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Frontiers in Dermatology and Venereology

Centenary theme issues in Volume 100 of *Acta Dermato-Venereologica*
An overview

Current issue

Genodermatoses
Theme editors: Anette Bygum and Matthias Schmuth

- An Early Description of a "Human Mosaic" Involving the Skin: A Story from 1945, *R. Happle*
- Spectrum of Genetic Autoinflammatory Diseases Presenting with Cutaneous Symptoms, *H. Bonnekoh, M. Butze, T. Kallinich, N. Kambe, G. Kokolakis, K. Krause*
- Dental Manifestations of Ehlers-Danlos Syndromes: A Systematic Review, *I. Kapferer-Seebacher, D. Schnabl, J. Zschocke, F. M. Pope*
- Legius Syndrome and its Relationship with Neurofibromatosis Type 1, *E. Denayer, E. Legius*
- Diagnosis and Management of Inherited Palmoplantar Keratodermas, *B. R. Thomas, E. A. O'Toole*
- Molecular Genetics of Keratinization Disorders – What's New About Ichthyosis, *J. Uitto, L. Youssefian, A. H. Saeidian, H. Vahidnezhad*
- Genetics of Inherited Ichthyoses and Related Diseases, *J. Fischer, E. Bourrat*
- Ichthyosis: A Road Model for Skin Research, *A. Vahlquist, H. Törmä*

Forthcoming issues

Cutaneous and genital infections
Theme editors: Roderick Hay and Kristian Kofoed

- Skin Infections Caused by *Staphylococcus aureus*, *P. Del Giudice*
- A Hundred Years of Diagnosing Superficial Fungal Infections: Where Do We Come From, Where Are We Now and Where Would We Like To Go? *Y. Gräser, D. M. L. Saunte*
- The Management of Scabies in the 21st Century: Past, Advances and Potentials, *C. Bernigaud, K. Fischer, O. Chosidow*
- Skin Disease in the Tropics and the Lessons that can be Learned from Leprosy and Other Neglected Diseases, *R.J. Hay*
- The Changing Spectrum of Sexually Transmitted Infections in Europe, *A. Stary*

Skin malignancies
Theme editors: Veronique Bataille and Nicole Basset Seguin

- Melanoma Genomics, *J. Newton-Bishop, D. T. Bishop , M. Harland*
- Update in the Management of Basal Cell Carcinoma, *N. Basset-Seguin, F. Herms*
- Melanoma Risk and Melanocyte Biology, *J. U. Bertrand, E. Steingrimsson, F. Jouenne, B. Bressac-de Paillerets, L. Larue*
- Melanoma Epidemiology and Sun Exposure, *S. Raimondi, S. Gandini*
- Biomarkers Predicting for Response and Relapse with Melanoma Systemic Therapy, *S. J. Welsh, P. G. Corrie*
- Cutaneous Melanoma – A Review of Systemic Therapies, *K. A. Lee, P. Nathan*
- It's Not all Sunshine: Non-sun-related Melanoma Risk-factors, *V. Bataille*
- Update of the Management of Cutaneous Squamous-cell Carcinoma, *E. Maubec*

Atopic dermatitis
Theme editors: Magnus Lindberg and Carl-Fredrik Wahlgren
Content details TBA

Previous issues

Itch and pruritic disorders
Theme editors: Elke Weisshaar, Tasuku Akiyama and Jacek Szepietowski

- The Challenge of Basic Itch Research, *E. Carstens, T. Follansbee, M.I. Carstens*
- Mechanisms and Management of Itch in Dry Skin, *C. Sagita Moniaga, M. Tominaga, K. Takamori*
- Non-dermatological Challenges of Chronic Itch, *A.E. Kremer, T. Mettang, E. Weisshaar*
- Itch and Psyche: Bilateral Associations, *R. Reszke, J.C. Szepietowski*
- A New Generation of Treatments for Itch, *E. Fowler, G. Yosipovitch*
- Challenges in Clinical Research and Care in Pruritus, *M.P. Pereira, C. Zeidler, M. Storck, K. Agelopoulos, W.D. Philipp-Dormston, A.G.S. Zink, S. Ständer*

Psoriasis
Theme editors: Lone Skov and Enikö Sonkoly

- Psoriasis and Genetics, *N. Dand, S. Mahil, F. Capon, C.H. Smith, M.A. Simpson and J. Barker*
- The Woronoff Ring in Psoriasis and the Mechanisms of Postinflammatory Hypopigmentation, *J. Prinz*
- Psoriasis and Treatment: Past, Present and Future Aspects, *C. Reid, C.E.M. Griffiths*
- Psoriasis and Co-morbidity, *M. Amin, E.B. Lee, T-F. Tsai, J.J. Wu*
- Pustular Psoriasis: the Dawn of a New Era, *H. Bachelez*

Blistering skin disorders
Theme editor: Kaisa Tasanen

- Skin Fragility: Perspectives on Evidence-based Therapies, *L. Bruckner-Tuderman*
- Collagen XVII Processing and Blistering Skin Diseases, *W. Nishie*
- Drug Development in Pemphigoid Diseases, *K. Bieber, R.J. Ludwig*
- Current Concepts of Dermatitis Herpetiformis, *T. Salmi, K. Hervonen*
- Bullous Drug Reactions, *M. Mockenhaupt*

GENODERMATOSES

Theme Editors:

Anette Bygum and Matthias Schmuth

TABLE OF CONTENTS

REVIEW ARTICLE

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

Rudolf HAPPLE
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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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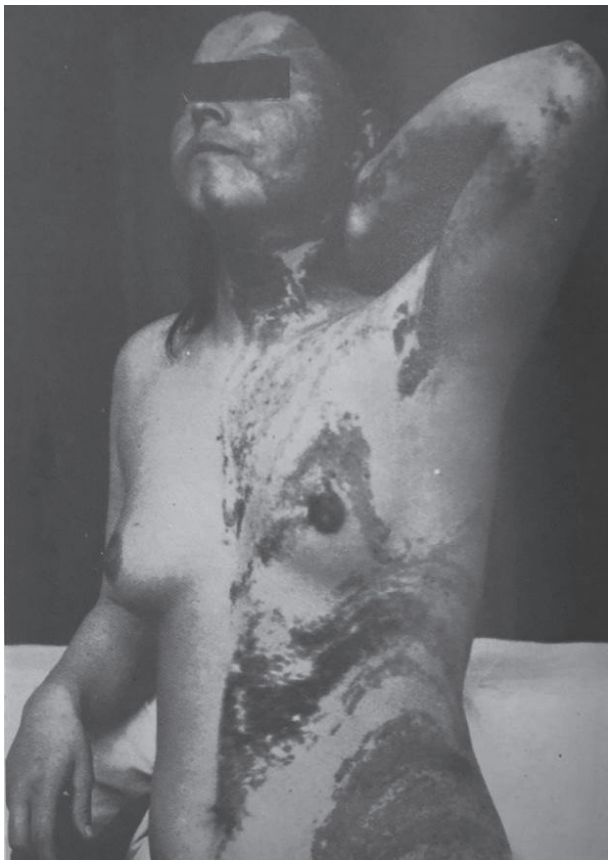

Fig. 1. A 24-year-old woman with systematized linear epidermal naevus, described by Zlotnikoff as "naevus pigmentosus pilosus et mollusciformis unilateralis", with a sharp separation down the midline (1). (Reproduced with permission from the American Genetic Association, USA, and Oxford University Press,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

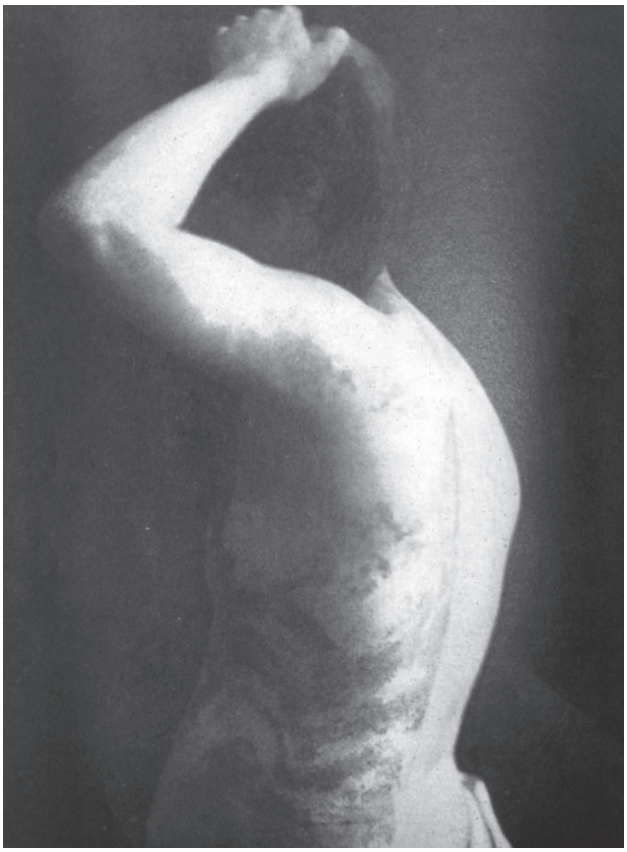

Fig. 2. The systematized linear lesions on the patient's back, likewise show an exact midline separation. (Reproduced with permission from the American Genetic Association, USA, and Oxford University Press, UK).

