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Atopic Eczema and the Filaggrin Story

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The discovery that null mutations in the filaggrin gene (*FLG*) are associated with atopic eczema represents the single most significant breakthrough in understanding the genetic basis of this complex disorder. The association has been replicated in multiple independent studies during the past 2 years with the use of various methodologies, from populations in Europe, the United States, and Japan. Filaggrin plays a key role in epidermal barrier function, and its association with atopic eczema emphasizes the importance of barrier dysfunction in eczema pathogenesis. This review aims to summarize the current state of knowledge regarding the role of *FLG* mutations in ichthyosis vulgaris, atopic eczema, and other skin disorders, with an emphasis on potential clinical applications. Further research is needed to clarify the precise role of filaggrin in skin and systemic atopic disease, to pave the way for novel therapeutic interventions.
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Atopic eczema[1] is a complex disorder, ie, multiple genetic and environmental factors contribute to its etiology.[2] The recent discovery that mutations within the filaggrin gene (*FLG*) are strongly associated with atopic eczema represents a very significant breakthrough in understanding the genetic basis of this common disorder. Many publications relating to filaggrin have appeared in rapid succession during the past 2 years and form the basis of this review.

Genetic Factors in Atopy and Eczema

There is substantial evidence in support of a strong genetic component in the etiology of atopic eczema. Studies in twins show concordance rates of approximately 0.8 in monozygotic twin pairs compared with 0.2 in dizygotic twin pairs (that is to say an identical twin has an 80% chance of developing eczema if their twin is affected whereas a fraternal twin has an approximately 20% chance of developing eczema if their twin is affected).[3-5] Eczema and other atopic disorders show clustering within families[6] and children whose parents have atopic eczema have a greater risk of developing eczema than children whose parents have asthma or hay fever.[7,8] These observations suggest that the genetic risk of eczema may be mediated through tissue-specific factors, ie, polymorphisms in genes encoding proteins important in the structure and function of the skin, rather than through systemic immune or "atopy" risk genes. Eczema can occur with increased severity along Blaschko's lines,[9] and this mosaicism further supports the concept that skin-specific eczema risk genes may be relevant. Genome-wide linkage screens[10] and DNA microarray analysis[11] have also indicated a role for genes expressed locally in the skin and there is a growing understanding of the importance of epithelial barrier dysfunction in atopic eczema.[12,13] However, many promising preliminary discoveries in the field of eczema genetics have failed to be replicated in subsequent studies.[2]

In May 2006, a group led by Professor Irwin McLean (University of Dundee, UK) with collaborators in Ireland, Scotland, and Demark, reported that 2 common polymorphisms in the filaggrin gene (*FLG*) are strong predisposing factors for atopic eczema.[14] This finding arose from the study of ichthyosis vulgaris, demonstrating that the study of Mendelian disorders can shed light on complex traits.[15]

Filaggrin: Basic Science

Filaggrin (*fil*ament-*agg*regating prote*in*) plays a key role in epidermal barrier function. The gene *FLG* is encoded within the epidermal differentiation complex on chromosome 1q21, a cluster of genes involved in the terminal differentiation of keratinocytes.[16] In the skin, *FLG* is expressed in the granular layer of the stratum corneum during terminal epidermal differentiation. In the gastrointestinal system, *FLG* is expressed in the oral[17] and upper esophageal[18] mucosa. In the respiratory system, it is expressed in the cornified epithelium of the nasal vestibulum but not within the transitional epithelium

