ONLINE MUTATION REPORT

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

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Monilethrix (MIM #158 000) is an autosomal dominant hair disorder that can cause scarring alopecia in affected individuals.[1] 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.[2] In 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*.[2–14] 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.[3 6 10 15] Mutations affecting the helix initiation motif have also been found.[7] 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.[16] 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.

Key points

- Monilethrix is an autosomal dominant disorder characterised by periodic beading of hair shafts and pronounced hair fragility, leading to hair loss and eventual scarring alopecia.
- Several familial cases of monilethrix were previously shown to be caused by mutations in the basic (type II) hair keratins hHb1 and hHb6. Disease causing mutations have so far not been described in the related type II hair keratin family member hHb3.
- A heterozygous missense mutation is reported, associated with monilethrix in exon 7 of *hHb3* and leading to the substitution of a glutamic acid by a lysine (E407K). This mutation is located in the keratin helix termination motif, which is a mutational hotspot in *hHb1* and *hHb6*. In two other patients with a typical monilethrix phenotype, a known mutation in hHb6 (E402K) was found in one, while no mutations in hHb1, hHb3, or hHb6 were found in the other.
- These findings, as well as those from other studies, indicate genetic heterogeneity in monilethrix.

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. On 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

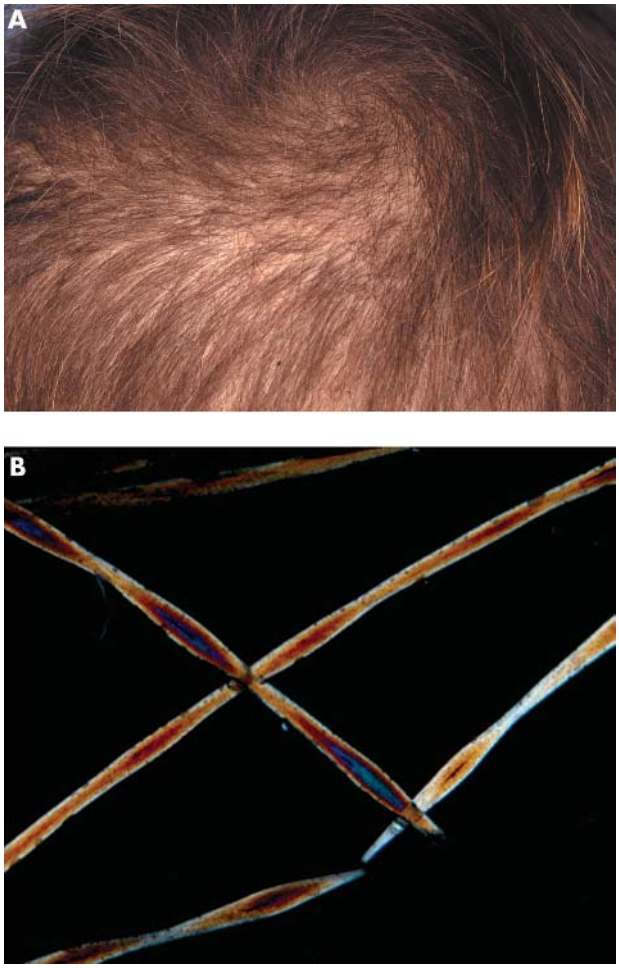

Figure 1 *hHb3* monilethrix phenotype. (A) Short hair with a weathered appearance. (B) Polarisation microscopy of plucked hair from the index patient. Evident periodic beading of the hair (original magnification ×20) with variability.

blood lymphocytes using protocols described elsewhere.[17] 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.[18–20]

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.