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-

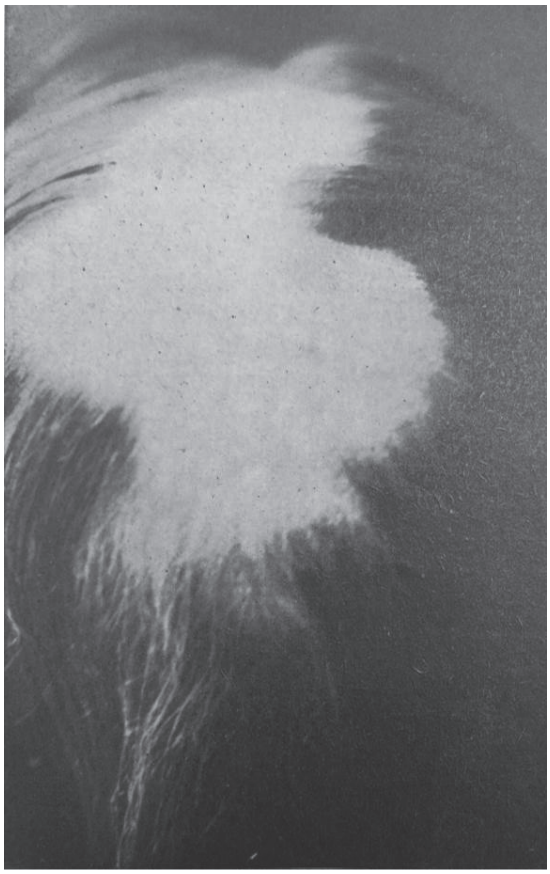

Fig. 3. Hair is lacking to the left of the midline, though part of the left side of the skull is unaffected. There is also a bald area above and back of the ear. (Reproduced with permission from the American Genetic Association, USA, and Oxford University Press, UK).

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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Centenary theme section: GENODERMATOSES

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).

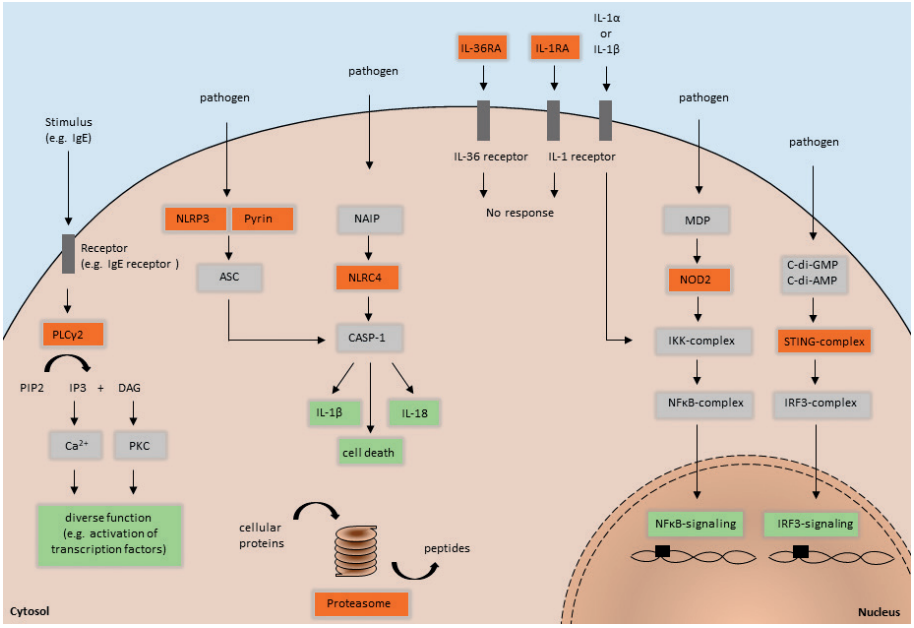

Fig. 1. Schematic representation of innate immune pathways and related pathomechanisms of the described autoinflammatory diseases. *Red squares* highlight the position of mutations associated with PLCG2-associated antibody deficiency and immune dysregulation (PLCγ2), autoinflammation and PLCG2-associated 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

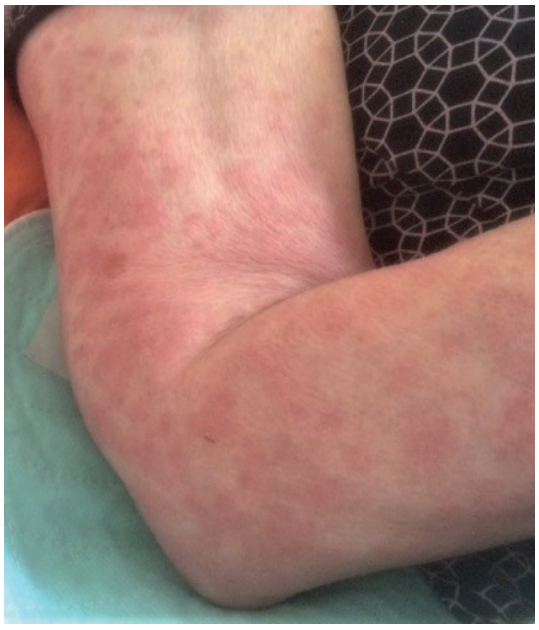

Fig. 2. Urticarial rash on the right arm in a 77-year-old woman with cryopyrin-associated periodic syndrome.

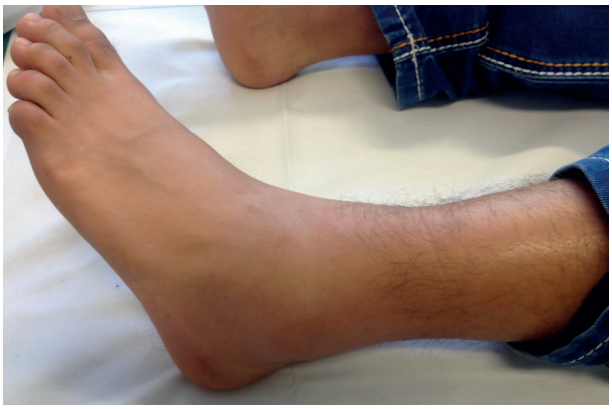

Fig. 3. Patient with familial Mediterranean fever with erysipelas-like lesion of the left lower leg and accompanying arthritis of the left ankle joint.

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

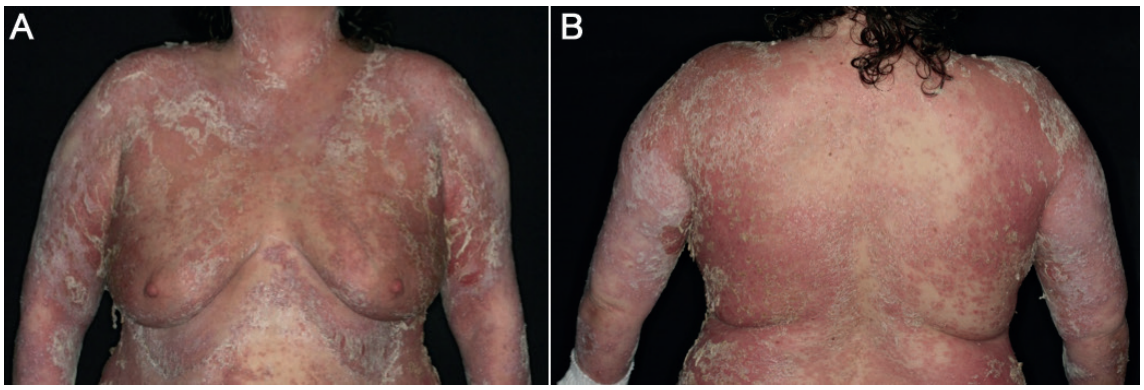

Fig. 4. (A and B) Front and back of a female patient with acute flare of generalized pustular psoriasis presenting with erythroderma, pustules, pustular lakes and erosions.

