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Netherton Syndrome in One Chinese Adult with a Novel Mutation in the SPINK5 Gene and Immunohistochemical Studies of LEKTI

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Abstract

Background:

Netherton syndrome (NS) is a severe autosomal recessive ichthyosis. It is characterized by congenital ichthyosiform erythroderma, trichorrhexis invaginata, ichthyosis linearis circumflexa, atopic diathesis, and frequent bacterial infections. The disease is caused by mutations in the SPINK5 (serine protease inhibitor Kazal-type 5) gene, a new type of serine protease inhibitor involved in the regulation of skin barrier formation and immunity. We report one Chinese adult with NS. The patient had typical manifestation of NS except for trichorrhexis invaginata with an atopic diathesis and recurrent staphylococcal infections since birth.

Aims:

To evaluate the gene mutation and of its product activity of SPINK5 gene in confirmation of the diagnosis of one Chinese adult with NS.

Materials and Methods:

To screen mutations in the SPINK5 gene, 33 exons and flanking intron boundaries of SPINK5 were amplified with polymerase chain reaction (PCR) and used for direct sequencing. In addition, immunohistochemical staining of LEKTI (lymphoepithelial Kazal-type-related inhibitor) with specific antibody was used to confirm the diagnosis of NS. The results were compared with that of healthy individuals (twenty-five blood samples).

Results:

A G318A mutation was found at exon 5 of patient's SPINK5 gene which is a novel missense mutation. The PCR amplification products with mutation-specific primer were obtained only from the DNA of the patients and their mother, but not from their father and 25 healthy individuals. Immunohistochemical studies indicated there was no LEKTI expression in NS patient's skin and there was a strong LEKTI expression in the normal human skin.

Conclusion:

In this report, we describe heterozygous mutation in the SPINK5 gene and expression of LEKTI in one Chinese with NS. The results indicate that defective expression of LEKTI in the epidermis and mutations of SPINK5 gene are reliable for diagnostic feature of NS with atypical clinical symptoms.

Keywords: *Mutation, Netherton syndrome, SPINK5 gene*

Introduction

What was known?

The defective gene for NS has been cloned on chromosome 5q32 and it is termed SPINK5.

Netherton syndrome (NS; MIM 256500) is a rare severe autosomal recessive disease characterized by congenital ichthyosiform erythroderma (CIE), ichthyosis linearis circumflexa (ILC), "bamboo hair" (trichorrhexis invaginata, TI), and atopic diathesis with high serum IgE levels. Most patients with NS are typically born with a severe scaly erythroderma which can persist throughout life or vary into a milder phenotype known as ILC.[1,2] ILC is characterized with migratory, polycyclic or serpiginous patches with double-edged scaling borders. The defective gene for NS has been cloned on chromosome 5q32 and it is termed SPINK5 (serine protease inhibitor Kazal-type 5).[3] The SPINK5 gene consists of 33 exons and encodes a putative serine protease inhibitor called LEKTI (lymphoepithelial Kazal-type-related inhibitor) which harbors 15 potential inhibitory domains.[3] LEKTI is a new type of serine protease inhibitor with antitrypsin activity. It is expressed in epidermal and mucosal surfaces, tonsils, and thymus. In the epidermis, LEKTI is strongly expressed in the uppermost spinous and granular layers, and is considered to play an essential role in skin barrier formation through inhibiting activities of several proteases[4,5] and may play a role in local anti-inflammatory and/or antimicrobial effects. Chavanas *et al.*, reported pathogenic mutations of SPINK5 in NS.[3] To date, more than 40 pathogenic mutations of NS have been reported in the SPINK5 gene,[3,4,6–9] resulting in premature termination codons. Such mutations often cause extensive degradation of the SPINK5 transcripts probably due to nonsense-mediated mRNA decay[3,6] which is predicated to lead to LEKTI deficiency.

