

***TGFBI* gene mutations analysis in Chinese families with corneal dystrophies**

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Abstract. The aim of the present study was to examine the clinical features of three Chinese families with autosomal dominant corneal dystrophy (CD) and examine transforming growth factor- β -induced (*TGFBI*) gene mutations in these families. The *TGFBI* gene mutations were detected using direct sequencing of the whole coding regions and exon-intron boundaries of the *TGFBI* gene in the affected members from the three families with CD. The phenotypes of all affected individuals in the three families were observed via slit lamp examination. Sections of the cornea were used for biopsy following keratoplasty. Three types of *TGFBI* gene mutations, R124C, H626R and R124H, were detected in the patients from these three families. One family, with the R124C mutation, was diagnosed with lattice corneal dystrophy type 1, and the family with the H626R mutation was diagnosed with lattice corneal dystrophy type IIIB. The family with the R124H mutation was diagnosed with granular corneal dystrophy type 2. The *TGFBI* gene mutations were considered underlying factors in the molecular mechanism underlying the pathogenesis of cornea dystrophy. Therefore, the detection of *TGFBI* gene mutations may be useful in the differential diagnosis of CD.

Introduction

Corneal dystrophies (CDs) are a group of heterogeneous inherited diseases with non-inflammatory, bilaterally progressive corneal opacities, which often lead to recurrent corneal erosion and visual impairment. According to the affected layer in the cornea, CDs can be categorized as epithelial, subepithelial, Bowman's layer, stromal or endothelial using slit-lamp imaging, confocal microscopy

or histological staining. However, it has been found that the clinical classifications of CDs based entirely on ophthalmological and histopathologic examinations are limited in scope and effectiveness (1).

Advances in molecular genetics have mapped CDs to eleven chromosomes: 1, 2, 5, 9, 10, 12, 13, 16, 17, 20 and X (2). Several genes, including the transforming growth factor β -induced (*TGFBI*) gene (OMIM 601692), carbohydrate sulfotransferase 6 gene, gelsolin gene, keratin 3 gene, keratin 12 gene and surface marker 1 gene, have so far been identified as responsible for CDs (3,4). Among these, *TGFBI*-associated CDs have provided the most reliable evidence. To date, at least five autosomal dominant CDs have been confirmed to be associated with mutations in the *TGFBI* gene: i) Reis-Bücklers CD (RBCD), ii) lattice CD type 1 (LCDI), iii) Thiel-Behnke CD, iv) granular CD type 1 (GCD1) and v) GCD2 (5,6).

As CD has significant genetic heterogeneity, different *TGFBI* gene mutations can lead to the same phenotype, for example, R124C, V625D, V505D, H626R and other mutations are associated with LCDI (7-9); whereas the same *TGFBI* mutation in a different ethnic population can exhibit different clinical phenotypes, for example, the R124C mutation is responsible for GCD2, LCDI and RBCD (10-12). Due to the complexity of phenotypic and genetic heterogeneity, the ability of ophthalmologists and geneticists to correctly diagnose and classify CDs is a challenge, as is understanding their phenotype-genotype aspects. In the present study, three Chinese families affected by different types of CD were recruited, and the gene mutations responsible for the disease were identified. The results aimed to provide an insight into the clinical-molecular correlations of the disease.

Materials and methods

Patients and subjects. In the present study, three Chinese pedigrees comprising individuals diagnosed with LCDI, LCD IIIB and GCD2, respectively, were recruited from the Tianjin Eye Hospital (Tianjin, China) between August 2013 and July 2014. The study was approved by the Ethics Committee of Tianjin Eye Hospital. Informed, written consent was obtained from each of the participants in accordance with the Declaration of Helsinki prior to the collection of their peripheral blood. The participating members underwent careful ophthalmic examination, including vision tests, slit-lamp biomicro-examination

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and anterior segment imaging. In addition, 100 unrelated subjects without CD were recruited from the Tianjin Eye Hospital as normal controls for the present study.

