A novel mutation I522N within the TGFBI gene caused lattice corneal dystrophy I

Chunmei Zhang, Guang Zeng, Hui Lin, Dandan Li, Liming Zhao, Nan Zhou, Yanhua Qi

Department of Ophthalmology, Harbin Medical University the 2nd Affiliated Hospital, Harbin, China

**Purpose:** To identify mutations within the TGFBI gene in a Chinese family with lattice corneal dystrophy type I (LCD I).

**Methods:** Genomic DNA of three affected, four unaffected family members and 50 normal individuals was extracted from peripheral leukocytes. All exons of TGFBI were amplified by polymerase chain reaction (PCR) methods and direct sequencing was carried out for mutation analysis.

**Results:** A missense mutation (1565T→A) in exon12 of TGFBI led to an amino acid substitution I522N in the TGFB-induced protein in all affected family members, but the mutation was not detected in normal subjects of the family and control individuals.

**Conclusions:** We conclude that the novel mutation I522N causes lattice corneal dystrophy type I in the studied family. This is the first report of the I522N mutation within TGFBI in LCD I worldwide.

Corneal dystrophies are hereditary diseases characterized by corneal opacities on different layers of the cornea. Lattice corneal dystrophy is known as an autosomal dominant disease. Histological examination of corneal specimens shows amyloid deposits. The typical clinical appearance of LCD I (LCD I; OMIM 122200) is characterized by subepithelial and stromal branching lattice lines. Generally, the clinical symptoms become evident in the patient’s first or second decade, with the appearance of white-grayish opacities in the superficial stromal layer of the cornea. Thereafter, the lesions tend to become larger, aggregate, and extend deeper and toward the periphery over time.

The transforming growth factor-beta-induced gene (TGFBI) is located on the human chromosome 5q31 [1]. Münier et al. [1] reported that mutations in TGFBI caused lattice corneal dystrophy type I. To date, the mutations reported in TGFBI as the causation of LCD type I include R124C, V505D, L518P, A546D, P551Q, L569R, H572R, and V625D [2-4]. The following mutations in TGFBI causing LCDI have been identified in Chinese families: R124C, V625D, and V505D [5,6]. In this study, we described a novel mutation I522N in TGFBI inducing LCDI in a Chinese family. None of the previously reported mutations in the TGFBI gene were found in this family.

**METHODS**

**Patients:** This study was approved by the Institutional Review Board of Harbin Medical University (Harbin, China). Four affected and six unaffected individuals from a Chinese family (Figure 1) were enrolled in this study after obtaining informed consent. Fifty unrelated healthy individuals were selected as the control group and they were all Chinese. All subjects, including control individuals, underwent clinical ophthalmologic examination and slit lamp photographs of affected eyes were taken.

**Molecular genetic analysis:** Peripheral blood (5 ml) was taken from patients, unaffected family members, and 50 healthy controls. Genomic DNA was extracted from peripheral leukocytes, according to the manufacturer's (Tiangen Biltech Co. Ltd, Beijing, China) standard methods. All 17 exons of TGFBI were amplified by polymerase chain reaction (PCR) using the primers described previously [7]. PCRs were

![Figure 1. Pedigree analysis and pedigree symbols. The squares indicate males and the circles indicate females. A filled symbol indicates a person affected with LCD I. The arrow indicates the proband.](http://www.molvis.org/molvis/v15/a267)
performed in a 50 μl volume containing 10× PCR buffer, 10–200 ng of genomic DNA, 0.2 mM of each dNTP, 1 unit of Taq polymerase, and 1 μl of 1 mM forward and reverse primers. The primer annealing temperature was adjusted separately for each PCR reaction, which was based on those described by Li et al. [7]. After pre-denaturation at 95 °C for 5 min, DNA fragments were amplified for 35 cycles of denaturation, annealing, and extension, followed by a final extension step at 72 °C for 10 min. PCR products were analyzed in 2% agarose gel, from which the bands with the amplified templates were examined and subsequently purified with a TIANgel Midi Purification Kit (Tiangen Biotech Co. Ltd) and sequenced with an ABI BigDye Terminator Cycle Sequencing kit v3.1 (ABI Applied Biosystems, Foster City, CA).

Nucleotide sequences of PCR products were manually compared with NCBI TGFBI Gene Reference Sequences (NM_000358.2).

RESULTS

Clinical findings: The common feature of corneal lesion in the pedigree was that the onset of the disease occurred in adulthood, approximately in the second decade of life, and is characterized by ocular pain, photophobia, and progressive visual defect. Clinical data of patients from the family were shown in Table1. Slit lamp examination revealed typical symmetric branching lattice lines in the central anterior stroma of the proband. The proband's mother showed new corneal vessels with recurrent corneal erosion and multiple

| Individual case | Age | Age at onset | Visual acuity at presentation |
|------------------|-----|--------------|-----------------------------|
| III-1            | 16  | 12           | OD 0.3, OS 0.4              |
| II-6             | 38  | 13           | OD 0.06, OS 0.12           |
| II-1             | 46  | 15           | OD HM/50 cm, OS HM/30 cm    |
| II-2             | 43  | 13           | OD 0.1, OS HM/50 cm        |

The Snellen visual acuity scale was used in these studies. In the table, HM indicates hand movement.
thick subepithelial, stromal lattice opacification (Figure 2). All of the affected individuals were referred to appropriate specialists for a work-up for systemic amyloidosis. However, no features of this discovery were detected.

