Identification of Novel Mutations in Basic Hair Keratins hHb1 and hHb6 in Monilethrix: Implications for Protein Structure and Clinical Phenotype

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Monilethrix is an hereditary hair dystrophy recently shown to be due to mutations in the helix termination motif of two type II (basic) human hair keratin genes, hHb1 and hHb6. It has been suggested that mutation in hHb1 produces a less severe phenotype. We have studied hair keratin genes and clinical features in 18 unrelated pedigrees of monilethrix from Germany, Scotland, Northern Ireland, and Portugal, in 13 of which mutations have not previously been identified. By examining the rod domains of hHb1, hHb3 and hHb6, we have identified mutations in nine of the new pedigrees. We again found the glutamine-lysine substitution (E413K) in the helix termination motif of hHb6 in two families, and in another, the corresponding E413K substitution in the hHb1 gene. In four families a similar substitution E402K was present in a nearby residue. In addition two novel mutations within the helix initiation motif of hHb6 were found in Scottish and Portuguese cases, in whom the same highly conserved asparagine residue N114 was mutated to histidine (N114H) or aspartic acid (N114D) residues, respectively. In four other monilethrix pedigrees mutations in these domains of hHb1, hHb3, and hHb6 were not found. The mutations identified predict a variety of possible structural consequences for the keratin molecule. A comparison of clinical features and severity between cases with hHb1 and hHb6 mutations does not suggest distinct effects on phenotype, with the possible exception of nail dystrophy, commoner with hHb1 defects. Other factors are required to explain the marked variation in clinical severity within and between cases. ***Key words: hair diseases/hair keratins/intermediate filaments/monilethrix, protein structure. J Invest Dermatol 113:607–612, 1999***

Monilethrix (Mt) is an autosomal dominant hair dystrophy, characterized by typical beaded or fragile hair shafts, and prominent follicular keratoses in many patients (Tietze, 1995). Nail defects, often subtle, have been reported (Heydt, 1963; Tietze, 1995). The effect on scalp hair is variable and even within families ranges from normality or mild occipital hair loss to near total alopecia (Birch-Machin *et al*, 1997). In some cases alopecia persists throughout life; in others regrowth of apparently normal hair may occur at the time of puberty or in pregnancy (Alexander and Grant, 1958).

Following ultrastructural demonstration of vacuolation of cortical trichocytes (Ito *et al*, 1990) and defects in the microfibrillar structure of the hair cortex (De Berker *et al*, 1993), the cysteine-rich "hard" keratins of hair and nail, were good candidate genes for Mt. Severalgroups reported linkage of Mt to the type II keratin gene cluster at 12q13 (Healy *et al*, 1995; Stevens *et al*, 1996)[1] where the human type II hair keratin genes also reside (Rogers *et al*, 1995). Subsequently, several groups identified point mutations in the basic hair keratins of the hair cortex, hHb1 and hHb6. Mutations in residue 117 of the 2B domain in hHb6 causing Glu413Lys (E413K) appear to be the most common (Winter *et al*, 1997a, b; Korge *et al*, 1998, 1999),[2,3] but Glu413Asp has also been reported (Winter *et al*, 1997a; Zlotogorski *et al*, 1998). Mutation in the corresponding

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Abbreviations: hHb, human hair basic keratin; IF, intermediate filament; Mt, monilethrix.

[1]Korge BP, Richards G, Puenter C, *et al*: Monilethrix links to the keratin type II cluster at 12q13 and cloning of a possible candidate gene. *J Invest Dermatol* 106:843, 1996 (abstr.)

[2]Korge BP, Healy E, Traupe H, *et al*: Monilethrix is caused by mutations in the helix termination peptide of human type II hair keratin hHb6 in three families. *J Invest Dermatol* 109:409, 1997 (abstr.)

[3]Korge BP, Hamm H, Traupe H, *et al*: Recurrent point mutations in human type II hair keratins hHb1 and hHb6: A frequent cause of monilethrix. *Arch Dermatol Res* 291:122, 1999 (abstr.)

