

RESEARCH ARTICLE

A Subset of Patients With Epithelial Basement Membrane Corneal Dystrophy Have Mutations in *TGFBI/BIGH3*

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Epithelial basement membrane corneal dystrophy (EBMD), also known as Cogan microcystic epithelial dystrophy or map-dot-fingerprint dystrophy, is a common bilateral epithelial dystrophy. Usually, this disease is not considered to be inherited although several families with autosomal dominant inheritance have been described. We report the analysis of two families with an autosomal dominant pattern of inheritance as well as the analysis of single affected individuals; we identified two different point mutations in the *TGFBI/BIGH3* genes, genes known to be associated with other corneal dystrophies. This is the first report of a molecular mutation in individuals with EBMD and it increases the spectrum of mutations in the *TGFBI/BIGH3* gene. Based on our screening, up to 10% of EBMD patients could have a mutation in this gene. *Hum Mutat* 27(6), 553–557, 2006. © 2006 Wiley-Liss, Inc.

KEY WORDS: corneal dystrophy; epithelial basement membrane corneal dystrophy; EBMD; Cogan dystrophy; *TGFBI*; *BIGH3*

INTRODUCTION

Epithelial basement membrane corneal dystrophy (EBMD), also known as Cogan microcystic epithelial dystrophy or map-dot-fingerprint dystrophy (MIM# 121820) is a common bilateral epithelial dystrophy. This disease was first described by Vogt [1930]. Later, Cogan et al. [1964] and Rodrigues et al. [1974] provided a thorough evaluation of the pathology. Sheet-like areas of basement membrane originating from the basal epithelial cells of the corneal epithelium and extending superficially into the epithelium are the hallmarks of EBMD. This condition represents by far the most common corneal dystrophy; it received its name from the characteristic appearance of slit lamp findings.

The pattern of clinical manifestations varies among patients. Most patients with basement membrane dystrophy are asymptomatic before the age of 30 years, but 10% of them may have recurrent idiopathic erosions and a loss of vision due to surface irregularity. Slit lamp examination may reveal dots, maps, grayish epithelial fingerprint lines, blebs, nets, or any combination of these patterns. Histological analysis shows abnormal redundant basement membrane and intraepithelial lacunae filled with cellular debris. There is usually no hereditary pattern, but some cases presented with an autosomal dominant inheritance [Laibson and Krachmer, 1975]. Age of onset may be younger (between 4 and 8 years of age) in familial cases, and the frequency of erosive attacks gradually decreases with age, being quite rare after the age of 50 years.

Mutations in *TGFBI/BIGH3* (MIM# 601692) cause various forms of corneal dystrophies, including lattice type I (CDLI; MIM# 122200), type IIIa (MIM# 608471), Groenouw granular type I (CDGGI; MIM# 121900), Reis-Bücklers' (CDRB; MIM# 608470), Thiel-Behnke (CDTB), Avellino (CDA; MIM# 607541), and polymorphic corneal amyloidosis [Munier et al., 1997; Yamamoto et al., 1998; Eifrig et al., 2004]. As some of these clinical entities are characterized by recurrent erosions with epithelial detachment and are notoriously heterogeneous, we considered the *TGFBI/BIGH3* gene as a potential candidate gene for Cogan corneal dystrophy, despite the fact that no mutation in this gene was observed in one single patient by Aldave et al. [2004].

PATIENTS AND METHODS

Familial and sporadic cases were identified in the cornea clinics of the Quinze-Vingts National Center of Ophthalmology, the

