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# A Novel SPINK5 Gene Mutation Associated with Netherton Syndrome in an Omani Patient

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**Abstract**

Netherton syndrome (NS) is an autosomal recessive primary immunodeficiency which is characterized by substantial skin barrier defects and is often misdiagnosed as severe atopic dermatitis or Hyper IgE syndrome. Although, over 80 pathogenic mutations in the SPINK5 gene have been reported worldwide in association with NS, only one NS-associated mutation has been reported in Arab populations to-date. This case report presents a novel association between the c.1887+1G>A mutation in the SPINK5 gene and NS in an Omani-Arab patient born in 2014. Accurate genetic diagnosis facilitated tailored clinical management of the index patient and enabled the provision of genetic counseling and offering of future reproductive options to the related individuals of the index patient.

***Keywords:*** Netherton syndrome, autosomal recessive, Serine Peptidase Inhibitor Kazal-Type 5, Congenital Ichthyosiform Erythroderma, genetics

## Introduction

Netherton syndrome (NS) is defined as an autosomal recessive primary immunodeficiency which is characterized by a substantial skin barrier defect leading to ichthyosis being present at birth with erythroderma and subsequently evolving into ichthyosis linearis circumflexa.[1] NS is caused by mutations in the serine peptidase inhibitor gene SPINK5, which encodes the serine protease inhibitor lymphoepithelial kazal-type inhibitor 1 (LEKTI) protein. NS patients exhibit severe atopic diathesis and a predisposition to allergies, asthma, and eczema, with hypereosinophilia, elevated serum IgE, and trichorrhexis invaginata (hair-shaft abnormality) leading to bamboo-like hair[2]. Owing to its dermatological manifestations, NS is often misdiagnosed as severe atopic dermatitis[3] or Hyper IgE syndrome.[4] In NS patients, early genetic testing and diagnosis can prove critical to the provision of correct clinical management and decreased mortality.

Over 80 pathogenic mutations in the SPINK5 gene have been reported worldwide in association with NS.[5] While there have been reports of NS patients from the Middle-east, to the best of our knowledge, only one NS-related mutation has been reported so far in Arab populations[6]. This case report presents a novel NS-associated mutation in an Omani-Arab family with multiple affected individuals (Figure 1).

## Case Report

Our index patient was a male infant born in 2014, who presented at our pediatric immunology clinic at 34 days of age. He was born to consanguineous parents and was affected with generalized erythematous desquamative skin rash from birth. He had normal lymphocyte count, mildly elevated eosinophil and normal IgE initially, which rose to >1000 [iU]/mL on follow-up (Table 1). At four months of age, the patient had normal IgG, IgA and IgM levels. Repeated immune-panel testing indicated normal complement C3, C4 & CH50 levels.

Immunophenotyping indicated 27% of HLA-DR positive cells within the lymphocyte subset and the patient was reactive for antibody-specific response to hepatitis B vaccine. The patient had suffered recurrent infections from the age of 1 month, which worsened in frequency and severity over time. Cultures from his blood, ears, CSF (cerebrospinal fluid) and eyes showed colonization by MRSA (Methicillin-Resistant Staphylococcus Aureus). At 2 years of age, our patient also

presented with failure to thrive, global developmental delay, severe anemia and food allergy indicated by high Immunocap and RAST (Radioallergosorbent test) values to cow's milk and wheat (Table 1). He required peripheral red blood cell transfusion six times.

An older deceased male sibling had also presented with a skin condition similar to the index patient. This sibling had been born by spontaneous vaginal delivery at home and was rushed to the nearest hospital with delay in crying, cyanosed and in distress. He was intubated and ventilated for about 4 weeks in the newborn intensive-care unit (NICU), where he was diagnosed by a dermatologist to have non-bullous congenital ichthyosiform erythroderma. During his stay in NICU, he developed sepsis with Coagulase-negative staphylococci (CoNS) and candida. By 70 days of age, the sibling had rapidly developed septic shock and died while in admission.
On further probing, the parents of the index patient revealed a history of infant deaths of paternal cousins of the index patient who had died of sepsis before the ages of 1 year (Figure 1) and had received diagnoses of atopic dermatitis. Given this family history and the propensity for recurrent infections, the index patient was then suspected to have a primary immunodeficiency. Later, hair biopsy and histopathology evaluations of our index patient at 5 months of age (Figure 2) indicated a clinical diagnosis of Netherton syndrome (NS).