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).

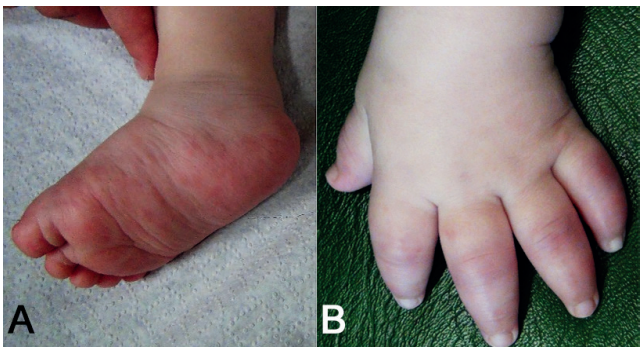

Fig. 5. Skin symptoms in an infant with chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature. (A) Erythematous papules and plaques on the right foot and right lower leg. (B) Purplish oedematous plaques on the fingers that resemble perniotic lesions with accompanying swelling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

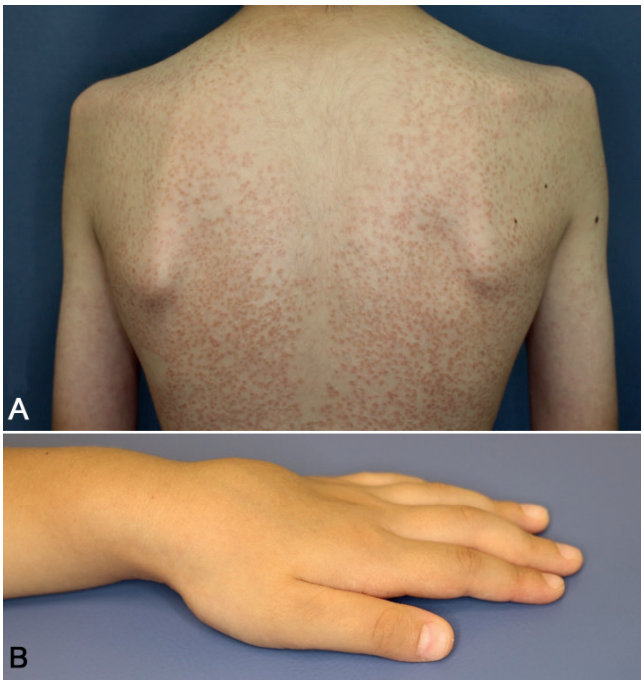

Fig. 6. Blau syndrome. A) A 12-year-old boy with disseminated small scaly solid papules with onset at age 6 months. These asymptomatic eruptions improve spontaneously, but relapse again without specific events. (B) A 4-year-old boy showing joint involvement with cystic swelling of the dorsal sides of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

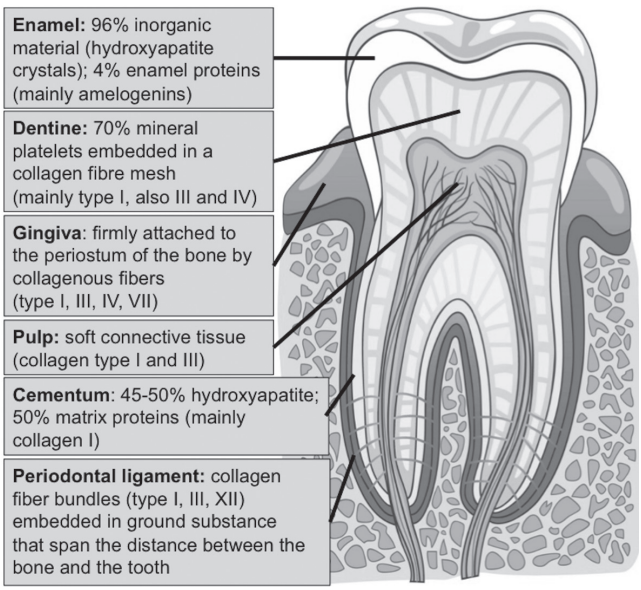

Fig. 1. The tooth and its supporting tissues. The biology of dental tissues implies that tooth abnormalities might occur in various subtypes of Ehlers-Danlos syndrome.

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

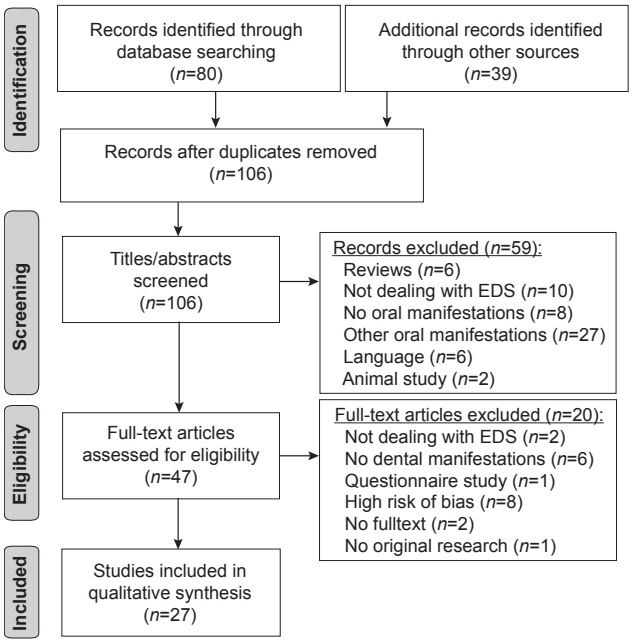

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Fig. 2. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram illustrating the study literature search and selection process.

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-

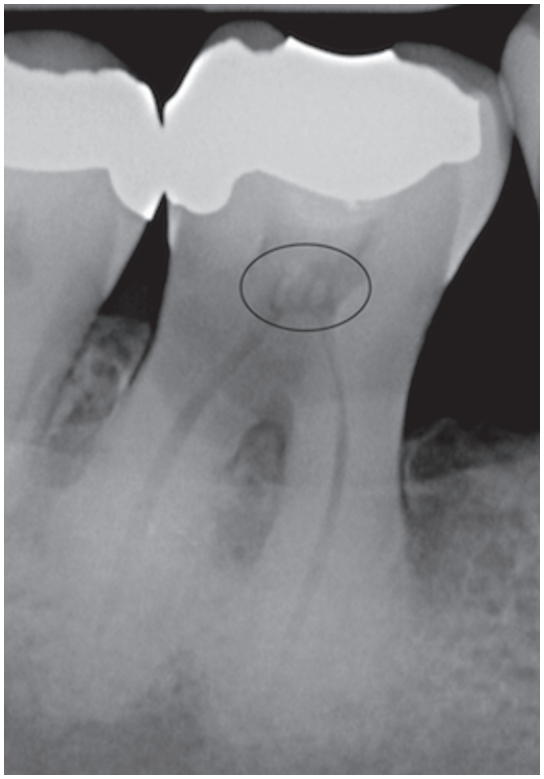

Fig. 3. Pulp stones. Dental radiograph of a 41-year-old woman, clinically and genetically diagnosed with periodontal Ehlers-Danlos syndrome (pEDS), showing pulp stones (black circle) in the mandibular right first molar.

