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LETTERS TO THE EDITOR

Common *IL-31* Gene Haplotype Associated with Non-atopic Eczema is Not Implicated in Epidermolysis Bullosa Pruriginosa

Nikoletta Nagy, Akio Tanaka, Tanasit Techanukul and John A. McGrath
St John's Institute of Dermatology, King's College London, Floor 9 Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT, UK. E-mail: john.mcgrath@kcl.ac.uk
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Epidermolysis bullosa pruriginosa (EBP; OMIM #604129) is an unusual variant of autosomal dominant (or occasionally recessive) dystrophic epidermolysis bullosa (DEB) in which intense itching and scratching impacts upon the phenotype (1, 2). Although trauma-induced blistering often occurs, and toe-nail dystrophy is almost universal, the skin lesions can often resemble nodular prurigo, lichen simplex chronicus, hypertrophic lichen planus, dermatitis artefacta or other acquired itchy dermatoses (1, 3, 4). EBP therefore can be difficult to diagnose clinically and its precise pathophysiology is not known. Of note, the nature of the underlying mutations in the type VII collagen gene, *COL7A1*, does not differ substantially from those delineated in other non-itchy cases of DEB (3–5). Moreover, parameters such as IgE levels, atopy, biochemical or endocrinological abnormalities, iron deficiency, filaggrin gene pathology, and matrix metalloproteinase-1 gene promoter polymorphisms, have all been excluded as potential disease-modifying factors (1, 3, 4, 6).

Itch is a common, complex, and only partially understood clinical symptom (7). Nevertheless, recent data have demonstrated that interleukin-31 (IL-31), a cytokine belonging to the IL-6 family, may be relevant to some pruritic disorders (8–11). Notably, over-expression of IL-31 in transgenic mice (ubiquitous or lymphocyte-specific promoter) induces severe itching, normal IgE levels and a phenotype similar to non-atopic eczema (8). Expression of the *IL-31* gene is also up-regulated in the skin in several itchy human skin disorders, including atopic dermatitis, allergic contact dermatitis, psoriasis and nodular prurigo (9–11). IL-31 signals via a receptor complex that is composed of IL-31 receptor A (IL-31RA) and oncostatin M receptor (OSMR) subunits (12), and naturally occurring mutations in both these receptor components may underlie familial primary localized cutaneous amyloidosis, a pruritic autosomal dominant disease (13). Polymorphisms in the *IL-31* gene have also been linked to eczema susceptibility. Schulz et al. (14) have shown that a particular *IL-31* gene haplotype may be associated with altered regulation of *IL-31* gene expression, and that this can have functional consequences and be more common in subjects with non-atopic eczema.

Schulz et al. (14) identified three principal *IL-31* gene haplotypes, which they termed A, B and C, that are present in >90% of the white Caucasian population. These could be distinguished by genotyping three single-nucleotide polymorphisms: IL-31 2057 G>A (rs6489188; chromosomal position 121226729), IL-31 1066 G>A (rs11608363; chromosomal position 121225738), and IL-31 IVS2+12 A>G (chromosomal position 121224332). The A, B and C haplotypes reflected the following combination of polymorphisms: GAA, AGA and GGG, respectively. Functional studies showed that peripheral blood mononuclear cells from individuals who were homozygous for haplotype A released more IL-31 following stimulation with CD3 or CD28 antibodies (14). Haplotype A carrier status was then shown to correlate with an increased risk of non-atopic dermatitis. Of the three haplotypes, only haplotype A is associated with an A nucleotide for IL-31 1066 G/A. We therefore decided to assess whether this particular single nucleotide polymorphism, in homozygotes or heterozygotes, might also be implicated in the pathophysiology of EBP.

