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REVIEW ARTICLE

Netherton Syndrome: A Genotype-Phenotype Review

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Abstract Netherton syndrome (OMIM #256500) is a rare but severe autosomal recessive form of ichthyosis that affects the skin, hair, and immune system. The identification of *SPINK5*, which encodes for the serine protease inhibitor LEKTI, as the gene responsible for Netherton syndrome, enabled the search for causative mutations in Netherton syndrome patients and families. However, information regarding these mutations and their association with the pathological Netherton syndrome phenotype is scarce. Herein, we provide an up-to-date overview of 80 different mutations in exonic as well as intronic regions that have been currently identified in 172 homozygous or compound heterozygous patients from 144 families. Genotypes with mutations located more upstream in LEKTI correlate with more severe phenotypes compared with similar mutations located towards the 3′ region. Furthermore, splicing mutations and post-transcriptional mechanism of nonsense-mediated mRNA decay affect LEKTI expression in variable ways. Genotype–phenotype correlations form the basis of prenatal diagnosis in families with a history of Netherton syndrome and when consanguinity is implied.

Key Points

We provide an analysis of the genetic background of Netherton syndrome.

A correlation of the syndrome's clinical severity with the position and the nature of the *SPINK5* mutations are explored.

1 Introduction

Netherton syndrome (OMIM #256500) is a rare autosomal recessive disorder that is characterized by congenital ichthyosis, trichorrhexis invaginata, erythroderma, atopic manifestations, high immunoglobulin E levels, hypereosinophilia, and failure to thrive. Although its incidence is reported to be 1 per 200,000 births, it is thought that the incidence might be higher (~1 per 50,000), the underestimation may be due to the difficulty of diagnosis as the symptoms that resemble those of atopic dermatitis, congenital erythodermas, and some forms of ichthyosis [1, 2]. The prognosis of Netherton syndrome is poor for the most severely affected infants, mainly due to dehydration and infections.

The affected gene, serine peptidase inhibitor, Kazal type 5 (*SPINK5*), which codes for the serine protease inhibitor LEKTI, has been identified through linkage analysis and homozygosity mapping [3, 4]. *SPINK5* maps at 5q32 in a cluster of *SPINK* genes that also includes *SPINK* 14, 6, 13, 7, and 9, which are located more distally (NC_000005.9) and most of which are not functionally characterized.

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SPINK5 is subjected to alternative splicing in human keratinocytes, producing three transcripts (designated *SPINK5f-l*, *SPINK5sh*, and *SPINK5l*) that are translated into different LEKTI precursor isoforms of 145, 125, and 148 kDa, respectively. All three transcripts share the same sequence corresponding to the first 28 exons (domain 13). The splicing of exon 28 to exon 29 generates the *SPINK5f-l* isoform, which consists of 33 exons (15 domains). The *SPINK5sh* isoform is composed of 13 domains. The use of an alternative polyadenylation signal within intron 28 produces this shorter isoform of 28 exons. Finally, *SPINK5l* carries a 30 amino-acid residue insertion encoded by cryptic exon 28a (34 exons), as compared with the full-length transcript [5, 6]. The three alternative transcripts have similar expression patterns, while the expression levels of the main transcript, *SPINK5f-l*, are about 40-fold higher [5]. At the protein level, the main isoform of LEKTI is localized in the granular and uppermost spinous layers of the epidermis as well as in the most differentiated layers of all stratified epithelia [7].

The precursor main polypeptide is composed of 1064 amino acids and contains 15 putative serine protease inhibitory domains (D1–D15) of the Kazal type (Fig. 1) [8], two of which (domains D2 and D15) contain the highly conserved motif with the canonical 6 cysteine residues, whereas the others have a Kazal-related structure that are devoid of one disulfide bridge. Nevertheless, all of the domains are able to generate the typical Kazal-binding loop hairpin structure [9]. The LEKTI precursor is subjected to proteolytic cleavage, by a furin-driven proteolytic activation cascade, into fragments that contain single or multiple inhibitory domains which are secreted from the cell (Fig. 1) [10].

Proteolytic activation of LEKTI gives five final bioreactive fragments. The first proteolytic cleavage at Arg355 gives two fragments. The first fragment contains D1–D5 and is not cleaved further. The second consists of D6–D15 and is subjected to further proteolysis at Arg625, generating fragments D6–D9 and D10–D15. The fragment containing D10–D15 is not processed further, while D6–D9 is subjected to proteolysis at Arg425, producing fragments consisting of D6 and D7–D9. The latter gives the final fragments containing D7 and D8–D9 by proteolytic cleavage at Arg489 (Fig. 1) [10].

LEKTI major targets for inhibition are the most abundant kallikreins in the stratum corneum—kallikrein (KLK)5, KLK7, and KLK14 [10–13]—as well as trypsin, plasmin, subtilisin A, cathepsin G, and human neutrophil elastase [14]. Each form of LEKTI exhibits its own inhibitory activity with specific as well as overlapping targets [13]. LEKTI*f-l* has been shown to inhibit trypsin, plasmin, subtilisin A, cathepsin G, and neutrophil elastase but not chymotrypsin [14]. A partial recombinant form of LEKTI consisting of D6–D9 has been shown to exhibit inhibition against trypsin, subtilisin A, chymotrypsin, KLK5, and KLK7 but not cathepsin G, plasmin, or elastase [15, 16]. Multidomain D6–D9 and derived fragments D7–D9 and D8–D9 are strong inhibitors of KLK5 and KLK14 but weak inhibitors of KLK7 [10]. Single domain 6 is a potent inhibitor of both KLK5 and KLK7, and trypsin [11], although single-domain fragments (D6 and D7) show weaker inhibition than corresponding multidomain fragments [10]. The fragment D10–D15 seems to be a weak inhibitor of KLK5 and KLK14, but presents a more efficient inhibition against KLK7 [10]. The balance between serine proteases and their inhibitors ensures the integrity of the stratum corneum, the lipid barrier and the envelope as well as the proper desquamation in the epidermis. In Netherton syndrome, the reduction of LEKTI inhibition on serine proteases can explain the increased desquamation and the clinical manifestations of the skin.

The generation and analysis of the knockout *Spink5*$^{-/-}$ mice [17–19] that reproduce key features of the syndrome have shed light on the pathogenesis of the disease. The *SPINK5* loss of function mutations that have been identified in patients with Netherton syndrome cause epidermal protease hyperactivity, resulting in detachment of stratum corneum, skin barrier defect, and over-desquamation. In addition, the upregulated kallikrein KLK5 induces skin inflammation and allergy [19].

