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INVESTIGATIVE REPORT

Analysis of *SPINK 5, KLK 7* and *FLG* Genotypes in a French Atopic Dermatitis Cohort

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The role of a genetically impaired epidermal barrier as a major predisposing factor in the pathogenesis of atopic disorders is currently under closer investigation. Variants on three candidate genes (*SPINK5, KLK7* and *FLG*) have been associated with atopic dermatitis. A functional relevance has already been established for filaggrin variants, but not for *SPINK5* and *KLK7* polymorphisms. The objectives of this study were to confirm the association between *SPINK5, KLK7, FLG* variants and atopic dermatitis and to assess how variants influence selected phenotypic traits. This cross-sectional study was carried out over 20 months in 99 children and adults with atopic dermatitis (median age 7 years). The following items were analysed: SCORAD, TEWL, ichthyosis vulgaris, presence of asthma, total IgE serum levels. The *SPINK5* E420K SNP, the *KLK7* 4bp insertion polymorphism and the filaggrin mutants (R510X and 2282del4) were analysed as described previously. The control group for genetic analysis was recruited in an ethnically matched, phenotypically anonymous cohort (*n*=102). The allelic frequencies were 0.525 for *SPINK5*, 0.26 for *KLK7* polymorphisms, 0.101 and 0.075 for 2282del4 and R501X *FLG* mutants, respectively. The association of atopic dermatitis with filaggrin variants was confirmed, but not that of *SPINK5* or *KLK7* polymorphisms. SCORAD and TEWL measurements were not influenced by any of the variants. The *SPINK5* polymorphism was associated with high IgE serum levels (*p*=0.011). Abnormal barrier genes do not influence the severity of atopic dermatitis. The *SPINK5* gene polymorphism may modulate systemic immune effects favouring the IgE response to atopens. TEWL does not allow the characterization of subsets of patients with or without abnormal barrier genes. *Key words: atopic dermatitis; filaggrin; KLK7; SPINK5; TEWL.*

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A skin barrier defect has been proposed as a primary event in the pathogenesis of atopic disorders based primarily on clinical and allergy testing observations as well as morphological and biochemical data in infantile atopic dermatitis (AD) (1). The integrity of the stratum corneum is essential to prevent epidermal water loss, but also to limit the penetration of aeroallergens, infectious organisms or toxic chemicals (2). An underlying skin barrier defect in predisposed individuals could lead in the first months of life to epicutaneous sensitization to atopens, which, in a second step, may trigger a Th2 immune response in a subset of those individuals. Since this hypothesis has been put forward, genome-wide scans have shown several loci associated with the AD phenotype, especially in the epidermal terminal differentiation cluster on chromosome 1 (3). In parallel, the candidate gene approach has linked several polymorphisms or mutations affecting genes involved in the skin barrier structure or function to AD. *SPINK5* encodes LEKTI, a protease inhibitor that is defective in Comel-Netherton syndrome. AD has been associated with a polymorphism in exon 14 (E420K) in one British and two Japanese studies (4–6). The relevance of this single nucleotide polymorphism (SNP) in AD pathogenesis is based mainly on the phenotype of Netherton's syndrome, which associates immediate skin and mucosal allergic features, ichthyosis, AD-like features and developmental anomalies. The involvement of this polymorphism in AD itself is still debated. A polymorphism in the human kallikrein 7 gene (*KLK7*) characterized by a 4-bp insertion in the 3'UTR has been reported in a British AD cohort (7). *KLK7* encodes the protease kallikrein 7, also named stratum corneum chymotryptic enzyme (SCCE), which is involved in the desquamation process by degrading corneodesmosomes (8). According to Vasilopoulos et al. (7), this polymorphism may result in a gain of function of the protease, leading to a premature degradation of the corneodesmosomes and subsequent defect of the skin barrier. The functional relevance of this polymorphism has, however, not yet been demonstrated.

Recently Palmer et al. (9) showed that two independent loss-of-function genetic variants (R501X and 2282del4) in the gene encoding filaggrin (*FLG*) are associated with AD. Filaggrin is essential in the formation of the stratum corneum barrier (2). The same mutations were first identified as causative of ichthyosis

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vulgaris (10). In several Caucasian-based cohorts, these mutations are associated both with AD, influencing both severity and early onset of the disease, and asthma in the context of AD (9, 11–16).

