

A novel keratin 12 mutation in a German kindred with Meesmann's corneal dystrophy

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Abstract

Aim—To study a kindred with Meesmann's corneal dystrophy (MCD) to determine if a mutation within the cornea specific K3 or K12 genes is responsible for the disease phenotype.

Methods—Slit lamp examination of the cornea in four members of the kindred was carried out to confirm the diagnosis of MCD. The region encoding the helix initiation motif (HIM) of the K12 polypeptide was polymerase chain reaction (PCR) amplified from genomic DNA derived from affected individuals in the kindred. PCR products generated were subjected to direct automated sequencing. Restriction enzyme analysis employing *Ban I* was used to confirm the presence of the mutation in affected individuals of the family.

Results—Sequencing of the K12 gene in an affected individual from the family revealed a novel heterozygous missense mutation (413A→C), predicting the substitution of a proline for a glutamine at codon 130 (Q130P) in the HIM of the K12 protein. The mutation was excluded from 50 normal, unaffected individuals by restriction enzyme analysis and was therefore unlikely to be a common polymorphism.

Conclusion—A novel missense mutation in the K12 gene leads to MCD in a German kindred. Missense mutations have now been identified within the region encoding the helix initiation motif of the K12 protein in eight of 11 MCD kindreds analysed at the molecular level.

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Meesmann's corneal dystrophy (MCD) (OMIM 122100) is an autosomal dominantly

inherited disorder affecting the corneal epithelium of both eyes.¹⁻³ It manifests in early childhood and is characterised by myriads of intraepithelial microcysts of variable distribution and density. The microcysts are best seen by retroillumination and slit lamp examination.^{4,5} Further clinical signs of the disease are recurrent punctate erosions with lacrimation and photophobia. Symptoms include blurred vision and foreign body sensation. Histologically, the corneal epithelium appears irregularly thickened and contains numerous vacuolated suprabasal cells.⁶⁻⁸ In addition, there is intraepithelial cyst formation with accumulated periodic acid Schiff (PAS) positive material. By electron microscopy, epithelial cells have been shown to contain fibrogranular material often referred to as "peculiar substance".^{6,7,9}

Keratins K3 and K12 are specifically expressed within all cell layers of the adult central corneal epithelium¹⁰⁻¹³ and are regarded as markers of advanced corneal epithelial differentiation.^{14,15} K5 and K14 are also expressed within the corneal epithelium, though to a lesser extent than either K3 or K12.^{15,16} Comparative studies of autosomal dominantly inherited blistering disorders of human epidermis and other epithelial tissues have provided ample evidence that pathogenic keratin gene mutations exert dominant negative effects on keratin filament assembly which result in intracellular keratin aggregation.¹⁷⁻¹⁹ In vitro biochemical assays²⁰ and protein expression experiments in cultured cells²¹ have shown that mutations in keratin rod domains exert powerful dominant negative effects. Lack of a functional keratin cytoskeleton appears to critically reduce the mechanical resilience of the affected epithelial cells and tissues.²²

Mice have been genetically engineered in which the K12 gene has been ablated.¹⁶ The corneal epithelium of these mice is very fragile and easily removed by wiping the surface of the eye with a microsponge.¹⁶ No mutations within the K3 or K12 genes have yet been found to underlie a recessive corneal epithelial disease in humans. However, this research group recently found the first dominant negative mutations within the K3 and K12 genes in MCD patients.⁴ Further heterozygous missense mutations in the K12 gene have since been reported.^{5,23,24}

Here we investigated a southern German family with MCD and detected a novel mutation within the region encoding the highly conserved helix initiation motif of the K12 protein. In addition we review the mutations published in the K3 and K12 genes to date.

