

Mutations in *SPINK5*, encoding a serine protease inhibitor, cause Netherton syndrome

We describe here ¹¹ different mutations in *SPINK5*, encoding the serine protease inhibitor LEKTI, in 13 families with Netherton syndrome (NS, MIM256500). Most of these mutations predict premature termination codons. These results disclose a critical role of *SPINK5* in epidermal barrier function and immunity, and suggest a new pathway for high serum IgE levels and atopic manifestations.

Netherton syndrome is a severe, autosomal recessive disorder characterized by congenital ichthyosis with defective cornification, a specific hair shaft defect (trichorrhexis invaginata or 'bamboo hair') and severe atopic manifestations including atopic dermatitis and hayfever, with high serum IgE levels and hypereosinophilia¹. Failure to thrive, infections and hypernatraemic dehydration result in high post-natal mortality.

We recently localized the NS gene locus to *D5S463–D5S2013* on chromosome 5q32 (ref. 2), in a region where the gene encoding serine protease inhibitor LEKTI (for lympho-epithelial Kazal-type related inhibitor) has previously been mapped³.

LEKTI was initially described in thymus and mucous epithelia as the precursor of two proteolytic fragments, one of which was found to exert an anti-trypsin activity *in vitro*³. As proteolysis is critical in cell activation and communication⁴, and because some serine protease inhibitors have been shown to downregulate the pro-inflammatory NF- κ B pathway^{5,6}, we assumed that LEKTI was a plausible candidate for NS. We therefore investigated steady-state levels of the mRNA encoding

LEKTI in cultured epidermal keratinocytes from a healthy control and five NS patients from whom skin biopsies were obtained (Fig. 1a). Northern-blot analysis showed a 3.7-kb marked hybridization signal in the control and a marked reduction of signal in the extracts from patients, suggesting non-sense-mediated decay of mutated transcripts, as is frequently observed in recessive diseases⁷.

We therefore initiated a search for mutations in the gene encoding LEKTI in NS patients. By a combination of database mining and PCR, we elucidated the intron-exon layout of this gene, which we named *SPINK5* (for serine protease inhibitor, Kazal-type 5). Mutation analysis identified 11 different mutations in 13 families (Table 1 and Fig. 1b–f), at least 9 of which generate premature termination codons of translation and predict mRNA