Our study analyses simultaneously the three different genotypes in the same AD cohort including infants, children and adults. Our primary objective was to analyse the association between gene variants and AD, and to assess simultaneously skin barrier function using trans-epidermal water loss (TEWL) assay, scoring of atopic dermatitis (SCORAD) index (severity), and Th2 skewing (total IgE serum levels). Since AD is a multifactorial disease, it can be assumed that environmental influences combined with genetic background produce the AD phenotype (17). In this context, the assessment of a potential stratum corneum gene effect influencing barrier dysfunction, clinical severity of AD or associated asthma was our secondary objective.

METHODS

This cross-sectional study was carried out in the Department of Dermatology at Bordeaux University Hospital between December 2004 and September 2006.

All patients met the criteria for AD of the UK Working Party (18) and a clinical diagnosis of ichthyosis vulgaris was made by a dermatologist (TH, FB, CL, AT). An ethnically matched control group made of phenotypically anonymous subjects served as control for allele frequencies (KM).

Procedures

A complete dermatological examination was performed by a trained dermatologist and ichthyosis vulgaris features (palmar hyperlinearity, xerosis, scaling on legs) were noted. Information about familial and personal atopic history was obtained using a standardized questionnaire. Physician-diagnosed asthma was registered. Disease severity was assessed using the SCORAD index (19, 20). After written informed consent, blood sample was obtained for genetic testing and IgE measurement.

Trans-epidermal water loss measurement (TEWL)

TEWL measurements ($g \bullet m^{-2} \bullet h^{-1}$) were performed with a vaporimeter (Delfin Technologies ltd, Kuopio, Finland) following published guidelines (21). In particular, the measurements were performed at constant temperature and moisturizers were avoided in the previous 24 h. The probe was applied on the medium part of uninvolved volar forearm.

Total IgE serum levels

The total IgE serum level was determined using the Pharmacia CAP system (Pharmacia Diagnostics, Uppsala, Sweden). Total IgE serum levels higher than 150 $kU \bullet l^{-1}$ were classified as abnormal.

Genetic analysis

DNA was extracted using the Wizard genomic kit (Promega, Lyon, France). The genotypes were determined by restriction fragment length polymorphism (RFLP) analysis (E420K SNP of the *SPINK5* gene and R501X mutation of the *FLG* gene) or by fluorescent fragment size analysis (4 bp insertion of the *KLK7* gene and 4 bp deletion of the *FLG* gene) using an automated gene analyser and the Gene mapper software (Applied Biosystems 3130). The RFLP analyses were performed as described by Walley et al. (4) and Palmer et al. (9), respectively. The fragment size analysis techniques were adapted from Palmer et al. (9). The following sequences were used as amplimers: 5'- TGC AAT TGT GAG GAT TTC ACA G -3' (forward *SPINK5*), and 5'- CCT GAA CAT GAT CTG TGG ATC -3' (reverse *SPINK5*); 5'- CTC ACT GAC TCT TCT CCA GCA C -3' (forward *KLK7*) and 5'- GAA AAT GCA CAG GAG TGA GGA CG -3' (6FAM-labelled reverse *KLK7*); 5'- TCC CGC CAC CAG CTC C -3' (forward *FLG* 2282del4) and 5 - TG GCT CTG CTG ATG GTG A -3' (6FAM-labelled reverse *FLG* 2282del4); 5'- ACA GCC TGA CTC TGC CCA TG -3' (forward *FLG* R501X) and 5'- GCA CTT CTG GAT CCT GAC TG -3' (reverse *FLG* R501X). Polymerase chain reaction (PCR) conditions were as follows: 100 ng genomic DNA, 2 mmol/l $MgCl_2$, 200 μmol/l each dNTP, 200 nmol/l each amplimer, 1X Taq Gold buffer and 1 unit Taq Gold enzyme (Perkin Elmer, France). Cycling conditions were as follows: 10 minutes at 95°C (initial denaturation) then 25 (*KLK7* and *FLG* 2282del4) or 35 cycles (*SPINK5* and *FLG* R501X) including denaturation (30 sec at 94°C) annealing (30 sec at 58°C, 60°C or 65°C for *SPINK5*, *FLG* and *KLK7*, respectively), extension (30 sec at 72°C) and a final extension step (5 min at 72°C). PCR products were run on a 1.5% agarose gel prior to RFLP or fragment size analyses. Amplicon sizes were 304, 177–184, 312 and 193–197 bp for *SPINK5, KLK7, FLG* R501X and 2282del assays, respectively. Restriction digests using NlaIII (*FLG* R501X) and HphI (*SPINK5*) were performed on 1/10 of the PCR product during 2 h at 37°C according to the supplier's instrucitons (New England Biolabs, Saint Quentin Yvelines, France) and run on a 3% agarose gel. The *FLG* R501X and the *SPINK5* variant alleles exhibited a new restriction site for Nla III and Hph I enzymes, respectively. For fragment size assays, 6FAM-labelled PCR products (*KLK7* and *FLG* 2282del) were diluted 1/2 to 1/40 and the Genescan Rox 350 fluorescent marker was added prior to analysis on the automated gene analyser.

