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R501X and 2282del4 Filaggrin Mutations Do Not Confer Susceptibility to Psoriasis and Atopic Dermatitis in Italian Patients

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Key Words

Psoriasis • Atopic dermatitis • Filaggrin • Complex disease

Psoriasis (PS) is a chronic inflammatory skin disorder characterized by keratinocyte hyperproliferation and altered differentiation. Atopic dermatitis (AD) is a common chronic inflammatory skin disease characterized by itchy inflamed skin. Genome-wide linkage studies for PS/AD revealed a significant linkage to a region on chromosome 1q21 [1, 2], containing the epidermal differentiation complex (EDC). EDC is a cluster of genes with a key role in terminal differentiation of human epidermis. Recently we demonstrated a co-localization of the PS (PSORS4) and AD (ATOD2) susceptibility loci in a 42-kb interval in Italian patients [3].

Most recently, it has been reported that two loss-of-function mutations – R501X and 2282del4 – within the filaggrin gene (*FLG*) located in the epidermal differentiation complex are associated with AD in several populations [4–6]. Loss-of-function mutations in *FLG* predispose to ichthyosis [7], which in its mild form shares clinical characteristics with PS and AD such as dryness and altered differentiation of keratinocytes. In the outer granular layer of the epidermis, filaggrin is associated with keratin intermediate filaments, supports their packing into bundles and plays a critical role in carrying out the protective task of epidermis. In terminally differentiated keratinocytes, filaggrin is cross-linked to the cornified cell envelope, which constitutes an insoluble barrier of the stratum corneum protecting the organism against environmental agents and preventing epidermal water loss. The loss of epidermal barrier function represents one of the first steps in the development of PS and AD and therefore *FLG* can be considered an obvious predisposing gene to both diseases.

Although *FLG* is located 1 Mb from the susceptibility haplotype associated to both PS and AD in Italian patients [3], *FLG* null mutations were genotyped in a PS and AD cohort in order to evaluate either their involvement in the pathogenesis of both diseases

or the existence of an epistatic relationship between Italian shared susceptibility haplotype (MIDDLE-ENDAL16) and *FLG* mutations. AD and PS patients selected either from trios analyzed in the previous work or newly recruited AD/PS patients were screened. A total of 195 PS patients and 178 AD patients were analyzed; 210 blood donors from the same ethnic background and without a history of PS, AD or other autoimmune disorders were selected as controls. All PS patients had a dermatologist-confirmed diagnosis of chronic plaque psoriasis and most of them had type I psoriasis (70%). As for AD patients a consensus diagnosis of the disease was assessed either by an expert dermatologist or by a pediatric allergologist. Written consent was obtained for all the patients or their parents.

AD was extrinsic in 68% of the cases with 52% of the patients showing early onset of the disease (<2 years old). Specific sensitization was defined to be present if at least one of the specific IgE antibodies was positive (CAP-RAST class ≥ 1 , corresponding to ≥ 0.35 kU/l). A raised total serum IgE was considered greater than 100 kU/l. Extrinsic AD was considered as AD with sensitization and/or IgE levels ≥ 100 kU/l.

The 2282del4 mutation was typed by sizing a fluorescently labeled PCR fragment on an Applied Biosystems 3130xl as previously described [7]. Genotyping of R501X was performed by TaqMan allelic discrimination assay (primer F: 5'-GCA CTG GAG GAA GAC AAG GAT-3'; primer R: 5'-CTC TTG GGA CGC TGA ATG C; probe 1: 5'-CTG TCT CGT GCC TGC-3'; probe 2: 5'-CTG TCT CAT GCC TGC-3'). Genotype assessment of both 2282del4 and R501X was confirmed by direct sequencing of 10% of the samples. Screening of *FLG* mutations was performed as previously described [5]. A PCR fragment of 3697 bp was amplified from human genomic DNA using forward primer FILF3 (5'-GCT GAT AAT GTG ATT CTG TCT G-3') and reverse primer RPT3P10R (5'-GAC CCC GAT GAT TGT TCC TGT-3'). This PCR fragment encompasses the 5'-end of exon 3 of the profilaggrin gene, repeats 1 and 2 and 401 bp of repeat 3. PCR conditions were as follows: (94°C 5 min) 1, (94°C 30 s, 61°C 45 s, 72°C 3 min 20 s) 31 cycles, and a final extension at 72°C for 5 min. The 3,697-bp PCR fragment was sequenced with primers FILr3.F1 (5'-GGG TCA GGA CAC CAT TCG TGC-3') and RPT3P10R.

Genotyping of *FLG* mutations in PS patients revealed that the frequency of risk alleles of R501X and 2282del4 was 0.0%, i.e. no mutant allele has been observed (table 1). Genotyping of R501X and 2282del4 mutations in AD patients and controls showed similar frequency of risk alleles: 0.6 vs. 0.0% and 0.9 vs. 0.5%, respectively. Risk allele frequencies observed in the Italian AD patients were strongly reduced in respect to those described in other patients of European origin [4, 6]. Following the above-mentioned results we searched for additional mutations in *FLG*. We resequenced 120 AD patients and 100 PS patients for 5'-end of exon 3 of the profilaggrin gene, repeats 1 and 2 and 401 bp of repeat 3. We did not observe additional mutations in *FLG* in either AD or PS patients. These results taken together with very recent data [8] seem to rule out an involvement of R501X and 2282del4 in PS and

Table 1. Frequencies of wild-type/risk alleles of R501X and 2282del4

Variant	Study group	Allele frequency	
		wild-type	risk allele
R501X	psoriasis	390 (100%)	0 (0%)
	atopic dermatitis	354 (99.4%)	2 (0.6%)
	controls	420 (100%)	0 (0%)
2282del4	psoriasis	390 (100%)	0 (0%)
	atopic dermatitis	353 (99.1%)	3 (0.9%)
	controls	418 (99.5%)	2 (0.5%)

AD in the Italian population. Although it should be further considered that another mutation located elsewhere in *FLG* could be involved in the susceptibility of PS and AD in the Italian population, we would like to outline that it will be important to investigate on the extremely low frequency of R501X and 2282del4 in the Italian samples. Previous reports showed that these two highly prevalent null mutations (R501X and 2282del4) in the beginning of *FLG* significantly predispose European individuals to early-onset, severe, and persistent AD and also to the form of asthma associated with AD [4]. These results were replicated in several other European case-control association studies with similar conclusions [9]. The lack of association of R501X and 2282del4 in PS/AD Italian cohorts may reflect the existence of a negative selection of these *FLG* mutations in the Italian chromosomes or the existence of a different chromosome pool. In this perspective, the study of Italian as well as other 1q21-linked samples can be particularly useful both to disclose population-specific pathogenetic pathways for *FLG* and other 1q21 genes. It would be fundamental in the future to evaluate the worldwide distribution of *FLG* mutations in order to understand the genetic heterogeneity underlying the susceptibility to AD/PS mediated by *FLG*.

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