Gene Corner

Novel and recurrent mutations in the 1B domain of keratin 1 in palmoplantar keratoderma with tonotubules

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Palmoplantar keratodermas (PPKs) are a large group of disorders characterized by hyperkeratosis of palms and soles. They can be classified by their mode of inheritance, the morphology and distribution of the hyperkeratosis (diffuse, focal or punctate), the involvement of other ectodermal structures, the presence or absence of associated nonectodermal features and the morphological findings at light and electron microscopic levels.[1] Mutations in a variety of proteins are associated

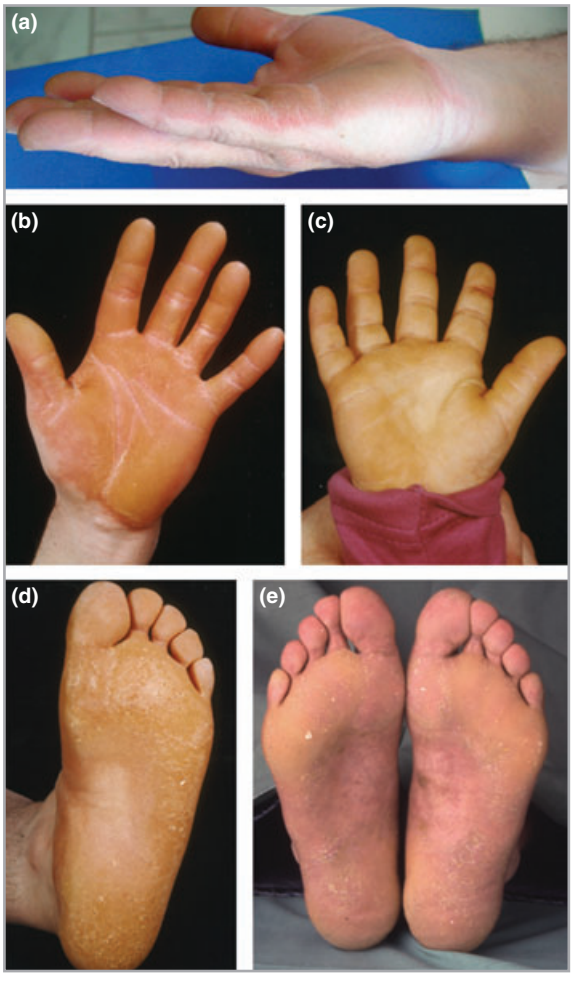

Fig 1. Clinical presentation of palmoplantar keratoderma with tonotubular keratin. The diffuse yellowish hyperkeratosis on palms and soles is clearly bordered without progression and is demarcated by an erythematous rim: (a) family 1; (b–d) affected daughter and father of family 2; (e) family 3.

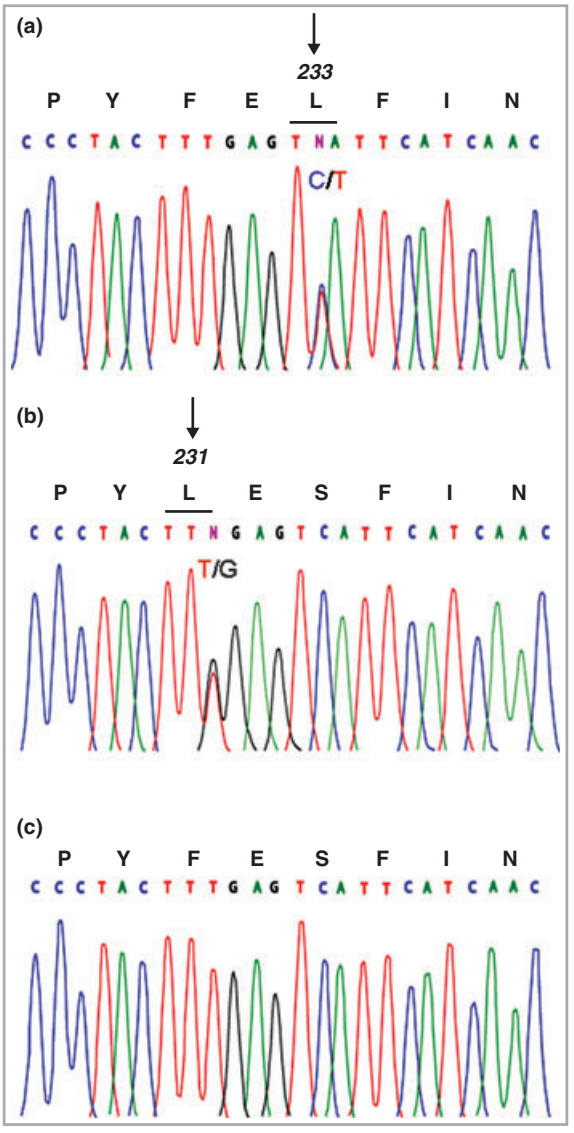

Fig 2. Mutation analysis showing the p.S233L mutation (a; family 1, family 3) and the p.F231L mutation (b; family 2) which are both located in the 1B domain of keratin 1. (c) Wild-type sequence.

