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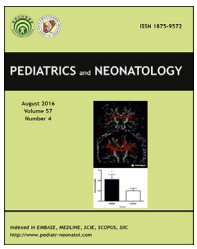

Original Article

Phenotype and genotype features of Vietnamese children with pachyonychia congenita

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Key Words
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pachyonychia congenita

Background: Pachyonychia congenita (PC) is a group of autosomal dominant disorders caused by mutations in one of five keratin genes (*KRT6A*, *KRT6B*, *KRT6C*, *KRT16*, or *KRT17*). PC is an extremely rare condition. To our knowledge, this is the largest genotype—phenotype study of PC in a Vietnamese population to date.
Materials and methods: We investigated keratin gene mutations and clinical features of seven Vietnamese children with PC.
Results: The seven Vietnamese patients were from six different families (two patients in the same family) from across Northern, Central, and Southern Vietnam. All children displayed PC symptoms before 1 year of age, but diagnosis was delayed in 4/7 patients. Thick fingernails, thick toenails, oral leukokeratosis, and follicular hyperkeratosis were the most common features recorded by all seven patients. Plantar keratoderma and thick fingernails were the clinical features associated with the most significant effect on daily function. All patients had mutations in *KRT6A* (PC—K6a) focused on the 1A and 2B domains. We found three distinct types of mutations (K6a R466P, K6a N171K, and K6a N172del). One mutation (N172del) was common to 5/7 (71.4%) of the patients.

Abbreviations: PC, Pachyonychia congenita; IPCRR, The International Pachyonychia Congenita Research Registry.

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Conclusions: Individuals displaying nail dystrophy, oral leukokeratosis, follicular hyperkeratosis, and plantar keratoderma should be referred for genetic testing given the high likelihood of a PC-K6a-related mutation in patients with this constellation of clinical signs.

1. Introduction

Pachyonychia congenita (PC) is a very rare hereditary skin disorder characterized by severe, painful, and highly debilitating plantar keratoderma, variable hypertrophic nail dystrophy, epidermal cysts, leukokeratosis, and other features.[1] PC is associated with mutations in any one of five keratin genes, *KRT6A, KRT6B, KRT6C, KRT16,* or *KRT17,* encoding the differentiation-specific keratins K6a, K6b, K6c, K16, and K17, respectively. Due to the rarity of PC, many other genodermatoses with overlapping clinical features can be misdiagnosed as PC.[1,2] Therefore, genetic testing of patients with PC is critical for diagnostic confirmation and genetic counseling.[3,4] The International Pachyonychia Congenita Research Registry (IPCRR, WCG IRB #20040468), sponsored by the Pachyonychia Congenita Project, was created in 2004 and has since collected data on over 2000 patients suspected of having PC. Free genetic testing is provided for patients in the IPCRR. Research based on IPCRR data has shown significant overlap among previously identified types of PC. Therefore, a new classification system was proposed in 2011 that grouped PC into five types representing the specifically affected genes.[5–8]

There have been few reports of PC in developing countries. Vietnam is a tropical country where infectious diseases are common in the pediatric population. After many years of war and low socio-economic conditions, we often diagnose PC based on clinical manifestations and family history, so it is easily misdiagnosed. In this study, we report on seven Vietnamese children with PC confirmed by keratin mutation analysis. To our knowledge, this is the largest genotype–phenotype study of PC in a Vietnamese population to date attempting to improve the diagnosis and management of PC in Vietnam.

2. Methods

2.1. Patients

We studied seven Vietnamese children with PC. The diagnostic process involved the following steps: (1) we performed preliminarily screening of children with nail abnormalities to select patients with a PC-compatible phenotype; and (2) each selected patient was required to complete a detailed questionnaire provided by the IPCRR (www.pachyonychia.org) and provide information regarding whether and to what extent they were affected by the clinical signs of PC. Patients were also asked for additional information, such as the age of symptom onset and the impact that each feature had on their quality of life. To complete the questionnaire, patients were required to submit photographs of visible nail and skin changes to the IPCRR. All patients were diagnosed using a detailed clinical questionnaire and identification of mutation in a PC-associated gene according to genetic testing results. The study was conducted based on the principles of the Declaration of Helsinki and all patients provided written informed consent.

