

## ONLINE MUTATION REPORT

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

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**M**onilethrix (MIM #158 000) is an autosomal dominant hair disorder that can cause scarring alopecia in affected individuals.<sup>1</sup> 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.<sup>2</sup> 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*.<sup>2-14</sup> 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.<sup>3 6 10 15</sup> Mutations affecting the helix initiation motif have also been found.<sup>7</sup> 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*.<sup>16</sup> 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

## 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.

examination of hairs taken from mother and grandmother showed no beading (not shown).

## Case 2

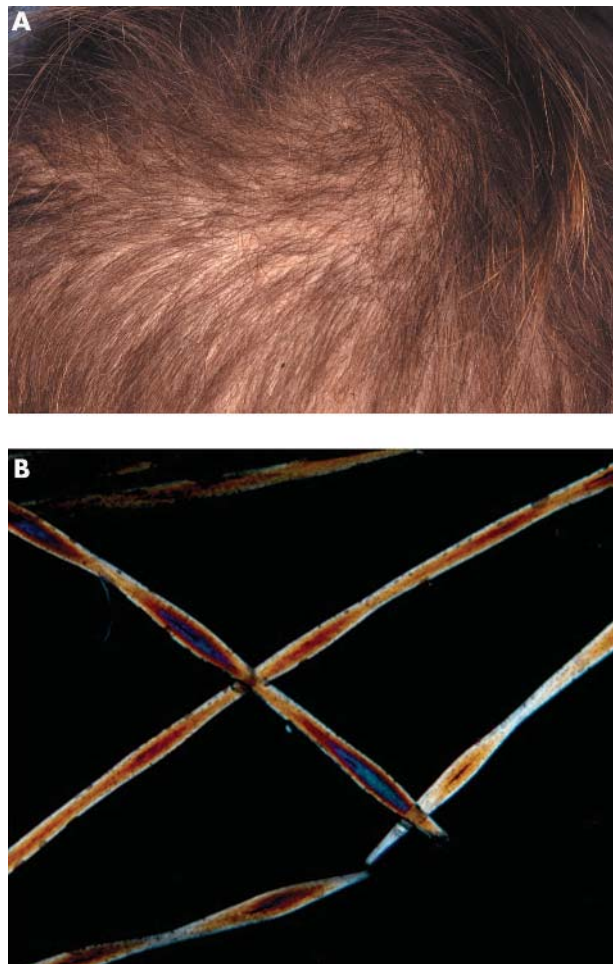
The second patient, an 18 year old woman of Turkish descent, presented with more severe hair loss. She had no hair at birth. Growth had started around the age of one year and had always been slow and sparse. As in patient 1, mechanical stress exacerbated hair loss. Upon examination, we found short, weathered, 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  $\times 20$ ) with variability.

blood lymphocytes using protocols described elsewhere.<sup>17</sup> 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.<sup>18–20</sup>

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

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

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

bp, base pairs.

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.

**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
<i>hHb1</i>	416/exon2	A/C	Coding-nonsynon	Q139P	2	<i>PstI</i>	A=0.83, C=0.17
<i>hHb1</i>	IVS4+4	C/T	Intron	–	–	<i>Cac8I</i>	C=0.76, T=0.24
<i>hHb3</i>	445/exon2	C/T	Coding-nonsynon	C149R	1	<i>BsUI</i>	C=0.88, T=0.12
<i>hHb3</i>	558/exon2	C/T	Coding-synon	N186N	3	<i>BsI</i>	C=0.74, T=0.26
<i>hHb6</i>	197/exon1	G/A	Coding-nonsynon	R66H	2	<i>BsrBI</i>	G=0.96, A=0.04
<i>hHb6</i>	348/exon1	G/A	Coding-synon	R116R	3	<i>TfiI</i>	G=0.86, A=0.14
<i>hHb6</i>	416/exon2	A/C	Coding-nonsynon	Q139P	2	<i>PstI</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<sup>21</sup> 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 males, the mild phenotype might be mistakenly diagnosed as androgenetic alopecia, a condition that can be treated with finasteride.<sup>22</sup> 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.<sup>6–23</sup> 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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