Use of Articles in the Pachyonychia Congenita Bibliography

The articles in the PC Bibliography may be restricted by copyright laws. These have been made available to you by PC Project for the exclusive use in teaching, scholarship or research regarding Pachyonychia Congenita.

To the best of our understanding, in supplying this material to you we have followed the guidelines of Sec 107 regarding fair use of copyright materials. That section reads as follows:

Sec. 107. - Limitations on exclusive rights: Fair use
Notwithstanding the provisions of sections 106 and 106A, the fair use of a copyrighted work, including such use by reproduction in copies or phonorecords or by any other means specified by that section, for purposes such as criticism, comment, news reporting, teaching (including multiple copies for classroom use), scholarship, or research, is not an infringement of copyright. In determining whether the use made of a work in any particular case is a fair use the factors to be considered shall include - (1) the purpose and character of the use, including whether such use is of a commercial nature or is for nonprofit educational purposes; (2) the nature of the copyrighted work; (3) the amount and substantiality of the portion used in relation to the copyrighted work as a whole; and (4) the effect of the use upon the potential market for or value of the copyrighted work. The fact that a work is unpublished shall not itself bar a finding of fair use if such finding is made upon consideration of all the above factors.

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.
Delayed-onset pachyonychia congenita associated with a novel mutation in the central 2B domain of keratin 16

J.B.CONNORS, A.K.RAHIL,* F.J.D.SMITH,† W.H.I.McLEAN† AND L.M.MILSTONE

Yale University School of Medicine, Department of Dermatology, 500 LCI, PO Box 208059, New Haven, CT 06520-8059, U.S.A.
*Department of Dermatology and Cutaneous Biology, Jefferson Medical College, 233 South 10th Street, Philadelphia, PA 19107, U.S.A.
†Epithelial Genetics Group, Human Genetics, Department of Molecular and Cellular Pathology, Ninewells Medical School, Dundee DD1 9SY, U.K.

Accepted for publication 12 December 2000

Summary

A young girl with clinical features of pachyonychia congenita type 1 was unusual in that the typical skin and nail changes were not noted until the age of 6 years. Direct sequencing of the KRT16A gene, encoding keratin K16, revealed a novel mutation K354N in the central 2B domain of the K16 polypeptide. The mutation created a new BsmI restriction site and therefore, the mutation was confirmed in the patient and excluded from both parents and 50 normal, unrelated individuals by BsmI digestion of KRT16A polymerase chain reaction products. This is the first time a mutation has been described in this location in a keratin other than K14, where similar mutations cause the milder Weber–Cockayne and/or Köbner types of epidermolysis bullosa simplex.

Key words: ectodermal dysplasia, genodermatosis, intermediate filaments, nail dystrophy

Pachyonychia congenita (PC) is a rare form of ectodermal dysplasia.1–4 It is most often inherited in an autosomal dominant fashion and typically presents at birth or in the early neonatal period. Symmetrically thickened, dystrophic fingernails and toenails are the defining characteristic of pachyonychia congenita. Other signs, such as cuticular and follicular hyperkeratosis, hair abnormalities, palmoplantar keratoderma, corneal dyskeratosis and angular chelitis are variably expressed.5 Two major clinical subtypes of PC are generally recognized: type 1 (PC-1, MIM no. 167200, Jadassohn–Lewandowsky type1) and type 2 (PC-2, MIM no. 167210, Jackson–Lawler type1).2,4 Oral leucokeratosis has been described as a distinguishing feature of PC-1;2 however, we have observed that this does occur in PC-2 and shows incomplete penetrance in both disorders.6–9 The presence of multiple pilosebaceous cysts is the best hallmark of PC-2, although these appear at puberty and therefore young patients are more difficult to classify.6–9 Hair abnormalities and natal teeth are additional features of the PC-2 variant, which show incomplete penetrance. Some patients exhibiting the phenotype of PC-1 have findings that only appear late in childhood or adulthood10 and this group has been called ‘pachyonychia congenita tarda.’

The two major clinical subtypes of pachyonychia congenita, PC-1 and PC-2, have been shown to be caused by mutations in four differentiation-specific keratin genes. Specifically, PC-1 has been shown to be caused by mutations in keratins K6a,11–13 and K16.9,14–16 In contrast, PC-2 has been associated with mutations in K6b7 and K17.6,8,9 Genetic mutations in cases of delayed onset pachyonychia congenita have not previously been reported. Here, we report a case of late onset pachyonychia congenita occurring in association with an unusual type of mutation in the central portion of the K16 rod domain.

