Identification of a Germline Mutation in Keratin 17 in a Family with Pachyonychia Congenita Type 2

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Pachyonychia congenita type 2 (PC-2), also known as Jackson-Lawler type PC, is an autosomal dominant disorder characterized by hypertrophic nail dystrophy associated with focal keratoderma and multiple pilo-sebaceous cysts. It has been demonstrated that PC-2 is associated with germline mutations in the keratin 17 (K17) gene and in its expression partner keratin 6b.

In this report, we describe a novel germline mutation in K17, M88T, in a family with PC-2. Key words: K17/pachyonychia congenita. Journal of Investigative Dermatology 113:848–850, 1999

RESULTS

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Abbreviations: K17, keratin 17; PC, pachyonychia congenita; SM, steatocystoma multiplex.

Keratins are intermediate filament proteins that are coexpressed as specific type I and type II keratin pairs. Together, these pairs are expressed in epithelial tissues at different stages of development and differentiation. Keratins possess a 310 amino acid residue α-helical rod domain, flanked by nonhelical amino head and carboxy tail domains. The rod domain consists of four segments referred to as helix 1A, 1B, 2A, and 2B. The sequences, at the beginning of the helix 1A and at the end of the helix 2B, are highly conserved and are the most critical for the assembly of the 10 nm keratin filaments in vivo and in vitro. Therefore, mutations in these segments can disrupt filament formation and filament stability (Letai et al, 1992; Fuchs and Weber, 1994; Corden and McLean, 1996).

Pachyonychia congenita (PC) is a group of autosomal dominantly inherited diseases characterized by nail dystrophy and by varying features of ectodermal dysplasia. Based on the clinical phenotype, several different classifications exist. There are two major subgroups of the disease. Jadassohn-Lewandowsky type PC (PC-1) presents with nail dystrophy accompanied by focal palmpoplantar keratoderma, follicular keratoses, and oral leukokeratosis (Gorlin et al, 1976). PC-1 is associated with mutations in the keratin 16 gene (McLean et al, 1995), or its expression partner keratin 6a (Bowden et al, 1995). By contrast, Jackson-Lawler type PC (PC-2) is characterized by the presence of nail dystrophy and cysts arising from the hair follicle infundibulum (epidermoid or infundibular cysts), and those arising from the sebaceous duct epithelium (eruptive vellus hair cysts and steatocystomas) (Jackson and Lawler, 1951). Additional findings in this group include natal teeth, hair abnormalities (pili torti), and hidradenitis suppurativa (Moon et al, 1994). Linkage analysis of a single family with 25 affected members exhibiting PC-2 phenotype was mapped within the type I keratin gene cluster on chromosome 17q12–21 (Munro et al, 1994). Subsequently, a germline mutation in keratin 17 gene (K17) was identified in this kindred (McLean et al, 1995). Soon after, K17 gene mutations were found in other families with PC-2 (Smith et al, 1997), as well as in families presenting with steatocystomas in the absence of nail changes, also known clinically as steatocystoma multiplex (SM) (Covello et al, 1998).

K17 is a type I keratin expressed in the outer root sheath of hair follicle, sebaceous gland, nail bed, as well as other appendages (Troyanovsky et al, 1989, 1992). Until recently, the expression partner of K17 had been unknown. Smith et al demonstrated colocalization of K17 with keratin 6b, and described a family with PC-2 in whom a keratin 6b mutation was identified (Smith et al, 1998). To date, 12 families with PC-2 or SM phenotype were shown to exhibit germline mutations in the K17 gene (McLean et al, 1995; Smith et al, 1997; Covello et al, 1998; Fujimoto et al, 1998). All of the mutations have been in exon 1, which encodes the highly conserved sequences of helix 1A.

In this report, we describe a novel heterozygous missense mutation in the helix initiation domain of the K17 gene in a family with the PC-2 phenotype.

MATERIALS AND METHODS

Genomic DNA was extracted from whole blood using a QIAamp Blood Maxi Kit (Qiagen, Valencia, CA). Exon 1 of the K17 gene was amplified by PCR with primers specific for the K17 gene, which do not amplify either of the two pseudogenes, as described previously (McLean et al, 1995). For mutation detection, PCR products were sequenced using an Applied Biosystem 310 automated sequencing system.

The K17 mutation, M88T, destroys a restriction site Nla III, which was used to confirm the mutation in the affected individuals. The PCR products were digested with with Nla III at 37°C for 2 h and analyzed on a 2% agarose/TBE mini gel.