An additional consistent feature of Netherton syndrome patients is failure to thrive and/or growth retardation. This could be explained by the dysregulation of human growth hormone (hGH). *SPINK5* is expressed in the pituitary gland where it inhibits many kallikreins, which are mainly

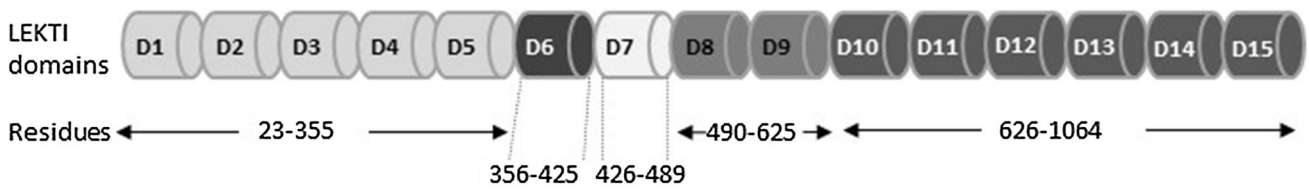

Fig. 1 The 15 serine protease inhibitory domains of LEKTI (D1–D15) and the five bioreactive fragments that are produced by proteolysis of the precursor protein (shown in different scales of *grey*)

expressed in tissues that are regulated by steroid hormones [20]. These kallikreins in turn participate in the proteolytic cleavage of hGH. hGH exists as a heterogeneous population of molecules in the human body. The major fraction consists of a 22 kDa single-chain form (single-hGH), which can be altered post-translationally by proteolytic cleavage. The proteolysis of single-hGH creates a two-chain form linked by a disulfide bond. This form shows a slower rate of metabolism in the circulation. Splice variants and fragments of 17 (or 16), 12, and 5 kDa have also been found in the pituitary as well as in the periphery. Patients with Netherton syndrome usually lack domains D6–D15, thus having increased kallikrein activity and hGH proteolysis [21]. Recently, a good response to hGH administration has been reported in three patients with Netherton syndrome who had growth retardation and growth hormone deficiency [22].

Currently, there is no proven cure or consensus on treatments (see, for example, Maatouk et al. [23] and Gallagher and Patel [24]). Different antibacterials are used for skin infections, emollients for the treatment of dryness, and topical corticosteroids for eczema. Furthermore, inhibitors of kallikreins involved in skin diseases have been designed and have tested negative for cytotoxicity toward healthy human keratinocytes [25]. In addition, in a recent case report, administration of the humanized antibody omalizumab resulted in the improvement of allergic skin symptoms [26]. Finally, a phase I trial study protocol has been described [27] for grafting ex vivo gene-corrected keratinocyte stem cells onto patients carrying mutations in *SPINK5* (still ongoing, EU Clinical Trials Register, EudraCT number: 2011-003212-22).

In this review we summarize all of the mutations that have been identified to date in *SPINK5* and their consequences at the molecular level as well as clinical details of attempting to identify phenotype–genotype correlations.

2 Methodology

Fifty publications were identified that referred to the genetic background of Netherton syndrome and we also consulted the Human Gene Mutation Database (HGDB) [28–31]. Apart from the patients' genotypes, data regarding their clinical phenotype were also collected. In order to evaluate and compare the clinical phenotype of all patients described, we used a clinical severity score system (see Sect. 2.1). Mutations that affect the expression and/or sequence of LEKTI in different ways were reported. In the case of missense mutations, we performed pathogenicity prediction using Polyphen-2 [32].

2.1 Clinical Severity Score

Severity assessment for the Netherton patients found in the literature was performed independently by two clinical dermatologists (EZ, AP) and was based mainly on clinical manifestations, as described in the published articles, taking into account the severity of each symptom, alone and in combination with the others in order to designate the severity status. A classification of clinical severity score with increasing severity was followed in scale from 1 to 5 where 1 is given to milder phenotypes, such as faintly detectable erythema, as they were designated in the primary studies and 5 to deceased patients. A score of 2 was given to patients with skin manifestations and additional symptoms such as trichorrhexis invaginata, high immunoglobulin E, and allergies, but not infections or developmental derogations. A score of 3 corresponded to intermediate disease severity. Recurring infections was the main extra characteristic of patients in this category. However, patients without an incidence of infections who showed growth retardation or failure to thrive were also included in this class. Finally, the more severe (but not deceased) patients were classified as a 4 on the clinical severity scale. They usually suffered from severe skin manifestations in combination with growth retardation and/or failure to thrive and a burdened clinical state.

3 Results and Discussion

There are registered 84 mutations of *SPINK5* that are associated with Netherton syndrome in the HGDB. However, mutations registered using the database codes CM014760, CS001465, CS121225, and CD013874 are not described in the articles cited (for more information visit the HGDB). So far, 80 different mutations in exonic as well as intronic regions have been identified in patients from 144 families (Tables 1, 2, 3). Among them, 18 mutations are nonsense, two are non-synonymous, and 34 are insertion and/or deletion mutations of one or a few nucleotides altering the reading frame and resulting in the generation of premature termination codons (PTCs). Furthermore, 24 mutations disrupting the normal splicing pattern have been found in both splice sites and distant splicing elements. The distribution of the mutation in the genomic sequence of *SPINK5* in combination with their effect is shown in Fig. 2c. The absence of interfamilial gene variation in over 100 families leads to the assumption that no other gene(s) in these patients play a noticeable role affecting the clinical phenotypes.

As shown in Table 1, 86 of the 172 patients in which mutations have been described are homozygous and over 40% of them came from consanguineous families. These