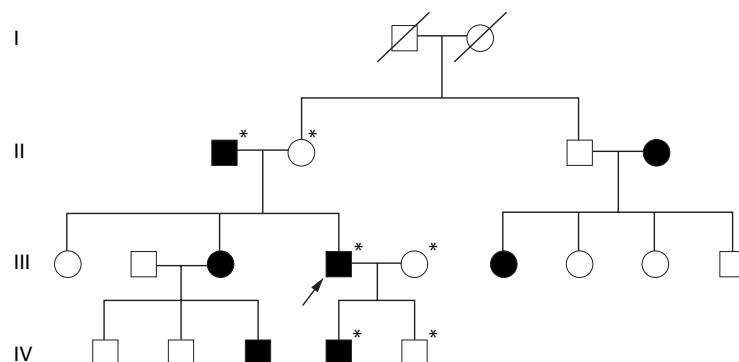


Figure 1 Pedigree of the kindred studied, displaying typical autosomal dominant inheritance. Family members from whom DNA was obtained are marked with an asterisk. Arrow indicates the proband.

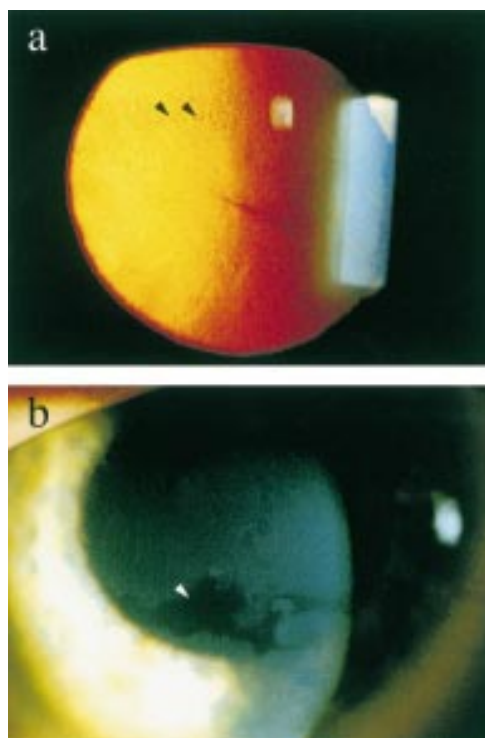


Figure 2 Corneal changes in two patients in the family studied as shown by (a) retroillumination and (b) slit lamp examination. (a) Right eye of individual II-1 (see Fig 1), showing relatively even distribution of microcysts (black arrowheads). Grey lines in the corneal epithelium were also seen in this patient by slit lamp analysis (not shown). This phenomenon is not readily observed by retroillumination. (b) Left eye of individual III-4 (see Fig 1), showing intraepithelial blebs and an irregularly shaped area devoid of microcysts (white arrowhead). The underlying mechanism producing this area of phenotypic reversion is unknown.

Materials and methods

SUBJECTS

We investigated a four generation family with MCD from southern Germany. The family history indicated that seven of 18 living family members were affected. The pedigree was consistent with an autosomal dominant mode of inheritance (Fig 1). A full ophthalmological check up including slit lamp examination was performed in four affected family members. Three of them showed typical signs of MCD. Their corneal surfaces were studded with numerous intraepithelial microcysts, as shown in Figure 2. Irregularly shaped areas devoid of microcysts were noted in all affected corneas (Fig 2b). One of the patients (II-1) showed additional changes in the form of curved grey lines (not shown). All of the patients complained about foreign body sensation and recurrent painful erosions accompanied by photophobia, lacrimation, and blurred vision. Signs of MCD were noted in early childhood and tended to progress with age of patient. No further ocular abnormalities were noted.