Table I. Pedigrees studied and mutations found, by gene

ID	Origin	Original report	Cases	Affected gene	Mutation
M2	Germany	Korge *et al*, 1996[1]	7	hHb1	E402K
M6	Germany	Tietze, 1995	4	hHb1	E402K
M8	N Ireland	Not previously reported	3	hHb1	E402K
M11	Germany	Tietze, 1995	7	hHb1	E402K
L	Scotland	Alexander and Grant, 1958	2	hHb1	E413K
D	Scotland	Birch-Machin *et al* 1997	5	hHb6	N114H
M9	Portugal	Not previously reported	1	hHb6	N114D
S	Scotland	Alexander and Grant, 1958	17	hHb6	E413K[a]
O	Scotland	Healy *et al*, 1995	9	hHb6	E413K[a]
K	England	Birch-Machin *et al* 1997	11	hHb6	E413K[a]
M1	Germany	Tietze, 1995	1	hHb6	E413K[a]
M4	Germany	Tietze, 1995	5	hHb6	E413K[a]
M10	Scotland	Anderson, 1883	6	hHb6	E413K
M12	Germany	Not previously reported	6	hHb6	E413K
I	Spain	Birch-Machin *et al* 1997	7	Not known	
M3	Germany	Tietze, 1995	3	Not known	
M5	Germany	Tietze, 1995	3	Not known	
M7	Germany	Tietze, 1995	4	Not known	

[a]Mutations reported in Korge *et al*, 1998.

residue in hHb1 (E413K; Winter *et al*, 1997b)[3] has been reported but E402K (residue 106 of 2B domain) in hHb1 is also found (Winter *et al*, 1998).[3,4] From knowledge of diseases due to epithelial keratin mutation, defects in the 1A domains would also be predicted but have not hitherto been reported. It has been suggested on the basis of a small pedigree (Winter *et al*, 1998) that mutations in hHb1 may produce a less severe phenotype.

Hair keratins and their patterns of expression in humans are less well understood than epithelial keratins, and the reasons for the considerable number of hard keratin genes is obscure. As with epithelial keratins, hair keratin intermediate filaments (IF) are composed of heterodimers containing paired acidic and basic keratins, with a highly homologous central rod domain, and functional specificity conferred by the variable N- and C-terminal domains. In humans, evidence to date suggests nine acidic and at least four basic hair keratins (Rogers *et al*, 1997, 1998), but more may exist. Four basic keratins have been cloned (Rogers *et al*, 1995, 1997; Bowden *et al*, 1998),[1] of which human basic hair keratin 5 (hHb5) is expressed in the proliferative pool of the hair matrix, and hHb1, hHb3, and hHb6 in the cortex. These genes, and in particular hHb1 and hHb3, have a very high degree of sequence homology.

We have previously found the E413K mutation in five families (Korge *et al*, 1998). Here, we report the search for mutations in the rod domain of hHb1, hHb3, and hHb6 in 13 more unrelated cases or families with Mt. In addition to identifying known types of mutation in six families, we have found two novel mutations (N114D and N114H) in a highly conserved asparagine residue within the helix initiation motif of the 1A domain of hHb6. In five other Mt cases mutations in the rod domains of hHb1, hHb3, and hHb6 have not been identified. We discuss the likely consequences of the known mutations for keratin structure. To address the possible distinct effects of mutations in different keratins on phenotype, we have compared the clinical features in previously and newly reported families with mutations in hHB1 and hHb6.

MATERIALS AND METHODS

Subjects Most of the 18 pedigrees have previously been reported (**Table I**); these include the descents of a Scottish pedigree which was the first to be described (Anderson, 1883). In four of the 13 pedigrees in whom mutation had not previously been found, linkage analysis confirmed or was consistent with linkage to 12q13 (Korge *et al*, 1998). In all families most members affected displayed the typical phenotype of beaded and broken hairs, although in many patients this was limited to the occiput. Some patients presented only with follicular keratoses, or were known to be affected only as obligate carriers (Birch-Machin *et al*, 1997). In two cases, the presence of a mutant gene was only identified after mutation analysis. In 54 clinically or genetically confirmed cases, data on the phenotype of the disease were gathered by personal examination, or where family members were not accessible, by postal questionnaire. A simple scale was used to assess severity: apparently normal hair, localized thinning, generally thinning, or severe alopecia. Severity in childhood was assessed retrospectively using the same four-point scale. A history of change with age and pregnancy, and evidence of associated features such as fragile or soft nails, or follicular keratoses, were also sought.

