



# Pachyonychia Congenita Project

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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.

## Novel Keratin 17 Mutations in Pachyonychia Congenita Type 2

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**Pachyonychia congenita type 2 is an inherited ectodermal dysplasia characterized by hypertrophic nail dystrophy and multiple pilosebaceous cysts. Focal nonepidermolytic palmoplantar keratoderma, natal teeth, and pili torti may also be present. Epithelial tissues affected in pachyonychia congenita type 2 express the keratin pair K6b/K17. Here, we report three novel heterozygous mutations in the K17 gene**

**(KRT17A) in patients presenting with pachyonychia congenita type 2. These mutations, R94–98del (deletion of the peptide sequence RLASY) and missense mutations R94P and L95Q, are all within the 1A domain hotspot for pathogenic keratin mutations. Key words: genetics/genodermatosis/intermediate filaments/keratinizing disorder/mutation. J Invest Dermatol 116:806–808, 2001**

**P**achyonychia congenita (PC) includes a range of ectodermal dysplasias where hypertrophic nail dystrophy is the main clinical feature (Gorlin *et al*, 1976). PC is normally inherited in an autosomal dominant manner and is subdivided into two main types: PC-1 (Jadassohn and Lewandowsky, 1906) and PC-2 (Jackson and Lawler, 1951). In the PC-2 variant (OMIM number 167210), characteristic multiple pilosebaceous cysts develop during puberty. Patients may also have natal teeth, *pili torti*, mild focal nonepidermolytic palmoplantar keratoderma, or oral leukokeratosis; however, these features show variable expression (Munro *et al*, 1994).

Keratins are structural proteins found in all epithelial cells (Lane, 1993). They are divided into type I (K9–20) and type II (K1–8) keratins, and are normally expressed in pairs consisting of one of each type, in a tissue- and differentiation-specific manner. Specific pairs of type I and type II keratins form heteropolymeric 10 nm intermediate filaments that serve to protect epithelial cells from physical damage (McLean and Lane, 1995). K17 is expressed in the nail bed, sebaceous glands, hair follicles, and other epidermal appendages (Troyanovsky *et al*, 1989; McGowan and Coulombe, 2000). Several keratins are thought not to possess a unique partner and, until recently, K17 was thought to fall in this category. It has now been shown by *in situ* hybridization, however, that the K6b isoform of K6 colocalizes with K17 in epidermal adnexae (Smith *et al*, 1998).

Pathogenic mutations in keratin genes are now known to underlie a range of human epithelial fragility disorders with the majority of mutations occurring in the helix boundary motifs, highly conserved regions at either end of the rod domain (Fuchs

and Cleveland, 1997; Irvine and McLean, 1999). Four keratin genes are associated with PC. Mutations in type I K16 or type II K6a cause PC-1 (Bowden *et al*, 1995; McLean *et al*, 1995) and mutations in type I K17 or type II K6b lead to PC-2 (McLean *et al*, 1995; Smith *et al*, 1998). The discovery of a mutation in K6b in a family with PC-2 provided evidence that K6b and K17 are a *bona fide* keratin pair (Smith *et al*, 1998). Mutations in K17 also cause steatocystoma multiplex, a variant of PC-2 characterized by multiple pilosebaceous cysts but with very little or no nail involvement (Covello *et al*, 1998). In some cases, the same mutation in K17 has been reported to give rise to both PC-2 and steatocystoma multiplex demonstrating phenotypic variation regardless of the specific mutation (Covello *et al*, 1998).

Here, we report three novel mutations in the K17 gene in patients presenting with the PC-2 phenotype.