**Gene analysis:** Exon 12 of TGFBI of the affected and unaffected individuals was analyzed by direct sequencing (Figure 3). The sequence of the patients revealed a heterozygous T→A mutation at nucleotide 1565. This nucleotide substitution resulted in an amino acid replacement from isoleucine (ATC) to asparagine (AAC) at codon 522 (I522N). The reverse sequence of the mutation showed a corresponding base replacement A>T, which further confirmed the mutation. No other non-pathogenic SNPs were observed in the other remaining TGFBI exons of the family. The homologous sequences within the TGFBI protein from diverse species were listed in Figure 4. The alignment shows that the mutation I522N is located at a highly conserved site.

**DISCUSSION**

In 1992, TGFBI (BIGH3) was first isolated by Skonier et al. [8] in a study of genes induced by the transforming growth factor-β (TGFβ) in a human adenocarcinoma cell line derived from the lung. TGFBI (BIGH3) was found to be expressed in the corneal epithelium [9]. So far, all the pathogenic mutations reported on TGFBI are only related to corneal dystrophies, which are characterized by progressive accumulation of deposits in the cornea [10]. It has been shown that corneal deposits are made of the mutant KE protein [11]. The genetic studies revealed specific missense mutations in TGFBI, causing diverse hereditary corneal dystrophies. The most frequent types of these dystrophies result from mutations at two hot spots, which are localized at codons Arg124 and Arg555 [12].

The TGFBI gene product is a secreted protein of 683 amino acids, consisting of four regions of internal homology of approximately 140 amino acids and a carboxy terminal Arg-Gly-Asp (RGD) sequence. The tandem repeated regions are known as FAS1 domains. These four FAS1 domains correspond to amino acids 134-236 (FAS1-1), 242-372 (FAS1-2), 373-501 (FAS1-3), and 502-632 (FAS1-4) [13]. All the mutations of TGFBI that cause corneal dystrophy are located in FAS1 domain 4, with the exception of Arg124 [13]. Clout et al. [14] made a homology model of the FAS1 domain 4 of TGFBI protein, which suggested the common mutations of TGFBI at positions 124 and 555 are likely to affect protein-protein interactions directly, whereas the rare mutations are likely to cause misfolding of the protein within the cell.

The mutation described in this study is located in residue 522 and in the fourth FAS1 as well. This residue is conserved among several species and thought of as a distinct functional and/or structural sequence of the protein induced by TGFβ gene. According to the model of Clout et al. [14], Ile522 is a hydrophobic residue and situated in helix α1. The mutation alters highly conserved residue and leads to the substitution of hydrophilic residue for hydrophobic residue. The nucleotide replacement may alter the protein's physicochemical property and affect proper folding of the protein within the cell, which results in abnormal corneal deposits and corneal dystrophy.
To date, five types of LCD have been established according to the stromal location of deposits, the characteristics of lattice lines, and the age of onset. The five types are I, II, III, IIIA, and IV [15]. Only LCD type II is caused by mutations in the GSN gene, while the others are all linked to mutant TGFBI and different phenotypes resulting from distinct mutation points in the same gene. In addition, several atypical forms of LCD, which cannot be classified as LCD I, II, III, IIIA, and IV, have been observed to be associated with various TGFBI mutations [16-18]. Genetic testing of TGFBI mutations in LCD patients will contribute to the clinical classification, correct diagnosis, prevention and treatment level of hereditary disease. For those individuals, genetic testing at an early age may improve clinical outcomes by encouraging frequent ocular examination and thus laying a solid foundation for genetic therapy.

This is the fourth genetic mutation reported in association with the classic type I of LCD. The novel gene mutation expands the mutation spectrum of TGFBI and contributes to the study of molecular pathogenesis of corneal dystrophy.

ACKNOWLEDGMENTS

The authors are grateful to all patients, the family, and normal volunteers for their participation in this investigation. This research was supported by the Young Scientists Fund of National Natural Science Fund of China (30801274).