Mutation detection Linkage and DNA studies were approved by the local research ethics review committee. DNA was extracted from blood or salivary samples obtained from family members, and polymerase chain reaction (PCR) amplification performed by standard methods, using the Expand Long template PCR system (Boehringer Mannheim, Germany). The primers were picked from the published sequences (EMBL/GenBank: No X81420 or Y13621 for hHb1; No X99142 or AJ000263 for hHb6).

Gene region	+ primer	– primer
hHb1 exon 1/9	cta tcc tgt cct ctg caa cc	cag gag tggg agg ggt ctt t
hHb1 exon 7/9	agt gat gcc cgc tgc aag ct	cag gag tgg gag ggg tct tt
hHb3 exon 1/2	gtt cca tcc tct gcc atc tac tcc	ggg gct cca ggt tac tct ggc agc
hHb3 exon 7/9	agt gat gcc cgc tgc aag ct	agt ctc aca gtg ctt ctt cca
hHb6 exon 1/7	cac agc gtg tgc gga ggc ttt cg	ctg gtt gca ggg tgg gga ggt ta
hHb6 exon 7	ccc tca gcg atg ccc gct gca ag	ctg gtt gca ggg tgg gga ggt ta

PCR was performed at 94°C, then 35 cycles (94°C, 1 min; 65°C, 1 min; 68°C, 2 min) followed by a 7 min extension at 68°C. After gel purification of the PCR products obtained, direct sequencing was performed using ^{33}P-labeled ddNTPs and the PCR Product and Sequencing Kit (USB/Amersham Life Science, Cleveland, OH).

RESULTS

Codon 413 of hHb6 and hHb1 and codon 402 in hHb1 are hot spots for mutations in Mt Most mutations reported to date have been at the end of the 2B domain in the helix termination motif of cortically expressed hair keratins, and hence we began by screening these regions of hHb1, hHb3, and hHb6 in the 13 families without known mutation. In two families, one German (M12) and one British (M10), we again found the E413K substitu-

[4]Pearce EG, Smith SK, Bowden PE: Different hair-specific keratin (hHb6) mutations in two families with monilethrix. *J Invest Dermatol* 112:593, 1999 (abstr.)

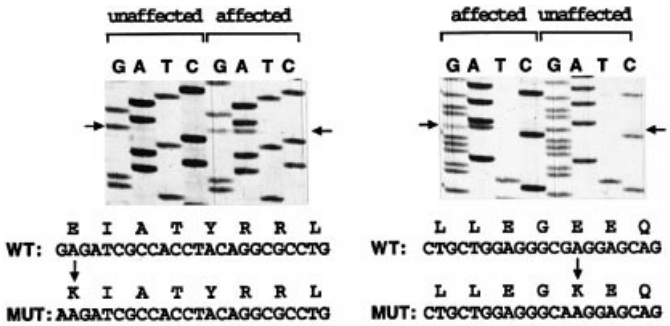

Figure 1. Part of DNA sequencing gel showing G to A transversion (*arrow*) in 2B domain of hHb1 gene of an affected and unaffected family member. The glutamic acid to lysine substitution in the mutant allele due to this point mutation is indicated for both mutations below. *Right side*, E402K substitution in family M2; *left side*, E413K substitution in family L.

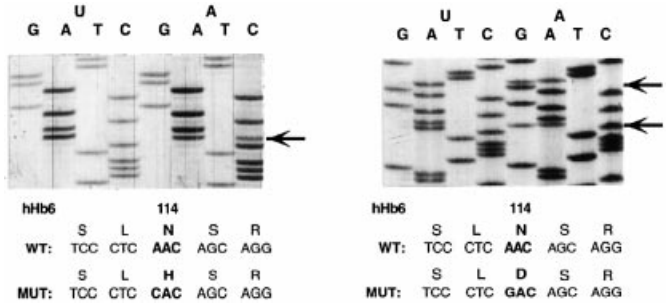

Figure 2. Part of DNA sequencing gel. *Left side*: A to C transversion (*arrow*) in 1A domain of hHb6 gene of an unaffected and affected family member (family D). The asparagine to histidine substitution in the mutant allele due to this point mutation is indicated below. *Right side*: A to G transversion (*lower arrow*) in 1A domain of hHb6 gene of an unaffected and affected family member. The asparagine to aspartic acid substitution in the mutant allele due to this point mutation is indicate below. The *top arrow* indicates a polymorphism which is present in the unaffected and affected individual of this family. This G to A transversion does not change the amino acid encoded.

