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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.
Abstract  Monilethrix is a rare dominant hair disease characterized by beaded or moniliform hair which results from the periodic thinning of the hair shaft and shows a high propensity to excess weathering and fracturing. Several cases of monilethrix have been linked to the type II keratin gene cluster on chromosome 12q13 and causative heterozygous mutations of a highly conserved glutamic acid residue (Glu 410 Lys and Glu 410 Asp) in the helix termination motif of the type II hair keratin hHb6 have recently been identified in monilethrix patients of two unrelated families. In the present study, we have investigated two further unrelated monilethrix families as well as a single case. Affected members of one family and the single patient exhibited the prevalent hHb6 Glu 410 Lys mutation. In the second family, we identified in affected individuals a lysine substitution of the corresponding glutamic acid residue, Glu 403, in the type II hair keratin hHb1, suggesting that this site represents a mutational hotspot in these highly related type II hair keratins. Both hHb1 and hHb6 are largely coexpressed in cortical trichocytes of the hair shaft. This indicates that monilethrix is a disease of the hair cortex.

Introduction

Monilethrix is a rare congenital hair disease that is inherited as an autosomal dominant condition with variable expression. The disease has been named for the regular beaded appearance of affected hairs, which exhibit elliptical nodes of normal thickness alternating with narrow, dystrophic constrictions. These internodes possess a high tendency to weathering and fracture, leading to the characteristic clinical appearance of dystrophic alopecia, i.e., short, stubby hair associated with follicular hyperkeratosis and perifollicular erythema. In the mildest forms, the disease involves only the occipital regions and the nape of the neck, but in its more severe form, the entire scalp, secondary sexual hair, eyebrows, and eyelashes may also be involved. In addition, monilethrix is occasionally associated with fragility and splitting of nails (McKee and Rosen 1961; Guummer et al. 1981; Zimmermann 1983).

Previous ultrastructural hair studies of monilethrix patients suggested local edematous degeneration in matrix cells of the hair bulb, together with cytolysis and disrupted keratin filament packing in cortex cells of the hair shaft (Ito et al. 1990; De Berker et al. 1993). More importantly, several pedigrees of monilethrix have recently been linked to the type II keratin gene cluster on chromosome 12q13 (Healy et al. 1995; Korge et al. 1996; Stevens et al. 1996). More importantly, several pedigrees of monilethrix have recently been linked to the type II keratin gene cluster on chromosome 12q13 (Healy et al. 1995; Korge et al. 1996; Stevens et al. 1996), which also harbors the genes for type II hair keratins (Rogers et al. 1995a). Although there is evidence for genetic heterogeneity in monilethrix (Richard et al. 1996), the disease has therefore been suggested to be caused by a hair keratin defect (Healy et al. 1995; Korge et al. 1996; Stevens et al. 1996).

A large number of hereditary diseases of skin, skin appendages, and oral mucosa have been shown as being due to mutations in keratin genes (for review, see Steinert 1995; Fuchs 1996). Keratins are grouped into type I, acidic pro-
teins, and type II, basic to neutral proteins, which form the 10-nm intermediate filament network in cells of epithelial origin by obligatory association of equimolar amounts of distinct pairs of type I and type II keratins (Steinert 1995; Fuchs 1996). Keratins possess highly homologous central \( \alpha \)-helical rod domains, flanked by non-\( \alpha \)-helical head and tail domains of variable size. The short regions at the extremities of the rod domain, designated helix initiation motif (HIM) and helix termination motif (HTM), are particularly conserved in keratins, and disease-causing mutations are prevalent in these regions (Steinert 1995; Fuchs 1996).

