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A Novel Mutation in the Second Half of the Keratin 17 1A Domain in a Large Pedigree with Delayed-Onset 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 pilosebaceous cysts. We report a large Chinese pedigree of typical delayed-onset PC-2 that includes 19 affected members. Direct sequencing of PCR products revealed a novel heterozygous 325A → G mutation in the affected members. This mutation predicts the substitution of asparagine by aspartic acid in codon 109 (N109D) located in the second half of the keratin 17 1A domain, where similar mutation in keratin 5 is associated with the mild Weber–Cockayne form of epidermolysis bullosa simplex.

Key words: ectodermal dysplasia/genodermatosis/intermediate filaments/nail dystrophy
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Pachyonychia congenita (PC) consists of a group of inherited ectodermal disorders characterized by hypertrophic nail dystrophy. Two main clinical subtypes of PC are generally recognized, PC-1 (Jadassohn–Lewandowsky syndrome, MIM no. 167200) and PC-2 (Jackson–Lawler syndrome, MIM no. 167210). In PC-1 pachyonychia is accompanied by severe palmoplantar keratoderma (PPK), follicular keratoses, and oral leukokeratosis. In PC-2 pachyonychia is also associated with focal PPK and follicular keratoses but is readily distinguished by the presence of multiple steatocysts. Usually, since the cysts normally only appear at puberty, PC-2 is difficult to distinguish from PC-1 in childhood. The genetic cause of PC is mutations in four differentiation-specific keratin genes that are expressed by the affected epithelia. PC-1 is due to mutations of keratin 16 (K16) gene (McLean *et al*, 1995) or its expression partner K6a (Bowden *et al*, 1995). Similarly, PC-2 is due to mutations in keratin 17 (K17) gene (McLean *et al*, 1995; Smith *et al*, 1997) or the K6b (Smith *et al*, 1998).

In classical PC-2, thickening of the nails usually begins within the first months of life. Clinical observation of some patients with the onset of the characteristic nail changes of PC during the second and third decades of life has been reported in the literature, which are designated "PC tarda" (Paller *et al*, 1991). To our knowledge, keratin mutation detected in delayed onset PC has been reported in only one case of PC-1, which occurred at the central 2B domain of K16 (Connors *et al*, 2001). Here, we report a Chinese pedigree with typical delayed-onset PC-2. A novel mutation in the second half of the K17 1A domain underlies the affected members' phenotype.

Results

The nested PCR produced expected 200 bp DNA fragment. Direct sequencing of the PCR products revealed a heterozygous 325A → G mutation in the nine affected members. This mutation predicts the substitution of asparagine by aspartic acid in codon 109 (N109D) located in the second half of the 1A domain (N25D in terms of the 1A domain) of K17. No such mutation was found in the unaffected member and 50 unrelated controls (Fig 1). Sequencing of 50 unrelated and unaffected controls' PCR products excluded the polymorphism.

Discussion

Keratins are heterodimeric proteins that form the intermediate filament cytoskeleton of epithelial cells. They have a similar protein structure consisting of a central helical rod domain, which is responsible for polymerization of these proteins to form keratin tonofilaments. This rod domain is subdivided into 1A, 1B, 2A, and 2B segments by flexible linkers L1, L12, and L2. 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 intermediate filaments *in vivo* and *in vitro* (Fuchs and Weber, 1994; Irvine and Mclean, 1999). K17 is expressed in the nail

Abbreviations: EBS, epidermolysis bullosa simplex; K5, keratin 5; K14, keratin 14; K16, keratin 16; K17, keratin 17; PC, pachyonychia congenita; PPK, palmoplantar keratoderma

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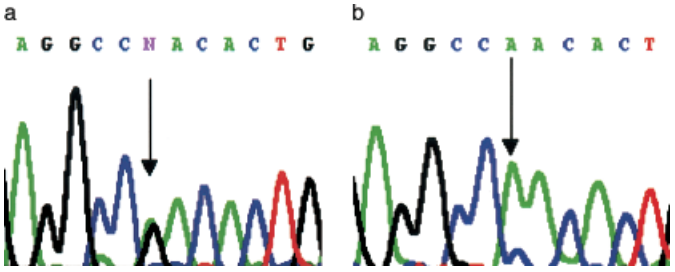

Figure 1
Direct automated sequencing of KRT17 gene, showing codon 109 in exon 1. (*a*) Heterozygous missense mutation 325A → G. This mutation predicts the amino acid change asparagine to aspartic acid at codon 109 (N109D). (*b*) Sequence in normal subjects. Green, A; black, G; red, T; blue, C.

