

## MUTATION REPORT

# Meesmann Corneal Dystrophy (MECD): Report of 2 Families and a Novel Mutation in the Cornea Specific Keratin 12 (*KRT12*) Gene

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**Purpose:** Meesmann corneal dystrophy (MECD) is an autosomal dominant disorder affecting the corneal epithelium. It is caused by heterozygous mutations in *KRT3* or *KRT12* gene. Actually, 14 mutations have been reported, 1 in *KRT3* and 13 in *KRT12*. These genes were screened in several patients suffering from MECD. **Methods:** Patients from 2 families were screened for mutation in *KRT3* and *KRT12*. Exons were PCR-amplified and directly sequenced. The new mutation was checked by DHPLC in 51 control individuals of Swiss origin. **Results/Conclusions:** In one family, the M129T heterozygous mutation was observed in *KRT12*. In the second family, we identified a novel I426S heterozygous mutation in exon 6 of *KRT12*.

**Keywords** Corneal dystrophy; Meesmann; keratin 12; mutation

## INTRODUCTION

Meesmann epithelial corneal dystrophy (OMIM 122100; MECD) is an autosomal dominant disorder causing fragility of the anterior corneal epithelium and was initially described in a German family.<sup>1,2</sup> It manifests in childhood and usually affects both eyes. Clinically, the disease is characterized by a corneal epithelium with myriads of fine punctate opacities and intraepithelial vesicles, which can be visualized by retroillumination and slit lamp examination. Vision is only rarely impaired to a serious degree. Histologic examination reveals an irregularly thickened epithelium and vacuolated keratinocytes.

Keratin proteins are important for the structural integrity of the corneal epithelium. They are expressed specifically in the cytoplasm of epithelial cells where they form a dense meshwork of 10 nm intermediate filaments.<sup>3</sup> Keratins form obligate het-

eropolymers consisting of either type I (acidic, KRT9 to KRT21) or type II (basic, KRT1 to KRT8) intermediate filaments. Basically, keratins are composed of two parallel  $\alpha$ -helical segments. The expression of various keratin pairs is tissue specific, differentiation dependent and developmentally regulated. During cornea differentiation, the acidic keratin 12 (*KRT12*) pairs with the basic keratin 3 (*KRT3*) to become the major keratins in this tissue.<sup>4</sup>

MECD is due to heterozygous mutations in *KRT3* on chromosome 12 or in *KRT12* gene on chromosome 17.<sup>5–12</sup> In the literature, only 14 mutations have been reported so far, 13 on *KRT12* and 1 on *KRT3* (Table 1). All these mutations fall either in *KRT3* exon 7 or in *KRT12* exons 1 and 6. We report on two families, one showing a novel I426S mutation in *KRT12*, the other exhibiting the already known M129T mutation in *KRT12*.<sup>8</sup>

## MATERIALS AND METHODS

### Patients

The first patient belongs to a family from the USA and DNA from one member was referred to the laboratory of molecular

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TABLE 1  
List of reported mutations in Meesmann's epithelial corneal dystrophy

Exon	Nucleotide	Codon	Reference
<b>KRT3</b>			
7	1525G > A	E509K	5
<b>KRT12</b>			
1	410T > C	M129T	8
1	413A > C	Q130P	9
1	423T > G	N133K	10
1	427A > G	R135G	6
1	428G > T	R135I	6
1	428G > C	R135T	2, 8
1	429A > C	R135S	12
1	433G > C	A137P	11
1	443T > G	L140R	6
1	451G > C	V143L	5
6	1222ins27	400ins- 9 <sup>ISNLEAQLL</sup>	12
6	1300A > G	I426V	7
6	1301T > G	I426S	Novel mutation
6	4064T > G	Y429D	6

ophthalmology for mutation analysis. This patient was reported to have typical lesions of MECD. The second family, originating from Switzerland, included 12 individuals over 4 generations (Fig. 1). Several members were examined at the Jules Gonin Eye Hospital in Lausanne and blood samples were obtained from participating family members after informed consent. Affected members suffered from photophobia and the index patient had a best correct visual acuity of 0.8 in both eyes. A complete ophthalmologic evaluation, including slit lamp examination, was performed in these patients. Biomicroscopical examination, iris scatter micrographs and thin optical sections revealed typical microcystic intraepithelial lesions over the whole cornea (Fig. 2). The corneal surface was studded with numerous intraepithelial vesicles.

