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Clinical and genetic characterization of Netherton syndrome due to *SPINK5* founder variant in Latvian population

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Abstract

Objective: Netherton syndrome (NS) (OMIM:256500) is a very rare autosomal recessive multisystem disorder mostly affecting ectodermal derivatives (skin and hair) and immune system. It is caused by biallelic loss-of-function variants in the *SPINK5* gene, encoding the protease inhibitor lymphoepithelial Kazal-type-related inhibitor (LEKTI).

Material, Methods and Results: Here, we describe NS clinical and genetic features of homogenous patient group: 9 individuals from 7 families with similar ethnic background and who have the same *SPINK5* variant (NM_006846.4: c.1048C>T, p.(Arg350*)) in homozygous or compound heterozygous states, suggesting that it is a common founder variant in Latvian population. Indeed, we were able to show that the variant is common in general Latvian population, and it shares the same haplotype among the NS individual. It is estimated that the variant arose >1000 years ago. Clinically, all nine patients exhibited typical NS skin changes (scaly erythroderma, *ichthyosis linearis circumflexa*, itchy skin), except for one patient who has a different skin manifestation—epidermodysplasia. Additionally, we show that developmental delay, previously underrecognized in NS, is a common feature among these patients.

Conclusions: This study shows that the phenotype of NS individuals with the same genotype is highly homogeneous.

1 | INTRODUCTION

Netherton syndrome (NS) (also known as Comel–Netherton syndrome, OMIM:256500) is a very rare autosomal recessive multisystem disorder mostly affecting ectodermal derivatives (skin, hair) and the immune system. The typical clinical signs of NS are severe skin barrier defects (e.g., inflammatory skin lesions), atopic manifestations such as bamboo hair (*trichorrhexis invaginata*), *ichthyosis linearis circumflexa*, erythroderma, and atopic predisposition with high serum levels of immunoglobulin (Ig) E. However, a wide phenotypic variability has been reported among NS patients.[1]

In addition to skin barrier defects, NS is defined as a primary inborn error of immunity. Its immune system defects predominantly manifest as antibody deficiency with a reduced number of memory B cells and selective antibody insufficiency, e.g., reduced responses to pneumococcal vaccinations but can also include decreased

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numbers of natural killer (NK) cells or an immature phenotype of NK cells with reduced lytic function.[2] However, it is currently not known whether the immune abnormalities are primary or are a result of chronic (skin) inflammation and infections.[3]

NS is caused by biallelic loss-of-function variants in the *SPINK5* gene, encoding the protease inhibitor lymphoepithelial Kazal-type-related inhibitor (LEKTI). To date, more than 60 different (likely) pathogenic *SPINK5* variants have been reported (literature reviewed on 25.02.2022).[4] Most of the variants result in the complete loss of protein expression, but there are known severity differences among the variants, which likely contribute to the variable expressivity of NS.[5]

In this study, we investigated the clinical, immunological, and genetic features of NS based on a population-wide, ethnically and genetically homogeneous NS patient cohort in Latvia.

Key message

This study highlights that the phenotype of Netherton syndrome individuals with the same genotype is highly homogeneous and highlights underrecognized features of Netherton syndrome. Additionally, we show that the identified *SPINK5* variant is a common founder in Latvian population and is responsible for the condition's high prevalence in Latvia.

2 | MATERIALS AND METHODS

2.1 | Ethics

The study was approved by the Central Board of the Ethical Committee of the Health Ministry of the Republic of Latvia (No. 01–29.1/2878), which complies with the Declaration of Helsinki. Written informed consent was obtained from each patient's parent/legal guardian (all the participating NS patients were minors). Photographs or additional biological samples (e.g., hair and blood) were taken only with explicit consent.

