bacterial community composition and select for antibiotic resistance among skin bacteria (85–87).

CONCLUSION

Multiple studies have shown that increased abundance of *S. aureus* and loss of bacterial diversity on skin are associated with disease severity and flares in children and adults with AD. The enhanced burden of *S. aureus* skin colonization is probably facilitated by AD-related changes in the skin, including reduced levels of filaggrin and NMFs leading to increased skin pH and skin barrier impairment. In addition, deficiency of commensal bacterial strains with *S. aureus* inhibitory properties may contribute to the increased density of *S. aureus* on AD skin. Functional assays indicate that cutaneous *S. aureus* can exacerbate AD by expressing virulence factors that can induce skin inflammation and skin barrier disruption. Thus, changes in the composition of the skin bacterial community may be an important inducer of the clinical manifestations in AD patients with established disease. Whether bacterial community dysbiosis is also considered to be present prior to AD development is still unclear, and needs further investigation. Increasing knowledge regarding *S. aureus* as a potent promoter of AD exacerbation, has highlighted the skin microbial community as a potential target for future treatment strategies, and is a research field of great interest. Future studies are needed to explore the potentials, efficiency and safety of these novel anti-bacterial treatment approaches.

The authors have no conflicts of interest to declare.

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Centenary theme section: ATOPIC DERMATITIS

REVIEW ARTICLE

A Therapeutic Renaissance – Emerging Treatments for Atopic Dermatitis

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Atopic dermatitis (AD) is a chronic, inflammatory cutaneous disease that is characterized by complex immune dysregulation and skin barrier dysfunction with a wide variety of clinical phenotypes. Until recently, conventional therapeutic modalities for AD remained rather non-specific despite AD's complex etiology. Failing to take into account the underlying inflammatory pathways led to treatments with inadequate efficacy or unacceptable long-term toxicities. We are currently in the midst of a therapeutic renaissance in AD. Recent progress in molecular medicine provides us a better understanding of the AD pathogenesis, suggesting a dominant helper T cell (Th) 2/Th22 response with a varying degree of Th1/Th17 overexpression. Targeted therapeutic agents including biologics and small molecule inhibitors in development hold promises for more effective and safer therapeutic approaches for AD. A better understanding of individual differences amongst AD patients will allow for a more tailored approach in the future. This review aims to cover the most promising emerging therapies in the field of atopic dermatitis utilizing recently published manuscripts and up-to-date conference abstracts and presentations.

Key words: atopic dermatitis; targeted therapeutic agents; biologics; small molecule inhibitors.

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SIGNIFICANCE

Effective treatment of atopic dermatitis is complicated due to its chronic nature, multifaceted pathophysiology, and variable clinical manifestations. The success of dupilumab confirms the importance of type 2 cytokines in the pathophysiology of atopic dermatitis. Besides type 2 cytokines, certain phenotypes of atopic dermatitis may be driven by additional cytokine pathways. However, data to date attempting to target specific cytokines outside of the type 2 axis have been largely unsuccessful. Further data using large-scale and long-term clinical trials are needed in order to create tailored and personalized treatments for atopic dermatitis.

With an increasing prevalence worldwide, atopic dermatitis (AD) is a chronic, pruritic inflammatory skin disease that often presents in infancy and may persist or re-emerge in adulthood (1). The pathophysiology of AD is complex and involves genetic predispositions, environmental factors, skin barrier dysfunction, immune dysregulation, and disruptions in the skin microbiota (2, 3). Approximately one third of all AD patients have moderate-to-severe disease with symptoms including pruritus, increased risk of sleep disturbances, mental health comorbidities, and suicidal ideation, all of which contribute to a poor quality of life (QoL) (4, 5). Selecting treatments for AD in the clinical setting is often challenging due to a variety of AD phenotypes, which may be due to the various cytokine profiles of AD (6). Conventional systemic immunosuppressive agents including corticosteroids, cyclosporine, methotrexate, azathioprine, and mycophenolate mofetil provide inadequate long-term control in many patients who require systemic therapy due to inadequate efficacy or adverse drug reactions. Thus, there remains a large unmet need for an effective and safe long-term systemic treatment for AD. Considering the multifactorial etiology of AD, the ideal therapeutic treatment should target the specific molecular defect or defects underlying the particular patient's disease. Over the past few years, our increasing knowledge of the immunopathogenesis and heterogeneity of AD has initiated an era of targeted therapeutics, such as biologics and small molecule inhibitors. We can expect to see a more personalized therapeutic treatment approach for AD in the future.

PATHOPHYSIOLOGY OF ATOPIC DERMATITIS

Analysis of the skin and blood of patients with AD reveal an array of adaptive and innate immune derangements. For many years, AD pathophysiology was thought to be driven by a predominant helper T (Th) 2 response in the acute phase of the disease, and a skewed Th1 response in the chronic phase (7). This acute (Th2) and chronic (Th1) paradigm emerged from studies involving inhalant allergen patch tests – an artificial model system with questionable relevance to AD. In this model, Th2 cells and interleukin (IL)-4 messenger RNA (mRNA) were predominantly observed in acute lesions, while Th1 cells and recombinant interferon (IFN)-γ mRNA were prima-

rily seen in chronic lesions (8). Recent findings using patients with AD, not patch tests, have suggested that AD has a stronger association with a Th2/Th22 response and a much more variable Th1/Th17 response throughout both the acute and chronic stages of the disease (9–11). In the acute phase, lesions display overactivation of Th2/Th22 related signals and to a lesser degree Th17 related signals (12, 13). Intensification of these axes, along with an upregulation of Th1 cells, recruit and coordinate the chronic phase of the disease (9).

In AD skin, disruption of the epidermal barrier by irritants, allergens, and pathogens give rise to the activation of nonlymphoid cells like Langerhans cells (LC) and keratinocytes. Epidermal disruption may also occur via genetically driven alterations in skin barrier function such as loss-of-function mutations in the *FLG* gene that encodes for the skin barrier protein filaggrin (14). Disrupted keratinocytes initiate or potentiate inflammation via the release of cytokines and chemokines, including thymus- and activation-regulated chemokine (TARC), thymic stromal lymphopoietin (TSLP), IL-25, and IL-33. These cytokines drive local tissue inflammation and activate a series of Th2-mediated events such as immunoglobulin (Ig) E class switching and recruitment of IL-5 dependent eosinophils into the skin (**Fig. 1**) (15, 16). Th2 cells release IL-4, IL-5, IL-13, and IL-31, which mediate the activation of additional inflammatory cells like mast cells and eosinophils. They also inhibit the expression of barrier proteins such as filaggrin, and barrier lipids such as ceramides (17, 18). Notably, IL-4 and IL-13 induce keratinocytes to secrete additional TSLP, which results in Th2 polarization and a positive feedback loop (19). IL-31, an interleukin that induces itching via sensory nerves, is upregulated in AD lesions and triggers scratching behavior, which may further drive inflammation (20). Group 2 innate lymphoid cells (ILC2s), which are activated by keratinocyte mediators, release both IL-5 and IL-13 that perpetuates Th2 immunity (21, 22). In conjunction with IL-17 released by Th17 cells, IL-22 released by Th22 cells promotes epidermal hyperplasia and aberrant epidermal differentiation (9).

By identifying a growing number of immune pathways underlying AD, numerous targeted and broad-acting drugs are currently in the therapeutic pipeline. Given the critical role of the Th2 axis in AD, anti-Th2 agents like dupilumab, which represents the first biologic drug approved for AD, have been developed (23, 24). Multiple targeted drugs involving the Th22 and Th17 pathways, as well as broader T cell inhibitors, are also currently under investigation. The aim of this review is to provide up to date information regarding this unique and promising era of innovation and novel therapeutic development.

CLINICAL AND MOLECULAR HETEROGENEITY OF ATOPIC DERMATITIS

Recent research reveals several AD subtypes classified by different endotypes and phenotypes including age, chronicity, ethnicity, filaggrin gene mutational status, IgE status, *S. aureus* colonization status, and underlying molecular signaling abnormalities (25–28). Subtypes of various ethnic backgrounds such as European American decent, African American decent, and Asian origin have also been identified. Other AD classifications include pediatric patients versus adult patients, subjects with acute versus chronic disease, and patients exhibiting intrinsic versus extrinsic type. In spite of a similarity in clinical presentation and response to therapy, extrinsic AD was historically defined as patients with high serum IgE levels, personal and family atopic background, while the intrinsic phenotype having normal IgE levels shows female predominance and lack any other atopic diathesis (25).

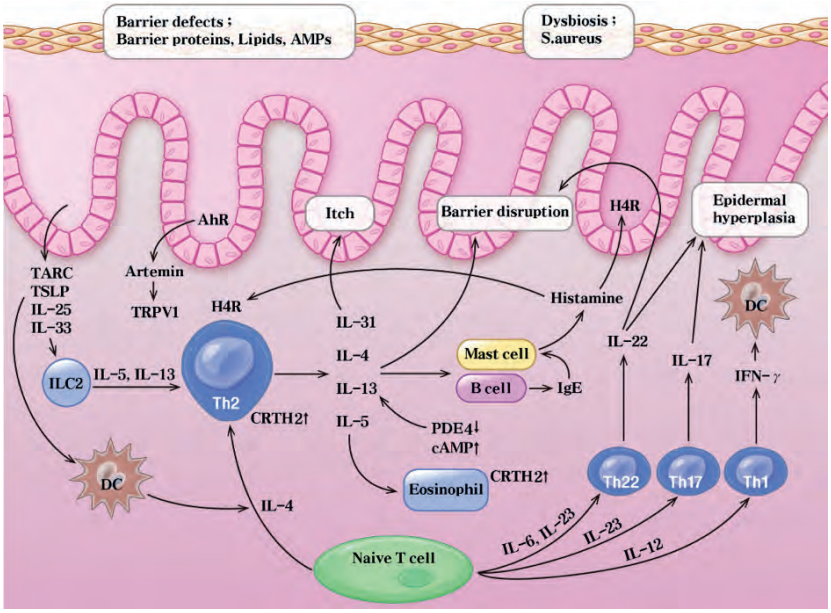

Fig. 1. Immune pathophysiology of atopic dermatitis (AD). In AD skin, epidermal disruption initiates or potentiates inflammation through the release of cytokines and chemokines, including thymus- and activation-regulated chemokine (TARC), thymic stromal lymphopoietin (TSLP), interleukin (IL) -25, and IL-33. These cytokines drive local tissue inflammation and activate a series of Th2 cytokines such as IL-4, IL-5, IL-13, and IL-31, thereby leading to immunoglobulin (Ig) E class switching and accumulation of inflammatory cells into the skin. Together with IL-17 released by Th17 cells and IL-22 released by Th22 cells, epidermal hyperplasia and barrier disruption are intensified throughout the acute and chronic stages of AD. AD: atopic dermatitis; Th: helper T; AMPs: antimicrobial peptides; AhR: aryl hydrocarbon receptor; ILC2: group 2 innate lymphoid cells; TRPV1: transient receptor potential cation channel subfamily V member 1; H4R: histamine receptor type 4; DC: dendritic cell; CRTH2: chemoattractant receptor-homologous molecules expressed on Th2 lymphocytes; PDE4: phosphodiesterase4; cAMP: cyclic adenosine monophosphate; IFN-γ: interferon-γ.

