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2017

http://hdl.handle.net/10138/189055
https://doi.org/10.2340/00015555-2578

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The Value of FLG Null Mutations in Predicting Treatment Response in Atopic Dermatitis: An Observational Study in Finnish Patients

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The contribution of FLG null mutations to predicting atopic dermatitis (AD) treatment response is not clear, nor have such mutations been studied in the Finnish population. This study tested the association of the 4 most prevalent European FLG null mutations, the 2 Finnish enriched FLG null mutations, the FLG 12-repeat allele, and 50 additional epidermal barrier gene variants, with risk of AD, disease severity, clinical features, risk of other atopic diseases, age of onset, and treatment response in 501 patients with AD and 1,710 controls. AD, early-onset AD, palmar hyperlinearity, and asthma showed significant associations with the combined FLG null genotype. Disease severity and treatment response were independent of patient FLG status. Carrier frequencies of R501X, 2282del4, and S3247X were notably lower in Finns compared with reported frequencies in other populations. This data confirms FLG mutations as risk factors for AD in Finns, but also questions their feasibility as biomarkers in predicting treatment response.

Key words: atopic dermatitis; filaggrin gene; skin barrier; tight junction; biomarker; treatment response.

Accepted Nov 10, 2016; Epub ahead of print Nov 14, 2016


The movement of substances between cells is regulated by tight junctions (TJ), which form the major paracellular barrier in the human epithelia (20). The permeability of TJs is regulated mainly by claudins. Claudin 1 (CLDN1) and Claudin 23 (CLDN23) expressions are reduced in AD and the reduction in CLDN1 has been shown to diminish the integrity of TJs and correlates inversely with Th2 cytokines (21, 22). Certain mutations of CLDN1 are associated with increased risk of AD in North American populations (21). The possible roles of many other TJ proteins, and proteins involved in the structure and function of the cell envelope structure in the pathogenesis of AD are yet to be elucidated.

It seems plausible that AD consists of several different endophenotypes, which may have an effect on response to treatment (23). Studies on the effect of FLG null mutations or other barrier gene mutations on the treatment response are sparse.

doi: 10.2340/00015555-2578

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Finland is an isolated founder population with a unique genetic composition differing from that of other European populations (24). Over the centuries, founder effect, genetic drift and isolation have shaped the gene pool of the population. Isolated populations provide special opportunities for studying the disease contribution of low-frequency and rare variants. Genetic bottlenecks alter the allele distribution in the population, leading to enrichment of some alleles and loss of others. Thus, deleterious variants are present at higher frequencies, some of which contribute to disease susceptibility. A recent study by Lim et al. (25) explored this potential by comparing exome sequence data on 3,000 Finns and 3,000 non-Finnish Europeans and discovered that the average Finn has more low-frequency (0.5% < minor allele frequency (MAF) ≤ 5%) LoF variants and complete gene knockouts.

The primary aim of the current analysis was to assess the frequencies and the effect of the 4 most prevalent European FLG null mutations (2282del4, R501X, R2447X, S3247X), 2 FLG mutations (S1020X, V603M) enriched in the Finnish population, and the 12-repeat allele (rs12730241) on the risk of AD in a case-control setting. In addition, to further characterize their effect on response to standard treatment, clinical features, and the risk of other atopic diseases, and evaluate their usefulness as possible biomarkers in a real-life observational clinical cohort setting.

The secondary aim of this study was to estimate the effect of additional selected potential liability variants within 10 essential epidermal barrier genes on the risk of AD in a case-control setting, and to assess their effect on response to standard treatment, clinical features, and the risk of other atopic diseases in an observational clinical cohort setting. The main focus in the selection of additional epidermal barrier genes was in genes involved in TJ structure or function. These TJ-associated genes were claudin-1 (CLDN1), claudin-4 (CLDN4), claudin-20 (CLDN20), claudin-23 (CLDN23), occludin (OCLN), junctional adhesion molecule A (JAM1), and tight junction protein 1 (TJP1). Other investigated genes were cell envelope genes involucrin (IVL) and loricrin (LOR), and Filaggrin-2 (FLG2). Taking into account the population-specific spectrum of FLG mutations, the aim was to explore the distribution and potential medical impact of Finnish-enriched LoF mutations in FLG, FLG2 and CLDN20 (see Table SI for a complete list of variants).

