See related commentary on pg 504

Null Mutations in the Filaggrin Gene (*FLG*) Determine Major Susceptibility to Early-Onset Atopic Dermatitis that Persists into Adulthood

Jonathan N.W.N. Barker[1], Colin N.A. Palmer[2], Yiwei Zhao[3], Haihui Liao[3], Peter R. Hull[3], Simon P. Lee[2], Michael H. Allen[1], Simon J. Meggitt[4], Nicholas J. Reynolds[4], Richard C. Trembath[5] and W.H. Irwin McLean[3]

Atopic dermatitis (AD) is a common disease with a complex etiology in childhood and adult life. A significant proportion of childhood AD is transient, but in many cases it persists into adulthood. We have recently shown that null mutations in the filaggrin gene (*FLG*) are an important predisposing factor for childhood eczema and eczema-associated asthma, but persistence to adulthood has not been analyzed. Here we studied a cohort of adult patients with persistent AD, which had been present since early childhood. In this cohort, the combined allele frequency of the two common *FLG* null variants was 0.270 (cf. population frequency 0.046). This represents an odds ratio of 7.7 with 95% confidence interval of 5.3–10.9 and a χ^2 *P*-value of 1.7×10^{-53}. Our data conclusively demonstrate that identification of *FLG* null alleles is an indicator of a poor prognosis in AD, predisposing to a form of eczema that starts in early infancy and persists into adulthood. This study helps to further define the nature of the AD phenotype associated with *FLG* null alleles.

Journal of Investigative Dermatology (2007) **127,** 564–567. doi:10.1038/sj.jid.5700587; published online 21 September 2006

INTRODUCTION

The filaggrin gene, *FLG*, is situated in the epidermal differentiation complex 1q21 (Mischke *et al.*, 1996), a dense cluster of genes encoding proteins involved in terminal differentiation of the epidermis and a shared susceptibility locus for AD (*ATOD2*; OMIM #605803) and psoriasis (*PSORS4*; OMIM #603935). The stratum corneum consists of tightly compacted, highly chemically crosslinked layers of dead cells that form a physical barrier to reduce water loss and protect the body from its chemical, antigenic, and allergenic environment (Candi *et al.*, 2005). Profilaggrin is the main protein component of the keratohyalin granules within the last living cell layers of the epidermis (Irvine and McLean, 2006). Upon terminal differentiation of these granular cells to form the squames, the >400 kDa polyprotein profilaggrin is proteolytically processed into 10–12 copies of the 37 kDa filaggrin peptide, the functional end-product of the *FLG* gene (Steinert *et al.*, 1981; Dale *et al.*, 1985; Gan *et al.*, 1990). These peptides rapidly aggregate the keratin cytoskeleton and bring about squame formation. Thus, filaggrin is essential for the cell compaction process that precedes chemical cross-linking and skin barrier formation.

FLG has an unusual structure where the vast majority of profilaggrin is encoded by the third and final exon, which consists of 10–12 tandemly repeated copies of the ~1 kb filaggrin sequence (Smith *et al.*, 2006). Recently, we found that about 10% of people of European origin carry one of two loss-of-function mutations in *FLG*, designated R501X and 2282del4 (Smith *et al.*, 2006). Both of these mutations lead to premature termination codons within repeat 1 and were shown biochemically to cause complete absence of processed filaggrin in the epidermis (Smith *et al.*, 2006). Less common mutations, located centrally in the filaggrin repeat domain, have also been identified (Sandilands *et al.*, 2006). These mutations were proven to cause ichthyosis vulgaris (dry, scaly skin) with a mild phenotype in heterozygotes or a much more marked phenotype in homozygotes or compound heterozygotes (Sandilands *et al.*, 2006; Smith *et al.*, 2006). Importantly, it was subsequently shown that these mutations are a major predisposing factor for atopic dermatitis (AD; Palmer *et al.*, 2006).