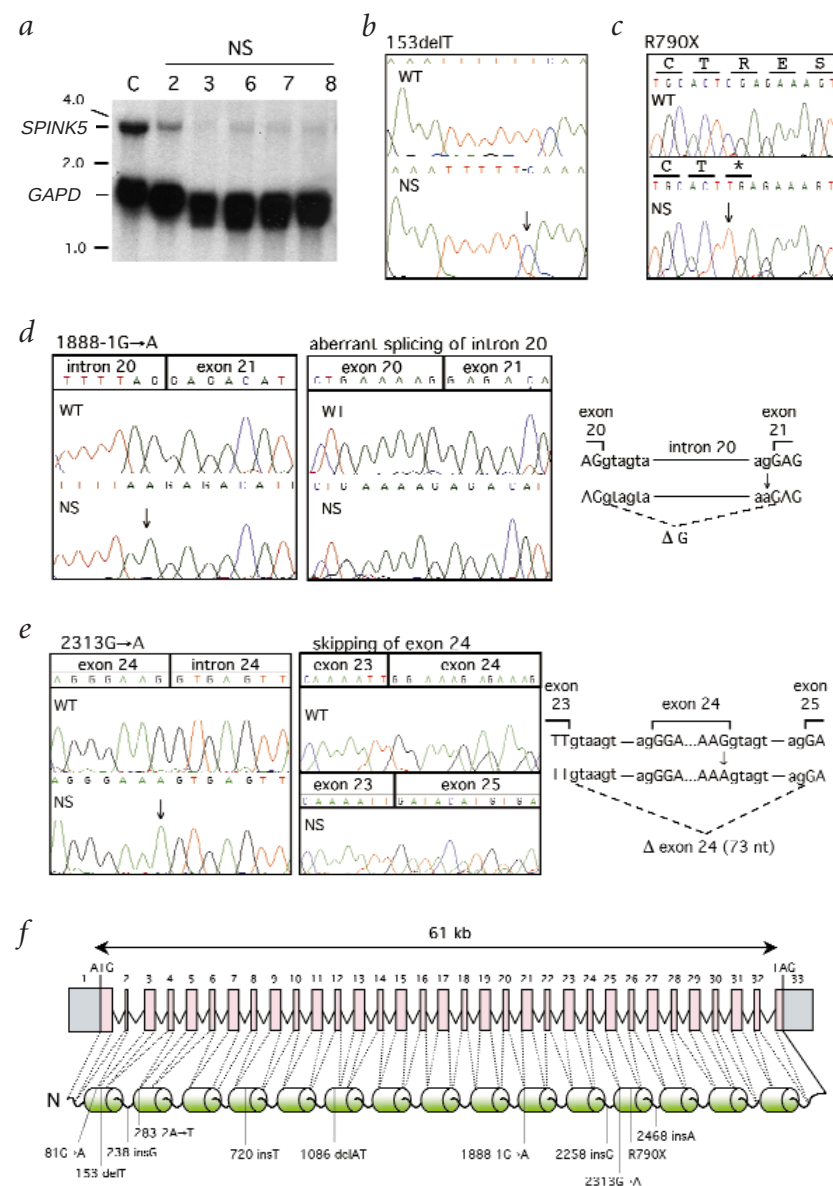


Fig. 1 Northern-blot analysis and *SPINK5* representative homozygous mutations in NS families. **a**, Northern-blot analysis of *SPINK5* expression in cultured epidermal keratinocytes from a control individual (C) and five NS patients (NS). A *SPINK5*-mRNA-specific probe shows a 3.7-kb hybridization signal in the control and a reduction of signal in NS keratinocytes (exposure time 8 h). Family numbers are indicated for each lane. RNA molecular weight markers are represented on the left (kb). **b**, Mutation 153delT in two families (F2 and F3) not known to be related is a thymidine within exon 3 that creates a *Xmn*I site. **c**, Mutation R790X in family 10 is a cytidine-to-thymidine transition at a CpG dinucleotide that generates a premature translation termination codon (*). **d**, Mutation 1888-1G→A in family 8 disrupts the intron 20 acceptor splice site, resulting in deletion of guanines 1888 in the *SPINK5* cDNA through the activation of the cryptic splice site aG located 1 nt downstream of the mutation. **e**, Mutation 2313G→A in family 9 occurs at the wobble position of codon 771 and alters the last base of exon 24, resulting in the skipping of exon 24 (73 nt). **f**, Schematic representations of *SPINK5* and the predicted protein. *SPINK5* (61 kb, top) comprises 33 exons and 32 introns. Exons 1 and 33 include the start codon (ATG) and the natural stop codon (TGA) of translation, respectively. 5' and 3' UTR, coding regions and introns are denoted by grey boxes, pink boxes and broken lines, respectively. Exon sequences have been submitted to EMBL (accession numbers AJ27094, AJ391230–54, AJ276577–80). The 15 highly homologous Kazal-type modules of LEKTI (1,064 residues, bottom) are depicted in green. The mutations detected in the NS families are indicated along the predicted polypeptide.

Table 1 • SPINK5 mutations in NS

Family	Location	Mutation ^a	Nucleotide change ^b	Verification ^c	Consequence ^d
1	exon 2	81G→A	AGgttagt→AAgttagt	CSGE + sequencing	altered splicing
	exon 24	2258insG	AGAA→AGGAA	+ <i>Mnl</i> I	frameshift (PTC+3)
2	exon 3	153delT	A(T) ₆ C→A(T) ₅ C	+ <i>Xmn</i> I	frameshift (PTC+4)
3	exon 3	153delT	A(T) ₆ C→A(T) ₅ C	+ <i>Xmn</i> I	frameshift (PTC+4)
4	exon 4	238insG	AGGGC→AGGGGC	+ <i>Nla</i> IV	frameshift (PTC+18)
5	intron 4	283-2A→T	cagCTG→ctgCTG	– <i>Pvu</i> II	altered splicing
	exon 26	2468insA	T(A) ₁₀ G→T(A) ₁₁ G	CSGE + sequencing	frameshift (PTC+4)
6	exon 9	720insT	CACG→CATCG	CSGE + sequencing	frameshift (PTC+4)
7	exon 12	1086delAT	TCAATATG→TCATG	+ <i>Nla</i> III, – <i>Nde</i> I	frameshift (PTC+6)
8	intron 20	1888-1G→A	tagGAG→taaGAG	+ <i>Mse</i> I	altered splicing (PTC+85)
9	exon 24	2313G→A	AGgtgagt→AAgtgagt	– <i>Hph</i> I	skip exon 24 (PTC+10)
10	exon 25	R790X	ACTCGA→ACTTGA	– <i>Xho</i> I	nonsense
11	exon 26	2468insA	T(A) ₁₀ G→T(A) ₁₁ G	CSGE + sequencing	frameshift (PTC+4)
12	exon 26	2468insA	T(A) ₁₀ G→T(A) ₁₁ G	CSGE + sequencing	frameshift (PTC+4)
13	exon 26	2468insA	T(A) ₁₀ G→T(A) ₁₁ G	CSGE + sequencing	frameshift (PTC+4)