MATERIALS AND METHODS

Patients
A total of 501 cases with dermatologist-confirmed AD were recruited consecutively for the planned 12-month follow-up in a tertiary referral centre setting at Helsinki University Skin and Allergy Hospital between June 2011 and December 2012 (Fig. 2). Detailed data on the course of their diseases, therapies used, clinical features (palmar or dorsal hand eczema, keratosis pilaris, palmar hyperlinearity, dermographismus), other manifestations of atopy (asthma, allergic rhinitis and allergic conjunctivitis), and history of herpes simplex virus (HSV) symptoms were collected. AD symptoms were scored with the validated Eczema Area and Severity Index (EASI) (26), Rajka & Langeland Severity Index Score (27), and Investigator’s Global Assessment (IGA) score. Evaluation of the clinical picture and symptoms was conducted both at baseline and during follow-up, ensuring a reliable assessment of the long-term response to treatment. DNA sample collection was conducted at baseline, but all samples were analysed together after completion of follow-up. Dermatologists involved in the study were therefore not aware of the patients’ genotype, and treatment interventions were made solely on a clinical basis. Treatment modalities used were topical corticosteroids and topical calcineurin inhibitors, and, in rare cases, systemic methotrexate and systemic cyclosporine. Treatment response was defined as an alleviation of symptoms and a decrease in clinical scoring. Serum total IgE levels were measured in connection with the clinical evaluations (CAP system-specific IgE fluorometric enzyme immunoassay).

Control population
A total of 1,710 control DNA samples were obtained from the Health 2000 (H2000) GenMets Study, representing the general Finnish population (Fig. 2). H2000 was a national health exami-
nation survey that was carried out in Finland from autumn 2000 to spring 2001 to obtain comprehensive data on general public health issues, and on the functional and working capacity of the population (see http://www.terveys2000.fi/indexe.html). A Gen-Mets subset was obtained by collecting all those who fulfilled the International Diabetes Federation (IDF) definition of metabolic syndrome criteria, and selecting a matched control for each (28). A subset of AD-negative H2000 controls (n = 1,243) and a subset of asthma-positive H2000 controls (n = 137) were used in the association analysis (Fig. 2). Data on AD status and asthma status was collected from extensive questionnaires, interviews, and clinical examinations conducted by physicians working according to written instructions and applying preset diagnostic criteria.

For determining the FLG allele frequencies in the Finnish population data from the Sequencing Initiative Suomi (SISu) project (http://www.sisuproject.fi) were also utilized. The SISu data-set comprised 1,941 whole-genome sequenced individuals from FINRISK (29) and H2000 cohorts selected on the basis of metabolic and neuropsychiatric traits and 400 healthy individuals from the Kuusamo region at the time when selection of variants was performed (Table SII \(^*\)). In addition, allele frequencies for the most common FLG mutations were checked from The Exome Aggregation Consortium (ExAC) database (http://exac.broadinstitute.org/gene/ENSG00000143631) (30).

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the local ethics committee. All subjects signed an informed consent form.

Genotypic analysis

DNA samples were screened for the 4 FLG null mutations most prevalent in the European population (R501X, 2282del4, R2447X, S3247X), 2 Finnish enriched FLG null mutations (S1020X, V603M), and the 12-repeat allele (rs12730241). An additional set of 59 functional variants predicted to be deleterious were genotyped within 10 barrier genes (CLDN1, CLDN4, CLDN20, CLDN23, OCLN, IVL, FLG2, LOR, JAM-1, TJFP) Table SII \(^*\)). Altogether, 7 out of 59 genotyped variants within FLG, FLG2 and CLDN20 were drawn from a systematic survey of distribution and medical impact of LoF variants identified as rare, likely deleterious, and markedly enriched in the Finnish population (25). Genotyping was performed using the Sequenom MassARRAY system and the iPLEX Gold assays (Sequenom Inc., San Diego, CA, USA) based on primer extension with single mass-modified nucleotides followed by matrix assisted laser desorption ionization-time of flight mass spectrometry for allele discrimination. Genotyping reactions were performed on 20 ng dried genomic DNA in 384-well plates according to the manufacturer’s recommendations and with their reagents. Both PCR and extension primers were designed using MassARRAY Assay Design software (Sequenom). The data was collected using the MassARRAY Compact System (Sequenom) and the genotypes were called using TyperAnalyzer software (Sequenom). For quality control reasons, the genotype calls were checked manually. Genotyping quality was examined by a detailed quality control procedure consisting of success rate checks, duplicated samples, water controls and Hardy-Weinberg Equilibrium (HWE) testing. All significantly associated genotypes were validated by capillary sequencing.

