Coexistence of 2282del4 FLG gene mutation and IL-18 –137G/C gene polymorphism enhances the risk of atopic dermatitis

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Abstract

Introduction: Atopic dermatitis (AD) pathogenesis appears in the context of the correlation between cornified envelope proteins and immunological factors.

Aim: To estimate the association between FLG R501X and 2282del4 gene mutations, –137 G/C IL-18 and –1112 C/T IL-13 gene polymorphisms and their influence on AD course and the risk in the Polish population.

Material and methods: One hundred and fifty-two AD patients and 123 healthy volunteers were included into the study. Amplification refractory mutation system – polymerase chain reaction method was used.

Results: 2282del4 FLG mutation, predominant (p = 0.04) in Polish AD patients, enhanced the risk of AD (OR = 2.35; p = 0.01) and was associated with itch (p = 0.023). GG genotype of IL-18 was prevailing in AD (p < 0.0001), associated with elevated IgE levels (p = 0.00074) and pruritus (p = 0.0001). GG genotype and G-allele in –137 position of IL-18 increased AD risk (OR = 5.4; p = 0.0001, respectively, OR = 5.3; p = 0.000029). –1112 C/T polymorphism of IL-13 was associated with elevated IgE levels (p = 0.00049), pruritus (p = 0.0005), SCORAD score (p = 0.02), concomitant asthma (p = 0.0087) and AD risk (OR = 2.02; p = 0.012). Coexistence of 2282del4 or R501X FLG gene mutation with GG genotype of IL-18 was associated with a 6-fold higher risk of AD (OR = 5.8; p = 0.000013), contrary to combined occurrence of FLG mutations with T-allele in –1112 position of IL-13 gene (OR = 0.12; p = 0.1).

Conclusions: 2282del4 FLG mutation similarly to GG genotype and G-allele in –137 position of IL-18 gene enhance the risk of AD in the Polish population. Coexistence of FLG mutations with GG genotype of IL-18 may be helpful to estimate chances of AD development.

Key words: filaggrin, interleukin 18, interleukin 13, atopic dermatitis.
ing IL-18 is located on chromosome 11q22.2–22.3, which has been designated as a candidate region for atopy [12, 13, 18, 19]. The crucial role of interleukin 13 (IL-13) in AD was confirmed by different studies [20–22]. This cytokine is mainly responsible for enhanced IgE levels. Expression of IL-13 correlates with elevated IgE levels and AD severity [23]. It is thought that the polymorphism of IL-13 gene in the promoter region may be related to the increased transcription of that gene and susceptibility to development of AD and increased serum levels of IgE [24].

In the light of recent publications, pathogenesis of AD appears in the context of two major groups of genes: encoding epidermal proteins and major elements of the immune system [2]. Taking this into account, the association between cornified envelope protein gene mutations and cytokine genotyped gene polymorphisms and their influence on AD risk seems to be interesting.

Aim

The aim of this study is to estimate the association of –137 G/C IL-18 gene polymorphism, –1112 C/T IL-13 gene polymorphism, FLG R501X and 2282del4 gene mutations with AD risk and course of the disease in the Polish population and answer whether –137 G/C IL-18 polymorphism or –1112 C/T polymorphism of IL-13 enhances the risk of AD development in AD patients with FLG R501X or 2282del4 mutations.

