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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.
Mutations in the hair cortex keratin hHb6 cause the inherited hair disease monilethrix

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Pathogenic mutations in a large number of human epithelial keratins have been well characterized. However, analogous mutations in the hard α-keratins of hair and nail have not yet been described. Monilethrix is a rare autosomal dominant hair defect with variable expression. Hairs from affected individuals show a beaded structure of alternating elliptical nodes and constrictions (internodes). These internodes exhibit a high propensity to weathering and fracture. Strong evidence that trichocyte keratin defects might underlie this hair disorder was provided by genetic linkage analyses that mapped this disease to the type-II keratin gene cluster on 12q13. All affected individuals from a four-generation British family with monilethrix, previously linked to the type-II keratin gene cluster, as well as three unrelated single monilethrix patients, exhibited a heterozygous point mutation in the gene for type-II hair cortex keratin hHb6, leading to lysine substitution of a highly conserved glutamic acid residue in the helix termination motif (Glu 410 Lys). In a three-generation French family with monilethrix of a milder and variable phenotype, we detected another heterozygous point mutation in the same glutamic acid codon of hHb6, which resulted in a conservative aspartic acid substitution (Glu 410 Asp). These mutations provide the first direct evidence for involvement of hair keratins in hair disease.

In addition to the epithelial or soft α-keratins, the keratin multigene family also comprises the smaller family of hard α-keratins, which, according to their most frequently investigated site of expression, are commonly referred to as hair keratins. Previous protein studies indicated that, independent of the species, the hair keratin family consists of four individual members per subfamily, which were designated Ha1-4 (Hair acidic, type-I keratins) and Hb1-4 (Hair basic, type-II keratins), respectively. Together with a so-called minor hair keratin pair, Hax/Hbx, the hair keratin family was thought to comprise 10 members, which, according to their patterns of expression in the hair follicle, could be grouped into cuticular or cortical keratins.

We recently began to characterize human hair keratins and were able to show that the hair keratin family is distinctly more complex than previously assumed. There are seven type-I human hair keratins (hHa1 (ref. 5), hHa2 (ref. 6), hHa3-1 (ref. 7), hHa3-2 (ref. 7), hHa4 (unpublished), hHa5 (ref. 8) and hHa8 (unpublished)) and four type-II hair keratins (hHb1 (ref. 6), hHb3 (ref. 9), hHb5 (ref. 9) and hHb6 (ref. 9). The type-II subfamily is still incomplete, missing at least the partners for hHa2 and hHa8. (For the currently used human hair keratin nomenclature, see ref. 9.) Human hair keratin genes co-localize with epithelial keratin genes on chromosomes 17q12-1q21 (type-I genes) and 12q13 (type-II genes), respectively. Expression studies showed that hair keratin synthesis is not restricted to the hair cuticle and the cortex but clearly begins earlier in matrix cells of the hair bulb. Whereas both matricial and cuticular trichocytes seem to express only one keratin pair each, keratin expression in the cortex involves at least four sequentially synthesized, structurally highly related keratin pairs.

A large number of hereditary disorders of skin, skin appendages and oral mucosa have now been shown to be due to mutations in keratin genes. Keratins have highly homologous central α-helical rod domains flanked by variable-sized head and tail domains. The boundary peptides at the extremities of the rod domain (the helix initiation motif, HIM, of the 1A subdomain and the helix termination motif, HTM, of the 2B subdomain) are highly conserved and crucial for structure and function. Pathogenic mutations appear most disruptive in the HIMs or HTMs of keratins. Monilethrix is a rare congenital hair disease that is inherited as an autosomal dominant condition with variable expression. Clinically, it is characterized by a dystrophic alopecia of varying size and rough follicular papules. Affected hairs have uniform elliptical nodes of normal thickness and intermittent constrictions, interrupting...
nodes at which the hair easily breaks.12,13 Usually, only the scalp is involved, but in its severe forms the secondary sexual hair, eyebrows, eyelashes and nails may also be affected.14,15

Several pedigrees of monilethrix have been linked to the type-II keratin gene cluster on chromosome 12q13 (refs 14–16). As this locus also harbours the type-II hair keratin genes, monilethrix has been suggested to be caused by a hair keratin defect. Further evidence came from previous ultrastructural hair studies of monilethrix patients, which suggested local occluding lamellar degeneration in matrix cells of the hair bulbs along with cytological and structural alterations of hair cortex cells, including disrupted keratin filament packing.17,18 We therefore analysed the genes of the matrix keratin hHb6 (ref. 9) and the three cortical keratins hHb1, hHb3 and hHb6 (ref. 9) in a four-generation British monilethrix family with known linkage to 12q13 (family 1, Fig. 1).15 The gene regions encoding H1M and HTM were amplified and sequenced. A heterozygous G→A point mutation in the first position of the glutamic acid 410 codon (GAG) in the exon encoding the HTM of the cortical keratin hHb6 was detected in affected members of the family. This mutation leads to a non-conservative Glu→Asp substitution (Fig. 2a,b). Surprisingly, the corresponding sequence analyses of both matrixal and cortical type-II keratin genes in a three-generation French monilethrix family (family 2, Fig. 1) in which linkage analysis had not been performed also revealed a heterozygous point mutation in the same codon of the hHb6 gene of affected individuals. In this case, a G→T transversion occurred at the third base of the codon and yielded a conservative glutamic acid 410 aspartic acid substitution (Fig. 2a,b). These mutations were not found in the unaffected members of pedigrees 1 and 2 (Fig. 2a,b) or in 60 unrelated healthy individuals (results not shown).