In 45% of children with AD, onset occurs within the first 6 months of life; in 60%, AD occurs during the first 12 months; and at least 85% are affected before their fifth birthday (Kay *et al.*, 1994). In children with onset at age less than 2 years,

[1]*St John's Institute of Dermatology, King's College London, St Thomas's Hospital, London, UK;* [2]*Biomedical Research Center, Population Pharmacogenetics Group, Biomedical Research Centre, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK;* [3]*Division of Pathology and Neuroscience, Epithelial Genetics Group, Human Genetics Unit, Division of Pathology and Neuroscience, University of Dundee, Ninewells Hospital and Medical School, Dundee, UK;* [4]*Department of Dermatology, University of Newcastle upon Tyne, Newcastle upon Tyne, UK and* [5]*Deparment of Medical and Molecular Genetics, King's College London, London, UK*

Correspondence: Professor William H.I. McLean, Epithelial Genetics Group, Human Genetics Unit, Division of Pathology and Neuroscience, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK
E-mails: w.h.i.mclean@dundee.ac.uk; jonathan.barker@kcl.ac.uk

Abbreviation: AD, atopic dermatitis

Received 27 July 2006; revised 22 August 2006; accepted 23 August 2006; published online 21 September 2006

20% will have persistent AD and a further 17% will have intermittent symptoms by age 7 years (Illi *et al.*, 2004). In contrast, onset after adolescence is only recorded in 16.8% of adults with AD (Williams and Strachan, 1998; Ozkaya, 2005). Here, we present data strongly confirming that filaggrin mutations are a major genetic factor in AD. Significantly, we show that carrying one or two filaggrin null alleles can lead to early-onset AD that persists well into adulthood.

RESULTS AND DISCUSSION

In our cohort of 163 adults with childhood onset persistent AD, we observed a greatly elevated frequency of the two *FLG* null alleles that are common in European populations, R501X and 2282del4. Specifically and strikingly, the combined allele frequency for these two *FLG* null variants was 0.270 in the AD cohort in contrast to 0.046 in the ethnically matched population controls, that is, there was almost 6-fold over-representation of the null alleles in the AD cohort. Another way of looking at this data is that 8.8% of the general population carry one or more *FLG* null alleles, whereas 42% of the AD cohort carry one or more of these *FLG* alleles. The *FLG* genotype data in the AD cohort and in a group of 1,463 ethnically matched population controls of anonymous phenotype are summarized in Table 1. The association between the *FLG* null variants and AD was highly statistically significant for each of the two common null variants. Specifically, for R501X, the χ^2 *P*-value was 1.0×10^{-27}, and for 2282del4, the *P*-value was 3×10^{-26}. The fact that each of the individual *FLG* variants produced highly significant *P*-values gives within-study replication. Combining both null alleles gave an extremely significant χ^2 *P*-value of 1.7×10^{-53} compared to the control population. The combined genotype data also gave an odds ratio of 7.7 with 95% confidence interval of 5.3–10.9 using Fisher's exact test, showing that the association is highly robust using more than one of the recognized statistical methods for analysis of this type of case:control association study.