Table 1 Homozygous Netherton patients

Family/ patient	Genotype	Age	Ethnic origin	Skin manifestations	Growth retardation	FTT	Inf.	High IgE	TI	Atopy	Others	Severity score	References
1	c.81+2T>A	12 years	IE/UK	CIE-like ichthyosis	+	+	+	+	+	+	Deh	4	[1]
2[c]	c.153delT	4 months	TR								Death	5	[33]
3[c]	c.153delT	n	TR								Death	5	[34]
4[c]	c.153delT	n	TR								Death	5	[34]
5_1[c]	c.153delT	n	TR								Death	5	[34]
5_2[c]	c.153delT	11 years	TR	ILC, SE, atopic dermatitis	+	+		+	+		Deh, Ent, He, AO	4	[34]
6[c]	c.153delT	5 years	TR	E, SE	+	+	+	+	+		Deh, Ent, HF	4	[34]
7[c]	c.153delT	3 months	TR								Death	5	[35]
8[c]	c.153delT	n	TR								Death	5	[35]
9[c]	c.153delT	2 months	TR								Death	5	[35]
10	c.153delT	7 years		ILC, E				+	+	+		2	[36]
11[c]	c.153delT	n									Death	5	[37]
12	c.153delT	n	TR	Ichthyosis, erythema, exfoliation							↑Na	2	[38]
13_1	c.238insG	1 month	TR								Death	5	[39]
13_2	c.238insG	3 months	TR								Death	5	[39]
14[c]	c.238insG	8 years	US (Puerto Rico)	Gen. CIE-like ichthyosis	+	+	+		+			4	[1]
15[c]	c.238insG	3 years	IR	Gen. CIE-like ichthyosis		+		+	+	+		4	[1]
16	c.268_269insT	<1 year		Gen. SE		+	+	+	+		Deh, ↑Na	4	[37]
17[c]	c.375delAT	16 months	JP								Death	5	[40]
18	c.391insCGTG	18 years	DO	Gen. CIE-like ichthyosis	+	+	+	+	+	+	Ent	4	[1]
19[c]	c.392insGTGC	1 years	DO	Erythroderma	+				+			3	[67]
20_1	c.462insGT	9 years	Asian	CI, exfoliative erythroderma	+				+			4	[41, 42]
20_2	c.462insGT	12 years	Asian	CI, exfoliative erythroderma	+				+			4	[41, 42]
21	c.581_82delGT	4 years (tw)		Gen. eczematous and erythematous lesions	+			+	+	+		4	[43]
22	c.581_82delGT	6.5 years (tw)		Ichthyosis	+				+	+	P	3	[22]
23	c.C628T (p.R210X)	17 years		ILC								1	[36]
24[c]	c.C628T (p.R210X)	8 years	PK	ILC, E				+	+		P	2	[34]
25[c]	c.C628T (p.R210X)	5 years	PK	ILC, E, SE	+	+		+	+		He, P, A	4	[34]
26	c.C649T (p.R217X)	4 months	Northern European								Death	5	[44]
27	c.652C>T (p.R218X)	6–11 years	FI	ILC, patchy erythema and scaling	+		+	+	+		AO, P	4	[45]

Table 1 continued

Family/ patient	Genotype	Age	Ethnic origin	Skin manifestations	Growth retardation	FTT	Inf.	High IgE	TI	Atopy	Others	Severity score	References
28	c.652C>T (p.R218X)	6–11 years	FI	ILC, patchy erythema and scaling	+		+	+	+		AO, U, A, rhinoconjuctivitis, ↑Na, P, diarrhea	4	[45]
29_1	c.652C>T (p.R218X)	6–11 years	FI	ILC, patchy erythema and scaling	+		+	+	+		↑Na, diarrhea, P	4	[45]
29_2	c.652C>T (p.R218X)	6–11 years	FI	ILC, patchy erythema and scaling				+	+		P	3	[45]
29_3	c.652C>T (p.R218X)	3–5 years	FI	ILC, patchy erythema and scaling				+	+		P	3	[45]
30	c.652C>T (p.R218X)	<1 year	FI	ILC, episodic erythema	+				+		↑Na, P	3	[45]
31_1	c.652C>T (p.R218X)	1–3 years	FI	ILC, patchy erythema and scaling, episodic erythema	+		+	+	+		U, AO, A, ↑Na, P	4	[45]
31_2	c.652C>T (p.R218X)	1–3 years	FI	ILC, patchy erythema and scaling, episodic erythema	+		+	+			↑Na, P	4	[45]
32	c.691delC	15 years	IQ	ILC or eczema of >30% of body surface, erythroderma			+					2	[44]
33[c]	c.715insT	10 months	TR	CIE	+	+	+	+	+		Ent, He	4	[46]
34	c.720insT											NPA	[4]
35_1	c.997C>T (p.Gln333X)	42 years	TR	ILC, erythematous xerotic patches	+			+	+			4	[47]
35_2[c]	c.997C>T (p.Gln333X)	20 years	TR	ILC, erythematous xerotic patches	+			+	+			4	[47]
35_3[c]	c.997C>T (p.Gln333X)	16 years	TR	ILC, erythematous xerotic patches	+			+	+			4	[47]
35_4[c]	c.997C>T (p.Gln333X)	22 years	TR	ILC, erythematous xerotic patches	+			+	+			4	[47]
35_5[c]	c.997C>T (p.Gln333X)	16 years	TR	ILC, erythematous xerotic patches	+			+	+			4	[47]
35_6[c]	c.997C>T (p.Gln333X)	12 years	TR	ILC, erythematous xerotic patches	+			+	+			4	[47]
35_7[c]	c.997C>T (p.Gln333X)	10 years	TR	ILC, erythematous xerotic patches	+			+	+			4	[47]
35_8[c]	c.997C>T (p.Gln333X)		TR								Death	5	[47]
36[c]	c.1048C>T (p.R350X)	n		Erythroderma	+	+	+	+	+		Deh, S	4	[48]
37	c.1111C>T (p.R371X)	9 months									Death	5	[49]
38	c.1430+2T>G		CN	SE				+	+	+		2	[50]
39_1[e]	c.1431–12G>A		Romani								Death	5	[51]
39_2[e]	c.1431–12G>A		Romani								Death	5	[51]

