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We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.
Identification of sporadic mutations in the helix initiation motif of keratin 6 in two pachyonychia congenita patients: further evidence for a mutational hot spot


Abstract: Pachyonychia congenita (PC) is a rare, autosomal dominant, ectodermal dysplasia characterized most distinctly by the presence of symmetric nail hypertrophy. In the Jadassohn–Lewandowsky form, or PC-1, additional cutaneous manifestations may include palmoplantar hyperkeratosis, hyperhidrosis, follicular keratoses, and oral leukokeratosis. Mutations have previously been identified in the 1A helix initiation motif of either keratin 6 or keratin 16 in patients with PC-1. In the current study, we have identified 2 sporadic, heterozygous mutations in the 1A helix region of the K6 isoform (K6a). The first mutation identified was a 3 base pair deletion (K6aΔ N171). The second mutation was a C-to-A transversion resulting in an amino acid substitution (K6a N171K). These data, in combination with previous reports, provide further evidence that this location is a mutational hot spot.

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Introduction

Pachyonychia congenita (PC) is an autosomal dominant genodermatosis characterized by ectodermal dysplasias, of which the hypertrophic nail dystrophy is the most prominent. Clinically, pachyonychia congenita has been divided into 2 subsets. In the Jadassohn–Lewandowsky form, or PC-1, the pachyonychia may be accompanied by palmoplantar hyperkeratosis, hyperhidrosis, occasional blistering, follicular keratoses, and oral leukokeratosis (1). The rarer Jackson–Lawler form, or PC-2, is also characterized by pachyonychia, but affected individuals lack the oral lesions commonly seen in PC-1 and, unlike PC-1, often exhibit multiple epidermal cysts. Individuals with PC-2 may also have recurrent flexural infections, hair abnormalities, corneal abnormalities, and natal teeth (2).

Keratins are the major structural proteins of the epidermis and associated appendages. These proteins are characterized by the presence of a highly conserved central rod domain consisting of 4 alpha-helical regions (1A, 1B, 2A and 2B). Approximately 30 different keratin genes have been identified, and these genes are differentially expressed at specific anatomic locations and epithelial surfaces (3). Keratin 6 (K6) and keratin 16 (K16) are normally restricted to the palmoplantar epidermis and the keratinoceytes en-
comprising hair follicles but not in the interfollicular regions (4, 5). However, upon irritation of the epidermis by stressors such as trauma, skin tumors, or psoriasis, these keratin genes are activated and the keratins are expressed (6). Keratins K6 and K16 are also expressed in numerous internal epithelia including the tongue, esophagus, trachea, and oral mucosa (5, 7). K17 is structurally very similar to K16, and its tissue distribution is somewhat similar but more restricted (8).

Mutations in K6, K16, and K17 have been identified in individuals with PC. PC-1 has been associated with mutations in K6 and K16, while PC-2 has been associated with mutations in K17. All of the mutations reported thus far have involved the 1A helix initiation motif of these keratin genes (9). Both single base pair substitutions and three base pair deletions have been reported (10–12). Here, we report two cases of PC-1 resulting from mutations in the 1A helix region of the K6 isoform (K6a).

Materials and methods

Direct sequencing of the 1A region of K6a

Human genomic DNA was purified from proband blood as previously described (13). The 1A region of K6a was amplified from genomic DNA using specific oligonucleotides HK6p14 (5’ CTG GGG GGC TCC AGG AGG ATC TCC 3’) and reverse primer HK6p13R (5’ CGC ACC TGA AAG AGA GAC AAG ATG 3’) (11). The amplified DNA fragment was captured onto streptavidin-coated magnetic beads via a biotinylated primer. Following denaturation, the resultant single-stranded DNA was used as a template in a manual DNA sequencing reaction (13). The sequencing primer used was HK6p11 (5’ CCA AGA GGT CAC CGT CAA CCA GAG 3’) (11).

Allele-specific sequencing

PCR-amplified DNA prepared as previously described was cloned into a pGEM-T vector (Pro-
mega Madison, WI, USA) (14). Cloned DNA corresponding to each allele was sequenced by fluorescence automated sequencing using primer HK6p11.

Automated sequencing of DNA from other family members

Human genomic DNA was purified from the blood of family members as previously described (13). The 1A region of K6a was amplified from genomic DNA using specific oligonucleotides HK6p14 (5’ CTG GGG GGC TCC AAG AGG ATC TCC 3’) and reverse primer HK6p ROEL (5’ CTT GTC GAT GAA GGA GGC AA 3’). The amplified DNA fragment was purified with Promega Magic PCR Preps (Promega). The PCR products were sequenced by fluorescence automated sequencing using primer HK6p11.

Figure 2. Mutation identification. (A) Note the double bands occurring after N171 in proband A. This is the result of a 3 base pair deletion at N171, in 1 of the alleles. (B) Automated sequencing of cloned DNA corresponding to each allele reveals a 3 base pair deletion (CA-A: N171) in the mutant allele and a normal sequence in the other allele. (C) Note the double band (C+ A) at N171 in proband B, who is heterozygous for the substitution of C-to-A in the mutant allele. (D) Automated sequencing of allele specific DNA shows a C-to-A transversion in the mutant allele, and a normal sequence in the other allele.