We have recently characterized four human type II hair keratins. One of them, hHb5, is synthesized in matrix cells, whereas the other three, hHb1, hHb3, and hHb6, are sequentially expressed in the cortex of the hair shaft (Rogers et al. 1995a, 1997). In our laboratory, genetic analyses of these four hair keratin genes in two unrelated British and French monilethrix families revealed two different heterozygous point mutations in the hHb6 hair keratin gene (Winter et al. 1997). The mutation in the British family led to a non-conservative lysine substitution of a highly conserved glutamic acid residue (Glu410Lys) in the HTM of hHb6, whereas in the French family, a different point mutation in the same glutamic acid codon resulted in a conservative substitution by aspartic acid (Glu-410Asp). These mutations provided the first evidence for hair keratins being involved in a hereditary hair disease (Winter et al. 1997).

In this study we have investigated two more unrelated monilethrix families as well as a single case. We confirm the Glu410Lys mutation in the HTM of hHb6 in one family and in the single patient, and describe a positionally identical Glu to Lys mutation in the type II hair keratin hHb1 in affected individuals of the second family.

Materials and methods

Genomic DNA isolation

After Ethic Committee approval, venous blood was drawn from affected and unaffected members of two unrelated German and Canadian monilethrix families, from a single patient of an Israeli monilethrix family, as well as from 40 healthy, unrelated individuals. Informed consent was obtained from all subjects after the reason and nature of the study had been explained. Genomic DNA was isolated using a blood and tissue culture DNA extraction system (Qiagen, Hilden, Germany).

PCR amplification and direct sequencing

The gene segments encoding the \( \alpha \)-helical 1A and 2B subdomains of the type II hair keratins hHb1, hHb3, hHb5, and hHb6 were amplified by PCR using the previously described gene-specific primer pairs for these regions (Winter et al. 1997). PCR was carried out with the Expand Long Template PCR system (Boehringer, Mannheim, Germany) using standard conditions (Winter et al. 1997). The PCR products were separated by agarose gel electrophoresis, purified using silica gel beads (Boehringer) and sequenced directly according to the Thermo Sequenase radiolabeled chain terminator cycle sequencing protocol (Amersham, Braunschweig, Germany) using the corresponding gene-specific forward primers as sequencing primers.

**Results**

The pedigree of the four-generation German monilethrix family 1, is indicated in Fig. 1. The affected individuals presented were two girls (patient IV-1, 13 years and patient IV-2, 12 years) and their brother (patient IV-3, 8 years). At birth, all patients had normal hair growth, but showed diffuse alopecia in the first year of age, which was particularly pronounced in patient IV-1. At present, hair growth has improved in patients IV-2 and IV-3, whereas their sister IV-1 still exhibits areas of sparse and short, 2- to 3-mm-long hair, particularly over the nape of the neck. The patients were afflicted with follicular hyperkeratosis of the knees, which was also seen in the neck of individual IV-1. In all three cases, light microscopic inspection of hair revealed typical features of monilethrix. The children’s father III-2, their uncle III-4, grandmother II-2, grand-aunts II-3 and II-4, as well as descendents III-6 and IV-4 of the latter, all suffered from early diffuse alopecia. Hair with monilethrix phenotype could clearly be confirmed in individuals III-2, III-4, III-6, and IV-4 using light microscopy. The case presented in the Canadian three-generation family (Fig. 1) was a 5-year-old girl, III-2, who was born with complete alopecia and later was noted to have poor scalp hair growth associated with follicular

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**Fig. 1** Pedigrees of the German monilethrix family 1 and the Canadian monilethrix family 2. Clinically affected individuals are indicated by solid symbols, unaffected members by open symbols. Clinically, individual I-1 of pedigree 2 (solid triangle) appeared unaffected, but carried a mutation in the hHb1 gene. Individuals whose DNA was analyzed in this study are marked by an asterisk. ? in pedigree 1 denotes unknown disease status
hyperkeratosis. Light microscopy revealed short hairs with prominent fusiform, regularly alternating segments of shaft thickening and thinning, typical of monilethrix. Clinically, all other members of the family seemed unaffected and none had noticeable alopecia or hair loss early in life. However, systematic scanning electron microscopy analyses clearly revealed moniliform hairs in the father, II-4, of patient III-2, whereas hairs of the rest of the family were interpreted as normal. The single Israeli patient (26 years) investigated presented typical monilethrix hair in the posterior lower scalp with only minor tendency to fracture. The patient also exhibited keratotic papules on the neck. His mother was not afflicted by the disease but both his sister (33 years) and his father (died at age 44 years) exhibited a distinctly more severe phenotype with short, stubbly hair and keratotic papules covering the entire scalp. In all monilethrix patients investigated in this study, the secondary sexual hair, eyelashes, and eyebrows as well as the nails appeared normal.