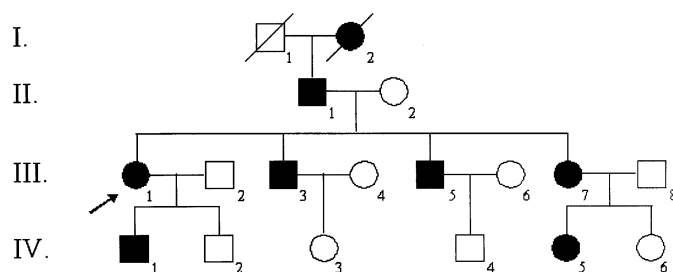


FIG. 1. Pedigree of the Swiss family studied, displaying typical autosomal dominant inheritance. Arrow indicates the proband.

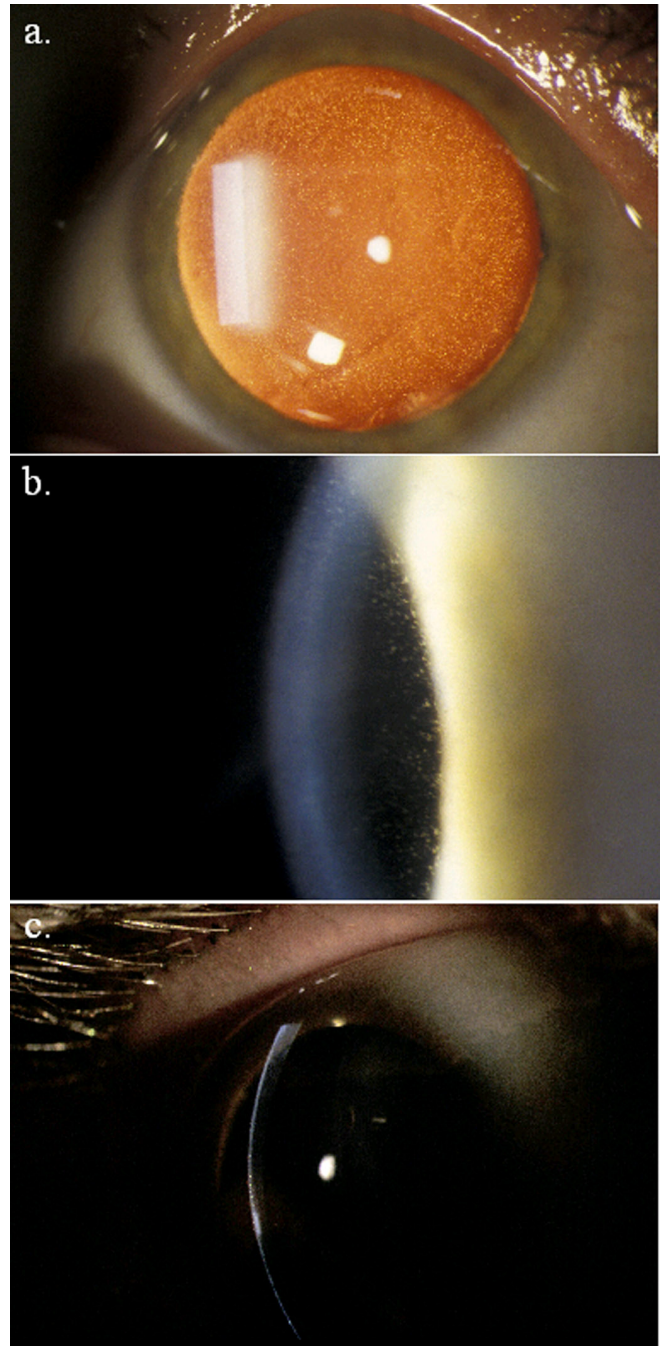


FIG. 2. Clinical photographs of the index patient (III-1) from the Swiss family. a. Biomicroscopy showing corneal intraepithelial microcysts over the whole cornea (retroillumination). b. Iris scatter. c. Thin optical section

#### Mutation Analysis

Genomic DNA was isolated from blood peripheral leukocytes by organic extraction (Nucleon; Amersham, Amersham, UK). Exon 7 of *KRT3* and exons 1 and 6 of *KRT12* were PCR-amplified using the following conditions: 1 cycle at 94°C

