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Lelp-1, Its Role in Atopic Dermatitis and Asthma: Poland and Portugal

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Abstract

Background: Atopic dermatitis (AD) that begins in childhood and is the first step of the so-called 'atopic march'. The chromosome 1q21 region has been associated with AD and psoriasis, with a peak in Epidermal Differentiation Complex (EDC) in a region of 2.05 Mb. The aim of this work was to study LELP-1 (late cornified envelope-like proline-rich 1) polymorphism [rs7534334] located within the EDC, in AD and asthma in two European populations: Portugal and Poland.

Methods: We studied 110 individuals in the control group and 129 asthmatics in the Portuguese cohort; 100 controls and 45 patients with AD and asthma in the Poland cohort. Written informed consent was obtained from all participating individuals. LELP-1 genotypes were determined by the PCR-RFLP technique. All statistical analyses were carried out using SPSS 21.0 software.

Results: The results were considered statistically significant with $p<0.05$. We found that the CC genotype was more frequent in Poland's cohort with AD and asthma when compared with controls (p=0.004), (OR: 2.80 [1.34-5.82]; adjusted p=0.006) and the C allele was also a risk factor (OR: 2.40 [1.35-4.28]; adjusted p=0.003) to both diseases in this group. When compared the cohort from Portugal with Poland, there was a trend for TT genotype to be a risk for asthma in the Portuguese cohort (OR=7.49 [0.92-60.91], adjusted p=0.06). C allele was more frequent in the cohort from Poland and T allele, in the cohort from Portugal (p=0.047).

Conclusion: These findings demonstrate that genetic variation of skin barrier genes like LELP-1 might contribute to allergic diseases.

Keywords: LELP-1; Atopy; Atopic dermatitis; Asthma; Portugal; Poland

Introduction

Epidermal keratinocytes undergo a terminal differentiation and programmed cell death (physiological apoptosis) known as cornification [1-3]. Cornification leads to the cornified layer, and different genes proceed in an organized sequence to provide this outermost skin barrier in the spinous and granular layers that express proteins like keratins (namely: K1, K2 and K10) and non- keratin proteins like filaggrin (FLG), loricrin (LOR), involucrin (IVL) and small proline rich proteins (SPRRs) [4-7]. These proteins are cross-linked in the cornified cell envelope by transglutaminase enzymes, and this insoluble envelope associated with the keratin-containing macrofibrils fills corneocytes and with the lipids ,forms the skin barrier that protect from dehydratation and environment allergens [4].

Atopic dermatitis (AD), or eczema, is a skin disease often associated with other allergic diseases, such as, allergic rhinitis and asthma [8,9]. AD is very common in westernized societies, where it affects about 20% of children and 3% of adults [8-12]. In children with AD about 60% will develop asthma, being a strong predictor of subsequent asthma development and the natural history of atopic march. The biological approach of AD implies a defective barrier defect, and overexpression of inflammatory mediators associated with immune dysregulation [9].

FLG mutations predispose significantly to an increased risk to develop atopic eczema. Apart from FLG other proteins involved in skin barrier functions such as SPRR, lipids synthesis and metabolism, protease and protease inhibitor function, all seem to play a role. Besides skin barrier function, immune deviation versus a Th2 dominance and increased IgE production is also genetically determined. Polymorphisms have been found in genes encoding IL-4, IL-13 and STAT-6, and recently a polymorphism on the high-affinity IgE receptor gene has been found. Using genome-wide association studies, new genes with yet unknown functions have been determined to be associated with atopy and atopic eczema [13,14,15,16].

The molecular signature of AD is mainly associated with Th2 [8,17-19] IgE high (extrinsic) and IgE low (intrinsic) mediated by keratinocyte thymic stromal lymphopoietin (TSLP) regulating dendritic cells. This Th2 activation contributes to barrier disfunction by impairing FLG and other skin barrier genes expression [20-23]. IL-22 and IL-33 play also its role, [24,25] in this Th2 driven inflammation by allergens, associated with FLG and other EDC gene polymorphisms, and are also important in other allergic diseases such as asthma, besides AD. Biphasic T cell response in the skin (Th2 cells in acute AD; Th1 cells in chronic AD) [8] and reduced skin innate immune response [26,27] are characteristics of this disease.