Materials and Methods

Subjects

An 18-year-old Chinese girl with scaly erythroderma came to our hospital in 2006. At her birth, she had generalized erythroderma with exfoliative scaling which accentuated in the cheeks, scalp and the flexor surface of elbows and knees. However, the generalized erythroderma disappeared gradually in five months after her birth and remained in the palmoplantar regions. The patient had profuse amount of fine, dry, flaky, branny desquamation, involving the entire scalp and ILC remained localized along with atopy. The patients didn't display characteristic hair shaft abnormalities, particularly trichorrhexis invaginata (bamboo hair) and torsion twists. The patient showed good responses to corticosteroid hormone and irritation to acitretin. Laboratory blood tests were normal except for the serum IgE levels (2176 IU/ml) and IgE antibody. No other abnormalities were observed by other physical and radiological examinations. Her parents were unaffected and there was no consanguinity.

Source of DNA

After obtaining signed informed consent from her parents and the approval of the ethics commission in Department of Guangzhou Institute of Dermatology, peripheral leukocyte DNA was prepared from peripheral blood of the patient and her parents using standard protocols. Genomic DNA was also extracted from 25 blood samples obtained from healthy individuals as controls. The study was conducted in accordance with the Declaration of Helsinki guidelines.

Analysis of the SPINK5 gene

The genomic DNA samples from the patients, their parents, and healthy individuals were then subjected to mutation screening by amplifying the segments of SPINK5 gene with PCR. All 33 exons and flanking intron boundaries of the SPINK5 gene were amplified for direct sequencing. The primers for the SPINK5 gene were designed as previously reported[6,10] and synthesized on the basis of

intronic sequences. For polymerase chain reaction (PCR) amplification, approximately 200 ng of genomic DNA, 12.8 pmol of each primer, 10 μ mol of deoxyribonucleoside, and 1.25 U of Ampli Taq Gold (Perkin Elmer, Roche Molecular Systems, Inc., Branchburg, NJ, U.S.A.) were used in a total volume of 50 μ L. PCR reaction were performed at 94°C for 5 min followed by 35 cycles of 94°C for 45 s, annealing 45 s at 60°C and 45 s at 72°C with a final extension at 72°C for 10 min. The amplified PCR fragments were run on 1.5% agarose gels. The PCR products were examined on 2% agarose gel [Figure 1] and purified by QIAquick columns (Qiagen, Chatsworth, CA, U.S.A.) followed by direct DNA sequencing with an ABI 377 automatic sequencer (Advanced Biotechnologies, Columbia, MD, U.S.A.) with both forward and reverse primers.

Immunohistochemistry

All reagents and chemicals were purchased from Sigma (Poole, UK) unless otherwise stated. Formalin-fixed paraffin-embedded skin samples from patient and normal Chinese individual were cut into 5- μ m sections and mounted on silane-coated slides. Immunohistochemistry using mouse monoclonal antibodies (Zymed laboratories, South San Francisco, CA) against N-terminal D1-D6 domains of LEKTI was performed by the following protocol described previously.[5] Tissue samples were fixed in 10% neutral buffered formalin. Tissue sample with 4- μ m were prepared from paraffin-wax-embedded tissues and their reactivity with LEKTI antibodies was studied by immunohistochemistry. Prior to immunodetection, specimens were deparaffinized, rehydrated and processed as described.[5] Antigen retrieval of dewaxed sections was performed by heat-treatment for 40 min using a water bath at 95°C in 10 mM citrate buffer (pH 6.0) containing 1% Tween 20, or using the Target Retrieval Solution (Dako, Trappes, France). Sections were immunostained for 30 min at room temperature with polyclonal-N (11 μ g/ml) or polyclonal-C (4 μ g/ml) antibodies diluted in PBS containing 0.3% BSA or with the undiluted-N monoclonal antibody (5 μ g/ml). Tissue sections were incubated with the streptavidin-biotin-peroxidase complex (ABC method) using the StrepABComplex/HRP Duet (mouse/rabbit) kit (Dako). Extensive washing with PBS containing 0.3% BSA was performed between each step. Labeling was revealed using diaminobenzidine tetrahydrochloride and hydrogen peroxide, and nuclei were counterstained with hematoxylin. Negative controls were included for each sample by omitting the primary antibody. The specificity of labeling was verified by using the corresponding pre-immune sera at the same concentrations and by competition experiments using 10-fold excess (weight) of the recombinant antigens.

