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Hereditary palmoplantar keratoderma – phenotypes and mutations in 64 patients

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ABSTRACT

Background: Hereditary palmoplantar keratodermas (PPK) represent a heterogeneous group of rare skin disorders with epidermal hyperkeratosis of the palms and soles, with occasional additional manifestations in other tissues. Mutations in at least 69 genes have been implicated in PPK, but further novel candidate genes and mutations are still to be found.

Objectives: To identify mutations underlying PPK in a cohort of 64 patients.

Methods: DNA of 48 patients was analyzed on a custom-designed in-house panel for 35 PPK genes and 16 patients were investigated by a diagnostic genetic laboratory either by whole exome sequencing, gene panels, or targeted single gene sequencing.

Results: Of the 64 PPK patients, 32 had diffuse (50%), 19 focal (30%) and 13 punctate (20%) PPK. None had striate PPK. Pathogenic mutations in altogether five genes were identified in 31 of 64 (48%) patients, the majority (22/31) with diffuse PPK. Of them, 11 had a mutation in *AQP5,* five in *SERPINB7,* four in *KRT9* and two in *SLURP1*. *AAGAB* mutations were found in nine punctate PPK patients. New mutations were identified in *KRT9* and *AAGAB.* No pathogenic mutations were detected in focal PPK. Variants of uncertain significance (VUS) in PPK-associated and other genes were observed in 21 patients that might explain their PPK. No suggestive pathogenic variants were found for 12 patients.

Conclusions: Diffuse PPK was the most common (50%) and striate PPK was not observed. We identified pathogenic mutations in 48% of our PPK patients, mainly in five genes: *AQP5, AAGAB, KRT9, SERPINB7,* and *SLURP1*.

INTRODUCTION

Hereditary palmoplantar keratodermas (PPK) are a heterogeneous group of rare skin disorders characterized by thickening of the epidermis of palms and soles. PPK can be divided into four different types according to the appearance of the hyperkeratosis: diffuse, focal, punctate and striate PPK.[1] PPK may be restricted to the palms and soles (isolated PPK), or include other skin areas or adnexal tissues (complex PPK), but may also involve other organs (e.g., heart). Syndromes with PPK are also known (e.g. ichthyoses; syndromic PPK).[1,2] Diffuse PPK is most common, and mutations in Keratin 9 (*KRT9*) are regarded the most common factor underlying autosomal dominant diffuse PPK.[2] Next generation sequencing (NGS) has enabled the identification of mutations causing various PPK subtypes.[3] Mutations in at least 69 genes are currently associated with PPK (https://panelapp.genomicsengland.co.uk/panels/556/, last accessed March 12 2021).[1,4] The exact incidence and prevalence of hereditary PPK is not known.[2] We searched for genetic causes in a large cohort of 64 PPK patients treated in a dermatological outpatient clinic.

MATERIALS AND METHODS

63 patients with likely hereditary PPK, based on clinical characteristics (type of hyperkeratosis, possible extracutaneous manifestations), age of onset and affected family members, were recruited from the Department of Dermatology at Helsinki Hospital and one patient referred from Oulu University Hospital, Finland, based on clinical information and histopathological findings. Patients with clinically and/or histologically confirmed PPK related to a concomitant skin disease (e.g. atopic dermatitis, psoriasis, eczema, lichen planus, pityriasis rubra pilaris), keratoderma climacterium, drugs, system disease, infection, malignancy or chemical association were excluded from the study.[5] All patients except the one from Oulu were clinically evaluated by at least one of the authors and additional data

were collected from patient records. Written informed consent was obtained from the patients and/or parents. This study was approved by the Coordinating Ethical Review Board of the Helsinki and Uusimaa Hospital District, Helsinki, Finland and conducted according to the principles expressed in the Declaration of Helsinki. The DNA of 48 patients was sequenced on a customized in-house NGS panel for 35 genes associated with non-syndromic or syndromic PPK. Ten patients were analyzed at Blueprint Genetics (Blueprint Genetics, Helsinki, Finland). Based on their phenotypes, four patients were sequenced for targeted genes/gene regions. Whole-exome sequencing (WES) was performed for two patients prior to the availability of gene panels and additional WES was performed for six patients initially with no in-house panel findings at Blueprint Genetics. Detailed description of all sequencing methods can be found in the supplemental material (Appendix S1, Table S1, Supporting Information).

