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CASE REPORT

IS c.1431-12G>A A COMMON EUROPEAN MUTATION OF *SPINK5?* REPORT OF A PATIENT WITH NETHERTON SYNDROME

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ABSTRACT

Netherton Syndrome (NS) is a very rare genetic skin disease resulting from defects in the *SPINK5* gene (encoding the protease inhibitor lympho-epithelial Kazal type inhibitor 1, LEKTI1). In this report, we provide a detailed clinical description of a Polish patient with two *SPINK5* mutations, the novel c.1816_1820+21delinsCT and possibly recurrent c.1431-12G>A. A detailed pathogenesis of Netherton Syndrome, on the basis of literature review, is discussed in the view of current knowledge about the LEKT1 molecular processing and activity.

Keywords: Mutation; Netherton Syndrome (NS); *SPINK5* gene.

INTRODUCTION

Netherton Syndrome (NS) (OMIM 256500) is a rare genetic skin disease with a prevalence of one in 200,000 live births, resulting from defects in the *SPINK5* gene, encoding the protease inhibitor lympho-epithelial Kazal type inhibitor 1 (LEKTI1). As a result of LEKTI deficiency, the skin becomes red and scaly. The epidermal barrier function is also affected; dryness of the skin results from increased permeability of the skin, and its decreased capacity to bind water [2]. Recurrent infections, IgG abnormalities, enteropathy, elevated IgE levels, and hypo-albuminemia are other common features of the disease [3]. Chronic skin inflammation results in scaling and exfoliation, predisposing these patients to life-threatening infections, sepsis, hypernatremic dehydration or pneumonia. Disease manifestations vary considerably among NS individuals [4]. Many children suffer from a failure to thrive in the first year of life and most of them remain underweight and have short stature. Other reported features include intellectual disability (15.0% of cases), seizures (<10.0%), recurrent infections or IgG abnormalities (15.0%) and aminoaciduria (25.0%) [5]. The reasons of phenotypic diversity in NS are complex and not fully understood. Herein, we present a case of NS in Polish male infant with two heterozygous *SPINK5* mutations: c.1431-12G>A and the novel c.1816_1820+21delinsCT.

CASE REPORT

A boy [weight 2750 g, length 49 cm, occipital-frontal circumference (OFC) 30 cm] was born prematurely at the 37th week of gestation to a 43-year-old mother by cesarean section due to the transverse position. A skin examination of the proband, conducted shortly after birth, revealed generalized erythroderma covered by fine, translucent scales all over the body and scalp (Figure 1). The eyebrows and eyelashes were thin and sparse, nails with normal conformation and shape. Moreover, hypotonia was observed. An ultrasonography examination did not detect any defect of the internal organs. Starting from birth, the infant has had ongoing problems with hypernatremic dehydration.

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Soon after birth, the skin infections caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa* were diagnosed. The boy then developed symptoms of sepsis [maximum C-reactive protein (CRP) levels of >400 mg/L] and meningitis was diagnosed. The baby developed respiratory failure that was treated with mechanical ventilation. Chronic diarrhea as an enteropathy symptom was revealed. Moreover, in the course of general infection intensifying skin lesions were observed. The immunological examination showed a decreased concentration of IgG (4.59 g/L) and C3 (0.76 g/L) components. The levels of IgG and C3 were taken 3 weeks after birth, which was 1 week prior to sepsis. Tricuspid and pulmonary valve insufficiency was identified in the echocardiography and the abnormalities of intraventricular repolarization and conduction were present on the electrocardiogram (ECG). At the age of 7 months, the child was diagnosed with bilateral hypoacusia.

The boy was admitted to the Department of Genetics, Wroclaw Medical University, Wroclaw, Poland with the suspicion of NS at the age of 8 months. The physical examination showed facial dysmorphic features resulting from ichthyosis, sparse hair with abnormal hair shafts and alopecia (Figures 2 and 3). His psychomotor development was delayed. Completing the medical history, the child's mother revealed the absence of hair on the scalp, the absence of eyebrows and eyelashes as well as axillary and pubic hair from her early childhood. After the child's delivery, mother's skin was clean, smooth, and no keratinizing change was observed. Written informed consent was obtained from the patient for publication of this case study and any accompanying images.

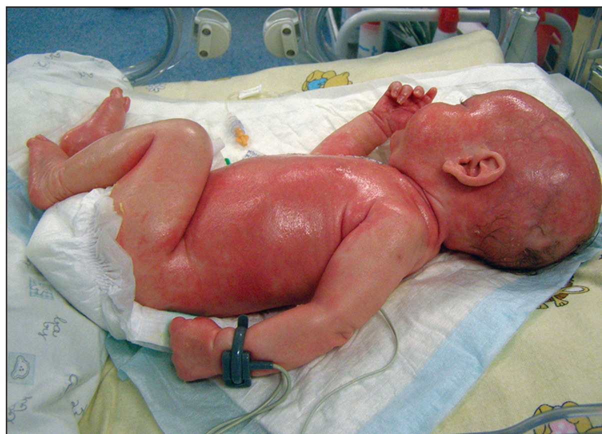

Figure 1. Generalized erythroderma covered by fine, translucent scales all over the body and scalp.