SEQUENCING THE HELIX INITIATION MOTIF OF THE K12 GENE

Most of exon 1 of the K12 gene, incorporating the HIM of the K12 protein, was amplified from the proband and an unaffected individual in the family using the primers K12e1.L (5' ATG GAT CTC TCC AAC AAC ACC ATG

3') and K12e1.R (5' GTA ATC GCT CTG TGA AGC ATC TGC 3'). "Touchdown" polymerase chain reaction (PCR) conditions were used, consisting of: 94°C for 5 minutes for 1 cycle; 94°C for 30 seconds; 65/63/61°C for 45 seconds; 72°C for 45 seconds for two cycles at each annealing temperature; 94°C for 30 seconds; 58°C for 45 seconds; 72°C for 45 seconds for 30 cycles; and 72°C for 5 minutes for one cycle. PCR products were purified using QIAquick columns (Qiagen, Chatsworth, USA) and sequenced with both forward and reverse primers, using the ABI PRISM Ready Reaction System (Perkin-Elmer, Foster City, CA, USA). Sequencing ladders were analysed on an ABI 377 automated sequencer.

MUTATION CONFIRMATION

Mutation 413A→C did not alter any known restriction enzyme site and so a mismatch primer was designed which in conjunction with the mutation, generated a *Ban* I site. PCR amplification was carried out using the mismatch primer K12M3 (5' CTG GAT CAG AAA AAG AAA CGG TGC 3') (mismatches underlined), with K12e1.R (as above), in Promega PCR buffer supplemented with 1 mM magnesium chloride and the following

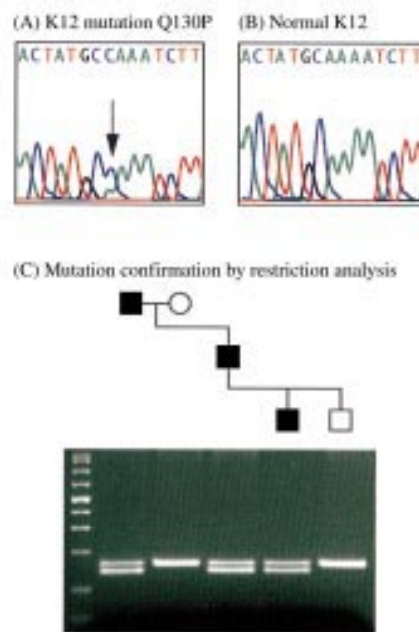


Figure 3 Automated sequencing of K12e1.L/K12e.R PCR products derived from the proband (III-4) and an unaffected individual (II-2) from the kindred, codons 128–132 of K12 shown. (A) In the proband, CAA is replaced by CCA (413A→C) in one allele, predicted to lead to the substitution of a proline residue instead of the normal glutamine at codon 130 (Q130P). (B) Sequence derived from a normal, unaffected individual (II-2) revealing the nucleotide sequence in the region of codon 130, located within the helix initiation motif of the K12 protein. CAA encodes a glutamine at this position. (C) *Ban* I restriction enzyme digestion of PCR products spanning the mutant region. Through use of a mismatch primer, a *Ban* I restriction enzyme site is generated within the mutant product. Upon digestion, the 164 bp full sized product is cut into bands of 144 bp and 20 bp (the latter does not resolve on 3% agarose gel). DNA molecular weight markers are shown on the left, with bands at 2000 bp, 1500 bp, 1000 bp, 700 bp, 525 bp, 500 bp, 400 bp, 300 bp, 200 bp, 100 bp, and 50 bp (Cambio, Cambridge).

Table 1 Mutations in K3 and K12 (current to October 1999)

Amino acid substitution	Nucleotide substitution	Keratin and domain	Amino acid change	Reference
E509K	1525G→A	K3 (HTM)	Acidic → basic	Irvine <i>et al</i> ⁴
I426V	1300A→G	K12 (HTM)	Neutral (apolar) → neutral (apolar)	Coleman <i>et al</i> ²³
Y429D	4064T→G	K12 (HTM)	Neutral (polar) → acidic	Nishida <i>et al</i> ⁵
M129T	410T→C	K12 (HIM)	Neutral (apolar) → neutral (polar)	Corden <i>et al</i> ²⁴
Q130P	413A→C	K12 (HIM)	Neutral (polar) → neutral (apolar)	This report
R135T	428G→C	K12 (HIM)	Basic → neutral (polar)	Irvine <i>et al</i> ⁴ ; Corden <i>et al</i> ²⁴
R135G	427A→G	K12 (HIM)	Basic → neutral (polar)	Nishida <i>et al</i> ⁵
R135I	428G→T	K12 (HIM)	Basic → neutral (apolar)	Nishida <i>et al</i> ⁵
L140R	443T→G	K12 (HIM)	Neutral (apolar) → basic	Nishida <i>et al</i> ⁵
V143L	451G→C	K12 (HIM)	Neutral (apolar) → neutral (apolar)	Irvine <i>et al</i> ⁴

