CONCISE COMMUNICATION

Two novel mutations of SERPINB7 in eight cases of Nagashima-type palmoplantar keratosis in the Chinese population

Tong Xiao | Yan Liu | Tian Wang | Junru Ren | Yumin Xia | Xiaopeng Wang

Abstract
Nagashima-type palmoplantar keratosis (NPPK) is a diffuse, autosomal recessive, and non-epidermolytic palmoplantar keratosis caused by mutations in the SERPINB7 gene, a member of the serine protease inhibitor superfamily. Genetic studies and case reports suggest that NPPK is the most common palmoplantar keratosis in East Asia but rare in Western countries. This study reports eight NPPK patients in seven pedigrees of the Chinese Han ethnicity with two novel (c.530T>C and c.643A>G) and two recurrent mutations (c.796C>T and c.455G>T) in SERPINB7. The diagnosis of NPPK is now well-defined because of the typical manifestations and pathogenic gene tests. However, its pathomechanism is still obscure, and treatment remains a challenge. This study reviewed all 15 pathogenic mutations and related data in the 1000 Genomes Project to elucidate the founder effect of SERPINB7. Also, several latest cases of NPPK in areas outside East Asia are presented, including France, Finland, and Thailand. Further clinical investigation and genetic studies are crucial for identifying the pathomechanism of NPPK. Also, large-scale control studies are required to determine the safety and curative effects of available therapies.

Keywords
founder effect, Nagashima-type palmoplantar keratosis, novel mutation, SERPINB7

1 | INTRODUCTION
Nagashima-type palmoplantar keratosis (NPPK; Online Mendelian Inheritance in Man [OMIM] #615598), first described by Nagashima in the Japanese literature in 1977, was recently established as a non-syndromic diffuse autosomal recessive palmoplantar keratosis (PPK). For years, NPPK was misdiagnosed as mal de Meleda (MDM; OMIM #248300), a type of transgressive diffuse hyperkeratosis. NPPK was later considered a milder form of MDM that is nonprogressive after the second decade and does not involve flexion contractures or constricting bands. Besides, the SLURP1 mutation responsible for MDM was never found in NPPK patients. Therefore, NPPK was recognized as an independent category of PPK effective 2008. In 2013, Kubo et al. identified SERPINB7 as the gene responsible for NPPK, following which NPPK became widely known in Asia. Molecular diagnosis in Japan, China, and South Korea reported hundreds of NPPK cases associated with 13 distinct pathogenic SERPINB7 mutations in a homozygous and compound heterozygous state. This case series report presents eight patients in seven pedigrees of NPPK cases with variant clinical manifestations and heredity presentation, especially with two novel mutations and two recurrent mutations in the SERPINB7 gene.

2 | CASE REPORT
Here, we report eight cases of NPPK of the Chinese Han ethnicity, of which one relevant family history occurred in patients 7 and 8. No consanguinity or relevant family history was noted in the other six
cases. Most of the patients presented with diffuse, reddish palmoplantar hyperkeratosis extended to the wrists, Achilles tendons, and dorsum of hands/feet. The onset age ranged from birth to 1 year. Genomic DNA was extracted from the peripheral blood of patients and their parents, following their written, informed consent. The institutional review board approved this study in adherence to the principles of the Declaration of Helsinki. Sanger sequencing was performed to screen mutations in SERPINB7.

Clinical features of the seven pedigrees are summarized in Table 1. Clinical pictures, heredity patterns, and mutations are presented in Figure 1. The heredity pattern in pedigrees 1–6 is an autosomal recessive inheritance. Interestingly, the heredity pattern in pedigree 7 seems to be an autosomal dominant inheritance because both father and son presented with typical manifestations of NPPK. However, genetic tests confirmed these two patients to be NPPK, an autosomal recessive disorder. So, this special heredity pattern is called pseudodominant inheritance as previously described in NPPK and other heredity disorders.