RESULTS

PPK subtypes and mutations in the cohort

Of the 64 PPK patients studied, 32 presented with diffuse (50%), 19 with focal (30%) and 13 with punctate (20%) PPK (Table 1). Striate PPK was not observed. Thirty-two patients were female (50%). The age of onset was below 10 years in almost half (45%, 29/64) and the majority (56%, 36/64) had affected family members with similar PPK: 62% (20/32) with diffuse, 42% (8/19) with focal, and 69% (9/13) with punctate PPK.

We found pathogenic mutations in 48% (31/64) of patients clustered to five genes previously associated with PPK (Table 2). Four genes in diffuse PPK: Aquaporin-5 *(AQP5),* Keratin 9, type 1 *(KRT9*), Serpin peptidase inhibitor, clade B, member 7 *(SERPINB7)* and Secreted Ly6/plaur domain-containing protein 1 (*SLURP1)*, and one gene in punctate PPK, Alpha-and

gamma-adaptin binding protein (*AAGAB)*. No pathogenic mutations were found in any focal PPK patients.

Potential mutations or variants of uncertain significance (VUS) in genes associated with PPK or dermatological diseases were found for 21 (33%) patients (12 focal, five diffuse and four punctate) (Table 1 and S2, Supporting Information). Six patients who failed to reveal mutations or VUSs in the in-house PPK gene panel, but all considered highly likely to have hereditary PPK with either childhood onset of symptoms and/or affected family members, underwent WES. Additional VUSs were identified for two punctate and focal patients studied, but not for one focal and diffuse patient. The VUSs located to genes previously associated with PPK and *COL5A1* (Table S2). Overall, no mutations or VUSs were found for 12 (19%) patients (seven focal and five diffuse) of which five had family members with similar PPK.

Insert Table 1 and 2 here.

Characterization of diffuse PPK patients

A disease-causing mutation was found in 68% (22/32) of the diffuse PPK patients (Table 1). Eleven had mutations in *AQP5,* five in *SERPINB7,* four in *KRT9* and two in *SLURP1* (Table 2). Five of these patients were born with PPK (two *KRT9*, one *SLURP1*, one *SERPINB7* and one *AQP5)* and five (one *KRT9*, two *SERPINB7,* two *SLURP1*, one *AQP5*) developed symptoms before the age of 2 years. Early onset of symptoms by 10 years was seen in 77% (17/22), and the majority 95% (21/22) developed symptoms by age 20. Only one patient with an *AQP5* mutation developed symptoms later, at age 32.

Eleven patients were identified with a heterozygous *AQP5* c.113C>A p.(Ala38Glu) (rs398123054) mutation in exon 1, enriched in the Finnish population (gnomAD total allele frequency 0.000006570, Finnish allele frequency 0.00009414). This mutation is 14 times more common in Finland than elsewhere, suggesting a Finnish founder mutation. All eleven had at least one and eight had several affected family members in multiple generations with clinically similar PPK (data not shown). All had mild diffuse PPK limited to their palms and soles with aquagenic whitening. Secondary dermatophyte infections and hyperhidrosis were typical, whereas nail changes (brittle, longitudinal ridges) were less common (Table 3, Fig. 1a-b). Four patients also carried heterozygous variants in three other PPK genes: *PKP1, KANK2,* and *GJB2* (Appendix S1, Supporting Information). Their phenotypes were comparable to the other *AQP5* patients (data not shown); thus the variants were likely clinically irrelevant.