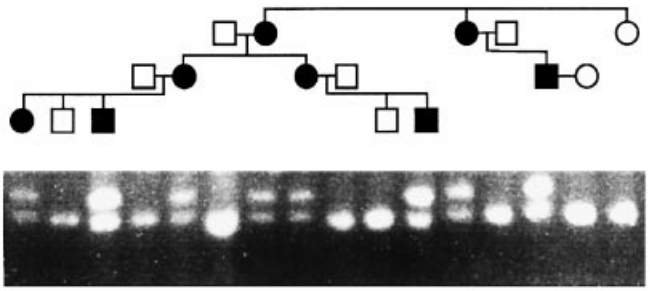

Figure 3. RFLP for the *Taq*I enzyme in a 1138 bp PCR fragment amplified from the C-terminus of the hHb1 gene in family M2, showing incomplete cleavage in affected members due to loss of the restriction site in the mutant allele. 1% agarose gel.

tion at residue 117 of the 2B helix in hHb6. Fifteen pedigrees or cases with this mutation have now been reported (**Fig 4**). In one Scottish family (L) we found the corresponding substitution E413K (residue 117 of the 2B helix) in hHb1, the second report of this mutation in Mt (**Fig 1**), and in three unrelated German and one Northern Irish family (M2, M6, M8, M11) an E402K substitution at residue 106 of the 2B helix (**Fig 1**). The latter mutation is now the second most frequently reported in Mt, with a total of six pedigrees or cases. All the above mutations were found in affected family members and were absent in 50 alleles of unrelated healthy control individuals. To date we have not identified disease causing

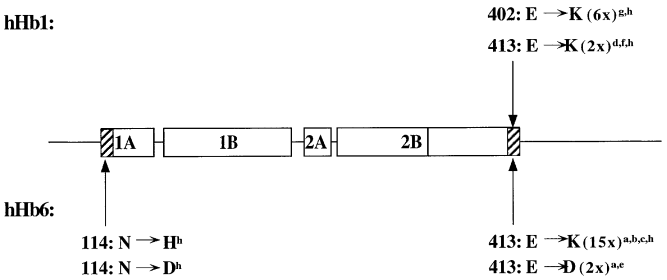

Figure 4. Schematic diagram of the central α-helical rod domain summarizing the point mutations reported in Mt. The numbers relate to the amino acid residue within the keratin chain. The single letter amino acid code is used to indicate the amino acid substitution. Numbers in parenthesis refer to the frequency for that mutation reported to date. The small letters refer to the literature where these mutations were described: (*a*) Winter *et al* (1997a); (*b*) footnote 2; (*c*) Korge *et al* (1998); (*d*) footnote 3; (*e*) Zlotogorski *et al* (1998); (*f*) Winter *et al* (1997b); (*g*) Winter *et al* (1998); (*h*) this report.

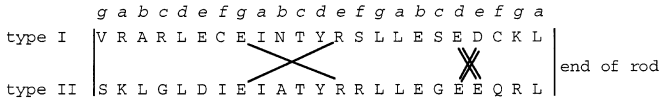

Figure 5. End of the 2B helix of a type I and II hair keratin. Single line, positive/attractive interaction; double line, negative/repulsive interaction.

mutation in the helix termination motif of hHb3, but in five families mutations have yet to be identified.