Results

Proband A

The first family, family A, was a Hispanic family from Texas whose daughter, proband A, originally presented at 2 months of age with oral leukokeratosis, mistakenly diagnosed as oral candidiasis (Fig. 1A). When the lesions were unresponsive to anti-fungals, the physician noted the hypertrophic nail changes (Fig. 1B) and referred the patient to a pediatric dermatologist who made the clinical diagnosis of PC-1. The patient was started on tretinoin 0.025% gel and lactic acid 12% lotion for the fingernails and toenails. The patient was also to use the tretinoin gel and clobetasol 0.05% gel on the tongue lesion. At 9 months of age, hyperkeratotic papules became evident on the elbows and knees that were not present on initial presentation (Fig. 1C). At 15 months of age, the hypertrophic
nail changes, oral leukokeratosis, and follicular hyperkeratoses were still evident. No palmoplantar hyperkeratoses were seen. Neither parents nor siblings had any hypertrophic nail changes, palmoplantar hyperkeratoses, oral hyperkeratosis, or any other skin dysplasias.

To identify the mutation, genomic DNA segments spanning the IA helix domain of K6 a were amplified by PCR and manually sequenced. Direct sequencing of DNA from proband A revealed a heterozygous 3 base pair deletion at codon 171, resulting in the deletion of an asparagine (Asn) residue from the amino acid sequence (Fig. 2A). Allele-specific DNA sequencing of proband A DNA revealed that 1 allele had the normal sequence, L-N-N-K-F, while the sequence of the other allele was L-N-K-F-A, confirming an Asn (N) deletion (Fig. 2B). Automated sequencing of parental DNA revealed no mutation in the IA helix domain of K6 (data not shown).

**Proband B**

The second family, family B, was from the East Coast of the United States whose son, proband B, had the clinical features of PC-1. These features included hypertrophic nail changes (Fig. 1D), oral leukokeratosis (Fig. 1E), and plantar hyperkeratosis (Fig. 1F). This patient was treated with a variety of topical keratolytics, as well as retinoic acid cream, but with minimal diminution of the hyperkeratosis of the skin. Neither parent had any clinical evidence of PC-1.

As in the previous family, DNA segments from the IA helix domain of K6 a were amplified by PCR and manually sequenced. Direct sequencing of DNA from proband B revealed a heterozygous C to A transversion (Fig. 2C). This nucleotide change resulted in the substitution of an asparagine (AAC) by a lysine (AAG) at codon 171 (N171K). Allele-specific DNA sequencing of proband B DNA confirmed the heterozygous mutation (Fig. 2D). No corresponding mutations were found in the K6a gene by automated sequence analysis of parental DNA (data not shown).

**Discussion**

In proband A, the trimucleotide deletion identified in the IA region of K6a has been previously described in several PC-1 patients by Bowden et al. (11) and Haley et al. (12). Interestingly, a trimucleotide deletion resulting in loss of Asn has also been reported in the same region of the structurally similar keratin 4 (15). The mutational mechanism has been speculated to be slipped mispairing and has been described as the possible cause of single and multiple base pair deletions in many genetic disorders (3, 15, 16). The deletions occur in regions with nucleotide repeats when DNA is mispaired onto similar adjacent nucleotide sequences during replication and a portion of the sequence is excised (16).

In proband B, the mutation detected was a transversion, also in the IA helix domain of K6a, resulting in an amino acid substitution of an asparagine by a lysine (N171K). In recent work by Haley et al. (12), an identical point mutation was identified. They also identified an additional point mutation in the IA helix at codon 174, which results in a phenylalanine to serine amino acid substitution. The occurrence of multiple mutations within the highly conserved helix initiation motif of K6a further supports the claim of mutational hot spots in keratin genes (3, 9).

One of the primary concerns of the parents was the risk of genetic transmission of PC-1 in future pregnancies for both themselves and their children. Genetic counseling in newly diagnosed autosomal dominant disorders becomes problematic when no additional family members are affected. For each family described in this article, the absence of clinical signs of PC in both families and the absence of mutations in the parents suggests both proband A and proband B were most likely affected by spontaneous mutations. In this case, the risk of the parents having another child with PC would be no greater than that of the general population. However, the risk for genetic transmission of PC would be significantly higher if either parent were affected by a phenomenon known as gonadal mosaicism. In gonadal mosaicism, a mutation occurs early in gametogenesis in the parent and a bimodal population of germ cells is produced. The risk for transmission would then be proportional to the percentage of germ cells carrying the mutation. Although gonadal mosaicism is rare, it has been reported or suspected in several ectodermal dysplasias, including epidermolysis bullosa simplex localisata (17), epidermolysis hyperkeratosis (18), and leukonychia totalis (19). With regard to the risk of either proband transmitting PC-1 to his or her offspring the risk is equivalent to transmission of any autosomal dominant trait.

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References