### MATERIALS AND METHODS

**Mutation detection and confirmation** Genomic DNA was extracted from whole blood by standard procedures. A 978 bp fragment spanning exon 1 of the K17 gene was amplified from genomic DNA using a primer pair specific to K17 (K17P8 and K17P10) in order to avoid pseudogene contamination as previously described (McLean *et al*, 1995), except that 1 mM MgCl<sub>2</sub> and 4% dimethylsulfoxide (DMSO) were used, greatly improving this reaction. Polymerase chain reaction (PCR) products were purified using QIA quick PCR purification kit (Qiagen, Crawley, England) and directly sequenced on an ABI 377 Genetic Analyzer (ABI, Foster City, CA) using forward primer K17P3 5' TAT GGC AGC AGC TTT GGG and reverse primer K17P4 5' GGT ACC AGT CAC GGA TCT TCA 3'. Each mutation was excluded from 50 normal, unrelated individuals by restriction enzyme analysis or conformation sensitive gel electrophoresis (CSGE; Ganguly *et al*, 1993).

For mutation R94–98del, the deletion was confirmed by cloning the 978 bp fragment (K17P8–K17P10) into pCR2.1 vector (Invitrogen, Groningen, The Netherlands). This mutation was excluded from a normal population by CSGE. Genomic DNA was amplified as above and the PCR products were analyzed on 8% CSGE gels without further purification. Gels were stained with ethidium bromide (0.05 µg per ml) for 15 min and fragments were visualized under ultraviolet light.

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Abbreviations: PC, pachyonychia congenita; PC-1, pachyonychia congenita type 1; PC-2, pachyonychia congenita type 2.

Mutation R94P does not alter any known restriction enzyme site. Using a primer with a mismatch base, a new *Sma* I site was created in combination with the mutation. Nested PCR reactions were performed to avoid pseudogene contamination. Genomic DNA was first amplified as above and a second round of PCR was performed to amplify a 282 bp fragment with primers K17P12 5' CAC CTC CTC CAG CTC CAT CAA G 3' and K17PGG 5' ACC TTG TCC AGG TAG GAG GCC CGG 3' (mismatch underlined). PCR was performed in 1 × PCR buffer (Promega, Madison, WI) containing 1 mM MgCl<sub>2</sub> and 4% DMSO. PCR conditions were 94°C 5 min × 1, followed by 30 cycles of 94°C 30 s, 55°C 45 s, 72°C 1 min, and 72°C 5 min × 1. Diluted PCR products were digested overnight at 27°C with 5 U *Sma* I (Promega) and analyzed on 3.5% agarose gels.

Mutation L95Q creates a new *Stu* I site, which was used to exclude the mutation from normal individuals. Using primers K17P8 and K17P10 (above), genomic DNA was amplified, followed by a second round of PCR to amplify a 338 bp fragment using primers K17P12 and K17P4 (above). Reaction conditions were as for K17P12–K17PGG (above). PCR products were diluted and digested overnight at 37°C with 5 U *Stu* I. Digests were analyzed on 3% agarose gels.

**DNA fingerprinting** For paternity testing in sporadic cases, the Second Generation Multiplex (SGM) set of seven short tandem repeat markers was used (Sparkes *et al.*, 1996). Microsatellite markers were labeled with fluorescent dyes and analyzed on an ABI 377 Genetic Analyzer using ABI Genescan and Genotyper software (ABI).

## RESULTS

**Clinical findings** Family 1 were of Italian Caucasian ethnicity and showed typical autosomal dominant inheritance of PC-2 through two generations. The proband is the second daughter of unrelated parents. Her mother was affected with PC-2 as was her older sister, who died at 4 mo of age from bronchopneumonia, presumably unrelated to the pachyonychia phenotype. The clinical findings of this family have been described previously (Clementi *et al.*, 1986).

The proband in family 2 was a female Australian Caucasian presenting with typical features of PC-2 as a sporadic case. Interestingly, the proband's paternal grandfather and paternal uncle had been diagnosed with epidermolysis bullosa simplex, although the patient's father showed no signs of either the latter disorder or pachyonychia.