Genetic analysis. Genomic DNA was extracted from the peripheral blood samples using a genomic DNA miniprep kit for blood (Roche Diagnostics, Indianapolis, IN, USA). DNA integrity was evaluated using 1% agarose gel electrophoresis. The DNA fragments, which encoded regions of the *TGFBI* gene, were amplified using polymerase chain reaction (PCR). The sequences of the primers were designed according to a previous study by Stix *et al* (13) (Table I). Standard PCR reactions were preformed in a 50 μ l reaction mixture including 1 μ l of 10X PCR buffer, 50 ng template DNA, 0.1 μ M of each of the forward and reverse primers, 300 μ M dNTP, 5 mM MgCl₂ and 0.3U Hotstart Taq. The PCR program used for DNA amplification was as follows: 95°C for 3 min; followed by 20 cycles at 95°C for 30 sec, 53–64°C for 30 sec, and 72°C for 45 sec; additional 20 cycles at 95°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec; and a final extension at 72°C for 7 min, using the Gene Amp PCR 9700 system (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Each PCR fragment was purified with a TIANGel Midi DNA Purification kit (Tiangen Biotec Co., Ltd, Beijing, China), and the two strands were subsequently sequenced on an ABI 3130 automated DNA sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc.). The sequence results were compared with the wild-type *TGFBI* sequence (GenBank NG_012646.1; https://www.ncbi.nlm.nih.gov/nuccore/NG_012646.1). The other family members and 100 Chinese control individuals were also screened for the presence of *TGFBI* mutations by PCR amplification and direct sequencing of PCR products.

Histopathological examination. The corneal specimens obtained using penetrating keratoplasty were processed for examination using light microscopy. The tissues were fixed in 10% formalin and embedded in paraffin. Cross sections of each button were prepared by free-hand sectioning at a thickness of ~10 μ m and were stained with Congo red.

Results

Family with LCDI. In the first family, comprising three generations, four affected members and five unaffected members were examined (Fig. 1A). The symptoms of all patients began with episodes of acute ocular pain, redness and photophobia at ~20 years of age. Slit-lamp examination revealed dots and fine lattice lines in the anterior corneal stroma (Fig. 1B). The small lattice-shaped opacity is a typical clinical feature LCDI, which can be observed using direct and retroillumination. The mutation analysis showed that a heterozygous c.371 C>T mutation (R124C) in exon 4 of *TGFBI* was present in the affected members, but not in the unaffected members or controls (Fig. 1C).

Family with LCDIIB. In the second three-generation family, six affected members and eight unaffected members were examined (Fig. 2A). Thick and ropy lattice lines of amyloid were visible under direct illumination of the corneas of the proband patient II-1 (Fig. 2B). This patient was referred to

hospital with visual acuity loss to 0.2 and underwent perforating keratoplasty on her right eye at 56 years old. The corneal specimens obtained during surgery were submitted for histologic examination in the present study, which revealed large patchy amyloid deposits in the subepithelial layer and deeper corneal stroma (Fig. 2C). The clinical symptoms of the other affected family members were similar to those of the proband. Mutation analysis showed that a heterozygous c.1877 A>G mutation (H626R) in exon 14 of *TGFBI* was present in the proband and other affected members, but not in the unaffected members or 100 controls (Fig. 2D).

Family with GCD2. In this three-generation family, five affected patients and seven unaffected members were examined (Fig. 3A). The proband (a 22-year-old female patient; III:2) had recurrent corneal epithelial erosion with visual impairment, and symptoms of redness, photophobia, lacrimation and foreign-body sensation in both eyes. The visual acuity recovered to 1.0 when corneal epithelial erosion disappeared. Slit-lamp examination showed bilateral scattered grayish small dot, annular and snow flake-like opacities in the superficial mid-stroma of the central cornea (Fig. 3B). Mutation analysis showed that a heterozygous c.371 G>A mutation (R124H) in exon 4 of *TGFBI* was identified in the proband and other affected members, but not in the unaffected members and healthy controls (Fig. 3C).

Discussion

The *TGFBI* gene (also known as *BIGH3*) was first identified by Skonier *et al* in 1992 (14), and has been closely associated with inherited CDs. In the present study, mutations of *TGFBI* were identified in three unrelated Chinese families affected with LCDI, LCDIIB and GCD2, respectively. Mutations were identified in all of the families, and there were perfect correlations between LCDI and R124C, between LCDIIB and H626R, and between GCD2 and R124H.

LCD is generally divided into three subtypes, and classification has been assigned according to clinical and pathological findings. LCDI is an autosomal dominantly inherited corneal amyloidosis, which is characterized by a network of delicate linear opacities within the superficial corneal stroma. The disease usually begins in the first decade of life with symptoms of recurrent painful epithelial erosions. Lattice lines and diffuse opacification of the central cornea develop gradually subsequent to the formation of amyloid accumulations or erosions. Several studies from different ethnic groups have demonstrated an R124C mutation of the *TGFBI* gene as the most common cause of LCDI (11,15,16). In the present study, the clinical features of the affected members of the family with LCDI were similar, however, Liu *et al* (17) reported that the clinical features of Chinese patients with the same R124C mutation were variable, even within the same family. Therefore, the mechanism underlying phenotypic variability with the same mutation and within the same family remains unclear and requires further investigation.