Atopic disease (atopy) affects up to 20% of children in the developed world (Holgate, 1999). Atopy is characterized by AD, which is often present from early childhood and often accompanied later in life by a range of allergic diseases, importantly including asthma, food allergy and allergic rhinitis (hay fever). Recent genetic evidence in the form of highly prevalent *FLG* null alleles in atopic individuals suggests that the primary defect is a failure of skin barrier function, allowing abnormally enhanced cutaneous presentation of antigens, allergens and chemicals to the immune system (Hudson, 2006; Palmer *et al.*, 2006; Weidinger *et al.*, 2006). About 9% of individuals in a number of European populations studied to date carry one of the two common *FLG* null alleles, that is, the population allele frequency is about 0.045. These null alleles have now been shown to strongly associate with AD in several distinct cohorts. In a small Irish cohort of children attending a pediatric dermatology department with severe AD, the *FLG* null-allele frequency was 0.336; in a large German cohort of almost 500 families, ascertained through probands attending hospital with AD and including their siblings, the null-allele frequency in individuals with AD was 0.249 (Weidinger *et al.*, 2006); and in a cohort of Scottish children, ascertained through asthma, who had both asthma and AD, the allele frequency was 0.254. A lower carrier rate of 0.190 of AD-diagnosed individuals was seen in children born to mothers with asthma in a community-based prospective cohort from Denmark (Palmer *et al.*, 2006). In this current cohort of adult patients with persistent AD attending hospital dermatology departments, the *FLG* null-allele frequency was strikingly high at 0.270. These data showing high representation of *FLG* null alleles in this well-phenotyped adult AD cohort help to better define AD. In particular, *FLG*-null or *FLG*-haploinsufficient AD appears to be a key factor in 20–25% of pediatric AD cases in several communities and may be associated with increased severity in childhood (Palmer *et al.*, 2006; Weidinger *et al.*, 2006). Our data here suggest that *FLG*-associated AD represents a significant proportion of persistent disease through to adulthood as 42% of the childhood-onset, adult-persistent AD cohort examined here carried one or more *FLG* null alleles. A large population-based study will be necessary to further validate these early interpretations and to gain detailed information about the penetrance and age milestones for the various atopic phenotypes associated with *FLG* variants.

Table 1. *FLG* null variant genotypes in adults with atopic dermatitis

	R501X		2282del4		Combined null genotype	
Genotypes	**Control population**	**AD cohort**	**Control population**	**AD cohort**	**Control population**	**AD cohort**
AA	1,381	121	1,414	129	1,334	94
Aa	79	33	49	30	124	49
aa	3	9	0	4	5	20
Totals	1,463	163	1,463	163	1,463	163
		$P=1.0 \times 10^{-27}$		$P=3 \times 10^{-26}$		$P=1.7 \times 10^{-53}$

AD, atopic dermatitis; AA, homozygous wild type for *FLG* null variant; Aa, heterozygous for either of the common *FLG* null alleles R501X or 2282del4; aa, homozygous or compound heterozygous for *FLG* null alleles R501X or 2282del4.
For the combined genotype data, note that compound heterozygotes ($n=7$) are counted as ''aa''. For example, in the AD cohort, the total is 20 (nine R501X homozygotes, four 2282del4 homozygotes, and seven R501X/2282del4 compound heterozygotes).

Table 2. Demographic data of AD cohort

Number of patients	163
Ethnicity	White British (51 NE England; 112 SE England)
Mean age at examination (years)	36.4 (range 16–82)
Mean age of AD onset	3.9 years (3 months–44 years)
Age of onset breakdown (years)	
Birth–2	115 (73%)
3–5	21 (13%)
6–10	9 (5%)
11–15	2 (1%)
16–20	4 (2%)
>21	7 (4%)
<18 but not exact age of onset unknown	5 (3%)
IgE	Mean=5,752 kU/l, range 23–71,000 kU/l 94% cases had elevated IgE (>70 kU/l)

AD, atopic dermatitis; NE, northeast; SE, southeast.

In biological terms, filaggrin is essential for bringing about a cascade of biochemical and cellular events leading to the formation of the stratum corneum – the dead, chemically crosslinked cell layers within which the skin barrier function resides (Listwan and Rothnagel, 2004). We have shown previously that the stratum corneum is poorly formed in filaggrin-null individuals (Smith *et al.*, 2006) and we hypothesize that this leads to increased passage of antigens, allergens, and chemicals through the epidermis where one or more of these agents invoke an immune response, manifesting as the various features of atopy (Irvine and McLean, 2006). Since skin barrier formation is impaired from birth in individuals carrying one or more *FLG* null alleles, it is logical to expect that the onset of AD is both early and persistent into adulthood. Similarly, longitudinal studies of patient behavior and/or micro-environment (Williams *et al.*, 2004) may help identify trigger factors that act upon the *FLG*-null or *FLG*-haploinsufficient genotypes to produce AD and other allergic phenotypes. Such data will inform future genetic testing regimens and perhaps shed light on beneficial behavioral or environmental interventions.