Statistical analysis

The prevalence estimates are reported as proportions with their 95% confidence intervals (CI). Differences of prevalence according to different patient's characteristics were compared using Pearson's χ^2 test or Fisher's exact test (according to group size). In order to increase the potency of statistical analyses, for each gene studied, the alleles were gathered in two groups, either wild-type or variant. In the variant allele group, patients had one (heterozygous status) or two (homozygous or compound heterozygous) mutant alleles. Quantitative variables were reported as medians or means, depending on their distribution. Comparisons were made using *t*-test or Wilcoxon rank test. Statistical analysis used SAS software, version 8.2 (SAS Institute Inc., Cary, North Carolina, USA). A p-value <0.05 was considered statistically significant.

RESULTS

Characteristics of the cohort

Ninety-nine patients with atopic dermatitis (34 females, 65 males) were enrolled. The median age was 7 years, ranging from 2 months to 68 years old. The clinical characteristics of the patients are summarized in Table I. The samples used as population controls belong to a phenotypically anonymous cohort of French

Table I. *Characteristics of the patients with atopic dermatitis (AD)*

	Available data (*n*)	AD group	Children <15 years	Adults >15 years
Group size	99	99	59	40
Median age (years) (IQ)	99	7 (2.0–23.0)	3	30
Sex ratio (% male)	99	65%	71%	60%
Mean SCORAD (CI)	89	24 (20.8–28.0)	23	25
Mean TEWL, ($g•m^{-2}•h^{-1}$) (CI)	60	12.4 (10.5–15.4)	13.4	11.4
Median total IgE, $kU•l^{-1}$ (IQ)	72	565.0 (57.5–3991.0)	138.5	2888
Asthma prevalence, % (CI)	51	41.2 (27.7–54.7)	39	42

SCORAD: scoring atopic dermatitis; TEWL: trans-epidermal water loss; CI: 95% confidence interval; IQ: interquartile interval.

adult men, which has already been tested in population studies concerning atopic dermatitis (9). The frequency of AD is known to be approximately 5% in the general population (22).

Allelic frequencies of candidate genes

The allelic frequencies of the *SPINK5*-SNP, the *KLK7*-4bp insertion, R501X and 2282del4 *FLG* variants in the AD group and in the control group are shown in Table II. Three homozygous (22282del4/2282del4) and one compound heterozygous (2282del4/R501X) patients were identified. The allelic frequencies of *FLG* 2282del4 and R501X mutants were higher in the AD group than in the control group ($p<0.001$ and $p=0.004$, respectively). The apparent linkage disequilibrium between 2282del4 and R501X mutants needs further analysis on a larger population of mutants. No association between AD and *SPINK5*-SNP or *KLK7*-4bp insertion was found according to allele frequencies (Table II) or genotypes (data not shown).

Correlation between phenotype and genotype

As shown in Table III, the presence of a gene variant in the AD group was neither associated with severity (SCORAD index) nor with TEWL readings or combined asthma. The same observation was made when considering two distinct subgroups, according to age, i.e. adults and children (data not shown). However, we found a significant association between high IgE serum levels (>150 $kUI•l^{-1}$) and *SPINK5*-SNP ($p=0.011$).