Whole exome sequencing (WES) of the index patient's DNA in 2016 revealed a homozygous mutation c.1887+1G>A (NM_001127699.2; hg19:Chr5:147492498) in intron 20 of the SPINK5 gene associated with autosomal recessive NS. This mutation affects the highly conserved consensus donor splice-site at the exon 20-intron 20 junction. This variant is present in the dbSNP database (rs1042707088) and was detected only twice in heterozygous form within a human longevity project from 2016, but was never associated with any clinical presentation. Sanger sequencing technique further validated the WES result and confirmed the segregation of the c.1887+1G>A mutation in heterozygous form in each parent (Figure 3A). The c.1887+1G>A variant was initially classified as a 'Variant of Uncertain Significance' (VUS) due to the lack of population frequency data or functional evidence regarding this variant. However, the ever-increasing volume of exome sequencing data compiled since 2016 at the two laboratories participating in this study and the absence of this variant in online population databases, enabled us to ascertain that this mutation was indeed rare and novel with respect to NS. Moreover, an

unaffected sibling of the index patient born six months ago was also screened and found to carry only normal SPINK5 alleles (Figure 3A).

The potential impact of the c.1887+1G>A mutation was further analyzed using the in-silico HSF (Human Splice Finder) Suite from GENOMNIS[7] which evaluates candidate splice site mutations using both the HSF matrix[8] and the MaxEntScan matrix[9] developed by the Burge Lab at MIT, USA. Both matrices predicted the c.1887+1G>A mutation observed in our index patient to 'probably affect splicing'. An image of the actual result from the HSF Suite is shown in Figure 3B.

Interestingly, another mutation, c.1888-1G>A [hg19:Chr5:147493924] affecting the same intron 20 at the opposite end, i.e. the acceptor splice site at the intron 20-exon 21 junction had already been reported as being pathogenic in NS patients.[10,11] On comparison, it was observed that the previously reported c.1888+1G>A mutation and the c.1887+1G>A mutation scored 3.83 and 3.85 respectively, on measurements of evolutionary conservation by two algorithms (PhastCons and PhyloP) from the PHAST package generated using the bioinformatic tools in the UCSC/Penn State Bioinformatics comparative genomics alignment pipeline.[12] This indicated that both mutations (separated by >1.4kbp) were located at equally and highly conserved splice sites at opposite ends of intron 20 of the SPINK5 coding sequence. Given all of the above evidence, the c.1887+1G>A mutation was hence re-classified as 'pathogenic' and predicted to result in aberrant LEKTI protein expression in homozygous patients.

Informed consent for testing and publication of anonymized data was collected from all patients/guardians involved in this study and appropriate ethical standards were employed in all procedures.



**Discussion**

We report here a novel association of the c.1887+1G>A mutation in the SPINK5 gene with NS. Although this mutation was observed in our index patient in 2016, we had initially classified it as a VUS. However, with access to growing data from subsequent exome sequencing studies over the past years, it became evident that the c.1887+1G>A mutation was indeed rare and likely to

be pathogenic in association with NS in our index patient. Given that over 50% of marriages in Oman are reported to be consanguineous unions,[13] the prospect of this being a founder mutation, cannot be ignored. Hence, the detection of novel variants in rare disorders like NS, facilitates a targeted screening approach for quicker diagnosis in future cases.