The same held true for the HTM-encoding 2B gene regions of hHb3 and hHb5 (results not shown). In contrast, analyses of the HTM-encoding regions of the hHb6 and hHb1 gene led to the detection of a heterozygous G to A point mutation either of the hHb6 gene in family 1 and the single patient or of the hHb1 gene in family 2 (Fig. 2). In all cases, the mutation site concerned the first position of the same glutamic acid codon, GAG, within the HTM coding region of the two genes and led to the substitution of glutamic acid by a lysine residue (hHb6, Glu410Lys; hHb1, Glu403Lys; Fig. 2). The hHb6 gene mutation was found in all clinically affected family 1 members from whom blood samples could be obtained (seven individuals; Fig. 1). This was also true for the hHb1 gene mutation in individuals II-4 and III-2 of family 2. Surprisingly, however, the G to A mutation was also present in the clinically unremarkable paternal grandmother I-1 of individual III-2, but not in the remaining family members. Both the hHb6 and hHb1 gene mutations were absent in 40 unrelated, healthy control individuals.

Discussion

We were recently able to show that the hereditary hair disease monilethrix is due to mutations in a hair keratin gene (Winter et al. 1997). We identified two types of causative mutations in the same GAG codon in the HTM coding region of the type II hair keratin hHb6 in two unrelated monilethrix families. The triplet encodes a glutamic acid residue which is positionally conserved in virtually all type II and most type I keratins. One mutation, a G to A transition in the first position of the GAG codon, changed Glu to Lys, the other, a G to T transversion in the third position of the GAG codon, led to the substitution of glutamic acid by a lysine residue (hHb6, Glu410Lys; hHb1, Glu403Lys; Fig. 2). The hHb6 gene mutation was found in all clinically affected family 1 members from whom blood samples could be obtained (seven individuals; Fig. 1). This was also true for the hHb1 gene mutation in individuals II-4 and III-2 of family 2. Surprisingly, however, the G to A mutation was also present in the clinically unremarkable paternal grandmother I-1 of individual III-2, but not in the remaining family members. Both the hHb6 and hHb1 gene mutations were absent in 40 unrelated, healthy control individuals.
seemed to be confirmed in the large German monilethrix family (family 1), in which affected members showed the Glu410Lys mutation in hHb6 which was also seen in the single Israeli monilethrix patient. In the Canadian family (family 2), a mutation in a new hair keratin was found. While we still observed a Glu to Lys substitution, this mutation, however, occurred in the type II hair keratin hHb1 at Glu403, which is the position equivalent to that of Glu410 in hHb6.

Disease-causing lysine substitutions in the equivalent position of hHb6 Glu410 and hHb1 Glu403, have already been described in other keratins. This is, for example, the case for Glu493 of the late epidermal type II keratin K2e in the majority of families with ichthyosis bullosa of Siemens (Kremmer et al. 1994; McLean et al. 1994; Rothnagel et al. 1994; Jones et al. 1996), for Glu475 of the basal epidermal type II keratin K5 in an epidermolysis bullosa simplex family (Stephens et al. 1997), and for Glu509 of the type II corneal keratin K3 in families with Meesmann's corneal dystrophy (Irvine et al. 1997).

