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Published in:
Acta Dermato-Venereologica

DOI:
10.2340/00015555-0383

Publication date:
2008

Document Version
Publisher's PDF, also known as Version of record

Acta Derm Venereol 2008; 88: 15–19

INVESTIGATIVE REPORT

Loss-of-function Variants of the Filaggrin Gene are Associated with Atopic Eczema and Associated Phenotypes in Swedish Families

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Recent studies have identified 2 loss-of-function variants, R501X and 2282del4, in the filaggrin gene as predisposing factors in the development of eczema. In this study, representing the first analysis of the variants in a Swedish population, we analysed transmission in 406 multiplex eczema families with mainly adult patients. In accordance with previous studies we found association between the filaggrin gene variants and atopic eczema ($p = 9.5 \times 10^{-8}$). The highest odds ratio for the combined allele, 4.73 (1.98–11.29), $p = 3.6 \times 10^{-8}$, was found for the subgroup with a severe eczema phenotype, and association was also found with raised allergen-specific IgE, allergic asthma and allergic rhinoconjunctivitis occurring in the context of eczema. Our results support an important role for the filaggrin gene variants R501X and 2282del4 in the development and severity of atopic eczema and indicate a possible role for the subsequent progression into eczema-associated phenotypes. ***Key words: eczema; atopic dermatitis; genetic association; filaggrin; R501X; 2282del4.***

(Accepted August 27, 2007.)

Acta Derm Venereol 2008; 88: 15–19.

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Eczema (1), previously also referred to as atopic dermatitis (OMIM#603165), is a chronic relapsing inflammatory skin disorder that results from a complex interaction of genetic and environmental factors. Eczema currently affects 10–20% of children and 1–3% of adults in westernized countries (2). Patients with eczema often have increased levels of specific and/or total IgE (3) and may develop other allergic diseases such as allergic asthma and allergic rhinoconjunctivitis.

The disease has a high familial occurrence, with twin studies showing concordance rates of 0.72–0.77 in monozygotic and 0.15–0.23 in dizygotic twin pairs (4, 5). Emerging evidence shows that barrier dysfunction is a major component in the pathogenesis of eczema (6), and genetic linkage studies indicate that the epidermal differentiation complex (EDC), located on chromosome 1 (1q21), contains susceptibility genes for eczema (7).

A recently published study on the fused gene family member filaggrin (*FLG*), located in the EDC, showed that the repeats in this gene are polymorphic and that the genetic variants, R510X and 2282del4, cause a loss of these repeats. This, in turn, leads to a full ichthyosis vulgaris phenotype if in a homozygote or compound heterozygote form and a milder phenotype if in a heterozygote form (8). Furthermore, Palmer et al. (9) showed that these variants also increase susceptibility to eczema and eczema-associated asthma. The *FLG* gene encodes the profilaggrin protein, which is one of the main protein components of the keratohyalin granules within the upper cell layers of the epidermis. Upon terminal differentiation of the granular cells, the profilaggrin protein is proteolytically processed into 10–12 filaggrin peptides, which aggregate to the keratin cytoskeleton and bring about formation of squames (10–12). Thus, filaggrin is an essential component in the process leading to the formation of a fully functional skin barrier.

In the present study, we aimed to analyse the frequency of the R510X and 2282del4 variants and their association with eczema and associated phenotypes in a Swedish material. Using a well-characterized eczema family material consisting of 406 multiplex families with mainly adult eczema patients, we found a strong association with eczema, especially in patients with a severe phenotype. Association was also found with raised allergen-specific IgE, allergic asthma and allergic rhinoconjunctivitis occurring in the same families.

MATERIALS AND METHODS

Patients

Eczema families were recruited during 1995–1997 at the dermatology departments of the Karolinska University Hospital and the Danderyd Hospital in Stockholm, Sweden. Families with at least two affected siblings were included, resulting in 406 multiplex families with 1514 individuals including 921 siblings with eczema. These individuals are a subsets of a larger material described previously (13) and 109 of the families in this study were used in a genome-wide linkage analysis published previously (14).

doi: 10.2340/00015555-0383

All the siblings were diagnosed with eczema by the same dermatologist, based on clinical examination and according to the UK Working Party's Diagnostic Criteria (15). An arbitrary score for the disease severity of eczema was obtained using the classification shown in Table II. A severe eczema phenotype was defined as severity scoring ≥ 4. Subjects were classified as having allergic asthma or allergic rhinoconjunctivitis based on physician's diagnosis. Parents were included regardless of their atopic status, and clinical information was gathered through a questionnaire. Seventy-eight percent of the affected siblings had onset of eczema ≤ 2 years of age and the mean age of the affected siblings at the time of sampling was 29 years.

