Novel and recurrent mutations in keratin 1 cause epidermolytic ichthyosis and palmoplantar keratoderma

F. J. D. Smith,1,2 I. M. Kreuser-Genis,3 C. S. Jury,4 N. J. Wilson,1 A. Terron-Kwiatowski5 and M. Zamiri6

1Dermatology and Genetic Medicine, Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Dundee, UK; 2Pachyonychia Congenita Project, Holladay, UT, USA; 3Department of Dermatology, University Hospital Crosshouse, Kilmarnock, UK; 4Department of Dermatology, Glasgow Royal Infirmary, Glasgow, UK; 5East of Scotland Regional Genetics Service, Ninewells Hospital, Dundee, UK; and 6Alan Lyell Centre for Dermatology, Queen Elizabeth University Hospital, Glasgow, UK

doi:10.1111/ced.13800

Summary

Mutations in keratin genes underlie a variety of epidermal and nonepidermal cell-fragility disorders, and are the genetic basis of many inherited palmoplantar keratoses (PPKs). Epidermolytic PPK (EPPK) is an autosomal dominant disorder that can be due to mutations in the keratin 1 gene, KRT1. Epidermolytic ichthyosis (EI), the major keratinopathic ichthyosis, is characterized by congenital erythroderma, blistering and erosions of the skin. Causative mutations in KRT1 and KRT10 have been described, with PPK being present primarily in association with the former. We report four unrelated cases (one with sporadic EI and three with autosomal dominant PPK), due to two novel and two recurrent KRT1 mutations. Mutations in KRT1 are not only scattered throughout the keratin 1 protein, as opposed to being clustered, but can result in a range of phenotypes as further confirmed by these mutations, giving a complex genotype/phenotype pattern.

Keratins constitute the intermediate filament proteins of keratinocytes, with the keratin cytoskeleton acting as an important structural stabilizer of epithelial cells.1 Mutations in keratin genes underlie a variety of epidermal and nonepidermal cell-fragility disorders, and are the genetic basis of a number of the inherited palmoplantar keratoses (PPKs).2 Epidermolytic PPK (EPPK) (Vorner type; OMIM 144200) is an autosomal dominant disorder that can be caused by dominant-negative mutations in the keratin 1 gene, KRT1.3 Epidermolytic ichthyosis (EI) [OMIM 113800; also known as epidermolytic hyperkeratosis (EHK) and bullous congenital ichthyosiform erythroderma (BCIE)], the major keratinopathic ichthyosis, is characterized by congenital erythroderma, blistering and erosions of the skin.4 Causative mutations in KRT1 and KRT10 have been described, with PPK being present primarily in association with the former.

We report four unrelated cases with KRT1 mutations (one with sporadic EI and three with autosomal dominant PPK) due to two previously unreported and two reported KRT1 mutations.

Report

Genetic testing was performed following ethics approval by an institutional review board. The study complied with principles of the Declaration of Helsinki, and informed consent was obtained from all participants.

Genomic DNA was extracted from peripheral blood leucocytes or from saliva collected using an Oragene DNA sample collection kit (DNA Genotek, Ontario, Canada). PCR and Sanger sequencing was performed to screen the coding regions and exon/intron boundaries of KRT1 (primer sequences available on request). Variants were confirmed as pathogenic by reference to an in silico prediction tool (Mutation Taster; http://mutationtaster.org/) and by sequencing of other family
members when available. None of the variants was present on the database of Single Nucleotide Polymorphisms (dbSNP), 1000 Genome Project, NHLBI Exome Variant Server, Exome Aggregation Consortium (ExAC) or the Genome Aggregation Database (gnomAD).

Families 1 and 4 presented to their local dermatology services, while Families 2 and 3 were identified through the International Pachyonychia Congenita Research Registry. All reported PPK since early childhood with a positive family history for Patients 2, 3 and 4. None of the affected individuals had a history.

Figure 1 Family 1: the proband had (a) hyperkeratosis of the digits and palm, (b) diffuse plantar hyperkeratosis and (c) warty flexural hyperkeratosis. (d) Wild-type KRT1 sequence of exon 1 (nucleotides 553–567) and (e) the equivalent region of KRT1 from the proband. The arrow indicates a heterozygous mutation c.560T>C resulting in an amino acid substitution of leucine to proline, p.Leu187Pro, in the keratin 1 protein.
### Table 1  Molecular and clinical details of cases in this study (novel and recurrent) compared with previously reported cases.