Table 1 continued

Family/ patient	Genotype	Age	Ethnic origin	Skin manifestations	Growth retardation	FTT	Inf.	High IgE	TI	Atopy	Others	Severity score	References
40_1	c.1431–12G>A	3 years	BA	ILC	+				+	+		3	[46]
40_2	c.1431–12G>A	7 years	BA	ILC	+	+			+	+	Deh	4	[46]
41	c.1431–12G>A	15 years										NPA	[65]
42	c.1732C>T (p.R578X)	7 years		Erythematous scaly patches				+	+	+		2	[52]
43*	c.1732C>T (p.R578X)	10 years	JP	Ichthyosiform erythroderma, gen. scaly erythema				+	+	+		3	[53]
44	c.1732C>T (p.R578X)	13 years	Caucasian									3	[41]
45	c.1772delT	50 years	FI	ILC, patchy erythema and scaling, episodic erythema	+		+				U, AO, P, Al, diarrhea	4	[45]
46	c.1086delAT											NPA	[4]
47[c]	c.1346_1352insT	3 years		CI, E		+	+		+		Deh	4	[54]
48	c.1888–1G>A											NPA	[4]
49[c]	c.1888–1G>A	1 year		ILC			+	+	+			3	[37]
50[c]	c.1888–1G>A	5 years		Gen. SE		+	+	+	+			4	[37]
51	c.1888–1G>A	41 years		Gen. SE			+	+	+		Deh, ↑Na	3	[37]
52	c.2240+5G>A	34 years	AZ	ILC or eczema of >30% of body surface, erythroderma					+			2	[44]
53_1	c.2260A>T (p.K754X)	3 years	TW	Gen. erythema			+	+	+	+		3	[55]
53_2	c.2260A>T (p.K754X)	11 months	TW	Gen. erythroderma	+		+	+	+			4	[55]
54	c.2313G>A											NPA	[4]
55[c]	c.2313G>A	17 years		ILC, gen. SE		+	+	+	+		Deh, ↑Na	4	[37]
56_1[c]	c.2368C>T (p.R790X)	29 years	JP	ILC, erythroderma				+	+			2	[56]
56_2[c]	p.R790X	24 years	JP	ILC, erythroderma				+	+			2	[56]
57	p.R790X											NPA	[4]
58	p.R790X	7 years	JP	ILC	+			+	+		He	4	[57, 58]
59_1[c]	c.2441+3delGAGT	6.5 years	TR	Gen. skin eruption			+	+	+			3	[59]
59_2[c]	c.2441+3delGAGT	5.5 years	TR	Gen. skin eruption			+	+	+			3	[59]
60	c.2468insA	6 years		ILC, IE		+		+	+			3	[60]
61	c.2468insA	5 years		ILC, E				+	+	+		2	[36]
62[c]	c.2468insA	2 years	PK	ILC, SE	+	+	+	+	+	+	Deh, Ent, P	4	[34]
63[c]	c.2468insA	43 years	DE	CIE				+	+	+		2	[46]
64	c.2468delA	6 years	Polynesian	CI, E		+	+		+	+	A, R	3	[54]
65	c.2468delA	7 years	Polynesian	CI, E			+		+		R	3	[54]
66	c.C2557T (p.R853X)	6.5 years	GE	ILC or eczema of >30% of body surface, erythroderma						+		2	[44]

Table 1 continued

Family/patient	Genotype	Age	Ethnic origin	Skin manifestations	Growth retardation	FTT	Inf.	High IgE	TI	Atopy	Others	Severity score	References
67	c.C2557T (p.R853X)	19 years	GE	ILC or eczema of >30% of body surface, erythroderma			+			+		3	[44]
68[c]	c.2579delA	3 years		Mild SE				+	+			2	[37]

A asthma, *Al* alopecia, *AO* angioedema, *AZ* Azerbaijan, *BA* Bosnia and Herzegowina, *CI* congenital ichthyosis, *CIE* congenital ichthyosiform erythroderma, *CN* China, *DE* Germany, *Deh* dehydration, *DO* Dominican Republic, *E* eczema-like rashes, *Ent* enteropathy, *FI* Finland, *FTT* failure to thrive, *GE* Georgia, *gen.* generalized, *He* hypereosinophilia, *HF* high fever, *IE* Ireland, *IgE* immunoglobulin E, *ILC* ichthyosis linearis circumflexa, *Inf.* Infections, *IQ* Iraq, *IR* Iran, *JP* Japan, *n* neonatal, *NPA* no phenotype available, *P* pruritus, *R* rhinitis, *S* sepsis, *SE* scaly erythroderma, *TI* trichorrhexis invaginata, *TR* Turkey, *tw* twins, *TW* Taiwan, *U* urticaria, ↑*Na* hypernatremia

* Homozygous due to complete maternal isodisomy

** This patient was initially reported to be heterozygous, carrying c.2471-2475delAAGAGinsT and c.2039-2049delAGAGGAAAGAA [77]. However, sample from this patient was used for Western blotting in a latter study [61] in which the patient was described as homozygous for c.2471_2475delAAGAGinsT

[c] Consanguinity; [e] endogamy

patients comprise a valuable source of information for genotype–phenotype correlations as they provide pure genotypes. Patients 1–36 have mutations in the first catalytic fragment of LEKTI (D1–D5), theoretically resulting in the production of a truncated protein. Truncated LEKTI could have a decreased catalytic activity, depending on the domains that are contained in the final product. Sixteen mutations that affect the first fragment of LEKTI have been identified in homozygosity, 12 of which change the codon to PTC while one disrupts the splicing pattern. Clinical phenotype is available for 48 of 49 patients, and the severity score is 4 or 5 in 39 of 48 patients (Table 1). The lower severity score (1 or 2) of five patients (patients 10, 12, 23, 24, and 32) is probably due to the restricted data [34, 36, 38, 44]. In particular, the clinical outcome of over one-quarter of these patients is death. However, this observation is an under-estimation because the information provided corresponds to clinical observations at the time. Poor updating of patients' clinical data could decrease the real percentage of mortality in this group. Patients 37–41 produce the first catalytic fragment of LEKTI but lack either D6–D15 or D7–D15. The strongest inhibitory activity against KLK5 and KLK14 in vitro is demonstrated from D8–D11 [13]. This is in coordinance with the severity score of patients 1–41 in comparison with that of patients 42–68. Patients 1–43 (lacking D8–D15) display a score of 4 or 5 on the severity scale, while almost 75% of patients 42–68 have a score $\leq$3. It seems that there is a correlation between genotype and phenotype, indicating that the severity of the phenotype increases as the mutation is nearest to the –NH2 terminal of LEKTI.

Six homozygous splicing mutations have been identified (c.81+2T>A, c.1430+2T>G, c.1431–12G>A, c.1888–1G>A, c.2240+5G>A, and c.2441+3delGAGT). Immunostaining of LEKTI in patient 38 (mutation c.1430+2T>G) showed weak expression of the protein [32]. Similarly, c.1888–1G>A reduces mRNA levels by more than 75% [4] while immunostaining showed no expression of LEKTI in three homozygous patients [37]. The mutation c.1431–12G>A has been shown using immunoblotting to lead to no protein production [46]. Finally, immunostaining showed no signal of LEKTI in a patient homozygous for c.2441+3delGAGT [59]. There is no information about the functional consequence of c.81+2T>A or c.2240+5G>A on LEKTI. For splicing mutations, nonsense-mediated mRNA decay is probably not complete and occurs in different percentages between individuals. This phenomenon could result in different symptoms between patients carrying the same mutations. For example, patients 39_1 and 39_2 scored 5 on the severity scale while patients 40_1 and 40_2 scored 3 and 4, respectively.