Despite a strong polarization of Th2/Th22 identified in the general AD population, there appears to be a relatively dominant Th17 subtype in pediatric patients, patients of Asian descent, and patients with intrinsic AD. African-American patients with AD and pediatric patients with AD also appear to lack any Th1 activation (25). A Dutch study based on the analysis of serum biomarkers of 193 adult patients with moderate-to-severe AD identified 4 endotype clusters of AD (29). Clusters 1 and 4 show higher levels of Th2 cytokine expression in "erythematous" phenotypes, while clusters 2 and 3 show lower levels of Th2 cytokine expression in "lichenified" phenotypes. Although further studies are needed to confirm the reliability of these subtypes, these findings and others can serve as useful tools in developing targeted treatments for AD. The clinical relevance of emerging endotypes will be deemed clinically relevant if they identify patients that respond better to a particular therapeutic (i.e., precision medicine) or help predict the natural course.

TOPICAL THERAPIES

Despite the advent of new systemic agents, topical therapies are still an essential component in the management of AD. Topical anti-inflammatory therapies for AD include the use of topical corticosteroids (TCS) as first-line therapy with topical calcineurin inhibitors (TCI) as an alternative to TCS in areas where TCS use is not recommended. Moderate-to-severe patients with AD, however, are often inadequately controlled with these agents. Additionally, the prolonged use of TCS may cause telangiectasia, skin atrophy, dyschromia, and adverse events. The use of TCI is often limited by burning and stinging (30). Given these limitations in traditional topical therapies, there remains a significant unmet need for patients. New topical agents are now being studied to modulate phosphodiesterase (PDE) 4, Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway, aryl hydrocarbon receptor (AhR), and the skin microbiome (**Table I**).

PDE4 inhibitors

Hanifin and colleagues (31) first made the observation that AD monocytes display overactive phosphodiesterase enzyme activity. Inhibition of PDE4 leads to an increase in cyclic adenosine monophosphate (cAMP), resulting in the down-regulation of inflammatory cytokines in chronic inflammatory skin diseases such as psoriasis and AD (32). Crisaborole, a topical PDE4 inhibitor was first approved in 2016 by the US Food and Drug Administration (FDA) for patients with mild-to-moderate AD over the age of 2 years. Two phase III trials showed

Table I. Novel topical targeted therapies of AD (in or beyond phase II trial)

Target	Agent	Mechanism	Phase status	Clinical trials
Phosphodiesterase 4 (PDE4)	Crisaborole/AN2728	PDE4 inhibitor	I/II completed	NCT01652885
			II completed	NCT03233529
				NCT01602341
			II completed	NCT03954158
			III completed	NCT02118766
				NCT02118792
			III ongoing	NCT04040192
			IV ongoing	NCT03868098
				NCT03539601
	MM36/OPA-15406	PDE4 inhibitor	II completed	NCT02945657
				NCT02068352
				NCT02914548
				NCT03018691
			III ongoing	NCT03961529
			III completed	NCT03911401
				NCT03908970
	Roflumilast	PDE4 inhibitor	II completed	NCT01856764
				NCT03916081
	AN2898	PDE4 inhibitor	II completed	NCT01301508
	Lotamilast/RVT-501/E6005	PDE4 inhibitor	I/II completed	NCT01179880
				NCT02094235
			II completed	NCT01461941
				NCT02950922
				NCT03394677
	DRM02	PDE4 inhibitor	II completed	NCT01993420
	LEO29102	PDE4 inhibitor	II completed	NCT01037881
Janus kinase (JAK)	Tofacitinib	JAK 1/3 inhibitor	II completed	NCT02001181
	Delgocitinib/JTE-052/LEO124249	JAK 1/3 inhibitor	IIa completed	NCT01037881
	Ruxolitinib/INCB18424	JAK 1/2 inhibitor	II completed	NCT03011892
			III ongoing	NCT03745651
				NCT03745638
Aryl hydrocarbon receptor (AhR)	Tapinarof/ WBI-1001/benvitimod/ GSK2894512	AhR agonist	I/II completed	NCT00837551
			II completed	NCT02564055
				NCT01098734
S. aureus	Roseomonas mucosa bacteria	Commensal interaction	I/II completed	NCT03018275
	Coagulase-negative Staphylococcus	Commensal interaction	I/II completed	NCT03151148
			II ongoing	NCT02144142

significant efficacy with 51% clear and 48% almost clear in the Investigator's Static Global Assessment (ISGA) score (33). A large vehicle effect, however, leads to a relatively large number needed to treat (NNT), ranging between 8 and 14. (34). This translates to between 8 and 14 patients are needed to be treated before one person achieves success over vehicle treatment (35). Improved signs of pruritus and good drug tolerability were reported amongst patients. Limited adverse events included pain, burning, and stinging. However, the clinical prevalence of these events are seemingly more common in clinical practice than that reported in trials. A study of crisaborole over 48 weeks confirmed its safety for longer-term use (36) but comparative efficacy data with other topical agents is currently lacking. A new study has been initiated to evaluate the efficacy of crisaborole compared to other topical agents like TCS and TCI (NCT03539601). MM36 (OPA-15406), another PDE4 inhibitor with high selectivity for PDE4B, at higher concentration showed significant improvement in Eczema Area and Severity Index (EASI) score at week 1 compared to placebo and persisted for 8 weeks (37). Various PDE4 inhibitors including roflumilast, AN2898, lotamilast, DRM02, and LEO29102 are currently undergoing phase II and phase III trials. Overall, topical PDE4 inhibitors appear to be a safe approach to long-term management of selected mild-to-moderate AD without the potential for significant systemic absorption or cutaneous atrophy.

JAK and other kinase inhibitors

JAK inhibitors are small molecules that inhibit the JAK-STAT signaling pathway. Although they have been mostly studied as systemic therapeutics for AD, topical applications have also shown promise in clinical trials. The JAK -STAT pathway has been implicated in the signaling of multiple AD-related cytokines such as IL-4, IL-5, IL-6, IL-12, IL-13, IL-22, IL-23, IL-31, IL-33, and IFN-γ (38–40). A JAK family of 4 receptor associated kinases (JAK1, JAK2, JAK3, and tyrosine kinase (TYK) 2) phosphorylate intracellular receptors and increase the production of a group of STATs, leading to the activation of targeted gene expression (**Fig. 2**). JAK inhibitors target different combinations of kinases with variable selectivity, resulting in overlapping but distinct inhibitory effects on various cytokine pathways. Spleen tyrosine kinase (SYK) is a non-receptor tyrosine kinase involved in the release of pro-inflammatory cytokines including IL-17, B cell activation, and keratinocyte differentiation (40). The SYK pathway plays an important role in Th17 signaling by recruiting Th17 cells to the skin along with inducing the production of CCL (C-C motif chemokine ligand) 20 (41). Consequently, targeting the JAK-STAT and SYK pathways downregulates multiple immune axes involved in the pathogenesis of AD (Th1, Th2, Th17, and Th22). The broader immune modulation of JAK inhibition holds the potential to bring greater efficacy. However, this theoretically results in an increase in potential adverse events as well.

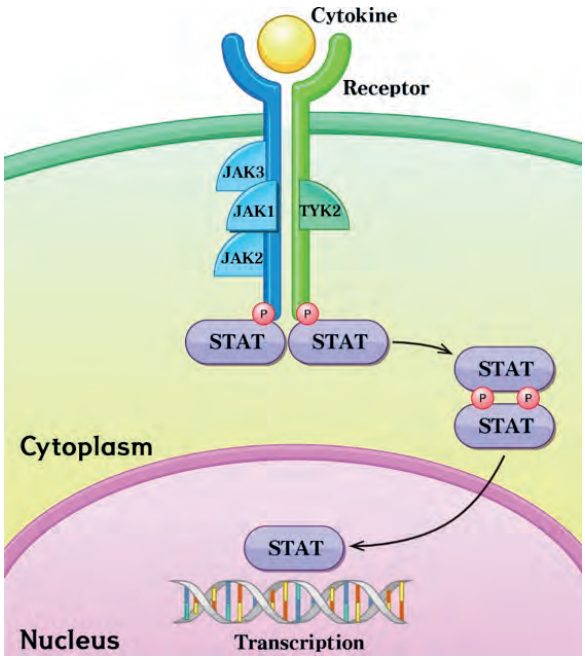

Fig. 2. JAK-STAT pathway. A cytokine binds to its cell surface receptor. A Janus kinase (JAK) family of four receptor associated kinases (JAK1, JAK2, JAK3, and tyrosine kinase (TYK) 2) phosphorylate intracellular receptors and increase the production of a group of signal transducer and activator of transcription (STAT). Phosphorylated STATs dimerize and translocate to the nucleus, leading to the activation of targeted gene expression.

Topical JAK inhibitors decrease IL-4 and IL-13 signaling pathways and enhance skin barrier functions in mouse AD models (42). A phase IIa trial investigating tofacitinib, a potent JAK1/JAK3 inhibitor, for patients with mild-to-moderate AD showed significant reduction of pruritus by day 2 and a large reduction in EASI score by week 4 (81% vs. 29% (placebo), $p<0.001$) (43). The application site reactions reported in two subjects were mild pain or mild pruritus. A controlled study of delgocitinib (JTE-052/LEO 124249), a pan JAK (JAK1-3,TYK2) inhibitor, showed significant improvement in the overall symptoms of AD by week 4, and low modified EASI (mEASI) and Investigator's Global Assessment (IGA) scores with a favorable safety profile (44). Improvements in pruritus were also observed by day 1, which was likely due to the inhibition of IL-31 signaling mediated by the JAK-STAT pathway (20) or possibly via direct effect of JAK inhibition on itch transmission by neurons (45). Improvements in mEASI score with the higher doses of delgocitinib were similar to the tacrolimus 0.1% ointment active control arm, although there was no statistical comparison (44). In an ongoing phase II trial, topical ruxolitinib (INCB018424), a potent JAK1/JAK2 inhibitor, showed significant efficacy in EASI score at week 4 in the cream 0.5% and 1.5% arms versus vehicle (46). Topical ruxolitinib at higher doses (1.5%) showed greater improvements in EASI score at week 4 than triamcinolone cream 0.1%. Other JAK inhibitors such as cerdulatinib (RVT-502), a

dual JAK and SYK inhibitor, and SNA-125, a JAK 3 and tropomyosin receptor kinase A (TrkA) inhibitor, are currently being evaluated in phase I/II trials of AD, however no data are available for review at this time.

