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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*), hornerin (*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 disturbance, 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 differences 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 proteins 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

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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 26.3 ± 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).

For genotyping and statistical analysis see Appendix S1[1]

RESULTS

Occurrence of HRNR SNP (rs11204937) and HRNR SNP (rs877776) in patients with atopic dermatitis

No associations were found between rs11204937 and rs877776 genotype/alleles and elevated levels of serum IgE, pruritus, SCORAD score, age, early onset of AD, eosinophilia, or concomitant asthma (see Appendix S2[1]).

Association of LELP1 *SNP (rs7534334) with course and risk of atopic dermatitis*

The CC genotype of *LELP1* (rs7534334) was predominant in the AD group, being present in 46% of patients with AD, and twice as frequent in the AD group as in the control group ($p = 0.046$) (Table I). rs7534334[C] was 1.7 times more frequent ($p = 0.029$) in the AD group than in healthy subjects (Table I). Comparison of *LELP1* (rs7534334) genotype and clinical factors indicated that the CC genotype ($p = 0.003$) and C-allele ($p = 0.001$) of *LELP1* (rs7534334) were associated with elevated levels

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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*

	Genotype and minor allele	Occurrence in patients with AD (%)	Occurrence in healthy subjects (%)	*p*	RR	OR	– 95% CI	+ 95% CI	*p*
LELP-1 rs7534334	CC	46	31	0.046	1.5	2.415118	1.021967	5.707418	0.043
	CT	44	56						
	TT	10	13						
	C	68	59						
	T	32	41						
FLG rs2282del4	Wild-type	80	93	0.0022	2.55	2.977568	1.234308	7.182902	0.015
	Heterozygotes	17	7						
	Homozygotes	3	0						
FLG mutation and CC genotype of *LELP-1*	NA	NA	NA	NA	2.74	3.148148	1.022209	9.695511	0.045
No *FLG* mutation and CC genotype of *LELP-1*	NA	NA	NA	NA	1.32	2.257998	0.9927243	5.135923	0.048

CI: confidence interval; OR: odds ratio; RR: relative risk; NA: not applicable.

of serum IgE (Fig. 1). Patients carrying the TT genotype of *LELP1* (rs7534334) presented a milder course of AD compared with those with CT genotype, who had a severe course of AD ($p=0.018$) (not shown). CC genotype and C-allele of *LELP1* (rs7534334) were associated with concomitant asthma ($p=0.03$ and $p=0.01$, respectively). In addition, significant associations of CC genotype and C-allele with eosinophilia ($p=0.002$ and $p=0.001$, respectively) and positive results of prick tests ($p=0.03$ and $p=0.02$, respectively) were noted (not shown). *LELP1* (rs7534334) enhanced the risk of AD nearly 2.5-fold ($p=0.043$, OR=2.41, relative risk (RR)=1.5) (Table I).

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 3 times ($p=0.015$, OR=2.97, RR=2.55) (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 *FLG* variants (R501X, S3247X, R2447X). The correlation between rs7534334 and 2282del4 variant of *FLG* was determined as D'=0.17 and $r^2=0.003$, 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 ($r^2=1$ 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

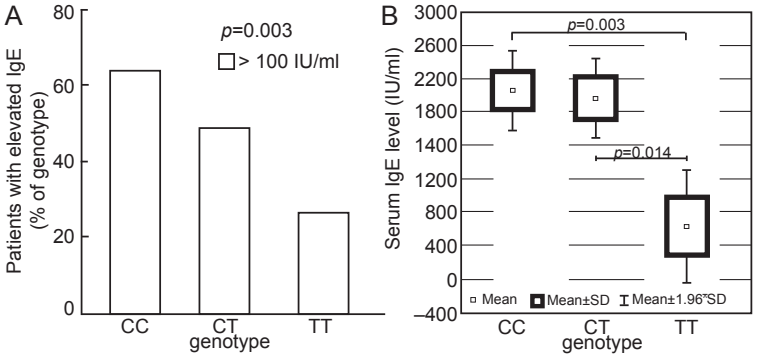

Fig. 1. Association of rs7534334 single nucleotide polymorphism (SNP) in the region of late cornified envelope-like proline-rich 1 (*LELP1*) with elevated level of total serum IgE in patients with atopic dermatitis (AD). (A) Relationship between *LELP1* SNP genotype and elevated total serum IgE (>100 IU/ml) in patients with AD (analysed by χ^2 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).

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 signification. The current study could not replicate the rs877776 association with AD phenotype, course and risk (see Appendix S2[1]). 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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