2.2. Mutation detection

After successful registration with the IPCRR, genetic testing was performed free of charge. In brief, saliva samples were collected using Oragene Dx OGD-500 kits (DNA Genotek, Ottawa, ON, Canada) and genomic DNA was extracted using a QiaAmp DNA mini kit (Qiagen, Germantown, MD, U.S.A.). In most cases, genetic testing was completed at the University of Dundee (School of Life Sciences, Dundee, U.K.) by polymerase chain reaction (PCR) and Sanger sequencing of the coding regions of five PC-associated keratin genes using primers specific to each gene. In cases where no mutation was identified in the keratin genes, patients were carefully re-examined to determine which other candidate genes would be screened by Sanger sequencing. The results for all cases were confirmed by independent genetic testing by a certified U.S. clinical laboratory (GeneDx, Gaithersburg, MD, U.S.A.).[1,6]

3. Results

3.1. Clinical details

The seven Vietnamese patients were from six different families from across Northern, Central, and Southern Vietnam (Table 1). All children displayed PC symptoms before 1 year of age, but only two patients were diagnosed at 1 year of age due to our experience with previous patients. Thick fingernails, thick toenails, oral leukokeratosis, and follicular hyperkeratosis were the most common features recorded by all seven patients (Table 2 and Fig. 1). Plantar and palmar keratoderma were observed in three and two of the seven patients, respectively. Cysts, hoarseness, and natal teeth were not documented in our patients.

The effects of the characteristic features of PC (thick fingernails, thick toenails, oral leukokeratosis, follicular hyperkeratosis, and plantar keratoderma) on the quality of

Table 1 Demographics of 7 Vietnamese children with pachyonychia congenita.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age at onset (Y)	<1	<1	<1	<1	<1	<1	<1
Age at diagnosis (Y)	1	4	5	1	3	2	7
Present age (Y)	4	8	10	4	8	5	10
Sex	M	M	F	M	F	F	M
Positive family history	No	No	No	Yes	No	No	Yes
Location of residence	N	C	N	S	N	S	S
Gene mutation	*KRT6A*	*KRT6A*	*KRT6A*	*KRT6A*	*KRT6A*	*KRT6A*	*KRT6A*
Specific change	N172del	N172del	N171K	N172del	R466P	N172del	N172del
Mutated domain	1A	1A	1A	1A	2B	1A	1A

Abbreviations: Y, year; M, male; F, female; N, Northern Vietnam; S, Southern Vietnam; C, Central Vietnam.

Table 2 Prevalence of selected clinical features in 7 children with pachyonychia congenita.

Phenotype	Total (n = 7)	%
Thick toenails	7/7	100
Thick fingernails	7/7	100
Oral leukokeratosis	7/7	100
Follicular hyperkeratosis	7/7	100
Plantar keratoderma	3/7	42
Palmar keratoderma	2/7	285
Hoarseness	0/7	0
Natal teeth	0/7	0

life (QoL) of the patients is summarized in Table 3. Plantar keratoderma and thick fingernails were the clinical features associated with the most significant impact on daily function; in contrast, oral leukokeratosis, thick toenails, and follicular hyperkeratosis were not associated with a significant effect on QoL or pain in our patients.

3.2. Mutation analysis

Following completion of the required steps, seven patients with PC were included in the registry. The demographic data are presented in Table 1. All patients had mutations in *KRT6A* (PC—K6a) focused on the 1A and 2B domains. We found three different types of mutations (K6a R466P, K6a N171K, and K6a N172del); these mutations have been identified in several other IPCRR cases. One mutation (N172del) was common to 5/7 (71.4%) of the patients.

In the K6a R466P mutation, one of the amino acid building blocks, arginine (written as Arg or R), is changed to proline (written as Pro or P). The number 466 indicates the exact position along the gene where the mutation is located. Because the mutated gene provides incorrect information, the correct protein cannot be produced. This mutation is in the 2B (HTM) domain of K6a (Table 1). In the K6a N171K mutation, one of the amino acid building blocks, asparagine (Asn or N), is changed to lysine (Lys or K); therefore, the correct protein cannot be produced. In the K6a N172del mutation, one of the Asn amino acids is deleted. The number 172 indicates the exact position along the gene where the mutation is located. Because the gene is giving wrong instructions, the correct protein is not produced.

