See related article on pg 1302

Breaking the (Un)Sound Barrier: Filaggrin Is a Major Gene for Atopic Dermatitis

Alan D. Irvine[1] and W.H. Irwin McLean[2]

We have recently shown that loss-of-function mutations in the filaggrin gene, carried by about 10% of people of European ethnicity, cause ichthyosis vulgaris and are strong predisposing factors for atopic dermatitis and asthma secondary to atopic dermatitis. These results demonstrate a prominent role for the epidermal barrier in atopic disease and have important implications for the study of complex traits.

Journal of Investigative Dermatology (2006) **126**, 1200–1202. doi:10.1038/sj.jid.5700365

Atopic dermatitis (AD) is one of the major problems in dermatology. It affects 15%–20% of children in developed nations and therefore represents an enormous burden on health care in general. AD has long been recognized as a complex trait where a number of genetic factors interact with environmental stimuli to produce the disease. Because the condition is characterized by inflammation of the skin, a lot of effort has gone into potential immunological mechanisms in AD. With the advent of molecular genetics, a number of genetic loci for AD have been mapped over the past decade, but, as with many stories in complex-trait analysis, definitively causative gene defects have been slow to emerge. More recently, a consensus has emerged that a barrier defect is likely to be a primary event in AD (Taieb *et al.*, 2006). This year, we identified the filaggrin gene (*FLG*) as a major player in AD, showing that a heritable epidermal barrier defect is responsible in many cases of AD. As the common variants we have identified in this gene result in complete loss of filaggrin expression, as opposed to being polymorphisms of uncertain function, this represents hard evidence for a primary keratinocyte defect in a large proportion of AD. We first identified filaggrin mutations in ichthyosis vulgaris (IV), itself a very common keratinizing disorder (Smith *et al.*, 2006). To our surprise, the two mutations we found in IV families, R501X and 2282del4, both of which lead to complete absence of filaggrin expression (Figure 1), are carried by about 10% of people of European origin. Because many of our IV families also had AD, we went on to show that AD is a transmissible monogenic trait with lower penetrance than IV in these families. Two association studies and a small prospective study gave extremely significant statistical association between these *FLG* mutations and AD. For example, in our pediatric AD cohort (Palmer *et al.*, 2006), the χ^2 *P* value was 3×10^{-17}.

We also showed that *FLG* mutations are a major risk factor for eczema-associated asthma, with lower penetrance than AD alone (Palmer *et al.*, 2006). On the basis of our current early data, we estimate that half or more of children with moderate to severe AD carry *FLG* mutations and that maybe as much as 20% of all asthma involves these gene defects, but, importantly, only asthma secondary to AD.

Filaggrin (filament-aggregating protein) was named by the late Peter Steinert, and its gene, *FLG*, is located with many others involved in terminal differentiation, in the epidermal differentiation complex on chromosome 1q21. As mapping of the epidermal differentiation complex was the work of the late Dietmar Mischke, we dedicate this short paper to these eminent erstwhile colleagues, who were well known by keratinocyte biologists and readers of the *Journal of Investigative Dermatology*. The initial product of the *FLG* gene, profilaggrin, is a 400-kilodalton polyprotein consisting of 10–12 tandem repeats of the filaggrin peptide. Profilaggrin is the main constituent of keratohyalin granules and, upon terminal differentiation, is proteolytically cleaved into multiple copies of the 37-kilodalton filaggrin peptide. Filaggrin binds to and aggregates the keratin cytoskeleton, which in these upper granular cells has been strongly anchored to the cell membrane by increasing numbers of desmosomal proteins. Filaggrin collapses the cytoskeleton, resulting in flattening of keratinocytes into squames. This condensed protein-lipid package is heavily cross-linked by transglutaminases to form the epidermal barrier. Filaggrin has been suspected as the causative gene for IV for about 20 years, with compelling biochemical and genetic evidence in humans and mice, so why has it taken so long to uncover its role in this disease and AD? The main reason is that this gene has a very unusual structure that is particularly difficult to sequence routinely. Most of the coding sequences

We estimate that half or more of children with moderate to severe AD carry *FLG* mutations.

[1]Department of Paediatric Dermatology, Our Lady's Hospital for Sick Children, Crumlin, Dublin, Ireland; and [2]Epithelial Genetics Group, Human Genetics Unit, Division of Pathology and Neuroscience, University of Dundee, Ninewells Hospital & Medical School, Dundee, United Kingdom

Correspondence: Prof. Irwin McLean, Human Genetics Unit, Ninewells Hospital & Medical School, Dundee DD1 9SY, United Kingdom. E-mail w.h.i.mclean@dundee.ac.uk