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Manchester Royal Eye Hospital, and the Jules Gonin Eye Hospital. After informed consent, blood samples were taken and genomic DNA was isolated by a standard phenol/chloroform extraction. All exons and adjacent intronic sequences of the TGFBI/BIGH3 gene were PCR amplified from genomic DNA with previously reported primers [Munier et al., 2002]. Amplicons were screened by DHPLC on a WAVE system (Transgenomic, Crewe, Cheshire, UK; www.transgenomic.com). Buffer A contained 0.1 M triethylammonium acetate (TEAA; Transgenomic). Buffer B contained 0.1 M TEAA (Transgenomic) and 25% acetonitrile HPLC grade (Sigma-Aldrich; www.sigmaaldrich.com). The flow rate was set at 0.9 ml/min and the Buffer B gradient was increased by 2% per min for 4 min. The optimum temperature was determined by the Wavemaker software (Transgenomic) for each DNA fragment. When multiple melting domains were established, each domain was analyzed at the appropriate temperature. Initial Buffer B concentration and temperature for each fragment are listed in Table 1. PCR products were purified using the QIAquick PCR purification kit (Qiagen; www.qiagen.com) or the Jet Quick system (Genomed, Löhne, Germany; www.genomed-dna.com). The PCR template was sequenced using ABI Dye Terminator (version 1

or 3; Applied Biosystems; www.appliedbiosystems.com) in a final reaction volume of 10 µl and run on a 3100 ABI genetic analyzer. Sequences were aligned using the Chromas chromatogram file editor (version 2.23; Technelysium; www.technelysium.com.au). The reference genomic sequence is available from NCBI (gi:4507466; NM_000358.1).

RESULTS

Family 1 is of French origin and, from its pedigree, autosomal dominant inheritance can be postulated (Fig. 1). The 59-year-old father (Patient III.2) presented with recurrent corneal erosion, from the age of 18 years. All laboratory examinations for corneal infection, including herpesviridae, were negative. Slit lamp examination (Fig. 2) revealed corneal maps. Corneal maps are irregularly shaped, faint gray-white patches between one and several millimeters in size. At the age of 51 years, visual acuity (VA) decreased to 20/100 for the right eye and 10/100 for the left eye. Both eyes were successfully treated by phototherapeutic keratectomy. The 26-year-old (Patient IV.1) and 24-year-old (Patient IV.2) daughters presented similar clinical features, with recurrent corneal erosions beginning at the age of 18 years. Maps were observed on slit lamp examination (Fig. 2B). Both daughters had normal VA (20/20 both eyes). The youngest daughter (Patient IV.3), who was 13 years old at the time of examination, suffered from spontaneous corneal erosions beginning at the age of 8 years. Slit lamp examination revealed EBMD with intraepithelial microcysts (Fig. 2C). The mother (Patient III.3) has never suffered from corneal disease and her eye examination was normal.

The second family is from Northern Ireland (Fig. 1). The proband was seen at 30 years of age with a 9-year history and symptoms of bilateral recurrent erosion syndrome. Examination revealed a visual acuity of 6/6 in the right eye and 6/5 in the left, Meibomian gland dysfunction and bilateral microcystic corneal epithelial changes in the absence of map-like defects. He was advised regarding lid hygiene and given ocular lubricants. This did not relieve his symptoms and required the use of a hydrogel bandage contact lens in the left eye. Eventually, this resulted in a diminution of the symptoms. One of his sisters was also suffering from recurrent corneal erosion. In view of these clinical findings and the family history, a provisional diagnosis of Cogan dystrophy was considered. Their father did not report any ocular symptoms.

TABLE 1. DHPLC Conditions for BIGH3

Exon	Temperature for each fragment (°C)	Initial Buffer B concentration (%) for each fragment
1	66.3; 67.3; 68.3	56.7
2	59.9	48.8
3	60.8; 61.8	54.1
4	62.9; 65.2	52
5	60.6; 62.3	53.4
6	61.4; 62	55.2
7	60.6; 62	59.5
8	61.7	58.7
9	58.9; 61	55.4
10	58; 59.5	51.1
11	60.8; 65	51.9
12	58.3; 62.1	55.2
13	59.2; 60.9; 64	54.7
14	51.6; 55.2; 62.4	53.4
15	59.5	56.2
16	59.1; 60.1	53.1
17	58.1	58.4