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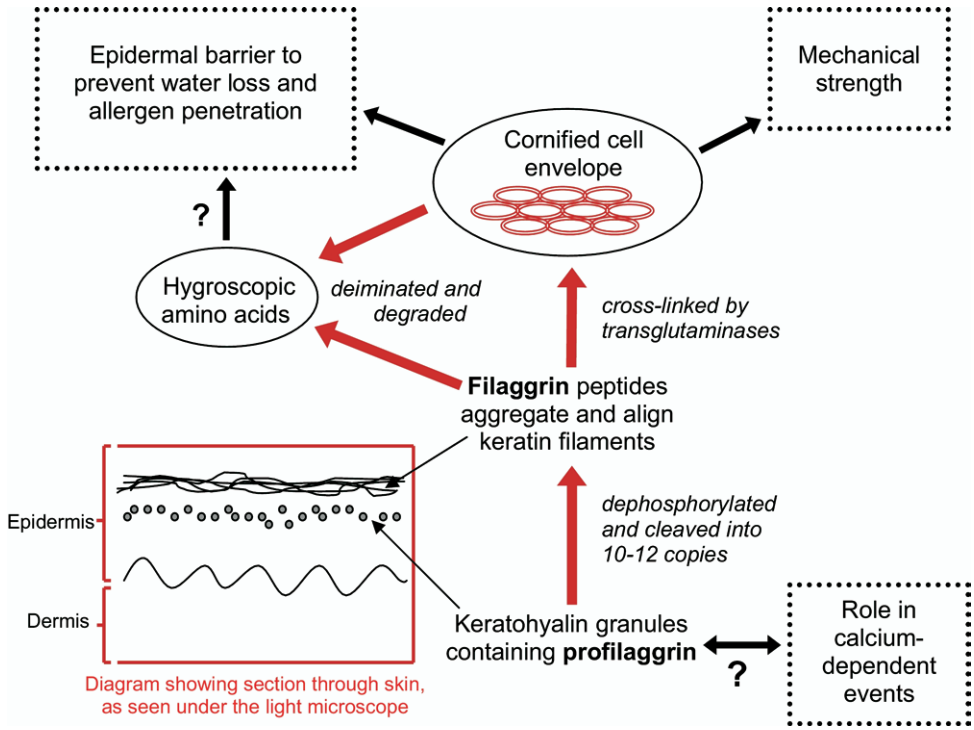

Figure 1 Diagrammatic representation of the processing and functions of filaggrin within the epidermis. Filaggrin plays a key role in facilitating epidermal differentiation and maintaining barrier function, but the relative importance of the different functions of filaggrin remains unclear. Filaggrin aggregates the keratin cytoskeleton by organizing intermediate filaments into tight bundles.[21] This facilitates the collapse and flattening of keratinocytes in the outermost skin layer to produce squamous cells. The protein-lipid cornified cell envelope is extensively cross-linked by transglutaminases and forms an important barrier,[22] helping to prevent water loss and minimizing the entry of allergens and micro-organisms. Filaggrin is subsequently deiminated (the positive arginine residues are converted to neutral citrulline) and degraded to produce a mixture of hygroscopic amino acids, as part of the so-called "natural moisturizing factor,"[23] which may also contribute to epidermal barrier function. Finally, the amino-terminal portion (S100 domain) of profilaggrin is a calcium binding domain[24] and may therefore play a role in the regulation of calcium-dependent events during epidermal differentiation; conversely, calcium may play a role in the control of profilaggrin processing. Genotypes resulting in relative or absolute filaggrin deficiency may therefore produce epidermal barrier dysfunction by more than one mechanism.[25] (Color version of figures is available online.)

covering the inferior turbinate bone.[19] Filaggrin is not expressed in the respiratory epithelium beyond this point and specifically it is not expressed in the bronchial mucosa.[19,20]

FLG encodes a large insoluble polyprotein, profilaggrin, which is the major constituent of keratohyalin granules (Fig. 1).[21-25] Profilaggrin is proteolytically cleaved to produce 10, 11, or 12 copies of the filaggrin peptide, according to our current understanding of population size polymorphisms.[26] Filaggrin aggregates keratin 1, keratin 10, and other intermediate filaments within the cytoskeleton of keratinocytes, helping to bring about their compaction into a squame shape during cornification, a unique form of programmed cell death.[22] The resultant cornified cell envelope replaces the keratinocyte cell membrane. It forms an important permeability barrier to water, microbes, and allergens and provides mechanical defense by maintaining skin integrity.[27] After keratinocyte compaction, filaggrin proteins are broken down to release hygroscopic amino acids, (part of the so-called "natural moisturising factor"), which may also contribute to epidermal barrier function by retaining water and, hence, increasing flexibility of the cornified layer. *FLG* null mutations are associated with reduced levels of hygroscopic amino acids in the stratum corneum and increased transepidermal water loss.[28]

Ichthyosis Vulgaris and Filaggrin

Ichthyosis vulgaris (OMIM #146700) is the most common inherited disorder of keratinization, with an estimated prevalence of between 1 in 80 and 1 in 250 in English school children.[29,30] Several convergent lines of reasoning led to study of the filaggrin gene as a cause for ichthyosis vulgaris. Skin histology from patients with ichthyosis vulgaris shows a reduction in keratohyalin granules[31,32] and immunostaining shows a reduction in filaggrin[31] and profilaggrin mRNA[33]; genome-wide screens have shown linkage of ichthyosis vulgaris to markers in the epidermal differentiation complex on chromosome 1q21[16,34]; finally, a murine model of ichthyosis vulgaris, the *flaky-tail* mouse, shows genetic linkage to the mouse epidermal differentiation complex.[32]