2.2 | Patient recruitment

NS patients were recruited from the Clinic for Medical Genetics and Prenatal Diagnosis at the Children's Clinical University Hospital in Riga, Latvia. The hospital is Latvia's only tertiary pediatric center (providing highly specialized medical care for immunological and dermatological conditions), and the clinic is the country's only referral medical genetic center and the national rare disease coordinating center. Patients were diagnosed in the outpatient clinic, and NS diagnosis was molecularly confirmed based on results from ichthyosis or immunodeficiency gene panel sequencing performed in a commercial laboratory using next-generation sequencing. All patients with identified homozygous or compound heterozygous *SPINK5* gene pathogenic variants were selected from the clinic's database. Genetic testing of the patients was performed in diagnostic settings as a routine investigation for individuals with suspected hereditary ichthyosis or immunodeficiency.

2.3 | Haplotype and age estimation analysis

For the haplotype and variant age estimation, exome sequencing (ES) was performed for five unrelated patients with the homozygous *SPINK5* variant. A high molecular weight genomic DNA sample was extracted from peripheral blood using an adapted phenol-chloroform method.[6] Exome sequencing was performed on an Illumina NovaSeq 6000 sequencing system using a PE100 protocol and Twist Comprehensive Exome enrichment kit (Twist Bioscience). Bioinformatic analysis was conducted using our laboratory's standard pipeline. Briefly, reads were mapped using BWA-MEM[6] and variants were called using DeepVariant.[7] Separate gVCFs were merged using GLnexus[8] and only high-quality variants were retained for further analysis.

For haplotype length estimation, we used the longest homozygous stretch per individual for the analysis. This information was uploaded to an *R Shiny* app based on the mutation dating method developed by Gandolfo et al.[9] and the analysis was performed according to the authors' recommendations.

Variant frequency estimation in the population was performed using Hardy–Weinberg equilibrium, based on the in-house database of ES data from individuals with various conditions but without immunodeficiencies or skin disorders.

2.4 | Patient investigations

For the present study, all known patients with NS in Latvia ($n = 9$ from seven unrelated families) were invited to participate. However, due to personal circumstances, two unrelated patients did not partake in the ensuing follow-up, which included additional peripheral blood and hair sampling.

For all patients, a review of all the available medical documentation was performed. For the 7/9 patients who agreed to a follow-up visit, we evaluated their health status, use of medications, daily skin appearance, growth rate, frequency of infections, hospital admissions, and allergies. During their outpatient consultation, patients were invited for a multidisciplinary evaluation by a pediatrician, dermatologist, immunologist, and clinical geneticist. Development delay was defined based on patients' development skill evaluation compared with those that are expected at the evaluation age.

2.5 | Immunological investigations

Immunological analysis was performed in an ISO 15189-certified laboratory as part of the outpatient consultation. Detailed methods

are provided in the Appendix S1. Shortly, the frequency and absolute numbers of NK cells ($CD3^-CD16^+/CD56^+$), T cells ($CD3^+$), helper T cells ($CD3^+CD4^+$), cytotoxic T cells ($CD3^+CD8^+$), B cells ($CD19^+$), naïve B cells ($CD19^+CD27^-IgD^+$), nonswitched memory/marginal zone-like B cells ($CD19^+CD27^+IgD^+$), and switched memory B cells ($CD19^+CD27^+IgD^-$) were performed in peripheral blood by Navios EX flow cytometer (Beckman Coulter). Total IgE, IgM, IgG, and IgA serum levels were measured by immunonephelometry with a NEPH 630 System (Siemens).

2.6 | Hair examination

Strands of hair were cut with scissors and examined under 100× magnification using an Eclipse E400 optical microscope.

3 | RESULTS

3.1 | Clinical characteristics of the patient cohort

The mean age of the nine NS patients (three males and six females) was 9 years (range 4 months–17 years). Molecular testing revealed that seven patients were homozygous and two were compound heterozygous genotypes. The clinical description and manifestations of the studied NS patients are shown in Table 1.