programme: 94°C for 5 minutes for one cycle; 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 1 minute for 35 cycles; and 72°C for 5 minutes for one cycle. Resultant PCR products were each digested overnight with 4 U of *Ban* I enzyme. An additional band was generated in members of the family carrying the mutation (Fig 3C). Fifty normal unrelated individuals were also screened using this *Ban* I restriction enzyme digest for presence of the mutation (data not shown).

Results

Sequencing of the region encoding the helix initiation motif of the K12 gene in an affected individual in the family revealed a heterozygous nucleotide substitution 413A→C (Fig 3A). This is predicted to substitute a proline residue for glutamine at codon 130 (Q130P). Sequencing in the reverse direction confirmed the presence of the heterozygous missense mutation in an affected individual (not shown). DNA from an unaffected individual from the family was also sequenced and did not contain the 413A→C nucleotide substitution

(Fig 3B). The mutation did not alter any known restriction enzyme site and so a mismatch primer was designed in order to generate a novel restriction site upon amplification of the mutant allele. This restriction fragment analysis showed that all affected individuals within the kindred harboured the mutation, whereas the unaffected individuals did not (Fig 3C). This test was used to exclude the mutation from 50 normal unrelated people (data not shown).

Discussion

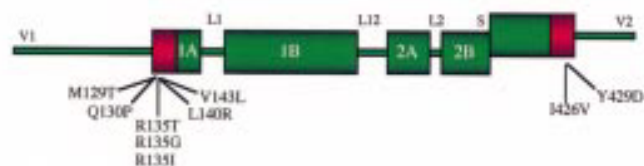
Meesmann's corneal dystrophy is an autosomal dominant disorder of the corneal epithelium. Its clinical and morphological characteristics were first reported in a family from northern Germany more than 60 years ago.¹⁻³ Previously, we demonstrated genetic linkage to the type I keratin locus on chromosome 17q and the first K12 mutation,⁴ in the descendants of the original Meesmann kindred.^{2, 25} Simultaneously, we described linkage to the type II keratin cluster on chromosome 12q and the first mutation in K3 in an Irish MCD family.⁴

Here, we have studied an MCD kindred from southern Germany and identified a novel heterozygous mutation within the region encoding the helix initiation motif of the K12 protein, Q130P. The 10 MCD mutations published to date are shown in Table 1 and the positions of these mutations in the K3 and K12 proteins is shown schematically in Figure 4. From the small number of mutations reported to date, the 1A domain of K12, where the mutation reported here is located, is emerging as a hot spot for MCD mutations.

The helix initiation and termination motifs are highly conserved regions of the keratin proteins and have been found to have an important role in normal filament assembly.²⁶⁻²⁹ Numerous mutations have been found within these regions underlying a wide range of epithelial diseases.¹⁷⁻¹⁹ From Table 1, it can be seen that the majority of published mutations involve substitution of an amino acid of a different group to the one which is present within the normal polypeptide. For example, the mutation E509K⁴ involves the substitution of a basic amino acid (lysine) for an acidic one (glutamic acid).