<table>
<thead>
<tr>
<th>Family</th>
<th>Mutation</th>
<th>DNA change</th>
<th>Exon</th>
<th>Keratin domain</th>
<th>Clinical description</th>
<th>First reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>p.Leu187Pro</td>
<td>c.560T&gt;C</td>
<td>1</td>
<td>1A</td>
<td>EI. Spontaneous case, with a history of blistering, scaling and erythema since birth. As an adult, diffuse hyperkeratosis on trunk, limbs and acral sites</td>
<td>Current study (novel mutation)</td>
</tr>
<tr>
<td></td>
<td>p.Leu187Phe</td>
<td>c.559C&gt;T</td>
<td>1</td>
<td>1A</td>
<td>EI. Spontaneous case with erythroderma, erosions, and blisters on the entire body surface at birth and palmoplantar and flexural areas of hyperkeratosis in the later stage</td>
<td>Szeverenyi et al., 2008⁵ (<a href="http://www.interfil.org">www.interfil.org</a>)</td>
</tr>
<tr>
<td></td>
<td>p.Leu187Phe</td>
<td>c.559C&gt;T</td>
<td>1</td>
<td>1A</td>
<td>EI. Spontaneous case, generalized hyperkeratosis involving palms and soles</td>
<td>Szeverenyi et al., 2008⁵ (<a href="http://www.interfil.org">www.interfil.org</a>)</td>
</tr>
<tr>
<td>2</td>
<td>p.Leu485Phe</td>
<td>c.1453C&gt;T</td>
<td>7</td>
<td>2B</td>
<td>PPK. Familial case of diffuse PPK with sharp demarcation developed in early childhood</td>
<td>Current study (novel mutation)</td>
</tr>
<tr>
<td></td>
<td>p.Leu485Pro</td>
<td>c.1454T&gt;C</td>
<td>7</td>
<td>2B</td>
<td>Generalized severe EI including PPK (mother had EI naevus)</td>
<td>Arin et al., 2011⁶</td>
</tr>
<tr>
<td>3</td>
<td>p.Ser233Leu</td>
<td>c.698C&gt;T</td>
<td>2</td>
<td>1B</td>
<td>PPK. Familial case with diffuse well-demarcated plantar hyperkeratosis with slightly erythematous borders since infancy. Fissuring hyperkeratosis of the palms and fingertips</td>
<td>Current study (recurrent mutation)</td>
</tr>
<tr>
<td></td>
<td>p.Ser233Leu</td>
<td>c.698C&gt;T</td>
<td>2</td>
<td>1B</td>
<td>EPPK (Vörner type). Familial case with diffuse PPK with a well-circumscribed erythematous margin. No hyperkeratosis affecting other body sites</td>
<td>Szeverenyi et al., 2008⁵ (<a href="http://www.interfil.org">www.interfil.org</a>)</td>
</tr>
<tr>
<td></td>
<td>p.Ser233Leu</td>
<td>c.698C&gt;T</td>
<td>2</td>
<td>1B</td>
<td>EPPK (Vörner type). Familial case with diffuse PPK with a well-circumscribed erythematous margin. No hyperkeratosis affecting other body sites</td>
<td>Szeverenyi et al., 2008⁵ (<a href="http://www.interfil.org">www.interfil.org</a>)</td>
</tr>
<tr>
<td></td>
<td>p.Ser233Leu</td>
<td>c.698C&gt;T</td>
<td>2</td>
<td>1B</td>
<td>EPPK (Vörner type). Familial case with diffuse yellowish hyperkeratosis on palms and soles, which developed shortly after birth, with a clear border and demarcated by an erythematous rim</td>
<td>Szeverenyi et al., 2008⁵ (<a href="http://www.interfil.org">www.interfil.org</a>)</td>
</tr>
<tr>
<td>4</td>
<td>p.Ser233Leu</td>
<td>c.698C&gt;T</td>
<td>2</td>
<td>1B</td>
<td>EPPK (Vörner type). Developed shortly after birth.</td>
<td>Holtz et al., 2016⁶</td>
</tr>
<tr>
<td></td>
<td>p.Gln418_Ile419ins18</td>
<td>c.1254 + 1G&gt;A</td>
<td>6</td>
<td>2B</td>
<td>PPK. Familial case with diffuse palmoplantar hyperkeratosis that developed shortly after birth</td>
<td>Current study (recurrent mutation)</td>
</tr>
<tr>
<td></td>
<td>p.Gln418_Ile419ins18</td>
<td>c.1254 + 1G&gt;A</td>
<td>6</td>
<td>2B</td>
<td>EPPK. Familial case, with hyperkeratosis restricted to the palms and soles, which developed in childhood. Varying severity between family members from patchy plantar keratoderma to confluent plantar keratoderma. A violaceous erythematous border was observed at the edge of the keratoderma in severe cases</td>
<td>Hatsell et al., 2001³</td>
</tr>
<tr>
<td></td>
<td>p.Gln418_Ile419ins18</td>
<td>c.1254 + 1G&gt;A</td>
<td>6</td>
<td>2B</td>
<td>EPPK. Familial case, with hyperkeratosis restricted to the palms and soles, which developed in childhood. Varying severity between family members from patchy plantar keratoderma to confluent plantar keratoderma. A violaceous erythematous border was observed at the edge of the keratoderma in severe cases</td>
<td>Hatsell et al., 2001³</td>
</tr>
<tr>
<td></td>
<td>p.Gln418_Ile419ins18</td>
<td>c.1254 + 1G&gt;A</td>
<td>6</td>
<td>2B</td>
<td>EPPK. Familial case, with hyperkeratosis restricted to the palms and soles, which developed in childhood. Varying severity between family members from patchy plantar keratoderma to confluent plantar keratoderma. A violaceous erythematous border was observed at the edge of the keratoderma in severe cases</td>
<td>Hatsell et al., 2001³</td>
</tr>
</tbody>
</table>