Statistical analysis

Power calculations for the primary discovery analysis were based on an estimated 10% prevalence of AD (31, 32). An individual FLG null mutation allele frequency of 0.01 predicted that recruitment of 500 AD cases, and 1,500 population controls would give a 97% power at a p-value of 0.001 to detect an allelic odds ratio (OR) of 3. Allele frequencies in case and control groups, and different phenotypic groups were compared using Fisher’s exact test, logistic regression, and linear model under the null hypothesis that there is no association with genotype. Logistic regression and Fisher’s exact test were used for the binary traits and linear model for the quantitative traits.

Statistical analyses were carried out using PLINK whole-genome association analysis toolset version 1.07 and R software version 3.1.2 (33, 34). Cases having 1 or 2 non-Finnish parents were excluded from the analysis. To adjust for multiple testing and correct for occurrence of false-positives Bonferroni correction was applied. p-value cut-off was set to p = 0.0016 for the initial association analysis of 32 variants, and p = 0.001 for the follow-up association analysis of 3 variants and 14 clinical features.
Genetic association analysis for clinical features palmar hyperlinearity and keratosis pilaris were performed within the clinical sample set \((n=445)\). Association with early-onset AD was tested using the H2000 supercontrols with no atopic diseases \((n=1,243)\) and early-onset AD cases. Into the association analysis of asthma, asthma-negative H2000 individuals were included as controls \((n=1,527)\), and asthma-positive H2000 individuals \((n=137)\) as cases. Supercontrol and asthma status were retrieved from questionnaires and clinical examination related to H2000 study. Association analysis using the combined \(FLG\) null genotype included carriers of R501, 2282del2, and R2447X (i.e. those \(FLG\) null mutations with association in the original screening).

### RESULTS

#### Demographic data

After applying the filtering criteria (Fig. 2), the study comprised 445 cases \((32.3 \pm 14.9\) years) and 1,664 controls \((50.6 \pm 10.7\) years), with a female preponderance in both groups \((62.8\% vs 51.5\%)\).

#### Allele frequencies

Allele frequencies of the 4 most prevalent European \(FLG\) null mutations \((R501X, 2282del4, R2447X, S3247X)\), 2 Finnish enriched \(FLG\) null mutations \((S1020X, V603M)\), and the 12-repeat allele \((rs12730241)\) were determined in Finnish AD cases and 2 separate population cohorts; the Health2000 GenMets cohort (genotype data, \(n=1,710)\) and the SISu cohort (whole-genome sequencing data, \(n=1,941)\). The allele frequencies of R501X, 2282del4 and S3247X were notably lower in Finns (H2000 GenMets cohort and SISu cohort) compared with other European populations. Allele frequencies are shown in Table SII1.

### Barrier genes and risk of atopic dermatitis

Screening of 32 variants yielded results for 445 cases, and 1,664 controls (Fig. 2). Twenty-six of 59 variants were either monomorphic or had unreliable clustering and were therefore excluded from the analysis. Marker rs116971953 \((CLDN20)\) failed Hardy-Weinberg equilibrium test in controls with \(p=0.0007\) and was excluded from the analysis (Table I).