All nine patients exhibited typical NS skin changes (scaly erythroderma, *ichthyosis linearis circumflexa*, itchy skin). The whole body was affected in four patients, whereas it was limited to the face, hands, and feet in four other patients. Recurrent skin infections and otitis were present in seven out of the nine patients, recurrent respiratory infections in five patients, and sepsis before 2 years old in three patients. One patient (#3) had wart-like skin lesions; however, the histological analysis indicated epidermodysplasia (Figure 1). Seven patients had an allergy; six had a food allergy; and one had a pet allergy. Eight patients have failure to thrive and seven of them have short stature. Additionally, one patient (#2) had sensorineural hearing loss (SNHL) and another (#8) had bronchial asthma. Interestingly, for three out of seven cases with available information developmental delay was observed.

3.2 | Hair examination

Visually, all patients had sparse and brittle hair bordering on alopecia. In order to observe the hair abnormalities in more detail, we examined hair from four patients under a light microscope. Surprisingly, only 1/4 showed typical NS bamboo hair with invagination of the distal part of the hair shaft into its proximal part (*trichorrhexis invaginata*) (Figure 2). Of note, the parents of the youngest patients did not consent to hair sampling due to alopecia. Table 2 summarizes the patients' hair features.

3.3 | Immunological features

All nine patients had an elevated serum level of IgE (for seven measured in the follow-up and for two measured in previous clinical testing). For one of the patients that did not take a part in the follow-up (#5), their medical records revealed an extremely high serum total IgE level (44,072 IU/mL, N < 94 IU/mL) and increased levels of IgA (2.42 g/L, N 0.53–2.04 g/L) and IgM (2.04 g/L, N 0.31–1.79 g/L). The other patient that did not take a part in the follow-up (#2) also had a markedly increased serum total IgE level (15,566 IU/mL, N 0–50 IU/mL); however, no other immunological test results were available. For all the other patients, normal levels of IgG, IgA, and IgM were measured and no antibody deficiency was found. However, we did not measure specific antibodies, e.g., to polysaccharide antigens.

For immune cell subtype counting, blood samples from seven NS patients were available. In 3/7 examined patients, nonswitched memory (IgD^+CD27^+) and switched memory (IgD^-CD27^+) B-cell numbers were decreased (similar to patients with common variable immune deficiency) but with normal antibody synthesis (excluding IgE). Naïve B-cell numbers (IgD^+CD27^-) were within the age-related normal ranges in all patients.[5] Similarly, all patients (7/7) had total, $CD4^+$ and $CD8^+$ T-cell numbers within the age-related normal ranges. However, NK cell counts ($CD3^-CD16^+/CD56^+$) were decreased in 2/7 patients. Appendix S2 details the immunological features of the patients.

3.4 | Genetic features

Among the Latvian patients with NS, the same nonsense variant predicting premature termination codon NM_006846.4 (*SPINK5*):c.1048C > T p.(Arg350*) in exon 12 was identified in all nine patients. Seven patients from the seven families included in the study were homozygous for this variant, suggesting that it is a common founder variant in our population. Additionally, two affected siblings were compound heterozygous for the same variant and a splice site mutation NM_006846.4 (*SPINK5*):c.1430 + 4A > G p.? (Table 1). Splice site variant is predicted to result in loss of the natural donor site (SpliceAI donor loss score = 0.91).

To establish whether this recurrent variant is in fact a founder variant in the Latvian population, we evaluated haplotype length using high-quality ES variants from five unrelated homozygous individuals. Indeed, all five individuals had homozygous stretches, confirming that both alleles have the same haplotype, ranging from 1.1 Mb to 1.9 Mb in size (with minimal overlap among all samples of 0.9 Mb)

Further, using the haplotype position and length, we estimated the mutation age. Assuming a "correlated" genealogy, the founder variant NM_006846.4 (*SPINK5*):c.1048C > T p.(Arg350*) arose 47.4 (95% CI 14.9–167.2) generations and 1175 years ago (assuming one generation = 25 years).

TABLE 1 Netherton syndrome patients' description and clinical manifestation.