bed, hair follicle, and sebaceous gland (Troyanovsky *et al*, 1989; McGowan and Coulombe, 2000). We have identified a novel mutation (N109D) located in the second half of the 1A domain (N25D in the 1A domain) of K17 in a Chinese pedigree with delayed-onset PC-2. To date and including this report, 24 independent mutations have been described in patients with either PC-2 or steatocystoma multiplex (Covello *et al*, 1998; Fujimoto *et al*, 1998; Celebi *et al*, 1999; Smith *et al*, 2001; Terrinoni *et al*, 2001; Wang *et al*, 2001; Hashiguchi *et al*, 2002; Feng *et al*, 2003; www.interfil.org). They were located in the helix initiation 1A domain of K17 (Fig 2). All previously reported mutations which cause PC have affected one of the highly conserved sequences at either end of this helical rod domain common to all keratin molecules (Munro, 2001), except a mutation in the mid-region of the 2B helical domain of K16 reported by Connors *et al* (2001) in a patient with delayed-onset PC-1.

Interestingly, mutations in the second half of the 1A domain have been associated with other keratin disorders, for example equivalent residue in keratin 5 (K5), N193K (N25K in the 1A domain) causes mild Weber–Cockayne form of epidermolysis bullosa simplex (EBS) (Humphries *et al*, 1996). In EBS, the site of mutation is a strong determinant of the severity of the phenotype. The most severe form of EBS with widespread clustered blistering is the Dowling–Meara variant (EBS-DM). This is predominantly caused by mutations affecting the helix boundary motifs of either K5 or K14. In the mild Weber–Cockayne form (EBS-WC), blisters are mainly found on easily traumatized sites such as the feet and hands. In this case, the mutations are located in clusters in the second half of the 1A and 2B domains, the L12 domain of both K5 and K14 (Irvine and Mclean, 2003). Furthermore, utilizing an established *in vitro* filament disassembly assay, Steinert's group have shown that inhibitory peptides analogous to sequences from the first half of the 1A domain interfere with keratin assembly, whereas those from the second half of 1A and other internal sites do not. This implies that the first half of 1A domain is more important in filament assembly/integrity (Steinert *et al*, 1993). Thus the identification here of a milder mutation giving rise to a late-onset form of PC-2 is consistent with the previous report of delayed onset of PC-1 (Connors *et al*, 2001), where a mutation was found in the central 2B domain of K16, a region also associated with milder EBS phenotypes. On this basis we speculate that the mutation occurring in the less critical site of the keratins may explain the delayed onset of PC. In addition to the location of the mutation within the keratin gene, other genetic or environmental factors could be important in the ultimate clinical expression (Stratigos and Baden, 2001). It is possible that modifier genes may promote or delay the onset of symptoms. A non-mutually exclusive possibility is that environmental factors may influence the age of disease presentation.

In conclusion, we report a novel mutation in the second half of 1A domain of K17 in a large pedigree with delayed-onset PC-2. More pedigrees of delayed-onset PC need to be studied to establish the correlation between the site of mutation in keratin and delayed-onset of pachyonychia.

Subjects, Materials and Methods

The Human Medical and Ethical Committee of Xian Jiaotong University approved the investigation presented here and all study subjects gave informed consent. We studied a Chinese pedigree of delayed-onset PC-2 (Fig 3) from the autonomous region of Inner

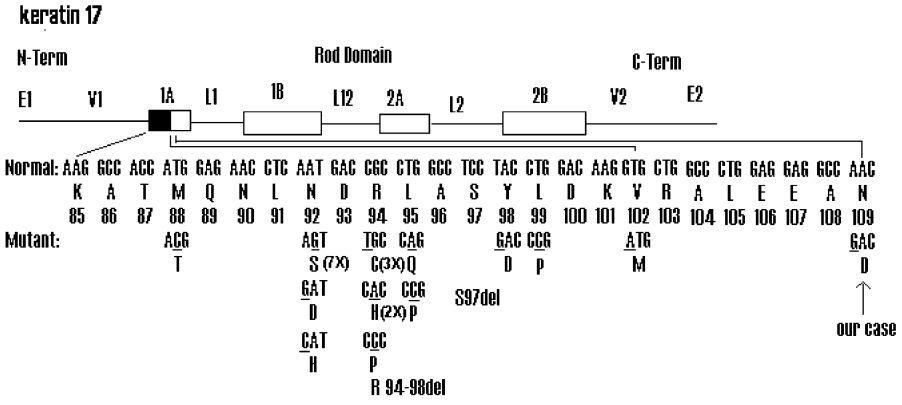

Figure 2
Schema of K17 protein structure and summary of reported K17 gene mutations in Pachyonychia congenita type 2 (PC-2). Keratin has helix domains (1A, 1B, 2A, 2B) separated by linker domains (L1, L12, L2). The DNA and deduced amino acid sequence of the 1A domain are shown. All previously reported mutations in PC-2 and steatocystoma multiplex are located in the first half (shaded area) of 1A domain of K17, e.g. the helix initiation 1A domain (www.interfil.org). The mutation reported here in the delayed-onset PC-2 is in the second half (blank) of the 1A domain of K17. Single-letter abbreviations for the amino acid residues: A, Ala; C, Cys; D, Asp; H, His; K, Lys; L, Leu; M, Met; N, Asn; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val.Y, Tyr. R94H(2 ×), R94C(3 ×), and N92S(7 ×) indicate that these mutations have been independently reported more than once.

