

Molecular Analysis of a Series of Israeli Families with Comèl-Netherton Syndrome

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Key Words

Comèl-Netherton syndrome · Ichthyosis · Hair · Linkage analysis · Serine proteinase inhibitors

Abstract

Background: Comèl-Netherton syndrome is a rare congenital autosomal recessive disorder characterized by congenital ichthyosis, hair shaft abnormalities and atopic diathesis. It is caused by mutations in *SPINK5*, which encodes the serine protease inhibitor LEKTI. **Objectives:** To delineate the spectrum of mutations carried by a series of Israeli patients in an attempt to establish an effective diagnostic strategy for this disease in Israel. **Methods:** Mutations were identified by direct sequencing of the entire coding sequence of *SPINK5* and confirmed using polymerase chain reaction-restriction fragment length polymorphism. **Results:** Three mutations were identified in seven families, of which two were novel. All mutations were predicted to result in premature termination of protein translation. **Conclusions:** This report presents the first case series of patients affected with Comèl-Netherton syndrome in Israel and suggests that some mutations reoccur in a substantial portion of cases in our country, a fact that should be taken into consideration when designing molecular analysis in new cases.

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Introduction

Comèl-Netherton syndrome (NTS; OMIM 256500) is a rare congenital autosomal recessive disorder described by Comèl in 1949 [1] and by Netherton in 1958 [2]. The disorder manifests at birth or soon after and is characterized by a clinical triad which includes localized or generalized ichthyosis, hair shaft abnormalities and atopic diathesis [1–4].

Cutaneous manifestations of the syndrome (fig. 1) include varying phenotypes ranging from ichthyosis linearis circumflexa (ILC), referring to migratory, serpiginous, erythematous plaques bordered by a double-edged scale [3, 5], up to life-threatening erythrodermic ichthyosis, which can be confused with inflammatory generalized peeling skin syndrome (OMIM 270300) or nonbullous congenital ichthyosiform erythroderma (OMIM 242100). Pruritus and secondary skin infections are common [3]. Hair abnormalities include trichorrhexis invaginata (bamboo hair), which is pathognomonic for the syndrome, as well as other hair anomalies such as trichorrhexis nodosa, pili torti and alopecia. A decrease in the conversion of cysteine to cystine leading to reduced cross-linking of hair keratins may be responsible for focal softening of the hair shafts and trichorrhexis invaginata [6].