Assessment of an additive gene effect on asthma, IgE, SCORAD and TEWL

In order to test the hypothesis of a phenotypic influence of the addition of compound gene variants/polymorphisms, we compared the frequency of associated asthma, mean SCORAD index, mean TEWL readings, respectively, with the presence of either zero, one, two or three variants of the studied genes (Table IV). No significant difference was found between the four classes of variants; however an increased prevalence of asthma was a non-significant trend in patients with compound variant genotypes.

DISCUSSION

Our data confirm the association between AD and FLG mutants, but fail to establish a correlation between AD and either *SPINK5*-SNP or *KLK7* 4bp insertion polymorphisms.

The allele frequency for *FLG* R501X variant was 0.075 in the AD cohort and 0.025 in the control group ($p=0.004$). The allele frequency of the 2282del4 variant was 0.101 in the patients with AD cohort and 0.010 in

Table II. *Allelic frequencies in atopic dermatitis (AD) and population controls*

	AD genotypes			Frequency of the variant allele			
				AD	Population controls	*p*-value	OR (95%CI)
SPINK 5	wt	E420K	K420K				
	19/99	56/99	24/99	0.525	0.520[a]	0.94	1.02 (0.95–1.11)
KLK 7	wt	ins/wt	ins/ins				
	53/99	40/99	6/99	0.26	0.22[a]	0.33	1.26 (1.13–1.40)
FLG	wt	del/wt	del/del				
	82/99	14/99	3/99	0.101	0.010[b]	<0.001	11.7 (5.4–25.3)
	wt	R501X	X501X				
	84/99	15/99	0/99	0.075	0.025[b]	0.004	3.2 (2.1–4.7)

[a]SPINK5 and KLK7 genotypes were determined in the same population controls previously analysed for FLG variants (9). [b]FLG allele frequency was extracted from (9).

CI: confidence interval; OR: odds ratio; wt: wild-type; E420K, ins/wt, del/wt, and R501X are heterozygous genotypes; K420K, ins/ins, del/del, and X501X are homozygous mutant genotypes.

Table III. *Correlations between phenotype and genotype*

	SPINK5 genotype		*KLK7* genotype		*FLG* genotype	
	wt	variant	wt	variant	wt	variant
Median age at AD onset (months) (IQ)	3 (2–30)	6 (2–12)	6 (4–12)	4 (2–24)	6 (3–24)	3 (2–6)
Mean SCORAD (CI)	29.4 (21.9–36.8)	23.2 (19.3–27.0	25.7 (21.0–30.3)	22.5 (17.2–27.7)	24.0 (19.7–28.3)	24.8 (18.9–30.7)
Mean TEWL (CI)	14.4 (8.8–19.9)	11.9 (10.0–13.9)	13.0 (10.3–15.6)	11.7 (8.9–14.5)	11.6 (9.38–13.8)	14.1 (10.4–17.2)
High IgE patients (prevalence)	$n = 4$ (4/11)	$n = 47$* (47/61)	$n = 28$ (28/40)	$n = 23$ (23/32)	$n = 37$ (37/50)	$n = 14$ (14/22)
Asthmatic patients (prevalence)	$n = 4$ (4/10)	$n = 19$ (19/41)	$n = 11$ (11/30)	$n = 12$ (12/21)	$n = 16$ (16/39)	$n = 7$ (7/12)

Mean TEWL values are expressed as $gm^{-2}h^{-1}$. High IgE is defined as IgE > 150 $kU.l^{-1}$.
Prevalence is the ratio: number of high IgE (or asthmatic) patients with a given genotype/ total number of patients with the same genotype.
*$p = 0.011$. Other p-values were non-significant
CI: 95% confidence interval; IQ: interquartile interval; AD: atopic dermatitis; TEWL: trans-epidermal water loss; SCORAD: scoring atopic dermatitis; wt: wild-type genotype; variant: heterozygous, homozygous mutant or compound heterozygous (*FLG*) genotypes.