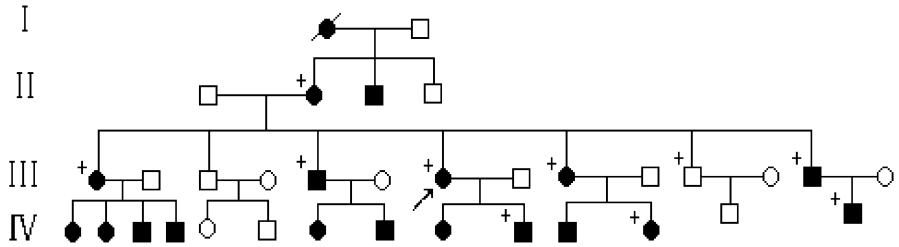

Figure 3
Pedigree of the delayed-onset pachyonychia congenita type 2 families studied. Arrow indicates the proband; I, II, III, IV: generation number; Cross indicates individuals from whom blood samples were obtained.

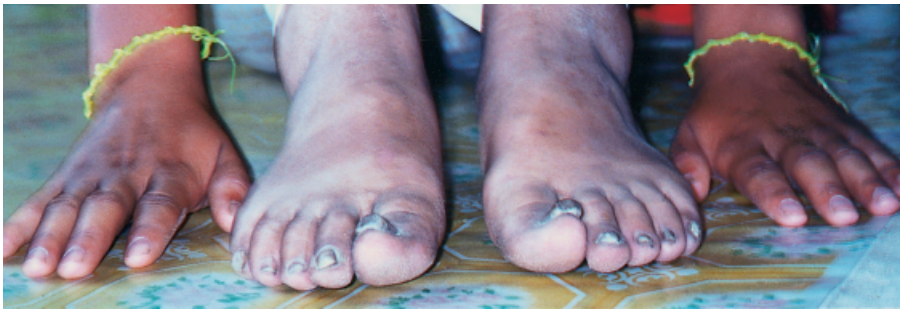

Figure 4
The hypertrophic toenail destrophy of the patient. Toenails show typical thickening of pachyonychia congenita, but the fingernails look normal.

Mongolian. The proband (female, aged 45 y) in this pedigree developed thickened toenails at 12 y of age and thickened fingernails at about 20 y of age. Multiple pilosebaceous cysts appeared in the axillae, groin area and on the chest and back at puberty. Most of the cysts are about 2–3 cm in diameter. Sometimes ulceration occurs and becomes infected. The ulcerated cysts healed with scars. Focal plantar hyperkeratosis appeared at about 14. The other 18 affected members had hypertrophic toenails at an age similar to the proband. Twelve of the 18 affected also had sebaceous cysts at puberty and focal plantar hyperkeratosis after thickened toenails appeared. Six of those are teenager patients whose toenails thickened, but fingernails still look normal (Fig 4). No natal teeth were found in all the affected members in the pedigree. Fungal examination under microscope and obvious family history exclude onychomychosis.

Five milliliters of peripheral blood was obtained from the proband, eight affected members, one unaffected member in the pedigree, and 50 unrelated and unaffected people. The genomic DNA was extracted with a whole blood genomic DNA extracting kit (Sino-American Bioengineer Company, Luoyang, China) and used as template for PCR-mediated amplification of exon 1 of the K17 gene. The primers (K17p8 and K17p10) and PCR conditions are described in the literature (McLean *et al*, 1995). The PCR products were diluted and used as template in the nested PCR whose primer sequences are as follows: the forward primer 5′-GCT GCT ACA GCT TTG GCT CT-3′ and the reverse primer 5′-CAC GAC GTT GTA AAA CGA CCA GTC ACG GAT CTT CAC C-3′. Sequence analyses were performed using Big Dye terminator chemistry on an ABI 377 genetic analyzer (Perkin-Elmer-Cetus Instruments, Norwalk, Connecticut). Sequences were compared with those of one unaffected, and 50 unrelated and unaffected samples.