Patient one showed compound heterozygous heredity with recurrent mutations (c.796C>T, c.455C>T). Patients 2–5 and the father of patient 7 were homozygous for the c.796C>T mutation. Patient 6 had compound heterozygous mutations of c.796C>T and c.530T>C (p.Phe177Ser). In addition, patient 7 had compound heterozygous mutations of c.796C>T and c.643A>G (p.Asn215Asp) and the father of patient 7 was homozygous for the c.796C>T mutation. This case series report presents the first case of mutations in c.530T>C and c.643A>G in SERPINB7.

3 | DISCUSSION

3.1 | Clinical features and diagnosis of NPPK

Clinically, NPPK is characterized as well-demarcated hyperkeratosis on the palmoplantar skin and other areas, such as the Achilles tendon. The affected skin shows a typical white and spongy appearance after exposure to water. Elbows and knees are also often affected because of the stress–strain induced by mechanical stress. The association of palmoplantar hyperhidrosis and dermatophytosis has also been reported in NPPK. Isolated cases of NPPK reported hyperkeratosis on the ears, toenail dystrophy, extensive erythema, and hyperkeratosis on the extremities and lumbar area. Sufficient information on NPPK manifestations, the pattern of autosomal recessive heredity, and the mutations in SERPINB7 can improve the precision of NPPK diagnosis.

3.2 | Pathogenesis of NPPK

SERPINB7, located on chromosome 18q21.3, encodes a subtype of clade-B serpins that inhibit serine proteases and prevent protease-mediated cell damage. SERPINB7 is abundantly expressed in the stratum granulosum all over the body, indicating its role in forming the stratum corneum. Currently, 13 distinct SERPINB7 mutations are reported, including mutations on exons and sequences at the exon–intron boundaries, which are predicted to trigger aberrant splicing of SERPINB7 mRNA or result in missense and frameshift variants or stop gaining protein synthesis. These SERPINB7 mutations may be equally pathogenic since none has undergone phenotype–genotype correlation. In NPPK skin lesions, loss-of-function of SERPINB7 mutations often induce overactivation of proteases, causing skin barrier defects with hyperkeratosis, mild inflammation, and increased water permeability. Although SERPINB7 expresses in the epidermis of the whole body, NPPK is restricted to the hands, feet, knees, and elbows, which suggests that chronic exposure to mechanical stress may cause the development of NPPK. In addition, more extensive manifestations, namely erythema and hyperkeratosis, on the extremities and lumbar may indicate other unknown modifier genes or environmental factors. Thus, mutations in SERPINB7 along with mechanical stress and other unknown factors cause NPPK-related cell damage.

3.3 | Mutations and founder effect of SERPINB7

To date, over 200 cases of NPPK are reported, and all of the 13 pathogenic mutations of SERPINB7 and the two novel mutations presented

| Affected individual | Sex/age (years) | Onset age | Transgressiens | Elbow/knee involvement | Hyperhidrosis | Dermatophytosis | Odor | White spongy appearance |
|--------------------|----------------|-----------|----------------|------------------------|---------------|----------------|-------|------------------------|
| 1                  | Female/27      | Birth     | +              | –                      | +             | –              | +     | +                      |
| 2                  | Male/9         | Birth     | –              | –                      | –             | –              | +     | +                      |
| 3                  | Male/43        | 6 months  | +              | +                      | +             | –              | +     | +                      |
| 4                  | Female/30      | 6 months  | +              | –                      | +             | –              | –     | +                      |
| 5                  | Female/20      | 3 months  | +              | –                      | +             | –              | +     | +                      |
| 6                  | Male/29        | 6 months  | –              | +                      | +             | –              | +     | +                      |
| 7                  | Male/11        | 5 months  | +              | +                      | +             | –              | +     | +                      |
| 8 (7F)             | Male/42        | 1 year    | +              | +                      | +             | –              | +     | +                      |