IgE quantification

In all affected siblings, the following measurements were performed:

- Total serum-IgE concentration using the Pharmacia CAP System, IgE FEIA (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). The phenotype "raised total IgE" was analysed as a qualitative variable, with age-specific cut-off values. The cut-offs were; 22.3 kU/l (9 months–5 years), 263 kU/l (5–20 years) and 122 kU/l (> 20 years).
- IgE antibodies to Phadiatop®, a mixture of inhalant allergens (Pharmacia CAP System Phadiatop®FEIA). The Phadiatop® was reported as either positive or negative.
- IgE antibodies to a mixture of relevant food allergens (hen's egg white, cow's milk, soya bean, peanut, fish and wheat flour) (fx5) (Pharmacia CAP System RAST® FEIA). The RAST mixture was divided into 6 classes, where a concentration < 0.35 kU/l represents a negative result (class 0).

Of the eczema-affected siblings, 38% had raised total IgE and 64% had increased allergen-specific IgE to food and/or inhalant allergens. On the basis of elevated specific IgE level, the siblings were divided into atopic eczema patients ($n = 558$) and non-atopic ($n = 333$), according to the revised nomenclature of the World Allergy Organization (1). The frequency of associated phenotypes and characteristics of the siblings with eczema are summarized in Table I.

The study was approved by the local ethics committee, conducted according to Declaration of Helsinki principles, and all subjects gave their informed consent. Where a sibling was less than 18 years of age the parents gave their consent.

Genotyping

Genomic DNA was extracted from peripheral venous blood using a standard protocol. Genotyping was performed for *FLG* variants R501X and 2282del4 on all 1514 individuals in the family material. R501X genotyping was performed using TaqMan-based allelic discrimination assay (Applied Biosystems, Foster City, CA, USA). Allele-specific Taqman MGB probes were labelled with fluorescent dyes FAM and VIC, respectively. Polymerase chain reactions (PCR) were carried out in 384-well plates with 5 ng of genomic DNA, 5 µl of a reaction mix containing the specific TaqMan assay solution (4 times diluted compared with the manufacturers manual) and 1X TaqMan Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems). Amplification was done following the Taqman Universal PCR protocol (95ºC for 10 min, followed by 40 cycles at 92ºC for 15 sec and 60ºC for 1 min). Allelic discrimination was performed with the ABI PRISM® 7900HT Sequence Detection System and the SDS 2.2.1 sequence detection system program (Applied Biosystems). The 2282del4 genotyping and allelic discrimination was performed using a TaqMan-based assay (Applied Biosystems) as previously described (16).

Table I. *Phenotype frequencies for the eczema affected siblings and sex ratios in the different phenotype groups*

Phenotype	Siblings *n* (%)	Sex ratio M:F
Eczema	921 (100)	1:1.5
Atopic eczema	588 (64)	1:1.3
Non-atopic eczema	333 (36)	1:2.2
Severe eczema (severity scoring ≥ 4)	139 (15)	1:1.3
Eczema and allergic asthma	347 (38)	1:1.5
Eczema and allergic rhinoconjunctivitis	613 (67)	1:1.5
Eczema and raised total IgE	345 (38)	1:1.6

For further details on phenotype definitions, see Materials and Methods section.

Table II. *Severity scoring of eczema*

Factor	Score
Age at onset ≤ 2 years	1
Hospitalization for eczema	1
Affected sites[a] on examination	
0	0
1–3	1
> 3	2
Raised total and/or allergen-specific IgE	1
Maximum score = 5	

[a]The presence of eczema at one or both sites in bilateral structures was regarded as presence at one site.

Data and statistical analysis

A Pedigree Disequilibrium Test (PDT) was performed in order to evaluate the evidence for association between variants and the different phenotypes (17). PDT and odds ratios (OR) estimates including 95% confidence interval (CI) were analysed using the UNPHASED program (3.0.7) (18). The OR for alleles was estimated relative to the most common allele. Population Hardy-Weinberg equilibrium (HWE) was evaluated using a χ^2 test as implemented in the zGenStat 1.128 software (Henric Zazzi), with a cut-off value of $p > 0.01$. None of the *p*-values presented are corrected.

RESULTS

We performed genotyping for *FLG* variants R501X and 2282del4 on all 1514 individuals in the 406 pedigrees, with a success rate above 91% for both assays. None of the variants analysed deviated from HWE.

