

Case Report

Identification of a Novel Missense *KRT12* Mutation in a Vietnamese Family with Meesmann Corneal Dystrophy

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Keywords

Corneal dystrophy · Meesmann corneal dystrophy · Corneal epithelial disease · *KRT12*

Abstract

Meesmann epithelial corneal dystrophy (MECD) is a rare dominantly inherited disorder that is characterized by corneal epithelial microcysts and is associated with mutations in the keratin 3 (*KRT3*) and keratin 12 (*KRT12*) genes. In this study, we report a novel mutation in the *KRT12* gene in a Vietnamese pedigree with MECD. Slit-lamp examination was performed on each of the 7 recruited members of a Vietnamese family to identify characteristic features of MECD. After informed consent was obtained from each individual, genomic DNA was isolated from saliva samples and screening of *KRT3* and *KRT12* genes was performed by Sanger sequencing. The proband, a 31-year-old man, complained of a 1-year history of eye irritation and photophobia. Slit-lamp examination revealed intraepithelial microcysts involving only the corneal periphery in each eye with clear central corneas and no stromal or endothelial involvement. Three family members demonstrated similar intraepithelial microcysts, but with diffuse involvement, extended from limbus to limbus. Sanger sequencing of *KRT3* (exon 7) and *KRT12* (exons 1 and 6) in the proband

revealed a novel heterozygous *KRT12* variant (c.1273G>A [p.Glu425Lys]) that was present in the three affected family members but was absent in the three family members with clear corneas. This study is the first report of a Vietnamese family affected with MECD, associated with an atypical peripheral corneal epithelial phenotype in the proband and a novel mutation in *KRT12*.

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Introduction

Meesmann epithelial corneal dystrophy (MECD; OMIM 122100) is a rare autosomal dominant inherited disorder of the corneal epithelium that is characterized by small round epithelial microcysts with onset as early as birth [1–3]. While MECD can be asymptomatic, affected individuals commonly experience ocular symptoms that include contact lens intolerance, foreign body sensation, lacrimation and photophobia. MECD is associated with mutations in the keratin 3 (*KRT3*) and keratin 12 (*KRT12*) genes, which encode cornea-specific keratins that form K3/K12 protein heterodimers responsible for imparting mechanical and structural integrity of the corneal epithelium. To date, 4 *KRT3* mutations and 24 *KRT12* mutations have been associated with MECD [3–23]. Each mutation is located in either the highly conserved helix initiation (located in exon 1 of *KRT3* and *KRT12*) or helix termination (located in exon 7 of *KRT3* and exon 6 of *KRT12*) motifs of the genes and is hypothesized to act in a dominant negative manner to cause MECD [3, 24]. *KRT3* and *KRT12* mutations have been identified in German, Chinese, Taiwanese, Japanese, Danish, Swiss, and American families affected with MECD but so far, there are no reports of mutations from Southeast Asia [4–7, 15, 17, 21, 22]. Here, we report a novel *KRT12* missense mutation in a three-generation Vietnamese family affected with MECD.

Case Presentation

A 31-year-old Vietnamese man (Fig. 1a, II-2) presented to the Vietnam National Eye Hospital with a 1-year history of recurrent episodes of ocular irritation and frequent photophobia. Uncorrected visual acuity measured 20/20 in each eye. Slit-lamp biomicroscopy revealed numerous small, clear, confluent intraepithelial cysts confined to the peripheral cornea of each eye, with a clear boundary between the involved and normal epithelium (Fig. 2a, b). The corneal stroma and endothelium were not involved, and the remaining of the eye examination was within normal limits. Slit-lamp biomicroscopy of the proband's 56-year-old mother (Fig. 1a, I-2), who was diagnosed with cataract in both eyes and had counting fingers at 1 m visual acuity in each eye, revealed diffuse intraepithelial cysts extending from limbus to limbus in each eye (Fig. 2c). The proband's 35-year-old brother (Fig. 1a, II-1), who denied ocular complaints and had uncorrected visual acuity of 20/20 in both eyes, presented with diffuse intraepithelial cysts extending from limbus to limbus in each cornea (Fig. 2d), similar to his mother. The proband's 4-year-old daughter (Fig. 1a, III-1), for whom visual acuity testing could not be performed, also demonstrated bilaterally diffusely distributed intraepithelial microcysts in both corneas.