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

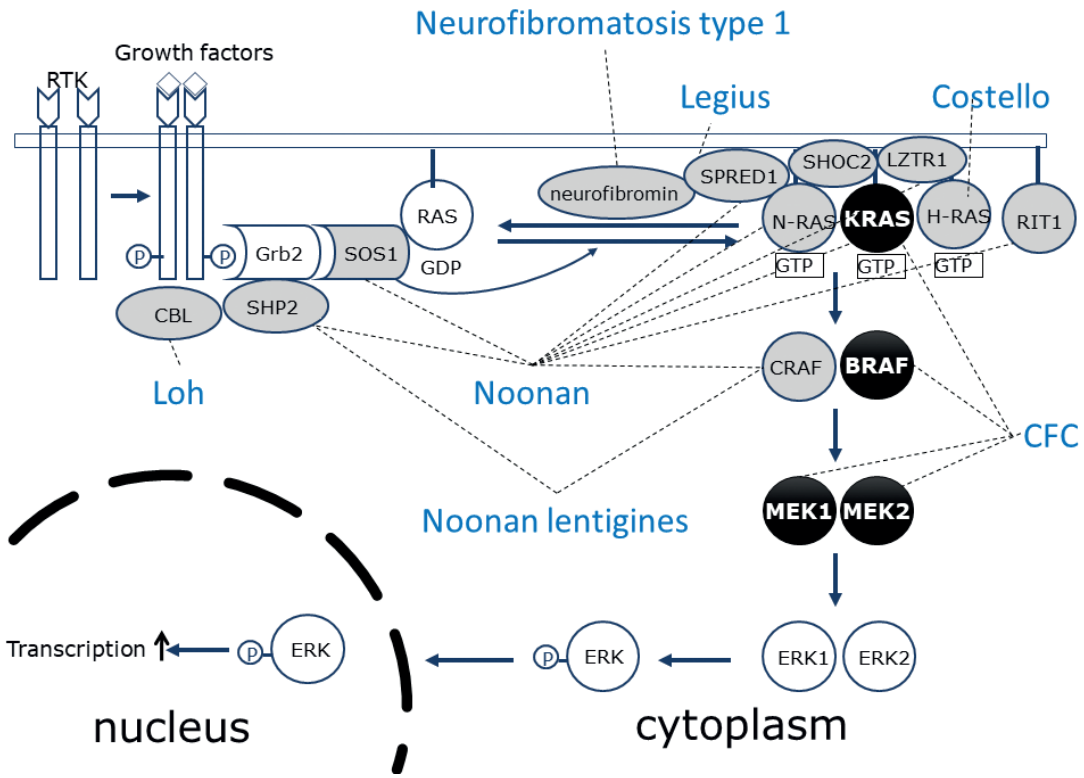

Fig. 1. The RAS-MAPKinase pathway and the proteins involved in the different RASopathies. SOS: son of sevenless; RAS: rat sarcoma; SHOC2: suppressor of clear homolog; LZTR1: leucine zipper like transcription regulator 1; N-RAS: neuroblastoma RAS; KRAS: Kirsten RAS; H-RAS: Harvey RAS; RIT1: RAS like without 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-

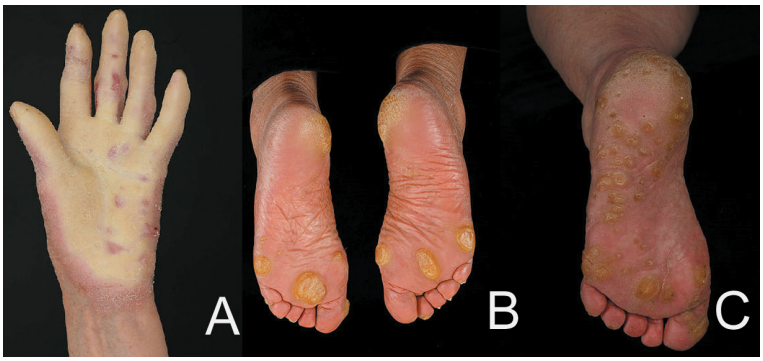

Fig. 1. Patterns of palmoplantar keratodermas. A) diffuse, B) focal and C) punctate.

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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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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

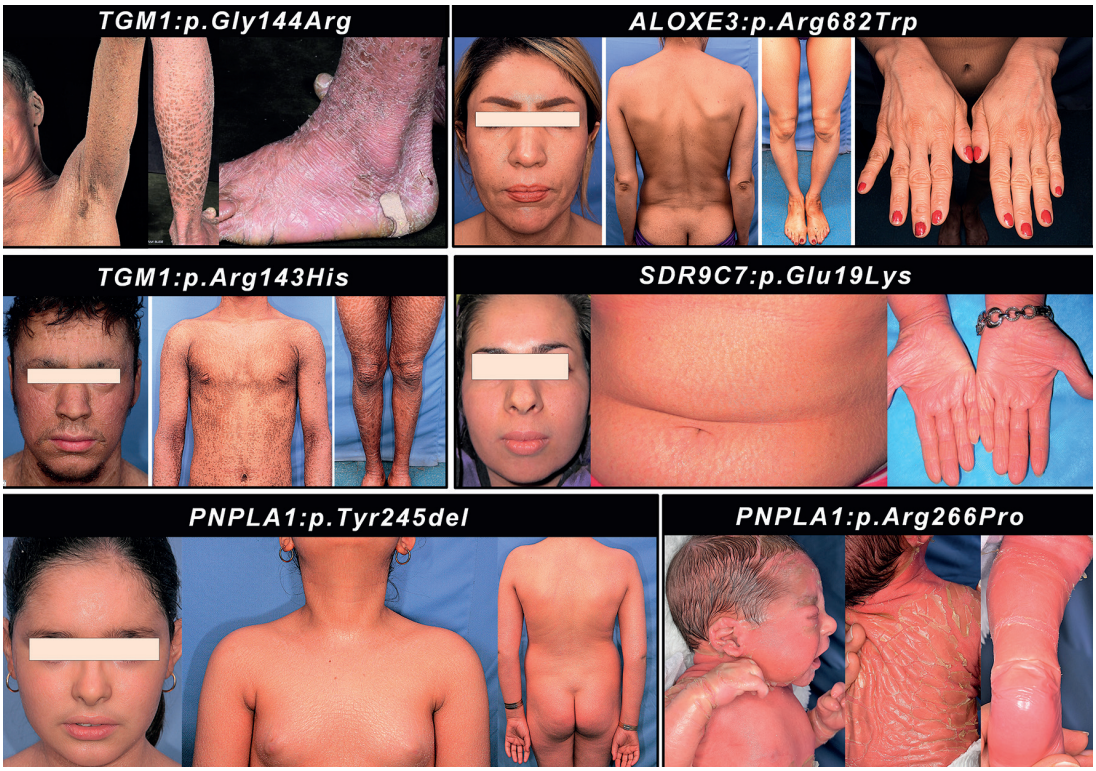

Fig. 1. Phenotypic variability in patients with autosomal recessive congenital ichthyosis associated with defects in different genes. Note the spectrum of severity in association of specific mutations indicated.

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

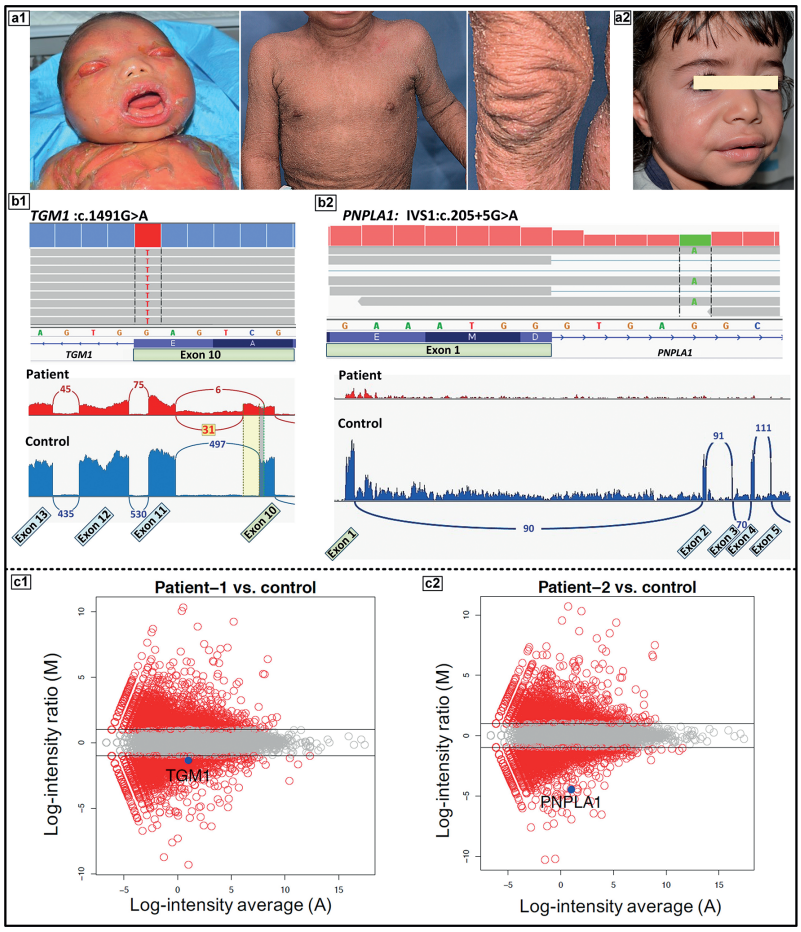

Fig. 2. Utility of RNA-based sequencing in identification and confirmation of a homozygous synonymous/splice-site mutation in *TGM1* (Patient-1) and a non-canonical splicing variant in *PNPLA1* in Patient-2. (a1) The proband, a female at neonate age (left panel) and at 3 years of age (right panel) showing 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).