The epidermal and dermal AD transcriptomes and their respective

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contributions to abnormalities in respective immune and barrier phenotypes have been highlighted recently in lesional and nonlesional AD skin [28].

The upregulated genes in lesional epidermal transcriptome consisted of proliferation-related, EDC, inflammatory antimicrobial genes and the upregulated genes dermal transcriptome included T-cell activation, IL-2 receptor α, Th2-related, Th22, Th17-related and collagen genes [24,25].

Studies of association of genes in AD put in evidence the cluster of the EDC [7,23] and other barrier candidates [29], but the most important associations were related to FLG (filaggrin) [13,30-32] and two null mutations (R510X and 2282del4) [33,34]. In this study we have studied the role of LELP1 (another EDC gene) polymorphism (late cornified envelope-like proline-rich 1) [rs7534334] (a polymorphism 258 bp downstream of the LELP1) using the HapMap database (HapMap data rel28 Phase II+III, August 10, NCBI B36 assembly) and its association with atopic dermatitis and asthma in a Portuguese and Poland's cohort.

LELP1 codes for a SPRR (cornifin) family protein, [35] assuming that many of those proteins (FLG, SPRR, loricrin, involucrin) are stored and released from keratohyalin granules in the granular layer .The cell membrane is then, covered with cross-linked intercellular proteins forming the cornified envelope [2,6,36,37]. Transglutaminases cross-link intercellular proteins and also link lipids to the cornified envelope, forming also the lipid envelope to provide a water barrier function [37].

The chromosome 1q21 region [38] has been associated with skin pathology like AD, ichthyosis vulgaris and psoriasis, in a region of about 2.05Mb (mega basis) in Epidermal Differentiation Complex (EDC).

Bronchial epithelial cells and keratinocytes were found to have a high degree of overlap in gene expression [39]. Bronchial epithelial cells, similar to keratinocytes, express components that are able to form a cross-linked protein envelope that may contribute to an effective barrier against noxious stimuli and pathogens [39]. SPRRs are part of the portfolio of genes expressed by both bronchial epithelial cells and keratinocytes in response to pro-inflammatory cytokines, suggesting the importance of these proteins in host defense [40,41]. There is an epithelial-specific molecular signature of gene expression in bronchial epithelial cells and keratinocytes comprising a family member of keratins, small proline-rich proteins and proteinase inhibitors [39].

It has become clear that epithelia and also epithelial tissues [42,43] have three main mechanisms to protect the organism from pathogens [39], pollutants and allergens. First, the epithelial cells form an impermeable barrier which both prevents pathogen entry and minimizes dehydration (xerosis). Secondly, epithelial cells are capable of producing defense molecules such as antimicrobial peptides and proteinase inhibitors. Finally, these cells are able to produce signaling molecules such as cytokines and chemokines, playing an active role in innate and adaptive immunity.

The aim of this work was to study the role of LELP-1 (late cornified envelope-like proline-rich 1) polymorphism [rs7534334] located on EDC, in atopic dermatitis and asthma in two different European populations: Portugal and Poland.

Material and Methods

The study population consisted of 110 individuals in the control group and 129 asthmatics from the Portuguese cohort and 100 controls and 45 AD with asthma from the Poland cohort.

Written informed consent was obtained from all participating individuals. The genetic study on EDC has been approved by the Independent Bioethics Commission for research.

Patients were diagnosed by physicians for asthma according to the guidelines of GINA, and as having atopy or not according to WAO/EAACI guidelines, they were examined for a self-reported history of breathlessness, wheezing, atopic dermatitis and family history, atopic individuals have a positive skin prick test (SPT) for at least one of the common environmental allergens or the presence of specific IgE ,associated with high serum IgE levels estimated using enzyme-linked immunosorbent assay and suffered from asthma, or AD and asthma. The SCORAD (SCOring Atopic Dermatitis) index was completed in all patients with AD. The demographic and clinical details of the study population are given in Table 1.

Genomic DNA Isolation

Whole blood samples from patients and controls were stored with EDTA at -20°C. The genomic DNA was isolated through a non-enzymatic method (salting out method) adapted from Lahiri, D. K., & Nurnberger, J. I et al., 1991 [44].