It is noteworthy that the non-conservative Glu→Asp mutation in the HTM of hHb6 of the British monilethrix family has also been reported in the analogous glutamic acid residue of the type-II epidermal keratin 2e in four unrelated families and in one sporadic case with ichthyosis bullosa of Siemen.19–21 The type of conservative Glu→Asp substitution in the French monilethrix family that shortens the side chain of the original amino acid residue by only one carbon atom has also been identified as a causative mutation of the respective K1e glutamic acid residue in affected members of an ichthyosis bullosa of Siemens family.22 The Glu410 residue mutated in our two families represents an amino acid residue that is positionally conserved in the HTM of all known type-II hair and epidermal keratins as well as in a variety of other intermediate filament proteins.23,24,25,26,27 Along the 6-helical heptad repeats, Glu410 of hHb6 occupies an internal d position and is therefore crucial for the stabilization of the coiled coils formed initially during filament assembly.28,29 Moreover, hHb6 expression in the upper cortical region of the hair shaft largely co-localizes with the area where keratin intermediate filament clumping has been described in monilethrix patients.30,31,32 In addition, cuticular and cortical type-II keratin sequence analyses in three single unrelated Scottish, French and German monilethrix patients invariably revealed the Glu410→Asp mutation in the HTM of hHb6 (results not shown). Taken together, these data suggest that the two mutations in the HTM of the late cortical hair keratin hHb6 (ref. 9) are causative mutations in monilethrix.

The phenotype of the disease in the British family was clearly more severe13 than that in the French family, in which one adolescent’s symptoms were virtually restricted to keratosis pilaris of the extremities (patient III.1, Fig. 1). It remains to be seen whether the non-conservative Glu410→Asp mutation is generally associated with a more severe phenotype of the disease, while the conservative Glu410→Asp mutation is typical for a milder phenotype with variable penetrance. Studies with synthetic HTM peptides indicate that the Glu→Asp substitution resulted in only mild disruption of protein structure33. Although keratosis pilaris represents an usual feature in monilethrix,15 it is also a common isolated finding with autosomal dominant transmission.34 Therefore, it might be worthwhile to investigate the mutational status of hHb6 in pure keratosis pilaris families.

Monilethrix has not yet been linked to the type-I keratin gene locus at 17q12–21 (ref. 25), and the present study seems to indicate a prevalence of causal mutations in a distinct codon of the late type-II hair keratin gene. However, by analogy with epidermal keratins,19,20,21 a monilethrix phenotype should also be expected from mutations in the type-I partner of hHb6. On the basis of our own

Fig. 3 a, Alopecia in the occipital and temporal areas of a 10-month-old monilethrix patient (individual III.3, family 2). b, Polarized light micrograph of hairs from the same patient at the age of 6 years. Note the variation in the extent of the typical peridigeral narrowing (arrowheads) of the hairs. Magnification 10.
expression studies of human type I hair keratins, we predict the similarity of late-expressed hair keratin hH64 (unpublished) to be the respective candidate gene. This is the first report of pathogenic mutations in a human hair keratin. This study supports the assumption that other hereditary hair disorders may also be due to mutations in hard ter-keratins.

Methods

Patients and biological materials. Family 1 is a four-generation British monolithe family (Fig. 1) whose clinical characteristics and genetic linkage to chromosome 12q13 have been described. Briefly, affected individuals uniformly showed normal hair at birth, but follicular cysts and hyperkeratosis developed over the occiput and the nape of the neck within the first few months of life. Subsequently the hair became brittle and was lost, leaving a stable 1-2 cm long over the whole scalp that persisted into adulthood. Scanning electron microscopy of plucked hair confirmed the typical features of monolithe. The secondary sexual hair, eyelashes, and eyebrows appeared normal. The skin, orogenital mucosa, and nails were normal.

Family 2 is a three-generation French monolithe family (Fig. 1) with variable expression of the disease. Males II.12 and II.3 developed alopecia with keratosis pilaris of the occipital and temporal areas in the first months of life (patient II.13, Fig. 3a). However, significant hair regrowth was obvious in both patients during adolescence and persisted into adulthood (current age: patient II.13, 64 years; patient II.12, 46 years). Light microscopy examination of plucked hairs showed typical monolithe (patient II.13, Fig. 4), with a very thick, pigmented and yellowish cortex and a normal cuticle. Alopecia in the first months of life and now exhibits nearly normal hair.

PCR and DNA sequencing. PCR was used to amplify genomic DNA fragments containing the coding sequences for either hH6 or hH6M of all known type II hair keratin genes. Amplification primers were designed from the published DNA sequences. A long-range PCR was carried out in a total volume of 50 μl using the Expand PCR system. The reaction consisted of 2 min at 94°C, followed by 30 cycles of 10 s at 94°C, 50 s at 60°C, and 50 s at 68°C. The PCR products were purified by agarose gel DNA extraction. Direct sequencing of double-stranded DNA was carried out according to the Thermo Sequenase radiolabelled chain terminator cycle sequencing protocol (Amersham) using either the PCR primers or gene specific nested primers.

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