4. Discussion

PC is a rare autosomal dominant keratinizing disorder characterized by severe, painful palmoplantar keratoderma and nail dystrophy and is often accompanied by oral leukokeratosis, cysts, and follicular keratosis. PC is associated with mutation in one of five keratin genes: *KRT6A*, *KRT6B*, *KRT6C*, *KRT16*, or *KRT17*.[5–7,9] Previously, PC was classified into two primary subtypes (type 1And type 2) based on clinical characteristics, leading to a significant phenotype overlap between cases. Now, PC subtypes are classified as PC-K6a, PC-K6b, PC-K6c, PC-K16, and PC-K17 for mutations in the *KRT6A*, *KRT6B*, *KRT6C*, *KRT16*, and *KRT17* genes, respectively. Due to the overlapping clinical features among the different PC subtypes, genetic testing is required to confirm the diagnosis of PC.[6,8] In the present study, we reported on seven Vietnamese children with PC and investigated the mutation characteristics of their keratin genes.

We found three different types of mutations in *KRT6A* (PC—K6a) focused on the 1A and 2B domains (Table 1). Our results suggested that mutation of K6a was highly common.[5,7,10,11] According to data from the IPCRR, the percentages of individuals with mutations in PC-K6a, PC-K16, PC-K17, PC-K6b, and PC-K6c were 40%, 33%, 15%, 9%, and 3%, respectively.[7] With estimates of 7000—10,000 cases worldwide, or about 0.9 cases per million people,[2] it is evident that many Vietnamese PC patients have not been correctly diagnosed. Historically, PC was diagnosed using clinical criteria,[12,13] but genetic testing is essential to confirm diagnosis and disease classification.[14–16]

The results of this study confirmed the common occurrence misdiagnosis of PC in pediatric patients. In the first year of life, all our patients manifested the key features of the disease, but a diagnosis was only made in two patients (28%). This delay in diagnosis may lead to inappropriate management or incorrect information about prognosis and inheritance.[17] We experienced many difficulties in diagnosing PC patients in Vietnam. Most patients were misdiagnosed and mistreated for many years; this could be explained by the rarity of the condition and the limited awareness of PC among Vietnamese medical professionals. Vietnam is a tropical country where infectious diseases are common in the pediatric population, and clinical features of rare conditions are frequently misdiagnosed as infections[18–21]: the oral leukokeratosis characteristic of PC patients was thought to be chronic oral candidiasis, and nail dystrophy was diagnosed as onychomycosis and subsequently