Material and methods

Two hundred and seventy-five subjects were included into the study: 152 AD patients diagnosed according to current criteria and 123 healthy volunteers with no previous history of allergic diseases. The M : F male and female) ratio was 0.8 : 1. The average age of the AD patients was 23.2 ±11.57 (age range: 5–56 years) and of the controls was 24.9 ±8.02 (age range: 8–52 years). Assessment of pruritus severity was performed using the visual analogue scale (VAS: 0–3 – mild pruritus, > 3–7 – moderate pruritus, > 7–9 – severe pruritus, > 9 – very severe pruritus). Atopic dermatitis severity was rated by SCORAD index (severe (SCORAD > 60, pruritus). Atopic dermatitis severity was rated by SCORAD (0–3 – mild pruritus, > 3–7 – moderate pruritus, > 9 – very severe pruritus). Atopic dermatitis severity was rated by SCORAD index (severe (SCORAD > 60, n = 67), moderate (SCORAD 25–60, n = 50), and mild (SCORAD < 25, n = 35)). The average SCORAD score was 47.5 (11.5–85). The patients taking immunosuppressive treatment or other immunotherapy were excluded from the study. Informed consent was obtained prior to enrollment in the study. The study was conducted with the consent of the local ethics committee.

Genomic DNA was isolated from peripheral blood samples using Blood DNA Prep Plus according to the instructions of the manufacturer (A&A Biotechnology, Gdansk, Poland).

Analysis of polymorphic variants of IL-18, IL-13 gene and FLG mutations was performed by the amplification refractory mutation system – polymerase chain reaction method (ARMS-PCR) using designed specific sequences of oligonucleotides. The samples tested in our study were evaluated (genotyping) with internal amplification control of growth hormone 1 (GHI). Serum total IgE levels were measured by fluoroimmunoenzyme assay using the Uni-CAP 100 System (Phadia, Uppsala, Sweden). The cut-off point for serum IgE was 100 kU/l.

All analyses were performed according to the manufacturer’s protocols.

Statistical analysis

The data from inquiry prepared specially for this study were statistically worked out using Excel 2003 (Microsoft Corp., Redmond, WA, USA), Statistica (Version 8.0; StatSoft, Tulsa, OK, USA). The W Shapiro-Wilk, U Mann-Whitney, Kruskal-Wallis and χ² were performed. A logistic regression model was used to calculate the odds ratio (OR) and 95% confidence intervals (CIs). The statistical significance were established for the p < 0.05.

Results

Filaggrin

2282del4 FLG mutation was the predominant one in AD patients with FLG mutations (p = 0.04). We have found 42 (28.6%) patients that were heterozygotes, and no homozygotes (Table 1). 2282del4 of FLG mutation coexisted with allergic rhinitis (p = 0.001) and pruritus (p = 0.03). There was no association of FLG 2282del4 mutation with elevated IgE levels (p = 0.16), early onset of the disease (p = 0.97), concomitant asthma (p = 0.14), SCORAD score (p = 0.97), eosinophilia (p = 0.65). 2282del4 FLG mutation enhanced the risk of AD over twofold (OR = 2.35; p = 0.01) (Table 2).

R501X heterozygous mutation was observed in 20 (13.2%) AD patients (p = 0.18) (Table 1). We have not disclosed any homozygous mutation. There was no association of R501X FLG mutation with pruritus (p = 0.14), elevated IgE levels (p = 0.09), early onset of the disease (p = 0.35), SCORAD score (p = 0.91), eosinophilia (p = 0.21). We have only observed the association of R501X mutation with concomitant asthma (p = 0.0047). R501X mutation had no influence on the AD risk (OR = 0.68; p = 0.38) (Table 2).

The presence of R501X or 2282del4 FLG mutation in AD patients was associated with: pruritus (p = 0.009), elevated IgE levels (p = 0.025), concomitant asthma (p = 0.01) and allergic rhinitis (p = 0.0014). The presence of any of FLG mutations enhanced the risk of AD (OR = 1.88; p = 0.016) (Table 2).

