INVESTIGATIVE REPORT

Association of a Single Nucleotide Polymorphism in a Late Cornified Envelope-like Proline-rich 1 Gene (LELP1) with Atopic Dermatitis

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There is some evidence that genes involved in the pathogenesis of atopic dermatitis, in addition to the filaggrin (FLG) gene, may be located at chromosome region 1q21. The aim of this study was to examine the association of single nucleotide polymorphisms in the region of the late cornified envelope-like proline-rich 1 (LELP1), horne- rin (HRNR) and FLG genes with the course and risk of atopic dermatitis. Single nucleotide polymorphisms and mutations were genotyped by PCR restriction fragment length polymorphism and real-time PCR in a group of 152 patients with atopic dermatitis and 104 healthy volunteers. CC genotype and C-allele of LELP1 rs7534334 were found in patients with atopic dermatitis and were associated with elevated levels of serum immunoglobulin E, severity of atopic dermatitis and concomitant asthma. LELP1 rs7534334 enhanced the risk of atopic dermatitis nearly 2.5-fold. This pilot study suggests that rs7534334 SNP, located in the LELP1 region, may be a potential genetic marker for the risk and course of atopic dermatitis. Key words: atopic dermatitis; polymorphism; cornified envelope proteins; filaggrin.

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Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease with a worldwide prevalence of 8.7–18.1% in children (1) and 1.5–10.2% in adults (2). The clinical features of AD, i.e. continual itchiness, flares and sleep disturb- ance, negatively affect the occupational activities and social relationships of patients, and the quality of life (QoL) of patients and their families (3). In addition to impairment of QoL, AD also has significant economic impact (3). The pathogenesis of AD is multifactorial; thus its analysis is difficult. Nevertheless, ongoing investigations have provided a number of clues to the background of AD. It has been shown that epidermal barrier dysfunction may be a key factor in the pathogenesis of AD (4–7). Genome- wide studies have indicated associations between different gene loci responsible for epidermal barrier function and AD (8–10). The defective skin barrier in AD is closely related to disturbances in cornified envelope (CE) proteins. Loss-of-function mutations (R501X and 2282del4) in the gene encoding filaggrin (FLG) are well-known risk factors for the development of AD, associated with severe course, early onset of the disease and elevated levels of immunoglobulin E (IgE) (11–14). However, FLG mutations are detected in only 10–50% of patients with AD, and similar FLG mutations are observed in approximately 9% of the European population with no concomitant inflammation (11, 13). Moreover, homozygotes for the 2282del4 and R501X FLG null mutation do not always develop dermatitis, and complete long-term remission can be observed (15). Ethnic differ- ences in FLG mutations have been reported, highlighting the need to determine the FLG mutations specific to given populations (16). The median prevalence of FLG mutation is 7.7% and 3% in European and Asian general populations, respectively, although, within the European population, there are regional differences; FLG mutations are much less common in people of southern European descent than those of northern European descent (17). Cytokines that are crucial for the pathogenesis of AD, e.g. interleukins (IL)-4, IL-13, IL-17, IL-22, IL-25, and IL-31, can influence FLG expression, even if there is no FLG mutation (18, 19). Similarly, exposure to environmental factors, such as water, low humidity, skin irritants, sunburn, and micro-organisms colonizing the skin, can also down-regulate the expression of FLG and accelerate degradation of this protein (18, 19).

The FLG gene is located within the epidermal differentiation complex (EDC), a 1.6 Mb region of chromosome 1, positioned at q21. The EDC contains 3 clustered gene families encoding CE proteins. One family is composed of genes that encode precursors of CE proteins (loricrin, involucrin, small proline-rich proteins (SPRR), and late envelope proteins (LEP, XP5 and SPRRL)). These prote- ins differ in their internal domain, characterized by short tandem peptide repeats in the central region. The second group of genes encodes proteins belonging to the S-100A family, i.e. calcium-binding proteins containing EF-hand domains. The third family of genes encodes S100-fused protein family evolved from the first and second family. In addition to FLG, trichohyalin, repetin, hornerin and cornulin also belong to this protein family (20). Accumulated evidence suggests that some hidden AD risk factors, other
than FLG mutations, are located within the EDC region (21, 22). Moreover, genetic analysis suggests that genetic variation in epidermal transglutaminase, which is a key player in the formation of the cornified envelope, and is linked to epidermal disorders, is not an important factor in susceptibility to AD (23). It has also been suggested that susceptibility genes other than FLG are likely to be involved in the development of late-onset AD (14).