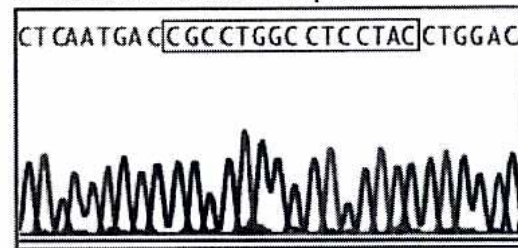
Family 3 was also a sporadic French Caucasian case who was born with three natal teeth, indicative of PC-2. Now at 3 y of age, she has pachyonychia of all fingernails and toenails, and oral leukokeratosis with two small white patches on the tongue. She has no signs of palmar or plantar hyperkeratosis.

**Identification and confirmation of novel mutations in K17** Direct sequencing of PCR products revealed mutations in exon 1 of the K17 gene in all three patients. In the proband of family 1, overlapping sequence traces were found, indicative of a deletion mutation (not shown). Cloning the K17 PCR fragment into pCR2.1 vector revealed two species of clones: one with the normal sequence and a second with a 15 bp deletion mutation, designated 279del15 (Fig 1a, b). This defect removes five amino acids (RLASY) from the helix initiation motif of the K17 protein (designated R94–98del). CSGE was used to exclude the mutation from 50 normal, unrelated individuals. In the proband, the smaller mutant allele could be separated from the normal allele and two bands were observed due to heteroduplex formation (Fig 2a). In contrast, all 50 control samples showed a single homoduplex band.

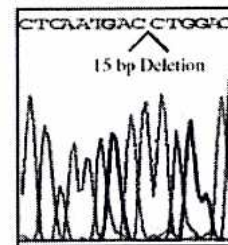
Heterozygous transversion mutation 281G > C was found in family 2 resulting in the predicted amino acid change R94P (Fig 1c). This mutation does not create or destroy any known restriction enzyme site so a primer with a mismatch base was designed that creates a new *Sma* I site in combination with the mutation. Analysis of PCR products digested with *Sma* I confirmed the mutation in the affected individual and excluded it from the unaffected parents and 50 normal, unrelated individuals (Fig 2b).

In the third family, the affected individual was heterozygous for the transition 284T > A leading to the predicted amino acid change L95Q (Fig 1d). This mutation creates a new *Stu* I site that