^aNumbering of the amino acids or nucleotides refers to the peptide or cDNA sequence, respectively, with the first nucleotide of ATG initiation codon as 1. All patients were found to be homozygous for *SPINK5* mutations, except patients in families 1 and 5, who were compound heterozygotes. ^bIntronic bases are denoted in lower-case, exonic bases in upper-case; bases altered by the mutation are underlined. ^cThe (–) and (+) symbols represent disruption or generation of a restriction site, respectively. ^d(PTC+n) denotes the occurrence of premature termination codon of translation 'n' residues downstream of the mutation. All parents were heterozygous carriers for the base changes, whereas the non-affected relatives were heterozygous or homozygous for the wild-type allele. Mutation 2468insA was detected in three families from Kashmeer (family 11 to 13), only two of which were known to be related. Mutation 153delT was detected in two Turkish families (families 2 and 3) not known to be related. Mutations 2468insA and 153delT occurred within 5 and 10 mononucleotide repeats, respectively, and may result from slipped mispairing at the replication fork. Four polymorphisms were also detected in patients and control DNA: 1113A→G (arginine 371), 1257C→T (glycine 439), 2358 T→C (leucine 786) and 3009C→T (glycine 1003).

instability, whereas the consequences of 2 of 4 splice-site mutations could not be assessed. The mutations include a non-sense mutation (R790X), four mononucleotide insertions (238insG, 720insT, 2258ins G, 2468insA), two mono/dinucleotide deletions (153delT, 1086delAT) and four splice-site mutations (81G→A, 283-2A→T, 1888-1G→A, 2313G→A). Although it is still unknown whether *SPINK5* transcripts undergo alternative splicing, these four splice mutations are likely to be pathogenic because they disrupt well-conserved splice elements or generate stop codons (Fig. 1*d,e*). Consistent with the recessive mode of inheritance of NS, these results predict null expression of the mutated *SPINK5* alleles through accelerated mRNA decay or loss of function of truncated LEKTI polypeptides if any were translated in the patients.

LEKTI is the first serine-protease inhibitor shown to be primarily involved in a skin disorder or hair morphogenesis. Together with the recent identification of cathepsin C mutations in Papillon-Lefevre syndrome⁸, our study underscores the importance of the regulation of proteolysis in epithelia formation and keratinocyte terminal differentiation. Whereas the other disorders of keratinization feature hyperkeratosis, a diminution or loss of the granular and horny layers has been described in NS patients⁹, suggesting that *SPINK5* mutations cause a distinctive defect in skin barrier function in NS. Defective cornification may favour recurrent infections and inflammation in NS,

but the observed tissue-specificity of LEKTI also suggests a specific role in the thymus³. Abnormal maturation of T lymphocytes in NS patients may disrupt the regulation of Th2 responsiveness to allergens, which drives the acute hypersensitivity response and IgE levels¹⁰.

Genetic variants of pro-Th2 interleukins (IL) 4 and 13 (5q31), the IL4 receptor α -subunit (16p12) and the IL13 receptor α 1-subunit (Xq13) have been shown to be associated with atopy, asthma and IgE levels^{11–13}. Mutations in the recombination-activating genes (RAG) 1 or 2, both located on chromosome 11p13, underlie Omenn syndrome, a severe immunodeficiency condition with erythroderma and a skewed Th2 lymphocyte profile¹⁴. Our findings indicate the existence of a novel pathway distinct from interleukin signalling and V(D)J recombination accounting for the regulation of Th2 lymphocytes.

SPINK5 may regulate extracellular matrix remodelling⁴ or yet-unknown protease-related signalling pathways, as described for thrombin¹⁵. Forthcoming functional studies and *in vivo* targeting of *SPINK5* should unravel new molecular bases of skin barrier morphogenesis and immunity.

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