These observations date from the 1980s and although the

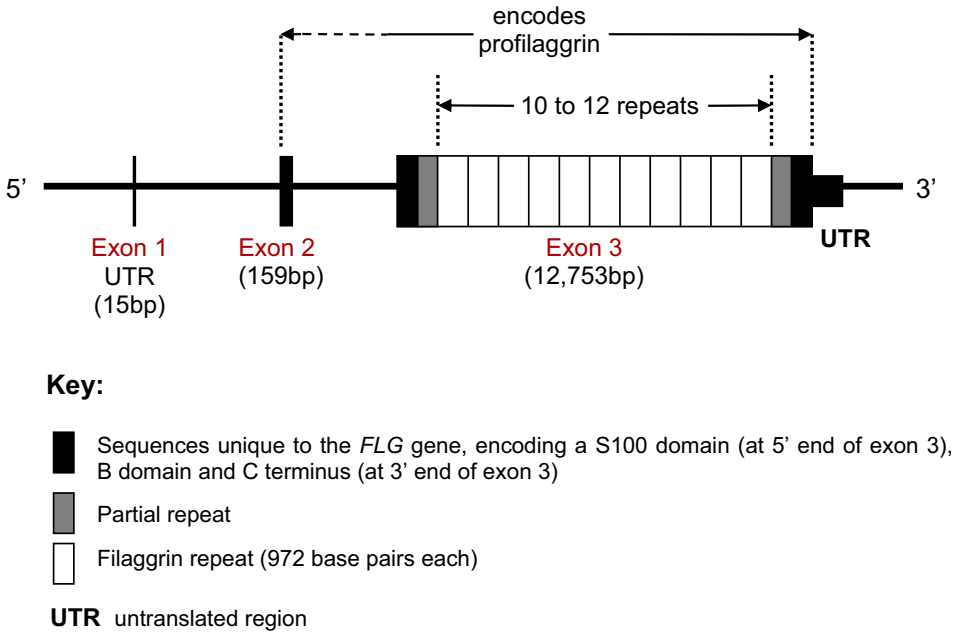

Figure 2 Structure of the filaggrin gene (*FLG*) and its corresponding protein. Modified diagram, from Smith and coworkers[32] and Sandilands and coworkers.[36,37] *FLG* is located on chromosome 1q21. Exons 1 and 2 showed no variation between individuals with and without ichthyosis vulgaris in 5 families.[32] Exon 2 contains the initiation codon. Exon 3 is unusually large and encodes most of the N-terminal domain as well as all of the filaggrin repeats and their linker sequences. Profilaggrin is polymorphic in that there are three alleles in the human population encoding 10,11 or 12 filaggrin repeats.[26] bp, base pairs; UTR, untranslated region. (Color version of figures is available online.)

profilaggrin gene was sequenced in 1992,[35] the identification of causative mutations in patients with ichthyosis vulgaris was delayed until 2006. This delay occurred in part because the inheritance pattern was unclear, with apparent autosomal-dominant inheritance in some families with ichthyosis vulgaris.[32] In addition, *FLG* is such a very large and repetitive gene (Fig. 2)[36,37] in which sequencing with the use of conventional polymerase chain reaction is technically difficult.

In 2006, Smith and coworkers finally succeeded in sequencing the *FLG* gene by using a long-range polymerase chain reaction technique to amplify the whole of exon 3.[32] They studied 15 kindreds of European and American origin with moderate-to-severe ichthyosis vulgaris and detected 2 recurrent null (nonfunctional) mutations, designated in short form: R501X and 2282del4, in repeat 1 of exon 3. Both of these mutations produce premature stop codons, resulting in a severely truncated form of profilaggrin and complete absence of processed filaggrin in the epidermis.[32] Individuals who are heterozygous for either of these 2 mutations tend to show a mild ichthyosis vulgaris phenotype, whereas homozygotes or compound heterozygotes (individuals with both mutations) usually show marked ichthyosis vulgaris. The best-fit inheritance pattern for these loss-of-function *FLG* mutations in ichthyosis vulgaris is therefore a semidominant pattern.

Filaggrin and Eczema

The association of ichthyosis vulgaris with atopy is well documented: 8% of eczema patients have features of ichthyosis vulgaris[29] and 37% to 50% of patients with ichthyosis vulgaris have atopic eczema.[29,32] Furthermore, because filaggrin expression is known to be reduced in atopic eczema (as shown by immunohistochemistry[38] and microarray analysis[11]) and genome-wide screens have shown linkage with the 1q21 region,[39] it was a logical step to investigate the frequency of R501X and 2282del4 in a cohort of patients with atopic eczema.