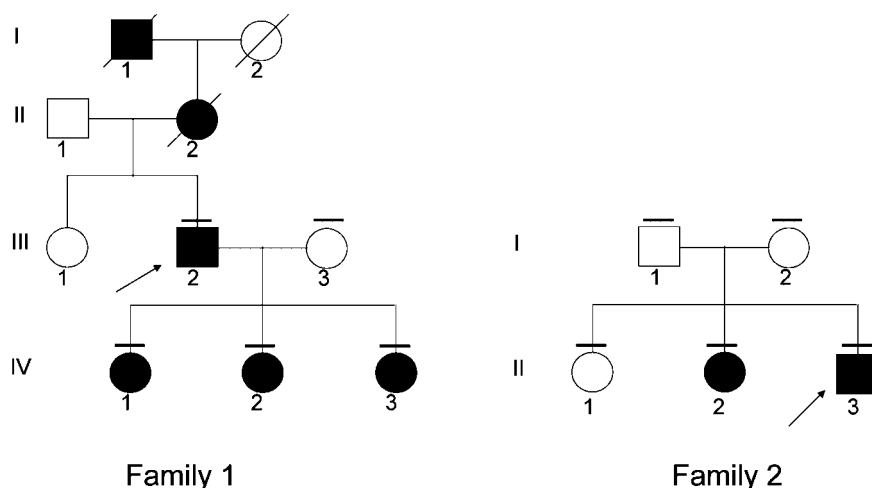


FIGURE 1. Pedigree of Families 1 and 2. Evaluated patients are indicated with a bar on top of the symbols.

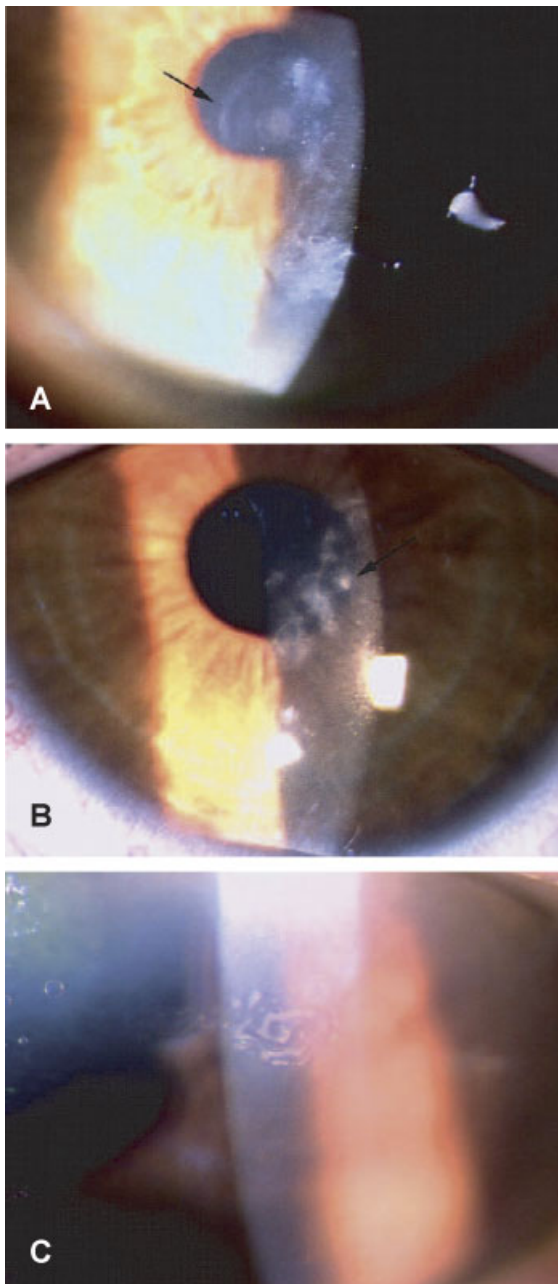


FIGURE 2. A,B: Slit lamp examination of Patient III.2; arrows point to map and dot. **C:** Fingerprint dystrophy in Patient IV.3.

Patient 3 was identified in a cohort of 30 sporadic cases and is of Swiss origin. He was diagnosed with the disease in his fourth decade. At the age of 39, he underwent excimer laser phototherapeutic keratectomy for recurrent corneal erosions in his right eye. The left eye was treated the following year.