The novel Q130P mutation described in this paper involves the substitution of a polar amino acid of molecular weight 146 Da (glutamine) by an apolar one of 115 Da (proline). Proline substitutions are especially disruptive to α helical tertiary structures in proteins.³⁰ This is

Type I keratin K12



Type II keratin K3



Figure 4 Model of the protein structure of the type I keratin, K12 and the type II keratin, K3, revealing the positions of published MCD mutations to January 2000. The α helical rod domain is composed of four subdomains termed 1A, 1B, 2A, and 2B. These subdomains are separated by non- α helical regions, the linkers L1, L12, and L2. The areas in red at the ends of the rod domain are regions of high sequence conservation, the helix initiation and termination motifs, which are critical in filament assembly. Only one mutation has been found within the K3 gene (E509K), in the region encoding the helix termination motif.⁴ There are two mutations within the region encoding the helix termination motif of the K12 protein; Y429D⁵ and I426V.²³ The remaining mutations are all found within the helix initiation motif of the K12 protein, with mutation of arginine-135 found in four kindreds.^{4, 5, 24}

because the nitrogen atom in proline which is involved in peptide bond formation is contained within a ring structure, which is not the case for other amino acids. In the case of intermediate filament proteins, proline mutations within the conserved ends of the α helical rod domain have been shown to have more dramatic effects on filament structure and assembly in cultured cells than similar mutations contained more centrally within the rod domain.³¹ This is the second instance of a proline substitution at the fifth residue of the α helical 1A region within a type I keratin. The substitution of a proline for a glutamine residue at codon five of the 1A domain (Q122P) has been previously reported in the K16 gene in a case of pachyonychia congenita.³²