In the 15 families studied for the ichthyosis vulgaris research,[32] it was noted that 13 of 29 (44%) of the cases with mild ichthyosis vulgaris had eczema, and all of these 13 were heterozygous for a *FLG* null allele. Eczema was even more prevalent in the cases of severe ichthyosis vulgaris, where 16 of 21 (76%) had eczema and all were homozygous or compound heterozygous for *FLG* null alleles.[14] Conversely, none of the individuals in these families who were homozygous wild-type had atopic eczema (n = 13). The authors then modeled atopic eczema as a Mendelian trait in these ichthyosis vulgaris families and statistical analysis of genetic linkage between atopic eczema and *FLG* null alleles gave an estimated LOD score (logarithm of the odds to the base 10) of 3.08-3.27, where a LOD score of ≥ 3 is considered to indicate significant linkage. For a complex trait with multiple genetic contributors this was a very striking finding.

The authors then proceeded to study 3 additional cohorts and control populations from other sources, in an attempt to replicate the association observed in the Irish families with ichthyosis vulgaris/eczema. Initially, 52 Irish pediatric patients from a hospital clinic with dermatologist-diagnosed atopic eczema were compared with an anonymous uns-

elected control population from Ireland (n = 189). This small group of Irish patients had a combined allele frequency of 0.330, which was significantly greater than the control population frequency of 0.042 ($P < 0.0001$, odds ratio 13.4, 95% confidence interval 6.2-27.5).

Second, 604 Scottish schoolchildren and adolescents from a cohort with asthma were compared with 1008 controls from a cohort of young Scottish schoolchildren from diabetes study. The control group (of unknown phenotype) showed that 5.8% were carriers of the R501X variant and 3.8% were carriers of the 2282del4 variant. This finding gives a remarkably high combined carrier frequency of 0.096, meaning that 9.6% of the Scottish population possesses one or more *FLG* null mutations. Both *FLG* variants were significantly over-represented in the Scottish asthma cohort. When we examine the influence on eczema, 72% (64/88) of all the children in the asthma cohort carrying a *FLG* null allele had atopic eczema, compared with only 46% (215/467) of those without a *FLG* mutation. Interestingly, the *FLG* null heterozygotes also had a substantial and significant association with atopic eczema ($P = 1.3 \times 10^{-5}$, odds ratio 3.1, 95% confidence interval 1.8-5.3).

Third, 372 Danish children from a birth cohort whose mothers had asthma were compared with controls from within the same cohort. Analysis once again showed that *FLG* variants were over-represented in children with eczema compared with others in the cohort without eczema (hazard ratio = 2.8, 95% confidence interval 1.7-4.5, $P < 0.0001$). In this study 17.5% (25/142) of all individuals with atopic eczema were carriers of *FLG* null alleles and the penetrance of *FLG* null alleles was very high: 63% of carriers had developed atopic eczema by the age of 3 years. Palmer and coworkers[14] therefore established the *FLG* null alleles R501X and 2282del4 as major predisposing factors for atopic eczema for the first time, albeit in four rather selected and arguably unusual case series and cohorts.

Increasing Weight of Evidence

Since this initial report,[14] multiple case/control and association studies have been published in a short space of time, some in collaboration with the original authors and others replicating and extending the findings independently. These studies are summarized in Table 1,[40-55] in approximate chronological order.

There has so far been only one negative study published relating to *FLG* mutations in atopic eczema.[49] This study found the R501X and 2282del4 mutations at such a low frequency in the Italian population (0.006 and 0.009, respectively) that they were not associated with eczema. In view of the multiple positive association studies in other European populations, this finding may be explained by the existence of different *FLG* null mutations in Italy, or possibly strong negative selection excluding *FLG* null alleles from this population.[49]

A total of 21 *FLG* null alleles have now been identified in ichthyosis vulgaris and atopic eczema cases. Some mutations are recurrent in either the European, Japanese, or Chinese populations and some are family- or population-specific. Their sites within the *FLG* gene are illustrated in Fig. 3.[56,57] Each null mutation appears to act with a similar effect, because biochemical and immunohistochemical studies indicate that the truncated profilaggrin cannot be processed into filaggrin,[36,37] so that even mutations occurring near the 3′ end of *FLG* result in a similarly severe phenotype and with a statistically similar effect.[55] This explains the rationale for statistical analysis using a "combined null genotype," that is, grouping together individuals with one or more of any of the known *FLG* null mutations.