MATERIALS AND METHODS

We investigated the same study population recently reported by Almaani et al. (4), which comprised individuals with autosomal dominant EBP ($n=23$), dominant DEB ($n=18$), recessive DEB ($n=20$) and normal control subjects ($n=25$). Each group comprised 70–80% white Caucasians, the remainder were of Middle-Eastern, South American, South-East Asian or Asian origins. There were no major differences between the ethnicity of any of the subject groups, including the controls. In subjects with EBP, clinical and laboratory investigations revealed no underlying cause for the itching.

Following informed consent, genomic DNA was amplified for the rs11608363 single nucleotide polymorphism, using the same oligonucleotide primers and the conditions reported previously (14). Direct sequencing of both forward and reverse strands was performed using a BigDye terminator Cycle Sequencing Kit (Applied Biosystems; Foster City, CA, USA). Characterization of this single marker does not permit distinction between haplotypes B and C, but it does identify individuals who are likely to harbour haplotype A on one or both alleles. The statistical significance between the frequencies of the alleles among the studied groups were determined by Fisher's exact probability test; the confidence intervals (CI) of the *p*-values and the odds ratios (OR) were also calculated using VassarStats (http://faculty.vassar.edu/lowry/VassarStats.html).

RESULTS AND DISCUSSION

The frequencies for homozygous AA, heterozygous AG or homozygous GG were determined with respect

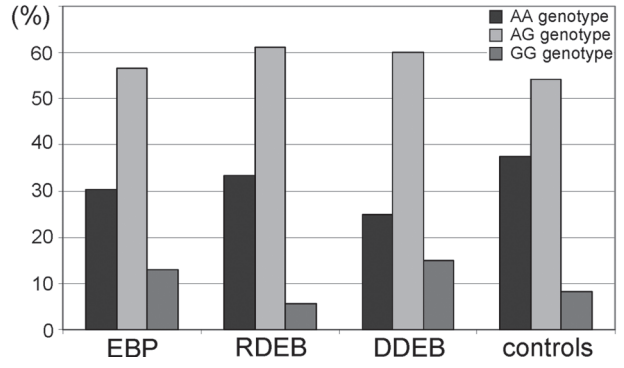

Fig. 1. Allele frequencies for the single nucleotide polymorphism IL-31 1066 A/G (rs11608363) in the different dystrophic epidermolysis bullosa (DEB) subtypes and controls. There are no statistically significant differences between the groups. EBP: epidermolysis bullosa pruriginosa; D: dominant; R: recessive.

to rs11608363 (Fig. 1). For the control group, the genotypes were: AA 38%, AG 54% and GG 8%. For the EBP population, the corresponding percentages were AA 30%, AG 57% and GG 13%; for dominant DEB subjects AA 33%, AG 61% and GG 6%, and for recessive DEB individuals AA 25%, AG 60% and GG 15%. None of the differences were statistically significant. We also performed a more comprehensive single nucleotide polymorphism analysis in these subjects (data not shown). We found that the A, B and C haplotypes defined by Schulz et al. (14) were present in ~96% of our subject cohort, but were unable to demonstrate that any particular *IL-31* gene polymorphisms were associated with the EBP phenotype.

Our study therefore failed to demonstrate an association between IL-31 haplotype and EBP, and thus the pathophysiology of this atypical variant of DEB remains unknown. Nevertheless, we cannot fully discount a role for IL-31 in EBP, since our investigation focused on examination of genomic DNA rather than IL-31 expression or signalling in skin. Treatment for EBP is difficult and various topical and systemic therapies have been used, including topical corticosteroids, topical tacrolimus, oral anti-H1 antihistamines, oral corticosteroids, ciclosporin and thalidomide (1, 3, 4). Most reports are anecdotal and responses have been variable and there is a need for more effective treatments for EBP. Modulation of IL-31 may have therapeutic benefits in other pruritic dermatoses, and use of IL-31 antibodies in the NC/NgA mouse model of itchy dermatitis has been shown to decrease scratching (15), and it is possible that targeting of IL-31 could also have benefits in patients with EBP, notwithstanding that functional (or other) polymorphisms in the *IL-31* gene do not appear to underlie this strikingly unusual clinical variant of DEB.

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