Filaggrin Null Alleles Are Not Associated with Psoriasis

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Psoriasis is a common skin disease with an etiology consistent with a multifactorial trait. Several psoriasis susceptibility loci are known, a number of which are also implicated in a predisposition to atopic dermatitis (AD), including the epidermal differentiation complex on chromosome 1q21. It has recently been shown in several replicate studies that prevalent null alleles for the filaggrin gene (*FLG*) on 1q21 are an important genetic factor in AD. Here, we examined the role of these *FLG* variants in psoriasis using case:control association studies comparing Irish and UK psoriasis cohorts (combined $n=691$) to ethnically matched populations (combined $n=2117$). No association was present for the two common European *FLG* mutations R501X and 2282del4 (combined χ^2 $P=0.989$). In addition, the 3′ end of the *FLG* open-reading frame was sequenced in a number of patients with differing types of psoriasis (plaque, guttate, palmoplantar, and late-onset), which excluded the possibility of a gain-of-function frameshift mutation such as those found in loricrin or certain keratin genes. These data suggest that *FLG* mutations are unlikely to be involved in genetic susceptibility to psoriasis and implies that there may be within-locus heterogeneity in chromosomal regions involved in both AD and psoriasis.

Journal of Investigative Dermatology (2007) **127,** 1878–1882; doi:10.1038/sj.jid.5700817; published online 5 April 2007

INTRODUCTION

Psoriasis is a chronic inflammatory skin condition affecting 2–3% of many human population groups and is therefore a considerable cause of morbidity worldwide (Griffiths *et al.*, 2004; Smith and Barker, 2006). The disease presents as a variety of distinguishable subtypes and appears to be a classic multifactorial disorder where genetic predisposition factors combine with environmental stimuli to produce the disease. Stable psoriasis is a heterogeneous disorder with a number of distinct clinical phenotypes suggesting possible different genetic backgrounds. These include chronic plaque psoriasis, guttate psoriasis, and palmoplantar pustular psoriasis (palmoplantar pustulosis). Two further groupings of chronic plaque psoriasis have been recognized, early-onset psoriasis (Type 1 psoriasis) with onset generally before the age of 40 years, and late-onset psoriasis (Type 2 psoriasis) with onset after the age of 50 years (Griffiths *et al.*, 2004). Late-onset and palmoplantar pustular variants have been shown to be genetically distinct from classic forms of psoriasis and perhaps should not be considered as psoriasis *per se* (Asumalahti *et al.*, 2003; Allen *et al.*, 2005). A number of susceptibility loci have been mapped by linkage analysis in large families or by genome-wide transmission studies, including genetic linkage in families, transmission disequilibrium testing, and sibling-pair analysis. The predominant locus for psoriasis vulgaris is *PSORS1*, which lies in the region of the major histocompatibility complex on chromosome 6p21.3, where about half of all psoriatic patients carry the HLA-Cw6 allele (Trembath *et al.*, 1997). Recent data suggest that the Cw6 allele may in fact be the causative variant; however, this awaits independent confirmation (Nair *et al.*, 2006). A number of other loci have been mapped for psoriasis, as reviewed recently (Bowcock and Cookson, 2004; Capon *et al.*, 2004; Bowcock

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Abbreviations: AD, atopic dermatitis; EDC, epidermal differentiation complex

Received 2 November 2006; revised 12 December 2006; accepted 14 January 2007; published online 5 April 2007

and Krueger, 2005; Kere, 2005) and interestingly, a number of the known susceptibility loci for psoriasis are also shared with susceptibility for atopic dermatitis (AD), another common inflammatory skin condition that is inherited as a multifactorial complex trait (Bowcock and Cookson, 2004). AD is more common than psoriasis, affecting up to 20% of children in the developed world. It is also associated with the progressive development of a range of other allergic conditions that make up the ''atopic march'' which include food allergy, asthma, rhinitis, and others (Spergel and Paller, 2003). The apparent colocalization of these two inflammatory skin diseases suggests two possible pathomechanistic hypotheses. Firstly, variations within a single gene located in these shared loci might predispose to both of these inflammatory skin diseases. Included in this hypothesis is the possibility that distinct classes of mutations in the same gene, for example, nonsense or missense mutations, are involved AD or psoriasis, respectively. The second, alternative, hypothesis is that some of these loci may be gene clusters where polymorphisms in two or more physically close and genetically similar genes, encoding functionally related but nevertheless distinct molecules, might give rise to either psoriasis or AD.