Significant associations were seen for the individual \(FLG\) null mutations R501X and 2282del4 \((OR=11.29, p=0.0022\) and OR=2.66, \(p=0.00016)\), and suggestive association for R2447X \((OR=3.10, p=0.0072)\) with AD (Table SIII1). S1020X had a very low allele frequency (only 2 cases and 2 controls) and was not associated with AD; thus it was not included in the combined analyses. The same was noted for mutation S3247X (no cases). In the combined analysis, having any of the \(FLG\) null mutations \((R501X, 2282del4, R2447X)\) was highly signi-

### Table I. Clinical data for cases in relation to \(FLG\) status

<table>
<thead>
<tr>
<th>Clinical phenotype</th>
<th>Total, (n=445)</th>
<th>(FLG) wild-type Total, (n=394)</th>
<th>(FLG) null combined(^a) Total, (n=49)</th>
<th>R501X Total, (n=8)</th>
<th>2282del4 Total, (n=30)</th>
<th>R2447X Total, (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rajka-Langeland score, baseline, mean (\pm SD)</td>
<td>5.8 (\pm 1.9)</td>
<td>5.8 (\pm 1.9)</td>
<td>5.9 (\pm 0.26)</td>
<td>6.6 (\pm 0.75)</td>
<td>6.1 (\pm 0.35)</td>
<td>5.1 (\pm 0.56)</td>
</tr>
<tr>
<td>EASI score, baseline, mean (\pm SD)</td>
<td>8.2 (\pm 10.2)</td>
<td>7.9 (\pm 9.9)</td>
<td>8.6 (\pm 1.5)</td>
<td>11.6 (\pm 7.2)</td>
<td>9.3 (\pm 2.0)</td>
<td>5.9 (\pm 3.2)</td>
</tr>
<tr>
<td>Total IgE, IU/ml, baseline, median (mean)</td>
<td>4,078 (571)</td>
<td>3,451 (515)</td>
<td>3,937 (1,184)</td>
<td>30,531 (5,908)</td>
<td>4,503 (768)</td>
<td>5,860 (816)</td>
</tr>
<tr>
<td>Age of onset, (n) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;2) years</td>
<td>297 (74.8)</td>
<td>254 (72.8)</td>
<td>41 (89.1)</td>
<td>7 (100.0)</td>
<td>25 (86.2)</td>
<td>10 (90.9)</td>
</tr>
<tr>
<td>(\geq 2) years</td>
<td>100 (25.2)</td>
<td>95 (27.2)</td>
<td>5 (10.9)</td>
<td>0 (0.0)</td>
<td>4 (13.8)</td>
<td>1 (9.3)</td>
</tr>
<tr>
<td>Palmar hyperlinearity, (n) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>143 (40.6)</td>
<td>110 (36.1)</td>
<td>32 (71.1)</td>
<td>6 (100)</td>
<td>19 (63.3)</td>
<td>8 (80.0)</td>
</tr>
<tr>
<td>No</td>
<td>209 (50.4)</td>
<td>195 (63.9)</td>
<td>13 (28.9)</td>
<td>0</td>
<td>11 (37.7)</td>
<td>2 (20.0)</td>
</tr>
<tr>
<td>Keratosis pilaris, (n) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>48 (14.2)</td>
<td>36 (12.2)</td>
<td>12 (28.6)</td>
<td>4 (66.7)</td>
<td>7 (25.9)</td>
<td>2 (20.0)</td>
</tr>
<tr>
<td>No</td>
<td>290 (85.8)</td>
<td>258 (87.8)</td>
<td>30 (71.4)</td>
<td>2 (33.3)</td>
<td>20 (74.1)</td>
<td>8 (80.0)</td>
</tr>
<tr>
<td>Dermographism, (n) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>173 (48.9)</td>
<td>152 (49.2)</td>
<td>20 (46.5)</td>
<td>3 (42.9)</td>
<td>9 (33.3)</td>
<td>8 (80.0)</td>
</tr>
<tr>
<td>No</td>
<td>181 (51.1)</td>
<td>157 (50.8)</td>
<td>23 (53.5)</td>
<td>4 (57.1)</td>
<td>18 (66.7)</td>
<td>2 (20.0)</td>
</tr>
<tr>
<td>Hand eczema, (n) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>321 (78.1)</td>
<td>287 (79.1)</td>
<td>33 (71.7)</td>
<td>6 (85.7)</td>
<td>21 (70.0)</td>
<td>7 (70.0)</td>
</tr>
<tr>
<td>No</td>
<td>90 (21.9)</td>
<td>76 (20.9)</td>
<td>13 (28.3)</td>
<td>1 (14.3)</td>
<td>9 (30.0)</td>
<td>3 (30.0)</td>
</tr>
<tr>
<td>Palmar hand eczema, (n) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>120 (34.5)</td>
<td>109 (35.4)</td>
<td>11 (28.5)</td>
<td>1 (16.7)</td>
<td>7 (29.2)</td>
<td>3 (33.3)</td>
</tr>
<tr>
<td>No</td>
<td>228 (65.5)</td>
<td>199 (64.6)</td>
<td>27 (71.5)</td>
<td>5 (83.3)</td>
<td>17 (70.8)</td>
<td>6 (66.6)</td>
</tr>
<tr>
<td>History of HSV symptoms, (n) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>128 (30.2)</td>
<td>114 (30.5)</td>
<td>13 (27.1)</td>
<td>5 (62.5)</td>
<td>7 (23.3)</td>
<td>2 (18.2)</td>
</tr>
<tr>
<td>No</td>
<td>296 (69.8)</td>
<td>260 (69.5)</td>
<td>35 (72.9)</td>
<td>3 (37.5)</td>
<td>23 (76.7)</td>
<td>9 (81.8)</td>
</tr>
<tr>
<td>Asthma, (n) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>202 (45.9)</td>
<td>173 (44.4)</td>
<td>28 (58.3)</td>
<td>7 (87.5)</td>
<td>17 (56.7)</td>
<td>5 (45.5)</td>
</tr>
<tr>
<td>No</td>
<td>232 (54.1)</td>
<td>217 (55.6)</td>
<td>20 (41.7)</td>
<td>1 (12.5)</td>
<td>13 (43.3)</td>
<td>6 (54.5)</td>
</tr>
<tr>
<td>Allergic rhinitis, (n) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>338 (77.2)</td>
<td>297 (76.5)</td>
<td>39 (81.3)</td>
<td>8 (100)</td>
<td>22 (73.3)</td>
<td>10 (90.9)</td>
</tr>
<tr>
<td>No</td>
<td>100 (22.8)</td>
<td>91 (23.5)</td>
<td>9 (18.8)</td>
<td>0 (0)</td>
<td>8 (26.7)</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Allergic conjunctivitis, (n) (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>307 (69.8)</td>
<td>270 (69.2)</td>
<td>36 (75.0)</td>
<td>5 (62.5)</td>
<td>22 (73.3)</td>
<td>9 (81.8)</td>
</tr>
<tr>
<td>No</td>
<td>133 (30.2)</td>
<td>120 (30.8)</td>
<td>12 (25.0)</td>
<td>3 (37.5)</td>
<td>8 (26.7)</td>
<td>2 (18.2)</td>
</tr>
</tbody>
</table>