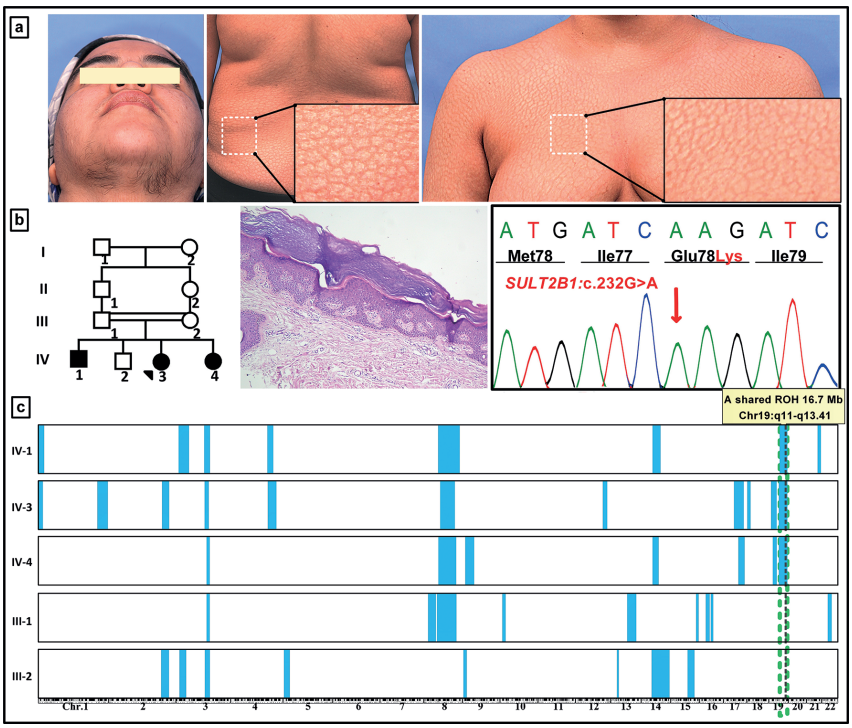

Fig. 3. Utility of homozygosity mapping in gene discovery in a consanguineous family with a *SULT2B1* mutations. (a) The proband, a 32-year-old woman, demonstrated extensive scaling present since birth and hirsutism due to mutations in *SULT2B1*. (b) Pedigree of the three affected individuals with first 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

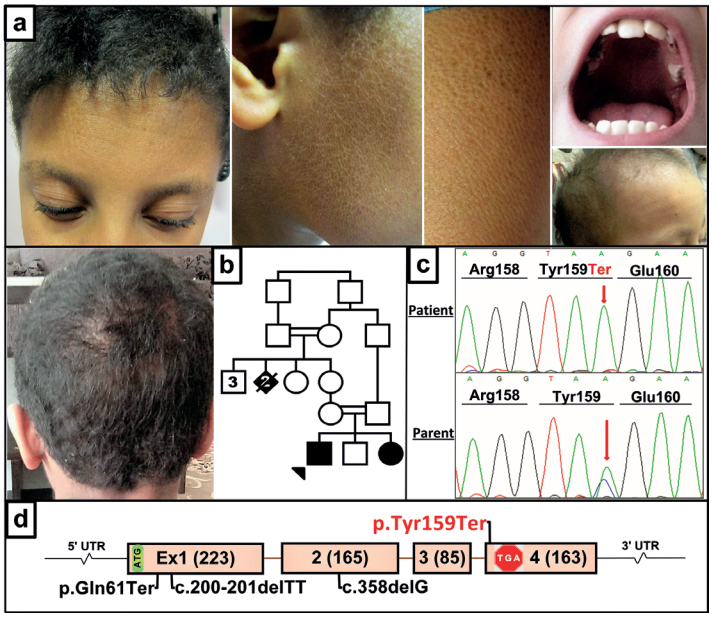

Fig. 4. Clinical features and mutation detection in a consanguineous family with NISCH syndrome with claudin-1 deficiency. (a) The patients with fine scaly ichthyosis and alopecia, oligodontia and enamel hypoplasia. (b) Family pedigree of the affected individuals with consanguineous parents. (c) Gene-targeted 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

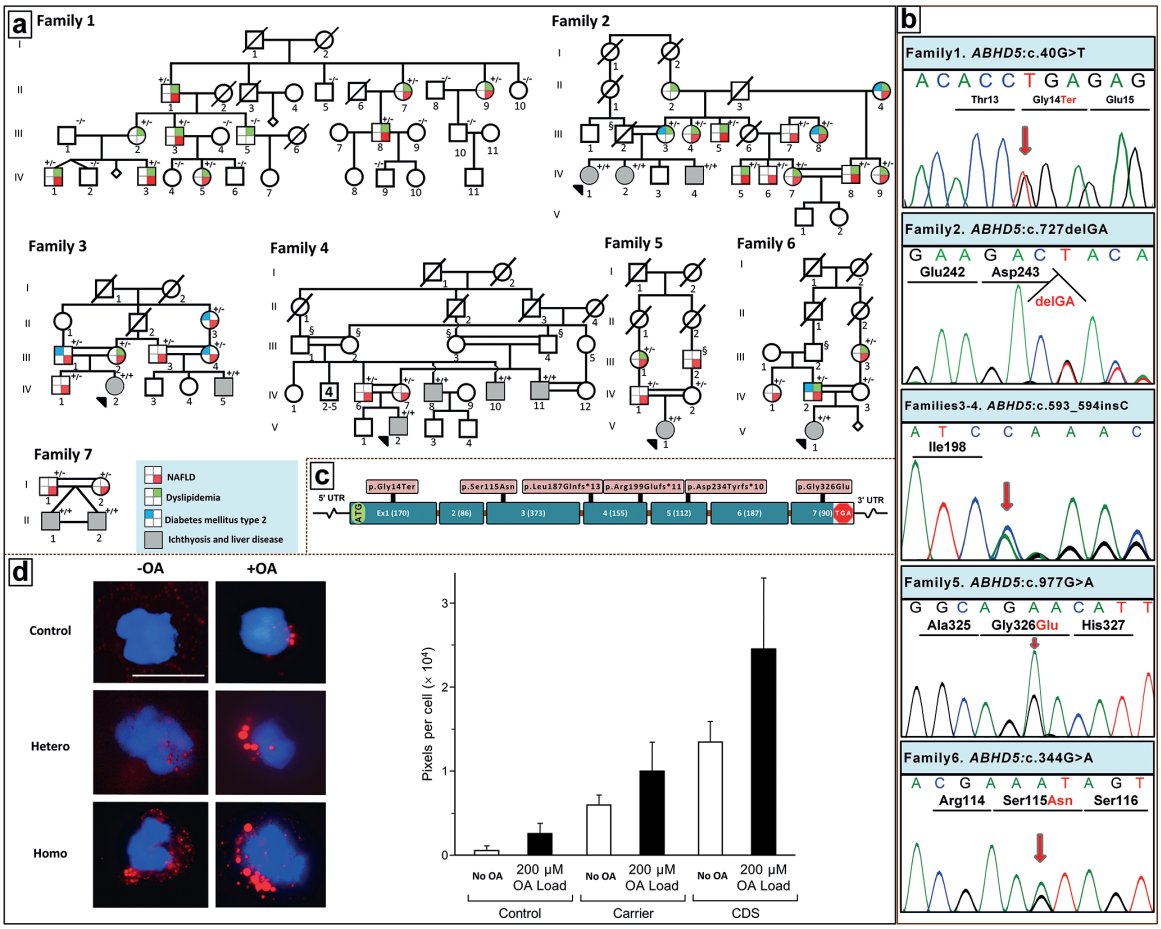

Fig. 5. Pedigree structures and clinical findings in NAFLD families with *ABHD5* mutations. (a) Family 1 is of nonconsanguineous Italian ancestry with a monoallelic mutation in *ABHD5*. Families 2–7 of Iranian ancestry with Chanarin-Dorfman syndrome (CDS) show extensive consanguinity. Heterozygous carriers (+/−) 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,