In Family 1, TGFBI/BIGH3 mutation screening showed an abnormal DHPLC profile, which when sequenced revealed a heterozygous T to G base change at position 1526 (first nucleotide of ATG = +1) that modifies the leucine codon 509 to arginine (c.1526T>G). This L509R mutation cosegregated in all affected individuals and was not observed in unaffected individuals. In Family 2, the screening of TGFBI/BIGH3 showed a heterozygous G to C transversion at position 1998 (c.1998G>C), modifying the arginine codon at position 666 into serine (Fig. 3). This mutation

was transmitted by the unaffected father and was not present in the unaffected sister. The same c.1998G>C mutation was observed in the Swiss patient. His parents were not available for clinical examination and molecular testing. Both mutations were not observed in 96 control individuals or in more than 200 cases affected with various forms of corneal dystrophy sent to our laboratory for mutation screening. The screening of the rest of cohort with EBMD did not identify any mutation in the coding regions of TGFBI/BIGH3.

DISCUSSION

Mutations in the TGFBI/BIGH3 gene cause a growing number of corneal dystrophies which have been called “keratoepithelino-pathy” (KEP), based on keratoepithelin (KE), the protein encoded by this gene [Munier et al., 2005]. To the best of our knowledge, this is the first time that a mutation has been associated with Cogan dystrophy.

TGFBI/BIGH3 was first identified as a transforming growth factor (TGF)- β -inducible gene in a lung adenocarcinoma cell line [Skonier et al., 1992]. The cDNA sequence encodes KE, a protein of 68 kDa molecular mass, consisting of four regions of internal homology of approximately 140 amino acids and a carboxy terminal Arg-Gly-Asp (RGD) motif, which in other proteins mediates binding to some integrins [Arnaout et al., 2002]. The repeated regions are known as FAS1 domains, reflecting the presence of four such domains in the insect cell adhesion molecule fasciclin I. KE is secreted to the extracellular matrix (ECM).

It appears that TGFBI/BIGH3 plays a role in corneal development and healing and has additional functions in other tissues [Skonier et al., 1994; Dieudonne et al., 1999; Kim et al., 2000a]. Biochemical studies suggest that TGFBI/BIGH3 is a component of the extracellular matrix, where it binds to fibrinogen and various forms of collagen (I, II, IV, and VI) [Hashimoto et al., 1997; Rawe et al., 1997; Billings et al., 2002] to support cell adhesion and spreading [LeBaron et al., 1995; Ohno et al., 1999]. The expression pattern in developing bovine tissues resembles that of type VI collagen [Gibson et al., 1997]. In the mouse embryo, it is mainly observed in the mesenchyme of many organs [Schorderet et al., 2000] and in the skin, especially the papillary dermis [LeBaron et al., 1995]. In another study using human corneal epithelial cells (HCE) and site-directed mutagenesis, Kim et al. [2000b] showed that TGFBI/BIGH3 mediates HCE cell adhesion through $\alpha 3\beta 1$ integrin and that a conserved Asp-Ile (DI) motif in the second or the fourth FAS1 domain was sufficient to mediate cell adhesion. This motif is also instrumental in transmitting apoptotic signals when various plasmid constructs containing disease-causing mutations of KE were overexpressed in HeLa or HCE cells [Morand et al., 2003]. Yet another integrin, $\alpha v\beta 5$, has been implicated in the adhesion of fibroblasts to TGFBI/BIGH3. All four FAS1 domains were independently capable of mediating cell adhesion and mutation of two amino acid residues; an adjacent conserved tyrosine and histidine abolished integrin binding [Kim et al., 2000b].