Genotyping of Lelp-1 [Rs7534334]

The LELP-1 genotypes were determined by the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) technique, the polymorphic region was amplified in a 50 µl reaction mixture: 10 mM of each primer (forward: 5´- CCTCCACCATGTACAACGCT-3´; and reverse: 5´-TTGCATTAAACCCATGCAGCC-3´), 200 ng of genomic DNA and 0.2 mM of PCR nucleotide Mix Thermo Scientific® DreamTaq Green containing 10 mM dNTPs, 1.5 mM $MgCl_2$, 1 U Taq polymerase. PCR conditions involved an initial denaturation of DNA at 94°C for 3 min,

Portugal	Controls	Asthma	p.
N (%)	110 (46.0)	129 (54.0)	n.a.
Female	42 (38.2)	82 (63.6)	**<0.001**
Male	68 (61.8)	47 (36.4)	
Age (years) †	110 (42.82 ± 10.88)	129 (38.40 ± 19.24)	**0.027**
<1515-30> 30	0 (0.0)	16 (12.4)	**<0.001**
	15 (13.6)	37 (28.7)	
	95 (86.4)	76 (58.9)	
Atopy	n.a.	111 (86.0)	n.a.
Asthma controlled	n.a.	92 (71.3)	n.a.
Poland	Controls	AD and Asthma	p.
N (%)	100 (69.0)	45 (31.0)	n.a.
Female	64 (64.0)	27 (60.0)	0.712
Male	36 (36.0)	18 (40.0)	
Age (years) ††	100 (25) [18-61]	45 (23) [7-59]	**0.027**
<15 15-30> 30	0 (0.0)	8 (17.8)	**<0.001**
	66 (66.0)	27 (60.0)	
	34 (34.0)	10 (22.2)	
Atopy	n.a.	38 (84.4)	n.a.
Asthma controlled	n.a.	45 (100.0)	n.a.
SCORAD †	n.a.	45 (54.7 ± 20.5)	n.a.

The values represent absolute frequencies (relative frequencies, %) for dichotomous dependent variables. Values statistically significant for p value <0.05; p, χ^2 test values. p, † Independent sample-test; and values are means ± standard deviation (SD). p, †† Mann-Whitney-test; and values are (median) and [range]. n.a., non applicable; AD, Atopic Dermatitis; SCORAD, SCOring AD index.

Table 1: Participant´s characteristics of Portugal and Poland with asthma, AD and controls.

followed by 35 cycles of amplification at 94°C for 30 s, 53°C for 45 s, 72°C for 1 min and 30 s and one cycle at 72°C for 5 min. The amplified fragments of 506 bp were then digested by the restriction endonuclease MwoI at 60°C for 3 hr according to the manufacturer's recommendations. The digestion products were analyzed by electrophoresis in 3% agarose gel stained with ethidium bromide (10 μg/mL) for 60 minutes, with 80 volts. With this process we are able to differentiate genotypes: the TT genotype gives rise to one single band of 506 bp; the CC genotype appears as two bands, one with 339 bp, and other with 167 bp; the CT genotype has all the three bands.

Statistical Analysis

Observed genotype frequencies were tested for deviation from Hardy-Weinberg equilibrium (HWE) with the Chi-square goodness-of-fit test. This test was also used to evaluate the significant differences between groups, in and within the two populations, in order to know if the odds ratio (OR) test was justifiable. In the two cohorts OR for patients risk and the corresponding 95% confidence intervals (95% CI) were calculated using logistic regression analysis. This test was applied to the polymorphism, to analyze its risk factor individually. The power of the sample was verified every time there were statistical differences among genotype distribution. All statistical analyses were carried out using the SPSS 21.0 software. The results were considered statistically significant for $p<0.05$.

Results

LELP-1 polymorphism [rs7534334] was evaluated in the 2 cohorts: Portugal and Poland, within 2 atopic diseases: AD and asthma. Table 1 shows the characteristics of participants of these two cohorts compared with controls.

In the Portuguese cohort there are differences in gender, being the females more frequent in asthmatics and males in the control group ($p<0.001$) (Table 1). The asthmatics in the Portuguese cohort were younger than the control group ($p=0.027$) (Table 1) and this was more evident when we stratified the groups being the older than 30 years more frequent in the control group ($p<0.001$) (Table 1). The asthmatic patients were in the majority of them atopic (86%) and had their asthma symptoms controlled (71.3%) (Table 1).