Two missense mutations have been reported in Netherton syndrome patients. p.D106N has been identified in

Table 2 Compound heterozygous Netherton patients. Family/patient numbering follows that of Table 1

Family/patient	Genotype	Age	Ethnic origin	Skin manifestations	Growth retardation	FTT	Inf.	High IgE	TI	Atopy	Others	Severity score	References
69	c.153delT/c,891C>T (p.Cys297Cys)	26 years	XK (Kosovo)	ILC, E	+				+		P	3	[61]
70	c.153delT/c.891C>T	10 months	XK (Kosovo)	E, SE	+		+	+	+	+	Deh, He, P, Al	4	[61]
71	c.153delT/c.891C>T	4 years	IT (Sicily)	Eczematous-like lesions	+			+	+	+	P, A, Al,	4	[62]
72_1	c.153delT/c.81+2T>A	14 years	XK (Kosovo)	ILC, E, SE	+		+	+	+	+	Deh, Ent, P, HF, AO	4	[34]
72_2	c.153delT/c.81+2T>A	6 years	XK (Kosovo)	E, SE	+		+	+	+	+	Deh, Ent, P, HF, AO	4	[34]
73[c]	c.153delT/c.81+2T>G	5 years		ILC, gen. SE		+	+	+	+		Deh, ↑Na	4	[37]
74	c.238insG/p.Q46X	5 years	IT	ILC, E			+	+	+	+		4	[1]
75_1	c.238insG/p.R217X (c.C649T)	29 years	IT	SE	+	+	+	+	+		Deh, P, A, AO	4	[34]
75_2	c.238insG/p.R217X (c.C649T)	15 years	IT	SE			+	+	+		P, AO	4	[34]
76_1	c.238insG/c.891C>T	5 years	GR	E			+	+		+	P	3	[61]
76_2	c.238insG/c.891C>T	9 months	GR	ILC, E			+	+		+	P, Al, B	3	[61]
77	c.238insG/c.891C>T	12 years	GR	ILC, E, SE				+	+	+	P, A, AO, Al, U	4	[61]
78	c.238insG/c.1431–12G>A	n	GR	Erythematous lesions	+	+	+		+		S, electrolytic disorders	4	[63]
79	c.238insG/p.Arg217X	27 years		Gen. SE		+	+	+	+		Deh, ↑Na	4	[37]
80	c.283-2A>T/c.2468insA	27 years		ILC, gen. SE		+	+	+	+			4	[37]
81	c.316_317delGA/c.2468insA	23 years		Gen. SE				+	+	+	P, A, R	2	[64]
82	c.354delTTGT/c.1432–13G>A	1.5 years										NPA	[65]
83	c.474G>A (p.Gln158Gln)/c.1732C>T (p.R578X)	2 years	JP	ILC				+	+			2	[66]
84	c.603+1G>A/c.282+1G>A	7 years		CI			+		+	+	A, R	3	[54]
85	c.652C>T/c.1220+1G>C	1–3 years	FI	ILC, patchy erythema and scaling, episodic erythema			+	+			A, P, diarrhea	3	[45]
86	c.652C>T/c.1220+1G>C	<1 year	FI	Episodic erythema			+	+	+			3	[45]
87	c.691delC/c.C649T (p.R217X)	26 years	Ashkenazi Jews	CIE, severe erythroderma			+	+	+	+		4	[68]
88	c.691delC/c.C649T (p.R217X)	20 years	Northern European	ILC or eczema of >30% of body surface and occasionally erythroderma	+	+	+				Deh, Ent	4	[44]
89_1	c.715insT/c.375delAT (p.Y126X)	10 years	JP	Consistent erythroderma	+		+	+			He, A	4	[58]
89_2	c.715insT/c.375delAT (p.Y126X)	n	JP								Death	5	[58]
90	c.891C>T/c.283-12T>A	16 months	GR	ILC, E, SE			+	+	+		Deh, He, P, Al	4	[61]
91	c.891C>T/c.410+1G>A	2 years	GR	ILC, E					+	+	P	2	[61]

Table 2 continued

Family/ patient	Genotype	Age	Ethnic origin	Skin manifestations	Growth retardation	FTT	Inf.	High IgE	TI	Atopy	Others	Severity score	References
92	c.891C>T/c.1820+53G>A	38 years	DK	ILC, E			+	+	+		P, A, AO, U	3	[61]
93	c.900A>G/c.1913delT	1 year										NPA	[65]
94	c.957–960dupTGGT/c.C649T (p.R217X)	22 years	IT	Ichthyosiform erythroderma, gen. erythema		+		+	+	+		3	[69]
95	c.966insC/375delAT (p.Y126X)	1 year	JP	Consistent erythroderma	+		+		+	+	He, U	4	[58, 70]
96	c.1024ins4/c.81+2T>A	13 years	Northern European	Gen. CIE-like ichtyosis	+	+	+	+	+	+	Deh, Ent	4	[1]
97	c.1048C>T (p.R350X)/c. 2098G>T	12–14 years	FI	Patchy erythema and scaling	+			+	+		R	3	[45]
98	c.1111C>T/c.81+2T>A	37 years	UK	ILC, E, SE	+		+	+	+		P, AO, HF, U	4	[34]
99_1	c.1111C>T/c.891C>T	10 years	FR	ILC, E	+		+	+	+	+	P, A, AO, Al	4	[61]
99_2	c.1111C>T/c.891C>T	2 years	FR	ILC, E	+				+	+	A, Al, P	4	[61]
100	c.1111C>T/c.1036insG(A)4	8 years	UK	ILC, E, SE	+	+	+	+	+		Deh, Ent, AO	4	[34]
101	c.1111C>T/c.2041delAG	10 years	IT	ILC, E, SE	+	+		+	+		Deh, He, P, A, HF	4	[34]
102	c.1302+4A>T/c.209+1G>T	16 years	Caucasian									1	[41]
103	c.1302+4A>T/c.2240+1G>A	29 months	Caucasian	ILC								1	[71]
104	c.1302+4A>T/c.2240+1G>A	19 months	Caucasian	ILC								1	[71]
105	c.1431–12G>A/c.354–357delTTGT	6 months		CI, E		+	+		+	+	Deh	4	[54]
106_1	c.1621G>T (p.E541X)/c.375delAT (p.Y126X)	21 years	JP	Consistent erythroderma	+		+	+			Deh, He, P, HI	4	[57, 58]
106_2	c.1621G>T (p.E541X)/c.375delAT (p.Y126X)	21 years	JP	Consistent erythroderma			+	+			Deh, He, P, HI	3	[57, 58]
107	c.2017del9/p.G103X	16 years	DE-IE	ILC, E				+	+	+	Deh	2	[1]
108	c.2041_2042del/p.Arg371X	12 years		ILC, gen. SE		+		+	+		Deh, ↑Na	4	[37]
109	c.2098G>T (p.G700X)/c.410+1G>A	1 year		CI, E		+	+		+	+	Deh	4	[54]
110	c.2240+1G>A/p.R218X	33 years	IT-FI	ILC, E			+	+	+	+		3	[1]
111	c.2240+1G>A/c.81+5G>A	4 years	FR	E, SE			+	+	+		Deh, P	3	[34]
112	c.2240+1G>A/c.316–317delAG	7 years	Caucasian									3	[41]
113	c.2258insG/c.81G>A											NPA	[4]
114_1	c.2264insA/c.391insCGTG	17 years	African-American	Gen. CIE-like ichtyosis		+	+	+	+	+		4	[1]
114_2	c.2264insA/c.391insCGTG	2 years	African-American	ILC, E		+	+	+	+			4	[1]
115	c.2264insA/c.475–2A>G	5 yeasr	Northern European	ILC, E		+	+	+	+	+		4	[1]
116	c.2368C>T (p.R790X)/c.375delAT (p.Y126X)	7 years	JP	Focal erythroderma	+		+	+		+	He	4	[58]
117_1	c.2368C>T (p.R790X)/c.398delTG	8 years	DE	CIE		+		+	+	+	Deh	4	[46]

