



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.

A novel point mutation of keratin 17 (*KRT17*) in a Japanese family with pachyonychia congenita type 2: an RNA-based genetic analysis using a single hair bulb

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Pachyonychia congenita type 2 (PC-2) (MIM 167210; Jackson-Lawler syndrome) is an autosomal dominant keratin disorder characterized by hypertrophic nail dystrophy with multiple pilosebaceous cysts. Genetic defects in PC-2 have been correlated with two different keratin genes: keratin 17 (*KRT17*) and keratin 6b (*KRT6B*).^{1,2} We report here a novel missense mutation, methionine at codon 88 to lysine (p.M88K), of *KRT17*, which was detected using RNA from only an individual hair bulb.

Patient and methods

A 12-year-old Japanese girl came to our clinic because of her thickened nails and palmoplantar hyperkeratosis with a complaint of plantar pain during exercising (Fig. 1b,c). She did not have hair abnormalities or eruptions on her arms or legs. Her teeth and tongue were normal at that time, but she had been born with natal teeth. Fungi were not identified in samples from nails or scales of her palms and soles. The family pedigree showed six affected persons over three generations (Fig. 1a), an inheritance pattern that is compatible with autosomal dominance. The affected adults had multiple subcutaneous nodules on the flexor sides of their arms (Fig. 1d).

For genetic analysis, one hair bulb and 3 mL peripheral blood were obtained from the patient and from family members. Each hair bulb was immersed in RNAlater solution (Applied Biosystems, Foster City, CA, U.S.A.) immediately after plucking and was stored at 4 °C until analysis. Total RNA was extracted from each hair bulb and cDNA was synthesized using a HighPure RNA paraffin kit (Roche Diagnostics, Basel, Switzerland) and ReverTra Ace reverse transcriptase (Toyobo Co., Ltd, Osaka, Japan) the mutation rate of which is about 1:56%. RNA purified from one hair bulb was insufficient for quantitative analysis but was sufficient for two to three rounds of cDNA synthesis by scaling down the reaction. The reaction products were used as templates for polymerase chain reaction (PCR) amplification of *KRT17* and *KRT6B* cDNA. The 1413-bp nucleotides covering the full coding sequence of the *KRT17* cDNA were amplified into overlapping upper (F1) and lower (F2) DNA fragments by PCR using the following primers: 5'-ATGACCACCTCCATCCGCCAGTT-3' and 5'-TCTTCTCATAC-

TGGTCACGCATCTCGTTG-3' for F1, and 5'-GAGGAGCTGGCC-TACCTGAA-3' and 5'-AAGCATGGGGAAGGGACTGA-3' for F2. PCR cycling conditions for F1 or F2 were 94 °C for 2 min; 40 cycles of 94 °C for 15 s, 65 °C or 60 °C, respectively, for 30 s, and 68 °C for 1 min, with a final extension step at 68 °C for 10 min. The analysis of *KRT6B* cDNA and *KRT17* genomic DNA was as reported previously.^{1,2} The protocol of this study was approved by the ethical committee of the Hyogo College of Medicine, and informed consent was obtained from all family members enrolled for the analysis and from healthy volunteers used as controls.

Results and discussion

Direct sequencing of *KRT17* and *KRT6B* cDNA derived from the single hair bulb of II-2 revealed a heterozygous T to A substitution at nucleotide c.263, resulting in the missense mutation p.M88K in *KRT17* (Fig. 1e). No other substitutions or deletions/insertions of bases were found in the coding sequence of *KRT17* and no mutation was detected in the *KRT6B* cDNA of II-2. The same mutation of *KRT17* was found in cDNAs derived from individual hair bulbs of affected family members. The mutation was verified by direct sequencing of exon 1 in the *KRT17* genomic DNA. The mutation-dependent loss of an NlaIII recognition site in exon 1 was not found in genomic DNA from 100 unaffected healthy volunteers (data not shown). The specific substitution p.M88K has not been reported as a single nucleotide polymorphism (SNP) in the Entrez SNP (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp>) or the dbProP (<http://dbprop.nirs.go.jp/Prop/index.html>) databases.

The novel mutation p.M88K is located in the helix initiation motif (HIM) of *KRT17*, where 13 of the reported 14 mutations of *KRT17* in PC-2 have been mapped, and the residue M88 of the HIM is also a mutation hotspot of other keratin disorders (<http://www.interfil.org/>).³ The mutation p.M88T at the same codon was reported in two previous patients with PC-2.^{4,5} Follicular keratoses reported in those patients with p.M88T were absent in our patients, and instead, the focal palmoplantar keratoderma seen in our patients was not documented in those earlier patients.