AhR agonist

The AhR is a cytosolic ligand-activated transcription factor that is involved in both pro- and anti-inflammatory signaling pathways (47). It has the potential to impact the balance of Th17 and regulatory T (Treg) cell production and can restore epidermal barrier function (48, 49). Tapinarof (benvitimod/GSK2894512/WBI-1001), an AhR agonist, is a naturally derived molecule produced by the bacterial symbionts of entomopathogenic nematodes (50). In two phase II trials, significant improvements in EASI and IGA scores were seen at week 4 in patients with mild-to-moderate AD and significant efficacy in IGA scores of both 0.5% and 1% dosing groups at week 6 in patients with mild-to-severe AD (51, 52). In earlier studies of higher dose tapinarof at 2%, headache, diarrhea, nausea and/or vomiting were observed. This suggests the potential for systemic absorption at higher concentrations (53). Phase 3 studies are anticipated.

Commensal organisms

Cutaneous dysbiosis, characterized by a reduction in microbial diversity and an increase in colonization of *S. aureus,* has been shown to initiate and worsen the flare of AD (54). Recent research suggests a unique phenotype and endotype for patients colonized with *S. aureus*. Characteristics of *S. aureus*-colonized patients include more severe skin disease, reduced barrier function, increased serum lactate dehydrogenase (LDH) levels, increased allergen sensitization, elevated IgE levels, elevated eosinophil counts, and increased levels of various Th2 biomarkers such as TARC, periostin, and CCL26 (55). Increased *S. aureus* colonization has been proposed as a potential mechanism for disease progression and flare-up of AD. A recent open-label trial with topical application of *Roseomonas mucosa* for patients with AD found that the commensal bacterium provided patients with clinical improvement in AD severity and pruritus, and a reduction of TCS use (56). Another study reported that autologous transplantation of coagulase-negative *Staphylococci* enriched with novel anti-*S. aureus* peptides leads to a decrease in *S. aureus* colonization and clinical improvements in AD (57). Currently, a phase I/II trial using *Roseomonas mucosa* and a phase II trial testing coagulase-negative *Staphylococcus* are underway. These studies will help elucidate whether the dysbiosis in AD is a primary driver of the disease or merely a consequence of barrier dysfunction or type 2 inflammation. Should this approach provide efficacy, it is intriguing to speculate that transplanting beneficial live commensals could theoretically yield a remittive effect on the disease.

SYSTEMIC THERAPIES

Systemic treatments may be appropriate for pediatric and adult patients with moderate-to-severe AD whose disease is inadequately controlled with appropriate amounts of topical therapies. According to an International Eczema Council (IEC) consensus paper, the decision to commence or offer systemic treatments should involve an assessment of disease severity, an understanding of the impact on QoL, and include individual factors such as patient preferences, prior treatment history, financial considerations, and comorbidities (58). Traditionally, systemic therapies include phototherapy or systemic immunomodulators such as corticosteroids, cyclosporine, methotrexate, azathioprine, and mycophenolate mofetil. Given the risk of potential toxicities with traditional immunosuppressant long-term treatments, there is still an unmet need for safe and effective long-term therapies. Dupilumab, the first biologic drug approved for AD, has filled this large void for a safe and effective therapy for long-term use. Since the advent of dupilumab, a number of biologics and small molecule inhibitors are now being developed and investigated to provide alternatives to dupilumab (**Table II**).

Targeting Th2 pathway

IL-4 and/or IL-13 antagonists. IL-4 and IL-13 are the key mediators of Th2 inflammatory responses and are responsible for the production of IgE. Cell culture studies reveal increased IL-4/IL-13 levels that not only lead to the recruitment of additional inflammatory cells, but also disturb skin barrier function by inhibiting the production of barrier structural proteins like filaggrin, lipids and antimicrobial peptides, and encourage *S. aureus* colonization (57, 59). IL-13 is overexpressed in both lesional and non-lesional AD, and correlates with disease severity (10, 60). Dupilumab, a fully human monoclonal antibody (mAb), inhibits both the IL-4 and IL-13 signaling pathway by blocking their shared IL-4Rα receptor subunit (61). Dupilumab was approved to treat moderate-to-severe AD in adults in the US and Europe in 2017, and its approval was extended to patients with moderate-to-severe AD over the age of 12 years in the US in 2019 (62). In a phase III trial of identical design (SOLO1 and SOLO2), adult patients with moderate-to-severe AD who received dupilumab every other week showed improvement in disease at week 16, with the proportion of patients achieving a 75% reduction in EASI score (EASI-75) ranging between 44–51% versus placebo (12–15%) (24). Patients also reported improvements in their symptoms including pruritus, anxiety, and depression. They also reported an overall improvement in QoL. In another phase III study (LIBERTY AD CHRONOS), a year-long trial of dupilumab showed an improved disease activity with a good safety profile when combined with TCS exhibiting only local injection reactions and conjunctivitis as adverse events (63). A LIBERTY AD CAFÉ study with concomi-

Table II. Novel systemic targeted therapies of atopic dermatitis (AD) (in or beyond phase II trial)

Target	Agent	Mechanism	Route	Phase status	Clinical trials
Biologics					
T-helper 2	Dupilumab	Anti-IL-4Rα mAb	Subcutaneous	IV ongoing	NCT03411837
					NCT03293030
					NCT03389893
					NCT03667014
	Pitrakinra/Aeroderm	Anti-IL-4 mAb	Subcutaneous	IIa completed	NCT00676884
	Lebrikizumab	Anti-IL-13 mAb	Subcutaneous	II completed	NCT02340234
					NCT03443024
					NCT02465606
					NCT04178967
				III ongoing	NCT04146363
	Tralokinumab	Anti-IL-13 mAb	Subcutaneous	II completed	NCT02347176
					NCT03562377
				III completed	NCT03363854
					NCT03160885
					NCT03131648
				III ongoing	NCT03587805
					NCT03761537
					NCT03526861
	Tezepelumab/AMG157/ MEDI9929	Anti-TSLP mAb	Subcutaneous	IIa completed	NCT02525094
				II ongoing	NCT03809663
	GBR830	Anti-TSLP mAb	Subcutaneous	II completed	NCT02683928
				IIb ongoing	NCT03568162
	KHK4083	Anti-OX40 mAb	Subcutaneous	II ongoing	NCT03703102
	Nemolizumab/CIM331	Anti-IL-31RA mAb	Subcutaneous	II completed	NCT01986933
					NCT03100344
				II ongoing	NCT03921411
				III ongoing	NCT03989206
					NCT03985943
					NCT03989349
	Mepolizumab	Anti-IL-5 mAb	Intravenous	II terminated	NCT03055195
T-helper22	Fezakinumab/ILV-094	Anti-IL-22 mAb	Subcutaneous	II completed	NCT01941537
T-helper 1/ T-helper 17	Ustekinumab	Anti-IL-12/23p40 mAb	Subcutaneous	II completed	NCT01806662
					NCT01945086
	Secukinumab	Anti-IL-17A mAb	Subcutaneous	II completed	NCT02594098
					NCT03568136
	MOR106	Anti-IL-17C mAb	Subcutaneous	II terminated	NCT03568071
					NCT03864627
IgE	Omalizumab	Anti-IgE mAb	Subcutaneous	II completed	NCT01179529
				IV completed	NCT02300701
					NCT00822783
	Ligelizumab/QGE031	Anti-IgE mAb	Subcutaneous	II completed	NCT01552629
Interleukin (IL)-1α	Bermekimab/MABp1	Anti-IL-1α mAb	Subcutaneous	II completed	NCT03496974
				II ongoing	NCT04021862
Small molecules					
Janus kinase (JAK)	Barcitinib	JAK1/2 inhibitor	Oral	II completed	NCT02576938
				III completed	NCT03334422
					NCT03733301
					NCT03334396
				III ongoing	NCT03559270
					NCT03435081
					NCT03334435
					NCT03428100
					NCT03952559
	Upadacitinib/ABT494	JAK1 inhibitor	Oral	II completed	NCT02925117
				III ongoing	NCT03607422
					NCT03569293
					NCT03568318
					NCT03738397
					NCT03661138
	Abrocitinib/PF-04965842	JAK1 inhibitor	Oral	II completed	NCT02780167
				II ongoing	NCT03915496
				III completed	NCT03349060
					NCT03575871
					NCT03627767
					NCT03422822
				III ongoing	NCT03720470
					NCT03796676
	ASN002/Gusacitinib	JAK/spleen tyrosine kinase inhibitor	Oral	II completed	NCT03531957
				II terminated	NCT03654755
Phosphodiesterase (PDE) 4	Apremilast	PDE4 inhibitor	Oral	II completed	NCT02087943
					NCT00931242
Chemoattractant receptor-homologous molecules expressed on Th2 lymphocytes (CRTH2)	OC000459/ODC-9101	CRTH2 mAb	Oral	IIa completed	NCT02002208
	Fevipiprant/QAW039	CRTH2 mAb	Oral	IIb completed	NCT01785602
Histamine receptor	ZPL-389	H4R inhibitor	Oral	II completed	NCT02424253
				II ongoing	NCT03948334
					NCT03517566
Neuropeptide substance P and neurokinin 1 receptor (NK1R)	Tradipitant/VLY-686	NK1R inhibitor	Oral	II completed	NCT02651714
				III completed	NCT03568331
				III ongoing	NCT04140695
	Serlopitant/VPD-737	NK1R inhibitor	Oral	II completed	NCT02975206
				III ongoing	NCT03540160

tant use of TCS exhibited an EASI-75 of 63% at week 16 in moderate-to-severe adult AD who were refractory or intolerant to cyclosporine (64). Translational studies reveal that dupilumab reduces expression of Th2 immunity markers, Th17/Th22-related epidermal hyperplasia, and inflammatory cell infiltrates. It also enhances the expression of genes that control epidermal differentiation and barrier function, including genes for loricrin and filaggrin (65). Two meta-analyses demonstrated statistically significant increased efficacy and a well-tolerated safety profile for patients with moderate-to-severe AD on dupilumab compared to placebo (66, 67).