Therefore, in addition to FLG mutations (2282del4, R501X, S3247X and R2447X), the current study investigated single nucleotide polymorphisms (SNP) of genes encoding other CE proteins, i.e. hornerin (HRNR): rs11204937, rs877776, and late cornified envelope-like proline-rich 1 (LELP1): rs7534334, which is located 255 bp downstream of LELP1. Associations between particular SNPs, FLG mutations, and the course and risk of AD were investigated, and the impact of the studied polymorphisms on the course of AD in patients with no FLG mutations was analysed.

METHODS
Patients
The study population comprised 256 subjects of Polish origin. Patients with AD were recruited from the Department of Dermatology, Venereology and Allergology on the basis of the diagnostic criteria of Hanifin & Rajka (24). A total of 152 patients with AD were included in the study (65 males (42.8%), 87 females (57.2%); male:female 0.7:1; mean ± standard deviation (SD) age 24.4 ± 12.1 years, age range 10–64 years). The mean Severity Score of Atopic Dermatitis (SCORAD) was 48.51 ± 22.09 (range 5–86). Mild course of AD was observed in 27 patients (17.8%), moderate in 72 (47.3%) and severe in 53 (34.9%). Mean ± SD age at disease onset was 4.8 ± 8.6 years. Early onset of AD (<2 years of age) was noted in 83 subjects (54.6%). Concomitant asthma existed in 42 patients with AD (27.6%). Patients undergoing immunosuppressive treatment or other immunotherapies were excluded from the study. The control group comprised 104 healthy, ethnically matched volunteers with no medical history of allergic, immunological diseases or malignancies (40 males (38.5%), 64 females (61.5%); male:female 0.6:1; mean ± SD age 24.4 ± 9.9 years, age range 15–61 years).

The study was conducted with the consent (NKEBN/486/2011) of the local ethics committee (Independent Bioethics Commission for Research at Medical University of Gdansk). Written consent was obtained from all patients prior to enrollment.

Determination of IgE level
Total serum IgE levels were estimated by fluorescent enzyme immunoassay using the Uni-CAP 100 System (Phadia, Sweden) according to the manufacturer’s instructions.

Determination of atopic disease severity
The SCORAD scale was used to measure the severity of AD: 0–20 points: mild AD; 21–60 points: moderate AD; over 60 points: severe AD. A visual analogue scale (VAS)/numeric rating scale (NRS) was employed to estimate the pruritus level (0–10 points).

Table I. Frequency of genotypes and alleles of late cornified envelope-like proline-rich (LELP1) and filaggrin (FLG) mutation in patients with atopic dermatitis (AD) and control group, with logistic regression analysis of association between single nucleotide polymorphism of LELP1, FLG mutations and AD

<table>
<thead>
<tr>
<th>Genotype and minor allele</th>
<th>Occurrence in patients with AD (%)</th>
<th>Occurrence in healthy subjects (%)</th>
<th>p</th>
<th>RR</th>
<th>OR</th>
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<tr>
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<td>FLG mutation and CC genotype of LELP-1</td>
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CI: confidence interval; OR: odds ratio; RR: relative risk; NA: not applicable.

1http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-2301
Mutations in LELP1 (rs7534334) CC genotype in patients without FLG (R501X, 2282del4, S3247X, R2447X)

FLG mutation (2282del4) was more frequent in patients with AD (p = 0.002) than in controls (Table I). Twenty-six heterozygotes (17.1%) and 5 homozygotes (3.3%) of FLG 2282del4 were found. Occurrence of FLG (2282del4) in patients with AD was associated with elevated levels of serum IgE (p = 0.035), eosinophilia (p = 0.016) and severity of the disease (p = 0.045). FLG (2282del4) enhanced AD risk nearly 2.5-fold (p = 0.015, OR=2.41, relative risk (RR)=1.5) (Table I). In contrast, no carriers of R501X or R2447X mutations, and only 2 heterozygotes of S3247X, were found in the subjects analysed.