Meta-analysis of 9 comparable studies has estimated, for the combined null genotype of R501X and 2282del4, an odds ratio of 4.09 (95% confidence interval 2.64-6.33) from case/control studies and an odds ratio of 2.06 (95% confidence interval 1.76-2.42) from family studies.[58] The association of *FLG* with atopic eczema therefore appears to be highly significant and robust in several populations and using different methodologies. These are important considerations for a candidate gene in a complex trait.[25,59]

An Emerging Picture of *FLG*-Related Eczema

Between 14% and 56% of eczema cases in the positive studies carry one or more *FLG* null mutations (Table 1).[14,42] Similarly, the presence of a *FLG* null allele confers a 1.2 to 13 times increased risk of developing atopic eczema (Table 1).

Given that "eczema" is a complex trait and a heterogeneous disorder, what type of eczema is most closely associated with *FLG* null mutations? To date, the most highly significant associations have been reported in severe eczema cases, particularly early-onset and persistent disease.[43-45,55] However, studies have not directly compared the association across mild, moderate, and severe eczema cases, and the limited data available are insufficient to support or exclude a role for *FLG* in determining eczema severity.[46,47,60]

Atopic (extrinsic) eczema, in contrast to intrinsic eczema, has shown closer association with *FLG* in some studies.[40,45,47] However, elevated immunoglobulin E levels are associated with *FLG* null alleles only in the presence of other atopic diseases[48] and hence may represent an artifact of gathering case series from hospital clinics,[46] where a greater percentage of cases have an elevated IgE compared with those collected from community-based series.

Almost all of the original reports focused on eczema cases recruited via hospitals and specialist clinics, representing moderate-to-severe and/or treatment-resistant eczema. Representative control populations are difficult to define for these selected cases because few control population sets have detailed phenotyping information on presence or absence and subtype of eczema. We, in collaboration with others, have now reported 3 separate population cohort studies that examine cases of atopic eczema in English and German children and give information on the importance of *FLG* at a population level.[19,30,51]

Table 1 Summary of Genetic Studies Contributing Data on the Prevalence and Significance of *FLG* Null Mutations in Atopic Eczema

Study Population	Method of Recruitment	% Eczema Cases with One/More *FLG* Null Mutations	*P* Value from χ^2 Analysis	Odds Ratio (95% CI)
Ireland[14]	52 pediatric patients at hospital clinic	56	3×10^{-17}	13.4 (6.2 to 27.5)
Scotland[14]	604 children and adolescents with asthma (204 had AE)	23	4.8×10^{-11}	3.3 (2.1 to 5.6)
Denmark[14]	307 in birth cohort from mothers with asthma (142 had AE)	17.5	<0.0001	HR 2.8 (1.7 to 4.5)
Germany[40]	476 parent-child trios from hospital clinics	22.75	5.1×10^{-8}	Not calculated
Europe[41]	490 nuclear families with AE (903 children had AE)	18.6	Sibling TDT: 1.9×10^{-9}	Not calculated
Germany[41]	871 from birth cohort (189 had AE)	16.7	3.5×10^{-5}	3.73 (1.98 to 7.02)
Germany[42]	272 pediatric patients at hospital clinic	35	2.01×10^{-8}	7.1 (3.41 to 14.78)
Germany[42]	338 parent-child trios	14.2 (R501X only)	0.0001	3.39 (1.75 to 6.58)
England[43]	163 adult patients at hospital clinic†	42	1.7×10^{-53}	7.7 (5.3 to 10.9)
Germany[44]	378 patients at specialist clinic (210 with onset before 2 years of age)	21.3 for AE onset before 2 years of age	0.001 for all ages; 7.6×10^{-7} for onset < 2 years	Not calculated
Germany[45]	274 adults at hospital clinic	21.1	4.9×10^{-5}	3.53 (1.92 to 6.48)
Japan[52]	7 patients with IV and 143 with AE at hospital clinic	NA	0.0015*	Not calculated
Northern Europe & Asia[47]	148 nuclear families with child at hospital clinic	26.4	0.002	2.03 (1.46 to 2.81)
Europe & South Asia[47]	278 nuclear families with child at hospital clinic	26.4	0.008 (LOD = 1.24)	2.03 (1.46 to 2.81)
Ireland[37]	188 pediatric patients at hospital clinic (includes 52 patients in the original discovery cohort)	47	2.12×10^{-51}	10.02 (6.75 to 14.89)
Germany[48]	56 adults from a population cohort enriched for atopy	Not calculated	Logistic regression: 3.0×10^{-5}	6.78 (2.76 to 16.64)
Italy[49]	178 AE cases	0.6	Not calculated	Not calculated
Sweden[50]	406 families with adult eczema cases	Not calculated	PDT: 9.5×10^{-8}	2.21 (1.50 to 3.25)

