A missense mutation in the type II hair keratin hHb3 is associated with monilethrix

M A M van Steensel, P M Steijlen, R S Bladergroen, M Vermeer, M van Geel

Monilethrix (MIM #158 000) is an autosomal dominant hair disorder that can cause scarring alopecia in affected individuals. Nail changes and keratosis pilaris of the skin of neck and arms have also been described. The hallmark hair abnormality in monilethrix is a beading of the hair shaft caused by periodic narrowing with the nodes separated by about 0.7 mm. The cause of the beading is unknown. The expression of monilethrix is variable. Mild cases, dystrophic hairs may be found only on the occiput, but severely affected individuals may suffer complete alopecia.

Most cases described so far are associated with mutations in the type II (basic) trichocyte keratin genes hHb1 and hHb6. Both genes have a mutational hotspot in the region coding for the helix termination motif. Most mutations seem to affect the same residues—glutamic acids at positions 413 and 402. Mutations affecting the helix initiation motif have also been found. From the phenotype, it is apparent that hHb1 and hHb6 are major hair cortex keratins. A third type II trichocyte keratin, hHb3, is expressed in much the same pattern as hHb1. From this, it may be expected that mutations of hHb3 may cause monilethrix as well. However, mutations in this gene have so far not been described.

We analysed three patients suffering from monilethrix for the presence of mutations in hHb1, hHb3, and hHb6. In one patient, we found a heterozygous missense mutation in hHb3 causing the substitution of a glutamic acid by a lysine at position 407 in the helix termination motif (E407K). This mutation corresponds to the E402K substitution in hHb1 and hHb6, clearly defining this particular residue as a trichocyte keratin mutational hotspot. In a second patient we identified the previously described hHb6 E402K mutation, whereas a third patient did not have any mutations in any of the three genes.

CASE REPORTS

Case 1

The first patient, a six year old girl of Dutch descent, visited our department for a complaint of increasing hair loss and inability to grow long hair. The hair loss was concentrated in the occipital region and was exacerbated by mechanical stress such as wearing a cap. During the first two to three years of life, the hair had been reportedly normal although it did not attain any great length. Upon examination, we found short, dark blonde hair with a weathered appearance (fig 1A). In the occipital region, there was partial alopecia. No scarring was evident. Light microscopy of the hairs revealed regular beading (fig 1B). A diagnosis of monilethrix was made based on the light microscopic findings, history, and clinical findings. The father and a younger sibling had normal hair. The mother kept her hair short and stated that she was unable to grow long hair. Upon examination of the mother, we noted a thin implant of the hair, particularly on the vertex, along with several broken hairs. Findings in the maternal grandmother were identical, though in addition some scarring was noted on the vertex. Light microscopic examination of hairs taken from mother and grandmother showed no beading (not shown).

Case 2

The second patient, an 18 year old woman of Turkish descent, presented with more severe hair loss. She had no hair at birth. Growth had started around the age of one year and had always been slow and sparse. As in patient 1, mechanical stress exacerbated hair loss. Upon examination, we found short, weathered, and thinly implanted hair over the entire scalp. There was a pronounced follicular hyperkeratosis on the neck and upper arms. Light microscopy showed pronounced beading of the hairs examined. A diagnosis of monilethrix was made.

Case 3

The third patient, a four year old Dutch girl, presented to our department with a lifelong complaint of slowly growing, thin, and brittle hair. Her parents were not affected. Upon examination, we noted thinly implanted short hair with a weathered appearance. Other skin abnormalities were not noted; in particular, follicular hyperkeratoses were absent. Light microscopy of the hair showed the beads on a string appearance typical of monilethrix.

METHODS

Informed consent for the studies was obtained from all the patients and their parents. DNA was isolated from peripheral...
blood lymphocytes using protocols described elsewhere. Initially, the known mutational hotspots of the type II hair keratin genes hHb1 and hHb6 (exons 1 and 7) were amplified by polymerase chain reaction (PCR) and subjected to direct sequencing using the BigDyeDeoxy terminator method and an ABI 3100 automated sequencer (Applied Biosystems, Foster City, California, USA). Sequencing primers were identical to the PCR primers. Patients lacking mutations in these hotspots were further screened for mutations in all coding exons and intron–exon boundaries of hHb1, hHb3, and hHb6. The sequences were assembled and analysed using the Phred-Phrap-Consed software tools.

PCR primer sequences for hHb1, hHb3, and hHb6 are listed in table 1. Reaction conditions were identical for all primer pairs: an initial denaturing step of 94°C for 1.5 minutes was followed by 35 cycles of denaturing at 94°C for 30 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 60 seconds.