\(^a\)Carriers of R501, 2282del2, and R2447X. \(^b\)Including 1 compound heterozygote 2282del4/R501X.

SD: standard deviation; EASI: Eczema Area and Severity Index; HSV: herpes simplex virus; Missing data not listed in the table.
ificantly associated with AD with OR 3.22, \( p = 3.16 \times 10^{-8} \) (Tables SIII and SIV). Screening of the additional set of variants, including genes implicated in skin barrier function and those enriched in the Finnish population (CLDN1, CLDN4, CLDN20, CLDN23, OCLN, IVL, FLG2, LOR, JAM-1, TJP1, CLDN20, and FLG2) did not provide significant results testing for association with AD (see Table SI1 for a complete list of variants).

**Associations of FLG with clinical phenotype**

The mean length of follow-up of the patients was 1.4 years, with 77% followed ≥ 6 months and 69% completed the planned follow-up 12 ± 1 months.

The combined FLG null genotype was significantly associated with early-onset AD (< 2 years of age) (OR 4.15, \( p = 1.82 \times 10^{-10} \)), asthma (OR 2.76, \( p = 1.57 \times 10^{-6} \)), palmar hyperlinearity (OR 4.67, \( p = 1.46 \times 10^{-5} \)), and suggestively associated with keratosis pilaris (OR 3.1, \( p = 0.0021 \)).

Although the number of mutation-positive cases is small for each of these individual mutations (Table SIV), mutation 2822del4 was significantly associated with early-onset AD (OR 3.38, \( p = 8.38 \times 10^{-6} \)) and showed suggestive association with asthma (OR 2.10, \( p = 0.0033 \)). R501X was associated with early-onset AD (OR 14.88, \( p = 0.00079 \)), asthma (OR 17.43, \( p = 0.00038 \)), and suggestively with keratosis pilaris (OR 13.09, \( p = 0.0035 \)). R2447X showed suggestive association with early-onset AD (OR 3.86, \( p = 0.0018 \)).