Heterozygous *KRT9* mutations were found in four patients with a thick and waxy diffuse PPK, plantar hyperhidrosis and aquagenic whitening (Tables 2 and 3). A 3-year-old boy with PPK since infancy had a novel heterozygous *KRT9* c.471G>T, p. (Met157Ile) mutation (not present in mutation databases). Sanger sequencing confirmed the mutation also in his affected father. A 9-year-old boy with PPK since birth had a heterozygous *KRT9* c.488G>A p.(Arg163Gln) (rs57758262) mutation (gnomAD total allele frequency 0.000006584).[6] An erythematous border was evident on his hands and feet and hyperkeratosis was noted also on knuckle pads; the PPK was transgredient and progressive. Slight conical tapering of fingers was present (Fig. 1c-d). Tactual sensation of the severely hyperkeratotic skin was reduced and bacterial infections were frequent. A 10-year-old boy, with PPK from a few months age and a heterozygous *KRT9* c.487C>T, p.(Arg163Trp) mutation (rs59616921), also had slightly clubbed nails.[6] A 24-year-old man of Middle-African origin, with PPK from the age of 16,

had a heterozygous *KRT9* c.482A>G p.(Asn161Ser) (rs56707768) mutation.[7] The PPK was transgredient, with hyperkeratotic knuckle pads, slightly conically tapered fingers and slightly clubbed nails (Table 3).

We report for the first time a patient of Vietnamese origin, compound heterozygous for the *SERPINB7* mutations c.796C>T p.(Arg266*) (rs142859678) and c.522dup p.(Val175Cysfs*46) (rs672601344). Both mutations have been reported only in Japanese, Chinese and Korean populations, with c.796C>T p.(Arg266*) a founder mutation in all three populations.[8-10] The patient had a typical transgredient diffuse PPK for *SERPINB7* mutations with plantar hyperhidrosis, aquagenic whitening and slight clubbing of the finger nails and horizontal ridges on toe nails.

In addition, four patients with mild PPK since infancy with hyperhidrosis were homozygous for the *SERPINB7* mutation c.1136G>A p.(Cys379Tyr) (rs201208667), some of which were previously reported (Tables 2 and 3, Fig. 1e-f).[11]

Two patients with *SLURP1* mutations c.178G>A p.(Glu60Lys) rs200727790 and c.218_220del p.(Cys73del) were previously reported (Table 2).[12] Both had diffuse, transgredient PPK since infancy with waxy yellow hyperkeratosis with an erythematous border, aquagenic whitening and nail changes. Hyperkeratosis was also present on the elbows, knees and knuckle pads (Table 3, Fig. 1g-h).

In addition, five diffuse PPK patients (16%) had possible causative mutations deemed as VUSs in other PPK genes (Tables 1 and S2). Two had symptomatic family members suggesting a genetic cause and one of them had had PPK from birth, but the other had onset at age 70 years. Another five diffuse PPK patients revealed no mutations. The overall onset of

symptoms was later in this group, as 3/5 (60%) patients developed symptoms over the age of 40 years. However, three of them had affected family members, suggesting hereditary PPK.

Insert Table 3 and figure 1 here.

Characterization of patients with focal PPK

Focal PPK was present in 19 patients, but none showed pathogenic mutations in any known PPK genes. However, VUSs were found in 63% (12/19) of patients (Table S2). Six patients had affected family members and they all developed symptoms before age 30, the youngest at one year, suggesting hereditary etiology. Six had no affected family members but four developed symptoms before age 30. No pathogenic mutations or VUSs in any of the PPK genes studied were detected in 37% (7/19) of the patients (Table 1). Two had affected family members and their onset of symptoms varied between birth and 70 years. Onset of symptoms in patients with no affected family varied from 15 to 55 years.