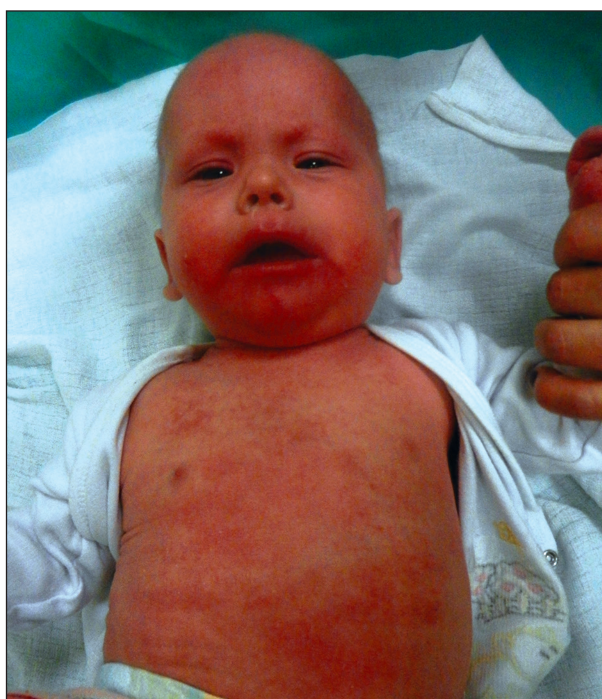

Figure 2. Facial dysmorphic features resulting from ichthyosis.

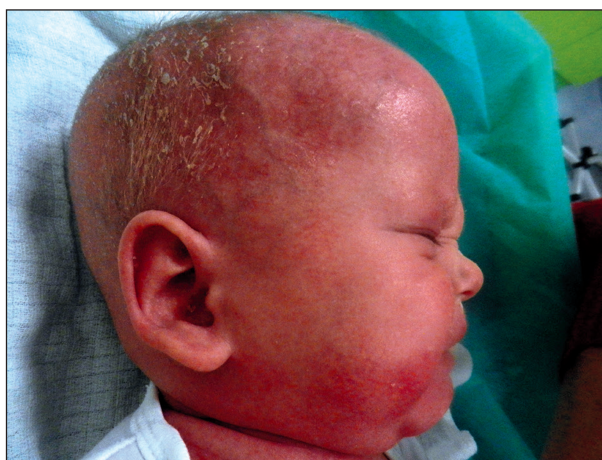

Figure 3. Sparse hair with abnormal hair shafts and alopecia.

GENETIC INVESTIGATION

Molecular analysis of all coding exons of the *SPINK5* gene (ref. NM_001127698.1) using Sanger sequencing revealed the following mutations: c.1816_1820+21 delinsCT (of maternal origin) and c.1431-12G>A. The origin of the latter mutation has not been established, as the father's DNA was unavailable, but it was absent in the mother, which indicated heterozygosity. The effect of both mutations on RNA and protein level was predicted using ALAMUT bioinformatics package (http://www.inter active-biosoftware.com/). The following prediction algorithms were used: SpliceSiteFinder-like, MaxEntScan, NNSPILICE, GeneSplicer, HumanSplicing Finder (http:// www. umd.be.HSF/), which employ various calculation methods, *i.e.,* position weight matrices, Maximum Entropy principle, neural networks and Markov models. The c.1816_1820+21delinsCT was uniformly evaluated as a mutation that removes natural

donor splicing site at c.1820. In the case of the c.1431-12G>A mutation, all algorithms indicated that a putative novel acceptor splicing site was generated in intron 15, 10 nucleotides before exon 16, while SpliceSiteFinder, MaxEntScan, NNSPILICE also showed that the introduction of c.1431-12G>A reduces the strength of the natural splice site at c.1431. Skin biopsy expression studies were not performed, due to the disagreement of the proband's mother, however, our results confirmed NS in the proband.

DISCUSSION

LEKTI1 is expressed in epithelial, thymus, tonsils, parathyroid glands, hair follicles and the trachea [3], but the vast majority of data concerning its biology and functions comes from epithelium research. The spectrum of LEKTI1 activity as a serine protease inhibitor is wide and it has been shown that LEKTI1 is not only expressed as full-length protein (145 kDa), but also as two other isoforms (a short one: 125 kDa, and a long one: 148 kDa). Furthermore, full length isoform in addition to its own activity, undergoes several proteolytic cleavages, resulting in generation of various LEKTI1 domains with different inhibitory specificities [2]. Various LEKTI1 derived poly-peptides inhibit KLK5, KLK7 and KLK14 (kallikrein-related peptidases) and it is hypothesized that deficient inhibition of those proteins is one of the major causes of epidermal symptoms observed in NS [1,4]. Hovnanian [2] proposed that as a result of LEKTI1 deficiency, KLK5, KLK7 and ELA2 (elastase 2) are improperly activated leading to abnormal skin desquamation, development of proinflammatory response together with abnormalities of lipids and improper profilaggrin processing. This mechanism triggers further actions, which, together with higher penetration of microbes and allergens, aggravate proin-flammatory response leading to development of NS symptoms [2,6,7].

