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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**

Department of Dermatology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, China

Correspondence
Xiaopeng Wang, Department of Dermatology, The Second Affiliated Hospital of Xi'an Jiaotong University, 157 Xiwu Road, Xi'an, Shaanxi 710004, China.
Email: wxpdoctor@mail.xjtu.edu.cn

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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.[1] In 2013, Kubo *et al*. identified *SERPINB7* as the gene responsible for NPPK, following which NPPK became widely known in Asia.[2] 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.[2–4] 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.[5]

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.[1] 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.[1] Isolated cases of NPPK reported hyperkeratosis on the ears, toenail dystrophy, extensive erythema, and hyperkeratosis on the extremities and lumbar area.[6,7] 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.[2,5]

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* pre-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.[8] 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.[2] 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.[6] 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

TABLE 1 Summary of clinical manifestations of eight patients

Affected individual	Sex/age (years)	Onset age	Transgrediens	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.

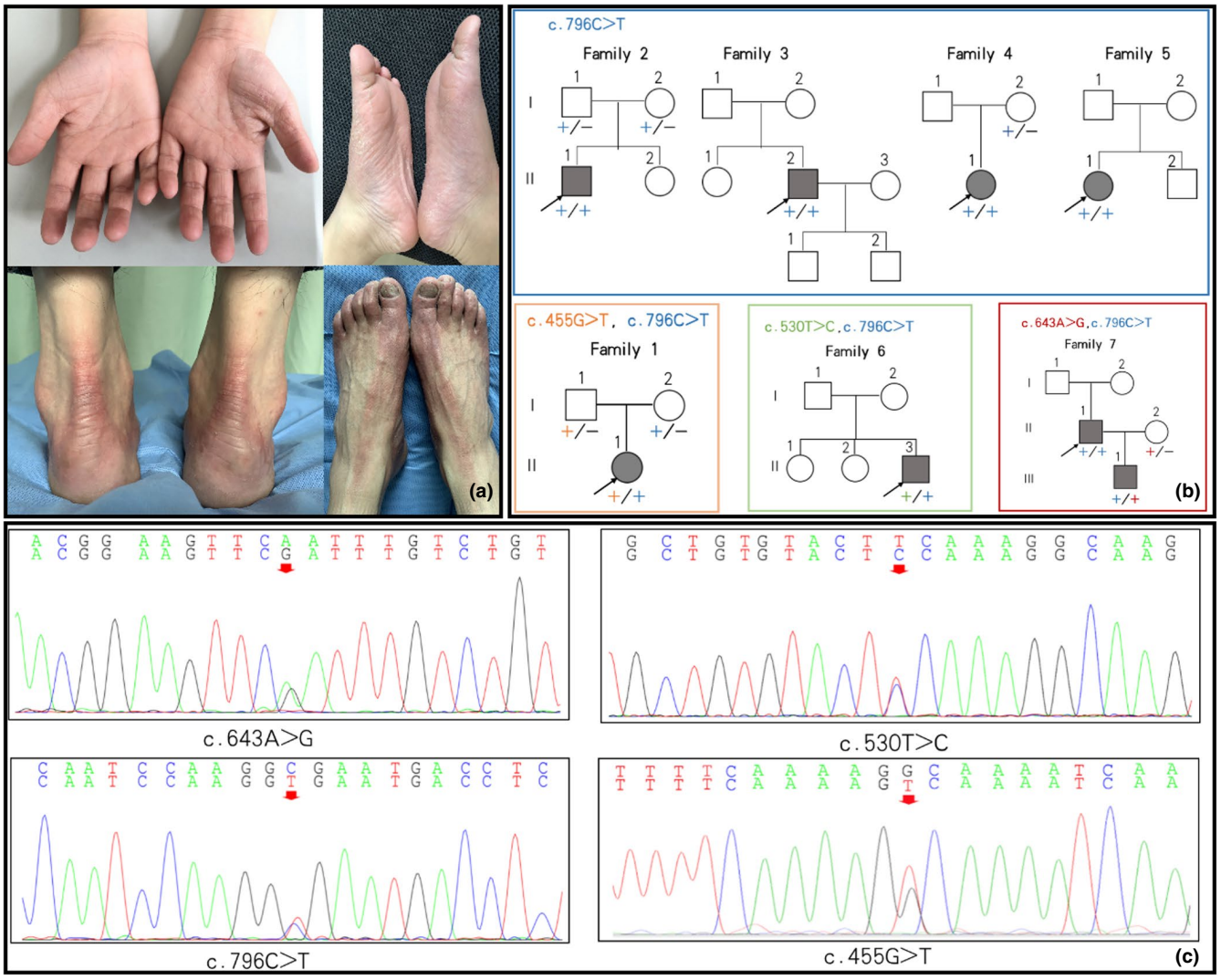

FIGURE 1 (a) Clinical presentation. Bilateral redness and mild hyperkeratosis of the palms and soles, extending to inner wrists, dorsal feet, and Achilles tendon, accompanied by mild desquamation. (b) Pedigrees for the eight patients. (c) Mutated sequences of affected individuals

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.[5] 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*.[9] 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
						Japanese	Chinese	Korean	
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_219delAGinsTAAACTTTACCT	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 alternation	Splice donor variant	+	+	−	Mizuno *et al.* (2014)
c.830C>T	rs1456356249	chr18:61471556	Exon 7	p.Pro277Leu	Missense	+	−	−	Shiohama *et al.* (2016)
c.122_127delTGGTCC	−	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	rs1433891736	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

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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	rs577442939 c.455-1G>A	rs200479020 c.643A>G	rs201208667 c.1136G>A