Dupilumab-induced conjunctivitis, or ocular surface disease, is a common (5–28% of patients) but poorly understood side effect (68). The conjunctivitis is usually mild to moderate in severity and can be treated with various topical anti-inflammatory approaches. For unknown reasons, the conjunctivitis associated with dupilumab therapy only occurs in patients with AD. This side effect was not observed in studies of asthma or chronic sinusitis (24). Ongoing mechanistic studies will hopefully shed light onto the etiology of this adverse effect.

Overall, dupilumab appears to be a safe therapy suitable for long-term use. Dupilumab does not appear to be immunosuppressive and has not been associated with increased overall infection rates. Studies reveal significantly reduced risk of serious or severe infections and bacterial non-herpetic skin infections compared to placebo (69). Dupilumab appears to correct AD skin dysbiosis – perhaps the mechanism that explains the observed protection against skin infections (65). Vaccination responses are also not affected by dupilumab therapy (70). No laboratory monitoring is required as no end-organ damage has been observed (70, 71). Dupilumab was also recently approved by the FDA for moderate-to-severe asthma with eosinophilic phenotype or oral corticosteroid-dependent asthma and chronic rhinosinusitis with nasal polyposis that are also driven by type 2 cytokines (62). Pitrakinra (Aeroderm), a biologic that targets only IL-4, has been tested in a phase IIa trial. However, no results have been reported and the status of further development is unknown.

IL-13 antagonists. IL-13 plays an important role in allergic inflammation and is expressed in both acute and chronic lesions of AD (72). Like IL-4, IL-13 induces keratinocyte to produce CCL26, thereby causing an accumulation of eosinophil at the inflammatory lesion (73). Lebrikizumab, an anti-IL-13 mAb, at 125 mg dose every 4 weeks achieved an 50% reduction in EASI score (EASI-50) of 82% at week 12 as compared to a placebo group response of EASI-50 of 62% at week 12 for patients with moderate-to-severe AD with concomitant mandatory TCS use twice daily ($p = 0.026$) (74) in a placebo-controlled phase II trial (TREBLE). In a recent press release from a phase IIb trial, patients treated with lebrikizumab at the 125 mg dose every 4 weeks and at the 250 mg dose every 2 or 4 weeks showed significantly dose- and frequency-dependent improvements in EASI scores compared to placebo at 16 weeks (75). Tralokinumab, another anti-IL-13 mAb, showed significant improvement in EASI and IGA scores in a phase II study, particularly in patients with high serum biomarker levels of IL-13 activity (76). Heavy use of concomitant TCS likely diminished the effect size when compared to placebo. Patients reported improvement in QoL and pruritus, and there were no significant adverse effects. A phase III trial (NCT03131648) using tralokinumab monotherapy without TCS is underway to better evaluate its efficacy. Overall, IL-13 inhibitors appear to be well tolerated and show an acceptable safety profile with limited adverse events, including upper respiratory infections (URIs), nasopharyngitis, and headaches that are common but mild and self-limited (74, 76). Phase III data will be important to reveal whether conjunctivitis is an IL-13 class effect or is limited to only certain biologics targeting the pathway.

Inhibitors of the TSLP-OX40 axis. The TSLP-OX40 axis is also known to play an important role in initiating the Th2 allergic inflammatory response (77). Keratinocyte-derived TSLP activates dendritic cells to induce the production of Th2 immunity cytokines such as IL-4, IL-5, IL-13, and tumor necrosis factor (TNF)-α (19). IL-33 appears to amplify TSLP's effect of inducing expression of OX40 ligand on dendritic cells (78, 79). Tezepelumab (AMG157/MEDI9929), an anti-TSLP mAb, is regarded to be a potential suppressor of the Th2 pathway. In a phase IIa trial (NCT02525094), however, it did not show a significant EASI-50 response compared to placebo at week 12 in patients with moderate-to-severe AD, presumably due to heavy concomitant TCS use in the placebo group (80). In a phase IIa trial, GBR 830, an anti-OX40 mAb, was well tolerated and showed an acceptable safety profile, decreased inflammatory serum biomarkers, and significant improvement in EASI-50 versus placebo (81). In a phase I trial (NCT03096223), patients treated with KHK4083, an anti-OX40 mAb, every 2 weeks for 6 weeks showed a continuous reduction in EASI score even at week 22 suggesting a long-lasting response (82). An additional phase II trial (NCT03703102) is underway. Currently, there have been several proof-of-concept (PoC) trials testing various TSLP-OX40 axis-related inhibitors including a TSLP receptor antagonist MK-8226 (NCT01732510), an anti-IL33 mAb Etokimab (ANB020) (NCT03533751).

IL-31 receptor antagonists. Interruption of the itch-scratch cycle is one of the main goals in managing AD. IL-31, dubbed the "itch cytokine" is predominantly produced by activated Th2 cells and mast cells. The IL-31 receptor (IL-31R) is expressed on C-fibers of peripheral neurons (83). IL-31 is significantly increased in acute and chronic AD and plays a critical role in pruritus and disease activity (84). Nemolizumab, an anti-IL-31RA

mAb, showed a significant reduction in visual analogue scale (VAS) scores for pruritus in patients with moderate-to-severe AD in a 12-week phase II trial (85). In another long-term phase II trial, it showed significant and continued itch suppression and was well-tolerated over the 64 weeks trial with limited adverse events, including nasopharyngitis, AD exacerbations, and URIs (86). A recent phase IIb trial revealed that nemolizumab significantly improved EASI, IGA and itch scores at week 24 versus placebo and was well tolerated, with the 30 mg dose being most effective (87). BMS-981164, an anti-IL-31 mAb, was completed as a phase Ib trial (NCT01614756), but results have not yet been published. KPL-716 is an anti-oncostatin M receptor beta mAb (anti-OSMRβ) inhibiting both IL-31 and oncostatin M, an inflammatory signal implicated in pruritus, Th2 inflammation, and fibrosis. KPL-716 showed good safety and tolerability as well as an anti-pruritic effect in patients with moderate-to-severe AD in a phase Ia/Ib study (88). Additional phase II studies (NCT03858634, NCT03816891) for chronic pruritic diseases and prurigo nodularis are currently underway.

IL-5 antagonist. Eosinophils are speculated to play a large role in the pathogenesis of AD due to their high prevalence in tissue and blood found throughout the course of the disease. IL-5 induces the migration of eosinophils within inflamed tissue of patients with Th2 allergic inflammatory diseases like asthma and eosinophilic esophagitis (89). Mepolizumab, an anti-IL-5 mAb recently approved for severe eosinophilic asthma, was tested in a pilot study for AD but did not reach statistical significance in SCORing Atopic Dermatitis (SCORAD) score, pruritus scoring, and TARC levels despite decreasing the peripheral blood eosinophilic count (90). Given its efficacy in treating eosinophilic asthma, a phase II trial for moderate-to-severe AD had been implemented to test the effectiveness in the AD subtype with eosinophilia but was terminated early, as this study reached pre-determined futility criteria following interim analysis.

Targeting Th22 pathway

IL-22 promotes epidermal hyperplasia and disrupts barrier function by inhibiting keratinocyte differentiation and tight junction production (91). IL-22 is significantly increased in AD lesions and expression levels correlate with disease severity (60). In a phase II trial funded by the National Institutes of Health, fezakinumab, an anti-IL-22 mAb, did not reach significance in reducing the SCORAD score compared to placebo, but a sub-analysis of severe AD (SCORAD score >50) showed significant improvement with fezakinumab versus placebo (92). It was overall well-tolerated with a limited safety profile, including URIs as adverse events. A recent study revealed fezakinumab had a better efficacy in patients with a higher IL-22 baseline, suggesting an effect of IL-22 blockade on multiple inflammatory pathways encompassing Th1, Th2, Th17, and Th22 axis (93). Treatment antagonizing IL-22 could be a promising option amongst African American, Asian, intrinsic, and pediatric AD subtype patients showing dominant Th22 polarization and/or psoriasiform Th17/Th22 endotypes (25).

Targeting Th17 pathway

Some phenotypes such as Asian, intrinsic, pediatric, and elderly AD show higher expression of Th17-related markers like those found in psoriasis (25). Thus, these patients may be potential candidates for IL-17/IL-23 targeting therapies. IL-23 initiates both Th17 and Th22 pathways and is significantly decreased after AD treatments (94). The IL-17 family consists of 6 members of interleukins, IL-17A-F. Among them, IL-17A and IL-17C show complementary cooperation between keratinocytes and T cells, leading to the amplification of cell immune responses (95). Unlike IL-17A which is produced by Th17 cells and innate immune cells, IL-17C appears to be a keratinocyte-derived cytokine (96). Despite showing promise in several reports of AD (97, 98), ustekinumab, a mAb antagonizing IL-12/IL-23p40 with efficacy in psoriasis, did not demonstrate significant improvements over placebo with concomitant TCS use in a phase II trial for AD (99). In another phase II trial in Japan, patients with severe AD treated with ustekinumab 45 mg and 90 mg did not show meaningful efficacy versus placebo, although it was generally well-tolerated (100). MOR106, an anti-IL-17C mAb, exhibited an EASI-50 of 83% at week 4 at the higher dose and the treatment response maintained over 2 months after stopping treatment in a phase I trial (NCT02739009) (101). MOR106 and secukinumab, an anti-IL-17A mAb, are being tested for AD in phase II trials.

IgE antagonists

IgE is a hallmark for atopic diseases and is a downstream product of the Th2 axis. It is implicated in basophilic activation and the initiation of sensitization in allergic inflammatory cascades. IgE is also present on the cell surface of inflammatory dendritic cells (IDECs) characteristic of AD (102). Extrinsic AD subtypes defined by high levels of IgE and pediatric AD subtype with a tendency for atopic march early on in life may be good targeted candidates for anti-IgE drugs (25). However anti-IgE treatments in AD have shown largely negative results. Omalizumab is a recombinant humanized monoclonal IgG1κ antibody used in chronic spontaneous urticaria and asthma. Despite some case series demonstrating favorable efficacy for AD, omalizumab did not show improved efficacy over placebo in an RCT (103). A phase IV trial for severe pediatric AD was completed, but results have not yet been posted. In a phase II trial, patients treated with ligelizumab (QGE031), a high affinity anti-IgE Ab,

every 2 weeks for 12 weeks did not show a significant reduction in the severity for AD compared to placebo (104). The phase I trials using other anti-IgE agents, such as MEDI4212 (NCT01544348) and XmAb7195 (NCT02148744) have been completed, but show limited potential (105, 106). To date, anti-IgE approaches do not appear to have significant clinical activity in AD.