Case report

An 8-year-old Haitian girl was first evaluated by a dermatologist at age 6 years because of pruritus, scaly patches, and hyperpigmentation over the face, arms, legs, trunk and feet (Fig. 1). She was diagnosed with atopic dermatitis, and the features documented at presentation waxed and waned over the next year in response to topical steroids. However, there was
progressive thickening of the toenails and fingernails. She gradually developed hyperkeratosis of the proximal nail folds while lichenified and keratotic papules appeared over the fingers, toes, knees, Achilles tendon and malleoli. She has had patches of perioral scaling, which have varied in severity, but she has had no mucous membrane abnormalities. There was no history of natal teeth. There has been no palmoplantar hyperhidrosis, no hair abnormalities, and no cutaneous blisters, cysts or milia. A biopsy specimen from a lichenified patch on the knee was compatible with chronic dermatitis and a biopsy specimen from a keratotic papule on the knee showed hyperkeratosis and papillomatosis, but no blisters or keratinocyte lysis were seen in either specimen. The parents and two siblings have no nail abnormalities and no keratotic papules. The father and brother have dyshidrotic eczema.

Materials and methods

Peripheral blood lymphocyte DNA was extracted by standard methods. Exons 1–7 of the KRT16A gene were amplified in a single fragment using the long-range polymerase chain reaction (PCR) method recently described, which avoids unwanted amplification of the two K16-like pseudogenes, ψKRT16B and ψKRT16C (ψ denotes a pseudogene). PCR products were purified using the QIAquick system (Qiagen, Crawley, U.K.) and directly sequenced with the amplification primers and additional

Figure 1. Clinical appearance of the patient. At the age of 8 years, the affected girl had hyperconvex fingernails (a) and toenails (b,c), showing thickening of the nail plate and nail bed. Accuminate, keratotic papules (some in linear arrays) were scattered over the knuckles (a,b), heels and sides of her feet (c).

Figure 2. Molecular genetic analysis of the patient reveals a novel K16 mutation. (a) Direct automated sequencing of the KRT16A gene, showing codons 352–356 in exon 6, derived from a normal control sample. (b) The same region of the KRT16A gene as shown in (a), derived from the proband, showing heterozygous missense mutation 1062A→T. This mutation predicts the amino acid change lysine to asparagine at codon 354 (K354N). (c) Confirmation of the mutation by restriction enzyme analysis. BsmI digestion of exon 6 polymerase chain reaction fragments derived from the proband reveals additional cut bands in the proband (lane 1), which were not seen in digests derived from the unaffected mother, father or sibling (lanes 2–4, respectively). Similarly, the mutation was excluded from a population of 50 normal, unrelated individuals (data not shown).
internal primers, as described. For confirmation of mutation K354N in the patient and exclusion of this sequence variant from normal individuals, a 494-bp fragment spanning the mutation was amplified using primers K16sp7 (5’ TAG TGG GCT AGC TTT TCG CC 3’, + strand) and K16sp3 (5’ GGA TTG GCC AGA TGC TTG CT 3’, – strand). PCR was performed in High Fidelity buffer (Boehringer-Mannheim, Lewes, U.K.) containing 1.5 mmol L⁻¹ MgCl₂ and 4% dimethyl sulphoxide. Reactions were subjected to a ‘hot start’ with 1 U Amplitaq polymerase (PE Biosystems, Warrington, U.K.) and the following PCR conditions were used: (94 °C, 2 min) x 1 cycle; (94 °C, 30 s: 58 °C, 45 s; 72 °C, 2 min) x 35; (72 °C, 5 min) x 1. Under these conditions, this PCR reaction was found to be specific for the functional KRT16A gene. PCR products were digested overnight with 1 U of BsmI without further purification and digests were analysed on 2% agarose/TBE minigel.