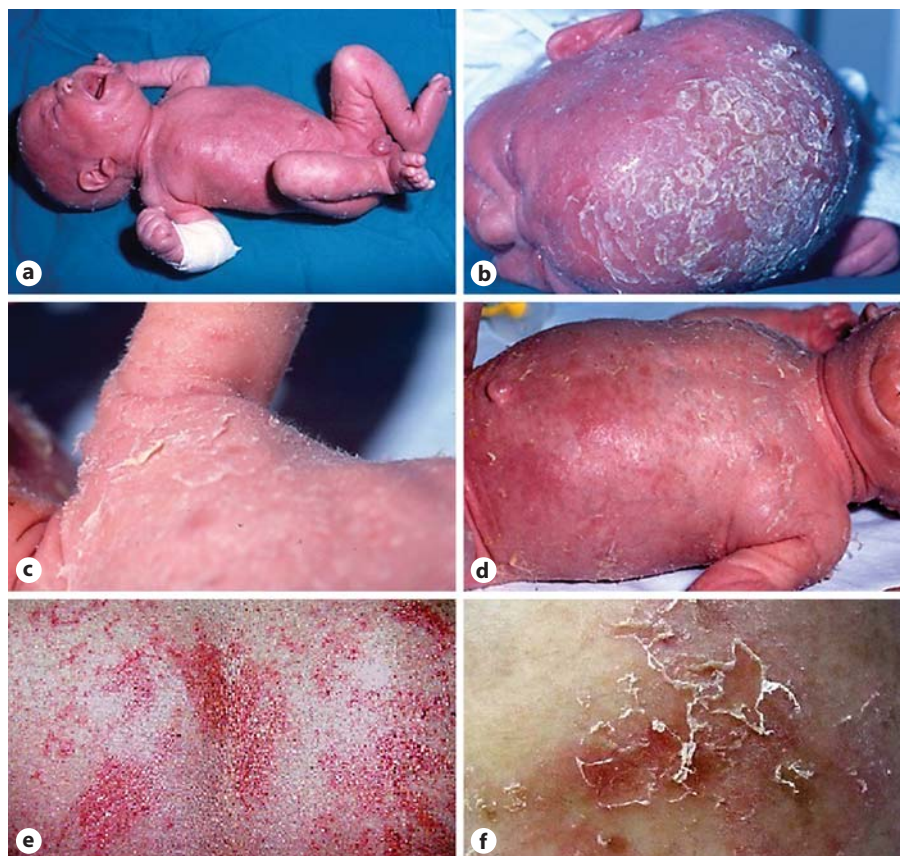


Fig. 1. Cutaneous manifestations of NTS in several patients. **a–d** Erythrodermic ichthyosis in patient No. 1 with severe skin involvement in the presence of p.Arg217stop (homozygous state), with involvement of the scalp with scaling plaques (**b**) and seriginous, erythematous plaques bordered by a double-edged scale (ILC) (**c, d**). **e** Eczematous plaques over the back in patient No. 2 with moderate skin involvement in the presence of p.Arg217stop (non-homozygous state). **f** ILC without erythroderma in patient No. 7 with c.2240+5G>A mutation.

Hair abnormalities and ichthyosis linearis circumscripta can manifest later in childhood, which may complicate the diagnosis [4]. Allergic and immunologic abnormalities are also typical of the disease, including atopic disorders, elevated IgE levels, multiple food allergies and recurrent infections [1–4].

In about 20% of patients the syndrome may present during the neonatal period with life-threatening complications such as electrolyte imbalance, hypernatremic dehydration, inability to regulate body temperature, failure to thrive and sepsis [3–5, 7, 8]. These complications are due to the loss of the epidermal barrier function [9]. Indeed, skin biopsies of patients demonstrate impaired maturation of lamellar membrane structures and premature secretion of lamellar body contents, leading to disorganization of the epidermal barrier [9].

At the molecular level, the syndrome is caused by mutations in *SPINK5* (serine protease inhibitor Kazal-type 5), a 61 kb-large gene comprising 33 exons, located on chromosome 5q32. *SPINK5* encodes the serine protease inhibitor LEKTI (lympho-epithelial Kazal-type related

inhibitor) [3, 10–12] whose function is essential to maintain the integrity of the epidermal barrier as demonstrated in a mouse model [13].

As the Israeli population is composed of numerous closed and small ethnic subgroups [14], recurrent population-specific mutations are often found to underlie monogenic disorders in this country. In fact, even common recessive diseases are characterized by specific mutation patterns. For example, the frequency of the common *CFTR* $\Delta F508$ mutation causing cystic fibrosis is about three times lower in the Jewish and Arab populations in Israel in comparison to Western populations [15, 16]. In contrast, some mutations in this gene are almost exclusively encountered in Middle Eastern populations [17]. These data imply that mutational search strategies and mutational databases developed in European and/or US populations may not be useful in genetically homogeneous populations such as those found in the Middle East region, which have over the years generated their own pool of specific genetic alterations. Over the past years, we have obtained evidence supporting this concept in skin

Table 1. Mutations and clinical features of NTS

Pa- tient	Ethnic origin	Age ^a	Mutation	Effect	Exon	First reported	Skin involvement				Hair shaft abnormalities		Associated features				
							1	2	3	4	1 mild	2 severe	postnatal hyper- natremic dehydration	failure to thrive	growth retardation/ enteropathy	severe recurrent infections	allergic reactions (food/ pollen)
1 ^b	Northern European	4 m	c.C649T/ c.C649T	R217stop	8	Bitoun et al. [12]			3		1		1	1	1 (~3 SD)	1	0
2	Northern European	20 y	c.C649T/ c.691delC	R217stop + Q231KfsX1	8+9			2			1		1	1	1 (~2 SD)	1	0
3	Georgia	6.5 y	c.C2557T/ c.C2557T	R853stop	27	here		2			1		0	0	0 (within SD)	0	1
4	Georgia	19 y	c.C2557T/ c.C2557T	R853stop	27	here		2			1		0	0	0 (within SD)	1	1
5	Georgia	10 m	c.C649T/ c.C2557T	R217stop + R853stop	8+27				3		1		1	1	1 (~2 SD)	0	1
6	Iraq	15 y	c.691delC/ c.691delC	Q231KfsX1	9	Sprecher et al. [21]		2				2	0	0	0 (within SD)	1	0
7	Azerbaijan	34 y	2240+5G>A/ 2240+5G>A	altered splicing	23	here		2			1		0	0	0 (within SD)	0	0

m = Months; y = years; SD = standard deviation.