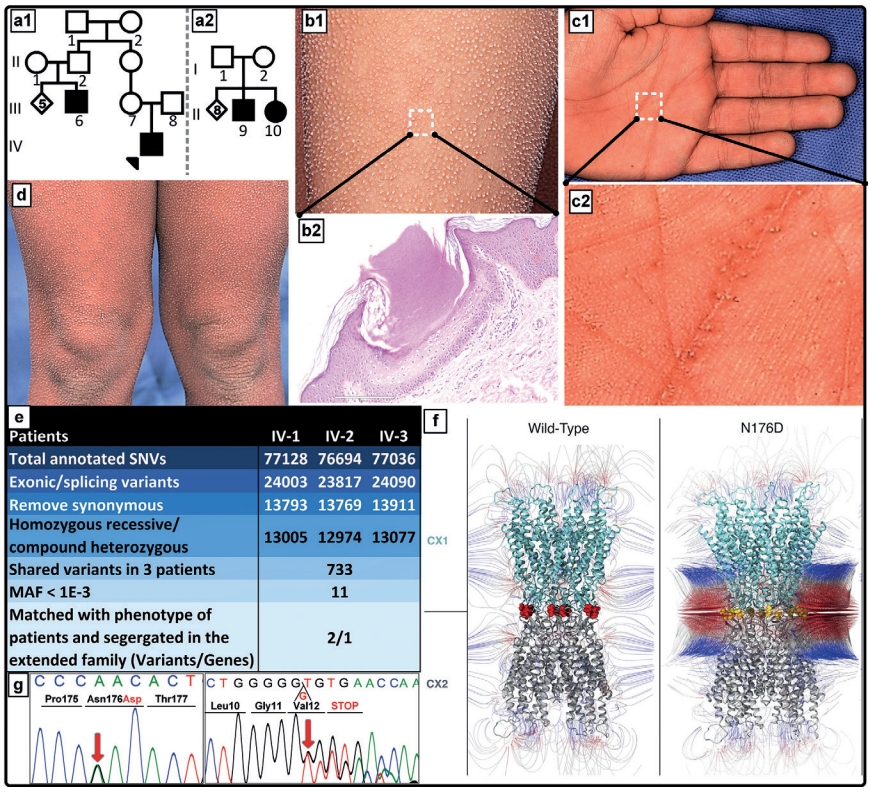

Fig. 6. Example of a novel *GJB2*-associated syndromic form of ichthyosis. Pedigree structure, cutaneous features, histopathology, alteration of the magnetic field and mutations in *GJB2* in families with autosomal recessive follicular hyperkeratosis, PPK, and bilateral sensorineural deafness. (a1, a2) 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

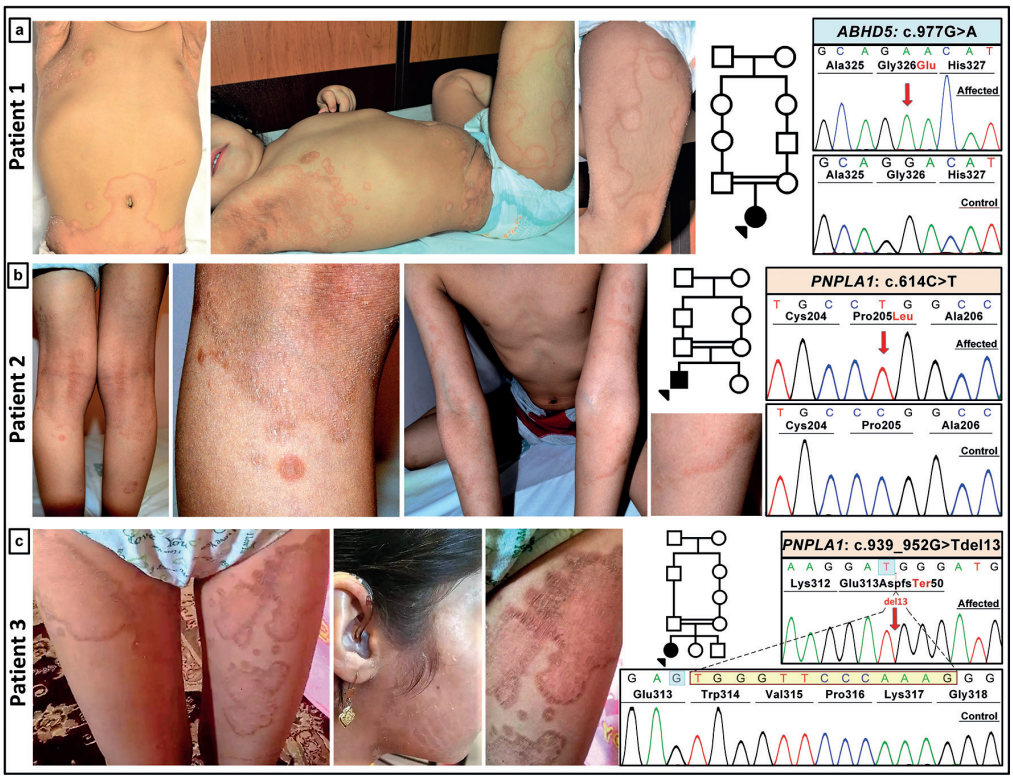

Fig. 7. Patients with ichthyosis and different genetic mutations presenting with erythrokeratoderma. (a) Patient 1, a 2-year-old female patient with erythrokeratoderma and a homozygous missense mutation in the *ABHD5* gene consistent with Chanarin-Dorfman syndrome. (b) Patient 2, a 7-year-old male with 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