TABLE 2  
Sequence of the primers for exon 1 and 6 of *KRT12*. Exon 1 was amplified in 2 overlapping fragments

Primer set	Forward primer sequence	Reverse primer sequence
<i>KRT12</i> exon 1a	5'-AGTGAACCTTTTCAACTGCGA-3'	5'-TGCCCGAGAGAATACCTAGA-3'
<i>KRT12</i> exon 1b	5'-AGGACTGGGTGCTGGTTTG-3'	5'-CTGCAAGTACAGCTAAATTGGA-3'
<i>KRT12</i> exon 6	5'-GCTCGTGCGCAAACAGACGT-3'	5'-CCCAGGCATATCTTTACTAGA-3'

for 5 min, followed by 30 cycles (94°C 1 min, 60°C 1 min, 72°C 1 min) and by a last extension step at 72°C for 10 min. The primers used are listed in Table 2. The size of the products were 526 bp for *KRT3* exon 7, 450 bp for *KRT12* exon 1a, 447 bp for the exon 1b and 490 bp for the exon 6 of *KRT12*. The PCR products were directly sequenced on both strands on an ABI 310 using the Big Dye Terminator Labeling Kit, version 1.0. The new mutation was screened by denaturing high-performance liquid chromatography (DHPLC) (WAVE, Transgenomic, Cramlington, UK) in 51 control individuals from Swiss origin. Five  $\mu$ l of the PCR reactions were screened using the following conditions: T°: 65°C, %B: 58.4, running time: 5 min. A time shift of 2.5 min was applied.

## RESULTS

Sequencing of the PCR products spanning exon 1 of *KRT12* in the affected patient from the USA and comparison with the reference sequence (NCBI sequence: NM.000233.2) revealed a heterozygous nucleotide substitution 410T > C. This predicts the substitution of the methionine (ATG) residue by a threonine (ACG) at codon 129 (M129T). The mutation does not alter any known restriction enzyme site.

In the patients studied in the Swiss family, a T to G transversion at position 1301 of the *KRT12* cDNA was observed. This base change generated a missense mutation substituting Isoleucine (ATT) for Serine (AGT) at codon position 426 (I426S) of the cDNA (Fig. 3a–b). A second method using DHPLC was used to confirm the presence of the mutation in the *KRT12* gene (Fig. 3c). The I426S segregated in all available affected family members and was never observed in 51 control individuals.

## DISCUSSION

First identified by Pameijer and Meesmann and Wilke in three German families in 1935 and 1939,<sup>1,2</sup> MECD is characterized by a great number of fine punctate opacities in the epithelium and sometimes in Bowman's layer. This autosomal dominant disease is caused by heterozygous mutations in either *KRT3* or *KRT12*. *KRT3* is a 65 kDa protein encoded by 9 exons on chromosome 12q13. *KRT12* is located on chromosome 17q12 and is composed of 8 exons which encode a 494 amino acids 53.5 kDa protein. The two proteins are specifically expressed together in the cornea where they form a functional heterodimer.

These heterodimers are important for the stability of the corneal keratinocytes as was demonstrated by Kao et al.<sup>13</sup> in a *KRT12* knock-out mouse model. Therefore, any modification in these structures will be associated with increased fragility. This could explain why mutations in any of the members of the heterodimer lead to the same phenotype.

The I426S heterozygous mutation observed in the Swiss family is located in the helix termination motif of the *KRT12* protein, a site where many pathogenic mutations have been reported in both type I and type II keratins.<sup>14</sup> The heterozygous mutation found in the American patient (M129T) falls also in the helix initiation motif of *KRT12*. This region appears to be a hot spot for MECD mutation.

Until now, fourteen heterozygous mutations have been associated with MECD. All these mutations are located within the conserved helix boundary motifs of the *KRT3* and *KRT12* proteins. The helix initiation and termination motifs are highly conserved in the keratin proteins. They play an important role in the protein–protein interaction during assembly of intermediate filaments. Mutations in these regions are most likely to alter filament stability.<sup>15</sup>

The mutation identified in the American family has already been described by Corden et al.<sup>8</sup> in a same ethnic origin. We don't know if these patients are related. The I426S heterozygous mutation observed in the Swiss family has not been reported so far. As MECD is a rare disease in Switzerland, it would argue that this mutation is rather young. Investigation of the whole family is difficult: the grand-mother of the index patient is dead and no older relatives are available. It is well possible that this represents a private mutation.