Results

Genomic DNA from the patient and her parents were used for DNA sequencing of all exons and exon-intron boundaries of the SPINK5 gene. It was found that a novel mutation was heterozygous G-A transition (318 G-A) in exon 5 of the SPINK5 gene which generated a premature termination codon (D106X) [Figure 2]. The PCR amplification products with mutation-specific primer were obtained only from the DNA of the patients and their mother, but not from their father and 25 healthy individuals. The patient's mother was a heterozygote for this mutation without phenotype of NS. The sequencing analysis of the parents' DNA indicated that the 318 G-A mutant allele was inherited from her mother. Allele-specific PCR performed by using either a wild-type-specific or a mutation-specific reverse primer, and a common forward primer, was used to confirm that the mutation is not a polymorphism.

Monoclonal antibodies against the N-terminal region of LEKTI did not detect LEKTI in the skin of the patient [Figure 3a]. In contrast, a positive staining in the granular layer of the epidermis was obtained in control skin [Figure 3b].

Discussion

In this study, we identified heterozygous mutation in the SPINK5 gene of one Chinese girl affected with NS. She had generalized erythroderma, ichthyosis linearis circumflexa, and radioallergosorbent tests were positive to cow's milk and egg IgE antibodies. The patients didn't display characteristic hair shaft abnormalities, particularly trichorrhexis invaginata (bamboo hair) and torsion twists which is

different from the reported case that most patients later display characteristic hair shaft abnormalities, particularly trichorrhexis invaginata (bamboo hair) and torsion twists (pili torti).[7] In addition, the expression of bamboo hairs is often delayed.[7] Medical treatment started after the age of two years and the patient had a good response to corticosteroid hormone. But the lesions were aggravated by acitretin therapy. We screened the gene mutations in the SPINK5 gene, all 33 exons and flanking intron boundaries of SPINK5 were amplified by PCR for direct sequencing. Immunohistochemical staining of LEKTI with specific antibody was used to confirm the diagnosis of NS.

Immunological detection of LEKTI in normal skin was confined to the granular layer giving a predominantly cytoplasmic reaction occasionally mixed with a pericellular signal. Recent studies have revealed that LEKTI deficiency influenced several other molecules in the epidermis of patients with NS[11] and SPINK5-knockout mice.[12,13] It is consistent with previous reports.[5,7,11] Immunohistochemistry using anti-LEKTI antibodies showed there was no LEKTI in patient's skin sample. Our studies show the identified D106X is sense mutation. Bitoun *et al.*, reported that there was no clear correlation between mutation and phenotype in NS.[6] The protein analyses with anti-LEKTI antibodies supported their suggestion because LEKTI was not detected in most skin samples of patients with NS regardless of location of mutations.[5,11,14,15] We have shown that there is no LEKTI expression in epidermis of the case. The result demonstrated that loss of LEKTI expression is the major molecular mechanism underlying NS. Because we had no cell cultures of NS patient available, we could not evaluate the effect of the novel mutation at mRNA level. Our immunohistochemistry analysis of NS skin confirmed that there is no SPINK5 gene product in NS patient analyzed. In contrast, normal or slightly reduced levels of LEKTI were detected in normal human skin.