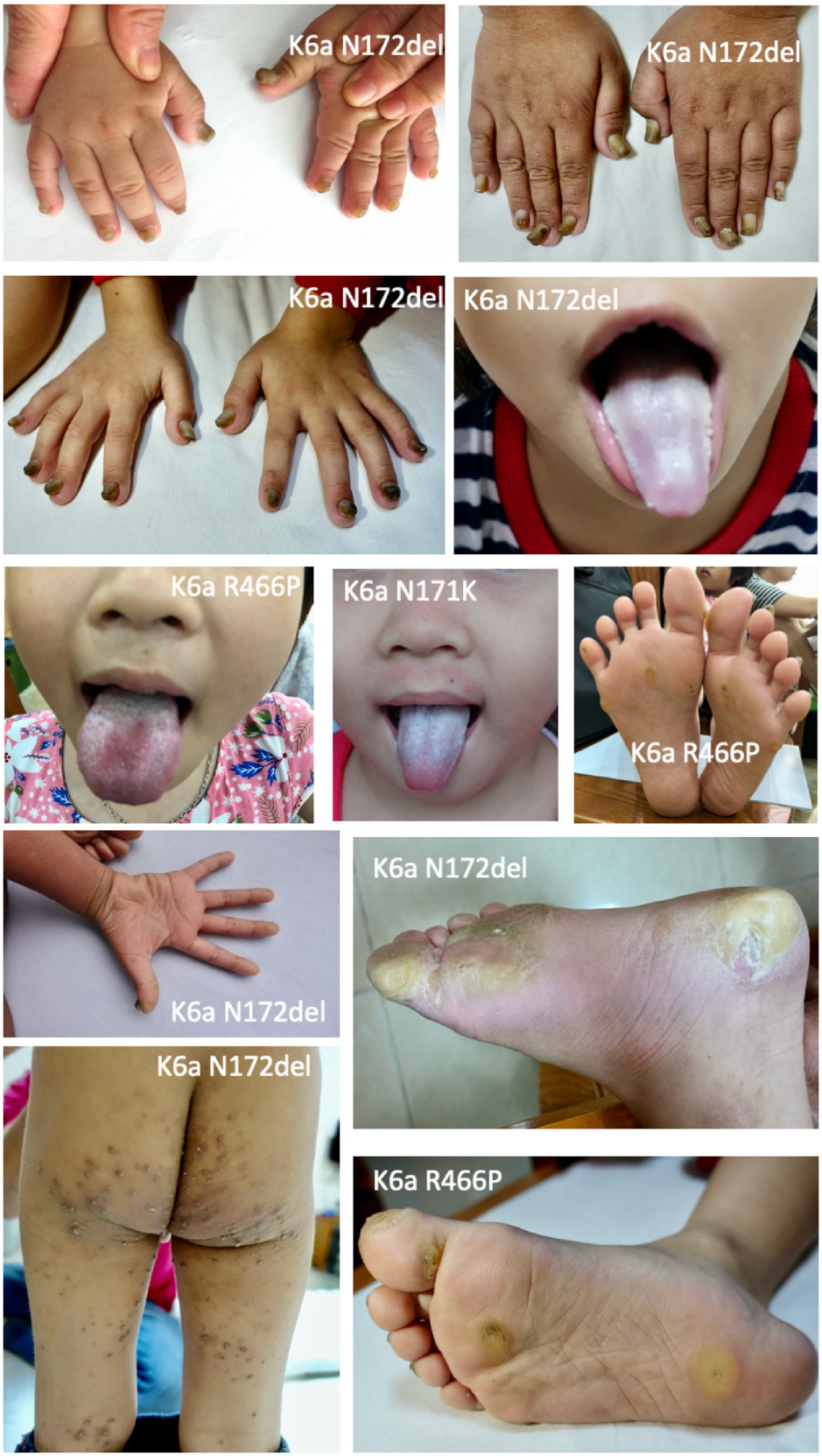

Fig. 1 Clinical features of pachyonychia congenita (PC). Nail dystrophy, oral leukokeratosis, follicular hyperkeratosis, plantar keratoderma, and palmoplantar keratoderma, and in PC patients with identified mutations.

Table 3 Impact of specific features of pachyonychia congenita on quality of life.

Impact on quality of life	Thick toenails	Thick fingernails	Oral leukokeratosis	Follicular hyperkeratosis	Planta keratoderma
No impact	2/7 (28.5%)	1/7 (14%)	3/7 (43%)	2/7 (28.5%)	0/3
Sometimes a problem	3/7 (43%)	3/7 (43%)	4/7 (57%)	3/7 (43%)	1/3 (33,3%)
Always a problem, but able to function	2/7 (28.5%)	3/7 (43%)	0/7	2/7 (28.5%)	2/3 (66.7%)
Unable to function	0/7	0/7	0/7	0/7	0/3

treated by systemic and local aggressive antifungal therapy to no effect. Due to the support of the IPCRR, subsequent Vietnamese PC patients were diagnosed in a timely manner.