Skin involvement: 1 = ILC or eczema of >30% of body surface and never erythroderma; 2 = ILC or eczema of >30% of body surface and occasionally erythroderma; 3 = mainly erythroderma and occasionally ILC or eczema; 4 = lifelong erythroderma.

Hair shaft abnormalities: trichorrhexis invaginata, pili torti, trichorrhexis nodosa.

^a Age at diagnosis. ^b Deceased.

disorders such as different forms of inherited epidermolysis bullosa [18–20].

The aim of the present study was therefore to delineate the molecular basis of all Israeli NTS patients referred to our laboratory over the past 12 years in an attempt to derive from these data a population-specific diagnostic algorithm.

Patients and Methods

Patients

We obtained written informed consent from all participants according to a protocol approved by the local Helsinki Committee and by the Committee for Genetic Studies of the Israeli Ministry of Health. The clinical diagnosis of NTS was based on the presence of three clinical manifestations: congenital ichthyosiform erythroderma-like ichthyosis or ILC, hair shaft abnormalities and immunologic abnormalities including elevated IgE levels, and/or history of recurrent bacterial infections, neonatal hypernatremic dehydration, allergies and failure to thrive. In order to compare the severity of the disease between the patients, we developed a severity score (table 1) based on the following criteria: skin involvement (ILC or eczema of >30% of body surface and never erythroder-

ma = 1, ILC or eczema of >30% of body surface and occasionally erythroderma = 2, mainly erythroderma and occasionally ILC or eczema = 3, lifelong erythroderma = 4); hair shaft abnormalities, such as trichorrhexis invaginata, pili torti, trichorrhexis nodosa or alopecia (none = 0, mild = 1, severe = 2); associated features including postnatal hypernatremic dehydration, failure to thrive, growth retardation, enteropathy and allergies (absent = 0, present = 1). DNA was extracted from peripheral blood samples following standard procedures.

Mutation Detection

Genomic DNA was amplified by polymerase chain reaction using primer pairs spanning the entire coding sequence and the intron-exon boundaries of the *SPINK5* gene as previously described [10]. Gel-purified DNA fragments were submitted to direct sequencing using the BigDye terminator system on an automated sequencer (ABI PRISM 3100 Genetic Analyzer; Applied Biosystems, Foster City, Calif., USA).

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism Analysis

The mutation was verified by polymerase chain reaction-restriction fragment length polymorphism analysis. For verification of the mutation c.C2557T, a 332 bp-long DNA fragment was amplified using forward primer 5'-TCT GTT TTT TTC CTG TGT TAT GAG T-3' and reverse primer 5'-GTG TGA TGC CAA GTA

TCT TAG G-3'. The mutation results in the loss of a recognition site for DNA endonucleases *BstBI*. To verify the mutation, genomic DNA from the patient and 50 controls (from the same ethnic background) were amplified by polymerase chain reaction using the same pair of primers. The resulting fragments were digested with *BstBI* endonucleases for 6 h, separated on 3% agarose gel and visualized with ethidium bromide. To verify mutation c.2240+5G>A, genomic DNA from the patient and 58 controls (from the same ethnic background) was amplified by polymerase chain reaction and the resulting fragments were digested with *SspI* endonuclease, separated on 3% agarose gel and visualized with ethidium bromide.

Results

Over a period of 12 years, seven NTS patients from seven different families were referred. Patients' mean age was 13.67 years at the time of diagnosis (range 4 months to 34 years). All patients were of Jewish descent. Two patients were of Northern European descent, three patients were of Georgian extraction, one patient originated from Azerbaijan and one patient was of Iraqi origin. Skin involvement was moderate to severe (score 2–3) in all patients, but most of them had only mild hair changes (table 1).

A total of four mutations were identified in our series: c.C649T resulting in p.Arg217stop, c.691delC resulting in frameshift (p.Q231KfsX1), c.C2557T leading to p.R853stop, and c.2240+5G>A resulting in aberrant splicing. p.Arg217stop and p.Q231KfsX1 have previously been reported [12, 21]; in contrast, the other two mutations have not been previously described (table 1). Segregation of the new mutations with the disease phenotype was confirmed in each family and the mutations were excluded from a panel of 50 population-matched controls.

Out of the seven families, we found that all patients who presented with postnatal hypernatremic dehydration, failure to thrive, growth retardation, enteropathy but without allergies carried mutation p.Arg217stop. These patients were of Northern European origin. In contrast, patients that presented with allergies and without postnatal hypernatremic dehydration, failure to thrive, growth retardation or enteropathy carried mutation p.Arg853stop and originated from Georgia. Of interest, one patient of Georgian origin was a compound heterozygous carrier of both mutations and displayed a clinical phenotype typical of both mutations.