Results

Genetic analysis was performed on DNA samples from the patient, the unaffected parents and one unaffected sibling using a previously described long-range PCR strategy to avoid coamplification of K16 pseudo-genes. A unique mutation, designated 1062A→T (numbering from the corrected K16 coding sequence), was identified in exon 6 of the KRT16A gene in the patient but not in her parents or brother (Fig. 2). This mutation predicts the amino acid change K354N in the first half of the helix 2B domain of the K16 polypeptide, prior to the helix inversion (stutter) motif (Fig. 3). The non-conservative substitution of a highly basic lysine residue for a neutral/polar asparagine residue in this coiled-coil domain is likely to be detrimental to keratin assembly and/or integrity. The mutation creates a recognition site for the restriction enzyme BsmI. A new PCR reaction was devised that specifically amplifies exon 6 of the KRT16A gene without pseudogene contamination. Digestion of exon 6 PCR products derived from the proband resulted in cutting at the single BsmI site created by the mutation, whereas no digestion was observed of analogous fragments derived from the unaffected family members (Fig. 2) or from 50 normal, unrelated individuals (not shown). Thus, the K354N mutation is consistent with the sporadic occurrence of the disorder in the proband and is also unlikely to represent a common polymorphism in the general population.

Discussion

The phenotypic similarity between our patient and other patients with PC-1 having mutations in K16 strongly suggests that the mutation in K16 is the cause of the disease in this case. The mutation was excluded from 100 normal unrelated chromosomes, which is a standard test for exclusion of a mutation as a neutral polymorphism. The location of our patient’s K16 gene mutation and the cutaneous trauma associated with rubbing her atopic dermatitis might have conspired to produce the delayed onset of her disease and the surprisingly extensive cutaneous findings.

Our patient’s mutation in the mid-region of the 2B helical domain of K16 might be expected to have a milder phenotype than mutations in K16 previously associated with PC-1. In epidermolysis bullosa simplex (EBS), an inherited blistering disease associated with mutations in K5 or K14, there is a strong correlation between the severity of disease and the location of the mutation within the keratin molecule. The most severely affected patients are those with the Dowling–Meara form of EBS (EBS-DM), who tend to have mutations at the highly conserved regions that delineate the start and end of the α-helical rod domain, which are termed the helix boundary motifs. In
contrast, less severely affected patients, with the Kärner (EBS-K) or Weber–Cockayne (EBS-WC) forms, have mutations in the non-helical domains or in more internal regions of the α-helical rod domain. The mutation reported here occurs in the centre of the 2B domain, in a region where analogous K14 mutations have been found in the milder EBS-K and EBS-WC patients. In the previous cases of PC-1 where keratin defects have been reported, the K6a and K16 mutations were identified within the helix boundary motifs (Fig. 3). These regions are entirely analogous to mutations in more severely affected EBS-DM patients.

In addition to the location of the mutation within the keratin gene, the varied clinical findings associated with mutations in K6a, K6b, K16 and K17 reported to date suggest that additional genetic or environmental factors could be important in the ultimate clinical expression. Mutations in K16 have been associated either with PC-1 or with non-epidermolytic palmoplantar keratoderma having minor or no nail findings. Likewise, mutations in K17 have been associated either with PC-2 or with steatocystoma multiplex having minor or no nail findings.

The role of trauma in the pathogenesis of individual lesions was reviewed by Jadassohn and Lewandowsky, who also noted that leucoplakia could have variable onset and be evanescent. The abrupt, but delayed, onset of our patient’s clinical findings suggests that rubbing her atopic dermatitis may have played an important part in her clinical presentation. Keratins K6, K16 and K17 are not constitutively expressed in interfollicular keratinocytes but their expression is rapidly induced in those cells following trauma. Although the mechanism of trauma-induced K16 expression and the physiological function of that induction are unknown, recent data point toward a close association between the epidermal growth factor receptor signalling pathway and K16 expression.

In conclusion, we report a patient with PC-1 in whom we have detected a novel mutation, K354N, in the centre of the K16 rod domain. Examination of additional pedigrees will be needed to establish whether the position of this mutation is sufficient to explain a late onset PC phenotype.

**Acknowledgments**

We are grateful to the patient and her family for their co-operation in this research. We wish to thank Hans-Jürg Alder and his staff, Nucleic Acid Facility, Kimmel Cancer Center, Jefferson Medical College, Philadelphia for DNA synthesis and sequencing. WHIM and FJDS are supported by a Wellcome Trust Senior Research Fellowship (to WHIM) and this work was also funded by The Dystrophic Epidermolysis Bullosa Research Association (DeBRA) U.K.

**References**

18. Letai A, Coulombe PA, Fuchs E. Do the ends justify the means? Proline mutations at the ends of the keratin coiled-coil rod