SPINK5 gene mutations can result in LEKT1 deficiency. LEKTI has been shown to inhibit several members of the serine protease family such as KLK5, KLK7 and KLK14, as well as trypsin, plasmin, subtilisin A, cathepsin G, and human neutrophil elastase.[14] Unhindered functioning of these serine proteases can trigger a cascade of events whereby increased proteolysis destroys the stratum corneum integrity, affects lipid barrier maintenance and exacerbates desquamation of the epidermis.[14] These effects conceivably compromise the antimicrobial-barrier function of the skin in NS patients, thereby leading to colonization of lesional and non-lesional skin by S. aureus; a presentation reported in both our index patient and his deceased sibling.

Unearthing the genetic etiology of the patient's symptoms was crucial in providing correct clinical management and likely avoided the severe complications observed in the index patient's older sibling and deceased paternal cousins (Figure 1). While there are multiple treatment modalities targeting skin manifestations and immune defects, symptomatic treatment with allergy control medication, moisturizers, steroid creams and antibiotics have limited effect on NS patients. However, intravenous immunoglobulin (IVIG) replacement therapy was previously reported to be quite effective in NS patients.[15] Hence, an accurate genetic diagnosis enabled a tailored clinical strategy, whereby IVIG therapy (0.4g/kg/month) was administered and led to decreased inflammation and itching of the skin in our patient. It also led to thicker hair with reduced hair shaft breakage and healthier scalp. At the last clinical follow-up, the index patient was 2 years of age. His skin condition had vastly improved, albeit with some generalized persistent redness. While he still had developmental delay, he had started to acquire milestones such as being able to sit without support.

Retrospective evaluation of the case histories of the previously deceased paternal cousins of the index patient indicated that they had likely been affected with NS as well. This was unsurprising

as all the affected offspring (Figure 1) were born of consanguineous unions. This led to mutation carrier screening being offered to other individuals within the extended family, especially in the context of pre-marital counseling for unmarried heterozygous carriers who intended to seek consanguineous unions. The family members with previous deceased offspring were also counseled with regards to alternative reproductive options to avoid the recurrence of affected offspring. In Oman, prenatal genetic screening combined with assisted reproduction is usually offered as a safe and culturally acceptable option to such affected families.

## Conclusion

In conclusion, NS is a rare and severe disease which can be life-threatening in infants without accurate diagnosis and tailored management. Genetic diagnosis of NS patients can have significant implications for their correct and effective clinical management using IVIG which helps in reduction of infections & even ameliorates skin and hair anomalies. Moreover, genetic testing for patients with rare diseases like NS also facilitates the provision of genetic counseling and selection of future reproductive options provided to affected families, particularly in societies which are highly consanguineous and opposed to abortion.

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**Table 1:** Immunological investigations done in the index patient

| PARAMETER | VALUE | UNIT | REFERENCE RANGE |
|---|---|---|---|
| Investigations done at age: 4months | | | |
| Lymphocyte Count | | | |
| Total lymphocytes | 7.91 | $10^9$/L | 3.6 - 8.8 |
| T-cells (CD3+) | 5.19 | $10^9$/L | 2.3 - 6.5 |
| B-cells (CD19+) | 1.32 | $10^9$/L | 0.5 - 1.5 |
| T-helper cells (CD3+/ CD4+) | 2.63 | $10^9$/L | 1.7 - 4.6 |
| T-cytotoxic cells (CD3+/ CD8+) | 1.46 | $10^9$/L | 0.7 - 3.5 |
| CD4:CD8 ratio | 1.79 | $10^9$/L | 1.2 - 3.5 |
| T-helper CD4+ count | 2630 | {Cells}/uL | |
| Eosinophils % | 2.6 | $10^9$/L | 0.1 – 0.8 |
| Immunoglobulins panel in Serum | | | |
| IgE | 193 | [iU]/L | 0 - 15 |
| IgG | 6.0 | g/L | 2.05 – 9.5 |
| IgA | 0.89 | g/L | 0.08 – 0.91 |
| IgM | 0.58 | g/L | 0.17 – 1.5 |
| Complement levels in Serum | | | |
| C3 | 968 | mg/L | 820 - 1850 |
| C4 | 283 | mg/L | 150 - 530 |
| CH50 | 71 | % | 70 - 140 |
| Investigations done at age: 2 years | | | |
| Immunoglobulin E in Serum | 1526 | [iU]/L | 0 - 60 |
| Allergy Testing - Specific IgE | | | |
| Cow's milk | 20.60 | kU/L | A result >0.1kU/L indicates being sensitized by the tested allergen |
| Wheat | 9.25 | kU/L | |

