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ORIGINAL CONTRIBUTION

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A *KRT16* mutation in the first Chinese pedigree with Pachyonychia congenita and review of the literatures

Qiaohu Xu MS[1,2] | Qun Zhang MS[1,2] | Lili Tang MD, PhD[1,2] | Ling Jin MS[1,2] | Xiaomeng Wang MS[1,2] | Lixin Kan PhD[3,4] | Xiaodong Zheng PhD[2] | Sen Yang MD, PhD[1,2]

[1]Department of Dermatology, the First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China

[2]Key Laboratory of Dermatology of Ministry of Education, Anhui Medical University, Anhui, China

[3]School of Basic Medical Sciences, Anhui Medical University, Anhui, China

[4]Department of Neurology, Northwestern University, Chicago, Illinois

Correspondence
Sen Yang, Department of Dermatology, the First Affiliated Hospital of Anhui Medical University, Key Laboratory of Dermatology of Ministry of Education, Anhui Medical University, Anhui, China.
Email: yang2004sen@163.com
and
Xiaodong Zheng, Key Laboratory of Dermatology of Ministry of Education, Anhui Medical University, Anhui, China.
Email: zhengxiaodong.1234@163.com

Summary

Background: Pachyonychia congenita (PC), a rare autosomal dominant disorder, is featured by significant hypertrophic nail, palmoplantar keratoderma, and plantar pain. It is caused by the mutation of KRT6A, KRT6B, KRT6C, KRT16, or KRT17.

Aims: To identify the gene mutation caused the PC in a Chinese family.

Patients/Methods: Genomic DNA was extracted from peripheral blood samples of five patients and six healthy individuals. Genomic DNA of three patients was sequenced by whole-exome sequencing (WES). Then, exons 6 of KRT16 of all samples were amplified by polymerase chain reaction (PCR), and PCR products were sequenced to identify potential mutations.

Results: We identified the proline substitution mutation p.Leu421Pro (c.1262T>C) in the 2B domain of K16 that is associated with PC in a Chinese family. The same mutation was not found in the six healthy individuals of the family.

Conclusions: The mutation found in this study is the first report in China. So far, 25 mutations in KRT16 have been reportedly associated with PC. Twenty-one mutations are located on exon 1, and four mutations on exon 6.

KEYWORDS

Chinese, *KRT16*, Pachyonychia congenita, palmoplantar keratoderma

1 | INTRODUCTION

Pachyonychia congenita (PC, OMIM #615726, #615728, #615735, #167200, #167210) is a rare, autosomal dominant disorder which is featured by hypertrophic onychodystrophy, palmoplantar keratoderma,[1] oral leukoplakia, cysts, follicular hyperkeratosis, hyperhidrosis, hoarseness, and fetal teeth.[2] It is estimated there are between 2000 and 10 000 cases of PC worldwide (http://www.pachyonychia.org/).[1] So far, PC has been reported to be caused by mutations of *KRT6A*, *KRT6B*, *KRT6C*, *KRT16*, and *KRT17*. The majority of these mutations are missense mutations, including deletions, insertions, and alternative splice site mutation.[3] Based on the reported five identified disease genes, PC is subdivided into five subtypes, namely PC-K6a, PC-K6b, PC-K6c, PC-K16, and PC-K17.[4] The PC-K6a mutation accounted for 38%, PC-K6b 9%, PC-K6c only 3%, PC-K16 33%, and PC-K17 17%.[1] It is worth to stress the clinical features of five subtypes are overlapping and the atypical symptoms could be easily overlooked or even misdiagnosed.

Here, we first report the identification of a proline substitution mutation p.Leu421Pro in the 2B domain of K16 that is associated with PC in a Chinese family. The patients were classified as PC-K16.

Xu, Zhang, and Tang contribute equally to this work.

2 | MATERIAL AND METHODS

2.1 | Clinical features

There were five male and one female patients in this three-generation pedigree (Figure 1). The 48-year-old male proband apparently has compact, thick and yellowish symmetrical, hyperkeratotic lesions since he was 1 year old, which was mainly on the heels and the frontal areas of the soles, as well as the external and internal side of the first and fifth toes, while the internal side of the fourth toe, the thenar, and hypothenar areas were largely spared. Some toenails appeared to be slight thickened or atrophy around at 10 years old. Parts of hyperkeratotic edges of fingernails were found to be slightly yellowish, and black lines were also found at the tip of some nails (Figure 2). The patient feels pain while walking and has sweaty feet. No signs of oral damage, hoarseness, abnormal hair, epidermal cysts, or blister were observed. Other patients of the pedigree were displayed similar clinical symptoms.

