Linkage of Monilethrix to the Trichocyte and Epithelial Keratin Gene Cluster on 12q11-q13

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Monilethrix is characterized by beaded or moniliform hair, which results from the periodic thinning of the hair shaft. The beaded hair thus produced is subject to excess weathering and premature fracturing at the internodes. Clinically, monilethrix presents with short, fragile, broken hair. The follicular abnormalities range from subtle perifollicular erythema and hyperkeratosis to horny follicular papule formation. At the ultrastructural level, cytolysis and keratin tonofilament clumping (epidermolysis) are seen in the cortical cells of the bulb of the hair follicle. Microsatellite markers flanking the keratin gene clusters at 17q12-q21 and 12q11-q13 were used to perform linkage analysis in a monilethrix pedigree. This study demonstrates linkage of monilethrix in a pedigree to microsatellite DNA loci mapping to the region on chromosome 12 containing the type II keratin cluster. A major group of structural hair proteins, the basic type II trichocyte keratins, map within this epithelial cytokeratin gene cluster. This study implicates a mutation in a trichocyte keratin gene in the pathogenesis of a structural hair disorder. ***Key words: hair disease/hard keratins/follicular hyperkeratosis/epidermolysis. J Invest Dermatol 106:795–797, 1996***

Monilethrix is a rare congenital defect of hair that is inherited as a fully penetrant, autosomal dominant condition with variable expression. Clinically, the hair is subject to excessive weathering and fragility with a tendency to fracture prematurely at the narrower internodes (Gummer *et al*, 1981). Affected individuals have normal hair at birth, but within the first few months of life this hair is replaced by fragile, brittle hair, which tends to fracture, producing bald patches. In the mildest forms, it involves only the occipital regions and the nape of the neck. Usually only the scalp is involved, but in its more severe form, the secondary sexual hair, eyebrows, and eyelashes may also be involved. Follicular hyperkeratosis with perifollicular erythema is characteristic, with horny follicular papules seen in the most severe forms of the disease.

Ultrastructurally, monilethrix is characterized by uniform elliptical nodes and intermittent constrictions, internodes, along the hair shaft (Gummer *et al*, 1981). Transverse sections of affected hair shafts at the node demonstrate a reduced number of cuticle cell layers compared with the internodal regions (Ito *et al*, 1984). The cuticle cell membrane appears normal, with a regular concentric arrangement of endocuticle and exocuticle. In the internodal regions, the cuticle is seen to degenerate progressively along the hair shaft. Distally along the hair shaft, there is increased ridging and fluting of the cuticle with cuticular cell breakage and shedding, until distally the cortex is completely exposed (Gummer *et al*, 1981). With the light microscope, the hair matrix has a pale and edematous appearance; with electron microscopy, the cortex of the hair bulb has an amorphous appearance with areas of trichocyte degeneration. In other areas of the cortex, cytolysis and tonofilament clumping are seen (Ito *et al*, 1990). The keratin tonofilament network appears relatively well preserved in the cuticular cells, with only the occasional tonofilament aggregate described (Ito *et al*, 1990).

Keratin mutations have now been demonstrated in a number of disorders characterized by varying degrees of epidermal fragility with secondary hyperkeratosis. Cytolysis and keratin tonofilament clumping have been demonstrated in most of the diseases in which human epithelial keratin gene mutations have been found: epidermolysis bullosa simplex (K5; K14) (Coulombe *et al*, 1991; Ishida-Yamamoto *et al*, 1991; Dong *et al*, 1993), ichthyosis bullosa of Siemens (K2e) (McLean *et al*, 1994b), bullous congenital ichthyosiform erythroderma (K1; K10) (McLean *et al*, 1994a), epidermolytic palmoplantar keratoderma (K9) (Navsaria *et al*, 1994), and pachyonychia congenita (K16; K6a) (Bowden *et al*, 1995; McLean *et al*, 1995). In two of the most recently described keratin diseases—pachyonychia congenita and focal palmoplantar keratoderma with orogenital hyperkeratosis (McLean *et al*, 1995; Shamsher *et al*, 1995)—follicular erythema and hyperkeratosis are characteristic features.