Although the number of MECD families investigated and reported is rather low, 2 amino acids, Arg135 and Ile426, have been mutated more than once. Arg135 has been mutated to glycine, isoleucine, threonine or serine in many families. Usually, mutations in arginine occur because of the CpG sequence and known higher mutability of this dinucleotide due to the deamination of the 5'methyl-cytosine of the codon. This is not the case here as the arginine codon is AGA. Therefore, another mechanism has to be responsible for this high mutability.

Molecular investigation of families with MECD and further analysis of the *KRT12* knock-out mouse are important in order to establish a good genotype-phenotype correlation and to understand the mechanisms implicated in the development of the disease.

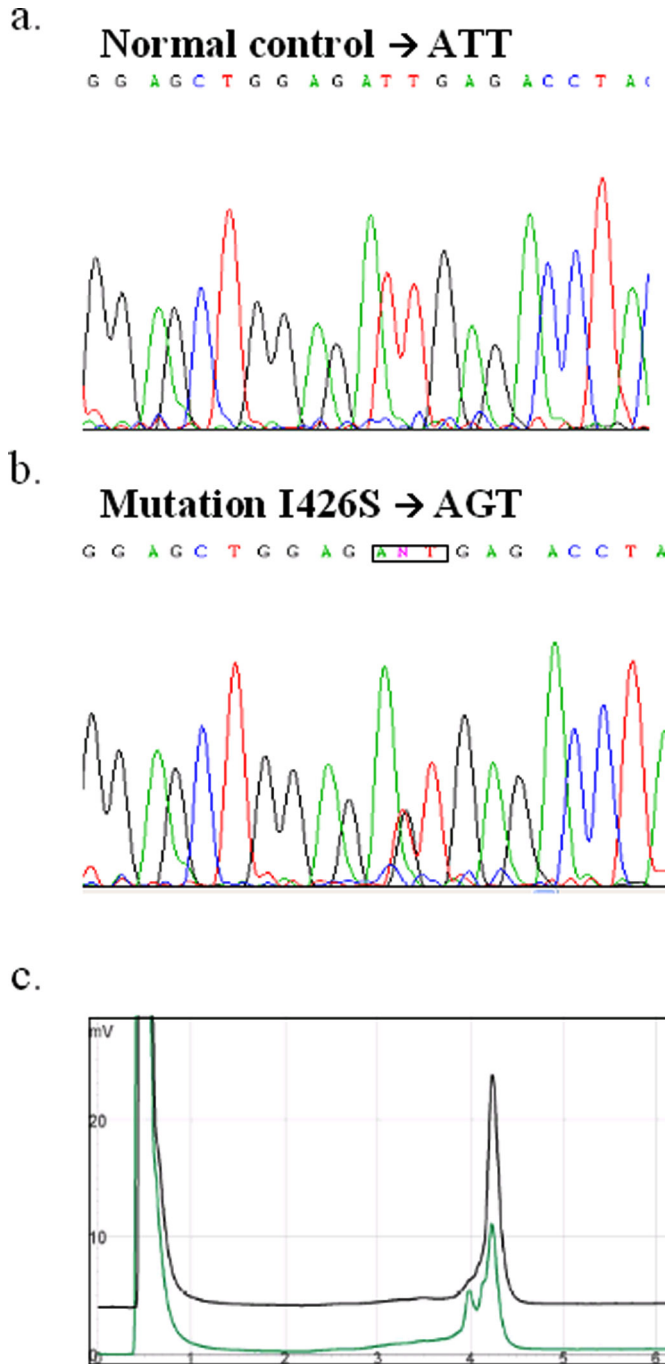


FIG. 3. Automated sequencing of KRT12 exon 6 amplified from the proband (III-1) and an unaffected Swiss control. a. Normal, unaffected individual showing nucleotide sequence around the region of codon 426. ATT encodes an Isoleucine at this position. b. Affected index patient (III-1) with MECD showing a 1301T > G (ATT > AGT) heterozygous mutation in exon 6 of KRT12. c. Confirmation of the I426S mutation by DHPLC. The profile of the unaffected control (black line) displayed one single peak, whereas several peaks, typical for a heterozygous mutation, are observed in patient III-1 (green line).

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