EI, epidermolytic ichthyosis; EPPK, epidermolytic palmoplantar keratoderma; PPK, palmoplantar keratoderma.
of collodion membrane, or of hair, teeth or cardiac abnormalities.

In Family 1, the proband was a 23-year-old man who was wheelchair-bound. He presented as an adult with diffuse hyperkeratosis on his trunk and limbs in addition to acral sites, having not sought medical attention for many years. Skin fragility and blistering were reported at sites of trauma, with a history of blistering, scaling and erythroderma being present from birth. Severe flexion contractures of the fingers and toes were evident, with surgical correction considered limited in terms of benefit (Fig. 1a–c). Osteopenia, chronic pain, and the psychological impact of his debilitating skin disease, resulting in social isolation and childhood bullying, necessitated a multidisciplinary management approach. There was no known family history, although the proband was estranged from most of his close relatives so further clinical and genetic information was unobtainable. Mutation analysis of this patient identified a previously unreported heterozygous missense mutation in exon 1 of the keratin 1, p.Leu187Pro; c.560T>C (Fig. 1d,e and Fig. 4). DNA was not available from any other family members. This mutation is within the 1A domain of keratin 1, and another pathogenic variant has previously been reported at the same codon, p.Leu187Phe; c.559C>T in two patients with typical EI (Table 1, www.interfil.org).5

The proband of Family 2 was a 39-year-old woman, who presented with a history since early childhood of diffuse keratoderma of bilateral palms and plantar feet with sharp demarcation and yellow hue, with milder callosities and fissuring affecting the palms and fingertips (Fig. 2a–c). Her older brother, mother and maternal grandmother all had similar findings. A previously unreported mutation, p.Leu485Phe; c.1453C>T (Fig. 2d,e and Fig. 4) was identified in exon 7 of KRT1 in this patient. The mutation was
present in two other affected family members: the patient’s brother and mother. Another pathogenic missense mutation, p.Leu485Pro; c.1454T>C, has previously been reported at this position in the 2B domain of keratin 1 in a patient with PPK (Table 1).