Abbreviation: 7F, father of patient 7.
in this research are summarized in Table 2. NPPK was long considered to be limited to China, Japan, and Korea. The founder effect was described for NPPK and mutations of SERPINB7 in NPPK populations. Data from the 1000 Genomes Project (http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) illustrated that most of the single nucleotide polymorphisms (SNP) only appear in East Asia. Among these mutations, the nonsense mutation c.796C>T is the most frequent with 2.43% allele frequency in the Chinese Han ethnicity in Beijing and 1.44% in Japanese ethnicity. Therefore c.796C>T was considered a strong potential founder mutation for East Asian ethnicities. Other SNPs considered as potential founder mutations in East Asia include c.455G>T, c.336+2T>G, and c.643A>G with 0.49% allele frequency in Chinese Han ethnicity in Beijing, while c.455-1G>A shows 0.48% allele frequency among the Japanese in Tokyo. This data suggests the potential founder effect of SERPINB7 in East Asia.

In 2020, two brothers with NPPK were reported in Thailand. Both showed compound heterozygous mutations for c.796C>T and c.650_653delCTGT in SERPINB7.8 Mutation c.796C>T was heterozygous in the Thai exome database with 0.6% allele frequency and c.650_653delCTGT with 0.4% allele frequency.9

For decades, no NPPK case was reported in areas other than Asia; thus, NPPK was scarcely known in Europe and the USA.10 One NPPK case of a young adopted Chinese girl living in France reported mutations in c.796C>T and c.650_653delCTGT of SERPINB7, which ignited the recognition of NPPK in Europe. So far, these case reports were limited to Asia until the newly reported Finnish case broke this balance.11 This novel Finnish mutation, c.1136G>A (rs201208667), has 0.51% allele frequency in Finnish Finland. Since both Asia and Europe belong to the Eurasian continent, the founder effect of SERPINB7 might provide evidence of the origin and migration of that population. Part of the pathogenic mutations of SERPINB7 is presented in Table 3.

Nagashima-type palmoplantar keratosis is characterized by an autosomal recessive inheritance, but a pseudodominant inheritance
TABLE 2  Summary of 15 mutations in SERPINB7 (data from NCBI, GRCh37)

| Nucleotide change | SNP name | Chromosome location | Genomic location | Amino acid change | Functional consequence | Population | References |
|-------------------|----------|---------------------|------------------|-------------------|------------------------|------------|------------|
| c.796C>T          | rs142859678 | chr18:61471522      | Exon 8           | p.Arg266*         | Stop gained            | +          | Kubo et al. (2013) |
| c.455-1G>A        | rs577442939 | chr18:61465837      | Intron 5         | p.Gly152Valfs*21  | Frameshift variant     | +          | Kubo et al. (2013) |
| c.218,219delAGinsTAACTTTACCT | rs797044479 | chr18:61459676–61459677 | Exon 3           | p.Gln73Leufs*17   | Frameshift variant     | +          | Kubo et al. (2013) |
| c.650,653delCTGT  | -        | chr18:61468152–61468155 | Exon 6           | p.Ser217Leufs*7   | Frameshift variant     | -          | Yin et al. (2014) |
| c.455G>T          | rs202182550 | chr18:61465838      | Exon 6           | p.Gly152Val       | Missense               | +          | Yin et al. (2014) |
| c.522_523insT (c.522dupT) | rs672601344 | chr18:61465905–61465906 | Exon 6           | p.Val175Cysfs*46  | Frameshift variant     | -          | Yin et al. (2014) |
| c.336+2 T>G       | rs201433665 | chr18:61460513      | Intron 5         | Predicted splicing alteration | Splice donor variant | +          | Mizuno et al. (2014) |
| c.830C>T          | rs1456356249 | chr18:61471556      | Exon 7           | p.Pro277Leu       | Missense               | +          | Shiohama et al. (2016) |
| c.122,127delITGGTCC | - | chr18:61449728–61449733 | Exon 2           | p.41_42del       | Codon mutation         | -          | Yao et al. (2016) |
| c.635delG         | rs773633666 | chr18:61468137      | Exon 6           | p.Lys213Serfs*12  | Frameshift variant     | +          | Nakajima et al. (2017) |
| c.382C>T          | rs143891736 | chr18:61463545      | Exon 4           | p.Arg128*         | Stop gained            | +          | Kubo et al. (2017) |
| c.271delC         | -        | -                   | Exon 3           | p.His91Thrfs*9    | Frameshift variant     | -          | Chen et al. (2018) |
| c.1136G>A         | rs201208667 | chr18:61471862      | Exon 8           | p.Cys379Tyr       | Missense               | -          | Hannula-Jouppi et al. (2020) |
| c.530 T>C         | rs769423314 | chr18:61465913      | Exon 6           | p.Phe177Ser       | Missense               | -          | Present case |
| c.643A>G          | rs200479020 | chr18:61468145      | Exon 7           | p.Asn215Asp       | Missense               | -          | Present case |
novel missense mutations confirmed their pathogenicity. Results of and −2.586 showed deleterious in both mutations. Using Mutation tivity, 0.14; specificity, 0.99). Relative Provean scores of −7.580 dictate the protein function in SERPINB7. Results in both c.530T (p.Phe177Ser) and c.643A >G (p.Asn215Asp) using PolyPhen-2 were found to be “probably damaging” with a score of 0.999 (sensitivity, 0.14; specificity, 0.99). Relative Provean scores of −7.580 and −2.586 showed deleterious in both mutations. Using Mutation Taster, disease causing and polymorphism were obtained relatively. Results of in silico analysis of three different software in the two novel missense mutations confirmed their pathogenicity.