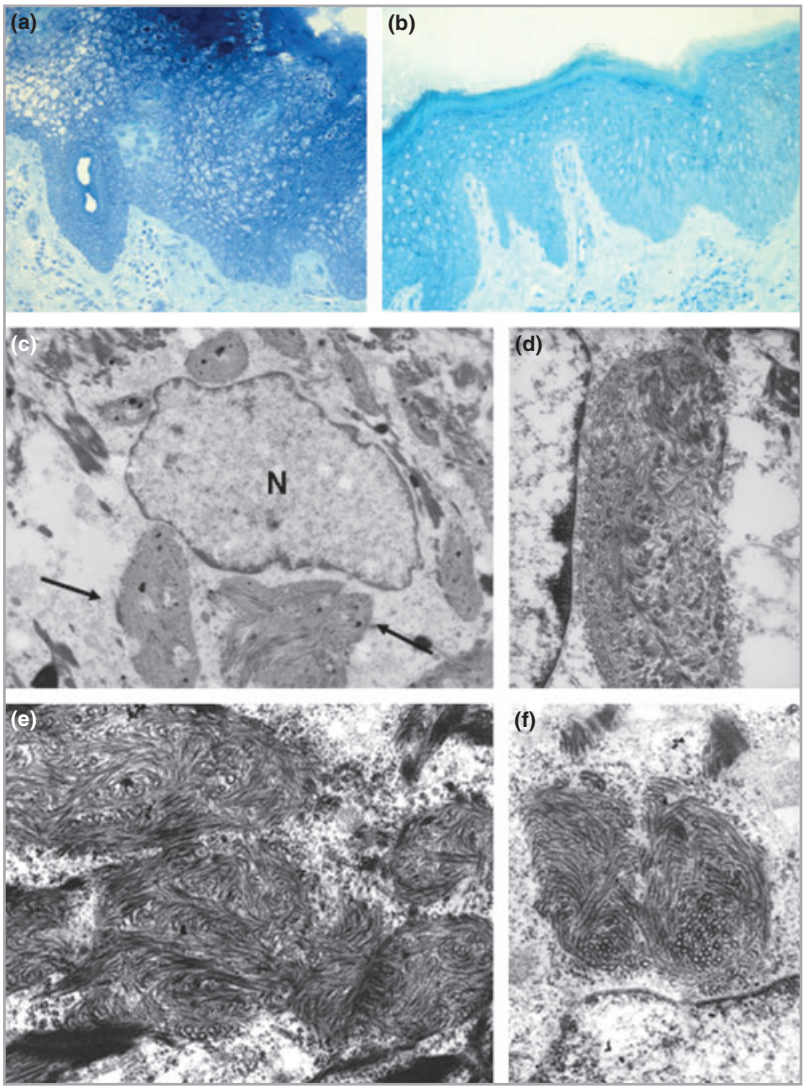

Fig 3. Light microscopy of a plantar biopsy shows epidermolytic hyperkeratosis in the suprabasal layer of palmoplantar epidermis in family 3 (a), which is not present in family 2 (b) (original magnification × 160). Ultrastructural analysis shows electron-dense aggregates in the cytoplasm of suprabasal cells (arrows) consisting of a highly specific conformation (c; original magnification × 7300). These whorls of keratins (e; original magnification × 17 000) contain tubular structures in hexagonal array and arranged in whorls which can be observed in transverse and longitudinal sections (d,f; original magnification × 24 000). N, nucleus.

with different types of PPK. These proteins include keratins, proteins of the cornified cell envelope (loricrin), and molecules for cohesion (desmoglein, desmoplakin), intercellular communication (connexins) and transmembrane transduction (cathepsin C).

PKK of Voerner is the most frequently inherited PPK.[2] It is transmitted in an autosomal dominant mode and starts within the first year of life. It is characterized by a diffuse, yellowish or erythematous hyperkeratosis, sometimes covering bullous lesions, that is sharply demarcated with an erythematous margin; typically there is no progression to the dorsal aspects of hands and feet. Additional features frequently seen are knuckle pad-like keratoses over the flexural areas of the finger joints, clubbing of the nails, and hyperhidrosis.[3] Mutations in the keratin 9 gene (*KRT9*) have been identified in this PPK.[3]

A peculiar subset that is clinically identical to Voerner type PPK but shows the characteristic ultrastructural finding of tonotubules has been termed PPK with 'tonotubular' keratin.[4] We have studied three families with this PPK subtype including the original family described in 1991 and have identified a novel and a previously described mutation affecting the 1B domain of keratin 1 (K1).