The follicular changes seen in monilethrix (de Berker *et al*, 1993), combined with the abnormal keratin tonofilament network, suggest a trichocyte keratin gene mutation in the pathogenesis of this disease. In this study, we focused on a single family with monilethrix. All the known epithelial and trichocyte keratins have been shown to map to two keratin gene clusters: the acidic keratin cluster

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Abbreviations: Ha, hard acidic keratin; Hb, hard basic keratin; LOD score, the logarithm (to base 10) of the ratio of the likelihood of the data at a specified value of Ø divided by the likelihood of the pedigree data if there were free recombination between the loci (Ø = 0.5).

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Table I. Linkage Analysis in Monilethrix With Microsatellite Markers Flanking the Keratin Gene Clusters on 17q and 12q[a]

	Recombination Fraction						
Locus	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D12S361	3.25	3.20	2.96	2.65	1.98	1.24	0.49
D12S368	2.86	2.80	2.57	2.27	1.64	0.98	0.38
KRT 9 'CA'	Infin.	−6.19	−3.45	−2.30	−1.19	−0.59	−0.22

[a] LOD scores were computed with FASTLINK version 2.2, assuming autosomal dominant inheritance.

(type II) at 17q12-q21 and the basic keratin gene cluster (type II) at 12q11-q13 (Rogers *et al*, 1995). Linkage analysis was performed in this pedigree using microsatellite DNA marker loci flanking the two keratin clusters.

MATERIALS AND METHODS

Scanning Electron Microscopy The plucked hair samples were examined by scanning electron microscopy after gold coating.

DNA Analysis DNA was extracted from the blood from all consenting individuals from the pedigree, using a Nucleon II kit according to the manufacturer's instructions (ScotLab, Lanark, OK). Microsatellite DNA marker loci were obtained and analyzed as described previously (Kelsell *et al*, 1993; Kelsell *et al*, 1995). The map order of markers used in this study was as follows: 12q11-q13 cen-D12S361-D12S368-D12S90-tel; 17q12-q21 cen-KRT'CA'-D17S855-tel.

Linkage Analysis Linkage analysis was performed with the MLINK module of the FASTLINK software package version 2.2 (Cottingham *et al*, 1993), assuming an autosomal dominant inheritance for monilethrix, with a gene frequency of 0.003 and a penetrance of 95%. Allele frequencies for the DNA markers were either computed from the data or estimated to be equifrequent.

RESULTS

Follicular and Hair Shaft Abnormalities The family was traced through four generations with 12 affected individuals. The same clinical characteristics were observed in all affected individuals. The hair was normal at birth, but follicular erythema and hyperkeratosis developed over the occiput and the nape of the neck within the first few months of life. Subsequently, the hair became brittle and was lost, leaving a stubble 1–2 cm in length over the whole scalp. Scanning electron microscopy of plucked hairs confirmed the features of monilethrix with node and internode formation and the longitudinal ridging and fluting. The secondary sexual hair, eyelashes, and eyebrows appeared normal. The skin, orogenital mucosa, nails, and teeth were all normal.

Monilethrix Linked to the Keratin Gene Cluster on 12q DNA extracted from family members was screened with polymorphic microsatellite DNA marker loci mapping to either 12q11-q13 or 17q12-21, where the two epithelial keratin clusters are located. Informative markers and LOD scores (logarithm to base 10 of the likelihood of the data) are listed in **Table I**.

Linkage was observed to the disease phenotype with the DNA marker D12S361, with a maximum two-point LOD score of 3.25 at a recombination fraction of 0.00. Haplotypes for microsatellite markers mapping to 12q11-q13 were constructed with the minimal number of recombinants possible in the pedigree. Clear cosegregation of a haplotype with the disease phenotype was observed **(Fig 1)**. There was clear evidence against linkage of the disease to 17q12-21, with a negative LOD score of −2.30 for the keratin 9 intragenic microsatellite at a recombination fraction of 0.1.

