

Fleshing Out Filaggrin Phenotypes

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The association of filaggrin null alleles with eczema has been replicated in several European populations. Three large, well-conducted studies confirm this association and offer insights into the phenotypic nature of eczema associated with these alleles. Early data suggest that *FLG*-associated eczema may be more persistent, more likely to have palmar hyperlinearity, and more likely to be associated with asthma. These initial hints will require further confirmation in cohort studies.

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Earlier this year, two recurrent null alleles in filaggrin (*FLG*) were observed to confer substantial risk of atopic dermatitis (AD; henceforth called eczema, according to the World Allergy Organization nosology (Johansson *et al.*, 2004)) and of asthma occurring in the context of eczema (Palmer *et al.*, 2006). Within a short time these two null alleles in *FLG* (R501X and 2282del4) have become the most widely and independently replicated and most strongly associated genetic factor conferring susceptibility to eczema in European populations to date, with odds ratios of between 3.73 and 7.1 reported in the larger cohorts (Marenholz *et al.*, 2006; Ruether *et al.*, 2006). In this issue of the *Journal of Investigative Dermatology*, three additional replication studies are reported (Barker *et al.*, 2007; Stemmler *et al.*, 2007; Weidinger *et al.*, 2007). Each of these studies reports strong association between eczema and *FLG* null alleles, and each allows further exploration of the genetic contribution of these common recurrent null alleles to the pathogenesis of eczema. A summary of the populations studied to date and relevant findings is presented in Table 1. Significantly, all studies have shown strong replication of association with eczema, and no negative or equivocal study in Europeans, in whom these mutations are prevalent, has been reported. The position of *FLG* as a major

gene in AD is further reinforced by the striking finding that these two common recurrent mutations have a penetrance of 42% in the German Multicenter Allergy Study and that the population attributable risk factor (the proportion of eczema that is directly attributable to these two recurrent null alleles at population level assuming a causal association) for eczema in this cohort was 11% (Marenholz *et al.*, 2006). These calculations of the total cumulative effect of *FLG* null alleles in eczema are likely to be an underestimate, as several additional recurrent *FLG* null alleles are present in European populations at lower levels with intra-European, regionally distinct allele frequencies (A. Sandilands, W.H.I. McLean, and A.D. Irvine, unpublished data).

Given such a strong gene effect, and given that these same alleles cause the distinct phenotype of ichthyosis vulgaris in a semidominant inheritance pattern (Smith *et al.*, 2006), it is reasonable to ask whether the phenotypic characteristics of eczema related to *FLG* null alleles can in any way be dissected out from the broad and inclusive concept of eczema suggested by current guidelines. Subcategorization of eczema based on elevated circulating specific IgE levels (into atopic and nonatopic, or extrinsic and intrinsic forms) has been widely suggested, but it has failed to increase our predictive ability in terms

of phenotype or course of disease (Flohr *et al.*, 2004). In particular, elevated IgE levels are less relevant in community-based studies than in hospital-based studies (Flohr *et al.*, 2004). Could the presence or absence of *FLG* null alleles help define sub-phenotypes and predict the course of eczema? Examination of the early data allows a degree of cautious interpretation as to the precise contribution of *FLG* null alleles to eczema phenotypes and to the consideration of possible genotype–phenotype correlates. Within cohorts that have been ascertained through attendance of people with eczema at dermatology clinics, some recurrent themes emerge. Two large series of adult patients with AD presenting to hospital clinics show a high frequency of *FLG* null carrier rates in adults who have persistent AD with onset within the first 2 years (29.7% in a German cohort (Weidinger *et al.*, 2007), and 42% in an English cohort (Barker *et al.*, 2007)). These observations suggest that individuals with eczema who carry *FLG* null alleles could be more likely to have early onset and persistent disease, although controls with late-onset eczema and cohort studies are needed for further

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proof of such a notion. In well-characterized eczema cohorts from Munich, these alleles predispose to elevated IgE as a secondary trait (Weidinger *et al.*, 2006; Weidinger *et al.*, 2007). This predisposition was also seen in the extended Genetic Studies in Nuclear Families with Atopic Dermatitis cohort (Marenholz *et al.*, 2006) and in relation to “extrinsic” AD (AD plus sensitization determined by positive skin-prick testing or specific IgE elevation) (Weidinger *et al.*, 2007). These findings may reflect the more severe spectrum of disease seen in hospital studies, where elevated