The LCDIIB subtype is characterized by bilateral progressive visual impairment and has an intermediate age of onset, compared with LCDI and LCDIIIA. The LCDIIB

Table I. Primers for amplification of transforming growth factor β 1 regions.

Exon	Sequence (5'-3')	
	Forward	Reverse
1	TC TCA CTT CCC TGG AG	GAC TAC CTG ACC TTC CGC AG
2	GGT GGA CGT GCT GAT CAT CT	AGC CAG CGT GCA TAC AGC TT
3	TTC ACC CAC CAT TCC TCT TC	GGT ACT CCT CTC TCC CAC CA
4	ATC CCT CCT TCT GCT TTC TG	GCA GAC GGA GGT CAT CTC AC
5	TTA AAC ACA GAG TCT GCA GC	TTC ATT ATG CAC CAA GGG CCA
6	TGT TGA CTG CTC ATC CTT GC	CTC TTG GGA GGC AAT GTG TC
7	CTT CAG GGA GCA CTC CAT C	AAT CTA GCT GCA CAA ATG AGG
8	CTT GAC CTG AGT CTG TTT GGA	GGA TGG CAG AAG AGA TGG TG
9	CCT GCT GAT GTG TGT CAT GC	CTG CCT CCA GGG ACA ATC TA
10	TCA TTG CAG GAG CAC ATC TC	CCC AGG AGC ATG ATT TAG GA
11	GAG GCC CCT CGT GGA AGT A	ACA TCC CAC TCC AGC ATG AC
12	CTG TTG ACA GGT GAC ATT TTC	ATG TGC CAA CTG TTT GCT GCT
13	GGG ATT AAC TCT ATC TCC TT	TGT GTA TAA TTC CAT CCT GG
14	TCA GTA AAC ACT TGC TGA GTG AA	ACT GCC ACA TGG AGA AAA GGA C
15	CCT CAG TCA CGG TTG TTA TG	CTC TAT GGC CCA AAC AGA GG
16	TTG TCA TAA GCA GTT GCA GG	GCT TGC TTG GGG GTA AGG
17	TCC TAG ACA GAC ATG GGG AGA	TGA GAG AAA TTG GCG GAG AG