One such shared locus is the epidermal differentiation complex (EDC) on chromosome 1q21.3, a cluster of many dozens of genes encoding molecules found in the uppermost layers of the differentiating epidermis (Bowcock and Cookson, 2004). The EDC region was identified as a susceptibility locus for psoriasis by analysis of Italian kindreds (Capon *et al.*, 1999, 2001) and was subsequently shown to be a locus for AD (Cookson *et al.*, 2001). Recently, we have shown that loss-of-function alleles of *FLG*, the gene encoding filaggrin, which is located in the EDC, cause the common monogenic skin disorder ichthyosis vulgaris (Sandilands *et al.*, 2006; Smith *et al.*, 2006). We went on to show that these same common mutations, carried by ~9% of white European populations, also determine major susceptibility to early-onset, persistent, moderate-to-severe AD and eczema-associated asthma (Palmer *et al.*, 2006). This association has already been replicated several times in a range of European populations (Marenholz *et al.*, 2006; Ruether *et al.*, 2006; Weidinger *et al.*, 2006; Barker *et al.*, 2007; Stemmler *et al.*, 2007) and so represents a highly robust association of an EDC gene in AD and the secondary allergic diseases that make up the atopic march (Spergel and Paller, 2003).

Intriguingly, there are reports in the literature of changes in the expression pattern of filaggrin in psoriatic epidermis, compared to both non-lesional skin and normal controls. Specifically, filaggrin expression is lost from the granular layer in psoriatic lesions although it is present in non-lesional epidermis (Watanabe *et al.*, 1991). However, there are also changes in the expression of other EDC-encoded proteins closely related to filaggrin, such as hornerin, which is upregulated in psoriatic lesions (Takaishi *et al.*, 2005) or trichohyalin, which is redistributed in psoriatic plaques (Ishida-Yamamoto *et al.*, 1997). Thus, it remains to be seen if the alterations in filaggrin expression in psoriasis are primary or secondary events in the pathogenesis of the disease. Here, we address the question as to whether or not the same filaggrin null variants found in up to half of AD individuals are also the 1q21 genetic predisposing factor for psoriasis.

RESULTS AND DISCUSSION

Two *FLG* mutations, R501X and 2282del4, are prevalent in the white European population (Palmer *et al.*, 2006). Both variants lead to premature termination of profilaggrin translation within the first filaggrin repeat domain and therefore are functional null alleles for filaggrin, the end product of the *FLG* gene (Smith *et al.*, 2006). A total of 271 Irish cases of psoriasis vulgaris and 654 unselected Irish controls were genotyped for R501X and 2282del4 using established methods (Palmer *et al.*, 2006). Comparison of the allele frequencies between the psoriasis cohort with the Irish population controls gave a Pearson χ^2 value of $P=0.075$ for the R501X variant and $P=0.932$ for 2282del4 allele (Table 1). Thus, there appeared to be a weak association of the R501X variant but did not reach significance. Combining both null allele genotypes resulted in χ^2 $P=0.169$ (Table 1) and therefore there was no significant association of these *FLG* null alleles with psoriasis in the Irish population.

By the same methods, 420 UK cases of psoriasis were compared with 1463 ethnically matched population controls. The R501X allele again gave a weak association that failed to reach significance, χ^2 $P=0.075$, whereas the 2282del4 allele showed no significant association ($P=0.366$). Combining the genotype data for the two null alleles gave no significant association overall ($P=0.419$; Table 2).

Combining the Irish and UK psoriasis cohorts ($n=691$) and comparing these with the combined Irish/UK population controls ($n=2117$) gave no significant association for either R501X ($P=0.515$), 2282del4 ($P=0.457$), or the combined genotype ($P=0.989$; see Table 3). Another way of considering these data is that the combined allele frequency was essentially identical in the psoriasis cases (0.041, 95% confidence interval 0.031–0.052) and the combined controls (0.041, 95% confidence interval 0.035–0.047); the larger number of controls ($n=2117$) giving slightly tighter confidence intervals compared to the patient cohort ($n=691$).

Table 1. Irish psoriasis case:control association study

	R501X		2282del4		Combined genotype	
	Population	Psoriasis	Population	Psoriasis	Population	Psoriasis
AA	634	256	633	262	613	247
Aa	20	15	21	9	41	24
aa	0	0	0	0	0	0
	654	271	654	271	654	271
	P=0.075		P=0.932		P=0.169	

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.