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Table 1. Summary of genetic studies of association of *FLG* null alleles with eczema and related phenotypes

| Cohort/ethnicity | Number of subjects and characteristics of cohort | Phenotype examined | P value (combined genotype) | Odds ratio | % patients with one or more <i>FLG</i> null alleles |
|--|---|---|---|--|---|
| Irish ¹ (Sandilands <i>et al.</i> , 2006) | 10 large multiplex families with ichthyosis vulgaris and AD | AD and ichthyosis vulgaris | LOD 3.96 | NA | NA |
| Irish ² (Palmer <i>et al.</i> , 2006) | 52 children, early onset, severe AD | AD | 3×10^{-17} | 13.4 (6.2–27.5) | 56 |
| English ³ (Barker <i>et al.</i> , 2007) | 163 early onset, persistent AD | AD | 1.7×10^{-53} | 7.7 (5.3–10.9) | 42 |
| German ⁴ (Ruether <i>et al.</i> , 2006) | 272 children with AD | AD | 2.01×10^{-8} | 7.1 (3.41–14.78) | 35 |
| German ⁵ (R501X only typed) ^f (Ruether <i>et al.</i> , 2006) | 338 trios | AD | 0.0016 | 3.59 (1.75–6.58) | 14.2 (R501X only) |
| Scottish (Dundee BREATHE) (Palmer <i>et al.</i> , 2006) | 279 (children recruited through asthma clinics) | AD + asthma | 4.8×10^{-11} | 3.3 (2.0–5.6) | 23 |
| German ⁶ (Weidinger <i>et al.</i> , 2006) | 476 trios (recruited through hospital-based dermatology clinics) | AD | 5.1×10^{-8} | Not calculated | 22.75 |
| | | Allergic sensitization | 2.3×10^{-7} | Not calculated | |
| | | Total IgE | 9.8×10^{-8} | Not calculated | |
| | | Asthma | 0.0003 | Not calculated | |
| | | Palmar hyperlinearity | 5.9×10^{-6} | Not calculated | |
| | | Extrinsic AD | 9.3×10^{-8} | Not calculated | |
| German ⁷ (Stemmler <i>et al.</i> , 2007) | 378 patients with AD (210 onset <2 years) | Onset <2 years All AD | 7.6×10^{-7} | Not calculated | 21.3 |
| German ⁸ (Weidinger <i>et al.</i> , 2007) | 274 adults | AD IgE Asthma Rhinitis Intrinsic eczema | 4.9×10^{-5} 0.010 2.5×10^{-6} 2.4×10^{-5} No association | 3.53 (1.92–6.48) Not calculated Not calculated Not calculated No association | 21.1 Early onset, 29.7 |
| German ⁹ (GENUFAD cohort) (Marenholz <i>et al.</i> , 2006) | 490 nuclear families 903 cases with moderate to severe eczema | Eczema | 1.9×10^{-9} | Not calculated | 18.6 |
| | | Eczema + asthma | 0.00042 | Not calculated | |
| | | Eczema + allergic rhinitis | 2.5×10^{-5} | Not calculated | |
| | | Eczema + increased IgE | 1.9×10^{-9} | Not calculated | |
| German ¹⁰ (MAS cohort) (Marenholz <i>et al.</i> , 2006) | 871 children, population-based, longitudinal study up to 10 years 189 eczema 118 asthma | Eczema ($n = 155$) | 3.5×10^{-5} | 3.73 (1.98–7.02) | 16.7 |
| | | Nonatopic eczema ($n = 63$) | 0.00065 | 3.94 (1.7–8.9) | Not recorded |
| | | Eczema + asthma ($n = 40$) | 5.4×10^{-8} | 6.21 (2.6–14.8) | 25 |
| | | Eczema + rhinitis ($n = 44$) | 1.5×10^{-5} | 4.75 (2.0–11.6) | 20 |
| Danish (COPSAC) ¹¹ (Palmer <i>et al.</i> , 2006) | 142 (children born to asthmatic mothers) | AD | <0.0001 | 2.8 (1.7–4.5) | 17.5 |

Genetic analysis models: ¹linkage analysis; ²case-control χ^2 ; ³case-control χ^2 ; ⁴case-control χ^2 ; ⁵case-control χ^2 ; ⁶transmission disequilibrium test; ⁷transmission disequilibrium test; ⁸case-control χ^2 ; ⁹case-control χ^2 ; ¹⁰family-based association; ¹¹case-control χ^2 . AD, atopic dermatitis; BREATHE; COPSAC, Copenhagen Prospective Study on Atopy in Children; GENUFAD, Genetic Studies in Nuclear Families with Atopic Dermatitis; MAS, Multicenter Allergy Study; NA, not applicable.