For mutation analysis of *KRT17* and *KRT6B*, we used RNA isolated from individual hair bulbs, because *KRT17* has two pseudogenes and *KRT6B* has several orthologues.^{6,7} King et al.⁸

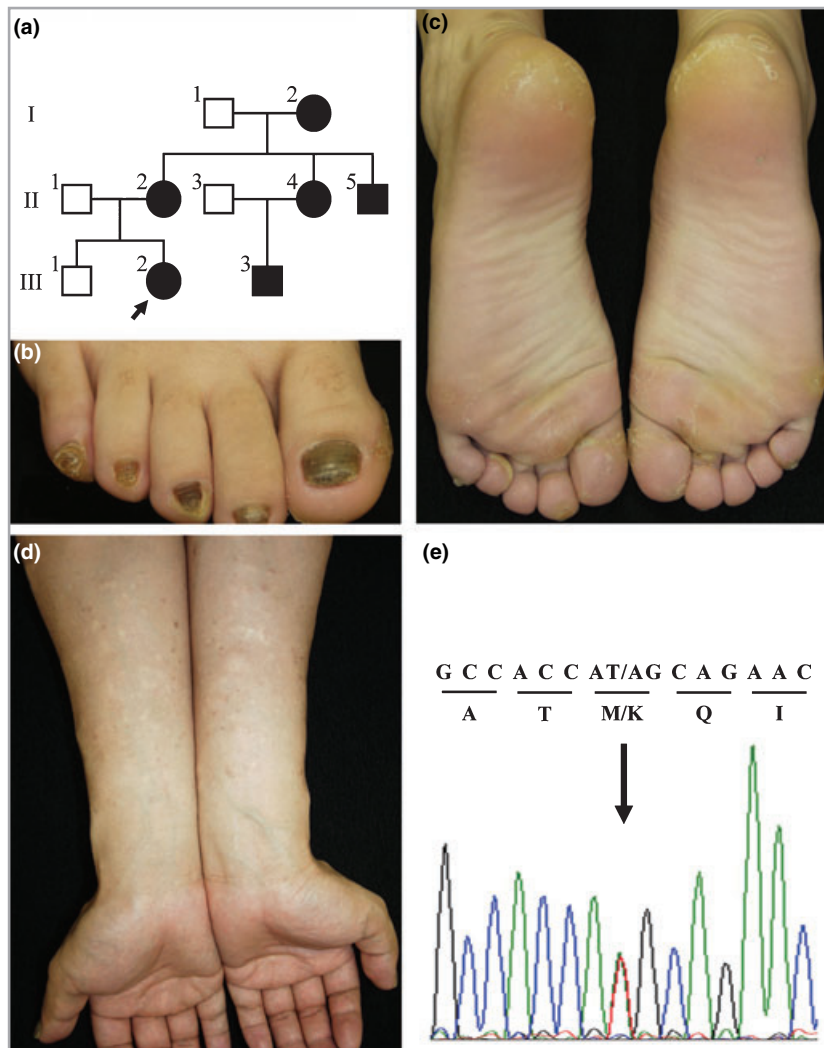


Fig 1. Clinical features of a family with pachyonychia congenita type 2 and a missense mutation p.M88K in *KRT17*. (a) Pedigree of the family. The arrow indicates the 12-year-old girl studied here. (b) Hypertrophic dystrophy of the right toenails of the patient. (c) Focal palmoplantar keratoderma of the patient. (d) Widespread multiple cysts on the forearms of affected family member II-5. (e) Heterozygous c.263T→A mutation identified by direct sequencing of *KRT17* cDNAs from all affected family members and in the genomic DNA of II-2 and III-2. This mutation was not found in genomic DNA from unaffected family members II-1 or III-1 or from 100 healthy volunteers.

and Tazón-Vega *et al.*⁹ have shown that RNA from 10–30 hair bulbs is sufficient for mutation analysis of *COL4A5* cDNA. Hair bulbs are more easily obtained and biologically safer to be handled than are blood samples. Plucking a few hair bulbs is not very painful and is less stressful for children. The method used in the present study requires only a single hair bulb for genetic analysis, although it will be necessary to repeat the analysis to avoid potential artifactual errors introduced by the reverse transcription or PCR steps, if genomic DNA is not used for confirmation.

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Conflicts of interest: none declared.