RESULTS

Clinical data The pedigree is illustrated in Fig 1(b). The proband (SD1–1) is a 27–y-old female of Caribbean origin, who developed thick dystrophic nails of the fingers and toes during the childhood period. In the second decade of life, she developed numerous cysts on the trunk as well as hidradenitis suppurativa in both axillae.
Figure 1. Clinical data, sequence analysis of exon 1 of the keratin 17 gene, and confirmation of the mutation by restriction endonuclease digestion. Hypertrophic finger nails of patient SD1–2. Pedigree of the family. A wild-type sequence and sequences of the affected individuals, SD1–1 and SD1–2. The Nla III digestion of the exon 1 cuts the 978 bp PCR product into 467, 411, and 100 bp fragments on the mutant allele (lanes 1 and 2 correspond to SD1–1 and SD1–2, respectively), whereas three cuts are observed on the wild-type allele resulting in 411, 261, 206, and 100 bp fragments (lanes 3 and 4 correspond to SD1–3 and a control sample, respectively).

Table I. Summary of keratin 17 mutations in families with pachyonychia congenita type 2 and steatocystoma multiplex

<table>
<thead>
<tr>
<th>#</th>
<th>Phenotype</th>
<th>Mutation</th>
<th>Predicted effect</th>
<th>Exon/K17 domain</th>
<th># of families</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PC-2</td>
<td>422 A&gt;G</td>
<td>N 92 D</td>
<td>1/IA</td>
<td>1</td>
<td>McLean</td>
</tr>
<tr>
<td>2</td>
<td>PC-2</td>
<td>423 A&gt;G</td>
<td>N 92 S</td>
<td>1/IA</td>
<td>6</td>
<td>Smith, Covello, Fujimoto</td>
</tr>
<tr>
<td>3</td>
<td>PC-2</td>
<td>440 T&gt;G</td>
<td>Y 98 D</td>
<td>1/IA</td>
<td>1</td>
<td>Smith</td>
</tr>
<tr>
<td>4</td>
<td>PC-2 or SM</td>
<td>422 A&gt;C</td>
<td>N 92 H</td>
<td>1/IA</td>
<td>1</td>
<td>Smith</td>
</tr>
<tr>
<td>5</td>
<td>PC-2 or SM</td>
<td>429 G&gt;A</td>
<td>R 94 H</td>
<td>1/IA</td>
<td>1</td>
<td>Smith</td>
</tr>
<tr>
<td>6</td>
<td>PC-2</td>
<td>428 C&gt;T</td>
<td>R 94 C</td>
<td>1/IA</td>
<td>1</td>
<td>Covello</td>
</tr>
<tr>
<td>7</td>
<td>SM</td>
<td>428 C&gt;T</td>
<td>R 94 C</td>
<td>1/IA</td>
<td>1</td>
<td>Covello</td>
</tr>
</tbody>
</table>

Skin biopsies of the cysts showed a variety of histologic findings including epidermoid cyst, eruptive vellus hair cyst, and steatocystoma. The proband’s 5-y-old son (SD1–2) was born with natal teeth and has similar cutaneous findings including thick dystrophic nails (Fig 1a), follicular keratosis, cysts, and hidradenitis, all of which are characteristic of pachyonychia congenita type 2. Neither the proband nor her son has had a keratoderma, leukokeratosis of the tongue, or hair abnormalities.

**Mutation detection and confirmation** Direct sequencing of the functional K17 gene with primers flanking exon 1 showed a T to C transition at nucleotide 411 (ATG → ACG). This transition results in the replacement of methionine by threonine (M88T) (Fig 1c). This heterozygous missense mutation was identified in both affected individuals (SD1–1 and SD1–2), but not in the proband’s unaffected mother (SD1–3). Unfortunately, the proband’s unaffected father was not available for testing. This sequence alteration destroys a restriction site, Nla III, which was used to confirm the mutation in the affected family members (Fig 1d). Additionally, the mutation was excluded from 50 unrelated controls by direct sequencing of exon 1.

**DISCUSSION**

Until recently, PC-2 and SM have been considered as separate entities with overlapping clinical features. PC-2 presents predominantly with nail dystrophy accompanied by focal keratoderma and pilosebaceous cysts, including steatocystomas, and other findings of ectodermal dysplasia, whereas SM is characterized by multiple steatocystomas as the only clinical finding. Recently, mutations in K17 have been demonstrated in both disorders. Moreover, individuals exhibiting both the clinical phenotypes of PC-2 and SM, within the same family associated with a single K17 mutation, R94C, has also been described (Covello et al, 1998). These data support the notion that, at least at the genetic level, PC-2 and SM are similar entities.

We have identified a novel missense mutation (M88T) in the 1A subdomain of K17 gene in a family with PC-2. The M88T substitution is located within the first 15 residues of the helix initiation motif that is highly conserved among all intermediate filaments. This substitution would be expected to lead to a distortion of the alpha-helical structure at the beginning of the 1A domain. To date, 12 mutations in K17 gene have been identified in families with either the PC-2 or the SM phenotype. As indicated in Table I, all of these mutations are heterozygous missense mutations, located in the helix initiation domain (1A) of K17. The clustering of the mutations in the helix initiation motif is not surprising, because this area is highly conserved and even subtle amino acid changes are likely to disrupt intermediate filament formation and assembly.

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