Characterization of patients with punctate PPK

Punctate PPK was found in 13/64 (20%) patients and nine of them (69%) revealed five different heterozygous *AAGAB* mutations compatible with punctate PPK type 1A, Buschke-Fisher-Brauer PPK (MIM 148600) (Tables 1 and 2). The majority of patients with *AAGAB* mutations (77%, 7/9) had affected relatives and onset of symptoms by age 20 (66%, 6/9) and all by the age of 41 years. The most common *AAGAB* mutation was c.370C>T p.(Arg124*) found in three unrelated patients.[13,14] The other mutations have not previously been associated with punctate PPK: c.(?_-84_(73+1_74-1)del leading to deletion of exon 1, c.73+2dup p.(?) located in the splice donor site in intron 1, c.315G>A p.(Trp105*) and c.335_345del p.(Leu112*) (rs1390240171) causing premature termination and all were deemed disease-

causing. They all are classified pathogenic by the American College of Medical Genetics and Genomics (ACMG) criteria. We performed additional cDNA analysis with Sanger sequencing for the c.73+2dup p.(?). No visible product with the c.73+2dup change could be detected, while the wild-type allele yielded normal sequence (not shown). The two patients with c.315G>A p.(Trp105*) are not known to be related but the two patients sharing *AAGAB* c.335_345del p.(Leu112*) turned out to be relatives. All patients except one with the c.370C>T p.(Arg124*) mutation reported aquagenic whitening with concomitant swelling of the punctate hyperkeratosis. A genotype-phenotype correlation could not be seen as only three patients with c.335_345del p.(Leu112*), c.315G>A p.(Trp105*) and c.(?_-84_(73+1_74-1)del mutations suffered from pain in the punctate PPK areas (Table 3, Fig. 1i-j).

VUSs were found in all remaining four punctate PPK patients (31%, 4/13) (Table S2). One developed symptoms at age 50 and had affected family members. One patient was born with punctate PPK, one developed PPK at age 8 and one at age 19, but none had affected family members (Table 1).

DISCUSSION

This is to our knowledge, the largest cohort of PPK patients studied for genetic etiology. Diffuse PPK was the most common clinical PPK subtype (50%), as generally observed.[1] Focal (30%) and punctate (20%) PPK were well represented, but no striate PPK patients were found. Our findings suggest that focal PPK is the second most common followed by punctate PPK. Striate PPK by itself appears to be rare, and it is associated with syndromes, such as skin fragility and woolly hair syndrome.[1,4] Altogether 48% of our patients revealed pathogenic mutations in known PPK genes (68% of diffuse, 69% of punctate, 0% focal PPK patients). An earlier onset of symptoms was seen for the genetically confirmed diffuse PPK patients (77% under 10 years and 95% by the age of 20) than punctate PPK patients (22%

under 10 years and 67% by the age of 20), as reported.[1,2] The majority of focal PPK patients (75%) had onset in childhood or adolescence.[1,2]

Interestingly, causative mutations clustered within only five genes. Diffuse PPK caused by *KRT9* mutations is regarded as the most common PPK, but in our cohort the most common form was diffuse PPK caused by one mutation in autosomal dominant (AD) inherited *AQP5* (17%), followed by punctate PPK with AD inherited *AAGAB* (14%) mutations and diffuse PPK with autosomal recessive (AR) inherited *SERPINB7* (7%), AD inherited *KRT9* (6%), and AR inherited *SLURP1* (4%) mutations.[1,2] The genetic isolation of the Finns and consequential genetic founder effects likely explain the high proportion of especially *AQP5* and *SERPINB7* mutations found.[15] The heterozygous *AQP5* c.113C>A p.(Ala38Glu) mutation was the most common finding overall in our cohort and in diffuse PPK patients. It has previously only been reported in diffuse PPK patients from Northern Sweden.[16-18] Finland and Sweden share a Northern border, so this is likely a shared founder mutation in both populations. Aquaporins are cell membrane proteins and they enable the osmotic movement of water but it is not yet clear how the defective epidermal-water-barrier leads to hyperkeratosis in PPK caused by *AQP5* mutations[17] Our patients' phenotypes matched the *AQP5* associated Bothnian type PPK (MIM 600231) with a childhood onset, diffuse, usually mild, transgredient hyperkeratosis and prominent aquagenic whitening.[1,16-18] Other observed diffuse PPK genes were *SERPINB7, KRT9* and *SLURP1*. The homozygous *SERPINB7* c.1136G>A p.(Cys379Tyr) mutation observed in three patients was also a Finnish founder mutation.[11] In addition, we identified for the first time a patient of Vietnamese origin compound heterozygous for the *SERPINB7* mutations c.796C>T p.(Arg266*) and c.522dup p.(Val175Cysfs*46), previously only reported in Japanese, Chinese and Korean patients.[8-10] Loss-of function of SERPINB7 might cause overactivation of proteasis in PPK lesions causing protein deterioration of keratinocytes and promote the water permeation in stratum corneum of the epidermis.[8,9] All