Genetic Screening

Sanger sequencing of *KRT3* (exon 7) and *KRT12* (exons 1 and 6) in the proband (individual II:2) revealed a novel heterozygous c.1273G>A (p.Glu425Lys) variant in *KRT12* exon 6

(Fig. 2b). Screening of *KRT12* exon 6 in 6 family members demonstrated segregation of the c.1273G>A variant with the affected phenotype, with its identification in the heterozygous state in three affected family members (Fig. 1a, individuals I:2, II:1, and III:1) and its absence in 3 unaffected family members (Fig. 1a, I:1, II:3, and III:2).

Methods

DNA Isolation

Saliva samples were collected with the Oragene Saliva Collection Kit (DNA Genotek, Inc., Ottawa, ON, Canada). Genomic DNA was isolated using the Oragene prepIT-L2P Kit (DNA Genotek, Inc.) according to the manufacturer's instructions.

PCR Amplification and Sanger Sequencing of *KRT3* and *KRT12*

Exon 7 of *KRT3* (Refseq Gene ID: 3850) and exons 1 and 6 of *KRT12* (Refseq Gene ID: 3859), the exons in which presumed pathogenic mutations associated with MECD have been identified, were amplified using previously published primers and polymerase chain reaction (PCR) conditions [4]. Briefly, DNA amplification by PCR was performed with KAPA 2G Robust DNA polymerase (Kapa Biosystems, Wilmington, MA, USA) according to the manufacturer's recommendations using 25–40 ng of genomic DNA and 240 nM of each primer per 25 µL reaction. The PCR protocol consisted of an initial denaturing step at 95°C for 3 min, followed by 36 cycles of denaturing at 95°C for 30 s, annealing at 60°C for 30 s, and elongation at 72°C for 30 s. Sanger sequencing was performed by Laragen, Inc. (Culver City, CA, USA).

Discussion

MECD can range from being asymptomatic to presenting with recurrent erosions leading to ocular irritation, photophobia and diminished visual acuity [3]. Similar to the *TGFBI* dystrophies, a genotype-phenotype correlation has been described for MECD. However, while different mutations in *TGFBI* are associated with distinct morphologies and distribution of the dystrophic deposits, the different mutations in *KRT3* and *KRT12* are associated with indistinguishable appearance of the corneal epithelial microcysts, although the distribution may be either interpalpebral or diffuse and patients may be either symptomatic or asymptomatic, depending on the associated mutation. In addition, MECD has been associated with “clear zones,” devoid of microcysts, located in various regions of the corneal epithelium, including the central cornea, as in the proband that we report. Intrafamilial phenotypic variability in regards to the presence or absence of clear epithelial areas has also been reported in other families affected with MECD [17]. In such cases of intrafamilial phenotypic variability in which the proband demonstrates an uncommon manifestation of MECD, as in this case, molecular genetic analysis provides a definitive means of confirming or refuting the suspected clinical diagnosis.

The identified novel p.Glu425Lys mutation resides in the helix termination motif of keratin 12, which is consistent with previous studies that report MECD-associated *KRT12* mutations localized to either the helix initiation or helix termination motifs. Additionally, the phenotypic variability of MECD observed in this family is also consistent with previous reports, which suggest that factors other than the identified *KRT3* or *KRT12* mutation are responsible for the observed intra- and interfamilial phenotypic variation [10].