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References

Bowden PE, Haley JL, Kansky A, Rothnagel JA, Jones DO, Turner RJ: Mutation of a type II keratin gene (K6a) in pachyonychia congenita. Nat Genet 10:363–365, 1995

Celebi JT, Tanzi EL, Yao YJ, Michael EJ, Peacocke M: Identification of a germline mutation in keratin 17 in a family with pachyonychia congenita type 2. J Invest Dermatol 113:848–850, 1999

Connors JB, Rahil AK, Smith FJD, Mclean WHI, Milstone LM: Delayed onset pachyonychia congenita associated with a novel mutation in the central 2B domain of keratin 16. Br J Dermatol 144:1058–1062, 2001

Covello SP, Smith FJ, Sillevis Smitt JH, *et al*: Keratin 17 mutations cause either steatocystoma multiplex or pachyonychia congenita type 2. Br J Dermatol 139:475–480, 1998

Feng YG, Xiao SX, Ren XR, Wang WQ, Liu A, Pan M: Keratin17 mutation in pachyonychia congenita type 2 with early-onset sebaceous cysts. Br J Dermatol 148:452–455, 2003

Fuchs E, Weber K: Intermediate filaments: Structure, dynamics, function and disease. Annu Rev Biochem 63:345–382, 1994

Fujimoto W, Nakanishi G, Hirakawa S, Nakanishi T, Shimo T, Takigawa M, Arata J: Pachyonychia congenita type 2: Keratin 17 mutation in a Japanese case. J Am Acad Dermatol 38 (6 Part 1):1007–1009, 1998

Hashiguchi T, Yotsumoto S, Shimada H, *et al*: A novel point mutation in the keratin 17 gene in a Japanese case of pachyonychia congenita type 2. J Invest Dermatol 118:545–547, 2002

Humphries MM, Mansergh FC, Kiang AS, *et al*: Three keratin gene mutations account for the majority of dominant simplex epidermolysis bullosa cases within the population of ireland. Hum Mutat 8:57–63, 1996

Irvine AD, McLean WHI: Human keratin diseases: The increasing spectrum of disease and subtlety of the phenotype–genotype correlation. Br J Dermatol 140:815–828, 1999

Irvine AD, Mclean WHI: The molecular genetics of the genodermatoses: Progress to date and future directions. Br J Dermatol 148:1–13, 2003

McGowan K, Coulombe PA: Keratin 17 expression in the hard epithelial context of the hair and nail and its relevance for the pachyonychia congenita phenotype. J Invest Dermatol 114:1101–1107, 2000

McLean WHI, Rugg EL, Lunny DP, *et al*: Keratin 16 and keratin 17 mutations cause pachyonychia congenita. Nat Genet 9:273–276, 1995

Munro CS: Pachyonychia congenita: Mutation and clinical presentations. Br J Dermatol 144:929–930, 2001

Paller AS, Moore JA, Scher R: Pachyonychia congenita tarda. A late-onset form of Pachyonychia congenita. Arch Dermatol 127:701–703, 1991

Smith FJ, Coleman CM, Bayoumy NM, Tenconi R, Nelson J, David A, McLean WH: Novel keratin 17 mutations in pachyonychia congenita type 2. J Invest Dermatol 116:806–808, 2001

Smith FJD, Corden LD, Rugg EL, *et al*: Missense mutations in keratin 17 cause either pachyonychia congenita type 2 or a phenotype resembling steatocystoma multiplex. J Invest Dermatol 108:220–223, 1997

Smith FJD, van Jonkman MF, Goor H, Coleman CM, Covello SP, Uitto J, McLean WHI: A mutation in human keratin K6b produces a phenocopy of the K17 disorder pachyonychia congenita type 2. Hum Mol Genet 7:1143–1148, 1998

Steinert PM, Yang JM, Bale SJ, Compton JG: Concurrence between the molecular overlap regions in keratin intermediate filaments and the locations of keratin mutations in genodermatoses. Biochem Biophys Res Commun 197:840–848, 1993

Stratigos AJ, Baden HP: Unraveling the molecular mechanisms of hair and nail genodermatoses. Arch Dermatol 137:1465–1471, 2001

Terrinoni A, Smith FJD, Didona B, *et al*: Novel and recurrent mutation in the genes encoding keratin K6a, K16 and K17 in 13 cases of pachyonychia congenita. J Invest Dermatol 117:1391–1396, 2001

Troyanovsky SM, Guelstein VI, Tchipysheva TA, Krutovskikh VA, Bannikov GA: Patterns of expression of keratin 17 in human epithelia: Dependency on cell position. J Cell Sci 93:419–426, 1989

Wang X, Shi Y, Ye Y, *et al*: Keratin 17 gene mutation in patients with steatocystoma multiplex. Zhonghua Yi Xue Za Zhi 81:540–543, 2001