Family Nr.	Family 1		Family 3		Family 3	Family 4	Family 5	Family 6	Family 7	TOTAL
Patient Nr. (Gender)	Patient #1 (F)	Patient #2 (F)	Patient #3 (F)	Patient #4 (M)	Patient #5 (M)	Patient #6 (F)	Patient #7 (F)	Patient #8 (M)	Patient #9 (F)	6F/3M
Age	1 y.o.	14 y.o.	8 y. o.	4 m.o.	15 y.o.	15 y.o.	9 y.o.	17 y.o.	2 y.o.	mean 9 y.o.
Genotype	c.[1048C > T];[1048C > T] p.[Arg350*];[Arg350*]		c.[1048C > T];[1430 + 4A > G] p.[Arg350*];[?]		c.[1048C > T];[1048C > T], p.[Arg350*];[Arg350*]	c.[1048C > T];[1048C > T], p.[Arg350*];[Arg350*]	c.[1048C > T];[1048C > T], p.[Arg350*];[Arg350*]	c.[1048C > T];[1048C > T], p. [Arg350*];[Arg350*]	*c.[1048C > T];[1048C > T]*, p. [Arg350*];[Arg350*]	7 homozygous and 2 compound heterozygous
Gestation (weeks + days)	39^{+5}	37	40	36 + 4	35	35	36	36	37	mean 36.7
Birth weight, g	3050	2700	3600	3420	3100	2660	2990	2810	3200	mean 3059
Neonatal hospitalization	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	8/9
Failure to thrive	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	8/9
Development delay	Yes[a]	Yes[b]	No	N/A	No	No	Yes[c]	N/A	No	3/7
Short stature	N/A	Yes	Yes	N/A	Yes	Yes	Yes	Yes	Yes	7/7
Sweating	N/A	Yes	Yes	No	N/A	N/A	N/A	Yes	N/A	3/4
High fever	No	No	Yes	No	N/A	No	No	N/A	Yes	2/7
Hearing loss	No	Yes-bilateral SNHL	No	N/A	No	No	No	No	No	1/8
External otitis	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	7/9
Recurrent respiratory infections	No	N/A	Yes	No	No	Yes	Yes	Yes	Yes	5/8
Bronchial asthma	No	No	No	No	No	No	No	Yes	No	1/9
Allergy	Yes	No?	Yes- dairy, fish, nuts, wheat	Yes- egg	No	Yes- cow milk	Yes-egg, milk, fish	Yes- cat, dog fur	Yes- milk, egg, wheat apple, potato	7/9
Prolonged a/b	No	N/A	No	No	No	Yes	Yes	N/A	Yes	3/7
Expressivity of the disease	Severe	Severe	Mild	Mild	N/A	Mild	Severe	Severe	Severe	5 Severe/3 Mild/ 1 N/A
Affected body parts with skin lesions	Whole body	Whole body	Face, top of hands and feet, legs, arms, back	Face, hands, feet	N/A	Face, hands	Face, scalp, hands, feet	Whole body	whole body	4 Whole body/4 parts/ 1 N/A
Recurrent skin infections	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	7/9
Urticaria	N/A	Yes	No	No	N/A	No	No	N/A	Yes	2/6
Erythroderma	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8/9
Scaly erythroderma	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9/9
Ichthyosis linearis circumflexa	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9/9
Itchy skin (Pruritus)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	8/9

[a]Does not crawl, nor sit, not walk at age 12 months; no language (related to hearing impairment).

[b]Crawling 12mo, walking 36 mo; delayed language development (related to hearing impairment).

[c]Crawling 13 mo; walking 15 mo; first words after 24 mo.