In Family 3, the proband was a 43-year-old woman, who had a history since infancy of diffuse well-demarcated plantar hyperkeratosis with slightly erythematous borders (Fig. 3a,b). Fissuring hyperkeratosis of the palms and fingertips was present, but no nail dystrophy, transgrediens or blistering. Her father and her paternal grandmother, great-grandmother and great-aunt all had similar symptoms. She was found to have a mutation in the 1B domain of KRT1.

| Figure 3 | (a,b) Family 3: the proband had diffuse well-demarcated plantar hyperkeratosis with a slightly erythematous border, and was found to have the mutation K1 p.Ser233Leu. Family 4: (c) the proband and her daughter had diffuse hyperkeratosis of the palms, and both had the mutation K1 p.Gln418_Ile419ins18. (d) Peeling of skin on the proband’s palm associated with oral acitretin therapy and (e,f) diffuse hyperkeratosis of the soles with some sparing of the arch. |
| Figure 4 | Schematic diagram showing the protein domain structure of keratin 1 and previously reported mutations. Those from this study are included; novel mutations are shown in red and recurrent ones in blue. |
p.Ser233Leu; c.698C>T, which has been previously reported (www.interfil.org) in several patients with EPPK (Table 1, Fig. 4).  

The proband of Family 4 was a 32-year-old woman, who had diffuse plantar and palmar hyperkeratosis that peeled significantly once acitretin 20 mg once daily was introduced (Fig. 3c–f). Her grandmother, mother, son and daughter were similarly affected. Genetic analysis identified the recurrent pathogenic mutation, c.1254 + 1G>A, causing a splice site mutation in exon 6, leading to utilization of a novel in-frame splice site 54 bases downstream, with subsequent insertion of 18 amino acids into the 2B rod domain, producing the mutation. p.Gln418_Ile419ins18, previously reported in association with EPPK (Table 1, Fig. 4).  

Keratin intermediate filaments are characterized by tissue-specific expression patterns, with the cell-type and tissue-specific expression profile of the mutated keratin dictating the cell type and body site that will be affected by the disorder. KRT1 and KRT10 confer structural integrity to suprabasal keratinocytes, and mutations in these genes impair the assembly of the keratin cytoskeleton. In some keratin disorders such as pachyonychia congenita, keratin mutations are predominantly clustered to the helix boundary domains at either end of the rod domain. However, the 84 cases previously reported with mutations in KRT1 (www.interfil.org) show that mutations in KRT1 are not only scattered throughout keratin 1 as shown in Fig. 4, but can result in a range of phenotypes as further confirmed by these mutations, giving a complex genotype/phenotype pattern. A wide range in phenotypic severity is also reported in association with KRT1 mutations, particularly in EI. Mutations in the keratin 9 gene (KRT9) also cause epidermolytic PPK; EPPK. With the development of new gene and mutation-specific therapies for inherited disorders of keratinization, it is becoming increasingly important to accurately document both genotype and phenotype. Our cases add to the mutational and clinical spectrum, demonstrating wide phenotypic variation in KRT1 mutations, despite their unifying molecular basis.

Acknowledgements

We thank the patients and their family members for their cooperation in this study. FJDS, NJW and MZ were supported by The Centre for Dermatology and Genetic Medicine at the University of Dundee, which is funded by a Wellcome Trust Strategic Award (098439/Z/12/Z to WHIMcL), and FJDS and NJW were also supported by a grant from the Pachyonychia Congenita Project (www.pachyonychia.org) to FJDS.

Learning points

- Mutations in keratin genes underlie a variety of epidermal and nonepidermal cell-fragility disorders, and are the genetic basis of a number of the inherited palmoplantar keratodermas.
- EPPK (OMIM 144200) is an autosomal dominant disorder usually caused by mutations in the keratin 9 gene (KRT9) but mutations in the keratin 1 gene (KRT1) have been reported in association with this phenotype.
- EI (OMIM 113800), the major keratinopathic ichthyosis, is characterized by congenital erythroderma, blistering and erosions of the skin.
- Causative mutations in KRT1 and KRT10 have been described in epidermolytic ichthyosis, with PPK being present primarily in association with the former.
- Mutations in KRT1 are not only scattered throughout the keratin 1 protein but can result in a range of phenotypes as further confirmed by these mutations, giving a complex genotype/phenotype pattern.
- This report adds to the mutational and clinical spectrum, demonstrating wide phenotypic variation in KRT1 mutations, despite their unifying molecular basis.

References