the control group ($p < 0.001$). Such allele frequencies are similar to those already published (9, 11–16). A multi-ethnic database has detected the *FLG* mutants only in Caucasian populations (9). Since the linkage between AD and the 1q21 region, containing the epidermal differentiation gene cluster where the *FLG* gene is located, has only been studied in Caucasian populations, one cannot extrapolate these genetic data to other ethnic groups (3, 23). However, a DNA microarray analysis of AD skin lesions in a Japanese cohort found significant down-regulation of the *FLG* (24). Our results confirm the central role of defective filaggrin in the skin barrier impairment of AD. The functional relevance of this finding was examined with TEWL measurements. As expected, mean TEWL in our study was higher in two homozygous patients for *FLG* variants who had the ichthyosis vulgaris phenotype. Although there was a trend for higher TEWL values in patients with one or two *FLG* variants, we could not show a significant association between *FLG* variants and TEWL. This trend needs to be confirmed in a larger group of patients. Alternatively, the absence of clear difference between carriers and non-carriers of the *FLG* mutations for TEWL suggest strongly the presence of other important constitutional barrier defects favouring AD not yet discovered (1). As shown recently in larger cohorts (12, 13, 16), a tendency for a younger age at onset of AD was noted in patients with a *FLG* variant. This may suggest that infants with defective barrier functions are more likely to develop atopen-induced skin lesions because of early epicutaneous sensitization (25). In a German cohort, filaggrin variants have been described as associated with high total serum IgE levels (11) suggesting a link between a genetically impaired epidermal barrier and Th2 skewing. The low number of FLG mutants in our cohort decreased the power of statistical analysis and could not confirm this finding.

Our results did not confirm the association between AD and the *KLK7* polymorphism (7). In a similar size cohort, these authors reported an allelic frequency of 0.56 in the AD group compared with 0.43 in the control group. This is in contrast with our findings, which show a much lower allelic frequency and no significant difference between allelic frequencies in AD and controls (0.26 and 0.22, respectively). This difference could be due to a marked genetic heterogeneity between the two populations. Nevertheless, *KLK7* is located on 19q13.3 and previous genome-wide scans have not found a linkage with AD at this locus (23, 26). Furthermore, increased mRNA or KLK7 proteolytic activity in AD

Table IV. *Analysis of compound gene variants effect on selected phenotypic traits*

	Number of gene variants			
	0	1	2	3
Mean TEWL (95%CI)	11.8 (8.6–19.5)	12.7 (8.8–16.5)	12.9 (9.9–15.8)	9.5 (5.4–13.5)
Mean SCORAD (95%CI)	27.2 (13.9–40.5)	27 (21.3–32.7)	21.3 (16.0–26.6)	24.2 (13.9–34.4)
High IgE patients (prevalence)	$n = 1$ (1/5)	$n = 22$ (22/28)	$n = 23$ (23/31)	$n = 5$ (5/8)
Asthmatic patients (prevalence)	$n = 1$ (1/5)	$n = 9$ (9/22)	$n = 10$ (10/20)	$n = 3$ (3/4)

0 is wt genotype for the 3 genes. 1, 2 and 3 are heterozygous or homozygous for 1, 2 and 3 genes, respectively.
Prevalence is the ratio: number of high IgE (or asthmatic) patients with 0, 1, 2 or 3 gene variants/total number of patients with the same number of gene variants.
p-values were non-significant.
TEWL: trans-epidermal water loss; SCORAD: scoring atopic dermatitis; CI: 95% confidence interval; IQ: interquartile interval.

skin has never been established. For Vasilopoulos et al. (7), the polymorphic allele would be responsible for an increased lifetime of KLK7 mRNA, leading to increased KLK7 enzymatic activity (7). Mice over-expressing KLK7 have an increased TEWL (27). Our heterozygous or homozygous patients for the KLK7 polymorphism had no higher TEWL readings compared with the wild-type genotype patients. However, this result does not rule out the involvement of epidermal kallikreins (KLK) in the pathogenesis of AD. KLK have a physiological role in desquamation, which is impaired in AD (28, 29). KLK are expressed in the stratum corneum and it has been shown that KLK5, KLK7 as well as KLK6, KLK8 KLK13 and KLK14 may act as desquamatory enzymes in the stratum corneum (8, 29–31). KLK5, KLK7, KLK14 are activated through a complex activation cascade regulated by pH (8, 32). Environmental influences may increase skin pH, leading to an increased activity of stratum corneum KLK (17).