Our data showed that 7/7 (100%) patients had thick toenails and fingernails, oral leukokeratosis, and follicular hyperkeratosis. Plantar keratoderma and palmar keratoderma were observed in 3/7 (42%) and 2/7 (28.5%) patients, respectively (Table 2, Fig. 1). The clinical appearance of nails varied among the patients and follicular hyperkeratosis was typically located in areas susceptible to friction (Fig. 1). Our results supported the notable features of PC associated with *KRT6A* mutation, including early onset and extensive nail dystrophies, oral leukokeratosis, and follicular hyperkeratosis, in addition to substantial disease manifestations in locations other than the palms and soles.[5,22] Our research also highlighted that dystrophy of the fingernails and toenails are initial indications of PC that are present in the first year of life; plantar keratoderma becomes evident at a later time.[1,17,23] We had initial success in combining the three main manifestations of PC (thick toenails and fingernails, oral leukokeratosis, and follicular hyperkeratosis) and presenting them on social media to increase awareness of PC among healthcare workers and the community. Through this success, we gathered patients with clinical manifestations resembling PC for detailed examination and genetic testing. Although PC does not affect lifespan, it dramatically impacts QoL.[6,24] Worldwide, IPCRR data showed that patients with mutations in PC-K6a reported painful plantar keratoderma as the most challenging feature of PC, and they were eager to find relief for the pain.[7] Our patients did not experience severe impact on their QoL (Table 3); however, this may change as the patients age. There is no cure for this condition, and current treatment options for PC symptoms are limited and palliative in nature.[25–28] The identification and documentation of additional PC cases will support and enhance the planning of future clinical trials for this rare disease.[10,29]

In conclusion, individuals displaying early-onset nail dystrophy, oral leukokeratosis, follicular hyperkeratosis, and plantar keratoderma should be referred for genetic testing given the high likelihood of a PC-K6a-related mutation. Early diagnosis by genetic analysis is essential for patients and families and allows for appropriate care and genetic counselling.

Ethics approval and consent to participate

Approval for the study was obtained from Medical Ethics Council of Haiphong University of Medicine and Pharmacy with No.: 955/QD-YDHP, and informed consent was obtained according to the Declaration of Helsinki. Written informed consent was obtained from the patient and his parents for publication of these data and for the accompanying images.

Author's contributions

HTC, QVV, DHL, TVL, DDTA and BBN participated in the study design, protocol development and performance, data analysis, interpretation of data, and writing of the manuscript and carried out the clinical data collection and data analysis. QVV and CVD reviewed and revised the manuscript, making important intellectual contributions. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no competing interests.

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References

1. Samuelov L, Smith FJD, Hansen CD, Sprecher E. Revisiting pachyonychia congenita: a case-cohort study of 815 patients. *Br J Dermatol* 2020;**182**:738–46.
2. Wu AG, Lipner SR. Distinctions in the management, patient impact, and clinical profiles of pachyonychia congenita subtypes. *Skin Appendage Disord* 2021;**7**:194–202.
3. Wilson N, O'Toole EA, Milstone LM, Hansen CD, Shepherd AA, Al-Asadi E, et al. The molecular genetic analysis of the expanding pachyonychia congenita case collection. *Br J Dermatol* 2014;**171**:343–55.
4. McLean WHL, Hansen CD, Eliason MJ, Smith FJD. The phenotypic and molecular genetic features of pachyonychia congenita. *J Invest Dermatol* 2011;**131**:1015–7.