RESULTS

In the first patient, we identified no mutations in the known mutational hotspots of exons 1 and 7 of hHb1 and hHb6. After screening all exons of hHb1, hHb3 and hHb6 in this patient, we identified a heterozygous G to A transition at nucleotide position 1219 of the hHb3 gene causing the substitution of a glutamic acid by a lysine (E407K). Her mother and the maternal grandmother had the same mutation. The mutation abolishes a TaqI site (New England Biolabs, Beverly, Massachusetts, USA), allowing distinction between mutant and wild type alleles of family members and control DNA by restriction analysis. The mutation was not present in the father or in 192 unrelated control alleles sampled from the Dutch population. In the second patient, we identified a heterozygous G to A transition in the hHb6 gene at nucleotide position 1204 causing a glutamic acid to lysine change at position 402 (E402K). This known mutation was not present in the father. Neither the mother nor other family members were available for analysis. As the mutation has previously been shown to be pathogenic we did not analyse controls for its presence. In the third patient we found no mutations in either hHb1, hHb3, or hHb6. However, we did locate previously undescribed polymorphisms in the coding and non-coding regions of these keratins (table 2). Interestingly, the PstI polymorphisms in hHb1 and hHb6 were linked in all controls and patients.

![Figure 1](image-url)  

**Figure 1** hHb3 monilethrix phenotype. (A) Short hair with a weathered appearance. (B) Polarisation microscopy of plucked hair from the index patient. Evident periodic beading of the hair (original magnification ×20) with variability.
DISCUSSION
We have shown that a missense mutation in the type II hair keratin hHb3 is associated with a mild monilethrix phenotype in a Dutch family. To our knowledge, this is the first report of an hHb3 mutation associated with a disease phenotype. It is of interest that the residue affected is equivalent to glutamic acid 402 in hHb1 and hHb6, a known hotspot for mutations in the latter genes. The extensive sequence conservation in the helix termination motif observed in all keratins \(^{21}\) indicates its importance for proper keratin assembly.

Why most mutations described so far have been in either hHb1 or hHb6 is subject to speculation. hHb1, hHb3, and hHb6 are clustered within a \(\pm 40\) kb region on chromosome 12q13.13 that is characterised by a relative paucity of repetitive elements (GoldenPath at http://genome.ucsc.edu, July 2003 freeze). Sequence comparison between the coding sequences of the three keratins shows extensive (>90%) conservation. There are no sequence differences around the hotspots that explain why mutations should occur less often in hHb3 than in hHb1 or hHb6. One explanation may be an ascertainment bias: hHb3 mutations may always cause a milder phenotype than mutations in hHb1 or hHb6 and as such go unnoticed because the patient does not seek medical help. The mild phenotype we observed in patient 1, her mother, and her grandmother supports this notion. The identification of more hHb3 mutations in the future will assist in settling this matter. It should be noted here that in females, the mild phenotype might be mistakenly diagnosed as androgenetic alopecia, a condition that can be treated with finasteride.\(^ {22}\) Obviously, monilethrix needs to be excluded by careful clinical examination. It will be of interest to examine how often the correct diagnosis is missed in cases of mild monilethrix.

Examination of the new polymorphisms we found showed that the PsiI polymorphisms in hHb1 and hHb6—sites that are approximately 13 kb apart—were linked in all controls and patients examined. Apparently, the polymorphisms are in linkage disequilibrium. One may speculate that both polymorphisms confer some kind of advantage to keratin structure, causing the polymorphisms to remain linked. Further studies will be needed to confirm this hypothesis.

As indicated by the absence of mutations in hHb1, hHb3, and hHb6 in patient 3, there is genetic heterogeneity in monilethrix, as previously noted by other groups.\(^ {22,23}\) Because the hair keratins hHb1, hHb3, and hHb6 are all basic, the obvious hypothesis is that mutations in the acidic partner keratins may be responsible for some monilethrix cases as well. As it is not known which acidic keratins pair with hHb1, hHb3, and hHb6, all known acidic hair keratins will have to be analysed. Any mutations found in the acidic keratins will suggest pairing with a monilethrix associated basic keratin. As such, finding and elucidating cases of monilethrix without mutations in hHb1, hHb3, and hHb6 will be of great value for our understanding of hair biology.

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REFERENCES

Table 2 Polymorphisms identified in hHb1, hHb3, or hHb6 and genotyping

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<th>Gene</th>
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