–137 G/C polymorphism of IL-18

GG genotype of IL-18 was prevailing in AD (p < 0.0001). It was observed in 94 (61.8%) AD patients (Table 1). GG genotype of IL-18 was dominant in the
Coexistence of 2282del4 FLG gene mutation and IL-18 –137G/C gene polymorphism enhances the risk of atopic dermatitis

Table 1. The occurrence of FLG mutations, genotypes of IL-18 –137 G/C promoter gene polymorphism and –1112 C/T IL-13 promoter gene polymorphism in atopic dermatitis patients and control group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>AD</th>
<th>P-value V^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2282del4 FLG mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>23 (18.7%)</td>
<td>42 (28.6%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Wild type</td>
<td>100 (81.3%)</td>
<td>105 (71.4%)</td>
<td></td>
</tr>
<tr>
<td>R501X FLG mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>10 (8.1%)</td>
<td>20 (13.2%)</td>
<td>0.18</td>
</tr>
<tr>
<td>CC</td>
<td>113 (91.9%)</td>
<td>132 (86.8%)</td>
<td></td>
</tr>
<tr>
<td>IL-18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>41 (33.3%)</td>
<td>94 (61.8%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>GC</td>
<td>51 (41.5%)</td>
<td>49 (32.2%)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>31 (25.2%)</td>
<td>9 (5.9%)</td>
<td></td>
</tr>
<tr>
<td>IL-13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>52 (42.3%)</td>
<td>34 (23.3%)</td>
<td>0.00028</td>
</tr>
<tr>
<td>CT</td>
<td>70 (56.9%)</td>
<td>102 (69.9%)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>1 (0.8%)</td>
<td>10 (6.8%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. The influence of FLG mutations, IL-18 and IL-13 genotypes and alleles on the AD risk

<table>
<thead>
<tr>
<th>Variable</th>
<th>P-value</th>
<th>OR</th>
<th>-95% CL</th>
<th>+95% CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2282del4 FLG mutation</td>
<td>0.01</td>
<td>2.35</td>
<td>4.519</td>
<td>1.223</td>
</tr>
<tr>
<td>R501X FLG mutation</td>
<td>0.39</td>
<td>0.684</td>
<td>0.289</td>
<td>1.616</td>
</tr>
<tr>
<td>2282del4 or R501X FLG mutations</td>
<td>0.016</td>
<td>1.885</td>
<td>3.179</td>
<td>1.118</td>
</tr>
<tr>
<td>IL-18</td>
<td>&lt; 0.0001</td>
<td>2.672</td>
<td>3.861</td>
<td>1.849</td>
</tr>
<tr>
<td>G-allele of IL-18</td>
<td>&lt; 0.0001</td>
<td>5.412</td>
<td>2.761</td>
<td>10.608</td>
</tr>
<tr>
<td>C-allele of IL-18</td>
<td>0.000029</td>
<td>5.351</td>
<td>2.429</td>
<td>11.804</td>
</tr>
<tr>
<td>–1112 C/T IL-13</td>
<td>&lt; 0.0001</td>
<td>0.308</td>
<td>0.508</td>
<td>0.178</td>
</tr>
<tr>
<td>T-allele of IL-13</td>
<td>0.013</td>
<td>2.021</td>
<td>1.159</td>
<td>3.526</td>
</tr>
<tr>
<td>C-allele of IL-13</td>
<td>0.038</td>
<td>0.111</td>
<td>0.014</td>
<td>0.892</td>
</tr>
<tr>
<td>2282del4 or R501X mutation and GG genotype of IL-18</td>
<td>0.00013</td>
<td>5.815</td>
<td>2.342</td>
<td>14.439</td>
</tr>
<tr>
<td>2282del4 or R501X mutation and T-allele of IL-13</td>
<td>0.12</td>
<td>0.625</td>
<td>0.348</td>
<td>1.123</td>
</tr>
</tbody>
</table>

group of patients with pruritus in contrast to CC genotype (p < 0.0001). We have observed the association of –137G/C IL-18 polymorphism with elevated IgE levels. GG genotype of IL-18 dominated in the group with enhanced IgE serum concentration comparing to CC genotype that dominated in the group with normal IgE level (p = 0.000074). We have observed an association of –137G/C IL-18 gene polymorphism with allergic rhinitis (p = 0.00027). We have not found any association of –137 G/C polymorphism of IL-18 with early onset of the disease (p = 0.48), concomitant asthma (p = 0.15) or eosinophilia (p = 0.26). –137G/C polymorphism of IL-18 gene enhanced the risk of AD, especially GG genotype (OR = 5.14; p < 0.0001) and G allele (OR = 5.35; p = 0.000029) over 5 times (Table 2).