3.5 | Expected NS and founder variant prevalence

Among the 694 in-house ES data from individuals from Latvia without immunodeficiencies or ichthyosis, five individuals were heterozygous for the NM_006846.4 (*SPINK5*):c.1048C>T p.(Arg350*) pathogenic variant. Therefore, based on our data, the variant is found in ~1/139 individuals from the Latvian population. Using Hardy–Weinberg equilibrium, we calculated the expected frequency of homozygous individuals based on the most recent Latvian population data (https://www.pmlp.gov.lv/lv/media/2881/download). Our calculation determined a total of 27 expected NS patients in Latvia, i.e., significantly higher than the total number of patients currently diagnosed with NS in Latvia ($n = 9$).

Based on the variant frequency data, NS prevalence in Latvia works out at 1:77,061. However, using only the data from diagnosed NS patients, the prevalence of NS in the Latvian population is 1:240,000. Additionally, birth prevalence, calculated based on the Latvian birth rate with data from the years 2004 to 2020, is 1:39,840 births. We selected the time period from our patient cohort starting from the birth year of the oldest patient through to the birth year of the youngest patient.

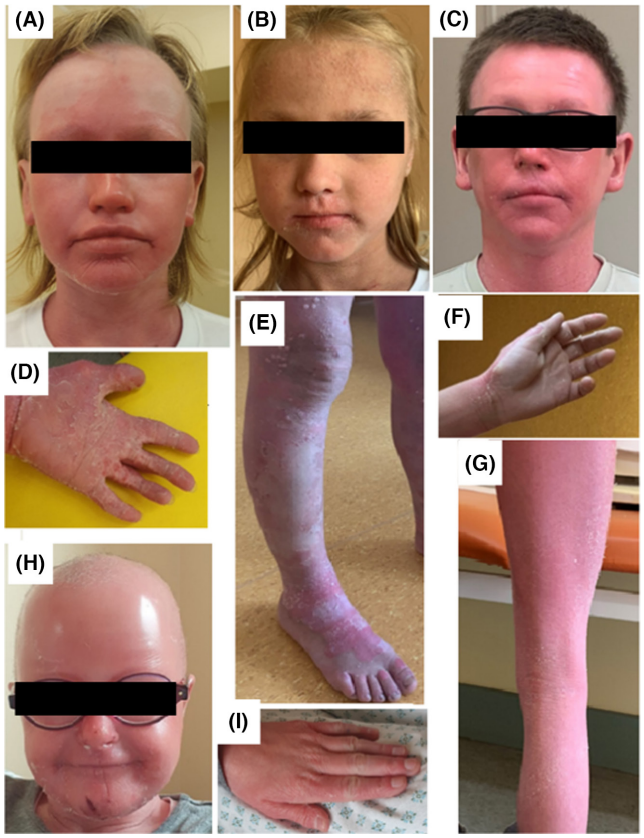

FIGURE 1 Patients affected skin parts A and D for patient No 6; B, E, F for the patient No3; C and G for patient No8; H and I for patient No7.

4 | DISCUSSION

In the present study, we provide clinical, immunological, and molecular features of clinically and genetically homogeneous NS patients from Latvia.

Clinically, although all patients displayed typical well-known NS features such as scaly erythroderma and common infections in early age, we also observed several other features in the large proportion of patients, e.g., developmental delay, failure to thrive, and short stature. In addition, some unique features not previously described for NS patients were noted—SNHL and epidermodysplasia—however, the association of these features with NS is currently uncertain.

Interestingly, while NS is considered to primarily affect the immune and ectodermal systems, the developmental delay was observed in three out of the seven patients. Two patients were not available for the assessment of developmental delay. At present, there is only limited information about the neuropsychological or psychosocial features of NS patients in the literature. Specifically, a couple of studies conducted in the 1970s and 1980s by Julias and Keeran (1971) and Caputo (1984) have described the cognitive problems (intellectual disability) in three male patients with NS.[10] To the best of our knowledge, no data on developmental delay in NS patients have been reported in previously published cohort studies. Furthermore, no systematic studies have been performed to investigate neuropsychological or psychosocial functioning in NS. Not surprisingly, due to this lack of information, *SPINK5* is not included in