Table 2 continued

Family/ patient	Genotype	Age	Ethnic origin	Skin manifestations	Growth retardation	FTT	Inf.	High IgE	TI	Atopy	Others	Severity score	References
117_2	c.2368C>T (p.R790X)/c.398delTG	6.5 years	DE	CIE		+	+	+	+	+		4	[46]
118	c.2368C>T (p.R790X)/c.1220+1G>C	6 months	JP	ILC	+			+	+	+		3	[72]
119_1	c.2368C>T (p.R790X)/c.2137C>T (p.Q713X)	2 years	JP	ILC				+	+			2	[73]
119_2	c.2368C>T (p.R790X)/c.2137C>T (p.Q713X)	2 months	JP	Focal ichthyosiform erythroderma								2	[73]
120	c.2368C>T (p.R790X)/c.C2423T (p.T808I)	3 years	TW	ILC			+	+	+		He	3	[74]
121	c.2368C>T (p.R790X)/c.375delAT (p.Y126X)	6 months	JP	SE	+			+		+	↑Na	3	[75]
122	c.2368C>T (pR790X)/c.1099dupT	5 years	JP	Diffuse erythemas			+	+			A, ↑Na	3	[75]
123	c.2468insA/c.283–2A>T											NPA	[4]
124	c.2468insA/c.1432–13G>A (c.1431–12G>A)	31 years	AT	CIE				+	+	+		2	[46]
125	c.2468delA/p.D106N	26 years	GR	ILC, erythematosus rash					+		P	2	This study data not shown
126	c.2468delA/c.1608–1G>A	8 years	FR	E, SE	+	+	+	+	+		Ent, P, HF	4	[34]
127	c.2468delA/c.2260A>T (p.K754X)	7 years	TW	ILC, CIE					+			2	[76]
128	c.2471–2475delAAGAGinsT/ c.2039–2049delAGAGGAAAGAA**		MG	ILC					+			1	[61, 77]
129	c.2473–2474delGA/c.375delAT	6 months		CI, E		+	+		+	+		4	[54]
130	c.2557C>T (p.Arg853X)/c.891C>T	13 years	IT	ILC, E, SE				+	+		He, P, A, Al	3	[61]
131	c.2557C>T (pArg853X)/c.C649T (p.R217X)	10 months	GE	Mainly erythroderma and occasionally ILC or eczema	+	+			+	+	Deh, Ent	4	[44]
132	c.2579del9/c.1888–1G>A	7 years	UK-IT	ILC, E			+	+	+	+		3	[1]

A asthma, *Al* alopecia, *AO* angioedema, *AT* Austria, *B* blepharitis, *CI* congenital ichthyosis, *CIE* congenital ichthyosiform erythroderma, *Deh* dehydration, *DE-IE* Germany - Ireland, *DK* Denmark, *E* eczema-like rashes, *Ent* enteropathy, *FI* Finland, *FR* France, *FTT* failure to thrive, *gen.* generalized, *GR* Greece, *He* hypereosinophilia, *HF* high fever, *HI* heat intolerance, *IgE* immunoglobulin E, *ILC* ichthyosis linearis circumflexa, *Inf.* Infections, *IT* Italy, *IT-FI* Italy - Finland, *JP* Japan, *MG* Madagascar, *n* neonatal, *NPA* no phenotype available, *P* pruritus, *R* rhinitis, *S* sepsis, *SE* scaly erythroderma, *TI* trichorrhexis invaginata, *tw* twins, *TW* Taiwan, *U* urticaria, *UK-IT* United Kingdom - Italy, *XK* Kosovo, ↑*Na* hypernatremia

** This patient was initially reported to be heterzygous, carrying c.2471–2475delAAGAGinsT and c.2039–2049delAGAGGAAAGAA [77]. However, sample from this patient was used for Western blotting in a latter study [61] in which the patient was described as homozygous for c.2471_2475delAAGAGinsT

[c] Consanguinity

Table 3 Netherton patients in whom just one mutation has been identified. Family/patient numbering follows that of Table 2

Family/patient	Genotype	Age	Ethnic origin	Skin manifestations	Growth retardation	FTT	Inf.	High IgE	TI	Atopy	Others	Severity score	References
133	c.56G>A	4 years	CN	E, SE				+	+		Deh, P	2	[34]
134	c.238_239insG	15 years		Gen. SE		+	+	+	+		Deh, ↑Na	4	[37]
135	c.238_239insG	21 years		Mild SE			+	+	+			3	[37]
136	c.316delAG	12 years										NPA	[65]
137	c.318G>A (D106N)*	18 years	CN	ILC, SE				+				2	[78]
138	p.Arg217X	17 years		ILC			+	+	+			3	[37]
139	c.882+1_882+3del	15 years	Caucasian									4	[41]
140	c.1151delGA	4 years	Northern European	ILC, E		+	+	+	+	+		4	[1]
141	c.1302+4A>T	12.5 years	Caucasian	Gen. erythema, atopic dermatitis	+				+	+		3	[22]
142	c.2212+1 G>A	12 years	AR	ILC, E		+	+	+	+	+	Deh	4	[1]
143	c.2469insA	2 years		Mild SE			+	+	+		Deh, ↑Na	3	[37]
144_1	Complete gene deletion	9 years	Asian									4	[41]
144_2	Complete gene deletion	12 years	Asian									4	[41]