The allelic frequency of the *SPINK5*-SNP polymorphism in our AD cohort is close to that published in the original British cohort (4). The analysis of unrelated patients did not show an association between the polymorphism and AD (Table II). A maternal transmission of the risk allele was first demonstrated in individuals affected with AD in the British cohort (4) and confirmed in a Japanese study (5). The results of association studies using a case-control design are conflicting (6, 33–35). Two German and one Dutch studies found no association between *SPINK5*-SNP and AD (33–35). Allelic frequencies are closely related (range 0.48–0.54) in the different studies. Several explanations of these contradictory results have already been put forward, including the study design (35). Finally the overall role of the *SPINK5*-SNP in AD remains unclear. This is mainly due to the high frequency of the minor allele in control populations. In our study, the presence of the *SPINK5*-SNP did not influence SCORAD, TEWL, or age of onset of AD. However, we found a significant association between the *SPINK5*-SNP and high IgE serum levels. How is it possible to reconcile these findings? On the cutaneous barrier side, the polymorphism reported is on exon 14, coding for LEKTI domain 7, and an *in vitro* study has shown that recombinant LEKTI containing domain 7 inhibits KLK7 and other KLKs: 5, 6, 13 and 14. But these desquamatory enzymes are also efficiently inhibited by recombinant LEKTI fragments containing other domains (31, 36, 37). LEKTI is not only expressed in the skin and appendages, but also in the thymus, and a central influence on T-cell maturation is also possible. A soluble form of LEKTI could also exert distant effects. It thus remains possible that the *SPINK5* gene influences more markedly the phenotype, by modulating systemic immune effects favouring the IgE response to atopens, than by local cutaneous barrier mediated effects.

No significant association was found in our study between the number of gene variants and TEWL, disease severity, serum IgE level or onset of asthma. However, our results for asthma, showing an increase in asthma prevalence according to the number of gene variants, even though not statistically significant, suggest that it would be useful to increase the database to address this issue in more depth. *FLG* variants are a predisposing factor for the clinical subtype of asthma that occurs in the context of existing AD. Asthma prevalence was lower in the ichthyosis vulgaris group (10%) than in the AD cohorts (49%) studied by Palmer et al. (9). This indeed suggests the involvement of other factors, including genetic influences, in the onset of asthma. The speculation on the role of *SPINK5* in the onset of asthma is based on reported associations between *SPINK5* SNP and asthma in patients with AD (4, 34). However, data on the association of *SPINK-5* SNP with isolated asthma are conflicting (34, 35), leading to consider that this genotype may only influence the onset of asthma in patients with AD (35). Thus, larger studies are needed to validate the concept that both *SPINK5* polymorphism and *FLG* variants are prognostic factors for asthma onset in children with AD.

In summary, the recent Copernican revolution, which has centred the pathophysiology of AD on skin itself, leads now to other questions (38). The patient with loss of function *FLG* mutations and who does not develop AD belongs to a subset that needs to be investigated as a priority in order to gain a better understanding of the steps beyond stratum corneum defects, which probably involve a dysregulated cross-talk between environmental irritants or pathogens and innate barrier immune/inflammatory responses. The abnormal barrier genes, such as *FLG* mutants, have a clear permissive effect on the early inflammatory steps that characterize infantile eczema. They are found at overall identical frequencies across age groups of patients with AD, suggesting that this cutaneous barrier defect has priming effects on disease expression, but affects also the chronicity of the disease. If abnormal barrier genes increase the accessibility of irritants and atopens to the innate and adaptive immune system, this effect needs to be better apprehended through methods more sensitive than the TEWL measurements obtained in this study, which failed to detect differences between carriers and non-carriers, and among carriers of one or several genetic variants. However, abnormal barrier genes do not seem, in isolation, to influence the severity of the AD phenotype. In the atopic diathesis, skin constitution is probably more important than previously thought, and skin should thus be considered as a primary objective for prevention (39). However, newer therapies, still badly needed in established disease, will have to address, in addition, other constitutional factors triggering non-remitting inflammation and mucosal allergy, which so far remain largely unknown.