Table 1 Continued

Study Population	Method of Recruitment	% Eczema Cases with One/More *FLG* Null Mutations	*P* Value from χ^2 Analysis	Odds Ratio (95% CI)
England[30]	811 children from unselected population birth cohort (195 had AE)	18.4	Fisher exact test: 1.2×10^{-4}	1.2 (0.7 to 1.9) for heterozygotes; 26.9 (3.3 to 217.1) for homozygotes
England[51]	6971 children from unselected population birth cohort (1445 had eczema)	20.7	3.96×10^{-20}	2.73 (1.87 to 3.99) for heterozygotes; 4.98 (9.99 to 382.5) for homozygotes
Germany[19]	3099 children from cross-sectional population study (540 had eczema)	15.8	2.5×10^{-14}	3.115 (2.326 to 4.173)

Figures relate to data for the combined null genotype, ie, combining data for all of the *FLG* null alleles in each study, since each of the null alleles have an equivalent biological effect.[36] P-values were calculated using the χ^2 test of association unless stated otherwise. Definitions of atopic eczema vary; studies used a combination of Hanifin and Rajka criteria,[41,42,44,52] UK diagnostic criteria,[30,40,43,45,47,50] parental report,[41,51] physician diagnosis,[14,41,48,49,51] dermatologist diagnosis,[14,30,37,40,42-45,47,48,50] elevated IgE[41,45,47,58,49,50,51] and/or skin prick tests.[47,48,51] Studies relating primarily to ichthyosis vulgaris rather than atopic eczema[32,36,37,53,54] have been excluded from this summary.

IV, ichthyosis vulgaris; AE, atopic eczema; LOD, logarithm$_{10}$ odds; CI, confidence interval; HR, hazard ratio; TDT, transmission disequilibrium test, a statistical tool to compare the rates of transmission of wild-type and mutant alleles between parents and children with/without the disease; PDT, pedigree disequilibrium test, analogous to the TDT; NA, not applicable.

*In these Japanese cases, R501X and 2282del4 were absent; data relate to the 3321delA and S2554X mutations.

†The analysis of these cases was extended to include all 6 of the most prevalent *FLG* mutations in a total of 186 individuals; statistical analysis then showed that 45.7% of cases had one/more *FLG* null mutations, Fisher exact test, $P = 1.3 \times 10^{-28}$, and odds ratio 5.6 (4.1-7.8).[55]

The first population-based case/control study (n = 811) showed a less strong association between *FLG* and the mild-to-moderate eczema phenotype that is most prevalent in the community (odds ratio 1.53, 95% confidence interval 0.99-2.37),[30] compared with that previously demonstrated in the moderate-to-severe case series (reported odds ratios between 2.03 and 13.4, Table 1). Interestingly, the association was only significant in children with 2 null alleles (homozygotes and compound heterozygotes) suggesting a "recessive" pattern of inheritance in these mild-to-moderate cases.[30] This is in contrast to the semidominant pattern found in moderate-to-severe cases.[14,55] A larger population-based longitudinal birth cohort study (n = 6971) supported a strong and significant association of *FLG* null alleles with eczema on a population level (odds ratio 3.12, 95% confidence interval 2.33-4.173, $P = 2.5 \times 10^{-14}$).[51] Furthermore, subgroup analysis of this data emphasizes the importance of *FLG* null mutations as a risk factor for a subtype of eczema that presents early in life, tends to persist in childhood, and is associated with wheezing in infancy as well as asthma and multiple allergic sensitizations.[51] Similarly, another large population cohort (n = 3099) confirmed the strong and significant association between *FLG* null alleles and eczema (odds ratio 3.12, 95% confidence interval 2.33-4.17, $P = 2.5 \times 10^{-14}$).[19] This study again demonstrated a strong association between *FLG* null mutations and the complex phenotype of eczema plus asthma (odds ratio 3.49, 95% confidence interval 2.00-6.08) and, for the first time, a significant association between *FLG* null mutations and allergic rhinitis independently of eczema (odds ratio 2.64, 95% confidence interval 1.76 to 4.00, $P = 2.5 \times 10^{-6}$).[19]

Possible Consequences of Filaggrin-Related Barrier Dysfunction

Filaggrin deficiency can, from our knowledge of its biochemistry, explain at least in part the barrier defect and xerosis associated with eczema (see Fig. 1). However, the marked inflammatory component of eczema remains to be explained. It has been postulated that skin inflammation occurs as a secondary phenomenon in filaggrin deficient skin, as a reaction to the entry of allergens, irritants, and pathogens.[61,62] Cytokines produced as part of an inflammatory response can then further reduce filaggrin expression.[63]