In this study, we identified heterozygous mutations in the SPINK5 gene of one Chinese adult with NS. The 318 G-A (D106X) is a novel mutation and it is hasn't been found in Europe[3,6,14] and Asia.[4,6–9,14] Together with previous reports, 15 of 44 mutations reported to date have been located between exons 22 and 26 of the SPINK5 gene indicating that this region would be one of the largest mutation clusters for NS. But in our study, we identified the 318 G-A (D106X) is a novel mutation which was located at exon 5 of the SPINK5 gene of one Chinese adult affected with NS.

Conclusion

In this report, we describe heterozygous mutation in the SPINK5 gene from one Chinese adult and confirm the diagnosis of NS with Immunohistochemistry. The study reveals that defective expression of LEKTI in the epidermis and mutations of SPINK5 gene are reliable for diagnostic feature of NS with atypical clinical symptoms.

What is new?

A G318A mutation was found at exon 5 of the Chinese patient's SPINK5 gene.

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Footnotes

Source of Support: Nil

Conflict of Interest: Nil.

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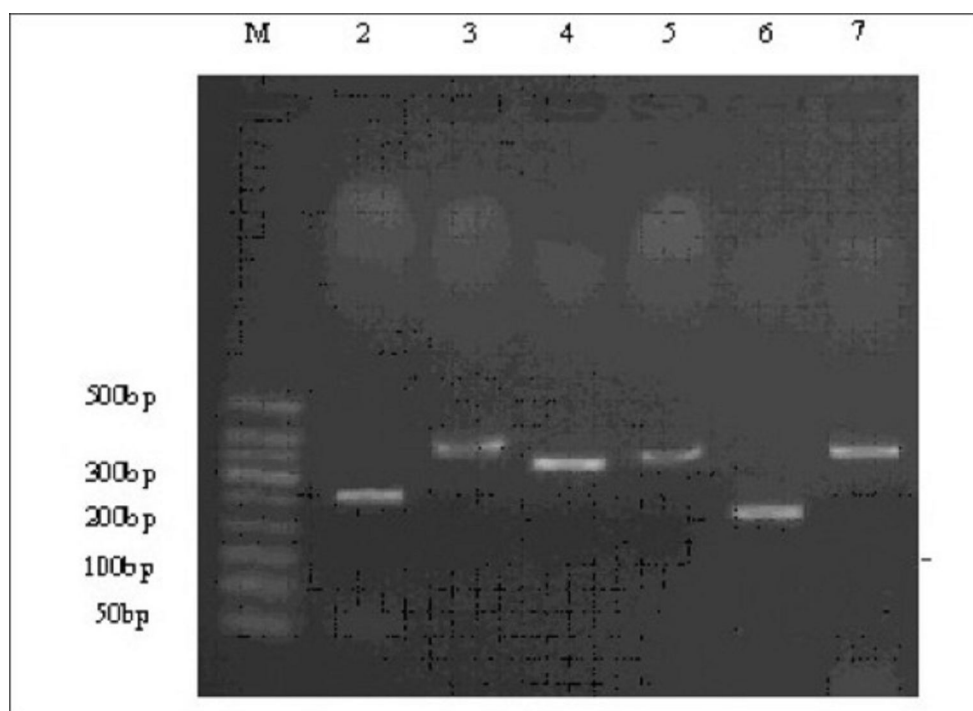
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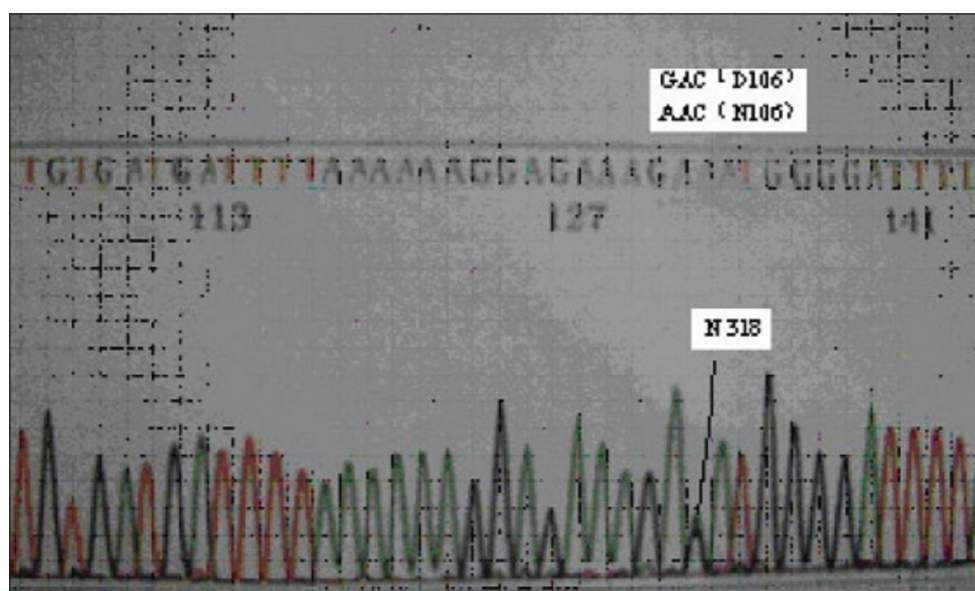
Figures and Tables