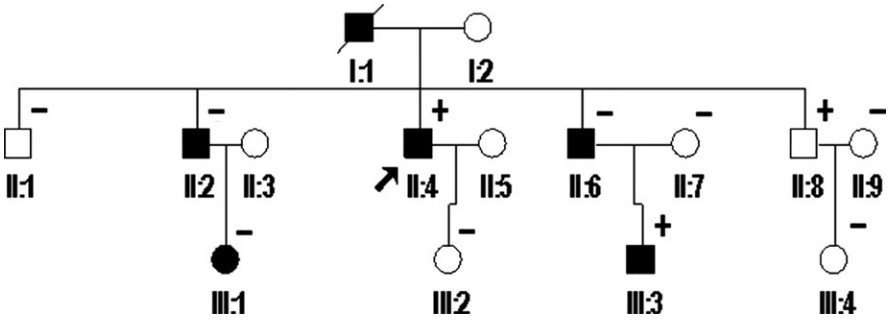

FIGURE 1 The pedigree of PC. "+" indicates the individuals who were examined by whole-exome sequencing. "–" indicates the ones who were examined by Sanger sequencing

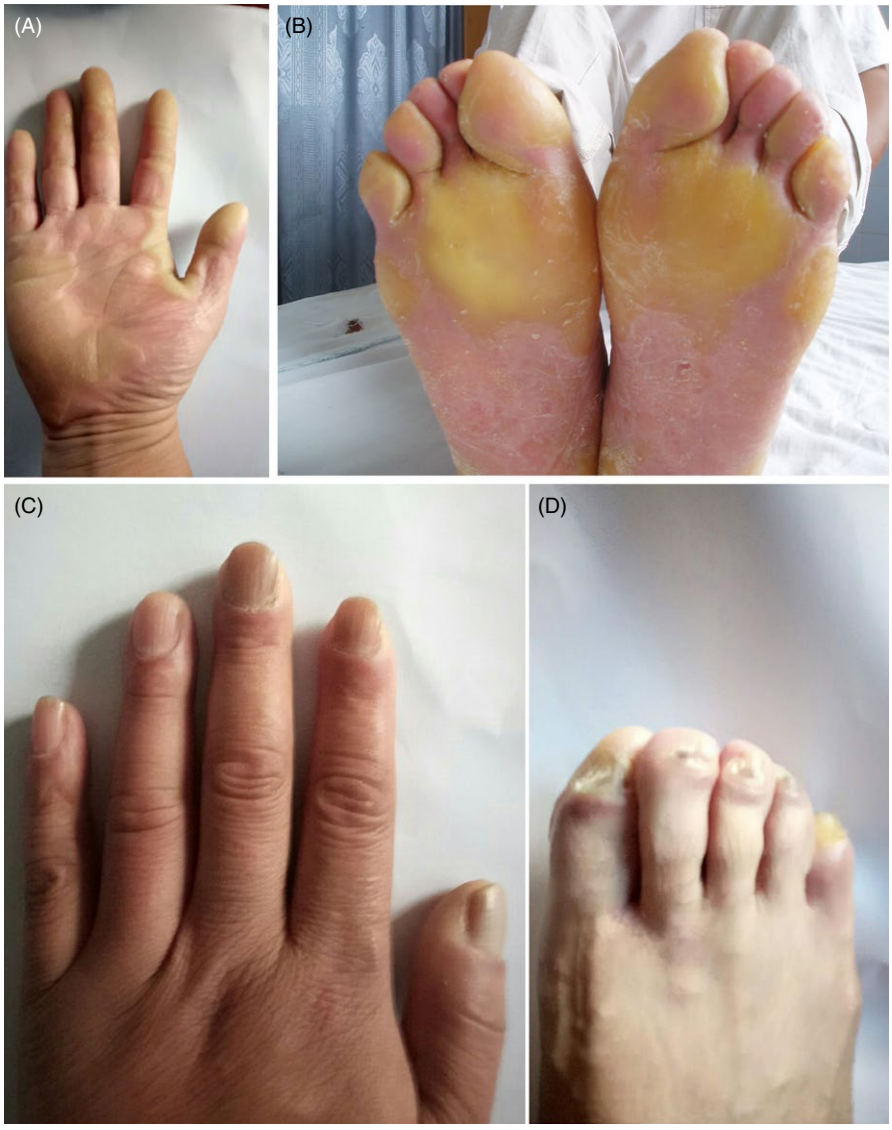

FIGURE 2 The clinical lesions of the proband. The images show thickening and hyperkeratosis on palms (A), soles (B), thicken nails (C), and dystrophy toenails (D)

2.2 | Blood sample

With written informed consent from all participants, blood samples were collected from eleven case for genomic DNA extracted and PCR.

2.3 | Sequencing analysis

Genomic DNA was extracted from peripheral blood samples of five patients and six healthy individuals (Figure 1). All exons of *KRT16* were sequenced by WES in the II:4, II:8, and III:3. *KRT16* of all samples were examined by Sanger sequencing to verify the results of WES. This mutation in PC-K16 was identified on exon 6 by WPS. We further analyzed exon 6 of the KRT16 gene with the following primers: forward 5′-TAG TGG GCT AGC TTT TCGCC-3′, reverse 5′-GGA TTG GCC AGATGC TTG CT-3′.

Polymerase chain reaction (PCR) conditions were initial denaturation at 95°C for 5 minutes, and 35 cycles consisting of 94°C for 30 seconds, 58°C for 35 seconds, 72°C for 40 seconds, and a final extension at 72°C for 10 minutes. The PCR products (400 bp) were visualized on 1.5% agarose gel with ethidium bromide staining before re-sequenced using ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA) at Shanghai Sangon Biotech Co., Ltd., China.

3 | RESULTS

A new heterozygous missense mutation (1262T>C) (NM_005557.3) was identified in exon 6 of KRT16 in proband and other patients, which resulted in substitution of cytosine for thymine, and substitution of proline acid for leucine acid at position 421 of the KRT16 protein. The same mutation was not found in the six healthy individuals of the family (Figure 3).

4 | DISCUSSION AND LITERATURE REVIEW

Pachyonychia congenita is a group of rare inherited ectodermal dysplasias disorder. We identified a p.Leu421Pro(c.1262T>C) in helix termination motif (HTM) of the 2B subdomain of K16 in a pedigree. This mutation has only been reported in a Spanish family before[5] therefore, this is the first report of this mutation in a Chinese PC Pedigree. Probands of both Spanish and Chinese families exhibit similar clinical features, including significantly focal plantar keratoderma where the primary stress-bearing part within the sole of the foot around two years old, toenails dystrophy occurred in childhood (about 10 years old), and the slightly affected fingernails. Both families had this mutation site without other accompanied mutations.[5] Interestingly, in this site, so far, no other mutation types have been reported.