Cases and methods

We describe three families with PPK that was clinically diagnosed as PPK Voerner. Investigation of the pedigree in family 1 revealed that approximately half of the members in five generations were affected and presence of affected individuals in each generation was consistent with autosomal dominant inheritance. In family 2 and family 3, two and three affected individuals in two generations were present, respectively.

Patient materials were obtained with informed consent. A skin biopsy was taken from plantar sites by excision biopsy under local anaesthesia and processed for light and electron microscopy. DNA was isolated from peripheral blood leucocytes as previously described.[5] All exons and neighbouring intronic sequences of *KRT1* and *KRT9* were amplified by polymerase chain reactions. Validation of novel single nucleotide polymorphisms (SNPs) was carried out by repetitive

sequencing from both directions. The identified novel mutation (p.F231L) was screened on 200 control chromosomes.

Results and discussion

The clinical picture of the affected individuals in families 1, 2 and 3 was similar. Diffuse hyperkeratotic lesions on palms and soles developed shortly after birth. During the first years of life the PPK worsened, but thereafter the clinical picture remained unchanged. The PPK was clinically diagnosed as PPK Voerner due to a diffuse yellowish keratoderma with a characteristic well-demarcated erythematous border both on palmar and on plantar surfaces (Fig. 1a). In family 2 both the father and his 4-year old daughter were affected, both complaining about disabling pain especially on the palms (Fig. 1b–d). In family 3, two brothers and their mother were affected. They showed thick well-demarcated yellowish hyperkeratoses on palms and soles that developed in childhood (Fig. 1e). In all patients studied, other body sites were not affected.

Sequence analysis was performed from affected individuals and unaffected family members (family 1, family 2, family 3). Affected family members of the original family described by Wevers *et al.*[4] (family 1) revealed a heterozygous base pair substitution at position 698 (c.698C>T) leading to a p.S233L substitution in the 1B domain of K1 (p.S233L) (Fig. 2a). The p.S233L mutation was also the underlying defect in family 3. Interestingly, the same mutation has recently been described in two Dutch kindreds.[6] In family 2 a novel heterozygous missense mutation two residues (p.F231L) upstream of this recurrent mutation (c.693T>G) was identified that leads to a phenylalanine to leucine substitution at position 231 of the K1 polypeptide (p.F231L) (Fig. 2b). These mutations were not present in unaffected family members (Fig. 2c) and screening of 100 ethnically matched normal controls did not show the SNP, suggesting that the identified novel mutation is pathogenic.

Palmoplantar keratoderma with 'tonotubules' is a specific subset of PPK which is clinically identical to PPK Voerner. This disorder was first described in a German kindred in 1991 (family 1) on the basis of the characteristic ultrastructural findings.[4] The authors found tonotubuli with an external diameter of 43 nm instead of 10-nm tonofilaments in patients with an epidermolytic PPK. Similarly, in family 3, histology was diagnostic of epidermolytic hyperkeratosis with vacuolization of keratinocytes in the granular layer and cytolysis in suprabasal keratinocytes (Fig. 3a). However, these tubulofibrillar structures had also been described by Anton-Lamprecht in patients without epidermolytic hyperkeratosis,[7] similar to the findings in family 2 (Fig. 3b). In all patients studied, electron-dense aggregates in the cytoplasm of suprabasal cells consisting of a highly specific conformation of keratin filaments in hexagonal array and arranged in whorls were observed upon ultrastructural examination (Fig. 3c,d). Higher magnification revealed the characteristic tubular structures that can be observed in transverse and longitudinal sections (Fig. 3e,f).

Most K1 mutations that have so far been described in PPK are localized in the central part of the rod domain or the variable end domains.[8–10] In epidermolytic PPK, mutations in the 2B domain have been identified, including missense mutations in the helix termination peptide of K1[10] and a splice site mutation that leads to insertion of 18 amino acids into the 2B domain.[8] As our patients were clinically diagnosed as having Voerner type PPK which produces hyperkeratosis strictly limited to ridged skin[11] and is usually caused by mutations in the palmoplantar-specific keratin gene KRT9[12] we performed sequence analysis of the entire KRT9 gene in all three families to exclude the presence of additional mutations.

In conclusion, both identified mutations are associated with tonotubular keratin, i.e. 'whorls' of aggregated keratin that form tubules as seen in transverse or in longitudinal sections (Fig. 3). These tubular structures have not been described in other keratin disorders and suggest a distinct role of the 1B domain in filament formation.

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