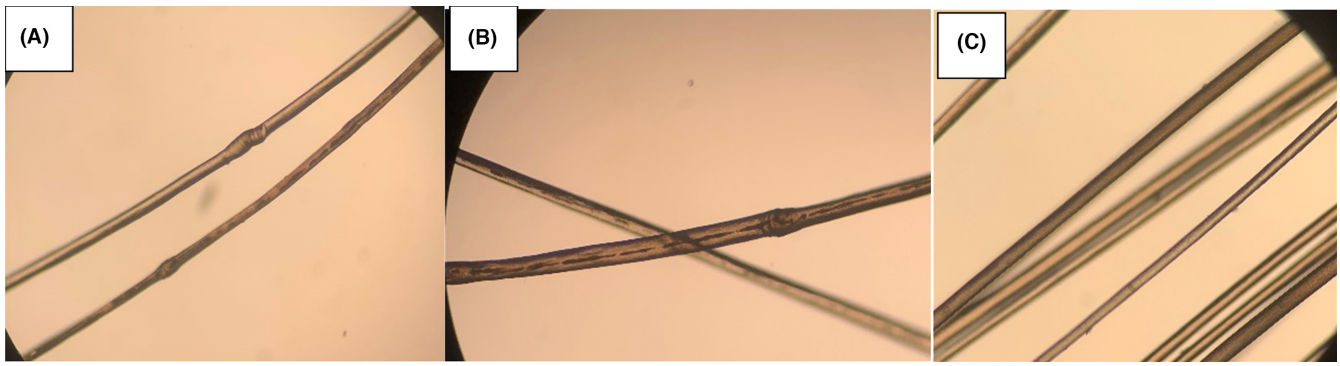

FIGURE 2 Microscopy A, B—typical "bamboo" hair (patients #5); C—normal hair (patient #3).

TABLE 2 Detailed hair description of NS patients.

Family Nr.	Family 1		Family 2		Family 3	Family 4	Family 5	Family 6	Family 7	TOTAL
Patient Nr. (Gender)	Patient #1 (F)	Patient #2 (F)	Patient #3 (F)	Patient #4 (M)	Patient #5 (M)	Patient #6 (F)	Patient #7 (F)	Patient #8 (M)	Patient #9 (F)	6F/3M
Age	1 y.o.	14 y.o.	8 y. o.	4 m.o.	15 y.o.	15 y.o.	9 y.o.	17 y.o.	2 y.o.	average 9 y.o.
Hair Sample available	No	No	Yes	No	No	Yes	Yes	Yes	No	4/5
Alopecia	Yes	Yes	No	Yes	N/A	No	Yes	Yes	Yes	6/8
Trichorrhexis invaginata	N/A	N/A	No	N/A	N/A	No	Yes	No	N/A	1/4
Brittle eyebrows/ eyelashes	Yes	Yes	No	N/A	N/A	N/A	Yes	Yes	Yes	5/6

the developmental disorder genes list created and maintained by the large cohort Deciphering Developmental Disorders (DDD) study,[10] which consequently could result in missed NS diagnosis in patients with developmental delay.

SNHL was observed in one patient in our study. Again, to the best of our knowledge, there are no data on hearing loss in NS patients in the literature. Therefore, we hypothesized that there was an alternative cause underlying the hearing loss in our patient. Accordingly, we analyzed a panel of genes related to hearing loss (from ES, analyzing PanelApp Hearing Loss Version 2.237),[11] but no pathogenic or likely pathogenic variants were found that could explain the SNHL. Thus, we propose that our patient's SNHL is a rare feature of NS, most likely resulting from frequent otitis media.