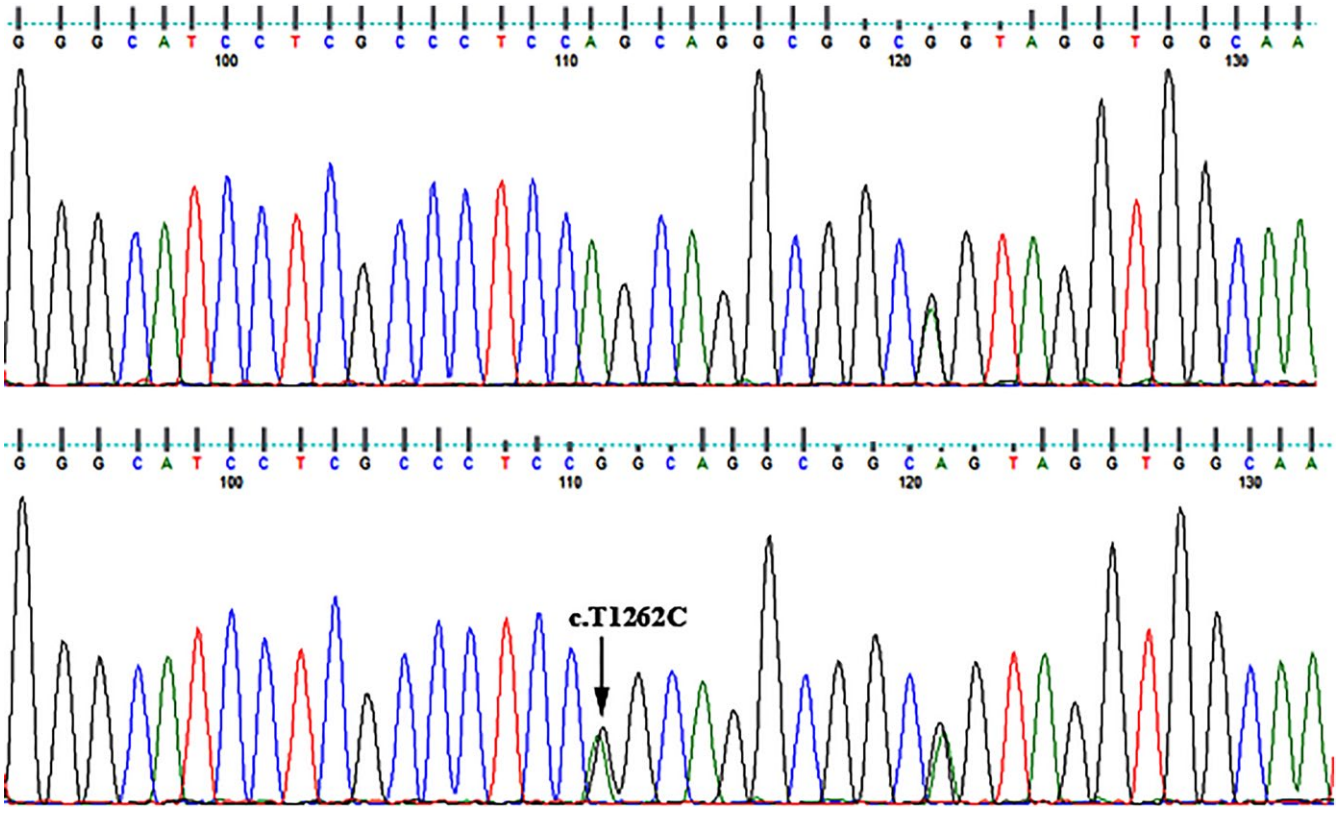

FIGURE 3 The first part is the KRT16 sequencing map of the unaffected members, and the second part is the mutation site map of the affected members in the family (C.1262T>C)

Till now, 25 mutations in *KRT16* have been reported to be causative of PC, of which 20 were missense mutations and 5 were deletions (Table 1). Twenty-one mutations are located in the 1A region and four in the 2B region. The *KRT16* mutation hotspots are currently concentrated in the 1A domain, such as the 124th, 125th, 127th, and 132th codons.[6,7] The missense mutations for c.374A>G, c.379C>T, c.395T>C, and c.380G>C accounted for 28.6%, 20.0%, 11.4%, and 7.6% of *KRT16* mutations, respectively. Generally, mutation of 2B area presented milder nail change compared with 1A area.[8] We also found that PC-K16 showed significant palmoplantar keratoderma and early-onset plantar pain, and the change of nail was later (around 10 years old).

Keratin is consisting of α-helical central rod domain comprising four domains (1A, 1B, 2A and 2B) connected by nonhelical regions (L1, L12 and L2). The majority of mutations causing PC are located in 1A and 2B which is evolutionary conserved in all intermediate filaments.[9] With the replacement of amino acids within the helix boundary motif domains of K16, it affects the structure of the peptide chain from two aspects. Firstly, amino acid structure alone affects the structure of the peptide chain. We found that 7 of the 25 mutant sites resulted in proline substitution. The bulky proline side chain is being strongly detrimental to the helical tertiary structures, which is particularly disruptive to the assembly and function of intermediate filaments.[10] Secondly, amino acid PH value as well as polarity also affects the structure of the peptide chain. It is essential that obligate heterodimers are formed by parallel alignment of type I acidic keratin and type II basic keratin (K16/K6a, K17/K6b; http://www.interfil.org). Most of the substituted amino acids are neutral or alkaline. For example, with the p.Lys354Asn mutation, the nonconserved substitution of a highly basic lysine residue for a neutral/polar asparagine residue in this coiled-coil domain is likely to be detrimental to keratin assembly and/or integrity.[10]