3.4 Prediction of protein function in two novel mutations

Here we chose PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml), PROVEAN (http://sift.jcvi.org/index.php), and Mutation Taster (https://www.mutationtaster.org) to predict the protein function in SERPINB7. Results in both c.530T>C (p.Phe177Ser) and c.643A>G (p.Asn215Asp) using PolyPhen-2 were predicted to be “probably damaging” with a score of 0.999 (sensitivity, 0.14; specificity, 0.99). Relative Provean scores of −7.580 and −2.586 showed deleterious in both mutations. Using Mutation Taster, disease causing and polymorphism were obtained relatively. Results of in silico analysis of three different software in the two novel missense mutations confirmed their pathogenicity.

3.5 Treatment of NPPK

As a genetic disorder, there is no complete curative strategy for NPPK. Despite being a mild and nonprogressive palmoplantar keratosis, the high frequency of founder mutations in Asia makes the alleviation of symptoms essential. Topical treatments include vitamin D3 and keratolytic agents to reduce hyperkeratosis. In a previous study, the topical application of a 10% aluminum potassium sulfate lotion, with or without the application of 2.5% benzoyl peroxide gel, improved the subjective symptoms and odor of palmoplantar hyperhidrosis.

Research on the application of gentamicin to manage NPPK is underway. According to Ohguchi et al., gentamicin restores full-length SERPINB7 via c.796C>T readthrough in CDNA and enhances in vitro production of full-length SERPINB7 protein in NPPK keratinocytes. Most recently, a double-blind vehicle-controlled study on topical application of gentamicin in 20 NPPK patients was carried out by Li et al. in 2021. Usage of both 0.1% and 0.3% gentamicin ointment showed significant improvement in hyperkeratosis and foul smell but not erythema in homozygous or heterozygous patients for c.796C>T. These in vivo and in vitro experiments proved the potency of gentamicin for therapies of nonsense mutations in SERPINB7. However, topical therapies only showed temporary effects and most patients relapsed after discontinuation of treatment.

In conclusion, this case report identifies two novel mutations (c.530T>C and c.643A>G), which explain the causative mutations in SERPINB7. However, evaluation of additional clinical cases and genetic studies of NPPK are necessary to identify the pathogenic mechanism of NPPK. A large case–control study and follow-up studies are required to evaluate the long-term safety and curative effect of promising therapies.