As mutation distribution across ethnic origin was highly suggestive of a founder effect in the Georgian and Northern European families, we established haplotypes in each family using intragenic single nucleotide poly-

morphisms (not shown). Unfortunately, the haplotypes were not informative enough to draw unequivocal conclusions.

Discussion

NTS has been reported in many populations worldwide. The diagnosis of the syndrome relies heavily upon clinical criteria and can be confirmed using either immunohistochemistry for LEKTI, which has been shown to be highly sensitive [22], or molecular confirmation. *SPINK5*, the NTS-associated gene, encodes LEKTI, a protein of 1,064 amino acids that contains 15 inhibitory domains (D1–D15), two of which (D2 and D15) match the classical Kazal-type pattern [3, 12, 15].

As in our series, NTS-causing mutations in *SPINK5* have been shown to result in loss of function of LEKTI, which functions in the epidermis as a potent serine threonine protease inhibitor. Accordingly, increased proteolytic activity within the upper epidermal layers is considered to be the cause of cutaneous manifestations in NTS [3]. Not only are proteases important for maintaining central homeostatic processes with the epidermis, such as desquamation and filaggrin processing [23], they seem to also play a role in other tissues including the immune system and the gut, which explains the high incidence of infectious and gastrointestinal complications typical of NTS [24]. LEKTI is expressed also in the thymus and mucous epithelia. The abnormal expression of LEKTI influences T cell differentiation and causes Th2 immune response, which explains the high levels of IgE [3]. In addition, loss of LEKTI function disrupts the epidermal barrier and facilitates penetrance of allergens through the skin, leading indirectly to a Th2-skewed response [12].

The considerable size of *SPINK5* and the lack of hot spot mutations in this gene are serious obstacles for the routine molecular diagnosis of NTS. Numerous presequencing screening methods have been used to circumvent this problem [3]. Population-tailored diagnostic strategies are another means to simplify the molecular analysis of NTS cases. In a previous report, Bitoun et al. [12] identified 18 mutations in the *SPINK5* gene in 21 NTS families of various geographic origins, 5 of which being related to ethnic origin.

The current study reports the result of the mutation analysis of all consecutive cases of Israeli NTS referred to our laboratory, in an attempt to identify recurrent mutations, which in turn may facilitate the molecular diagnosis of NTS in our region. Based on our data, we recom-

mend that the molecular analysis of Israeli cases of NTS should start with the direct sequencing of exons 8, 9 and 27 in *SPINK5*.

The availability of molecular data for a series of patients carrying recurrent mutations allowed us to assess phenotype-genotype correlations. Although no clear correlation has been defined in NTS, some investigators emphasize the predictive value of mutation analysis for disease clinical course [25]. In our series, we found a strong correlation between the presence of p.Arg217stop in patients of Northern European origin and postnatal hypernatremia, dehydration, failure to thrive, growth retardation and enteropathy with absence of food allergies. The skin involvement and growth retardation were more severe in the presence of p.Arg217stop, being most severe in the homozygous state (patient No. 1). In contrast, p.Arg853stop was predictive in affected individuals of Georgian origin with allergies and without postnatal hypernatremic dehydration, failure to thrive, growth retardation or enteropathy. Skin involvement was less severe in the presence of p.Arg853stop in the homozygous state (patients No. 3, 4) as compared to the presence of p.Arg217stop in the homozygous state. The patient who was a compound heterozygous carrier of both mutations (patient No. 5) had characteristics of both mutations and suffered from allergies as well as growth retardation and more severe skin involvement (table 1).

Since the two prevalent mutations in our study were carried in a population-specific fashion, the phenotype-genotype correlations observed could point either to a direct role of the causative mutation in determining the disease manifestations or to the co-inheritance of population-associated modifier traits. It is important to note that phenotype-genotype correlation does not mean necessarily whether certain features exist or not.

According to the location of stop codon mutations, each patient can generate a different number of LEKTI domains (in other words, different length of truncated LEKTI) until the stop codon. Hence genotype, that is how diverse LEKTI domains functions remain, might be correlated with the severity of each manifestation. However, the overwhelming majority of nonsense or frameshift mutations reported to date in *SPINK5*, whose effect has been studied at the RNA level, were shown to result in nonsense-mediated RNA decay [12]. In that sense, we do not think that in our study the association of different mutations with specific clinical features may be directly related to the number of functional LEKTI subdomains, but may be due to population-related modifier traits.

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