Failure to thrive and short stature were observed in 8/9 and 7/9 patients, respectively. During childhood, failure to thrive is a common consequence of malnutrition, prolonged diarrhea, metabolic disorders, chronic erythroderma, and persistent cutaneous infections. Short stature has previously been reported in NS, with undernutrition being a common problem among chronic patients and a possible reason for growth failure. Furthermore, deficiencies of selenium, iron, vitamin D, and zinc were described as a common finding in these patients.[12] Although growth retardation is common in NS patients, its pathogenesis is not known. LEKTI and kallikreins might be involved in the regulation of growth hormone processing. Growth retardation is possibly caused either by growth hormone over-processing or circulating bio-inactive forms in the pituitary gland due to a lack of inhibition of human tissue kallikrein proteases by LEKTI deficiency.[13] Growth hormone levels, however, were not measured in our patients.

Immunological evaluation of our patients revealed no evidence for a severe, clinically relevant systemic immunodeficiency. A similar conclusion was reported before by Stuvel et al, 2020,[14] where 16 NS patients from the Netherlands were immunologically examined. In our study, the main immunological disturbance identified was a decreased number of nonswitched memory and switched memory B cells (in 3/7 patients), similar to patients with common variable immune deficiency.[15] Previous studies have also reported a decreased number of nonswitched and switched memory B cells, as well as a decreased antibody response to polysaccharide antigens.[16] In a new classification of IEI 2022, the NS is described as a type of hyper IgE syndrome, which belongs to combined immunodeficiencies with associated or syndromic features.[17] Immunological characteristics described by experts in IEI: normal T cells, low-switched and nonswitched B cells, high level of IgE and IgA, antibody variably decreased. In our study, we do not study deeply T-cell compartment, but the investigated routine T-cell subpopulations were in normal ranges. Low-switched and nonswitched B cells were found just in 3/7 patients. Clinically, however, these 3 out of 7 patients did not have recurrent bacterial infections more often than the other examined NS patients, nor did they have an antibody deficiency. Additionally, for the most part, the total levels of IgM, IgG, and IgA have been reported to be normal or increased,[16,18] similar to the situation in our investigated patients. The effect of defective LEKTI expression on T-cell maturation is less well-known, but it could be a reason for T-cell immunity dysregulation leading to abnormal IgE overproduction. Therefore, it is likely that skin infections and other epithelial barrier defects in NS patients are secondary (LEKTI deficiency occurring in hair follicles and the granular layer of the epidermis) and not due to B- or T-cell deficiency in peripheral blood, as no significant immunological abnormalities were detected and *SPINK5* is not expressed in leukocytes.[3] In another study, Hannula-Jouppi with co-authors investigated 11 Finnish patients with Netherton syndrome and found elevated levels of IgG4, as well as a functional defect of NK cells (nonspecific immunity), which possibly can explain frequent skin infections in this group.[18] In our study, we did not examine the functional state of NK cells, just detected a number of NK cells, which was mostly in normal range (in 5 out of 7).

Immunologically, patient #3 had a high serum total IgE level. She also had an elevated level of IgD^+CD27^- cells/μL (%) and an undetectable level of IgD^+CD27^+ cells, similar to her sibling (patient #4). There were no other patients in our cohort with an undetectable level of IgD^+CD27^+ cells. Additionally, patient #3 had a different skin manifestation to the other patients (epidermodysplasia), suggesting another error of immunity probably not related to NS. Consequently, we checked all known immunodeficiency-associated genes for this patient (from ES, analyzing PanelApp Primary Immunodeficiency Version 2.539)[11] but did not find any additional genetic variants to explain the differences. This suggests that her distinctive genotype (compound heterozygous) may be the reason for the phenotypic variability.

A high serum total IgE level—a typical and very common clinical sign of NS—was observed in all nine patients. However, for two

patients, the IgE levels were extremely high: 15,566 IU/mL (patient #2, F, 14 years old) and 44,072 IU/mL (patient #5, M, 15 years old). Interestingly, these two patients were the only ones in the study population that did not have a food/pet allergy; no alternative reason for their high IgE level is known. We checked all known immunodeficiency-associated genes ($n = 581$) for patient #2 from ES but did not find any additional genetic variants to explain the extremely high serum IgE level. Patient #5 did not give consent for an additional follow-up blood sample, so his ES data were not available for the assessment. Nevertheless, a previous study has described NS patients with congenital ichthyosiform erythroderma-like ichthyosis with extremely elevated serum IgE levels (>10,000 IU/mL) without any reference to another pathology,[19] so this could be a rare feature of NS.