Chinese Dai in Xishuangbanna, China	C = 93/93 T = 1/93	G = 93/93 T = 0/93	T = 93/93 G = 0/93	G = 93/93 A = 0/93	A = 93/93 G = 0/93	G = 93/93 A = 0/93
Han Chinese in Beijing, China	C = 103/103 T = 5/103	G = 103/103 T = 1/103	T = 103/103 G = 1/103	G = 103/103 A = 0/103	A = 103/103 G = 1/103	G = 103/103 A = 0/103
Southern Han Chinese	C = 105/105 T = 2/105	G = 105/105 T = 0/105	T = 105/105 G = 0/105	G = 105/105 A = 0/105	A = 105/105 G = 0/105	G = 105/105 A = 0/105
Japanese in Tokyo, Japan	C = 104/104 T = 3/104	G = 104/104 T = 0/104	T = 104/104 G = 0/104	G = 104/104 A = 1/104	A = 104/104 G = 0/104	G = 104/104 A = 0/104
Kinh in Ho Chi Minh City, Vietnam	C = 99/99 T = 1/99	G = 99/99 T = 0/99	T = 99/99 G = 0/99	G = 99/99 A = 0/99	A = 99/99 G = 0/99	G = 99/99 A = 0/99
Other populations[a]	C = 2000/2000 T = 0/2000	G = 2000/2000 T = 0/2000	T = 2000/2000 G = 0/2000	G = 2000/2000 A = 0/2000	A = 2000/2000 G = 0/2000	G = 2000/2000 A = 1/2000[b]
Total	C = 2504/2504 T = 12/2504	G = 2504/2504 T = 1/2504	T = 2504/2504 G = 1/2504	G = 2504/2504 A = 1/2504	A = 2504/2504 G = 1/2504	G = 2504/2504 A = 1/2504

Abbreviation: SNP, single nucleotide polymorphism.

[a] Other populations include Yoruba in Ibadan, Nigeria; Toscani in Italia; 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 Divisions 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.

[b] The only sample count with a Finnish origin, Finland.

pattern occurs in populations with highly prevalent *SERPINB7* founder mutation. In 2014, Mizuno *et al*. reported a pedigree in an affected father and all three offspring, apparently in autosomal dominant inheritance. Whole-exome sequencing identified a homozygous *SERPINB7* mutation in this family with NPPK.[5] In our case report, family 7 shows a similar pseudodominant inheritance pattern in NPPK-affected father and son. This finding further demonstrates high frequencies of SNP in *SERPINB7*, especially the mutation c.796C>T.

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 D_3 and keratolytic agents to reduce hyperkeratosis.[12] 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.[13]

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.[14] 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.[15] 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.

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CONFLICT OF INTEREST

None declared.

ORCID

Yumin Xia https://orcid.org/0000-0002-3493-7198
Xiaopeng Wang https://orcid.org/0000-0001-6905-4734

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