The overall allele frequency of the 2 variants in the study population was 0.027 in the case of R501X (0.020 for parents and 0.032 for affected siblings) and 0.061 for 2282del4 (0.048 and 0.070, respectively). The combined allele frequency was 0.068 in the parents and 0.096 in the affected siblings. Four homozygotes for R501X, 7 homozygotes for 2282del4 and 2 compound heterozygotes were present among the siblings.

The results of the PDT, estimated OR and 95% CI are presented in Table III. Both R501X and 2282del4 were over-transmitted to the eczema affected siblings ($p = 1.5 \times 10^{-5}$ and $p = 2.9 \times 10^{-5}$), and the combined alleles ($p = 1.3 \times 10^{-6}$). Dividing the siblings into atopic

and non-atopic eczema subgroups, the atopic group showed an OR of 2.21 (1.50–3.25) for the combined allele ($p = 9.5\times10^{-8}$). In the non-atopic eczema group, there was no association with the 2 *FLG* variants.

Adding sex as a confounder in these analyses did not alter the association significantly (data not shown).

The *FLG* variants were even more over-transmitted to affected siblings in the severe eczema phenotype (severity scoring ≥4), where the combined allele showed an OR of 4.73 (1.98–11.29) with $p = 3.6\times10^{-8}$.

The association found with the early-onset phenotype was comparable with the eczema group as a whole (combined allele $p = 1.2\times10^{-6}$, OR 2.09 (1.46–2.97)).

In addition to the analysis of association with eczema, we analysed associated phenotypes in the eczema families. Association was found for increased total IgE, allergic asthma and allergic rhinoconjunctivitis, with the most significant result found for the R501X variant in allergic rhinoconjunctivitis, $p = 2.7\times10^{-12}$, OR 5.07 (1.47–17.46).

The effect of *FLG* null status on the severity of the eczema and associated phenotypes was further illustrated when we correlated the genotype of the affected siblings with the frequency of the phenotypes. For this analysis, one sibling from each family was randomly selected and only individuals with full genotype information were used. The frequency of the genotypes was, wild-type = 313, either heterozygotes = 67 and homozygotes (or compound heterozygotes) = 6. As shown in Fig. 1, there was an increase in the severity and the frequency of most of the eczema-associated phenotypes in the heterozygotes compared with wild-type, and in the homozygotes compared with the heterozygotes.

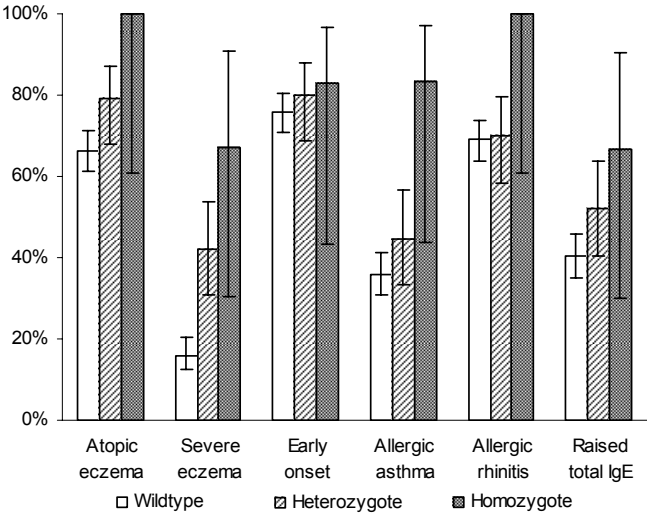

Fig. 1. Filaggrin genotype and correlation to phenotype frequencies with 95% confidence intervals in eczema-affected siblings.

DISCUSSION

Recently published studies on the EDC member *FLG* have identified 2 common loss-of-function variants, R501X and 2282del4, in this gene as causative factors in ichthyosis vulgaris and as major predisposing factors in the development of eczema and asthma in patients with eczema (8, 9). The association with eczema and associated phenotypes has now been replicated and extended in a number of populations in Western Europe (reviewed by Irvine (19)), and new predisposing variants have been identified including unique variants in the Japanese population (16, 20).

In the present study, representing the first analysis of *FLG* reported in a Swedish population, we present data that further reinforce the importance of *FLG* variants as susceptibility factors in eczema and eczema associated phenotypes.

In accordance with other family-based studies we found an over-transmission of the *FLG* variants in our material (21–24). There was a strong association with eczema, producing an OR of 2.68 (1.34–5.33) for the R501X variant ($p = 1.5\times10^{-5}$) and 1.85 (1.24–2.76) for the 2282del4 ($p = 2.9\times10^{-5}$).