5. Wilson NJ, Leachman SA, Hansen CD, McMullan AC, Milstore LM, Schwartz ME, et al. A large mutational study in pachyonychia congenita. *J Invest Dermatol* 2011;**131**:1018—24.
6. Eliason MJ, Leachman SA, Feng BJ, Schwartz ME, Hansen CD. A Review of the clinical phenotype of 254 patients with genetically confirmed pachyonychia congenita. *J Am Acad Dermatol* 2012;**67**:680—6.
7. *PC Data*. Available at, https://www.pachyonychia.org/pc-data/. Accessed January 20, 2022.
8. Chu HT, Le DH, Le TV, Nguyen BB, Schwartz J, Vu QV. Pachyonychia Congenita Type PC-K6a: the first report in the Vietnamese population. *Biomed Res Ther* 2021;**8**:4434—8.
9. Wu AG, Lipner SR. Distinctions in the management, patient impact, and clinical profiles of pachyonychia congenita subtypes. *Skin Appendage Disord* 2021;**7**:194—202.
10. Chiriac A, Rusu C, Murgu A, Chiriac AE, Wilson NJ, Smith FJD. First report of pachyonychia congenita Type PC-K6a in the Romanian population. *Maedica (Bucur)* 2017;**12**:123—6.
11. Smith FJD, Hansen CD, Hull PR, Kaspar RL, McLean WI, O'Toole E, et al. *Pachyonychia congenita*. Available at, https://www.ncbi.nlm.nih.gov/books/NBK1280/. Accessed March 21, 2021.
12. Smith FJD, McLean WHI. Keratin 6b variant p.Gly499Ser reported in delayed-onset pachyonychia congenita is a non-pathogenic polymorphism. *J Dermatol* 2017;**44**:e312.
13. Leachman SA, Kaspar RL, Fleckma P, Florell SR, Smith FJD, McLean WHI, et al. Clinical and pathological features of pachyonychia congenita. *J Invest Dermatol Symp Proc* 2005;**10**:3—17.
14. Wee JS, Smith FJD, Wilson NJ, O'Toole EA. Focal PPK secondary to a novel KRT6C mutation (Pachyonychia congenita-K6c). *J Eur Acad Dermatol Venereol* 2016;**30**:1415—6.
15. Duverger O, Carlson JC, Karacz CM, Schwartz ME, Cross MA, Marazita ML, et al. Genetic variants in pachyonychia congenita-associated keratins increase susceptibility to tooth decay. *PLoS Genet* 2018;**14**:e1007168.
16. *Pachyonychia congenita — GeneReviews® — NCBI bookshelf*. Available at, https://www.ncbi.nlm.nih.gov/books/NBK1280/. Accessed July 18, 2018.
17. Shah S, Boen M, Kenner-Bell B, Schwartz M, Rademaker A, Paller AS. Pachyonychia congenita in pediatric patients: natural history, features, and impact. *JAMA Dermatol* 2014;**150**:146—53.
18. Vu QV. Acne fulminans in a Vietnamese boy successfully treated with prednisolone and ibuprofen: a case report. *Biomed Res Ther* 2018;**5**:2708—11.
19. Vu QV, Wada T, Le HT, Le HT, Van Nguyen AT, Osamu O, Yachie A, et al. Clinical and mutational features of Vietnamese children with X-linked agammaglobulinemia. *BMC Pediatr* 2014;**14**:129.
20. Tran TT, Vu QV, Wada T, Yachie A, Le Thi Minh H, Nguyen SN. Novel HAX1 gene mutation in a Vietnamese boy with severe congenital neutropenia. *Case Rep Pediatr* 2018;**2018**:2798621.
21. Vu QV, Wada T, Tran TT, Ngo DN, Van Dinh T, Nguyen HC, et al. Severe congenital neutropenia caused by the ELANE gene mutation in a Vietnamese boy with misdiagnosis of tuberculosis and autoimmune neutropenia: a case report. *BMC Hematol* 2015;**15**:2.
22. Forrest CE, Casey G, Mordaunt DA, Thompson EM, Gordon L. Pachyonychia congenita: a spectrum of KRT6a mutations in Australian patients. *Pediatr Dermatol* 2016;**33**:337—42.
23. *Pachyonychia congenita: background, pathophysiology, epidemiology*. Available at, https://emedicine.medscape.com/article/1106169-overview. Accessed July 18, 2018.
24. Zieman AG, Coulombe PA. Pathophysiology of pachyonychia congenita-associated palmoplantar keratoderma: new insight into skin epithelial homeostasis and avenues for treatment. *Br J Dermatol* 2020;**182**:564—73.
25. Gruber R, Edlinger M, Kaspar RL, Hansen CD, Leachman S, Milstone LM, et al. An appraisal of oral retinoids in the treatment of pachyonychia congenita. *J Am Acad Dermatol* 2012;**66**:e193—9.
26. Goldberg I, Fruchter D, Meilick A, Schwartz ME, Sprecher E. Best treatment practices for pachyonychia congenita. *J Eur Acad Dermatol Venereol* 2014;**28**:279—85.
27. Wallis T, Poole CD, Hoggart B. Can skin disease cause neuropathic pain? A study in pachyonychia congenita. *Clin Exp Dermatol* 2016;**41**:26—33.
28. Abbas M, Schwartz ME, Smith FJ, McLean WH, Hull PR. PCQoL: a quality of life assessment measure for pachyonychia congenita. *J Cutan Med Surg* 2015;**19**:57—65.
29. Milstone LM, Fleckman P, Leachman SA, Leigh IM, Paller AS, van Steensel MA, et al. Treatment of pachyonychia congenita. *J Investig Dermatol Symp Proc* 2015;**10**:18—20.