IL-1α antagonist

IL-1α, a prototypical pro-inflammatory cytokine, is an attractive target as its major reservoir appears to be keratinocytes, which may play a key role in the initiation of the inflammatory cascade found in AD (107). IL-1α also enhances matrix metalloproteinases activity, thereby leading to epithelial barrier breakdown (108). Bermekimab (MABp1) is a naturally derived human mAb that shows immunomodulating activity by blocking IL-1α activity. The drug failed in a phase III for colorectal cancer, but is now being evaluated for inflammatory skin diseases like hidradenitis suppurativa and AD. A phase II trial of 38 patients with moderate-to-severe AD revealed significant improvements at all clinical endpoints (109). Controlled studies are needed to better assess the potential of this novel therapy in AD.

JAK inhibitors

JAK inhibitors potentially have a wide application in inflammatory skin diseases including AD. JAK is a key mediator in signaling numerous cytokines involved in the pathogenesis of AD, including IL-4 and IL-13. Notably, IL-4 requires signaling through JAK1/3 while IL-13 signals through JAK1/TYK2 (110). The JAK-STAT pathway may play an important role in mediating both inflammation and pruritus in AD (40). Baricitinib is a potent oral JAK1/JAK2 inhibitor approved in the EU and the US for the treatment of rheumatoid arthritis. In a phase II trial, patients with moderate-to-severe AD showed significant improvements in EASI-50 at week 16, 61% (4mg) versus 37% (placebo) when treated with baricitinib in combination with TCS (111). Patients also reported tolerating the medication well with improvements in pruritus and sleep. Dose-dependent adverse events including headache, increased creatine phosphokinase, and nasopharyngitis were reported. Two phase III trials BREEZE-AD1 and BREEZE-AD2 confirmed significant clinical efficacy in both baricitinib doses of 2 mg and 4 mg with a good safety profile for patients with moderate-to-severe AD (112). A number of phase III trials for baricitinib that include combination therapy with TCS and longer-term endpoints are still being recruited. Upadacitinib (ABT-494), a selective oral JAK1 inhibitor, is currently underway in clinical trials for rheumatoid arthritis, Crohn's disease, ankylosing spondylitis and psoriatic arthritis. In a phase IIb trial, upadacitinib showed reduction in pruritus as early as week 1 and a significant dose-dependent improvement in EASI score at week 2 in patients with moderate-to-severe AD (113). Adverse events included URIs and AD exacerbations. Further phase III trials including younger patients with moderate-to-severe AD are also currently underway. In a phase IIb trial, abrocitinib (PF-04965842), a selective oral JAK1 inhibitor, showed dose-dependent improvement in EASI and IGA scores at week 12 versus placebo (40). The top-line results detailed in a press release of a phase III trial of abrocitinib showed statistically significant results with good tolerability and no unexpected safety events (114). Other phase III trials with long-term treatment periods are now being investigated. In a short-term clinical I trial (NCT03139981), ASN002 (Gusacitinib), a dual inhibitor of pan-JAK (JAK1-3, TYK2) and SYK, showed improvement in clinical severity at week 4 with a reduction in Th2/Th22 biomarkers (115). Another phase II trial with longer duration is still ongoing. Oral tofacitinib in a small open-label study showed impressive reductions in SCORAD with no adverse events (116).

PDE4 inhibitor

PDE4 inhibitor increases intracellular cAMP levels, leading to a down regulation of a number of cytokines involved in AD including IL-2, IL-5, IL-13 IL-17, IL-22, IL-31, and IL-33 (117). PDE inhibitor also upregulates the anti-inflammatory cytokine IL-10. Apremilast, an oral PDE4 inhibitor approved for psoriasis and psoriatic arthritis, showed promising results in an AD pilot study (118). However, in a phase II trial, apremilast showed no significant change in EASI score at week 12 at a dose of 30 mg compared to placebo. Although apremilast at a dose of 40 mg showed clinical efficacy and decreased Th17/Th22 related biomarkers, it was discontinued due to serious adverse event like cellulitis (119).

Chemoattractant receptor-homologous molecules expressed on Th2 lymphocytes antagonists

Chemoattractant receptor-homologous molecules expressed on Th2 lymphocytes (CRTH2) is a prostaglandin D2 receptor that is expressed on Th2 cells, eosinophils, and basophils. It stimulates the initiation of Th2 cell migration in the skin (120). Two PoC phase II trials for two CRTH2 antagonists, OC000459 (ODC-9101) and fevipiprant (QAW039) had been completed, but results did not demonstrate efficacy (121, 122).

Histamine receptor type 4 antagonists

Histamine (H) is a known itch-inducing mediator. Yet, the roles of H1 and H2 blockade in AD and AD-associated itching has been rather disappointing (123). Histamine receptor type 4 (H4R) is expressed on Th2 cells, Th17 cells, keratinocytes, and sensory neural cells. H4 stimulation also stimulates IL-31 production (124). JNJ-

39758979, an H4R antagonist, was terminated early in a phase IIa trial due to serious adverse events including agranulocytosis (NCT01497119) although it did show significant reduction in pruritus compared to placebo (125). In a phase II trial testing ZPL-389, another H4R antagonist, significant reductions in EASI and SCORAD scores were found at week 8 compared to placebo for patients with moderate-to-severe AD with concomitant use of TCS. However, there was no significant reduction in pruritus (126). Additional phase II trials of ZPL-389 are still ongoing.

Neuropeptide substance P and neurokinin 1 receptor antagonists

Neuropeptide substance P and neurokinin 1 receptor (NK1R), the receptor for substance P, is associated with AD disease activity (127). The NK1R antagonist prompts decreased scratching behavior in AD mouse models (128). In a PoC phase II trial for patients with AD and chronic pruritus, patients treated with oral tradipitant (VLY-686) for 4 weeks experienced a significant reduction in pruritus VAS from baseline ($p < 0.0001$) (129). A phase III trial for tradipitant is currently underway. In a phase II trial involving AD patients with severe pruritus, subjects taking oral serlopitant (VPD-737) for 6 weeks revealed numeric differences in pruritus scores compared to placebo. However, the differences were not statistically significant (130).

CONCLUSION

Despite its high prevalence worldwide, effective management of AD is complicated due to its multifaceted pathophysiology, variable clinical manifestations, and chronic course of the disease. The success of dupilumab in AD confirms the central importance of type 2 cytokines in the pathophysiology of AD. In addition to type 2 cytokines, certain phenotypes of AD may be driven by additional cytokine pathways. However, data to date attempting to specifically target cytokines outside of the type 2 axis have largely been unsuccessful. Broad acting JAK inhibition may help patients with AD that are driven by more complex cytokine endotypes. Further data using large-scale and longer-term clinical trials with proper outcome measures that assess signs, symptoms, quality-of life and long-term control as recommended by the HOME initiative (www.homeforeczema.org) are needed in order to create tailored and personalized treatments for AD. The results of studies for several other promising approaches targeting inflammation, the microbiome, itch, and PDE4 are eagerly awaited.

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REVIEW ARTICLE

Prevention of Atopic Dermatitis

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Despite advances in atopic dermatitis (AD) treatments, research into AD prevention has been slow. Systematic reviews of prevention strategies promoting exclusive and prolonged breastfeeding, or interventions that reduce ingested or airborne allergens during pregnancy and after birth have generally not shown convincing benefit. Maternal/infant supplements such as Vitamin D have also not shown any benefit with the possible exception of omega-3 fatty acids. Systematic reviews suggest that probiotics could reduce AD incidence by around 20%, although the studies are quite variable and might benefit from individual patient data meta-analysis. Skin barrier enhancement from birth to prevent AD and food allergy has received recent interest, and results from national trials are awaited. It is possible that trying to influence major immunological changes that characterise AD at birth through infant-directed interventions may be too late, and more attention might be directed at fetal programming *in utero*.

Key words: atopic dermatitis; atopic eczema; eczema; prevention.

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SIGNIFICANCE

Just like we can prevent infectious diseases like polio, it should be possible to prevent eczema (atopic dermatitis), food allergy and asthma. Most things that have been tried so far to prevent eczema including exclusive breastfeeding, timing of starting solids, supplements like Vitamin D and reducing house dust mite do not seem to work. Taking probiotics (friendly gut bacteria) during pregnancy probably reduces the risk of eczema by around 20%, although we are still not sure what combination is best. New research is trying to find out if special creams that make a baby's skin barrier stronger can prevent eczema.

Despite the familiar adage that "prevention is better than cure", prevention of atopic dermatitis (AD) has been a relatively neglected topic of research until recently. A PubMed search (using the terms [atopic dermatitis OR eczema] AND treatment (August 14th 2019) revealed 19,755 hits, compared with just 3,150 when disease terms were combined with "prevention". Reasons for lack of research could include a lack of interest in population-based research in favour of basic science (**Fig. 1**), lack of research skill capacity in prevention research, lack of funding and a limited choice of identifiable risk factors that are amenable to public health manipulation. However, the number of AD prevention studies has increased over the last 10 years, especially in the field of probiotics and interventions to enhance the skin barrier. Basic science discoveries into the human microbiome and genetics of AD may have played a part in contributing to this recent trend (1, 2). Whilst identifying risk factors that can be manipulated is an essential part of prevention research, understanding the mechanisms by which the effects of prevention are mediated is interesting but not essential. For example, the benefits of stopping smoking to prevent lung cancer became apparent from simple epidemiological research long before the mechanisms and precise carcinogens were discovered (3). Prevention of disease is arguably a much more logical and cost-effective way to manage the burden of a disease such as AD than focussing solely on drug treatment of sick individuals who seek medical help after a long chain of irreversible pathological events (**Fig. 2**). Whilst some drugs such as

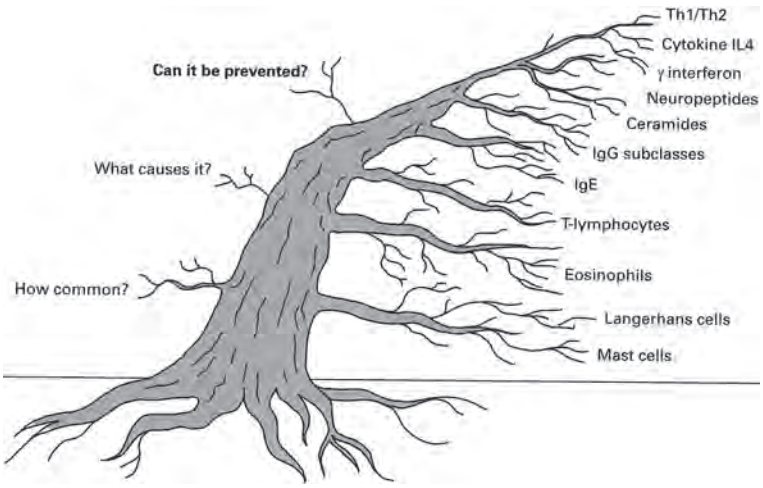

Fig. 1. A skewed interest toward cellular and molecular atopic dermatitis (AD) mechanisms relative to research into AD populations. Research into AD over the last 50 years has been dominated by interest in cells rather than broader questions such as whether disease prevention is possible.