Table 2. UK psoriasis case:control association study

	R501X		2282del4		Combined genotype	
	Population	Psoriasis	Population	Psoriasis	Population	Psoriasis
AA	1381	406	1414	402	1334	388
Aa	79	13	49	18	124	31
aa	3	1	0	0	5	1
	1463	420	1463	420	1463	420
	P=0.075		P=0.366		P=0.419	

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 are counted as 'aa'. For example, in the population cohort, the total is five (three R501X homozygotes, plus two R501X/2282del4 compound heterozygotes). For this reason, the figures in this table do not appear to add up at first glance.

Table 3. Combined UK/Irish psoriasis case:control association study

	R501X		2282del4		Combined genotype	
	Population	Psoriasis	Population	Psoriasis	Population	Psoriasis
AA	2015	662	2047	664	1947	635
Aa	99	28	70	27	165	55
aa	3	1	0	0	5	1
	2117	691	2117	691	2117	691
	P=0.515		P=0.457		P=0.989	

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 are counted as 'aa'. For example, in the population cohort, the total is five (three R501X homozygotes, plus two R501X/2282del4 compound heterozygotes). For this reason, the figures in this table do not appear to add up at first glance.

Thus, the very weak and opposing associations observed in the individual Irish and UK cohorts for R501X completely disappear in a larger case:control association study, and these results strongly suggest that filaggrin null alleles do not account for the 1q21 linkage observed in psoriasis. As it has been shown that the late-onset and palmoplantar pustular forms of psoriasis lack the strong HLA-Cw6 association seen in psoriasis vulgaris and guttate psoriasis (Asumalahti *et al.*, 2003; Allen *et al.*, 2005), we extracted the data for the former subtypes from the patient cohorts and considered these separately. This showed that the combined *FLG* null allele frequency did not vary significantly from the population controls for either palmoplantar pustular ($n=78$; $P=0.566$) or late-onset psoriasis ($n=93$; $P=0.329$). Therefore, neither of the two apparently genetically distinct psoriasis variants shows association with the two common filaggrin null variants.

Many of the genes in the EDC, including those encoding filaggrin and loricrin, consist of highly repetitive sequences both at the DNA and protein level. A consequence of this type of repetitive sequence is that frameshift mutations may not lead to premature termination codon mutations for a considerable distance following the frameshift. Depending on the precise sequence involved, this can lead to the expression of long foreign peptide fused to the N-terminus of the protein, which exerts a powerful dominant-negative effect. Such delayed termination codon mutations in the loricrin gene are the cause of the Camisa type mutilating keratoderma (Maestrini *et al.*, 1996) and similar defects in the final exon of the keratin 1 gene, which is structurally similar to loricrin, filaggrin, and other EDC genes, leads to a form of ichthyosis hystrix (Sprecher *et al.*, 2001). To exclude the possibility that this class of mutation might lead to psoriasis susceptibility, we undertook sequencing of the 3′end of the *FLG* exon 3, in four unrelated patients with each of the following subtypes: early-onset plaque, palmoplantar pustular, guttate, and late-onset plaque psoriasis (i.e., 16 patients in total). Specifically, the region of *FLG* exon 3 encoding filaggrin repeats 9, 10 and the partial repeat 11, was amplified using repeat-specific primers in three overlapping PCR fragments and fully sequenced using internal primers ending on unique non-polymorphic bases. No sequence changes were detected in these patients and so this type of C-terminal gain of function mutation was excluded.

Filaggrin is a highly abundant protein in the outermost layers of the epidermis. The keratohyalin granules which give the granular cells their name are mainly composed of the >400 kDa profilaggrin, which upon terminal differentiation is proteolytically cleaved into 10 or more copies of the 37 kDa filaggrin protein (Presland and Dale, 2000; Candi *et al.*, 2005). The liberated filaggrin binds to and aggregates the keratin cytoskeleton leading to keratinocyte compaction and formation of the stratum corneum. Patients homozygous for the R501X or 2282del4 mutations in *FLG* completely lack keratohyalin granules in their skin and have severe ichthyosis vulgaris and a high risk of atopic disease (Palmer *et al.*, 2006). Heterozygotes may show subtle signs of ichthyosis vulgaris and have a high risk of developing AD. It has also been suggested that filaggrin is further broken down in the stratum corneum into hygroscopic amino acids and derivatives thereof that may act as a natural moisturizing substance (Rawlings and Harding, 2004). Thus, complete loss of filaggrin or haploinsufficiency for the protein is consistent with the xerotic skin and impaired barrier function observed in ichthyosis vulgaris and AD. The inflammation of the skin seen in filaggrin mutation carriers is thought to be a secondary phenomenon following increased transepidermal antigen/allergen/irritant transfer through the defective skin barrier (Hudson, 2006). The coincident genetic linkage of psoriasis to 1q21 suggests that an analogous mechanism might also be a factor in this inflammatory skin disease.