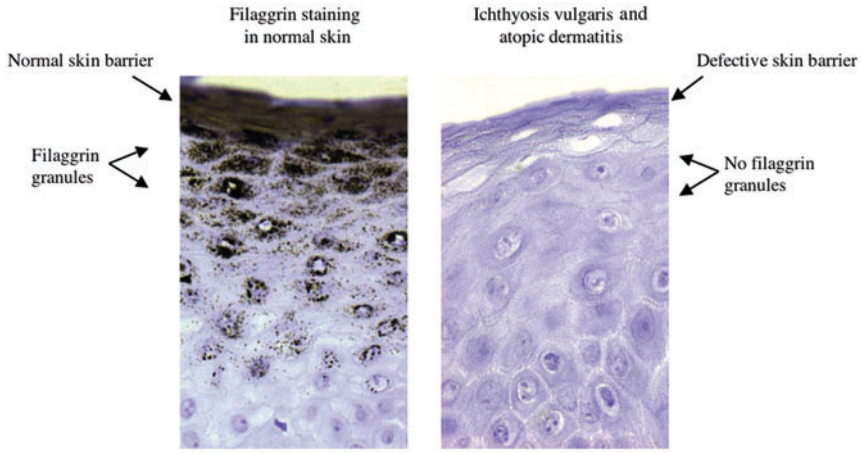

Figure 1. Homozygous *FLG* mutations lead to complete loss of filaggrin expression in skin.
Immunostaining with filaggrin repeat domain mAb 15C10 (Novocastra, Newcastle upon Tyne, United Kingdom) shows the abundant keratohyalin granules present in normal epidermis (left) In contrast, the granular layer is absent in an individual homozygous for a loss-of-function mutation in the filaggrin gene (right). This teenage individual is affected by ichthyosis vulgaris and also has had moderate to severe atopic dermatitis since infancy.

are contained in the third and last giant exon, which is composed of 10–12 almost perfect repeats about 1 kb in size, flanked by two additional imperfect repeats. Only a few isolated unique bases occur in each repeat that allow specific PCR, and in addition, we have found several of these to be polymorphic in the population, so that alleles can easily be lost during PCR. Some repeats contain no unique bases at all. Furthermore, there are size variants in the population, with some alleles having 10, 11, or 12 repeats. Frances Smith was finally able to crack filaggrin, using methods she and one of us (W.H.I.M.) devised for the cloning and diagnostic resequencing of the plectin gene in epidermolysis bullosa simplex with muscular dystrophy (McLean *et al.*, 1996). We can now routinely sequence about 90% of the filaggrin gene and are finding a range of IV- and AD-causing mutations other than the two common European variants.

Filaggrin deficiency in one in ten Europeans, or complete absence of filaggrin in one in 400, leads to mild or severe IV. These varying degrees of impaired keratinocyte differentiation and barrier formation allow increased transepidermal water loss and, importantly, increased entry of allergens, antigens, and chemicals from the environment. Thus, filaggrin-deficient individuals are chronically exposed to insult through the epidermis, which in many cases leads to inflammation of the skin — this is AD. We hypothesize that a percentage of these individuals go on to develop asthma when allergens, to which their immune system has already been primed via cutaneous exposure, later enter the lungs; this is one possible mechanism of AD-associated asthma.

Although this work confirms previous hypotheses of a primary barrier dysfunction in AD, these genetic mutations are ancient and clearly cannot explain the observed increase in AD and atopic disease in the past two decades. These recent increases in the prevalence of AD must be caused, at least in part, through environmental influences on the epithelial barrier. For example, different types of heating systems (Schafer *et al.*, 1999) and low-humidity environments are likely to exacerbate the effects of filaggrin deficiency and increase susceptibility to develop AD (or lower the threshold for developing it). Similar arguments may be advanced regarding hard-water areas, increased use of detergents, environmental pollutants, increased washing, and many other influences. A corollary is that current efforts to intervene early and to potentially prevent the "atopic march" (Hahn and Bacharier, 2005) are now supported by additional evidence that warrants a focus on improving barrier dysfunction. Although the precise population attributable risk of these mutations to AD and asthma will require replication and clarification in other cohorts, including longitudinal studies, it seems likely that carriers of *FLG* null alleles will represent a significant proportion of the at-risk population for AD and associated asthma. If early intervention measures in at-risk atopic children are shown to be effective, screening for *FLG* null alleles may well represent one mechanism of identifying cohorts likely to benefit from such measures.

Two independent genetic mechanisms have given rise to an epithelial barrier defect in around 10% of people of European origin. This frequency is not easily explained by genetic drift. The simultaneous emergence of these two functional polymorphisms to similar carrier frequencies of approximately 5% is consistent with the balanced selection that is seen where there is a heterozygote advantage. Similar mechanisms are widely accepted to underlie the simultaneous emergence of different sickle-cell anemia mutations, under selection pressure by conferring resistance to malaria, and are postulated for resistance to *Salmonella* infections in carriers of cystic fibrosis mutations. Could a minor barrier defect hold, or historically have held, an evolutionary advantage in European populations? One mechanism for this advantage might be through repeated low-level exposure of pathogens to epithelial antigen-presenting cells, effecting a "natural vaccination" and conferring increased immunity to infections such as tuberculosis, influenza, or even the plague. Is atopic disease a modern plague suffered by descendants of the survivors of ancient plagues? Although we have focussed on European populations, it seems likely that Asian and African populations will also have recurrent mutations in *FLG*. The population genetics of *FLG* mutations in these and other populations will be fascinating.