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REFERENCES

1. Taieb A. Hypothesis: from epidermal barrier dysfunction to atopic disorders. Contact Dermatitis 1999; 41: 177–180.
2. Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 2005; 6: 328–340.
3. Cookson WO, Ubhi B, Lawrence R, Abecasis GR, Walley AJ, Cox HE, et al. Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. Nat Genet 2001; 27: 372–373.
4. Walley AJ, Chavanas S, Moffatt MF, Esnouf RM, Ubhi B, Lawrence R, et al. Gene polymorphism in Netherton and common atopic disease. Nat Genet 2001; 29: 175–178.
5. Nishio Y, Noguchi E, Shibasaki M, Kamioka M, Ichikawa E, Ichikawa K, et al. Association between polymorphisms in the SPINK5 gene and atopic dermatitis in the Japanese. Genes Immun 2003; 4: 515–517.
6. Kato A, Fukai K, Oiso N, Hosomi N, Murakami T, Ishii M. Association of SPINK5 gene polymorphisms with atopic dermatitis in the Japanese population. Br J Dermatol 2003; 148: 665–669.
7. Vasilopoulos Y, Cork MJ, Murphy R, Williams HC, Robinson DA, Duff GW, et al. Genetic association between an AACC insertion in the 3'UTR of the stratum corneum chymotryptic enzyme gene and atopic dermatitis. J Invest Dermatol 2004; 123: 62–66.
8. Caubet C, Jonca N, Brattsand M, Guerrin M, Bernard D, Schmidt R, et al. Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/KLK7/hK7. J Invest Dermatol 2004; 122: 1235–1244.
9. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 2006; 38: 441–446.
10. Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet 2006; 38: 337–342.
11. Weidinger S, Illig T, Baurecht H, Irvine AD, Rodriguez E, Diaz-Lacava A, et al. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol 2006; 118: 214–219.
12. Barker JN, Palmer CN, Zhao Y, Liao H, Hull PR, Lee SP, et al. Null mutations in the filaggrin gene (FLG) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. J Invest Dermatol 2007; 127: 564–567.
13. Stemmler S, Parwez Q, Petrasch-Parwez E, Epplen JT, Hoffjan S. Two common loss-of-function mutations within the filaggrin gene predispose for early onset of atopic dermatitis. J Invest Dermatol 2007; 127: 722–724.
14. Marenholz I, Nickel R, Ruschendorf F, Schulz F, Esparza-Gordillo J, Kerscher T, et al. Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J Allergy Clin Immunol 2006; 118: 866–871.
15. Ruether A, Stoll M, Schwarz T, Schreiber S, Folster-Holst R. Filaggrin loss-of-function variant contributes to atopic dermatitis risk in the population of Northern Germany. Br J Dermatol 2006; 155: 1093–1094.
16. Weidinger S, Rodriguez E, Stahl C, Wagenpfeil S, Klopp N, Illig T, et al. Filaggrin Mutations Strongly Predispose to Early-Onset and Extrinsic Atopic Dermatitis. J Invest Dermatol 2007; 127: 724–726.
17. Cork MJ, Robinson DA, Vasilopoulos Y, Ferguson A, Moustafa M, MacGowan A, et al. New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene-environment interactions. J Allergy Clin Immunol 2006; 118: 3–21.
18. Williams HC, Burney PG, Pembroke AC, Hay RJ. Validation of the U.K. diagnostic criteria for atopic dermatitis in a population setting. UK Diagnostic Criteria for Atopic Dermatitis Working Party. Br J Dermatol 1996; 135: 12–17.
19. Kunz B, Oranje AP, Labreze L, Stalder JF, Ring J, Taieb A. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. Dermatology 1997; 195: 10–19.
20. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. Dermatology 1993; 186: 23–31.
21. Levin J, Maibach H. The correlation between transepidermal water loss and percutaneous absorption: an overview. J Control Rel 2005; 103: 291–299.
22. Coustou D, Brun-Strang C, Boralevi F, Taieb A. Prise en charge de la dermatite atopique en France en 2002. Annal Derm Venereol 2003; 130: 4S25–25.
23. Morar N, Willis-Owen SA, Moffatt MF, Cookson WO. The genetics of atopic dermatitis. J Allergy Clin Immunol 2006; 18: 24–34.
24. Sugiura H, Ebise H, Tazawa T, Tanaka K, Sugiura Y, Uehara M, et al. Large-scale DNA microarray analysis of atopic skin lesions shows overexpression of an epidermal differentiation gene cluster in the alternative pathway and lack of protective gene expression in the cornified envelope. Br J Dermatol 2005; 152: 146–149.
25. Boralevi F, Hubiche T, Leaute-Labreze C, Saubusse E, Fayon M, Roul S, et al. Epicutaneous aeroallergen sensitization in atopic dermatitis infants: determining the role of epidermal barrier impairment. Allergy (in press)
26. Yousef GM, Scorilas A, Magklara A, Soosaipillai A, Diamandis EP. The KLK7 (PRSS6) gene, encoding for the stratum corneum chymotryptic enzyme is a new member of the human kallikrein gene family – genomic characterization, mapping, tissue expression and hormonal regulation. Gene 2000; 254: 119–128.
27. Ny A, Egelrud T. Epidermal hyperproliferation and decreased skin barrier function in mice overexpressing stratum corneum chymotryptic enzyme. Acta Derm Venereol 2004; 84: 18–22.
28. Ekholm E, Egelrud T. Expression of stratum corneum chymotryptic enzyme in relation to other markers of epidermal differentiation in a skin explant model. Exp Dermatol 2000; 9: 65–70.
29. Descargues P, Deraison C, Prost C, Fraitag S, Mazereeuw-Hautier J, D'Alessio M, et al. Corneodesmosomal cadherins are preferential targets of stratum corneum trypsin- and chymotrypsin-like hyperactivity in Netherton syndrome. J Invest Dermatol 2006; 126: 1622–1632.
30. Kishibe M, Bando Y, Terayama R, Namikawa K, Takahashi H, Hashimoto Y, et al. Kallikrein 8 is involved in skin desquamation in cooperation with other kallikreins. J Biol Chem 2007; 282: 5834–5841.
31. Borgono CA, Michael IP, Komatsu N, Jayakumar A, Kapadia R, Clayman GL, et al. A potential role for multiple tissue kallikrein serine proteases in epidermal desquamation. J Biol Chem 2007; 282: 3640–3652.
32. Brattsand M, Stefansson K, Lundh C, Haasum Y, Egelrud T.