IgE is more commonly found (Flohr *et al.*, 2004), and it is interesting that in a birth-cohort study, *FLG*-null status was also strongly associated with nonatopic eczema (Marenholz *et al.*, 2006). Thus, elevated circulating IgE may be primarily an epiphenomenon that is a proxy for severity in eczema and is therefore more represented in hospital-ascertained series (Flohr *et al.*, 2004).

Although the relationship between *FLG* null alleles and asthma remains to be fully understood, in eczema cohorts where asthma status has been ascertained there is a consistent additional association with asthma as a secondary trait (Marenholz *et al.*, 2006; Weidinger *et al.*, 2006; Weidinger *et al.*, 2007). The more informative cohorts in this regard are those ascertained through a diagnosis of asthma, and population-based cohorts. In the Dundee BREATHE cohort, the association of *FLG* with asthma was entirely limited to the subgroup with a coincident history of eczema, with no association between *FLG* alleles and asthma in the absence of eczema (Palmer *et al.*, 2006). Similarly, in the German Multicenter Allergy Study cohort, *FLG* alleles had no association with asthma or allergic sensitization in the absence of prior eczema (Marenholz *et al.*, 2006). Importantly, this study also observed an additional risk of asthma in patients homozygous for *FLG* null alleles and calculated a striking population attributable risk factor of 20.6% for *FLG* null alleles for the phenotype eczema plus asthma (Marenholz *et al.*, 2006). These data suggest a sequence of phenotypic manifestations rather than individual susceptibilities to each of these manifestations of atopy and also allow speculation that early control of eczema may have an impact on future development of asthma, a concept that has already begun to be explored in studies such as the Early Treatment of the Atopic Child study (Warner, 2001).

Taken together, these early data hint at possible discriminating features of eczema associated with *FLG* null alleles: that the eczema is more strongly associated with asthma, has associated hyperlinearity of the palms, and is more likely to be persistent into adulthood. An additional association

with elevated IgE is also possible but not yet clear. This tentative analysis will doubtless be tempered as additional data become available, but it suggests a starting point to explore genotype-phenotype correlations. It should also be noted that although these carefully phenotyped cohorts where participants have been recruited through a diagnosis of eczema are very informative, they are unable to fully answer the question of what (if anything) is different about *FLG*-related eczema versus non-*FLG*-related eczema, nor can they determine the relative risk of *FLG* null alleles to asthma, atopy, and rhinitis (if any) per se. Large longitudinal or cross-sectional population-based cohorts that have been typed for all *FLG* variants will facilitate the dissection of these phenotypic characteristics and will enable researchers to compare and contrast *FLG* phenotypes versus others to test these speculations. Given the xerotic phenotype of ichthyosis vulgaris (Smith *et al.*, 2006), which is seen in many eczema sufferers, future studies observing additional physical parameters of skin barrier function, such as the degree of skin hydration, the rate of transepidermal water loss, and susceptibility to skin irritation, may help to test the hypothesis of a "dry or defective barrier" type of *FLG*-related eczema. The pharmacogenetic significance of filaggrin status in predicting possible treatment responses to interventions such as emollients, soap substitution, water softening, or pharmacological intervention, and whether such interventions alter the disease trajectory, will also be worth examining.

To date, filaggrin has not been shown to be a susceptibility factor for eczema in non-European populations. There are a few possible explanations for this, including the fact that the currently known recurrent mutations either are not present or are present at very low levels in many non-European populations (Palmer *et al.*, 2006). Also, except in Japan, few research groups have established data sets of non-European eczema patients for genetic analysis. The technical difficulties of sequencing filaggrin may mean that it will take some time to determine the role of *FLG* mutations in populations outside of

Europe. The incomplete penetrance of these alleles also merits further attention and is presumably explained by both genetic and environmental modifiers. Possible genetic modifiers of *FLG* null-allele penetrance include *SPINK5* (Walley *et al.*, 2001) and *SCCE* (Vasilopoulos *et al.*, 2004), both of which have been associated with eczema and both of which are plausibly involved in controlling filaggrin processing. Much has been learned in a short time about the role of *FLG* status in eczema. Much more needs to be understood about its function before this knowledge can be translated directly into patient benefit.