One can postulate that changes in the epidermal barrier lead to inflammation in the skin, which once established is difficult to reverse (Hudson, 2006). To this end, it is of note that prevalence of AD in adulthood is greater in individuals who undertake occupations that provoke irritation of the skin, for example, hairdressers or caterers (Holm and Veierod, 1994). Therapeutic strategies aimed at correcting this barrier early are therefore likely to have long-term beneficial effects.

MATERIALS AND METHODS

Study populations

All DNA samples were collected with institutional research ethics committee approval (King's College London and University of Newcastle) and informed consent that complies with all the Declaration of Helsinki Principles. One hundred and sixty-three adults with persistent AD were recruited from hospital-based dermatology clinics in England. All subjects were of white British ancestry. All study subjects met the UK Working Party definition of AD as interpreted by an experienced dermatologist (J.N.B., S.M., or N.J.R.) (Williams *et al.*, 1994a, b). The majority of individuals recruited had an age of onset of less than 16 years of age (92%). In five cases (3% of the cohort), the precise age of onset was <18 but was not precisely known. In seven cases (4%), the age of onset was >21 years. The AD cohort was reported previously (Veal *et al.*, 2005) and the demographics and clinical parameters of this study cohort are summarized in Table 2. A total of 1463 population controls were used, who were also of white British ancestry. DNA was prepared from whole blood using standard procedures.

Filaggrin genotyping

Genotyping for R501X was performed using a TAQMAN-based allelic discrimination assay (Applied Biosystems, Foster City, CA). Standard procedures were used based on Applied Biosystems reagents and 10 μl reaction volumes. Allelic discrimination was assessed using an Applied Biosystems 7700 sequence detection system. Probes and primers were as described previously (Palmer *et al.*, 2006). Mutation 2282del4 was genotyped by sizing of a fluorescently labelled PCR fragment on an Applied Biosystems 3100 or 3730 DNA sequencer as described previously (Palmer *et al.*, 2006).

Statistical analysis

All statistical analysis in the association study was performed using SPSS for Windows, v.11.5 or Instat 3 for Macintosh (Graphpad Software Inc., San Diego, CA). Allele frequencies were compared using Pearson's χ^2 tests of the three genotype frequencies (AA, Aa, aa); odds ratios for dominant models (AA vs aX) were determined using Fisher's exact tests and binary logistic regression. All *P*-values shown are not corrected for multiple testing as all the tests of the primary hypotheses firmly rejected the null hypothesis. All variants were in Hardy–Weinberg equilibrium. Note that when combining

the two null genotypes, ''AA'' refers to an individual who carries neither *FLG* mutation; ''Aa'' refers to an individual who is heterozygous for either R501X or 2282del4; and ''aa'' refers to individuals homozygous for R501X, or homozygous for 2282del4, or compound heterozygous for R501X/2282del4 (Table 1).

CONFLICT OF INTEREST

W.H.I. McLean has filed patents on genetic testing and therapy relating to filaggrin.

ACKNOWLEDGMENTS

We thank the patients and their families for their participation, which made this research possible. We also thank Alan D. Irvine, Our Lady's Children's Hospital, Crumlin, Dublin, for critical reading of this paper and to Jayne Mcfarlane, Epithelial Genetics Group, for clerical assistance. The Barker group was supported by The British Skin Foundation (N.J.R. and J.N.B.) and The Wellcome Trust (R.C.T. and J.N.B.). The McLean group is supported by grants from The Dystrophic Epidermolysis Bullosa Research Association, The Pachyonychia Congenita Project, and The British Skin Foundation/National Eczema Society. The Palmer group is supported by the Biotechnology and Biological Sciences Research Council (award D13460), Scottish Enterprise Tayside and the Gannochy Trust, and the Scottish Executive Genetic Health Initiative.