There are minor discrepancies in the definition of the fasciclin domain depending on the software used. The Protein families database of alignments (Pfam) and the Smart program located the FAS domain I at 107–230 and 131–231; domain II at 244–365 and 268–366; domain III at 379–492 and 403–493, and domain IV at 506–626 and 530–627, respectively. Skonier et al. [1992], in their initial publication, have even defined different boundaries for the FAS1 domains. With the exception of amino acid 124, all

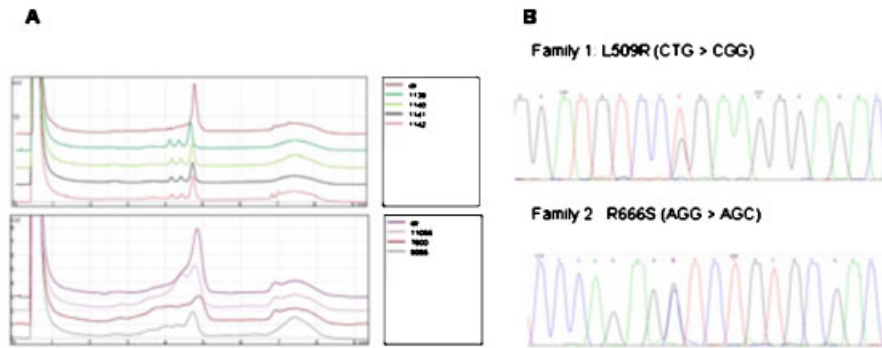


FIGURE 3. **A:** DHPLC retention curves from a control individual (ctr) and the four affected patients from Family 1 (top); retention curves from controls and individuals from Family 2 (bottom). ctr, control individual without SNP; 11055, affected individual without SNP; 7600, control individual with SNP; 9065, affected individual with SNP. **B:** Electropherograms of two affected patients showing the c.1526T > G (p.L509R) (top) and c.1998G > C (p.R666S) (bottom) mutations.

mutations described so far in KEPs have been localized to the fourth FAS domain. While L509R maps inside this last domain or very close to it, depending on the software used, and would therefore follow this rule, R666S is clearly located outside of the repeat, toward the C-terminus.

There is a body of evidence suggesting a pathologic role for the two mutations described in this report. First, the base changes described have never been observed in more than 300 control individuals and patients with corneal dystrophies and the L509R mutation segregates with the disease in the French family. Second, the L509 residue is conserved in the mouse, pig, and many other TGFBI/BIGH3 precursors. It is also conserved in the mouse and human periostin (PN), the rat and human stabilin 2, and the fasciclin I genes. Third, leucine is hydrophobic and has a bulky side chain that, together with aspartic acid and Isoleucine, is known to be important for interacting with integrins. Three other leucine residues (L518, L527, and L569) have been mutated to arginine in families with various forms of lattice corneal dystrophies [Munier et al., 2002; Fujiki et al., 1998; Warren et al., 2003]. The relationship between these four amino acids is not straightforward and it is not currently known whether they interact through integrin binding or through another mechanism. R666 is not well conserved among FAS1-related proteins. This is not surprising, as R666 lies outside of the fourth FAS1 domain. However, in mouse and rabbit *Tgfb1/Bigh3*, R666 is conserved. Therefore, this amino acid may have been selected for a function that is specific to TGFBI/BIGH3 but different from the FAS1 function. The role of the C-terminal part of TGFBI/BIGH3 is not well understood. It is possible that mutation in this region could induce a tertiary structure modification or a misfolding of the protein.

The proportion of Cogan dystrophies due to mutations in TGFBI/BIGH3 genes is difficult to evaluate. The diagnosis of EBMD is not easy because slit lamp corneal changes may be very mild and histologic and immunohistologic analyses are usually not performed. Nevertheless, it is probable that only a subset of EBMD is induced by TGFBI/BIGH3 mutations. Based on our mutation screening, this subset could represent as much as 10% of all EBMD. In addition, Family 2 shows reduced penetrance, as Patient I.1 is clearly a carrier of the mutation and is not affected. Investigation of additional familial and sporadic cases with Cogan corneal dystrophy will tell whether this estimation is correct. The rest of EBMD may be due to nongenetic causes or to mutations in other genes. Genes that are expressed in the cornea and have adhesion function would represent good candidates.

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