In the Polish cohort there were no differences by gender ($p=0.712$) (Table 1). The patients (AD and asthma) from Poland were younger than the control group ($p=0.027$) (Table 1) and this was more evident when we stratified the groups by age being the older than 30 years more frequent in the control group ($p<0.001$) (Table 1). The majority of asthmatic patients are atopic (84.4%), all of them had the asthma symptoms controlled under anti-asthmatic treatment. The SCORAD index has been done in all patients with AD with a mean (mean ± SD: 54.7 20.5) compatible with a more severe cutaneous disease (Table 1).There is a significant difference ($p=0.035$) being the value of SCORAD by genotype (mean ± SD): CC (52.96 ± 18.94); CT(59.72 ± 19.9) and TT (8 ± 0). We think that these findings might be indicative of a trend for those who express allele C to have higher values, but we think that we must increase the sample to have more robust results.

In the control group and between the two cohorts, there were statistical significances in gender and age; being the females more frequent among the controls of the Poland's cohort and the males among the Portuguese cohort ($p<0.001$) (data not showed); and the controls from Poland were younger than the control group from Portugal ($p<0.001$) (data not shown).

LELP-1 polymorphism [rs7534334]

For LELP-1 polymorphism [rs7534334] in the Portuguese cohort with asthma comparing with controls, there were no differences in genotype and allele frequencies ($p>0.05$) (Table 2). The genotype distributions in asthma and controls were in HWE ($p>0.05$) (data not shown).

The CC genotype was more frequent in the cohort from Poland with AD and asthma ($p=0.004$) (power sample>0.8) (Table 2) being a risk (OR: 2.80 [1.34-5.82]; adjusted $p=0.006$) to both diseases in this cohort when compared to controls (Table 2). The genotype distributions in patients and controls were in HWE ($p>0.05$) (data not showed). The C allele ($p=0.001$) was more frequent in the cohort from Poland with asthma and atopic dermatitis being a risk factor to both diseases in this group (OR: 2.40 [1.35-4.28]; adjusted $p=0.003$) (Table 2).

There were significant differences in the mean age between the two cohorts, being the patients in the Portuguese cohort older then

			OR [95% CI]				
			p	OR crude a	OR adjusted b	p a	p b
Portugal	**Controls**	**Asthma**					
LELP-1	*n=110*	*n=129*					
rs7534334							
CC	45 (40.9)	58 (45.0)		1.18 [0.71-1.97]	1.07 [0.62-1.83]	0.528	0.807
CT	50 (45.5)	55(42.6)	0.817	0.89 [0.53-1.45]	0.95 [0.55-1.62]	0.662	0.841
TT	15 (13.6)	15 (13.6)		0.90 [0.42-1.91]	0.90 [0.42-1.91]	0.777	0.95
C	140 (0.64	140 (0.64	0.565	1.12 [0.77-1.64]	1.04 [0.70-1.55]	0.546	0.834
T	80 (0.36)	87 (0.35)		0.89 [0.61-1.30]	0.96 [0.65-1.42]	0.546	0.834
Poland	**Controls**	**AD and Asthma**					
LELP-1	*n=100*	*n=45*					
rs7534334							
CC	32 (32.0)	26 (57.8)		2.91 [1.41-6.00]	2.80 [1.34-5.82]	0.004	0.006
CT	53 (53.0)	18 (40.0)	0.004	0.59 [0.29-1.21]	0.60 [0.29-1.24]	0.149	0.167
TT	15 (15.0)	1 (2.2)		0.13 [0.02-1.00]	0.14 [0.02-1.07]	0.051	0.058
C	117 (0.59)	70 (0.78)	**0.001**	2.45 [1.39-4.39]	2.40 [1.35-4.28]	**0.002**	**0.003**
T	83 (0.41)	20 (0.22)		0.41 [0.23-0.71]	0.42 [0.23-0.74]	**0.002**	**0.003**

The values for the genotypes and respective allele frequencies represent absolute frequencies (relative frequencies, %). Values statistically significant for p value < 0.05; AD, Atopic Dermatitis; OR, odds ratio; CI, confidence interval; p, χ^2 test values; p a, crude values; p b, values adjusted for age and gender (binary logistic regression).