Deh dehydration, *E* eczema-like rashes, *FTT* failure to thrive, *IgE* immunoglobulin E, *ILC* ichthyosis linearis circumflexa, *NPA* no phenotype available, *P* pruritus, *SE* scaly erythroderma, *TI* trichorrhexis invaginata, *↑Na* hypernatremia

* p.D106N: in the respective paper this mutation is referred as p.D106X

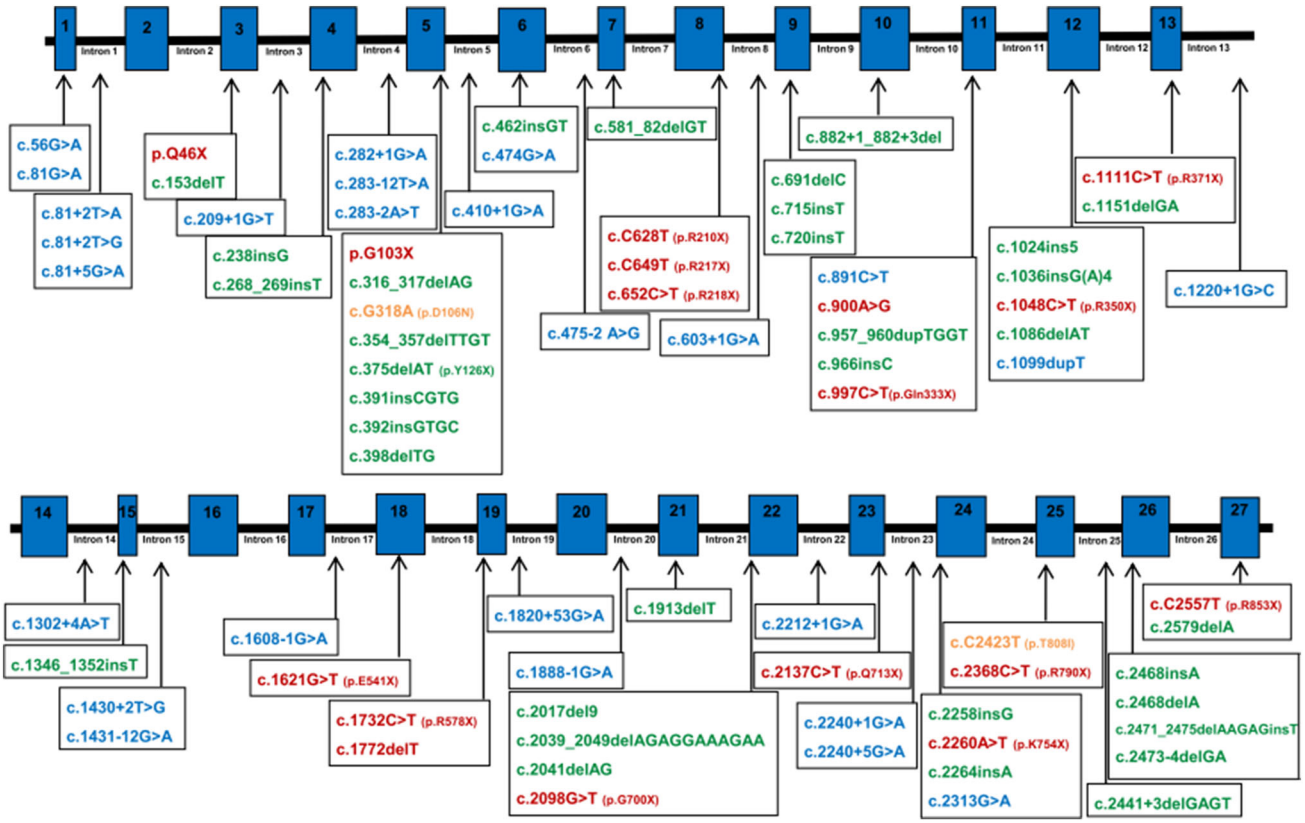

Fig. 2 The genomic organization and localization of the mutations in the *SPINK5* gene found in Netherton syndrome patients. Schematic representation of the exon–intron structure (introns are not in scale). The last exons/introns of the gene are not shown because mutations have not been reported there. *Coloring* represents the functional category of the mutations, i.e., *blue* indicates splicing, *green* indicates indel, *red* indicates nonsense, *orange* indicates missense

patients 125 and 137 in heterozygous patients: in patient 125, it was found in combination with c.2468delA, while in patient 137 a second mutation could not be identified. p.T808I has been reported once in heterozygosity with p.R790X (patient 120). Based on pathogenicity prediction by Polyphen-2, these mutations are considered to be rather benign substitutions. These patients had relatively low severity scores (2 and 3), which one could say is in agreement with Polyphen-2 prediction.

At this point, it is necessary to consider another important parameter during the evaluation of the clinical phenotype of the patients: the quality of the healthcare system in the patient's country. Developed countries have a higher quality level of healthcare system than developing or underdeveloped ones. As a result, patients in developing or underdeveloped countries may be at high risk of infections or poor treatment. This is mostly mirrored in the origin of the deceased patients. Nine mutations have been observed with lethal outcomes (c.153delT, c.238insG, c.375_376delAT, c.C649T, c.997C>T, c.1111C>T, c1431–12G>A, c.715insT, and 375delAT) in patients who originated from Turkey, Puerto Rico, Iran, Japan, North Europe, and Bosnia (additionally, two patients were Romanies). These mutations correspond to 23 patients (24 patients were homozygotes and one patient was heterozygous for c.715insT and c.375delAT). Mutations c.238insG and c.1431–12G>A were found to be homozygous in patients from different countries. Concerning c.238insG, two patients who originated from Turkey died in infancy. In contrast, two patients who originated from the USA and Iran presented with severe skin manifestations in combination with failure to thrive but did not die; in fact, at the time of publication of their cases, they were 8 and 3 years old, respectively. Similarly, two Romani patients carrying c.1431–12G>A died while two patients from Bosnia scored 3 (3 years old) and 4 (7 years old) on our severity scale. It is encouraging to note that if patients survive their first year of life, they have good chance of achieving a milder disease severity or even partial recovery. No deaths have been recorded in patients older than 9 months old.