An important lesson has emerged from this work: we should not completely neglect monogenic mendelian disorders in the quest for genes

involved in complex traits. A recent review article makes this point and goes on to show that, surprisingly, the rate of discovery of disease genes has actually decreased since the genome was completed, even though positional cloning and positional candidate genetics are easier than ever before (Antonarakis and Beckmann, 2006). Part of the reason is that funding agencies and, presumably, a good number of peer reviewers regard rare mendelian traits as easy to solve and not worthy of funding. The fact of the matter is that most disease genes, including many involved in complex traits or cancer, have emerged from monogenic disorders. For example, the *patched* gene was identified by study of the very rare Gorlin syndrome but is a major player in basal-cell carcinoma, the most common human cancer. We accept that filaggrin is an unusual example of the genre, and we agree that a large-scale approach to complex traits is required, but we would suggest that a more balanced view be taken, as so much of what we know about basic genetic mechanisms underlying both common and rare disorders continues to come from the mendelian camp.

Our work on filaggrin has firmly established an important role of the keratinocyte and the epidermal barrier formation in atopic dermatitis. As the epidermal differentiation complex locus on 1q21 is also known to harbor a psoriasis susceptibility gene (Bowcock and Cookson, 2004), we posit that a barrier defect is also involved in this complex trait. Furthermore, an autosomal dominant form of the follicular occlusion disorder hidradenitis suppurativa was recently linked to a large interval containing the epidermal differentiation complex (Gao *et al.*, 2006). Thus, the importance of maintaining a sound barrier in the skin and its appendages may come to the fore in genodermatology in the near future.

CONFLICT OF INTEREST
The authors state no conflict of interest.

REFERENCES
Antonarakis SE, Beckmann JS (2006) Mendelian disorders deserve more attention. *Nat Rev Genet* 7:277–82

Bowcock AM, Cookson WO (2004) The genetics of psoriasis, psoriatic arthritis and atopic dermatitis. *Hum Mol Genet* 13(Spec No 1): R43–55

Gao M, Wang P-G, Cui Y, Yang S, Zhang Y-H, Lin D *et al.* (2006) Inversa acne (hidradenitis suppurativa): a case report and identification of the locus at chromosome 1p21.1–1q25.3. *J Invest Dermatol* 126:1302–6

Hahn EL, Bacharier LB (2005) The atopic march: the pattern of allergic disease development in childhood. *Immunol Allergy Clin North Am* 25:231–46

McLean WHI, Pulkkinen L, Smith FJD, Rugg EL, Lane EB, Bullrich F *et al.* (1996) Loss of plectin causes epidermolysis-bullosa with muscular-dystrophy: cDNA cloning and genomic organization. *Genes Dev* 10:1724–35

Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP *et al.* (2006) Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 38:441–6

Schafer T, Heinrich J, Wjst M, Krause C, Adam H, Ring J *et al.* (1999) Indoor risk factors for atopic eczema in school children from East Germany. *Environ Res* 81:151–8

Smith FJD, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y *et al.* (2006) Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 38:337–42

Taieb A, Hanifin J, Cooper K, Bos JD, Imokawa G, David TJ *et al.* (2006) Proceedings of the 4th Georg Rajka International Symposium on Atopic Dermatitis, Arcachon, France, September 15-17, 2005. *J Allergy Clin Immunol* 117:378–90

See related commentary on page 1200

Epidermal Differentiation Complex Yields a Secret: Mutations in the Cornification Protein Filaggrin Underlie Ichthyosis Vulgaris

Julia A. Segre[1]

Ichthyosis vulgaris (IV), characterized by mild scaling on limbs and lower abdomen, has an incidence of 1 in 250. Smith, McLean, and colleagues demonstrate that common mutations in filaggrin underlie IV. Filaggrin aggregates keratin intermediate filaments and is cross-linked into the cornified envelope to form the epidermal barrier. These findings reinforce the importance of the epidermal barrier in pathogenesis of skin diseases.

Journal of Investigative Dermatology (2006) **126**, 1202–1204. doi:10.1038/sj.jid.5700367

Ten years ago the phrase "epidermal differentiation complex" (EDC) was first coined in this journal to describe the 1.6-Mb locus on human chromosome 1q21 that contains more than 30 genes encoding both epidermal cornification and S100 proteins (Mischke *et al.*, 1996). Now, finally, the EDC has revealed one of its most guarded secrets: Resident in this gene complex is the cause of the very common skin disorder ichthyosis vulgaris (IV) (OMIM 146700). In a recent report in *Nature Genetics*, Smith, McLean, and colleagues have convincingly demonstrated that mutations in the gene encoding filaggrin (*FLG*), the keratin filament-aggregating protein, underlie IV (Smith *et al.*, 2006). Twenty years ago, a defect in the synthesis of FLG was observed in IV patients (Sybert *et al.*, 1985). Four years ago,

[1]*National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA*

Correspondence: Dr. Julia A. Segre, National Human Genome Research Institute, National Institutes of Health, 49 Convent Drive, Building 49/Room 4A26, MSC 4442, Bethesda, Maryland 20892, USA. E-mail: jsegre@nhgri.nih.gov