Table 2: Distribution of *LELP-1* [rs7534334] genotype in asthma, AD and controls in the two cohorts (Portugal and Poland).

the Poland's patients (p<0.001) (Table 3). Comparing the 2 cohorts of patients there were no significant differences in gender distribution or atopic status (p>0.05) (Table 3).

When comparing the two cohorts, the CC genotype was more evident in the cohort from Poland and the TT genotype in the cohort from Portugal although it didn't reach the significance level (p=0.094) (Table 4). When considering all possible models of genotype analysis within these two cohorts, we found a trend for TT genotype to be a risk in asthma in the Portuguese cohort when comparing with patients with AD and asthma from Poland cohort (adjusted values: OR=7.49 [0.92-60.91], p=0.06) (Table 4).

For allele frequencies, there were significant differences in the 2 cohorts, being the C allele more frequent in Poland and the T allele in Portugal (p=0.047) (Table 4). These results were reflected in risk analysis being, the C allele protector and the T allele a risk for asthma in in the Portuguese population when compared with the cohort from Poland (OR=0.53 [0.29-0.95] and OR=1.90 [1.06-3.42], adjusted p=0.033, respectively) (Table 4).

Discussion

This study is part of a project which purpose is to identify novel polymorphisms in genes involved in skin barrier function and its association with atopic diseases.

LELP1, is a protein-coding gene located at chromosome 1q21 that belongs to the cornifin family (SPRR). The SPRR gene family, which includes the rs7534334- tag SNP of LELP1 is in the EDC complex that contains various other important genes such as IVL, LOR, FLG , trichohyalin (THH) and the S100 gene family [2,6,37].

Poland & Portugal	AD and Asthma	Asthma	p
N (%)	45 (25.9)	129 (74.1)	76 (58.9)
Female	27 (60.0)	82 (63.6)	0.722
Male	18 (40.0)	47 (36.4)	
Age (years) ††	45 (23) [7-59]	129 (38.0) [7-86]	**<0.001**
<15	8 (17.8)	16 (12.4)	
15-30	27 (60.0)	37 (28.7)	**<0.001**
>30	10 (22.2)	76 (58.9)	
Atopy	38 (84.4)	111(86.0)	0.807

The values represent absolute frequencies (relative frequencies, %) for dichotomous dependent variables. Values statistically significant for p value <0.05; p, χ^2 test values. p, †† Mann-Whitney-test; and values are (median) and [range]. AD, Atopic Dermatitis; n.a., non applicable.

Table 3: Participant´s characteristics of Poland and Portugal with asthma , AD.

Some authors [35], have found an association of this chromosome 1q21 tagged single nucleotide polymorphism (SNPs) within the LELP1 gene [rs7534334] with serum IgE levels. These results pointed to the need for research on LELP1 and other genes on EDC that could be related with many inflammatory diseases of the skin like AD and psoriasis.

However, most of the studies that have been done linking EDC with atopic disease involves the two null mutations in the FLG gene (R501X and 2282del4), that are associated with skin diseases like AD and asthma with AD [33,45]. As far as we know, this is the first paper studying LELP1 on the EDC, in 2 European cohorts with atopic disease (AD and asthma).

In the Portuguese cohort there were statistical differences within the control group by gender, being the females more frequent in asthmatics and males in the control group (p<0.001) and the asthmatics in the Portuguese cohort were younger than the control group (p=0.027).

In the Poland cohort there were no differences by gender (p=0.712) and the patients (AD and asthma) from Poland were younger than the control group (p=0.027).

According to authors that found differences in asthma and gender namely sex hormone estrogen and the physiopathology of asthma and increases in IL-4 and IL-13 production (46) we performed our analysis adjusted for gender and age between the controls and the patient groups.