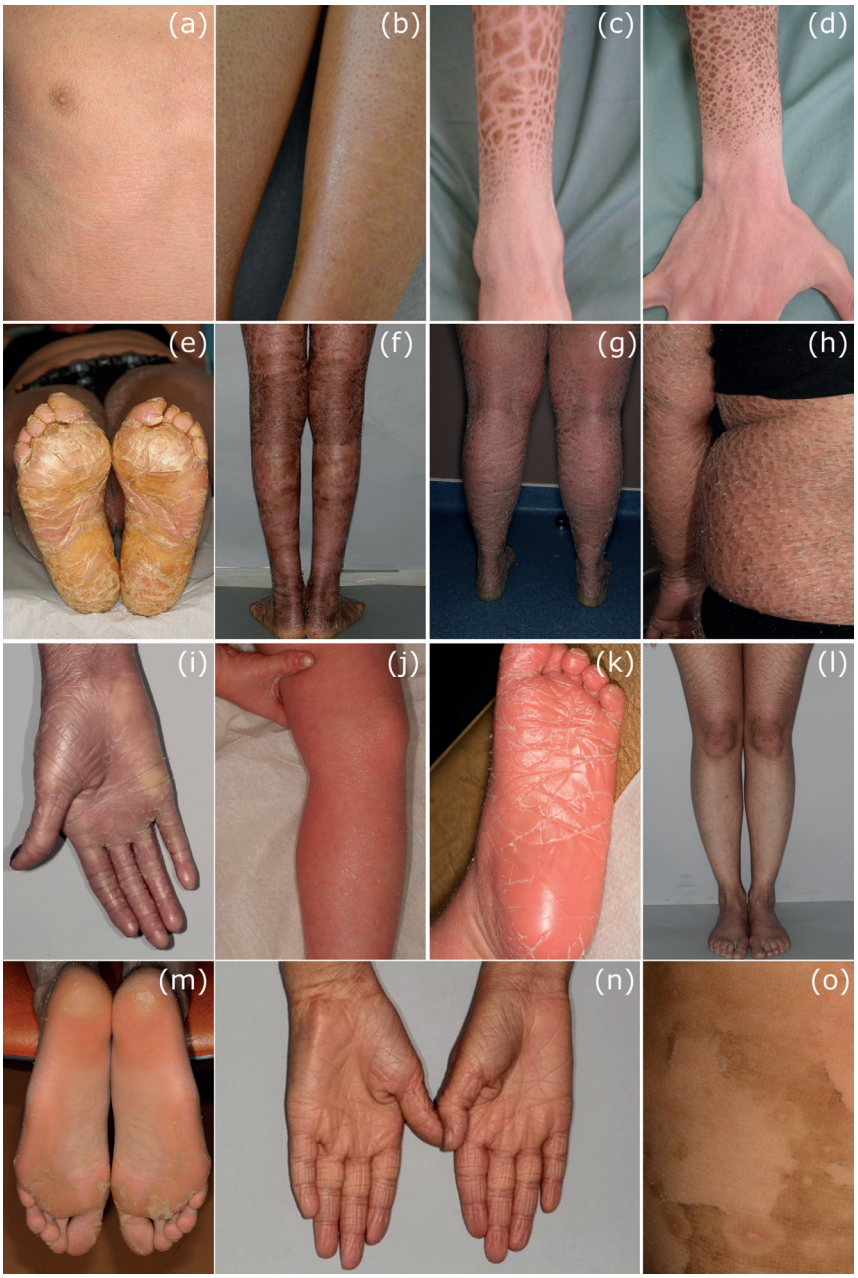

Fig. 1. Examples of skin signs in non-syndromic ichthyoses. (a, b) Fine, pale-grey scales of ichthyosis vulgaris on thorax and legs of a patient with compound heterozygous filaggrin (*FLG*) gene mutations. (c, d) Adherent, rhomboid, dark-brown scaling in X-linked recessive ichthyosis; hands and feet 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

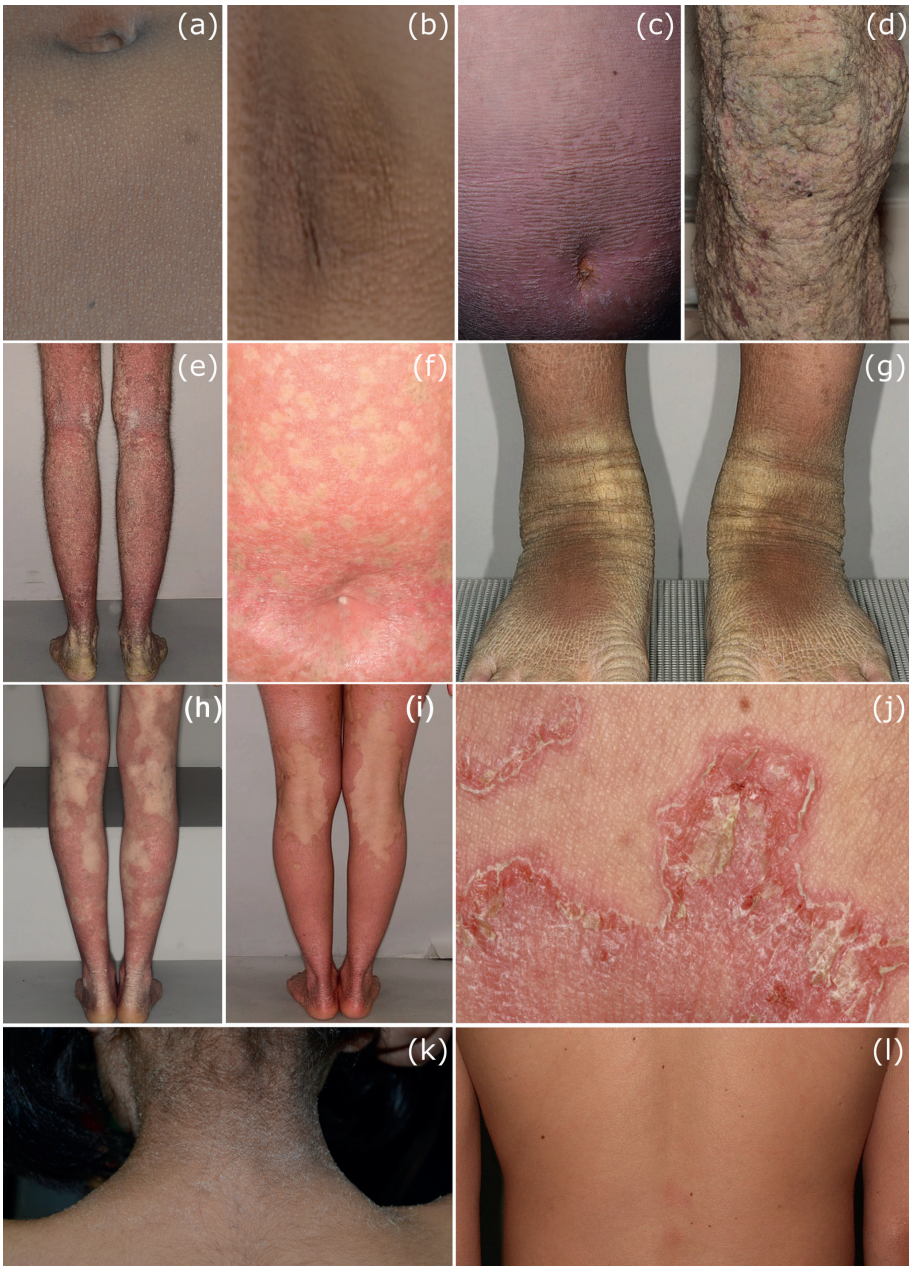

Fig. 2. Examples of skin signs in non-syndromic (continued from Fig. 1) **and syndromic ichthyoses.** (a, b) Follicular hyperkeratosis of the body and affected axilla in adults with ichthyosis prematurity syndrome (IPS). (c, d) Verrucous hyperkeratosis in epidermolytic ichthyosis (EI) of the abdomen 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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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

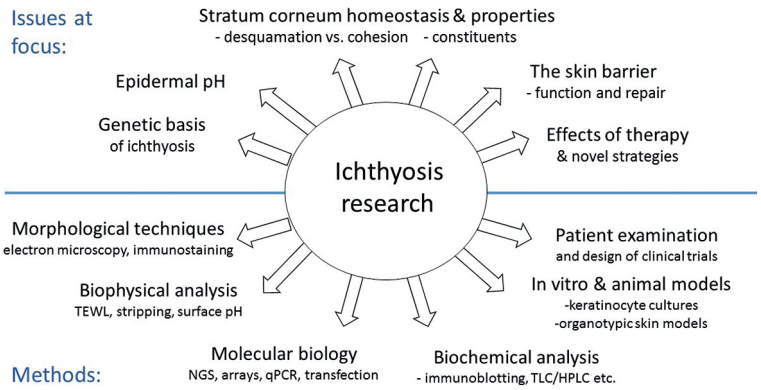

Fig. 1. Examples of scientific issues and methodological approaches in ichthyosis research. NGS: next generation sequencing; LC: liquid chromatography.

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).