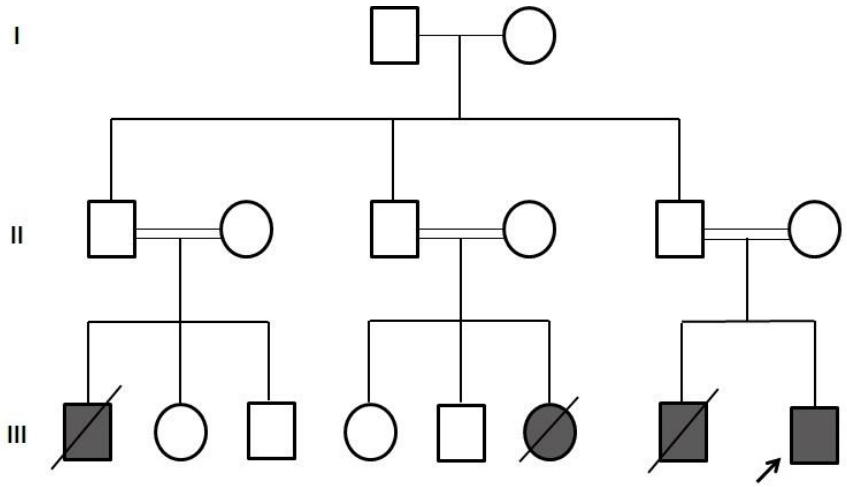



**Figure 1:** Family pedigree of the index patient. The sibling of the index patient (indicated by an arrow) was affected and had previously died of sepsis. Two paternal cousins who presented with features similar to the index patient had also died of sepsis before the ages of 1 year.