DISCUSSION

There are 20 known epithelial "soft" keratins and at least eight trichocyte "hard" keratins. The keratins may be divided on the basis of their migration on 2-dimensional electrophoresis into two main groups: the smaller, type I keratins, with an acidic isoelectric point, and the larger, neutral or slightly basic type II keratins (Fuchs *et al*, 1981; Moll *et al*, 1982; Schiller *et al*, 1982; Sun *et al*, 1984). Four trichocyte type I keratins (Ha1, Ha2, Ha3, and Ha4) are clustered with the epithelial type I keratins at 17q12-q21, whereas four trichocyte type II keratins (Hb1, Hb2, Hb3, and Hb4) (Heid *et al*, 1988a; Heid *et al*, 1988b; Heid *et al*, 1986) are clustered with the other type II keratins on 12q11-q13 (Rogers *et al*, 1995). In this study, we have demonstrated linkage of DNA markers mapping to the region 12q11-q13 with the monilethrix disease phenotype. As the marker locus D12S368 maps to yeast artificial chromosome, which contains a number of type II keratin genes (Yoon *et al*, 1994),

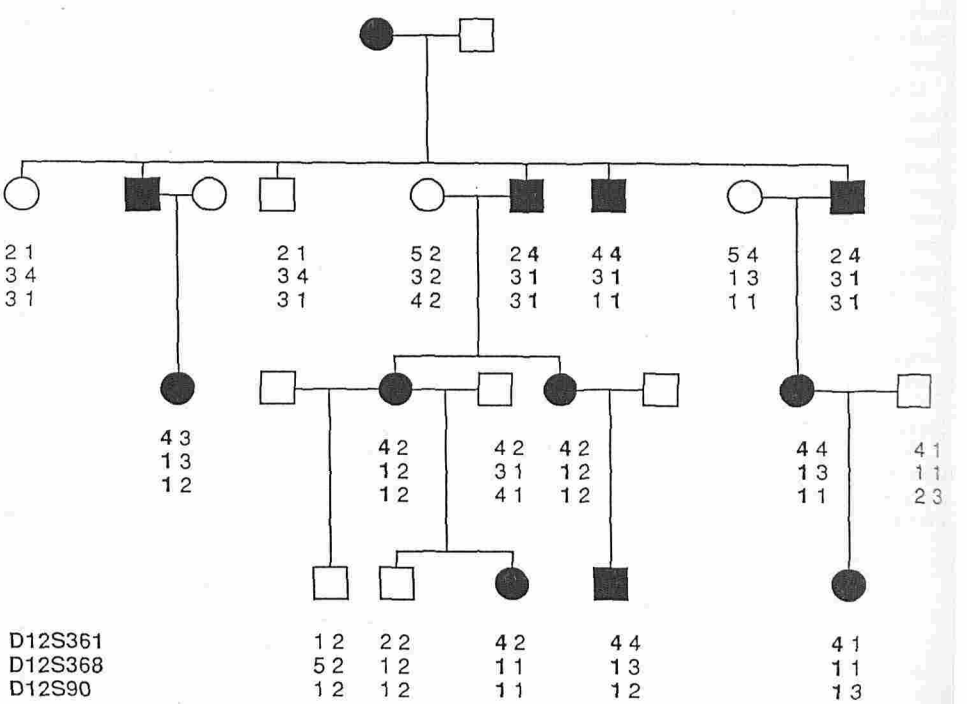

Figure 1. Segregation analysis in a monilethrix pedigree using markers flanking the 12q keratin gene cluster. Haplotypes constructed for the monilethrix pedigree show the consistent inheritance of a 12q haplotype (indicated in *bold type*; 4-1-1) with the disease for the DNA marker loci D12S361, D12S368, and D12S90.

it is possible that the disease in this family is due to a mutation in one of these keratins. The keratin gene cluster on 12q is relatively large, however, and the possibility of a mutation in a gene other than a keratin must remain. The moniliform changes seen in this family were restricted to the scalp, with normal body and secondary sexual hair. This regional distribution of hair involvement could be explained either in terms of a nonkeratin gene mutation or by proposing a regional localization of the trichocyte keratins in a manner analogous to that seen with the epithelial keratins.

Whereas the basal cells bordering on the apex of the dermal papilla and also in the cuticle may coexpress both trichocyte hard and epithelial soft keratins, there is no coexpression of the epithelial keratins within the cortex of the hair bulb (Heid *et al*, 1988a). The site of maximal cytolysis and keratin tonofilament aggregation in monilethrix is the cortex of the hair bulb (Ito *et al*, 1990). It is therefore probable that a mutation in a type II basic trichocyte keratin, and not an epithelial keratin, is the genetic basis of monilethrix in this pedigree.

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