ACKNOWLEDGMENTS

This work was supported by the District Science Foundation Program (NSFC no. 81974472) from the National Natural Science

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**TABLE 3** Sample counts in six SNP of SERPINB7 according to the 1000 Genomes Project (phase 3)

| Populations/mutations | rs142859678 c.796C>T | rs202182550 c.455G>T | rs201433665 c.336+2T>G | rs57744293 c.455-1G>A | rs200479020 c.643A>G | rs201208667 c.1136G>A |
|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Chinese Dai in Xishuangbanna, China | C = 93/93 T = 1/93 | T = 0/93 G = 0/93 | G = 0/93 A = 0/93 | G = 0/93 A = 0/93 | G = 0/93 A = 0/93 | G = 0/93 A = 0/93 |
| Han Chinese in Beijing, China | C = 103/103 T = 5/103 | T = 1/103 G = 1/103 | G = 1/103 A = 1/103 | G = 1/103 A = 1/103 | G = 1/103 A = 1/103 | G = 1/103 A = 1/103 |
| Southern Han Chinese | C = 105/105 T = 2/105 | T = 0/105 G = 0/105 | G = 0/105 A = 0/105 | G = 0/105 A = 0/105 | G = 0/105 A = 0/105 | G = 0/105 A = 0/105 |
| Japanese in Tokyo, Japan | C = 104/104 T = 3/104 | T = 0/104 G = 0/104 | G = 0/104 A = 0/104 | G = 0/104 A = 0/104 | G = 0/104 A = 0/104 | G = 0/104 A = 0/104 |
| Kinh in Ho Chi Minh City, Vietnam | C = 99/99 T = 1/99 | T = 0/99 G = 0/99 | G = 0/99 A = 0/99 | G = 0/99 A = 0/99 | G = 0/99 A = 0/99 | G = 0/99 A = 0/99 |
| Other populations* | C = 2000/2000 T = 0/2000 | T = 0/2000 G = 0/2000 | G = 0/2000 A = 0/2000 | G = 0/2000 A = 0/2000 | G = 0/2000 A = 1/2000 |
| Total | C = 2504/2504 T = 12/2504 | T = 1/2504 G = 1/2504 | G = 1/2504 A = 1/2504 | G = 1/2504 A = 1/2504 | G = 1/2504 A = 1/2504 | G = 1/2504 A = 1/2504 |

Abbreviation: SNP, single nucleotide polymorphism.

* Other populations include Yoruba in Ibadan, Nigeria; Toscani in Italy; Sri Lankan Tamil from the UK; Puerto Ricans from Puerto Rico; Punjabi form Lahore, Pakistan; Peruvians from Lima, Peru; Mexican Ancestry from Los Angeles USA; Mende in Sierra Leone; Luhya in Webuye, Kenya; Indian Telugu from the UK; Iberian Population in Spain; Gambian in Western Division in the Gambia; Gujarati Indian from Houston, Texas; British in England and Scotland; Finnish in Finland; Esan in Nigeria; Colombians from Medellin, Colombia; Utah Residents (Centre d’Etudes du Polymorphisme Humain) with North and Western European Ancestry; Bengali from Bangladesh; American of American Ancestry in southwest USA; and African Caribbeans in Barbados.

The only sample count with a Finnish origin, Finland.

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Foundation of China, and (2019JM-565) from the Natural Science Foundation of Shaanxi Province in China.

CONFLICT OF INTEREST
None declared.