A proteolytic cascade of kallikreins in the stratum corneum. J Invest Dermatol 2005; 124: 198–203.
33. Folster-Holst R, Stoll M, Koch WA, Hampe J, Christophers E, Schreiber S. Lack of association of SPINK5 polymorphisms with nonsyndromic atopic dermatitis in the population of Northern Germany. Br J Dermatol 2005; 152: 1365–1367.
34. Kabesch M, Carr D, Weiland SK, von Mutius E. Association between polymorphisms in serine protease inhibitor, kazal type 5 and asthma phenotypes in a large German population sample. Clin Exp Allergy 2004; 34: 340–345.
35. Jongepier H, Koppelman GH, Nolte IM, Bruinenberg M, Bleecker ER, Meyers DA, et al. Polymorphisms in SPINK5 are not associated with asthma in a Dutch population. J Allergy Clin Immunol 2005; 115: 486–492.
36. Egelrud T, Brattsand M, Kreutzmann P, Walden M, Vitzithum K, Marx UC, et al. hK5 and hK7, two serine proteinases abundant in human skin, are inhibited by LEKTI domain 6. Br J Dermatol 2005; 153: 1200–1203.
37. Deraison C, Bonnart C, Lopez F, Besson C, Robinson R, Jayakumar R, et al. Physiological LEKTI fragments inhibit epidermal kallikreins. J Invest Dermatol 2006; 216 suppl 3: 016.
38. Taieb A. Molecular genetics of the epidermal differentiation complex: perspectives for dermatology and medicine or the 'purloined letter syndrome'. Expert Rev Dermatol 2007; 2: 105–107.
39. Taieb A. Emollient therapy in atopic dermatitis. J Dermatol Treat 1998; (suppl 2): S7–SII.