Two novel mutations at codon 114 in the 1A domain of hHb6 causing Mt In six families/cases of Mt in whom we failed to identify mutations in the helix termination motif of cortical keratins we screened the beginning of the rod domain of hHb1, hHb3, and hHb6, including the helix initiation motif. Applying this approach we demonstrated mutations in this domain for the first time in Mt. In a Scottish family (D) we found an A to C transversion in the helix initiation motif of hHb6, encoding a histidine residue in place of asparagine at position 114 (N114H, residue 8 of the 1A helix). In the Portuguese case (M9) we found an A to G transversion, encoding a aspartic acid residue in place of asparagine at the same position (N114D).

***Bse*RI and *Taq*I-restriction fragment length polymorphism (RFLP) assay for mutation screening E413K and E402K** A suitable *Bse*RI-RFLP for E413K substitution in hHb1 and hHb6 was used as described (Korge *et al*, 1998). The 298 bp PCR fragment contains a cutting site for the restriction enzyme *Bse*R1 which is lost following the G to A transversion. A suitable *Taq*I-RFLP for the E402K substitution was used to confirm segregation of the mutation with the phenotype in the M2 pedigree (**Fig 3**), as well as to confirm its absence in 36 normal controls (data not shown). Using hHb1 7/9 +/– primers the 1138 bp PCR fragment amplified contains a cutting site in the wild-type allele shortening the PCR fragment by 122 bp. This site is lost in the affected allele. The RFLP methods we have developed will be of value in screening isolated cases or pedigrees for this common pathogenic mutation.

Clinical consequences of mutations in hHb1 and hHb6 are similar A comparison of the clinical effects of mutations in hHb1 and hHb6 does not suggest a general differential effect on severity (**Table II**). Improvement in hair growth generally occurred early in puberty, although in one case this was the time of disease onset. Although a smaller proportion of adults with hHb1 defects had the most severe alopecia, the spread of severity was otherwise similar, and there was no statistically significant difference between the groups. The only feature in which a difference was detected was the presence of soft or unusually ridged nails, which were significantly more common with hHb1 defects. Almost all the cases,

Table II. Clinical features in 54 cases of Mt

		All	Mutated gene		
			hHb1	hHb6	
Sex	M:F	24 : 30	10 : 8	14 : 22	
Severity – in childhood	No obvious defect	5	3	2	
	Locally (occipital) thin hair	12	4	9	
	Generally thin hair	11	5	8	n.s.[a]
	Severe alopecia	11	6	7	
	Data not available	10	–	10	
	All	55	18	37	
Severity – postpubertal	No obvious defect	13	5	8	
	Locally (occipital) thin hair	17	5	10	
	Generally thin hair	11	4	6	n.s.[a]
	Severe alopecia	7	1	7	
	All	47	15	32	
Improvement with age	Age > 11 affected as children	22/42	9/14	13/28	n.s.[a]
Follicular keratoses		30/54	11/18	19/36	n.s.[a]
Nail dystrophy		16/54	10/18	6/36	$p < 0.01$[a]
Improvement in pregnancy		3/15	0/2	3/13	n.s.[a]

[a]Fisher's exact test.

however, were from three German families, and the finding may reflect observer variation. There was no significant effect of gender on the likelihood of regrowth, or severity in adulthood.

DISCUSSION

The vast majority of pathogenic mutations in keratin genes lie in the domains encoding the helix boundary peptides, which are thought to be critical for higher order assembly of keratin intermediate filaments (Parry and Steinert, 1995). It has been suggested (Parry, 1995, 1996) that unlike epidermal keratins, hair keratin molecules in the IF, while maintaining the major overlaps characteristic of all IF, lack the head-to-tail overlap between similarly directed molecules. The hard α-keratins are believed instead to be stabilized by numerous intermolecular disulfide bonds. The helix termination sequence TYR(R/K)LLEGEE is highly conserved throughout both epidermal keratins and hair keratins. The recurrent E413K substitution in hHb6 in 15 of the 28 pedigrees/cases of Mt in which mutations have been identified supports our earlier contention that this is a hot spot for mutation (Winter *et al*, 1997a, b; Korge *et al*, 1998; this paper). Equivalent mutations in the corresponding nucleotide in keratin 2e represent a similarly high proportion of reported mutations in ichthyosis bullosa of Siemens (Kremer *et al*, 1994; McLean *et al*, 1994; Rothnagel *et al*, 1994; Jones *et al*, 1997). Equivalent mutations have been found also in the keratin 3 gene in Meesmann's corneal dystrophy (Irvine *et al*, 1997), in sporadic epidermolysis bullosa simplex cases (Stephens *et al*, 1997; Müller *et al*, 1999), and pachyonychia congenita.[5] Similar mutations E413K and E402K are apparently common in hHb1, with the latter predominating to date. The E482K mutation at position 106 of the 2B domain of the K2e gene is also the cause of ichthyosis bullosa of Siemens in one case.[6] The common mechanism is likely to be spontaneous deamination of a methylated cytosine in a CpG dinucleotide giving rise to thymine (Strachan and Read, 1996). As a result of ineffective mismatch repair the opposite strand is propagated as adenine instead of guanine.