Here, we have shown that although the EDC on 1q21.3, containing *FLG*, is a recognized psoriasis susceptibility locus, *FLG* null alleles are not predisposing factors in psoriasis. Furthermore, we have excluded mutations in the 3′ end of

filaggrin gene in 16 patients with four specific subtypes of psoriasis. Therefore, it appears that the EDC psoriasis gene, *PSORS4* (Capon *et al.*, 1999), is not *FLG*. Our data are consistent with the high-resolution linkage mapping of this locus using Italian families, which suggested that the causative variant for psoriasis is in the vicinity of the loricrin gene (Capon *et al.*, 2001), although the coding regions of this gene do not appear to harbor mutations or variants that fully co-segregate with psoriasis (Giardina *et al.*, 2004). Polymorphisms affecting the loricrin promoter may exist but are more difficult to prove as causative at the functional level compared to null mutations. The fact that *FLG* can be excluded as an EDC gene for psoriasis, although it is clearly very important in AD suggests that, in the 1q21 locus at least, the shared genetic susceptibility observed for these inflammatory skin conditions may be due to the clustering of genes with similar but non-identical functions rather than distinct clinical conditions arising from the same causative polymorphisms. It will be of interest to see if this trend also holds true for the other shared eczema/psoriasis loci on 3q21, 17p25, and 20p once the causative genes are determined. As filaggrin is a key player in epidermal barrier formation and hydration, will the psoriasis gene on 1q21 also be a barrier protein or will it have an immune regulatory function, as suggested for members of the S100 protein family nearby? Further fine mapping and molecular genetic characterization of genes within this locus should shed light on this question in the near future.

MATERIALS AND METHODS

Study populations

All DNA samples were collected with institutional research ethics committee approval (St James Hospital and Adelaide and Meath Hospital Ethics Committee, St Vincents's University Hospital Ethics Committee, and King's College London Ethics Committee) and informed consent that complies with the Declaration of Helsinki Principles. All subjects were of white Irish or British ancestry.

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 fluorescent-labelled PCR fragment on an Applied Biosystems 3100 or 3730 DNA sequencer as described previously (Palmer *et al.*, 2006). The region of *FLG* exon 3 encoding filaggrin repeats 9, 10 and the partial repeat 11 was amplified using repeat-specific primers in three overlapping PCR fragments and fully sequenced using internal primers ending on unique non-polymorphic bases (A. Sandilands and W.H.I. McLean, paper in preparation).

Statistical analysis

Power calculations were carried out assuming a disease prevalence for psoriasis of 2% and a gene dose multiplicative model (which is observed for AD). These calculations showed that the study had 89% power to detect a genotype relative risk of 2 at an alpha of 0.05, or 80% power to detect a genotype relative risk of 1.87 at an alpha of 0.05. That is, the study had ample power to detect the types of genetic associations being sought here. All statistical analyses in the association studies were 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 χ^2 tests of the three genotype frequencies (AA, Aa, aa). 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 either R501X or 2282del4 or, compound heterozygous for R501X/2282del4.

CONFLICT OF INTEREST

Irwin McLean has filed patents on filaggrin genetic testing and therapy development based on the filaggrin gene.

ACKNOWLEDGMENTS

We thank the patients and their families for their participation, which made this research possible. We also thank 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.), The Wellcome Trust (R.C.T. and J.N.B.), and the Psoriasis Association (J.N.W.N.B, C.E.M.G., and D.B.). The McLean group is supported by grants from The Dystrophic Epidermolysis Bullosa Research Association, The Pachyonychia Congenita Project, the British Skin Foundation/National Eczema Society (W.H.I.M and Frances J.D. Smith). Filaggrin research in Dundee is supported by a donation from an anonymous family from Tayside, Scotland. 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. We acknowledge the following clinicians for their support: Louise Barnes and Rosemarie Watson, St James Hospital, Dublin; Paul Collins, Barry Bresnihan, and Doug Veale, St Vincent's University Hospital, Dublin.

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