It has been suggested that the barrier defect associated with filaggrin deficiency may also facilitate the development of maladaptive hypersensitivity reactions in the systemic immune response and hence initiate and promulgate the atopic march.[61] *FLG* null mutations are not associated with asthma in isolation,[14,30,64] but have shown strong association with the subgroup of patients having asthma in the context of eczema.[14,19,30,41] *FLG* mutations may also predispose to a more severe asthma phenotype[65,66] but, at present, the pathogenic mechanisms linking epidermal barrier dys-

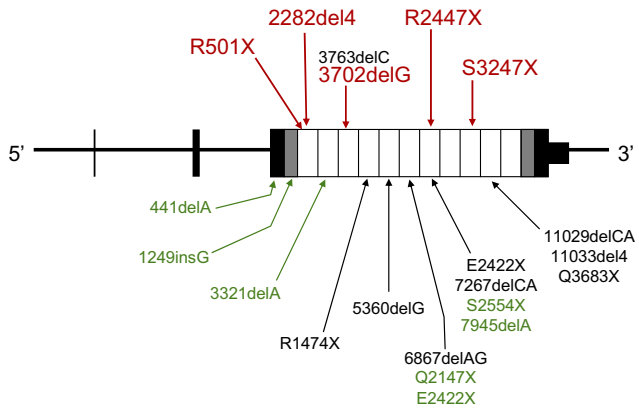

Figure 3 Diagram showing the *FLG* gene and sites of reported null mutations. Diagram modified from Sandilands and coworkers[37] with additional mutations reported by Nomura and coworkers,[56] and Chen and coworkers.[57] The mutations marked in red are prevalent in the European population, with a combined allele frequency of 0.09 (ie, approximately 9% of the population carry one/more of these mutations). The mutations marked in black are found in European and European American populations at much lower frequencies. The mutations marked in green have been identified in ichthyosis vulgaris and eczema cases from Chinese, Japanese and Singaporean Chinese populations. (Color version of figures is available online.)

function with a respiratory disorder remain purely speculative.

It has been reported that *FLG* null mutations are not associated with hand eczema or contact allergy,[67] but this study had insufficient statistical power to exclude an association.[68] A larger study designed to investigate the association between *FLG* null mutations and allergic contact dermatitis showed a significant association with allergic contact sensitization to nickel, but only when this phenotype was combined with self-reported intolerance to fashion jewelry.[48] This association may result from the impaired barrier function acting as a facilitator for allergen penetration and allergic sensitization. However, the statistical analysis did not control for comorbidity with atopic eczema and the same study did not show an association between *FLG* mutations and sensitivity to other contact allergens. This raises questions as to the true significance of the observed association and firm conclusions cannot be drawn without further investigation.

FLG Mutations in Other Skin Disorders

The association of *FLG* mutations with a variety of different skin disorders has been investigated because of theoretical pathogenic mechanisms. Psoriasis is another inflammatory skin disease with disordered keratinization. It has shown co-localization with eczema-susceptibility regions in the epidermal differentiation complex on chromosome 1q21 as well as other areas on whole genome screens.[39,69] However, case–control association studies have shown no association between R501X or 2282del4 and psoriasis[70,71] and gain-of-function frameshift mutations have not been identified in patients with several different types of psoriasis.[70] Hence, in the 1q21 locus, the shared genetic susceptibility to psoriasis and eczema appears to be the result of the close clustering of genes with similar functions, rather than to polymorphisms within the *FLG* gene itself.[70] Other inflammatory barrier diseases, including Crohn's disease and sarcoidosis, share common susceptibility loci[72] but do not show association with *FLG* null mutations.[42]

Alopecia areata is a tissue-specific autoimmune disease and genetic factors make a significant contribution to its etiology.[73] It is known to be associated with atopy and comorbidity with atopic eczema may predict a more severe form of alopecia areata.[74] A study of alopecia areata cases and unaffected controls showed no association between *FLG* null mutations (R501X and 2282del4) and alopecia. However these *FLG* mutations were significantly associated with the presence of atopic eczema among the alopecia cases. Furthermore, patients having one/more *FLG* null alleles as well as eczema plus alopecia areata, showed a significantly more severe form of alopecia than the wild-type individuals ($P = 0.003$, odds ratio 5.47, 95% confidence interval 1.59-18.76), in keeping with clinical observations.[74]

Keratinocytes show abnormal terminal differentiation within epidermoid cysts, so an immunohistochemical study was performed to investigate filaggrin staining as a marker of terminal differentiation to investigate the pathogenesis of these lesions. Filaggrin expression shows no abnormality in the pilosebaceous unit, but staining intensity is markedly increased in the epidermoid cyst wall.[75] *FLG* may also be overexpressed in the abnormal keratinisation associated with acne vulgaris[76] and naevus comedonicus,[77] but it remains to be shown whether altered filaggrin expression occurs as a primary, pathogenic event or as a secondary phenomenon.