–1112 C/T polymorphism of IL-13

CT genotype of IL-13 was predominant in AD (69.9%; p = 0.00028) (Table 1). T-allele was dominant in the AD group: 61.2% vs. 38.8% in controls (p = 0.0015). –1112 C/T IL-13 gene polymorphism was associated with pruritus (p = 0.0005) and elevated IgE levels (p = 0.00049),
SCORAD score ($p = 0.022$), eosinophilia ($p = 0.00029$) and concomitant asthma ($p = 0.0087$). We have not found any association of $-1112C/T$ polymorphism of IL-13 with early onset of AD ($p = 0.07$), allergic rhinitis ($p = 0.08$). $-1112 C/T$ IL-13 gene polymorphism was associated with the AD risk (OR = 2.02; $p = 0.013$). C allele decreased the risk of AD (OR = 0.111; $p = 0.038$). T allele increased the risk of AD (OR = 2.319; $p = 0.001$) (Table 2).

**Results of our study are consistent with the the one carried out in the German population [13], which revealed a significant association of SNPs in $-137G/C$ of IL-18 gene with AD. We have previously published the data, which have indicated that G-allele reveals susceptibility to AD development and C-allele seems to have protective properties [34]. Now we have more proofs because GG genotype of IL-18 is associated with elevated IgE levels and pruritus in contrast to CC genotype. Kruse et al. [19] have also observed an association of $-137 G/C$ IL-18 gene polymorphism with high IgE levels. By the way, we have found no association with SCORAD score, similarly to Novak et al. [13]. According to the latter study, the association of $-137 G/C$ IL-18 gene polymorphism with concomitant manifestation of allergic rhinitis or asthma. We have noticed the associations of $-137 G/C$ SNP of IL-18 with AD was not directly dependent on concomitant manifestation of allergic rhinitis, but not with asthma. The association of IL-18 serum levels with AD course was previously published [16, 17]. Now, in the context of FLG mutations, we have found no associations between elevated serum levels of IL-18 and 2282del4 FLG mutation as well as for R501X FLG.
mutation. Anyway, it is well documented that Th2 cytokines like IL-13, IL-4 influence the FLG expression even if no FLG mutation exists [10]. It seems to be interesting if IL-18 also inhibits FLG expression in AD patients, while they are not FLG mutations carriers. On the other hand, in our study AD 2282del4 carriers with homozygous GG genotype for –137 G/C polymorphism of IL-18 have a nearly 6-fold higher risk of AD development. We have previously published that elevated levels of IL-18 were associated with GG genotype and G allele [34]. Additionally, although –1112 C/T polymorphism of IL-13 gene and T allele enhance the risk of AD according to previous data [35], coexistence of T allele with any FLG mutation does not increase susceptibility to AD.

These results seem to indirectly indicate that there must be an interaction between FLG decreased expression and Th2 cytokines over-expression, as it was previously suggested [4, 36] and AD emerges in the light of innate and acquired immune response [37], genes and immunology interactive net.

Conclusions

2282del4 FLG mutation that dominates in the Polish population is a risk factor for AD development. GG genotype and G allele of IL-13, similarly to T allele of IL-13, seem to promote AD development. In contrast to combined occurrence of FLG mutations with T allele of IL-13, coexistence of FLG mutations with GG genotype of IL-18 is associated with a 6-fold higher risk of AD. Thus, our results indicate that this combined occurrence may be helpful to estimate chances of AD development and seems to be a useful parameter in separating patients from healthy persons.

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Conflict of interest

The authors declare no conflict of interest.

References