REFERENCES

1. Munier FL, Korvatska E, Djemai A, Paslier DL, Zografos L, Pescia G, Schorderet DF. Kerato-epithelin mutations in four 5q31-linked corneal dystrophies. Nat Genet 1997; 15:247-51. [PMID: 9054935]
2. Kannabiran C, Klintworth GK. TGFBI gene mutations in corneal dystrophies. Hum Mutat 2006; 27:615-25. [PMID: 16683255]
3. Atchaneeyasakul LO, Appukuttan B, Pingsuthiwong S, Yenchitsomanus PT, Tinavarat A, Srisawat C. A novel H572R mutation in the transforming growth factor-beta-induced gene in a Thai family with lattice corneal dystrophy type I. Jpn J Ophthalmol 2006; 50:403-8. [PMID: 17013691]
4. Tian X, Fujiki K, Zhang Y, Murakami A, Li Q, Kanai A, Wang W, Hao Y, Ma Z. A novel variant lattice corneal dystrophy caused by association of mutation (V625D) in TGFBI gene. Am J Ophthalmol 2007; 144:473-5. [PMID: 17765440]
5. Dong WL, Zou LH, Pan ZQ, Jin T, Yu J. Molecular genetic study on patients with lattice corneal dystrophy in China. Zhonghua Yan Ke Za Zhi 2005; 41:523-6. [PMID: 16608913]
6. Tian X, Fujiki K, Wang W, Murakami A, Xie P, Kanai A, Liu Z. A novel mutation (V505D) of TGFBI gene found in a Chinese family with lattice corneal dystrophy type I. Jpn J Ophthalmol 2005; 49:84-8. [PMID: 15838722]
7. Li D, Qi YH, Wang L, Lin H, Zhou N, Zhao L. An atypical phenotype of Reis-Bucklers corneal dystrophy caused by the G623D mutation in TGFBI. Mol Vis 2008; 14:1298-302. [PMID: 18636123]
8. Skonier J, Neubauer M, Madisen L, Bennett K, Plowman GD, Purchio AF. cDNA cloning and sequence analysis of beta ig-h3, a novel gene induced in a human adenocarcinoma cell line after treatment with transforming growth factor-beta. DNA Cell Biol 1992; 11:511-22. [PMID: 1388724]
9. Escribano J, Hernando N, Ghosh S, Crabb J, Coca-Prados M. cDNA from humanocular ciliary epithelium homologous to big-h3 is preferentially expressed as an extracellular protein in the corneal epithelium. J Cell Physiol 1994; 160:511-21. [PMID: 8077289]
10. El Kochairi I, Letovanec I, Uffer S, Munier FL, Chaubert P, Schorderet DF. Systemic investigation of keratoepithelin deposits in TGFBI/BIGH3-related corneal dystrophy. Mol Vis 2006; 12:461-6. [PMID: 16710170]
11. Korvatska E, Munier FL, Chaubert P, Wang MX, Mashima Y, Yamada M, Uffer S, Zografos L, Schorderet DF. On the role of kerato-epithelin in the pathogenesis of 5q31-linked corneal dystrophies. Invest Ophthalmol Vis Sci 1999; 40:2213-9. [PMID: 10476785]
12. Korvatska E, Munier FL, Djemai A, Wang MX, Frueh B, Chiou AG, Uffer S, Ballestrazzi E, Braunstein RE, Forster RK, Culbertson WW, Boman H, Zografos L, Schorderet DF. Mutation hot spots in 5q31-linked corneal dystrophies. Am J Hum Genet 1998; 62:320-4. [PMID: 9463327]
13. Munier FL, Frueh BE, Othenin-Girard P, Uffer S, Cousin P, Wang MX, Heon E, Black GC, Blasi MA, Ballestrazzi E, Lorenz B, Escoto R, Barraquer R, Hoeltzenthin M, Gloor B, Fossarello M, Singh AD, Arsenijevic Y, Zografos L, Schorderet DF. BIGH3 mutation spectrum in corneal dystrophies. Invest Ophthalmol Vis Sci 2002; 43:949-54. [PMID: 11923233]
14. Clout NJ, Hohenester E. A model of FAS1 domain 4 of the corneal protein beta(ig)-h3 gives a clearer view on corneal dystrophies. Mol Vis 2003; 9:440-8. [PMID: 14502125]

15. Klintworth GK. Advances in the molecular genetics of corneal dystrophies. Am J Ophthalmol 1999; 128:747-54. [PMID: 10612512]

16. Chau HM, Ha NT, Cung LX, Thanh TK, Fujiki K, Murakami A, Kanai A. H626R and R124C mutations of the TGFBI (BIGH3) gene caused lattice corneal dystrophy in Vietnamese people. Br J Ophthalmol 2003; 87:686-9. [PMID: 12770961]

17. Schmitt-Bernard CF, Guittard C, Arnaud B, Demaille J, Argiles A, Claustres M, Tuffery-Giraud S. BIGH3 exon 14 mutations lead to intermediate type I/IIIA of lattice corneal dystrophies. Invest Ophthalmol Vis Sci 2000; 41:1302-8. [PMID: 10798644]

18. Afshari NA, Bahadur RP, Elfrig DE Jr, Thoqersen IB, Enquild JJ, Klintworth GK. Atypical asymmetric lattice corneal dystrophy associated with a novel homozygous mutation (Val624Met) in the TGFBI gene. Mol Vis 2008; 14:495-9. [PMID: 18385782]