Finally, *FLG* null mutations can modify the effects of other genodermatoses. This was elegantly demonstrated by a study of 2 brothers: both children had X-linked ichthyosis (resulting from inactivating mutations in the steroid sulfatase gene), but one child showed a more severe ichthyotic phenotype and was found to carry the R501X mutation.[78]

What Is the Clinical Significance of These Findings?

FLG mutations appear to have both highly statistically and clinically significant effects. The estimated penetrance varies from 42% to 79%,[41,47] ie, between 42% and 79% of individuals with one or more *FLG* null mutations are likely to develop atopic eczema. The population attributable risk fraction has been estimated at 11% and 13.5% in German populations[19,41] and 15.1% in an English population.[51] These data indicate that, assuming that there is a causal association, 11% to 15% of eczema may be attributable to *FLG* null mutations on a population scale.

In the absence of a readily available screening test for *FLG* polymorphisms, can we predict from clinical examination that eczema patients may be carriers and furthermore is this

clinically relevant? Eczema in the context of ichthyosis vulgaris is very likely to be caused by *FLG* haplo-insufficiency (100% of cases in the original studies[14,32]). The presence of palmar hyperlinearity has shown very strong association with *FLG* null mutations,[30,46-48] with a positive predictive value of 71% for marked palmar hyperlinearity.[30] However, the mechanism by which filaggrin deficiency produces this clinical sign remains to be elucidated, occurring as it does to varying degrees with ichthyosis vulgaris, atopy, palmoplantar hyperkeratosis and also in the absence of skin disease.[79] Similarly, keratosis pilaris shows a highly significant association with *FLG* null mutations ($P = 2.2 \times 10^{-12}$) and this association is not dependent on comorbidity with ichthyosis vulgaris.[30] As described previously in this review, eczema that begins early in life (younger than the age of 2 years) and persists into adulthood has shown some of the most statistically significant associations with *FLG* mutations,[43-45] in contrast to adult-onset eczema.[42]

These observations may prove to be helpful both theoretically and practically in the clinic. Our current classification of eczema remains suboptimal[80] and is likely to continue evolving as our understanding of pathogenesis improves. A classification dividing *FLG* haplo-insufficient cases from other cases of eczema may well prove to be a useful distinction to predict prognostic factors such as natural history, associated disorders and response to treatment. Furthermore, the identification of patients with *FLG* mutations may facilitate the targeting of novel therapies to repair or replace the defective epidermal barrier. Timely intervention early in life may even halt the 'atopic march' and thus reduce the incidence of asthma and allergic rhinitis, though this exciting possibility is currently purely theoretical.

Unanswered Questions

The statistical estimates of the effects of *FLG* mutations are very striking, particularly in the context of a single gene in a complex trait. However, clearly not all eczema is caused by the *FLG* variants that have been studied to date. Even if further mutations are identified, the *FLG* gene cannot explain all eczema cases. Reanalysis of family data to estimate the linkage of eczema with a previously reported micro-satellite marker in the epidermal differentiation complex, as well as 2 *FLG* null mutations (R501X and 2282del4), showed a total LOD score of 3.57; the *FLG*-only LOD score was 1.54.[39] This leaves evidence of significant residual linkage to the region that includes the epidermal differentiation complex, although it remains to be seen whether this residual linkage signal persists after adjustment for all *FLG* mutations. Other genes, possibly within the epidermal differentiation complex or elsewhere in the genome, must also influence the phenotype of eczema. This may occur via mediation of filaggrin function and/or by independent mechanisms on skin barrier function as well as local and systemic immunity.

Other unanswered questions include the following:

- What are the most clinically important functions of filaggrin?
- Why does atopic eczema in childhood localize to the flexural skin?
- Which other genes and environmental factors modulate the effects of *FLG*?
- Can this increased understanding of the pathogenesis of eczema be utilized to develop novel therapeutic interventions for eczema and other atopic diseases?

Studies aimed at addressing some of these interesting and important questions are currently underway.

Conclusion

FLG is the single most significant genetic factor in atopic eczema that has been identified to date, demontrating the close link between atopic eczema and ichthyosis vulgaris as well as emphasizing the important role of epidermal barrier dysfunction in eczema pathogenesis.

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