Table 1 *hHb1, hHb3*, and *hHb6* primer sequences

Primer	Sequence (5′–3′)	Product length (bp)
KRTHB1		
hHb1F1	TCACAGCCAAGCCCCTTCAG	597
hHb1R1	GGCAGGCAGAGGTCTTTGTG	
hHb1F2	TGGGGGTTCCCATTAAAGCAC	404
hHb1R2	CATGGCCACTCACCCTACCTG	
hHb1F3	GGGCTGTGTGGAGGTGTTTC	314
hHb1R3	TGGGCAGTCCTAGCCTGCTG	
hHb1F4	CCCCTGGCTACATGGAATGG	657 (exons 4/5)
hHb1R5	GACCAGCATCCCCAGAAAAGG	
hHb1F6	AGGCTGAGCACTGTACAACCTG	301
hHb1R6	TCCAGGTAGGGCACACATAGGC	
hHb1F7	GCTTGGTGGGGAGTGTGGTC	381
hHb1R7	TCAGCCAAGGCCAAGGGTAG	
hHb1F8	TGACCAGTTGTGGACCCTACCC	477
hHb1R8	GAGCCTGACACGCAGAGGTC	
hHb1F9	CAGGAAGGCAGGATGGCTTG	494
hHb1R9	TAGGGTGGCCTTTCCCACTG	
KRTHB3		
hHb3F1	TCTTTGCTCCCTCTTTTAACACCAG	583
hHb3R1	TCAGTGCCCAGCCTGAGTTC	
hHb3F2	CTTTCAGGCCCTCGTGCTG	378
hHb3R2	CAGCTGCAGACTGGATACTCCTC	
hHb3F3	ACTTGGGGGCAGGAGACAAG	486 (exons 3/4)
hHb3R4	TCATGCCTGTGGGTGTCTGG	
hHb3F5	CCCGATGAGCCAAAAGGATG	395
hHb3R5	CTGCCACCCCAACATGAGAG	
hHb3F6	GGCTTCTTTGTCTAGGGATTCTG	436
hHb3R6	TGTCACCCATGAGACTGCACTG	
hHb3F7	CTATTGGAGACTGGCTGGGATTTC	401
hHb3R7	GATCCATAGAGGCAAGAATGTCACC	
hHb3F8	TGAGATGACCCATTTCAGCAC	316
hHb3R8	CCCTCCCCAACACATTCAGG	
hHb3F9	GCATCCTTCCCTGGATGGTC	485
hHb3R9	GGTCTATTCCCCAGCCACAGG	
KRTHB6		
hHb6F1	AAAAGGCCTACAGAGGTGCAAG	532
hHb6R1	GGCCCTGGCTGTGTAGGTG	
hHb6F2	TGGGGGTTCCCATTAAAGCAC	404
hHb6R2	CATGGCCACTCACCCTACCTG	
hHb6F3	TACTGTCCCCATGGCTGTGATG	261
hHb6R3	ATTGTGCCCCACGCTGAG	
hHb6F4	GGAATGGGTGGGAAGAGAGAGG	641 (exons 4/5)
hHb6R5	TGCATCCCCAGAAAAGGAAGC	
hHb6F6	GCTGAGCACTGCACAACCTG	299
hHb6R6	TCCAGATAGGGCACACATAGGC	
hHb6F7	AGCTTGGTGGGGAGCATGG	387
hHb6R7	AATGCTGCCAGGAGTGTGAGG	
hHb6F8	ATGGAGGGCCACAGATGTCC	574 (exons 8/9)
hHb6R9	GGAGGGCGCTTAGAGTGCAG	

bp, base pairs.

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 *Taq*I site (New England Biolabs, Beverley, 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.

Table 2 Polymorphisms identified in *hHb1*, *hHB3*, or *hHB6* and genotyping

Gene	Location	SNP allele	Type	Amino acid residue/ position	Codon position	Detection method	Allele frequency
hHb1	416/exon2	A/C	Coding-nonsynon	Q139P	2	*Pst*I	A=0.83, C=0.17
hHb1	IVS4+4	C/T	Intron	–	–	*Cac*8I	C=0.76, T=0.24
hHb3	445/exon2	C/T	Coding-nonsynon	C149R	1	*Bst*UI	C=0.88, T=0.12
hHb3	558/exon2	C/T	Coding-synon	N186N	3	*Bs*L1	C=0.74, T=0.26
hHb6	197/exon1	G/A	Coding-nonsynon	R66H	2	*Bsr*BI	G=0.96, A=0.04
hHb6	348/exon1	G/A	Coding-synon	R116R	3	*Tfi*I	G=0.86, A=0.14
hHb6	416/exon2	A/C	Coding-nonsynon	Q139P	2	*Pst*I	A=0.83, C=0.17

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 ± 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 males, 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 *PstI* 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.[6 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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Authors' affiliations

M A M van Steensel, P M Steijlen, R S Bladergroen, M van Geel, Department of Dermatology, University Hospital Maastricht, Maastricht, Netherlands
M Vermeer, Department of Dermatology, University Medical Centre Leiden, Leiden, Netherlands

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Correspondence to: Dr M A M van Steensel, Department of Dermatology, University Hospital Maastricht, PO Box 5800, 6202 AZ Maastricht, the Netherlands; mvst@sder.azm.nl

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