The mutations identified so far are mostly distributed in Europe and Asia. However, three mutations are found with increased frequency in patients who have originated from specific regions. Mutation c.153delT shows evidence of a founder effect in Turkey, based on haplotype analysis [35]. This mutation appears in eight Turkish families and has

also been identified in two compound heterozygous patients from Kosovo and Sicily. Similarly, c.392insGTGC was found in two patients from the Dominican Republic (unrelated families) both homozygous and in two African-American patients, both heterozygous. Based on haplotype analysis, it seems that c.392insGTGC is common among West Indies islanders. Finally, haplotype analysis in six families of Finnish origin carrying the c.652C>T (p.R218X) supports a founder effect in these families.

The molecular consequences of 11 different mutations on protein production, mRNA levels, and splicing pattern have been studied so far through Western blot, Northern blot, and reverse transcription–polymerase chain reaction (RT-PCR), respectively. Mutation c.715insT results in the absence of detectable LEKTI, as has been shown through Western blotting (patient 33, homozygous) [46]. Along the same lines, the c.2471_2475delAAGAGinsT mutation also inhibits LEKTI production, as it cannot be detected by Western blot in the patient 128 [61]. However, this patient is referred to as homozygous [61] and also as compound heterozygous (together with the c.2049delAGAGGAAAGAA mutation) [77]. Thus, a safe conclusion can be made only for the c.2471_2475delAAGAGinsT mutation. Mutation c.316delAG results in reduced SPINK5 expression, based on RT-PCR results in one heterozygous patient [65]. However, since no second mutation was identified in this patient, these results should be considered with caution. Splicing mutations c.283–12T/A and c.1820+53G/A cause the maintenance of the last 10 nt of intron 4 and of the first 54 nt of intron 19, respectively, in a proportion of the mature transcripts, as has been shown by RT-PCR [61]. However, the normal transcripts are possibly translated in wild-type LEKTI. Concerning the mutations found only in a heterozygous state, we can draw conclusions only for those that are found in compound heterozygosity in combination with mutations that have been studied in homozygosity and their consequences at the molecular level are known. It has been shown by Northern analysis in homozygous patients that mutation c.153delT reduces mRNA transcript levels by at least 27% [4, 34]. However, this mutation produces PTC in domain I of LEKTI, so no functional protein can be produced. Immunostaining in a homozygous patient showed no LEKTI signal [37]. Based on this, we can conclude the result of 891C>T mutation through a compound heterozygote, patient 90 (c.153delT/c.891C>T). c.891C>T has been shown by RT-PCR to cause skipping of exon 11 and PTC after 4 aa of exon 12, producing a truncated protein that contains domains 1–4, which can be identified by Western blot analysis [62]. However, the exon skipping is not complete (studied by RT-PCR), and a reduced amount of wild-type LEKTI is produced [62]. Mutation c.474G>A (p.Gln158Gln) (patient 83) also causes exon skipping (exon 6) as well as the production of other transcripts with parts of intron 6. However, in this case the exon skipping was complete (showed by RT-PCR) [66]. Interestingly, one of the mutant transcripts (in frame insertion of 108 bp) produced a protein that was expressed at a similar level to LEKTI (immunostaining results) [66]. Patient 83 also carried c.1732C>T (p.R578X), producing a truncated protein. However, no reduction of the transcript expression was observed (RT-PCR results) [66]. The mutation c.2468insA abolishes LEKTI expression as has been shown by immunostaining in the epidermis of a homozygous patient [36] and two heterozygous patients [37] and by Western blot in a compound heterozygous patient (c.2468insA combined with c.1432–13G/A) [46]. From the latter patient we can deduce that the c.1432–13G>A mutation also abolishes LEKTI expression, which is also confirmed by Western blotting [46] and immunostaining [51] in two homozygous patients (40_1, 40_2 and 39_1, respectively). Patient 80 is heterozygous for c.283−2A>T (combined with c.2468insA); due to the absence of LEKTI signal in his epidermis we can speculate that c.283−2A>T also abolishes LEKTI expression [37]. Several mutations seem to cause loss of LEKTI expression, although this is based only on immunostaining of LEKTI in the epidermis of six patients [37]. Two patients are homozygous for c.268_269insT and c.2313G>A, three are genotyped as c.238insG/p.Arg217X and c.2041delAG/p.Arg371X, and in three patients only one mutation has been identified in heterozygosity (p.Arg217X/nd and c.238insG/nd) [37]. Although immunostaining can give us a hint of the molecular consequences of the abovementioned mutations, further studies using more sensitive techniques are necessary in order to elucidate whether expression of LEKTI is significantly decreased or completely abolished.

4 Conclusions

Mutations that are located more upstream in LEKTI will produce a more severe phenotype than similar mutations located towards the 3′ region. The fact that mutations have not been identified in the 3′ region of the *SPINK5* gene (downstream of exon 27) could imply that these mutations remain undetected because they don't have a dramatic effect on LEKTI function. Mutations affecting the splicing pattern could be associated with a severe or a mild phenotype, which possibly depends on the proportion of normally spliced transcripts that are produced. The presence of normally spliced transcripts could conceivably be correlated with a milder phenotype. The post-transcriptional mechanism of nonsense-mediated mRNA decay (NMD) results in a decreased abundance of mRNAs that contain PTCs. This observation suggests that different PTC

mutations in *SPINK5* have variable effects on mRNA stability. Patients 133–143 are heterozygotes with different severity scores; no second mutation has been identified (Table 3). However, this could be due to hidden mutations, deeper in introns, in regions that have not been screened or, although there are no indications of this, an indication for mutations that result in the gain of function and are sufficient to cause the pathological phenotype. Additionally, several patients have a more severe phenotype than expected based on their genotype. This paradox could be explained if aberrant translation products are created that escape NMD and act in a dominant negative fashion, thus leading to the appearance of Netherton syndrome. NMD can act in either a beneficial or detrimental way; the latter if it prevents the production of proteins with some residual function and the former if it prevents the synthesis of toxic truncated proteins.

In this review of the mutations in *SPINK5* found in patients with Netherton syndrome, and their consequences at the molecular level we hoped to explain the wide variety of clinical phenotypes and to shed light on the gaps in our knowledge regarding the majority of the syndrome's mutations. Study of the functional impact of each mutation on LEKTI, and by extension on patients' phenotype, is crucial for prenatal diagnosis in cases of a family history of Netherton syndrome and when consanguinity is implied.

Compliance with Ethical Standards

Conflict of interest Authors C. A. Sarri, A. Roussaki-Schulze, Y. Vasilopoulos, E. Zafiriou, A. Patsatsi, C. Stamatis, P. Gidarokosta, D. Sotiriadis, T. Sarafidou, and Z. Mamuris declare that they have no conflicts of interest.

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