As reported in some previous studies, we found that the association is mainly to the atopic subgroup of eczema patients (22, 24, 25). The carrier frequency of *FLG* null alleles among the subjects in the non-atopic group was actually slightly lower than in the eczema group as a whole, indicating that these variants do not influence the development of non-atopic eczema in our adult material.

Table III. *Results of Pedigree Disequilibrium Test for filaggrin variants in the Swedish eczema families*

	R501X		2282del4		Combined	
Trait	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Eczema	2.68 (1.34–5.33)	1.5×10^{-5}	1.85 (1.24–2.76)	2.9×10^{-5}	1.81 (1.31–2.50)	1.3×10^{-6}
Atopic eczema	4.33 (1.88–9.94)	2.6×10^{-7}	2.22 (1.41–3.47)	4.1×10^{-6}	2.21 (1.50–3.25)	9.5×10^{-8}
Non-atopic eczema	2.07 (0.78–5.47)	NS	0.99 (0.54–1.80)	NS	1.07 (0.62–1.84)	NS
Early onset	4.37 (1.79–10.64)	2.8×10^{-7}	2.14 (1.41–3.23)	4.2×10^{-5}	2.09 (1.46–2.97)	1.2×10^{-6}
Severe eczema	5.29 (3.32–8.84)	1.3×10^{-12}	6.34 (1.90–21.2)	1.5×10^{-7}	4.73 (1.98–11.29)	3.6×10^{-8}
Allergic asthma	3.94 (1.12–13.87)	8.4×10^{-6}	3.85 (1.97–7.51)	2.4×10^{-7}	3.58 (1.99–6.42)	6.5×10^{-9}
Allergic rhinoconjunctivitis	5.07 (1.47–17.46)	2.7×10^{-12}	1.97 (1.28–3.03)	7.8×10^{-5}	2.03 (1.39–2.97)	7.0×10^{-7}
Raised total IgE	4.33 (1.26–14.81)	8.8×10^{-6}	1.85 (1.14–2.98)	0.0066	1.93 (1.24–2.99)	0.00054

OR: odds ratio of minor allele relative to major allele. OR for R501X in the severe eczema phenotype was calculated in a χ^2 test. NS: non-significant. CI: confidence interval,

We found a higher OR for 2282del4 (6.34 (1.90–21.2)) and R501X (5.29 (3.32–8.84)) when analysing the subgroup with the severe eczema phenotype, and 43.4% of the individuals in this subgroup were carriers of an *FLG* null allele. As to *FLG* null carriers, 67% of the homozygote carriers and 42% of the heterozygote carriers belonged to this phenotype group. Only 16% of wild-type carriers of *FLG* were classified into the severe-eczema phenotype (Fig. 1). Our results may therefore support the hypothesis that individuals with eczema who carry *FLG* null alleles could be more likely to suffer from a persistent severe disease. Two previous studies also present a high frequency of carriers of *FLG* null alleles, 29.7% and 42%, among patients with persistent eczema and an early onset (25, 26). In our material, where 78% of the patients had early onset, we found a null allele carrier frequency of 20.6% in the subgroup with early onset (≤2 years of age) and the effect of carrying a *FLG* null allele on early-onset phenotype was only marginal (Fig. 1).

Several studies have indicated that *FLG* variants may be susceptibility factors for asthma (9, 21, 22, 24, 25), rhinoconjunctivitis (21, 25) and raised total IgE (22, 25) in eczema patients.

A similar pattern of association to allergic asthma, allergic rhinoconjunctivitis and raised total IgE was also seen in our material. However, other studies have shown that there is no association with either asthma or rhinoconjunctivitis when eczema affected individuals are excluded from the study populations (9, 21, 24). Larger cohorts ascertained outside the context of eczema are needed to resolve the role of *FLG* variants in the pathogenesis of these phenotypes.

In conclusion, our results support an important role for the *FLG* gene variants R501X and 2282del4 in the development and severity of atopic eczema. Furthermore, by causing a dysfunctional skin barrier leading to increased epidermal allergen transfer, these variants could also play an important role in the subsequent progression into eczema-associated phenotypes.

ACKNOWLEDGEMENTS

Financial assistance is gratefully acknowledged from the Swedish Asthma and Allergy Association's Research Foundation, the Edward Welander-Finsen Foundation, the Swedish Foundation for Allergy Research and the Centre for Allergy Research, Karolinska Institutet. Colin N. A. Palmer is supported by the Scottish Executive as part of the *Generation Scotland* Initiative. The authors would like to thank all eczema patients and their families for contributing to the study.

Conflict of interest: None declared.

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