Mutations in K16 can also lead to focal nonepidermolytic plantar keratoderma (FNEPPK), which features mild to severe focal plantar keratoderma with mild nail changes.[8] This is very similar to the phenotype of patients in this study and requires genetic testing to confirm the diagnosis. Different amino acid substitutions at the same mutation site can cause PC or FNEPPK. For example, Liao et al[8] reported a case with mutation in *KRT16* which resulted in deletion of 24 bp c.1052_1059+16del24 across the exon 5/intron 5 junction, which caused a predominant FNEPPK phenotype.

TABLE 1 Summury of the mutations of KRT16 in PC patients

No.	cDNA variant	Protein variant	Variant types	Domain	Exon	Proportion (%)
1	c.25delA	p.Thr9ProfsX6	Deletion	1A	1	1.0
2	c.43A>T	p.Lys15X	Substitution	1A	1	1.0
3	c.362T>C	p.Met121Thr	Substitution	1A	1	1.9
4	c.362T>A	p.Met121Lys	Substitution	1A	1	1.9
5	c.365A>C	p.Gln122Pro	Substitution	1A	1	1.0
6	c.371T>G	p.Leu124Arg	Substitution	1A	1	1.9
7	c.371T>C	p.Leu124Pro	Substitution	1A	1	1.0
8	c.371T>A	p.Leu124His	Substitution	1A	1	2.9
9	c.371_373delTCA	p.Leu124_Asn125delinsHis	Indel	1A	1	1.0
10	c.373A>G	p.Asn125Asp	Substitution	1A	1	4.8
11	c.373_374delAAinsGG	p.Asn125Gly	Substitution	1A	1	1.0
12	c.374A>G	p.Asn125Ser	Substitution	1A	1	28.6
13	c.379C>T	p.Arg127Cys	Substitution	1A	1	20.0
14	c.379C>G	p.Arg127Gly	Substitution	1A	1	1.0
15	c.379C>A	p.Arg127Ser	Substitution	1A	1	1.0
16	c.380G>C	p.Arg127Pro	Substitution	1A	1	7.6
17	c.380G>A	p.Arg127His	Substitution	1A	1	1.0
18	c.383T>A	p.Leu128Gln	Substitution	1A	1	1.9
19	c.383T>C	p.Leu128Pro	Substitution	1A	1	1.0
20	c.389_391delCCT	p.Ser130del	Deletion	1A	1	4.8
21	c.395T>C	p.Leu132Pro	Substitution	1A	1	11.4
22	c.1062A>T	p.Lys354Asn	Substitution	2B	6	1.0
23	c.1253G>C	p.Arg418Pro	Substitution	2B	6	1.0
24	c.1253_1258delGCCGCC	p.Arg418_Arg419del	Deletion	2B	6	1.0
25	c.1262T>C	p.Leu421Pro	Substitution	2B	6	1.0

Therefore, we believe that the more influential of the structure of the peptide chain, the more severe the clinical symptoms of the mutation will cause.

Of note that not all PC patients show classical nail damage. For example, in our case, the proband's nails do not show typical hypertrophic nail dystrophy, and slight nail dystrophy only occurred around 10 years old with no other accompanying symptoms; therefore, this can be easily misdiagnosed as onychomycosis combined with callus and subsequently treated by systemic and local aggressive antifungal therapy. Although nail infection is sometimes a problem, it is not the main cause of his hypertrophic nail dystrophy.[1]

In summary, p.Leu421Pro (c.1262T> C) was first reported in Chinese PC pedigree, which is classified as PC-K16. The symptoms of this mutation were relatively mild, and the treatment was mainly to remove the hyperplasia cuticle skin regularly, wear soft shoes and socks, and avoid long walking.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest. The written informed consent form was obtained from patients.

ORCID

Qiaohu Xu https://orcid.org/0000-0002-8907-9688

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