Protein structural implications of the E413K substitution in hHb1 and hHb6 The lysine mutation at position 413 corresponds to residue 117 in the 2B helix of both hair keratins. It is thus located four residues from the C-terminal end of the coiled-coil rod domain. In hHb1 and hHb6 the mutant lysine residue (Winter *et al*, 1997a; Korge *et al*, 1998) replaces a glutamic acid residue which occupies an internal *d* position in the heptad repeat of the α-helix. The *d* position in type I, II, and III intermediate filament chains is often occupied by glutamic acid residues but never by lysine residues. In contrast, the *a* position is frequently occupied by lysine residues, but rarely by glutamic acid residues. This suggests that either glutamic acid in the *d* position stabilizes coiled-coil formation or that lysine interferes with it. Conversely, it is also possible that glutamic acid in position *d* destabilizes the coiled-coil and that lysine, if it was to occur in this position, would introduce unrequired stability. Either way the effect of the mutant would be expected to lead to changes in the intermediate filament assembly or its stability. Normally, residues 117 and 118 are both occupied by acidic residues and this leads to a potential destabilizing repulsive interaction (**Fig 5**). With the lysine mutation, however, the lysine is followed by an acidic residue, and this would allow a stabilizing interaction to be made instead. It is hard to predict the exact effect of this change only one to two turns from the end of the coiled-coil, but it would not be surprising if this region of the coiled-coil in the wild-type sequence was designed to have marginal stability, thus facilitating its termination or giving it greater flexibility. In contrast, the mutant form could lead to undesired stability arising from the lysine in the *d* position and the extra interchain ionic interaction that becomes possible. Irrespective of whether the lysine mutation stabilizes or destabilizes the structure of the keratin molecule it is absolutely clear that some structural rearrangement at this point in the rod domain would occur.

Protein structural implications of the E402K substitution in hHb1 The mutation at position 402 corresponds to position 106 in the 2B helix of hHb1. In the coiled-coil rod domain there are about nine residues that are almost totally conserved across all types of intermediate filament chains (Steinert and Parry, unpublished): one of these is E106. This alone implies the structural/functional importance of this particular residue. There are two obvious reasons why this residue has been so highly conserved. The first is that it forms part of one of only three almost perfectly conserved interchain ionic interactions across all IF chain types. It occurs between the glutamic acid in position 106 (position *g*) and a basic residue in position 111 (position *e*) and, very likely, has a special role in stabilizing the end of the coiled-coil rod domain (see **Fig 5**). The mutation results in the removal of two positive interchain ionic interactions by turning one of them into a repulsive interaction. Furthermore, *in vitro* studies with bacterially expressed wild-type and mutant keratin K5 and K14 chains have been tested in a stability assay in which the urea concentration at which a dimer

[5]Smith SK, Nogita T, Hashimoto T, Bowden PE: A novel K17 mutation in a patient with pachyonychia congenita type 2 (PC-2). *J Invest Dermatol* 110:618, 1998 (abstr.)

[6]Pitera R, Pitera JE, Eady R, *et al*: Novel keratin mutations causing ichthyosis bullosa of Siemens: phenotype variations and K2e. *J Invest Dermatol* 104:632, 1995 (abstr.)

molecule could be dissociated was measured.[7] Molecules where E106 was replaced dissociated at lower urea concentrations and formed shorter and more irregularly structured filaments. These *in vitro* data provide direct evidence that mutations at this site severely alter normal IF function. Secondly, the residues around E106 are part of the so-called trigger motif (Kammerer *et al*, 1998) which promote coiled-coil formation. Within this motif glutamic acid is a required component. Hence, it is hard to think of any residue that is more likely than E106 to have a detrimental effect on IF structure when mutated.