patients' diffuse PPK was in line with the autosomal recessive Nagashima type PPK (MIM 615598). Thus *SERPINB7* mutations are likely to be more common than expected. Alterations of secreted SLURP1, which is involved in keratinocyte differentiation leads to disturbance of skin development.[19] *SLURP1* mutations in two patients caused a slightly different phenotype sharing features of both Mal de Meleda (MDM) (MIM 248300) and less severe diffuse PPK Gamborg-Nielsen (GN) (MIM 244850) and also aquagenic whitening.[12,19,20] Autosomal dominant *KRT9* mutations cause diffuse PPK, with onset at birth or early childhood (MIM 144200).[21,22] Keratins constitute the intracellular keratin filament network in keratinocytes and mutations in *KRT9* might lead to the interruption of this filament network causing diffuse PPK.[21] All our four patients with *KRT9* mutations had a typical *KRT9* associated phenotype.[1,2] Interestingly, a later onset of symptoms at age 16 instead of typical early childhood was found in one patient. A novel *KRT9* c.471G>T, p. (Met157Ile) mutation in one patient and his father broadens the list of 85 known *KRT9* mutations in PPK (http://www.interfil.org/details.php?id=NM_000226, last accessed January 4 2021).[23] Surprisingly, punctate PPK with autosomal dominant *AAGAB* mutations, regarded as punctate PPK type 1A, or Buschke-Fisher-Brauer PPK (MIM 148600), was the second most common PPK subtype in our cohort.[13] Various mutations were found, and thus a founder effect is not plausible. At least 50 different *AAGAB* mutations associated with punctate PPK are currently known and we identified four novel unpublished *AAGAB* mutations: c.73+2dup p.(?), c.(?_-84_(73+1_74-1)del, c.315G>A p.(Trp105*) and c.335_345del p.(Leu112*).[14] All of them are classified as pathogenic, with nonsense mutations and frameshift deletions being a previously known mutation mechanism for *AAGAB*.[13,24,25] The fact that no visible product with the c.73+2dup change could be detected might be explained by efficient nonsense-mediated decay of the deficient mRNA product. The exact normal function of AAGAB and how its mutations cause punctate PPK is not entirely known but it is involved in membrane

trafficking, endocytosis and protein sorting.[14] All our patients' phenotypes matched the previously reported variable PPK punctate type 1A phenotypes.[26] We noticed a trend of punctate PPK worsening with age, which is typical for *AAGAB* mutations. None of our *AAGAB* patients had concomitant malignancies although such association has been suggested.[14]

Unexpectedly, we did not find any *KRT1* mutations in diffuse PPK, or mutations in other well-known genes associated with isolated PPKs e.g. *CTSB* in diffuse or *KRT6C* in focal PPK.[1,2,27] The number of publications on *CTSB* in PPK are few, suggesting that this gene is a rare cause of PPK, likely explaining why we did not detect it in our cohort. Mutations in *KRT1* and *KRT6C* cause isolated PPKs but also e.g. pachyonychia congenita (*KRT6C*) or epidermolytic ichthyosis (*KRT1*).[27] Our findings suggest that mutations in these and the other genes associated with PPK are not common contributors to isolated PPK.