The two type II hair keratins, in which mutations for monilethrix have now been found, are constituents of the hair cortex, with hHb1 being the earliest and hHb6 the latest and predominantly expressed differentiation-specific cortex keratin (Rogers et al. 1997). There is, however, a third type II cortex keratin, hHb3, that is synthesized in the region between these two keratins, so that three type II hair keratins are coexpressed in cortical trichocytes (Rogers et al. 1997). The expression of one mutated allele of either hHb1 or hHb6 appears apparently sufficient, however, to fatally weaken the particularly dense intermediate filament network that results from the association of the three type II keratins with their corresponding type I partners.

The high incidence of Glu410Lys mutations in hHb6 and the demonstration that the corresponding site in hHb1 can also be subject to lysine substitution, suggests that the corresponding GAG codon represents a mutational hot spot of the two type II cortex keratin genes. The base change observed might be promoted by a methylated CpG deamination mutation of a 5-methyl cytosine on the antisense strand in both genes, which leads to a CG to CA transition in the sense strand. Methylated CpG sequences are known to have a higher mutation rate than other dinucleotides (Cooper and Krawczak 1989). Considering that the HTM coding region in all three cortex keratin genes is absolutely conserved (Rogers et al. 1997), we do not exclude the possibility that further investigations could reveal monilethrix families with a lysine mutation in the corresponding glutamic acid residue of hHb3.

Up until now, pathogenic mutations in monilethrix seem to be restricted to type II cortex keratins. At first glance, this is reminiscent of a similar observation in ichthyosis bullosa of Siemens, which only seems to be associated with mutations in the late epidermal type II keratin K2e (Kremmer et al. 1994; McLean et al. 1994; Rothnagel et al. 1994; Jones et al. 1996; Yang et al. 1997). The latter is plausible in so far as, apparently, K2e does not possess a defined, similarly late expressed type I partner in the epidermis, but is thought to compete with K1 for filament assembly with K10 (Collin et al. 1992). Since K10 is already expressed in early spinous cells of the epidermis, pathogenic mutations in its gene invariably lead to genodermatosis epidermolytic hyperkeratosis (Steinert 1995; Fuchs 1996). In contrast, our studies have revealed at least four type I human hair keratins that are sequentially synthesized in the hair cortex (Fink et al. 1995; Rogers et al. 1995b; unpublished results). Of these, hHa1 and hHa4 show expression patterns deceptively similar to those of hHb1 and hHb6 (unpublished results), suggesting that they might be type I partners of the latter. In view of the general involvement of both type I and type II epithelial keratins in the etiology of skin fragility syndromes (Steinert 1995; Fuchs 1996), it is therefore intriguing that, up to now, neither mutations in type I cortex keratins nor linkage to the type I keratin gene cluster (Richard et al. 1996) have been found associated with monilethrix. A possible clue for this may be that the critical mutations in type II cortex keratins seem to be limited to their HTMs. There is evidence that single-residue substitutions in certain positions in the HTM of the type I keratin K14 do not visibly compromise filament assembly, while those in the type II keratin K5 heavily disturb this process (Letai et al. 1992, 1993; Wilson et al. 1992). This may indicate that HTMs of type II keratins are more susceptible to pathogenic mutations than those of type I keratins (Rothnagel et al. 1994). Provided type I cortex keratin mutations exhibit a similar HTM restriction, they may, therefore, not show up phenotypically and carriers of those mutations may remain undetected.

It should be emphasized that the extensive variation in the disease phenotype associated with the keratin hHb1 mutation in the Canadian family is reminiscent of a previously described French monilethrix family carrying a conservative Glu410Asp mutation instead of the non-conservative Glu410Lys mutation in hHb6 (Winter et al. 1997). Both families possess hair keratin-mutated members who lacked early alopecia and demonstrable moniliform hair at a later age, a feature that, up until now, has not been observed in families and single patients exhibiting the hHb6 Glu410Lys mutation. Whether these discrepancies depend on which of the type II cortex keratins is mutated or on the type of mutation in a distinct type II cortex keratin remains to be seen from further investigations.

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References