CONFLICT OF INTEREST

The author states no conflict of interest.

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disease hypothesis suggests that PXE is a metabolic disorder, and that, in the absence of MRP6 transport activity in the liver, the plasma levels of critical metabolic compounds are altered (Uitto *et al.*, 2001). Change in the plasma homeostasis would then promote, by currently unknown mechanisms, mineralization of the peripheral target tissues. In this context, it should be noted that MRP6 is a putative basolateral transporter in hepatocytes, and the direction of the transport in a physiologic situation is probably from the intracellular milieu of hepatocytes to plasma in circulation. Thus, one could argue that the main function of such metabolites in normal homeostasis is to prevent ectopic mineralization, and that if the MRP6 transporter is not functional, there is depletion of metabolites in the circulation, resulting in aberrant mineralization in the extracellular milieu (Figure 1).

The PXE cell hypothesis postulates that inactivation of *ABCC6* results in abnormalities in the resident cells in tissues affected in PXE, such as dermal fibroblasts in the skin and arterial smooth muscle cells in the vascular connective tissues. In support of this hypothesis, a number of activities have been shown to be altered in fibroblasts cultured from the skin of patients with PXE. For example, the cells harboring the mutations have been suggested to display abnormalities in cell–cell and cell–matrix adhesion properties, in their proliferative capacity, and in the rate of synthesis of connective tissue components, such as elastin, collagen, and proteoglycans (Boraldi *et al.*, 2003). These cells have also been suggested to suffer from mild chronic oxidative stress and to demonstrate increased degradative potential as reflected by elevated matrix metalloproteinase 2 expression (Quaglino *et al.*, 2005; Pasquali-Ronchetti *et al.*, 2006).

In support of the suggestion that compromised MRP6 activity in resident cells in affected tissues may alter the activity of such cells is the observation that low levels of the protein and mRNA have been detected by immunofluorescence staining, by highly sensitive RNase protection assays, and by multi-round, nested RT-PCR approaches (Beck *et al.*, 2003). It is clear, however, that the level

See related article on pg 581

Pseudoxanthoma Elasticum-Like Phenotypes: More Diseases than One

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The classic pseudoxanthoma elasticum (PXE) phenotype derives from mutations in *ABCC6*. PXE-like phenotypes have been observed in a number of disorders, with no evidence of mutations in *ABCC6*. Vanakker *et al.* report PXE-like skin findings in patients with mutations in *GGCX* critical for γ -carboxylation of gla-proteins. This report expands the clinical spectrum of PXE-like conditions and also provides potential insights into the ectopic mineralization process.

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Pseudoxanthoma elasticum (PXE), a mendelian autosomal recessive disorder, is characterized by ectopic mineralization of connective tissues in a variety of organs, including the skin, the eyes, and the cardiovascular system, with considerable morbidity and mortality (Neldner, 1988). PXE is now known to result from mutations in the *ABCC6* gene (Miksch *et al.*, 2005). This gene encodes multidrug resistance-associated protein 6 (MRP6), a putative transmembrane transporter, expressed primarily in the liver, to a lesser extent in proximal tubules of kidneys, and at very low levels, if at all, in tissues affected in PXE. Adding to the complexity of this disorder are the observations that there is considerable both inter- and intrafa-

miliar heterogeneity, the diagnosis is often delayed because of late onset of manifestations, and a number of environmental and lifestyle variables appear to modulate the progression and eventual outcome of the disease. Finally, the disease has been suggested to be more severe in women, and the female–male ratio of affected individuals has been reported to be approximately 2:1 (Neldner, 1988). It is conceivable, therefore, that a number of both genetic and environmental factors contribute to the phenotypic variability of PXE.

Two hypotheses have been advanced to explain the consequences of *ABCC6* mutations in PXE at the tissue level: the “metabolic disease hypothesis” and the “PXE cell hypothesis.” The metabolic

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