REFERENCES

Candi E, Schmidt R, Melino G (2005) The cornified envelope: a model of cell death in the skin. *Nat Rev Mol Cell Biol* 6:328–40

Dale BA, Resing KA, Lonsdale-Eccles JD (1985) Filaggrin: a keratin filament associated protein. *Ann NY Acad Sci* 455:330–42

Gan SQ, McBride OW, Idler WW, Markova N, Steinert PM (1990) Organization, structure, and polymorphisms of the human profilaggrin gene. *Biochemistry* 29:9432–40

Holgate ST (1999) The epidemic of allergy and asthma. *Nature* 402:B2–4

Holm JO, Veierod MB (1994) An epidemiological study of hand eczema. V. Prevalence among hairdresser trainees, compared with a general population of hairdressers. *Acta Dermatol Venereol Suppl (Stockholm)* 187:23–5

Hudson TJ (2006) Skin barrier function and allergic risk. *Nat Genet* 38:399–400

Illi S, von Mutius E, Lau S, Nickel R, Gruber C, Niggemann B *et al.* (2004) The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. *J Allergy Clin Immunol* 113:925–31

Irvine AD, McLean WH (2006) Breaking the (un)sound barrier: filaggrin is a major gene for atopic dermatitis. *J Invest Dermatol* 126:1200–2

Kay J, Gawkrodger DJ, Mortimer MJ, Jaron AG (1994) The prevalence of childhood atopic eczema in a general population. *J Am Acad Dermatol* 30:35–9

Listwan P, Rothnagel JA (2004) Keratin bundling proteins. *Methods Cell Biol* 78:817–27

Mischke D, Korge BP, Marenholz I, Volz A, Ziegler A (1996) Genes encoding structural proteins of epidermal cornification and S100 calcium-binding proteins form a gene complex (''epidermal differentiation complex'') on human chromosome 1q21. *J Invest Dermatol* 106:989–92

Ozkaya E (2005) Adult-onset atopic dermatitis. *J Am Acad Dermatol* 52:579–82

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

Sandilands A, O'Regan G M, Liao H, Zhao Y, Terron-Kwiatkowski A, Watson RM *et al.* (2006) Prevalent and rare mutations in the gene encoding filaggrin cause ichthyosis vulgaris and predispose individuals to atopic dermatitis. *J Invest Dermatol* 126:1770–5

Smith FJ, 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

Steinert PM, Cantieri JS, Teller DC, Lonsdale-Eccles JD, Dale BA (1981) Characterization of a class of cationic proteins that specifically interact with intermediate filaments. *Proc Natl Acad Sci* 78:4097–101

Veal CD, Reynolds NJ, Meggitt SJ, Allen MH, Lindgren CM, Kere J *et al.* (2005) Absence of association between asthma and high serum immunoglobulin E associated GPRA haplotypes and adult atopic dermatitis. *J Invest Dermatol* 125:399–401

Weidinger S, Illig T, Baurecht H, Irvine AD, Rodriguez E, Diaz-Lacava A *et al.* (2006) Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. *J Allergy Clin Immunol* 118:214–9

Williams HC, Burney PG, Hay RJ, Archer CB, Shipley MJ, Hunter JJ *et al.* (1994a) The UK Working Party's Diagnostic Criteria for Atopic Dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *Br J Dermatol* 131:383–96

Williams HC, Burney PG, Pembroke AC, Hay RJ (1994b) The UK Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation. *Br J Dermatol* 131:406–16

Williams HC, Strachan DP (1998) The natural history of childhood eczema: observations from the British 1958 birth cohort study. *Br J Dermatol* 139:834–9

Williams JR, Burr ML, Williams HC (2004) Factors influencing atopic dermatitis – a questionnaire survey of schoolchildren's perceptions. *Br J Dermatol* 150:1154–61