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penicillin for streptococcal infection can be curative, most only modify rather than cure chronic diseases like AD, they are often expensive, and all are associated with potential adverse effects.

This article attempts to critically review the current state of science on the prevention of atopic dermatitis. Throughout this article, we will refer to the disease of interest as AD, which is synonymous with atopic eczema or just "eczema" (4). We use the term atopic dermatitis to describe the clinical phenotype, rather than the scientific definition of clinical phenotype plus evidence of IgE sensitisation to environmental allergens. We start by introducing the reader to key considerations when designing or critically appraising studies of AD prevention, using our direct experience in designing and running a randomised controlled trial (RCT) of emollients to prevent AD. We then explore the main interventions that have been used to try and prevent AD such as maternal and infant dietary restrictions or supplements, aeroallergen avoidance and approaches designed to enhance the external skin barrier. The authors have chosen to use systematic reviews of evidence and RCTs as the evidence source where possible. Systematic reviews were harvested from the Centre of Evidence-Based Dermatology international collection of systematic reviews which is updated monthly by a senior information scientist (Dr. Douglas Grindlay) (5). Rather than summarise all 102 systematic reviews on AD prevention in this collection, we instead refer to overviews of systematic reviews or the most recent and comprehensive systematic reviews where possible (6, 7). We used the Global Resource for Eczema Trials (GREAT) database for RCTs that might not yet be included in systematic reviews (8).

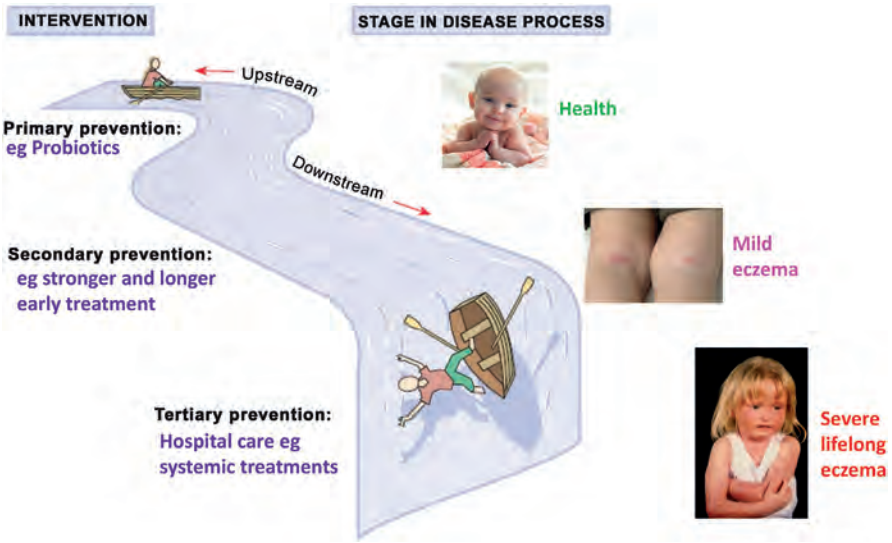

Fig. 2. Where is intervention most effective? Although the concept of prevention of atopic dermatitis is rarely discussed at international meetings, an upstream approach is a far more logical approach to reduce the burden of disease at a population level than the current approach of treating sick individuals with expensive drugs who present to secondary care after a long chain of pathological events.

SOME KEY BASIC CONSIDERATIONS

The power of prevention

Because prevention strategies act at a population level, their power is often not appreciated by individuals compared with treatments for a disease. Yet the power of prevention is potentially huge. In his article entitled "The power of prevention and what it requires" Woolf draws our attention to the fact that whereas new diabetes drugs that reduce glycohemoglobin levels by 0.5% often make the headlines, exercise, that can lower the incidence of diabetes by 50%, rarely achieves such publicity (9). The conquest of many infectious diseases such as diphtheria, smallpox, polio and measles are testament to the power of prevention, yet individuals who would have contracted these diseases are seldom "grateful" to those developing and implementing vaccines as it is unclear who would have contracted the disease in the first place. The recent re-emergence of measles due to misguided beliefs about vaccine safety, termed "vaccine hesitancy", are timely reminders of the "invisible" and powerful effects of population-based interventions (10).

Primary, secondary and tertiary prevention

Primary prevention typically refers to intervening before health effects occur. Secondary prevention implies detecting a disease at an early stage to prevent worsening, whereas tertiary prevention is the reduction of symptoms or improvement in quality of life of those with established disease – i.e. where health care professionals normally operate (11).

Application of the Participant, Intervention, Comparator and Outcomes framework to atopic dermatitis prevention studies

Participant, Intervention, Comparator and Outcomes (PICO) is a framework used in evidence-based medicine to understand the structure of RCTs and is useful when considering the design and critical appraisal of AD prevention trials (12).

Participants. Most AD prevention studies target a high-risk population e.g. babies born to families with a first-degree relative with AD or associated allergic diseases such as asthma, hay-fever or food allergy. The advantage of this approach is that parents who have experienced AD themselves or witnessed it in family members are often highly motivated (during pregnancy or soon after) to undertake interventions that could prevent AD in their new

baby. The disadvantage is that if the selected population is too narrow, the intervention may have a limited overall population impact. However, tackling an entire population such as all newborns is challenging, especially if the behaviour change modification is substantial, as parents will be less motivated to act on something that will be of little perceived benefit to their child. This phenomenon is known as the prevention paradox – a term coined by Rose to denote "a measure that brings large benefits to the community offers little to each participating individual" (13). **Fig. 3** illustrates the possible trade-off between high and low risk approaches to AD prevention suggested previously (14).

Intervention. An essential step in the prevention of any disease is a thorough knowledge of risk factors that can be manipulated. For example, filaggrin gene mutations cannot be directly manipulated *in utero* at present (although it may be possible in time) whilst a reduction in house dust mite in the home environment is achievable. Another key consideration is the acceptability of interventions given that healthy people are being asked to undergo elaborate changes to their lives in order to prevent disease in a proportion of people – the identity whom will remain unknown to them. Here, there is often a trade-off between intensity of intervention which might achieve a larger effect (such as applying emollient twice a day to their child for 2 years, wash only in soft water and use no soap) versus those that are likely to have wider population reach (such as advice to use emollients once daily for the first year of life as in the BEEP trial) (15). Testing acceptability of interventions is essential before proceeding to full scale evaluation (16). Assessing safety is paramount in prevention studies. Whilst individuals with severe AD might accept the risk of nausea and liver disease from methotrexate therapy, healthy individuals will have a low threshold for rejecting interventions with even small risks, such as the slipping on emollients spilt on a bathroom floor. Furthermore, minor adverse effects such as transient stinging after emollient application can reduce adherence to an intervention.

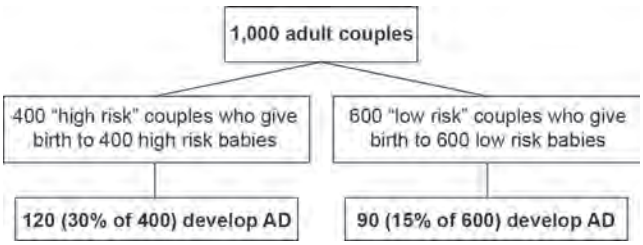

Fig. 3. Hypothetical example of the prevention yield from a high risk vs low risk prevention approach for atopic dermatitis. Depicts an average Western population where 40% of 1,000 adult couples have a strong family history of atopy and 60% do not. If 30% of the high risk babies develop AD compared with 15% without such a family history, a high risk approach would only prevent 57% (120/120+90) of AD cases at a population level. Adapted from Williams HC. Atopic Dermatitis. In: Williams HC, Strachan DP (eds). The Challenge of Dermato-Epidemiology. Boca Raton, CRC Press Inc., 1997.

Comparator. In the absence of a clear reference standard of an effective active treatment, control interventions for AD prevention trials are typically "standard care" (which is often not defined), an attention control, or some form of placebo (e.g. inactive probiotics). Convincing parents with a family history of AD to take part in a study with a 50:50 chance that their new baby will be allocated to the "no treatment" group can be challenging, and unless equipoise is carefully explained, parents may drop out if they don't get the "new active" intervention. Feasibility studies that test randomisation and retention are essential and offer the opportunity to develop patient information materials with patients that imply active monitoring and altruistic rewards to overcome the notion of "control neglect" that can result in resentful demoralisation (17).

Outcomes. Whereas clinical trials of people with AD (prevalent cases) seek to reduce disease severity, one is trying to prevent new (incident) cases from developing in a prevention study. There is a lack of research on defining an incident case of AD. Simpson et al. (18) undertook a systematic review of definitions of an incident case of AD used in prevention studies. Of 102 included studies, 27 did not define an incident case, 28 used the Hanifin & Rajka criteria (19), and 21 used definitions unique to that study without referencing the source. It is important to note that "chronic relapsing course" (a major criterion for the Hanifin & Rajka criteria), whilst acceptable for measuring cumulative incidence, is problematic when defining a new case which, by definition, has not yet become chronic. Yet diagnosing AD confidently in a baby on the first day they develop an eczematous rash is also fraught with problems as transient irritant eczematous dermatoses (which are probably not true AD) are common in infancy. Simpson et al. (20) suggested a compromise whereby the UK refinement of the Hanifin & Rajka criteria are used to denote a continuous or intermittent itchy skin condition lasting at least 4 weeks.

Ideally outcome assessment should be separated from the intervention period by a clear margin to separate treatment effects from prevention effects. For example, in the two small preliminary studies that suggested emollients might prevent AD, outcomes were assessed at the end of the intervention period, making it difficult to assess whether the apparent benefit was due to emollients preventing AD or actively treating new mild AD (16, 21). This is why the main BEEP trial of emollients used during the first year is assessing the primary outcome of AD (those fulfilling the UK refinement of the Hanifin & Rajka criteria in the last year) at the age of 2 years (15). Whilst complete prevention of disease is the ultimate goal, prevention of more severe forms of the disease (which cause the most morbidity and result in most healthcare usage) is also an important goal in AD prevention trials. Because the shape of AD prevalence in any population is skewed to the left (**Fig. 4**), even small shifts in the reduction of population severity can result in large gains in absolute terms for the number switching from severe to moderate or mild to very

mild/subclinical disease. Time to onset of AD is another outcome that can be considered although it is debatable whether simply delaying onset of a miserable disease to an older age is really a bonus. Given that AD is closely related to other "atopic" diseases such as food allergy, asthma and hay fever, AD prevention studies also need to evaluate whether benefits are seen in these diseases too. Measuring other atopic diseases present their own challenges, e.g. true food allergy has a low incidence making it unlikely that beneficial effects will be precisely measured even in large studies, and conditions like asthma have a later age of onset adding to the cost of following up individuals from RCTs that start at birth to older ages.