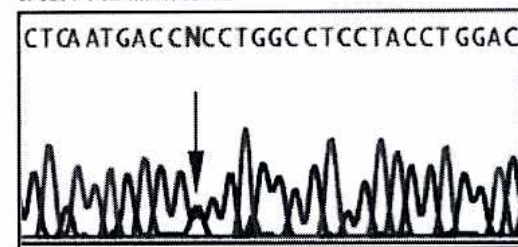
### a. Normal K17 1A Domain Sequence



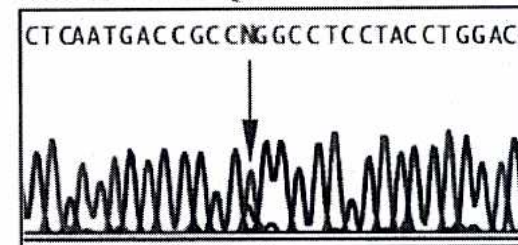
### b. K17 R94-98del



### c. K17 Mutation R94P



### d. K17 Mutation L95Q

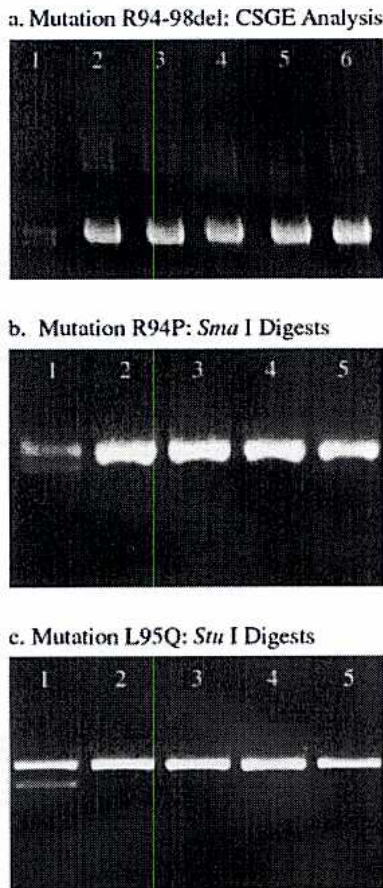


**Figure 1. Keratin 17 mutation analysis.** (a) Normal K17 sequence in exon 1, corresponding to codons 91–100, base numbers 271–300, inclusive. The 15 bases deleted in family 1 are boxed (b, below). (b) Sequence of a cloned mutant allele in pCR2.1 vector. The same region shown in (a), derived from the proband in family 1, showing the deletion 279del15 that removes five amino acids (R94–98del). (c) The equivalent sequence shown in (a) from the affected individual in family 2 showing heterozygous missense mutation 281G > C (arrow) that predicts the amino acid substitution R94P. (d) The equivalent sequence shown in (a) derived from the affected individual in family 3 showing missense mutation 284T > A (arrow) leading to amino acid change L95Q.

was used to exclude the mutation from the unaffected parents and 50 normal, unrelated individuals (Fig 2c).

## DISCUSSION

In the three PC cases studied, one familial and two sporadic, novel mutations were identified in the helix initiation motif of K17. The 279del15 mutation in family 1 is the first report of a deletion in K17 and leads to removal of the amino acid sequence RLASY (R94–98del) from the highly conserved 1A domain of the K17 protein (deletion of 1A domain residues 10–14). Missense mutations, R94P and L95Q, were detected in affected individuals of the other two



**Figure 2. Keratin 17 mutation conformation.** (a) Confirmation of mutation R94-98del by CSGE. Lane 1: PCR product derived from the patient shows a heteroduplex band representing the normal and mutant alleles; lanes 2-6 are homoduplex bands from unrelated unaffected individuals. (b) Confirmation of mutation R94P by *Sma* I digestion. Lane 1: digested PCR product from the affected individual gives an additional band due to introduction of an *Sma* I site in one primer that depends on the mutation. Lanes 2-5 are digested PCR products from normal unrelated controls. (c) Confirmation of mutation L95Q by *Stu* I digestion. Lane 1: digestion of PCR product from the affected individual shows an additional band due to a new *Stu* I site created by the mutation. Lanes 2-5 show digested PCR products from normal unrelated controls.

cases, again in the 1A domain (1A residues R10P and L11P). Previously, all reported mutations in PC-2, like those detected in families 2 and 3 here, have been heterozygous missense mutations in K17 (McLean *et al*, 1995; Smith *et al*, 1997; Covello *et al*, 1998; Fujimoto *et al*, 1998; Celebi *et al*, 1999). Interestingly, the 279del15 mutation produces a similar clinical phenotype to that seen with missense mutations in this region (Clementi *et al*, 1986). This is in contrast to a family reported with a 24 bp deletion in the 2B domain of K16, who presented with a very mild focal nonepidermolytic palmoplantar keratoderma phenotype (Smith *et al*, 2000). Larger deletions that completely remove a keratin helix boundary motif might be expected to have a milder dominant-negative effect due to loss of these critical molecular overlap regions (Steinert *et al*, 1993). Although this does not appear to be the case in family 1 here, it is possible that a larger deletion in this vicinity might result in a milder disease. Such unusual mutations are invaluable in defining the functionally critical regions of the keratin rod domain, thus emphasizing the need for continued mutation analysis.

Including this study, all reported K17 mutations are in the helix initiation motif at the start of the alpha helical rod domain (Irvine and McLean, 1999). Similar missense or small in-frame insertion/deletion mutations in the helix termination motif at the opposite end of the rod domain are also predicted to produce PC-2, although

none has been reported to date. K6b and K17 are now known to be coexpressed and with one report of a mutation in K6b it is likely that some PC-2 families where no mutation in K17 has been identified may have pathogenic mutations in K6b (Smith *et al*, 1998).

In conclusion, we report three novel mutations in K17 and show that in-frame deletions in this gene, in addition to missense mutations, can cause PC-2.

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