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REFERENCES
1. Kabashima K, Sakabe J, Yamada Y, Tokura Y. "Nagashima-type" keratosis as a novel entity in the palmoplantar keratoderma category. Arch Dermatol. 2008;144:375–9.
2. Kubo A, Shiohama A, Sasaki T, Nakabayashi K, Kawasaki H, Atsugi T, et al. Mutations in SERPINB7, encoding a member of the serine protease inhibitor superfamily, cause Nagashima-type palmoplantar keratosis. Am J Hum Genet. 2013;93:945–56.
3. Yin J, Xu G, Wang H, Zhao J, Duo L, Cao X, et al. New and recurrent SERPINB7 mutations in seven Chinese patients with Nagashima-type palmoplantar keratosis. J Invest Dermatol. 2014;134:2269–72.
4. Nakajima K, Ishiguro M, Shiohama A, Kubo A, Sano S. Novel frameshift mutation in SERPINB7 in a Japanese patient with Nagashima-type palmoplantar keratosis. J Dermatol. 2017;44:841–3.
5. Mizuno O, Nomura T, Suzuki S, Takeda M, Ohguchi Y, Fujita Y, et al. Highly prevalent SERPINB7 founder mutation causes pseudodominant inheritance pattern in Nagashima-type palmoplantar keratosis. Br J Dermatol. 2014;171:847–53.
6. Miyauchi T, Nomura T, Suzuki S, Ohguchi Y, Yamaguchi Y, Shinkuma S, et al. Extensive erythema and hyperkeratosis on the extremities and lumbar area as an unusual Mani-festation of Nagashima-type palmoplantar keratosis. Acta Derm Venereol. 2016;96:856–8.
7. Nakamizo S, Katoh N, Miyachi Y, Kabashima K. Atypical nail dystrophy in a possible case of Nagashima-type palmoplantar keratosis. J Dermatol. 2012;39:470–1.
8. Suzuki S, Nomura T, Mizuno O, Fujita Y, Shimizu H. Identification of previously unknown SERPINB7 splice variants in patients with Nagashima-type palmoplantar keratosis reveals the importance of the CD-loop of SERPINB7. Br J Dermatol. 2015;173:1288–90.
9. Songsantiphap C, Suwanwatana J, Ittiwut C, Asawanonda P, Rerknimitr P, Shotelersuk V. Nagashima-type palmoplantar keratosis with compound heterozygous mutations in SERPINB7. Case Rep Dermatol. 2020;12:241–8.
10. Chassain K, Croue A, Blanchard E, Leclerc-Mercier S, Fischer J, Martin L. Nagashima-type palmoplantar keratoderma: A little-known palmoplantar keratoderma in Europe. Ann Dermatol Venereol. 2019;146:125–30.
11. Hannula-Jouppi K, Harjama L, Einarsdottir E, Elomaa O, Kettunen K, Saarela J, et al. Nagashima-type palmoplantar keratosis in Finland caused by a SERPINB7 founder mutation. J Am Acad Dermatol. 2020;83:643–5.
12. Pasmooij AMG. Topical gentamicin for the treatment of genetic skin diseases. J Invest Dermatol. 2018;138:731–4.
13. Katsuno M, Shiohama A, Aoki S, Kitamura H, Sasaki T, Amagai M, et al. Novel nonsense mutation in SERPINB7 and the treatment of foot odor in a patient with Nagashima-type palmoplantar keratosis. J Dermatol. 2017;44:e146–7.
14. Ohguchi Y, Nomura T, Suzuki S, Takeda M, Miyauchi T, Mizuno O, et al. Gentamicin-induced readthrough and nonsense-mediated mRNA decay of SERPINB7 nonsense mutant transcripts. J Invest Dermatol. 2018;138:836–43.
15. Li Y, Yu X, Pan C, Wang Y, Han J, Yao Z, et al. Effect of gentamicin ointment in patients with Nagashima-type palmoplantar keratosis: a double-blind vehicle-controlled study. Acta Derm Venereol. 2021;101:adv00392.

How to cite this article: Xiao T, Liu Y, Wang T, Ren J, Xia Y, Wang X. Two novel mutations of SERPINB7 in eight cases of Nagashima-type palmoplantar keratosis in the Chinese population. The Journal of Dermatology. 2022;49:539–544. https://doi.org/10.1111/1346-8138.16310