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- 1 Meesmann A. Über eine bisher nicht beschriebene dominant vererbte Dystrophia epithelialis corneae. *Ber Zusammenkunft Dtsch Ophthalmol Ges* 1938;52:154-8.
- 2 Meesmann A, Wilke F. Klinische und anatomische Untersuchungen ueber eine bisher unbekannte, dominant vererbte Epitheldystrophie der Hornhaut. *Klin Monatsbl Augenheilkd* 1939;103:361-91.
- 3 Pameijer JK. Über eine fremdartige familiäre oberflächliche Hornhautveränderung. *Klin Monatsbl Augenheilkd* 1935;95:516-17.
- 4 Irvine AD, Corden LD, Swensson O, et al. Mutations in cornea-specific keratins K3 or K12 cause Meesmann's corneal dystrophy. *Nat Genet* 1997;16:184-7.
- 5 Nishida K, Honma Y, Dota A, et al. Isolation and chromosomal localization of a cornea-specific human keratin 12 gene and detection of four mutations in Meesmann corneal epithelial dystrophy. *Am J Hum Genet* 1997;61:1268-75.
- 6 Fine BS, Yanoff M, Pitts E, et al. Meesmann's epithelial dystrophy of the cornea. *Am J Ophthalmol* 1977;83:633-42.
- 7 Kuwabara T, Ciccarelli EC. Meesmann's corneal dystrophy: a pathological study. *Arch Ophthalmol* 1964;71:676-82.
- 8 Stocker FW, Holt LB. Rare form of hereditary epithelial dystrophy. *AMA Arch Ophthalmol* 1955;53:536-41.
- 9 Tremblay M, Dubé I. Meesmann's corneal dystrophy: ultrastructural features. *Can J Ophthalmol* 1982;17:24-8.
- 10 Liu CY, Zhu G, Westerhausen LA, et al. Cornea-specific expression of K12 keratin during mouse development. *Curr Eye Res* 1993;12:963-74.
- 11 Moll R, Franke WW, Schiller DL, et al. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell* 1982;31:11-24.
- 12 Sun T-T, Eichner R, Schermer A, et al. In: Levine AJ, Vande Woude GF, Topp WC, et al, eds. *The transformed phenotype*. Vol 1. New York: Cold Spring Harbor Laboratory, 1984:169-76.
- 13 Wu R-L, Zhu G, Galvin S, et al. Lineage-specific and differentiation-dependent expression of K12 keratin in rabbit corneal/limbal epithelial cells: cDNA cloning and northern blot analysis. *Differentiation* 1994;55:137-44.
- 14 Rodrigues M, Ben-Zvi A, Krachmer A, et al. Suprabasal expression of a 65-kDa keratin (No 3) in developing human corneal epithelium. *Differentiation* 1987;34:60-7.
- 15 Schermer A, Galvin S, Sun T-T. Differentiation-related expression of a major 64K corneal keratin in vivo and in culture suggests limbal location of corneal epithelial stem cells. *J Cell Biol* 1986;103:49-62.
- 16 Kao WW-Y, Liu C-Y, Converse RL, et al. Keratin 12 deficient mice have fragile corneal epithelia. *Invest Ophthalmol Vis Sci* 1996;37:2572-84.
- 17 Corden LD, McLean WHI. Human keratin diseases: hereditary fragility of specific epithelial tissues. *Exp Dermatol* 1996;5:297-307.
- 18 Fuchs E, Cleveland DW. A structural scaffolding of intermediate filaments in health and disease. *Science* 1997;279:514-19.
- 19 Irvine AD, McLean WHI. Human keratin diseases: increasing spectrum of disease and subtlety of phenotype-genotype correlation. *Br J Dermatol* 1999;140:815-28.
- 20 Coulombe PA, Chan YM, Albers K, et al. Deletions in epidermal keratins leading to alterations in filament organization in vivo and in intermediate filament assembly in vitro. *J Cell Biol* 1990;111:3049-64.
- 21 Letai A, Coulombe PA, Fuchs E. Do the ends justify the means? Proline mutations at the ends of the keratin coiled-coil rod segment are more disruptive than internal mutations. *J Cell Biol* 1992;116:1181-95.
- 22 McLean WHI, Lane EB. Intermediate filaments in disease. *Curr Opin Cell Biol* 1995;7:118-25.
- 23 Coleman CM, Hannush S, Covello SP, et al. A novel mutation in the helix termination motif of keratin K12 in an American family with Meesmann's corneal dystrophy. *Am J Ophthalmol* 1999;128:687-91.
- 24 Corden LD, Swensson O, Swensson B, et al. Molecular genetics of Meesmann's corneal dystrophy: ancestral and novel mutations in K12 and sequence of the human K12 gene. *Exp Eye Res* 2000;70:41-9.
- 25 Thiel HJ, Behnke H. Über die Variationsbreite der hereditären Hornhautepitheldystrophie (Typ Meesmann-Wilke). *Ophthalmologica* 1968;155:81-6.
- 26 Conway JF, Parry DAD. Intermediate filament structure: 3. Analysis of sequence homologies. *Int J Biol Macromol* 1988;10:79-98.
- 27 Steinert PM. Structure, function and dynamics of keratin intermediate filaments. *J Invest Dermatol* 1993;100:729-34.
- 28 Steinert PM, Marekov LN, Fraser RDB, et al. Keratin intermediate filament structure: crosslinking studies yield quantitative information on molecular dimensions and mechanism of assembly. *J Mol Biol* 1993;230:436-52.
- 29 Wilson AK, Coulombe PA, Fuchs E. The roles of K5 and K14 head, tail and R/KLLEGE domains in keratin filament assembly in vitro. *J Cell Biol* 1992;119:401-14.
- 30 Serrano L, Sancho J, Hirshberg M, et al. Alpha-helix stability in proteins I. *J Molec Biol* 1992;227:544-59.
- 31 Letai A, Coulombe PA, McCormick MB, et al. Disease severity correlates with position of keratin point mutations in patients with epidermolysis bullosa simplex. *Proc Natl Acad Sci USA* 1993;90:3197-201.
- 32 Smith FJD, Del Monaco M, Steijlen PM, et al. Novel proline substitution mutations in keratin 16 in two cases of pachyonychia congenita type 1. *Br J Dermatol* 1999;141:1010-16.