Protein structural implications of the two 1A helix mutations (N114H N114D) in hHb6 The mutation at position 114 corresponds to position 8 in helix 1A of hHb1. This asparagine, which lies in position *e* is also one of the nine almost totally conserved residues in all IF chains (Steinert and Parry, unpublished). As before, this implies special structural/functional significance, but as asparagine is not a charged or an apolar residue it seems possible that its role will be to provide key hydrogen-bonding interactions. Its role may also be to destabilize marginally the structure, but less so than for possible alternative conformations. The difference between asparagine and aspartic acid side chains lies in their relative charges and hydrogen-bonding capabilities. Whether the extra charge in aspartic acid stabilizes or destabilizes the end of helix 1A is not clear. Similarly, it is not possible to state whether the different hydrogen-bonding possibilities lead to stabilization of the wild-type or the mutant forms. Related comments are also pertinent to the asparagine to histidine mutations that occurs in the same position in helix 1A. In summary, therefore, it is possible either that asparagine destabilizes the wild-type structure and that the mutations (aspartic acid and histidine) actually introduce more stabilizing interactions that were previously possible or that the diametrically opposite explanation is true. Either way, the IF or the IF molecules will necessarily have altered stability or structure between the wild-type and the mutant forms. Keratin mutations involving position 8 of the 1A domain of K1 or K6a are also the cause for either epidermolytic hyperkeratosis (Mclean *et al*, 1994; Yang *et al*, 1994) or pachyonychia congenita (Bowden *et al*, 1995), respectively.

Genotype/phenotype correlation in Mt Knowledge of other keratin disorders (McLean and Lane, 1995; Korge and Krieg, 1996) predicts that defects in either partners in a keratin pair could produce the Mt phenotype, but to date only type II keratin defects have been found in Mt. On the basis of one small pedigree, it has been suggested that the hHb1 mutation may produce a milder, variant phenotype (Winter *et al*, 1998). It appears from our data, however, that essentially the same phenotype can occur with mutations in either hHb1 and hHb6. Nail defects may be more common with hHb1 mutation which may explain the prominence of these in some studies (Heydt, 1963; Tietze, 1995). It is clear, however, that the large variation in severity of hair dystrophy (Birch-Machin *et al*, 1997) applies to defects in either gene. These findings are intriguing, as they imply that Mt is unlike diseases due to mutations in epithelial keratins, where clinical phenotype is strongly determined by the keratin pair affected (Irvine and McLean, 1999). If the high degree of sequence homology between these genes (Rogers *et al*, 1997), particularly between hHb3 and hHb1, even in V domains and intron sequences, implies functional redundancy, it is further surprising that mutation in only one gene should have such a profound effect. *In situ* studies, however, indicate that whereas hHb1 and hHb3 have similar expression patterns in the emerging cortex, hHb6 mRNA starts and ends relatively higher in the emerging hair shaft (Rogers *et al*, 1997). It may be that the relative importance of each gene expressed varies at different stages in the hair cycle. Hair keratin hHa1 gene expression has been shown to be downregulated (Bowden *et al*, 1998) in telogen. We have previously suggested that the periodicity characteristic of Mt hairs results from a feedback loop in which dystrophic cortical keratinocytes cause a cytokine response indirectly modulating hair growth, perhaps switching to a different phase of the hair cycle, and inducing a corrective response. Perhaps defects in keratins expressed in differing proportions at different points in the cycle can initiate the same process of periodic dystrophy at these different points. The improvement of hair growth with age in many cases may reflect a change in the proportions or numbers of the various hair keratin genes being expressed.

[7]Wu K, Jang S-I, Idler W, Marekov LN, Parry DAD, Yang JM, Steinert PM: Conserved ionic pairs essential for the stabilization of coiled-coil molecules of intermediate filaments. *Mol Biol Cell* 9(Suppl.):250, 1998 (abstr.)

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