Reducing bias. In addition to standard approaches to reduce RCT biases such as registration of study protocols before recruitment starts and ensuring randomisation is truly random and concealed, two biases require special consideration in AD prevention trials. The first is performance bias which results from treating intervention and control groups differently. More attention given to the intervention group can result in different ancillary behaviours that can affect AD risk, so it is important that both groups are treated in the same way in terms of regularity of contact and incentives from the research team, and any post-randomisation behaviours that could confound the study result are recorded. Sometimes such behaviours can include contamination of the intervention in the control group (because they think they are missing out on something beneficial), which can be a particular problem if the intervention is something that can be easily accessed by participants without the need for healthcare professionals, such as reduction of house dust mites in the home. Contamination should therefore be measured and explored in the analysis. A second challenge lies in the fact that because many interventions such as emollient application or installing a water softener cannot be blinded, it is essential to include some form of objective outcome assessment (e.g. visible eczema recorded by investigators blinded to intervention status) to mitigate the risk of information bias. Studies should present findings as absolute risk reductions as well as the more impressive sounding relative risk reductions in order to provide a more realistic indicator of population benefit.

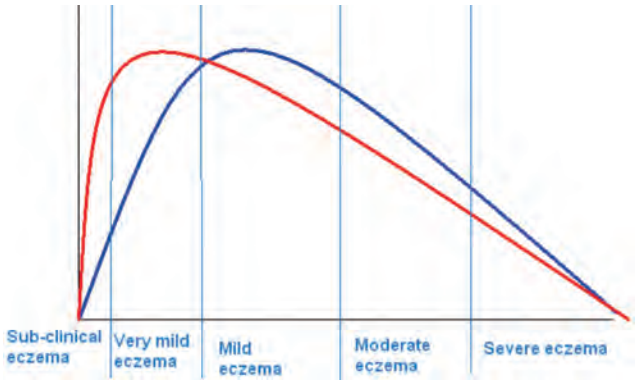

Fig. 4. Schematic representation of atopic dermatitis severity (x-axis) versus number with atopic dermatitis in two hypothesized populations. Even if atopic dermatitis cannot be prevented completely, shifting the population severity distribution of disease to the left (red curve) could have a huge impact on pushing more into subclinical disease and reducing the absolute proportion with severe disease who suffer the most and who consume most health resources.

THE EVIDENCE

Primary prevention

The 2011 overview of systematic reviews of primary prevention. In an attempt to reconcile the increasing number of Cochrane and non-Cochrane systematic reviews on AD prevention, a group (including the two authors) undertook an overview of all such systematic reviews in 2011 (search date up to August 2010). Quantitative and qualitative methods were used to collate and combine data where possible using Cochrane methods. Included reviews had to include some quantitative data that could be combined, search date within the last 5 years, and included participants between the ages of zero and 18 years. Seven systematic reviews containing 39 RCTs and 11,897 participants met the inclusion criteria. All 7 reviews were considered methodologically sound, although the data from the review on probiotics had to be re-analysed as data from one trial had been included more than once in the same meta-analysis. Interventions included use of hydrolysed formula milk (extensive and partial), extended duration of exclusive breastfeeding, dietary supplementation with omega-3 and omega-6 oils, maternal dietary antigen avoidance during pregnancy, lactation or both, soy formula milks, along with prebiotics and probiotics. Participants were from a mixture of high and lower risk families, although risk was rarely adequately defined. None of the pooled interventions showed clear evidence of benefit for AD prevention. A subgroup analysis of those at high risk of developing AD based on just one RCT found that prebiotics (ingested substances that favour the growth of beneficial bacteria in the gut) decreased AD incidence by 58% (RR: 0.42; 95% CI: 0.21, 0.84) compared with no prebiotics. Data on whether those developing AD were truly atopic was missing from most of the studies, and in those that did, there was no evidence that the interventions decreased atopy. One non-randomised study suggested that prolonged exclusive breastfeeding (at least 6 months) reduced AD incidence by 60% (RR 0.40, 95% CI 0.21 to 0.78). Despite the lack of any convincing signals for any of the interventions tested, the risk estimates for most interventions had low precision, indicating that some interventions with no evidence of benefit could still be useful.

The post 2011 overview era

Interventions that are ingested by mothers and/or infants. Also known as the "inside out" approach, ingested maternal/infant interventions include exclusive breastfeeding,

delay or early introduction of foods other than milk, dietary restrictions, and dietary supplements. Although breastfeeding (exclusive or prolonged) has clear benefits for infants, a systematic review of 16 moderate quality observational studies suggests that it does not appear to be protective of AD (22). One large cluster RCT (the PROBIT trial in Belarus) that promoted breastfeeding found a reduction in self-reported flexural eczema but not lung function, a finding that needs to be replicated (23). Around a half of milk feeding studies have been judged to be at high risk of bias (24). A Cochrane review of 5 trials failed to show any benefit of maternal avoidance of allergenic foods for AD prevention (25). A 2019 systematic review of mainly observational studies of complementary feeding (whereby other foods and drinks complement human or formula milk) found no clear evidence between the age at which complementary feedings is started and the risk of AD, food allergy or asthma (moderate evidence) (26). The same review found limited to strong evidence that introducing allergenic foods in year one of life to try and induce tolerance does not increase AD or food allergy risk, but may prevent egg and peanut allergy. The one well-conducted RCT included in the review found no benefit for AD prevention from early introduction of allergenic foods (27).

Interest in vitamin D supplementation as a possible preventative intervention stems from the association between low vitamin D levels and increased incidence and severity of AD. Vitamin D is also known to have a regulatory influence on skin barrier function and the immune system and skin barrier function, both of which are involved in AD development (28). A 2017 systematic review (search date January 2016) found one RCT and 3 non-RCTs that addressed vitamin D supplementation in women and children as a means of preventing allergic diseases found no clear evidence of benefit but with low certainty of evidence (29). A more recent and well conducted RCT found no clear benefit of infant vitamin D supplementation in the primary prevention of AD (30). A systematic review of omega-3 long-chain polyunsaturated fatty acids (such as from fish) intake during pregnancy found mixed results for AD prevention from observational studies, but a possible protective effect in the 3 included RCTs for early onset AD (31).

The evidence that ingested probiotics (non-pathogenic live bacteria or yeasts that can restore a dysfunctional pro-inflammatory gut microbiome) or prebiotics (non-digestible food ingredients that encourage beneficial bacteria to thrive) or both (synbiotics) can prevent AD is gathering momentum (32). The field is complicated as probiotics and prebiotics refer to a very wide range of ingredients, and they can be given to the mother during pregnancy, during lactation, to the infant after birth and various combinations of these and for different periods, leading to considerable heterogeneity which impacts on the ability to combine studies. One systematic review exploring the possible health benefits of yoghurt consumption among infants and toddlers that included two older cohort studies suggested a possible benefit for AD prevention, and called for new studies that evaluated such foods in a more contemporary setting (33). A systematic review in 2019 of 22 pooled trials published between January 2008 and May 2018 showed a reduction in AD incidence (RR 0.81, 95% CI: 0.70–0.93) for those receiving probiotic supplementation during pregnancy and/or infancy. Subgroup analysis suggested that benefits were strongest for those receiving *Lactobacillus* and *Bifidobacterium*, for those in whom probiotic supplementation occurred during pregnancy and infancy and in preventing AD developing in the first two years of life rather than later (34). Sources of study heterogeneity was also assessed and found to be mainly accounted by follow-up time (I^2 62.7%) and length of probiotic supplementation (I^2 53.5%). A more extensive systematic review that pooled 28 studies (27 good quality RCTs and one high quality cohort study, search date from inception to March 2018) showed a beneficial effect on AD prevention for probiotics compared with controls (OR 0.69; 95% CI 0.58–0.82, **Fig. 5**) (35). Analysis of studies whereby probiotics were provided only prenatally or postnatally did not show such benefit, prompting the authors to conclude that benefits are only realised when probiotics are started during pregnancy and continued in the infant for the first 6 months of life. A broader and high-quality systematic review of diet during pregnancy and infancy arrived at similar conclusions regarding a protective effect of probiotics on AD development from 19 probiotic trials (risk ratio 0.78; 95% CI 0.68–0.90; I^2 61% and an absolute risk reduction of 44 cases per 1,000; 95% CI 20–64) (24). Subgroup analysis suggested that it was maternal rather than infant probiotic supplementation that was important for realising a protective benefit. The evidence of prebiotics alone was weak due to high risk of bias, inconsistency, imprecision, and indirectness of study results.

Although the World Allergy Organisation guideline panel has determined that there is a net benefit of probiotics for AD prevention, concerns regarding the heterogeneity of studies remains (36). A comprehensive review of probiotics across all human diseases concluded that the evidence for benefit in allergic diseases was still uncertain and a stimulus for further studies rather than firm clinical recommendations (37). A high-quality individual patient data (IPD) meta-analysis – a type of systematic review that gathers and combines data belonging to individual patient who take part in clinical trials rather than aggregate data – would better identify who benefits most from probiotics, when and why (38).