Interestingly, only one of the compound heterozygous siblings (patient #3) had epidermodysplasia and an undetectable level of IgD^+CD27^+ cells. This could be due to another family-specific genetic variant or may indicate genotype–phenotype correlation in NS. It has been suggested in the literature that genotypes with mutations located more upstream in LEKTI correlate with more severe phenotypes compared with similar mutations located towards the 3′ region.[20] Furthermore, splicing mutations and the post-transcriptional mechanism of nonsense-mediated mRNA decay affect LEKTI expression in variable ways.[20] However, it is likely that other genetic and/or epigenetic factors contribute to the phenotype, which should be considered when NS families receive genetic counseling.

In this study, we included all known molecularly confirmed NS patients in Latvia with pathogenic *SPINK5* variants ($n = 9$). According to our calculations, the number of NS patients in the Latvian population should be three times higher ($n = 27$). There may be more than one reason for this: NS patients could have died in early age without a diagnosis as some variants have been reported to be lethal early in infancy[10,21]; or by contrast, they could have a mild phenotype or are misdiagnosed and do not receive genetic counseling and testing because the triad (congenital ichthyosiform erythroderma, hair shaft abnormalities, and immune dysregulation) is not always complete and other disorders have similar findings.

The identified common pathogenic variant (c.1048C>T p.(Arg350*)) in our study population has previously been described only once in an individual from the Baltic countries.[18] The variant is found eight times in gnomAD v2.1.1., mostly in individuals from the Estonian population (4/4822 individuals)[22] and is present only once from 9506 exomes of Russian population (based on http://ruseq.ru/ browser). According to our calculations, the mutation age is 1175 years, which fits the history of Latvia and Estonia in the Middle Ages being a single territory called Livonia (existing between the 13th and second half of the 16th century). Additionally, based on our in-house ES data, this variant is relatively common among individuals from the Latvian population, present in ~1%. These findings and the shared haplotype in all five tested unrelated NS patients of Latvian origin are extremely suggestive of the variant being a common founder mutation in the Latvian (and possibly Estonian) population. Unfortunately, there are currently no data on NS in Estonia.

Interestingly, different founder variants have been reported in several populations, including Finland,[18] Turkey,[21] and the Mediterranean region.[5] While the clinical features of individuals heterozygous for *SPINK5* variants are reported, such a common occurrence of different founder variants suggests advantages for carriers. However, there are no data available that describe such advantages.

5 | CONCLUSIONS

This study details a *SPINK5* founder mutation in the Latvian population. The phenotype of NS individuals with the same genotype is highly homogeneous. There were no significant immunological changes in the studied parameters observed, but that does not exclude immunological defect as a whole. More in-depth immunological investigation should be performed.

AUTHOR CONTRIBUTIONS

Inga Nartisa: Writing – original draft. **Rasa Kirsteina:** Data curation. **Katrina Daila Neiburga:** Writing – review and editing; software. **Sanita Zigure:** Writing – review and editing. **Lota Ozola:** Writing – review and editing; data curation. **Ineta Grantina:** Data curation; writing – review and editing. **Ieva Micule:** Data curation. **Daiga Murmane:** Data curation. **Baiba Slisere:** Writing – review and editing. **Linda Gailite:** Writing – review and editing. **Baiba Vilne:** Writing – review and editing; software. **Dmitrijs Rots:** Writing – review and editing; conceptualization; software. **Gita Taurina:** Data curation; writing – review and editing. **Natalja Kurjane:** Writing – review and editing; conceptualization; data curation; supervision.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/pai.13937.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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