Figure 1



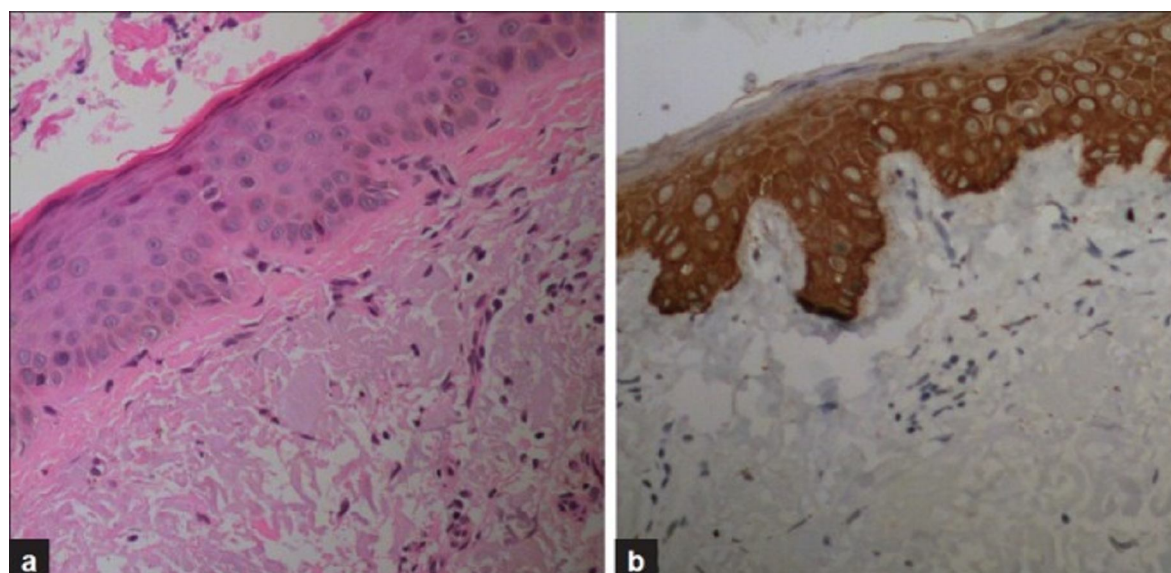
Result of exons of SPINK5 by PCR

Figure 2



G318A missense mutations was found at exon 5 of SPINK5 gene

Figure 3



(a) Patient skin showed absent staining indicating a dramatic reduction of epidermal LEKTI (hematoxylin and eosin, $\times 200$). (b) Normal skin showed a predominantly cytoplasmic, partially pericellular presence along the stratum granulosum (DAB stain, $\times 200$)

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