The Vietnamese family with MECD that we report is the first from South East Asia. Since the first case of MECD was reported in an 8-year-old Dutch boy 84 years ago [1], affected

individuals of other European (German and Swiss), American, and East Asian (Japanese, Chinese and Taiwanese) nationalities have been reported [2, 4, 7, 8, 14, 17, 18, 22, 25, 26]. The absence of reported MECD cases from South East Asia and other regions may be due to regional variations in the prevalence of MECD, reporting bias, or a combination of the two. Regional variation in the prevalence of other inherited corneal dystrophies is well recognized. For example, the prevalence of autosomal recessive dystrophies, such as congenital hereditary endothelial corneal dystrophy, is higher in regions where consanguineous marriages occur more commonly, such as in parts of the Middle East and India [27–30]. The prevalence of autosomal dominantly inherited corneal dystrophies also varies by region, with the prevalence of Fuchs endothelial corneal dystrophy, posterior polymorphous corneal dystrophy and granular corneal dystrophy type 2 reported to be higher in individuals from Northern Europe, Czech Republic and South Korea, respectively [25, 31–33]. However, it is likely that the geographic clustering of reports of MECD is due at least in part to the location of centers with more established research programs producing a higher number of publications. Therefore, it is important for developing countries such as Vietnam to report genotype-phenotype correlations, such as this, in order to be able to compare and contrast the molecular genetic basis of the corneal dystrophies across regions and nationalities and to gain insight into the variety of corneal dystrophies encountered worldwide.

Statement of Ethics

Informed written consent to participate in this study and permission to publish the results of the research were obtained from each family member (UCLA IRB # 11-000020).

Disclosure Statement

The authors declare that there are no conflicts of interest.

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Author Contributions

Pham Ngoc Dong, MD – Patient examination, collection of clinical data, preparation of the manuscript. Le Xuan Cung, MD – Patient examination, collection of clinical data, preparation of the manuscript. Tran Khanh Sam, MD – Patient examination, collection of clinical data, preparation of the manuscript. Do Thi Thuy Hang, MD – Patient examination, collection of clinical data, preparation of the manuscript. Doug D. Chung, PhD – Drafted and edited the manuscript; interpreted genetic screening results; prepared the figures. Turad A. Alkadi, MD – Edited the manuscript. Arjun Buckshey, MD – Performed genetic screening. Junwei Zhang – Performed

genetic screening. Alexa Kassels – Performed genetic screening. Anthony J. Aldave, MD – Edited the manuscript; interpreted the clinical findings and genetic screening results.

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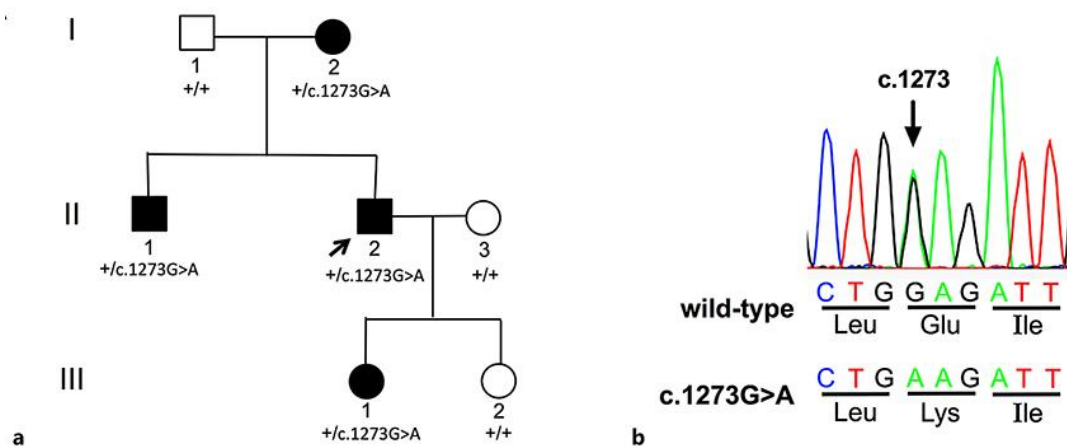


Fig. 1. **a** Pedigree of a Vietnamese family with Meesmann corneal dystrophy. Individuals heterozygous for the *KRT12* c.1273G>A variant are indicated by +/c.1273G>A and individuals who lack the mutation are indicated by +/+. The proband is indicated by a black arrow. **b** Sanger sequencing demonstrates that the *KRT12* c.1273G>A (p.Glu425Lys) variant is present in the proband and all affected family members.