Baseline IgE values were higher in patients with FLG null mutations (median 1,184 vs. 511 IU/ml) but the association was not significant (data not shown, combined \( p = 0.1274 \)). FLG null mutations were not associated with atopic hand eczema, dermographism, or HSV infections.

**FLG and treatment response**

There were no significant differences in the baseline AD severity between the FLG null and FLG wild-type groups when assessed by EASI, Rajka-Langeland score or IGA. Skin treatment for 56.9% of patients was monotherapy with topical calcineurin inhibitors (tacrolimus or pimecrolimus), for 12.2% of subjects monotherapy with topical corticosteroids, and for 26.2% a combination of topical calcineurin inhibitors and corticosteroids. Only 2.1% of the patients received systemic methotrexate or cyclosporine treatment during follow-up, and systemic therapy was combined with topical therapy. There were no significant differences in the relative frequencies of the use of different topical treatment modalities in relation to FLG null mutations. Neither was there a difference in the use of oral antibiotics during follow-up as a supportive flare medication or in treatment of secondary bacterial infections (14% in FLG wild-type vs. 13% in the FLG null patients). Antihistamines were also used in similar manner in both groups. Patients did not receive ultraviolet therapy during follow-up.

None of the FLG null mutations were associated with treatment response in the follow-up when assessed by EASI, Rajka-Langeland score or IGA (data not shown, R501X, \( p = 0.7724 \); 2282del4, \( p = 0.2851 \); R2447X, \( p = 0.3863 \); S3247X, \( p = NA \); combined \( p = 0.1863 \)). Positive treatment response (EASI) was similar in the FLG null carriers (70.9%) and non-carriers (72.2%). Complete response rate was the same (17.6%) both in the FLG null carriers and in the non-carriers. The number of patients hospitalized during follow-up was too small for estimates, but FLG null mutations did not seem to increase the lifetime risk of hospitalization due to AD (36.2% in FLG null group vs. 32.7% in FLG wild-type group, \( p = 0.64 \)).

The number of FLG null patients in different treatment groups was too small for a subgroup analysis to compare treatment response with different treatment interventions.

**FLG intragenic copy number variation**

Having 2 12-repeat alleles (24 copies) was more common in patients with AD than in subjects without AD (3.6% vs. 2.8%) and was significantly associated with a higher risk of AD (OR 1.96, 95% CI 1.36–2.81, \( p = 0.00056 \)) compared with subjects with <24 copies (analysis performed using complete set of controls, \( n = 1,664 \)). Early-onset AD was suggestively associated with 2 12-repeat alleles (data not shown, OR 1.47, \( p = 0.0076 \)). Having 2 12-repeat alleles was not significantly associated with disease severity, treatment response, or clinical phenotype in subjects with AD.

**DISCUSSION**

Several studies have shown a correlation between FLG null mutations and AD, allergic sensitization, rhinitis and asthma if eczema was present (8, 12, 13, 15, 35, 36). Studies on different European populations have demonstrated that 14–42% of patients with AD are carriers of a null mutation in the filaggrin gene (37–39). This study represents the first analysis of the FLG null and other barrier gene mutations in relation to AD in the Finnish population.

In this study group of mostly (88%) adult patients with AD, the frequencies of 2 FLG null mutations, R501X and 2282del4, were significantly higher compared with controls, which is in line with earlier studies (7, 8, 13). The combined allele frequency for the European prevalent FLG null mutations (R501X, 2282del4, R2447X and S3247X) was only 5.62% in Finnish patients with AD. We expected to observe higher frequencies due to the previous findings that FLG null mutations increase the persistence of AD symptoms and the majority of adult patients with AD have persistent disease (18). Moreover, population carrier frequencies for R501X, 2282del4 and
null mutations in FLG and disease severity. In previous studies the clinical correlation has been mostly seen in an experimental setting or in paediatric AD populations (40–42). It is possible that, despite the increased risk of AD caused by impairment of the epidermal barrier, the primary barrier defects do not play a significant role after triggering immunological changes in the pathogenetic cascades. This would explain the lack of correlation in clinical parameters in this patient group with persistent disease and a mean age of 32 years. We tested the usefulness of FLG null mutations as possible predictors of treatment response in a real-life prospective clinical setting. Unexpectedly, the long-term treatment outcome on standard topical AD therapy was not affected in any way by the FLG null status of the patients. Unlike serum total IgE, the assessed FLG and other barrier gene mutations do not appear to be useful treatment outcome-predicting biomarkers for clinical follow-up of patients with AD, unlike serum total IgE (43). However, an optimal setting for studying treatment response would be a randomized controlled trial.