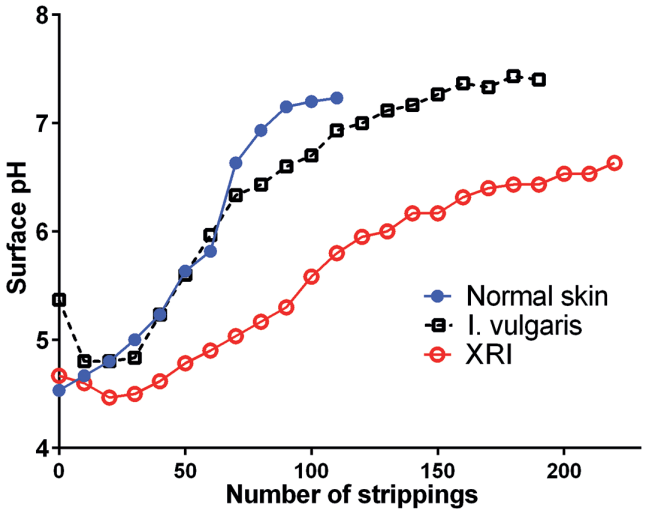

Fig. 2. The pH gradients over stratum corneum in healthy controls and patients with X-linked ichthyosis (XRI) or ichthyosis vulgaris (I. vulgaris). Mean pH values revealed by tape-stripping ($n = 10–11$ males in each group). When adjusting for the higher number of strippings required to remove SC in 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,

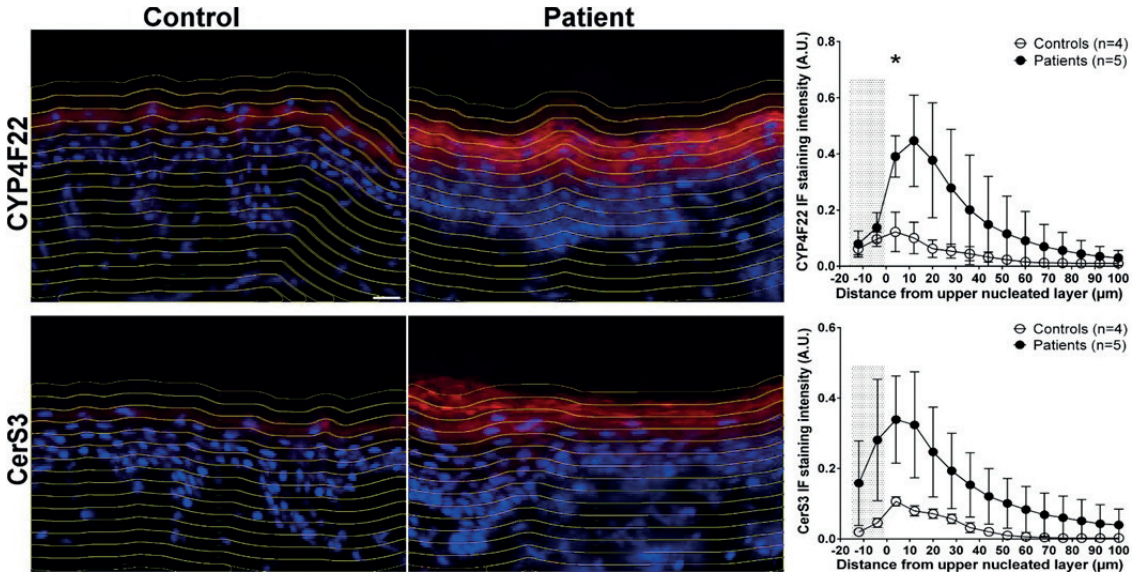

Fig. 3. Examples of immunofluorescence staining (left) and CellProfiler analysis (right) of the CYP4F22 and CERS3 proteins in patients with *TGM1* mutations versus healthy controls. The increased expression in patients' skin extends beyond the granular layer. The shaded area in the diagram correspond 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

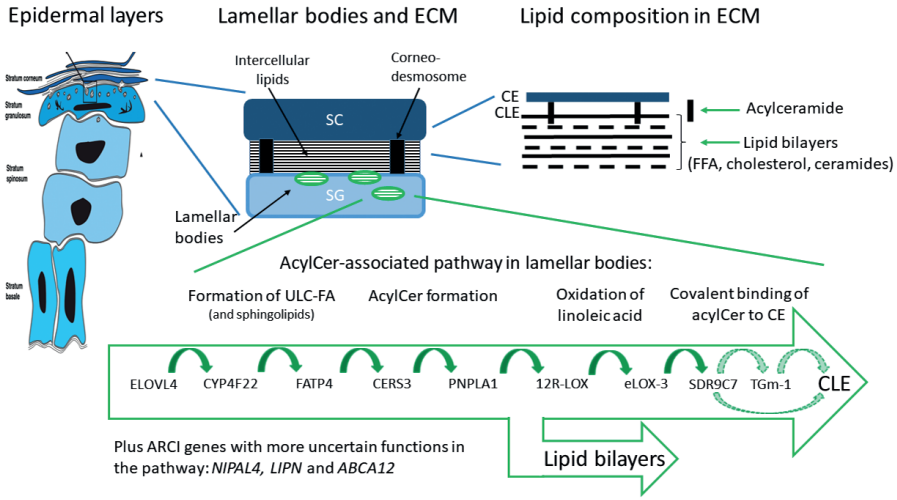

Fig. 4. Crucial components and biosynthetic steps in the formation of an epidermal lipid barrier. *Light green (hatched) arrows* in the box indicate two alternative pathways in cornified lipid envelope (CLE) formation (modified from Am J Clin Derm (4) with permission). SC: stratum corneum, SG: stratum granulosum, ECM: extracellular matrix; CE: cornified envelope, FFA: free fatty acids, ULCFA: 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.)

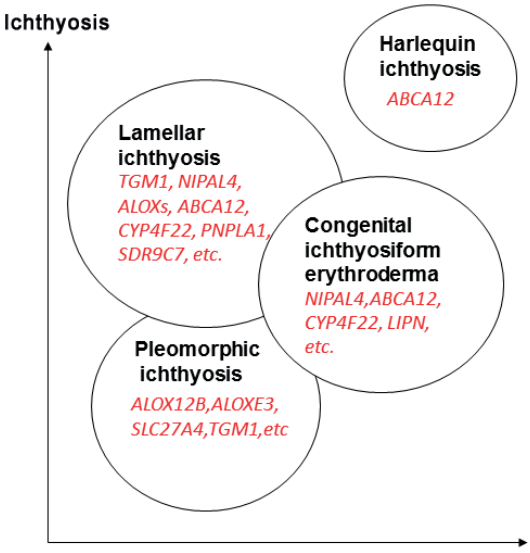

Fig. 5. Tentative correlation between ichthyosis and erythema severity 4 types of autosomal recessive congenital ichthyosis (ARCI) with partially overlapping phenotypes and culprit genes. (modified from refs. (4, 75) with permission).

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

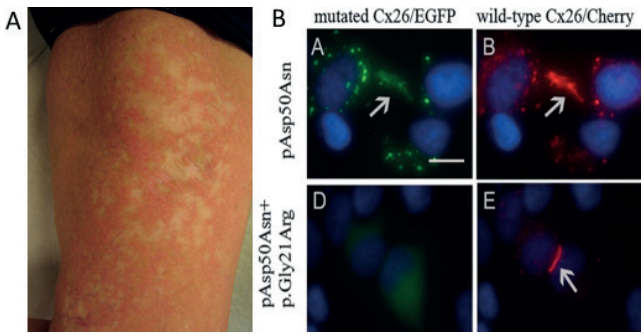

Fig. 6. Patient with keratitis-ichthyosis-deafness (KID) syndrome showing spontaneous revertant *GJB2* mutation restoring the normal phenotype of keratinocytes. (A) Gradually appearing white spots in erythrokeratodermic areas on the thigh, and (B) effects of the patient's somatic (silencing) mutation 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

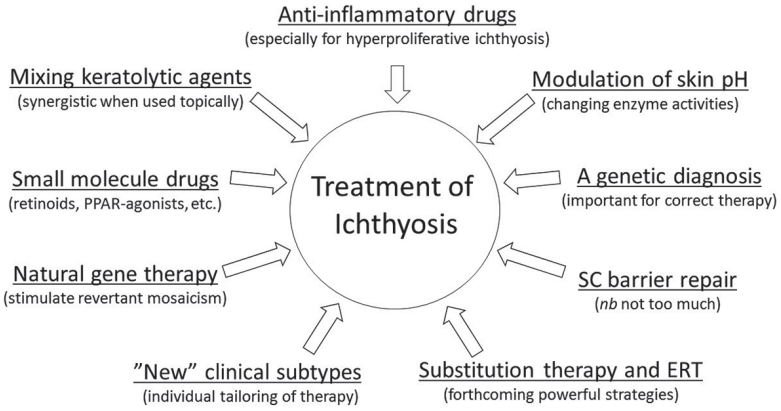

Fig. 7. Summary of ideas about future development of ichthyosis therapy. ERT: enzyme replacement therapy.

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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