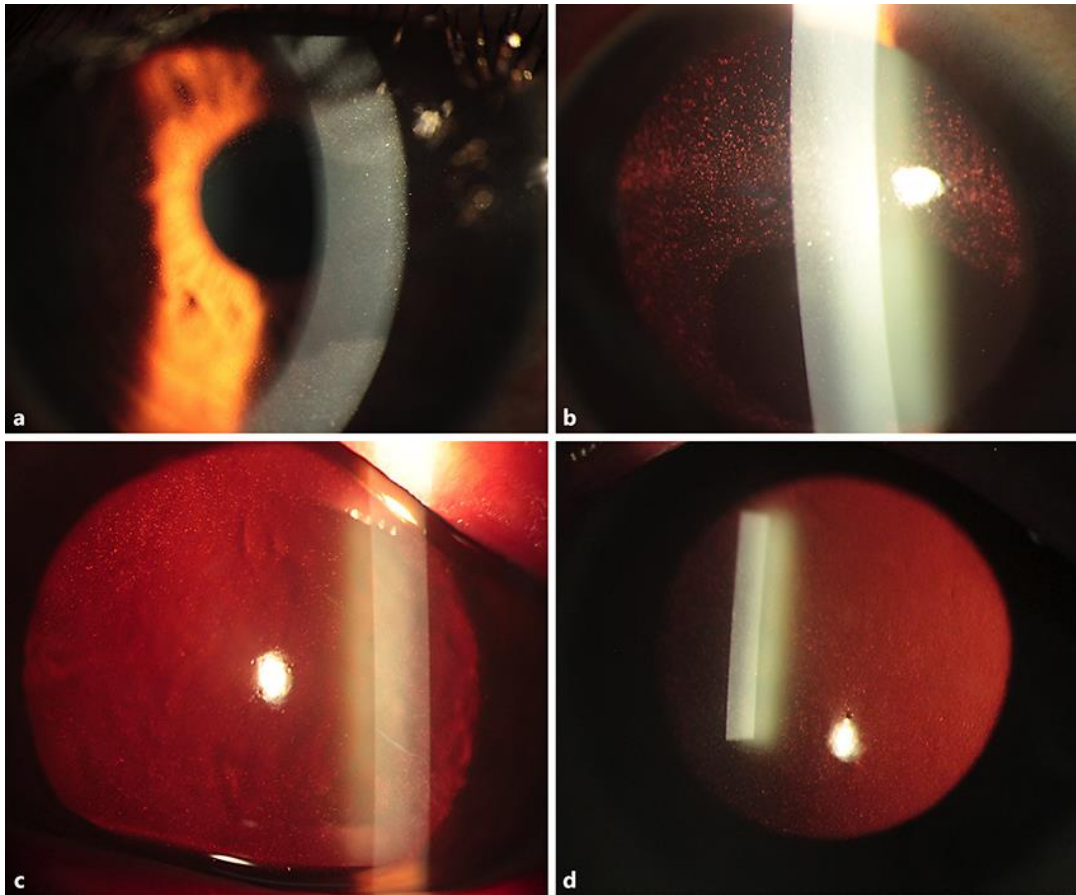


Fig. 2. Slit-lamp images of the 31-year-old proband's cornea, showing intraepithelial cysts in the periphery of the cornea sparing the central corneal epithelium, seen with both direct (**a**) and indirect (**b**) illumination. The proband's 56-year-old mother (**c**) and 35-year-old brother (**d**) demonstrated diffuse intraepithelial corneal microcysts on retro-illumination.