For 33% of patients we identified a VUS in 14 different genes all associated with PPK, except *COL5A1* associated with Ehlers-Danlos syndrome.[28] Further evaluation of the pathogenicity of these variants and possible novel genetic causes for PPKs is underway. For 12 patients (19%) with focal or diffuse PPK we were not able to find a causative mutation nor a VUS. Five patients had affected family members, indicating still unresolved genetic causes. However, novel *de novo* mutations may preside in patients with no affected family members. Furthermore, focal PPKs are associated with keratin genes *(KRT14, KRT17, KRT16, KRT6A, KRT6B, KRT6C)* which were difficult to cover fully by NGS due to numerous repetitive sequences.[29] Thus some pathogenic mutations might have been missed. It is also possible that all causative genes were not included in the panels, as at least 69 PPK related genes are known to date (https://panelapp.genomicsengland.co.uk/panels/556/, last accessed March 12 2021). At the time of the in-house panel design all relevant reported PPK genes were included but only 35 genes could be included. Moreover, PPKs might be caused by other than coding

region mutations, such as mutations in gene enhancers and other regulatory regions that were uncovered in our assays.[30] Thus further studies are needed for patients with no genetic findings. Some patients, especially those with no affected family and with a late onset of PPK might have acquired PPK although we tried to exclude these patients.[5]

In summary, our results confirm diffuse PPK the most common and striate PPK rare in isolated PPKs. Pathogenic mutations were clustered to common PPK genes and we broaden the mutation spectrum with novel mutations in *AAGAB* and *KRT9* genes. Gene panels are useful in PPK diagnostics, however many PPK patients were left without a genetic etiology and thus novel PPK genes are still likely to be found.

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FIGURE AND TABLE LEGENDS

Figure 1

Phenotypes of palmoplantar keratoderma patients with found mutations: (a)-(b) heterozygote *AQP5* c.113C>A p.(Ala38Glu), (c)-(d) heterozygote *KRT9* c.488G>A p.(Arg163Gln), e)-(f) homozygote *SERPINB7* c.1136G>A p.(Cys379Tyr), (g)-(h) homozygote *SLURP1* c.178G>A p.(Glu60Lys) and (i)-(j) (heterozygote *AAGAB* c.335_345del p.(Leu112*).

Table 1

Types of palmoplantar keratoderma (PPK) in patients with or without genetic findings, family history of similar PPK and the age of PPK onset. No striate PPK was found. n, number; VUS, Variant of Uncertain Significance.

Table 2

Mutations identified in palmoplantar keratoderma genes *AQP5, AAGAB, SERPINB7, SLURP1* and *KRT9*. n, number; - no rs-number available; Het, heterozygote; Hom, homozygote; ACMG, mutation pathogenicity by American Collage of Medical Genetics and Genomics; VUS, Variant of Uncertain Significance.

Table 3

Phenotypes associated with the identified palmoplantar keratoderma genes *AQP5, AAGAB, SERPINB7, SLURP1* and *KRT9*. n, number; –, no affected patients. No brachydactyly, pseudoainhum, visible hair or tooth abnormalities were detected.

SUPPORTING INFORMATION

Appendix S1

Supplementary material

Table S1

Primers and amplicon sizes used in Sanger sequencing or restriction enzyme analysis of the reported palmoplantar keratoderma mutations. §, Blueprint Genetics diagnostic gene laboratory high quality sequence variant and no Sanger sequencing confirmation; NA, primer data not available sequencing done by other commercial diagnostic gene laboratories; °, No amplicon size available, the analysis was done using probes d5-68: CTGGTGACTAACGCACAGGGTACGNA and d5-69 ACAGAACACTGAAAACCACGACCCT; -, no amplicon data available; Bp, Base pairs.

Table S2

Abbreviations of genes, zygosity and variants of uncertain significance (VUS) found in 21 PPK patients. Het, heterozygote; Hom, homozygote; -, no rs-number, REVEL score of pathogenicity or gnomAD total population frequency available.