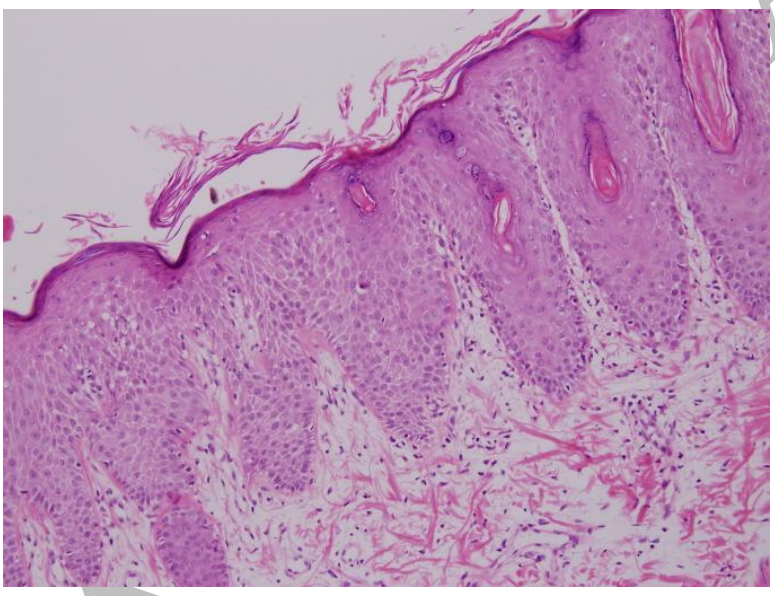
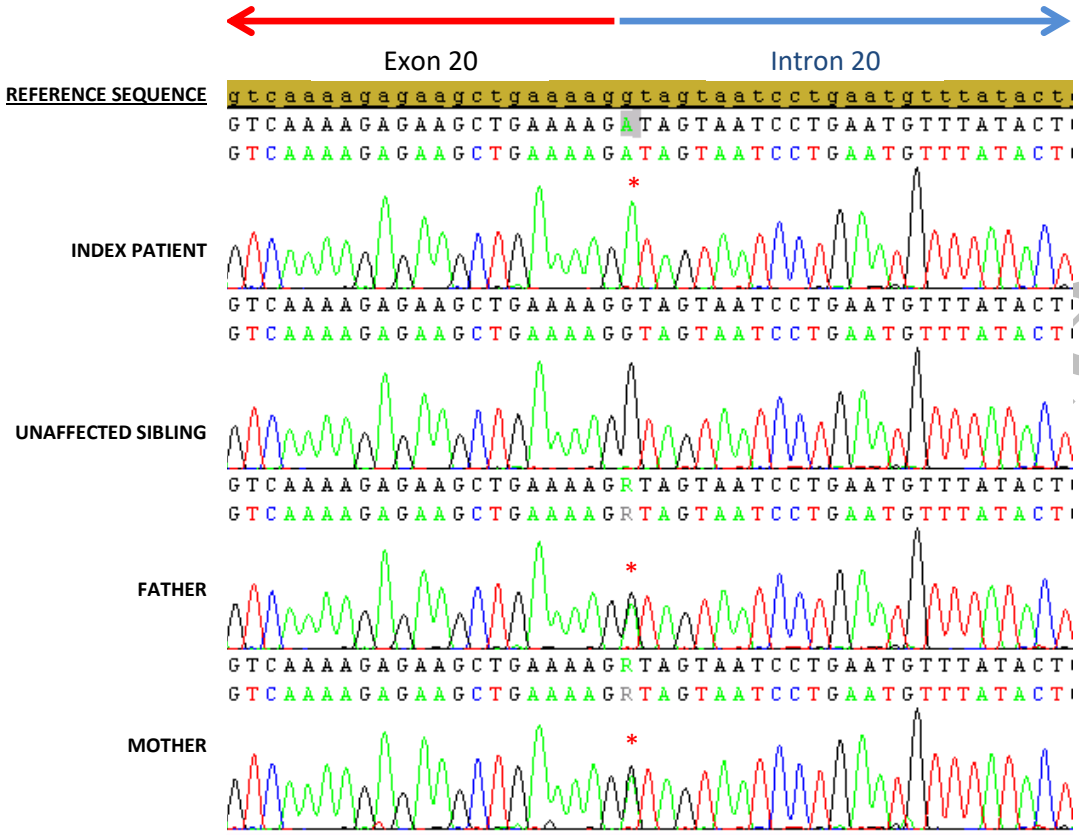


**Figure 2:** Immunohistopathology report of skin biopsy from the index patient. Skin biopsy section (H&E stain; 10X) showed psoriasiform acanthosis spongiosis, mild lymphocytic exocytosis with occasional dyskeratotic cells.

A.




B.

| Type | Interpretation |
|---|---|
| Broken WT Donor Site | Alteration of the WT Donor site, most probably affecting splicing |

| Algorithm/Matix | position | sequences | variation |
|---|---|---|---|
| HSF Donor site (matrix GT) | chr5:148112932 | - REF : AAGGTAGTA<br>- ALT : AAGATAGTA | 80.29 > 53.15<br>=> -33.8% |
| MaxEnt Donor site | chr5:148112932 | - REF : AAGGTAGTA<br>- ALT : AAGATAGTA | 5.69 > -2.49<br>=> -143.76% |

**Figure 3:** Evidence for pathogenicity of the c.1888+1G>A mutation. (A) Family segregation analysis using Sanger sequencing data of the exon 20-intron 20 junction shows the c.1888+1G>A mutation as homozygous in the index patient (location marked by an asterisk) and as heterozygous (R indicates G/A transition) in the parents of the index patient. The unaffected sibling of the index patient does not carry the mutated allele (B) Results from the in-silico analysis using the HSF suite indicate that the c.1888+1G>A mutation probably affects splicing at the exon 20-intron 20 junction.