According to Human Gene Mutation Database (HGMD) data, 72 distinct mutations were reported to cause NS, of which only two are missenses and others result in premature termination codon (PTC), frameshifts or splicing disruptions [8]. Similarly, the mutations identified in our patient are predicted to affect splicing. According to bioinformatics analyzes, the c.1431-12G>A introduces novel acceptor splicing site 10 nucleotides upstream of exon 16, while unreported before c.1816_1820+21delinsCT abolishes donor splicing site in intron 19, but also leads to a frameshift. The c.1431-12G>A mutation was reported by Raghunath *et al.* [9], who found it in two independent patients originating from Bosnia (homozygote) and Austria (heterozygote). Both parents of the Bosnian child were carriers of this mutation and were reported not to be consanguineous. Raghunath *et al.* [9] did not detect the LEKTI1 protein by immunohistochemical staining of skin biopsies and/or immunoblot analysis of plucked hair roots from these patients. These results and our data indicate that the majority of SPINK5 mRNA is truncated in our patient. Importantly, they do not rule out the possibility that small amount of full length mRNA may also be produced. According to Lacroix *et al.* [10], some deep intronic mutations activating cryptic splicing sites, may not completely abolish the natural splicing sites, allowing low level production of full length protein. This phenomena was observed in the case of mutations c.1820+ 53G>A and c.283-12T>A, and recognized to possibly be linked with a milder phenotype. This was also supported by analysis of residual expression of LEKTI1 in cultures of NS patients' (genotyped as c.1820+53G>A/p.Cys297 Cys and c.283-12T>A/p.Cys297Cys) keratinocytes, which proved that LEKTI1 fragments could be detected. The p.Cys297Cys is an exonic silent variant that causes disruption of exonic splicing enhancers and also introduces a cryptic splicing site. Although Lacroix *et al.* [10] hypothesized that p.Cys297Cys is fully penetrant, Fortugno *et al.* [11] showed that p.Cys297Cys also does not cause complete abolishing of natural splice sites and partial expression of LEKTI1 is preserved [11].

Moreover, Bitoun *et al.* [12] suggest that certain PTCs in *SPINK5* do not lead to mRNA degradation and may be used for truncated protein production. Primary structure of full length LEKTI1 consists of 15 different serine proteases inhibitory domains (D1-D15). As stated before, several isoforms of LEKTI1 with distinct inhibitory activities are expressed and/or subsequently generated. Thus, if truncated proteins (due to PTC) are produced, some domains would potentially be generated and functional. Komatsu *et al.* [13], despite unsuccessful attempts to identify truncated proteins in patients' tissues, performed a series of experiments aimed at differentiation of the profile of inhibitory activity of LEKTI1 in five NS patients. They showed that there is an adverse correlation between the number of retained LEKTI1 domains and clinical severity of NS. The more domains preserved, the less severe clinical features such as skin lesions, growth retardation, skin infections were noticed. Results of some biochemical tests also supported these findings. With regard to this theory, our patient may hypothetically have 1-7 and 1-9 domains retained. Although it is impossible to speculate about the potential effect of their preservation, the Komatsu *et al.* [13] results of an *in vitro* assay with recombinant polypeptides containing different LEKTI1 domains, suggest that D6-D9 seem to be responsible for dose-dependent inhibition of trypsin, such as Phe-Ser-Arg, and chymotrypsin-like activity.

Despite a growing number of findings explaining the molecular basis of phenotypic differences in NS patients, we still do not understand them. The LEKTI1 is involved in interaction with several proteins that play an important role in epidermal biology. Although the NS phenotype seems to be at least partially dependent on modifier genes and environmental factors [2], the hypothesis concerning preserved activity of LEKTI1 cannot be neglected. Correlation studies need to be performed on a larger cohort of patients that is always difficult in the case of orphan diseases. Thus, we are convinced that the case presented by us will provide further data for future studies. Furthermore, our patient is the third proband reported to carry the c.1431-12G>A mutation, thus our results provide evidence that c.1431-12G>A can be referred to as a frequent central-south European mutation of *SPINK5*. The majority of mutations in this gene is family-specific, however, several recurrent mutations have also been identified in different populations. With only a few exceptions, these mutations, similar to c.1431-12G>A, were mostly identified in two, three or four unrelated families [10,12,14,15].

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Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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