Combined occurrence of FLG 2282del4 mutation and CC genotype of LELP1 was predominant in patients with AD (p = 0.008) compared with healthy controls. Analyses performed on a group of subjects without FLG mutations (R501X, 2282del4, S3247X, R2447X) showed that the CC genotype of LELP1 (rs7534334) was predominant in patients with AD, whereas the TT genotype prevailed in healthy subjects. Risk of AD in the group of patients after excluding those with examined FLG mutations was increased more than 2-fold if the CC genotype of LELP1 was present (p = 0.048, OR=2.25, RR=1.32) (Table I). After excluding subjects with FLG mutation, the association of CC-genotype and C-allele of LELP1 with elevated IgE levels, and TT genotype and T-allele with normal levels of IgE (p = 0.015 and p = 0.005, respectively) remained.

No linkage disequilibrium (LD) was found in an analysis of association between rs7534334 and LELP1 variants (R501X, S3247X, R2447X). The correlation between rs7534334 and 2282del4 variant of FLG was determined as D’=0.17 and r²=0.003, indicating that these SNP are not correlated with each other.

DISCUSSION

Previous studies suggest that, in addition to FLG, other genes involved in the pathogenesis of AD might be hidden within the EDC (13, 21, 22).

Late cornified envelope-like proline-rich (LELP1) protein is a small protein of unknown function. According to some authors LELP1 may be regulated by STAT6, which is responsible for expression of IgE (26). The only published study of LELP1 found significant association of rs7534334 SNP, located 255 bp downstream of LELP1, with serum levels of IgE in patients with atopic asthma (26). To date there are no other reports of LELP1 SNP in subjects with AD. Our study observed statistically significant association of rs7534334 with elevated levels of serum IgE, more severe course of AD and eosinophilia. The risk of AD development associated with rs7534334 SNP was close to that associated with FLG (2282del4) mutation (27, 28) and was still present after excluding carriers of 2282del4. Our analysis of LD between rs7534334 and 2282del4 yielded r = 0.003, suggesting that these SNPs are not correlated with each other. It can be assumed that increasing the number of patients would make this more relevant. Therefore, it would be interesting to study this locus of LELP1 in a larger group of patients with AD. Based on the Ensemble Genome Browser information, we found 2 SNPs in HapMap data that are in LD (r²=1 and D’=1) with rs7534334. rs4845529 was found in a population of African ancestry in the southwest USA, and rs534994240 in an Esan population in Nigeria. However, analysis of data from genome-wide association studies (GWAS) showed that neither of these is associated with AD.

The current study observed a statistically significant association of rs7534334 SNP with concomitant asthma in the group of patients with AD. Further research will study the association between SNPs within and around the LELP1 and asthma risk in the group of asthma patients with atopic eczema. However, it should be noted that Sharma et al. (26) found no association with asthma.

**Fig. 1.** Association of rs7534334 single nucleotide polymorphism (SNP) in the region of late cornified envelope-like proline-rich 1 (LELP1) with elevated level of total serum IgE in patients with atopic dermatitis (AD). (A) Relationship between LELP1 SNP genotype and elevated total serum IgE (>100 IU/ml) in patients with AD (analysed by χ² test). (B) Relationship between LELP1 SNP genotype and total serum IgE level in patients with AD (analysed by Kruskal-Wallis analysis of variance (ANOVA) test and Dunn’s post-hoc test).