Interventions directed at the external skin surface. The main "outside in" approaches for preventing AD, sensitisation and food allergy have included attempts to reduce airborne allergens such as house dust mite at the time of birth, increasing exposure to an anthroposophic environment and measures to enhance the skin barrier. A systematic review of house dust mite avoidance strategies (alone or

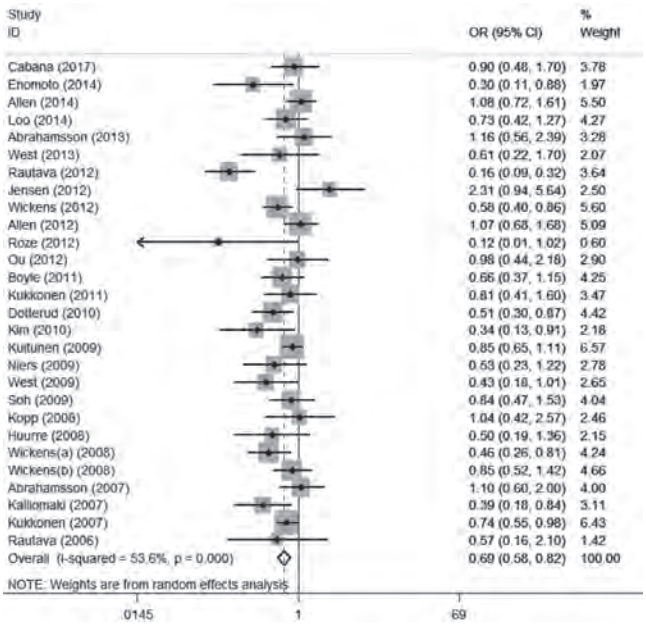

Fig. 5. The preventive effect of probiotics in atopic dermatitis. Forest plot depicting a meta-analysis that used a random effects model combining 28 evaluated studies. Although the summary odds ratio (OR) suggests clear benefit (OR 0.69; 95% confidence interval (CI) 0.58–0.82; $p < 0.0001$), there was considerable heterogeneity between the studies ($I^2 = 53.6\%$) (33). Reproduced with kind permission from the American Journal of Clinical Dermatology.

with allergen avoidance) that included 7 RCTs (search date October 2014) concluded such modalities do not decrease the risk of developing AD. Studies that have found strong associations between early exposures to anthroposophic environments such as farm animals have been limited to observational studies so far, but are a fruitful source of ideas for new possible primary interventions (39). Since the discovery of a strong association between AD and loss-of-function mutations in *FLG*, the gene encoding filaggrin – an essential protein for healthy skin barrier function, interest has increased on the potential benefits of skin barrier enhancement as a means of preventing AD and food allergy (40). Impaired skin barrier may precede eczema development and may be the route by which sensitisation to food allergens occurs (41, 42). Stimulated by the results of two small pilot RCTs that suggested a large benefit from using emollients on the skin of infants born to families with atopy, two large prevention RCTs have been set up to test the hypothesis that emollients from birth can prevent AD (15, 16, 21, 43). The first of these studies (Barrier Enhancement for Eczema Prevention (BEEP) trial) is investigating daily emollient for the first year of life in babies born to atopic families. The second, the Preventing Atopic Dermatitis and Allergies in children study (PreventADALL), is a factorial trial – a trial whereby two or more interventions are carried out and assessed simultaneously. The PreventADALL trial compares (*i*) no intervention with (*ii*) skin care (oil-bath at least 5 days/week to age 9 months) and (*iii*) consecutive introduction of allergenic foods (peanut, milk, wheat, and egg) between 3 and 4 months of age and (*iv*) both skin and complementary feeding strategies. Results of BEEP and PreventADALL are not available at the time of writing. Two trials were published in 2019, both of which used complex emollients containing ingredients such as ceramide designed to enhance the skin barrier (44, 45). The first study suggested that emollient therapy may reduce AD incidence, but this was not statistically significant, and there was no effect of emollient on barrier measurements (46). The second larger study was a factorial trial of emollient and synbiotics and found no evidence of a protective effect of either intervention (44). At least 10 other similar prevention trials that explore the potential of different skin barrier products to prevent AD in high and low risk populations (46). Together, most of these studies now form part of a prospectively-planned meta-analysis consortium called SCiPAD (Skin care intervention for prevention of atopic disease) (47, 48). Other direct to skin approaches such as "probiotic creams" that serve to influence the early skin microbiome towards one that is less favourable for the development of AD are also worthy of further research (49).

Combined approaches. Whilst it might be easier to implement one simple intervention to prevent AD, it might be possible to combine multiple interventions each of which has a small beneficial effect, especially if they interact to produce more than the sum of the whole. The hazard of a "throw in everything that might work" strategy is that they can become black boxes that are not amenable to replication, unless the components are separated using designs such as factorial trials as currently being done in the PreventADALL study (50).

Secondary prevention

Treating AD more aggressively when it first appears in an attempt to alter the subsequent course of disease in terms of remission or decreasing severity is an attractive notion. One such study of aggressive early treatment is underway in Japan, in which 650 infants who develop AD between the ages of 7–13 weeks old will be randomly assigned to enhanced topical anti-inflammatory treatment or conventional treatment with the aim of preventing food allergy and reducing AD severity (51). Poorly controlled disease resulting in skin damage from scratching can lead to a cascade that results in individuals developing autoimmunity towards their own skin components, a phenomenon that might be key to driving disease chronicity (52). Other non-pharmacological approaches such as behavioural methods to limit skin damage from scratching when AD first appears are also worth considering in this context (53). Like primary prevention, secondary prevention should not be taken lightly, especially with regards to safety. If for example, only 10% of those given early aggressive treatment with prolonged topical corticosteroids benefit from such therapy, then 90% arguably undergo "overtreatment" and incur side effects in order to benefit the few.

So far, prevention of related diseases such as food allergy and asthma have only been considered in the context of early interventions that primarily aim to prevent AD, but another important question to consider in relation to secondary prevention of AD is whether interventions that are initiated when AD is first identified can prevent the development of conditions such as asthma. Such a concept was the basis of the Early Treatment of the Atopic Child study (ETAC) whereby 795 children with new onset AD between 1 and 2 years of age were randomised to cetirizine or placebo for 18 months. Cetirizine was chosen because it might inhibit eosinophil tracking to the lungs as well as its anti-histamine effect. The ETAC study did not show that asthma could be prevented by such an approach (54). Although urticaria rates were less in the intervention group, severity of AD was not reduced in the cetirizine group either, throwing doubt on the value of anti-histamines in the treatment of AD – an observation that has been confirmed in a subsequent Cochrane review (55, 56). A follow-up RCT from ETAC called the EPAAC study explored the use of levocetirizine for the prevention of asthma in children with AD who were sensitised to grass and/or house dust mite was stopped due to lack of benefit (57).

Tertiary prevention

In its broadest sense tertiary prevention refers to disease treatment, prevention of deterioration, disease complications and sequelae. In relation to AD, one of the most important advances in disease treatment over the last 30 years has been the concept of proactive treatment (two consecutive days per week) for those who have been stabilised. This has been shown to dramatically reduce the number of subsequent flares (58). A meta-analysis by Schmitt et al. showed that topical fluticasone reduced the risk of further flares by around half (relative risk 0.46, 95% CI 0.38–0.55) with more modest reductions in flares with weekly topical tacrolimus (RR 0.78, 95% CI 0.60–1.00) (59). When considering prevention of flares, it is equally important to consider induction of remission before proactive therapy is initiated – the concept of "get control then keep control" as illustrated schematically in **Fig. 6** (60). Another review suggested that Vitamin D supplementation for early disease may have a small beneficial effect in reducing later disease severity (61). Given that AD is a chronic relapsing condition, prevention of flares and embracing the concept of overall disease control have become key considerations in improving quality of life of AD sufferers (62). Better prediction of flares in what often appears a random process offers exciting prospects for personalised medicine.

What about adult-onset atopic dermatitis?

Most of the evidence discussed relates to early life. This is with good reason as AD typically starts in the first few years of life. Recent studies have drawn attention to the importance of AD in adults, pointing out that around one in 4 of those with adult AD appear to develop it for the first time in adulthood (63). Less is known about the risk factors for adult-onset AD in order to identify candidates for prevention studies (64). One study of 67,643 US women postulated that niacin intake might protect against adult AD since niacin has been found to decrease transepidermal water loss. Instead, it found that adult AD was paradoxically increased with niacin intake, a finding that needs to be replicated (65).

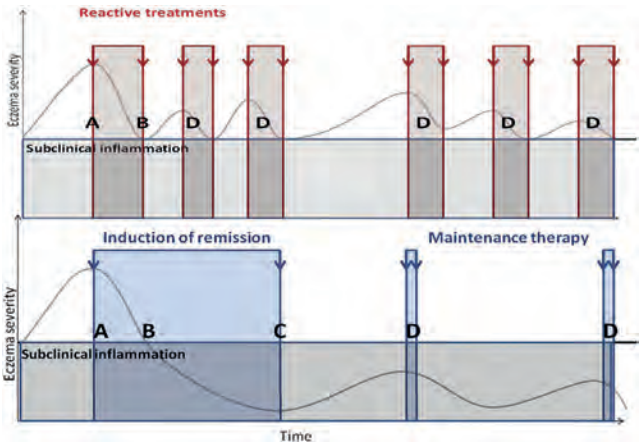

Fig. 6. The concept of getting control then keeping control in atopic dermatitis. A more subtle interpretation of tertiary prevention is the principle of inducing remission of atopic dermatitis with an initial blast of topical treatment followed by prevention of disease flares with weekly pulses of two consecutive days of topical treatment (also known as the Centre of Evidence-Based Dermatology "get control and keep control" approach). When contrasted against more traditional reactive approaches, the proactive approach results in more disease being pushed into a subclinical state and hence better overall disease control. Reproduced with kind permission from the Journal of Allergy and Clinical Immunology.

CONCLUSIONS

The last few decades of research into the prevention of AD have thrown up very few signals of simple, safe interventions that are likely to be effective at a population level. Errors in the design and reporting of studies tend to be repeated rather than learned, and the same old interventions are often tested again and again with little new insight. Past research has also been concerned with a rather fruitless obsession with allergic factors despite the fact that around half of people with "atopic" dermatitis are not atopic in the scientific sense (66). The main exception to the lack of positive findings for AD prevention has been the use of probiotics. Probiotic use has consistently shown modest benefit and good safety when tested in different populations around the world, prompting the World Allergy Organisation guideline panel to determine that there is a likely net benefit from using probiotics resulting primarily from prevention of eczema. The WAO guideline panel suggests using probiotics in: (*i*) pregnant women at high risk of having an allergic child; (*ii*) women who are breastfeeding infants at high risk of developing allergy; and (*iii*) infants at high risk of developing allergy. New evidence is likely to emerge on barrier enhancement as a strategy for AD

prevention over the next 5 years, especially through the SCiPAD prospectively planned meta-analysis.

In terms of future research, it is worth exploring new risk factors rather than doing more studies on the same interventions that do not look promising. The comprehensive overview of systematic reviews of epidemiology of allergic diseases conducted by Genuniet et al. (67) is a good place to start and by reconsidering the host of non-specific, specific and internal factors that make up the "exposome" for AD (67, 68). Rather than considering reduction of harmful exposures, exploration of increasing potentially beneficial substances might be considered. Given the inverse relationship between helminth exposure and allergic sensitisation, derivative products that switch off the dysfunctional immune response could be explored further (69). The foetal environment may be a better place to focus than the infant environment. Rather than conducting more probiotic trials, stopping and conducting a more refined analysis of the 28 or so existing studies using individual patient data meta-analysis may help to bridge the gap between cautious recommendation and implementation in order to benefit future generations of children who might otherwise be destined to a life with AD.

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