Study strengths and limitations

Strengths of this study included a large number of adulthood AD subjects with persistent disease and the prospective setting. The clinical follow-up was relatively long and it was conducted by a small group of dermatologists, limiting the variance in clinical evaluation of patients' phenotype and symptoms. Genotypic data were analysed after completion of the follow-up, thus it did not have any effect on treatment interventions. Due to the present Finnish medical expenses reimbursement system and prevalent reluctance towards systemic immunosuppressant medications among Finnish patients, most of the patients in the study were treated with topical treatments, applying also to those with a more severe disease. This made the study population suitable for investigating the effects of epidermal barrier defects. The data on healthy controls did not rely solely on questionnaires or other self-reported data, but were also collected from clinical examinations by trained physicians, ensuring a high reliability.

Many of the patients had been treated before the baseline and lack of treatment-naive patients may dilute the possible effects seen on treatment response. Patient adherence to treatment could not be measured in this study. The mean age of the controls was higher, which may lead to some recall bias and classification of subjects with non-persistent early childhood AD as healthy controls.

Conclusion

Despite the significant effect of FLG null mutations in the Finnish patients with AD, they explain only a portion of the total burden of AD. The genetic heterogeneity and multifactorial pathogenesis of AD are well established,
and the effect of acquired factors is clearly demonstrated by the marked increase in prevalence of AD in industrialized countries in the 20th century. Other barrier protein or junction protein defects can induce skin barrier impairment and changes in filaggrin content similar to those caused by FLG null mutations, and additionally immunological factors can downregulate the expression of epidermal barrier proteins (44, 45). Whether the primary aetiologies is immunological (“inside to outside”) or barrier deficiency (“outside to inside”), as the majority of the current evidence implies, additional acquired and environmental factors further compromising the barrier function are often required to trigger the disease. This study adds valuable data on the effect of barrier genes in adult patients with AD with moderate to severe disease and persistent symptoms. Our findings also further support the complexity of genetic and acquired factors in the pathogenesis of AD.

The results of the current study extend our knowledge of the effect of FLG null mutations by confirming variants R501X and 2282del4 as risk factors for AD in the Finnish population. Furthermore, FLG status does not associate with treatment outcome in a referral centre setting, and therefore its usefulness as a general predictive biomarker in the follow-up of patients with AD seems low.

In the future, targeted sequencing of FLG and other skin barrier or immunological genes and their related pathways may provide insights into the underlying genetic and immunological mechanisms that drive AD. Larger studies are needed to assess possible small differences between different treatment modalities in the search for genotype-driven intervention decisions.

ACKNOWLEDGEMENTS

The authors would like to thank all patients who participated in this study. Eija Hämäläinen (FIMM) and Harri Rissanen (THL) are recognized for their contribution to H2000 control data management. The authors are grateful to Kati Donner (FIMM) and Kaisa Kettunen (FIMM) for their contribution in iPLEX assay design. Suvi Kyttänen (FIMM) for performing sequencing experiments, Anti-Pekka Sarin (FIMM) for providing valuable technical support, and Katarina Hannula-Jouppi and Antti Lauherma (Skin and Allergy Hospital) for their intellectual input.

This work was supported financially by a research grant from Astellas Pharma.

Finally, in honour of late Professor Sakari Reitamo, and with cordial respect, the authors wish to dedicate this study to his lifelong enthusiasm and commitment to atopic dermatitis research, clinical work, and devoted patient care. Professor Reitamo’s life’s work laid the foundation for the present study.

SR acted as a consultant for Astellas Pharma. The other authors declare no conflict of interest.

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