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in a trio-based family (111 asthma patients) or in an independent case-control cohort (165 asthmatics, 166 nonasthmatics controls). Their study population mainly comprised patients with atopic asthma; patients with AD made up less than 5% of their cohort, therefore they may not have had pure AD (but rather were subjects with coexisting asthma). Conversely, in our group, there were no patients with pure asthma and only 27% of patients (n = 42) had concomitant asthma. Therefore, it is possible that differences in group design may explain the divergent results. Association of LELP rs7534334 with concomitant asthma may be present only in a group of patients with AD with coexisting asthma, similarly to FLG mutation, which was a strong predisposing factor for AD as well as for asthma, but for asthma occurring in the context of AD (11, 27, 28). Another question that requires further research is to what extent a given SNP alters the LELP1 gene and/or protein function. If such relations exist, genetic polymorphisms in LELP1 influencing asthma risk could be the next point confirming that epidermal barrier defects may play a role in the pathogenesis of asthma. In most studies associations of FLG mutations with asthma have been observed only in patients with concomitant AD (11, 27, 28). However, some studies have reported significant associations of FLG mutations and asthma in subjects without atopic eczema (29, 30). The current study revealed that the FLG mutation (2282del4) is a risk factor for AD. Most studies have reported that carriers of FLG gene mutations have a more severe course of AD (11, 16, 28, 31), and this trend was also noted in our study. Moreover, similarly to some authors (31, 32), we observed an association between the presence of 2282del4 and elevated levels of serum IgE. However, we have not observed a significant association of FLG (2282del4) with early onset of AD. The similar lack of correlation of 2282del4 with early onset of AD was also noted in a study in the German population (27); however, some studies have reported such a correlation (14, 16, 32). Rupnik et al. (14) suggest searching for other risk factors, and other susceptibility genes responsible for the development of AD in late childhood or adults. However, we have not found any association of SNP of LELP1 rs7534334 with late onset of AD (p = 0.7), even after excluding the subjects with FLG mutation (p = 0.2). The loss-of-function mutation (R501X) is another FLG mutation, detected most frequently among patients with AD in Austria, Germany and Ireland (12). Our study found no carriers of R501X mutation, which is in accordance with previous studies documenting that this mutation is rare in the Polish population (carriage rate 0.8%) (31). A very low frequency of R501X mutation (carriage rate 0.2%) has also been reported in the Croatian population (33). It is worth noting that, similarly to our results, in the Croatian population, no carriers of other FLG null mutations (R2447X, S3247X) were detected (33). However, the 4 FLG null mutations (2282del4, R501X, R2447X, S3247X) have been shown to be recurrent in Austrian, German, Scottish and Irish populations (11, 12, 32). All these findings may support the ethnic differences in occurrence of FLG mutations as well as a latitude-dependent distribution. Moreover, in populations with low penetrance of FLG mutations (R501X, R2447X, and S3247X), genetic alterations in other CE genes may play a dominant role in the pathogenesis of AD.

The route from FLG defects to airway disease is not yet understood. Filaggrin is expressed in the skin, and in the outer layers of the oral and nasal mucosa (11, 19), but not in mucosa of the lower airways (34). These data suggest that the development of FLG-associated asthma is mediated by a systemic, possibly immunological, mechanism and the impaired skin barrier function is caused by FLG mutations (28) and possibly by altered LELP1. Thus, further research is required into LELP1 expression in the airway epithelium and LELP1-associated asthma. A further unanswered question is whether rs7534334 SNP is associated with asthma susceptibility in patients with asthma who do not have AD.

SNP rs877776 located within the hornerin gene (HRNR), which encodes the hornerin protein, has been reported as a novel susceptibility factor for AD (22). HRNR is a protein with structural organization similar to profilaggrin. The function of this protein is not clear, but it appears to be similar or complementary to that of FLG (35). Esparza-Gordillo et al. (22) reported a significant association of rs877776[C] with AD compared with controls (without FLG mutation), but the trend lacked statistical significance. The current study could not replicate the rs877776 association with AD phenotype, course and risk (see Appendix S2). A similar lack of rs877776 association with AD was observed in the Irish paediatric population (36) and in Austrian patients with AD (37). Like others (36), we did not observe any significant differences in the rs877776 allelic or genotype distribution between patients with AD and controls. Conversely, we noted significant association of CC genotype of HRNR rs11204937 with elevated levels of serum IgE, early onset of AD and eosinophilia. However, the greater frequency of CC genotype and rs11204937[C] in the AD group was not statistically significant. This may be due to the relatively small group size or to ethnic limitations.

Reflecting the current views and concepts it should be mentioned, that beside CE proteins, abnormal lamellar body secretion and disorder in stratum corneum (SC) lipids, especially ceramides (CER) and chain length of free fatty acids (FFA), have a strong impact on impaired skin barrier function in AD (38, 39). However, no association has been found between properties of SC lipids and FLG mutations in patients with AD (38).

In conclusion, rs7534334 SNP, located 255 bp downstream of LELP1, may be an important factor in AD risk and course, at least in the Polish population. It would therefore be interesting to study this LELP1 locus in a larger group of patients with AD as well as in patients with asthma, and to determine whether
rs7534334 might be a robust biomarker for AD. Further research is needed into the significance of genes encoding other CE proteins for AD development, especially in the group of patients with no FLG mutations and in the context of their influence on lipid structures in the skin barrier in patients with AD.

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The authors declare no conflicts of interest.

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