In our study with LELP1 polymorphism [rs7534334] we found that the CC genotype was more frequent in Poland's cohort with AD and asthma when compared with controls (p=0.004), (OR: 2.80 [1.34-5.82]; adjusted p=0.006) and the C allele was also a risk factor (OR: 2.40 [1.35-4.28]; adjusted p=0.003) to both diseases in this group. When compared the cohort from Portugal with Poland, there was a trend for TT genotype to be a risk for asthma in the Portuguese cohort (OR=7.49 [0.92-60.91], adjusted p=0.06). C allele was more frequent in the cohort from Poland and T allele, in the cohort from Portugal (p=0.047).

The molecular basis for the skin barrier deficiency could be a secondary phenomenon associated with the epidermal differentiation complex (EDC) and barrier candidate genes like FLG (filaggrin) and LELP1 (late cornified envelope-like proline-rich 1) as we found in our results.

Other authors [35], refer a correlation of log10 serum IgE levels and rs7534334 in a group of asthmatic patients being the mutant genotype (TT) in patients, those with higher levels of IgE comparing with controls (TT) and comparing with wild type genotype (CC) in patients (3.49 ± 0.91 vs 2.43 ± 0.52 vs 2.92 ± 0.59) .This point to the works who

			OR [95% CI]				
			p	OR crude [a]	OR adjusted [b]	p [a]	p [b]
Portugal and Poland	AD and Asthma	Asthma					
LELP-1	n=45	*n=129*					
rs7534334							
CC	26 (57.8)	58 (45.0)		0.60 [0.30-1.19]	0.57 [0.28-1.17]	0.140	0.124
CT	18 (40.0)	55 (42.6)	0.094	1.12 [0.56-2.23]	1.12 [0.54-2.31]	0.758	0.766
TT	1 (2.2)	16 (12.4)		6.23 [0.80-48.40]	7.49 [0.92-60.91]	0.080	0.060
C	70 (0.78)	171 (0.66)	**0.047**	0.56 [0.32-0.98]	0.53 [0.29-0.95]	**0.043**	**0.033**
T	20 (0.22)	87 (0.34)		1.78 [1.02-3.12]	1.90 [1.06-3.42]	**0.043**	**0.033**

The values for the genotypes and respective allele frequencies represent absolute frequencies (relative frequencies, %). Values statistically significant for p value<0.05; AD, Atopic Dermatitis OR, odds ratio; CI, confidence interval; pa, χ^2 test values; p b, crude values; pc, values adjusted for age (regression binary logistic).

Table 4: Distribution of *LELP-1* [rs7534334] genotype between asthma and AD in the two cohorts (Portugal and Poland).

showed that when skin barrier function is compromised even without skin disease there is an increased incidence of atopic disease [28].

The clinical manifestations of atopic dermatitis in infancy are different from adults; first the lesions are on the cheeks and scalp, then the flexures, the posterior area of the scalp and popliteal region. In adults lichenified plaques of the flexures, head and neck are more frequent, with a chronic and relapsing skin inflammation, and a disturbance of epidermal-barrier function and IgE-mediated sensitization to allergens.

AD might be the first step of the so-called "atopic march" [9] that include other allergic disorders later in life such as asthma and allergic rhinitis.

Some authors proposed that in a "dual" allergen exposure hypothesis the low dose exposure through the lesional skin in AD of the allergens might interfere with the uptake of the Langerhan's cells that could polarize to a Th2 response and IgE diathesis. By other way: early high dose exposition could induce tolerance, being proposed that Th1 and Tregs might interfere with the gut-associated lymphoid tissue and develop tolerance.

In this hypothesis sensitization to allergens occur in the environmental exposure through the skin while the tolerance might occur when the allergens contact with the atopic patient via other route of absorption namely oral for food allergens [47].

This dual hypothesis point to the prioritization of the intensive treatment of AD in early infancy to decrease allergic sensitization and the atopic march with the emergence of asthma and allergic rhinitis [9,47-49].

LELP1 is a protein-coding gene located at chromosome 1q21 that belongs to the cornifin (SPRR) family and the allele T might be related with a poor prognosis of the disease, if we think that the families of small proline-rich proteins are present in epithelial cells of the airways and skin that utilize similar mechanisms in host defense [39]. The SPRR family are also induced in respiratory epithelia as a squamous cell marker metaplasia [41,50].

Being the skin barrier deficiency a secondary phenomenon associated with the EDC, FLG has demonstrated how the study of a monogenic trait could provide insight into a complex trait disease and the significance of *FLG* null mutations as a genetic risk factor for atopic dermatitis. This barrier defect could be present even in the absence of eczema [26,28,33,47], which could help us to understand the physiopathology of AD associated with LELP1 polymorphism [rs7534334] and other polymorphisms located on genes of EDC complex.

Different genes are expressed in a coordinated sequence to provide the structural component of cornification. Keratin intermediate filaments form a complex conglomerate in the cytoplasm and, after the removal of cell organelles, fill the cell interior. In addition, a number of proteins are cross-linked by transglutamination in the cell periphery to form the so-called cornified envelope where LELP1 as a member of epidermal differentiation complex (EDC) could be a barrier candidate gene.

As soon as keratinocytes are detached from the basement membrane of the epithelium, they change their gene profile under the control of many transcription factors [1,4].

In addition, keratins and the inflammatory profile can also regulate pathways involved in growth, proliferation, migration and apoptosis of epithelial cells [3]. The small proline-rich proteins are encoded by the EDC [2,37,40], where LELP1 play its role. The proteins that are encoded in this region share similarities, particularly in the glutamine- and lysine-rich regions that are involved in the action of the transglutaminases. Bronchial epithelial cells and keratinocytes not only share structural characteristics, but also share functional characteristics and that is why many barrier genes could be related with the "atopic march" and the pathophysiology of AD and respiratory diseases such allergic asthma.

The epithelial cells of the airways and the skin, utilize also similar defense mechanisms against infection [39], pollutants and allergens, despite the different structure of the epithelia.

Bronchial epithelial cells and keratinocytes have a high degree of overlap in gene expression and bronchial epithelial cells like keratinocytes, express proteins and other components that are able to form a cross-linked protein envelope that may contribute to a barrier against allergens, pollutants and pathogens. That could be compromised in patients with LELP-1 polymorphism [rs7534334].

It has been demonstrated that peptides that could be related with defense mechanisms besides barrier function, like LELP1 that codifies a protein belonging to the cornifin (SPRR) family [16] could be lower in skin with AD, and that the Th2 cytokines could also play a role by interfering with the expression of these peptides [20,40,43]. IL13 (a Th2 cytokine), could induce the expression of small proline-rich proteins (SPRR) in airway epithelium during allergic inflammation in the animal models [40].

LELP 1 which codes for one of the small proline-rich proteins (SPRR), expressed in both bronchial epithelial cells and keratinocytes in response to pro-inflammatory cytokines, might be related with the pathophysiology of atopic dermatitis and also with host defense against allergens, pollutants and microbes and might interfere with respiratory disease and "allergic march".

Our results point to the importance of the impaired skin barrier function on trans-epidermal entry of allergens and secondary development of allergic diseases like asthma and rhinitis. Some genome-wide association studies, point to the fact that FLG could partially tag some other mutations like those near LCE3E (rs61813875) [51,52] and according to this, we think that it might be important to study other polymorphisms in genes from the EDC complex namely FLG (loss-of-function mutations (R501X and 2282del4), in order to more accurately understand the importance of this complex in asthma, AD or other allergic diseases.

We also think that one of our limitations is the sample size, and we hope that in the future we could have larger cohorts to increase the robustness of our study.

These kind of studies are important because of the regulation of expression of epidermal barrier proteins and its clinical relevance on defense mechanisms in inflammatory disorders that affects epithelial surfaces [5,7,9] like AD and asthma and constitute an important therapeutic strategy for allergic diseases.

Conclusion

Altogether, these findings demonstrate that as much as immune mechanisms and IgE hipersensitivity, genetic variation of skin barrier genes might contribute to major atopic diseases such as atopic dermatitis and bronchial asthma.

We could then infer that we should readily treat atopic dermatitis in early childhood, reducing inflammation in the skin, permeability to allergens and so preventing allergen sensitization. In the same way the "dual hypothesis" could help us to understand how helpful it could be to decrease the environmental exposure to allergens in the "allergic march" and the development of allergic diseases.

These kinds of studies are important because of the regulation in expression of epidermal barrier proteins and its clinical relevance on defense mechanisms in inflammatory disorders that could affect epithelial surfaces in atopy and might constitute an important therapeutic target in allergic diseases.

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