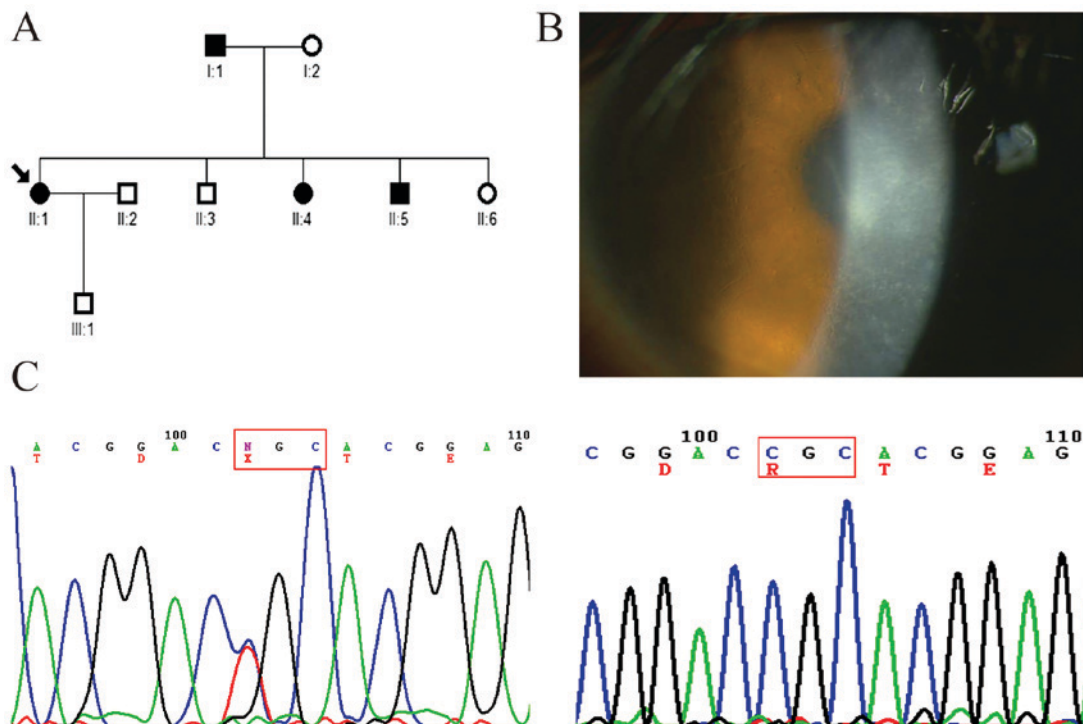


Figure 1. (A) Pedigree of family affected with LCDI. Open symbols indicate unaffected individuals; solid symbols indicate affected members. Arrows indicate the proband. (B) Corneal images of the proband show a network of linear opacities associated with other smaller opaque spots and refractile lattice lines. (C) Sequence around codon 124 within exon 4 in the patients with LCDI showing a nucleotide alteration at codon 124 (CGC→CTC), replacing an arginine with a cystine (R124C). LCSi, lattice corneal dystrophy type I.

subtype was first described in England (16), and was named by Chau *et al* (18). In 2010, Yang *et al* identified this mutation in a population in North China, which had clinical features of an intermediate subtype between LCDI and IIIA (19). In

2013, Wang *et al* first reported the *TGFBI* p.H626R mutation in a pedigree from South Chinese with LCDIIB (20). All these findings suggested that *TGFBI* p.H626R may be a mutation hotspot across different populations in LCDIIB.

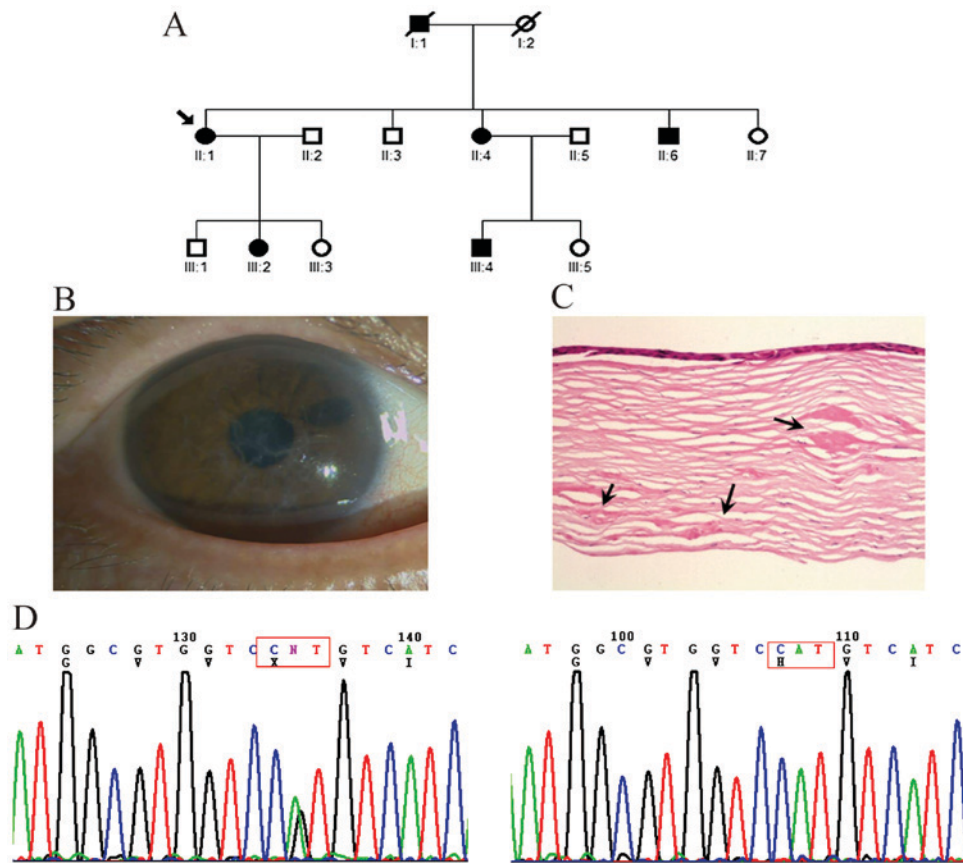


Figure 2. (A) Pedigree of family affected with the LCDIIB. Open symbols indicate unaffected individuals; solid symbols indicate affected members. Arrows indicate the proband. (B) Corneal images of the proband show thick lattice lines extending to the periphery. A superficial scar is seen as a result of recurrent corneal erosions. (C) Histopathological appearance of the corneal buttons obtained from the proband. Arrows indicate amyloid deposits. (D) Sequence around codon 626 within exon 14 in the patients with LCDIIB shows a nucleotide alteration at codon 626 (CAT→CGT), replacing a histidine with an arginine (H626R). LCDIIB, lattice corneal dystrophy type IIB.

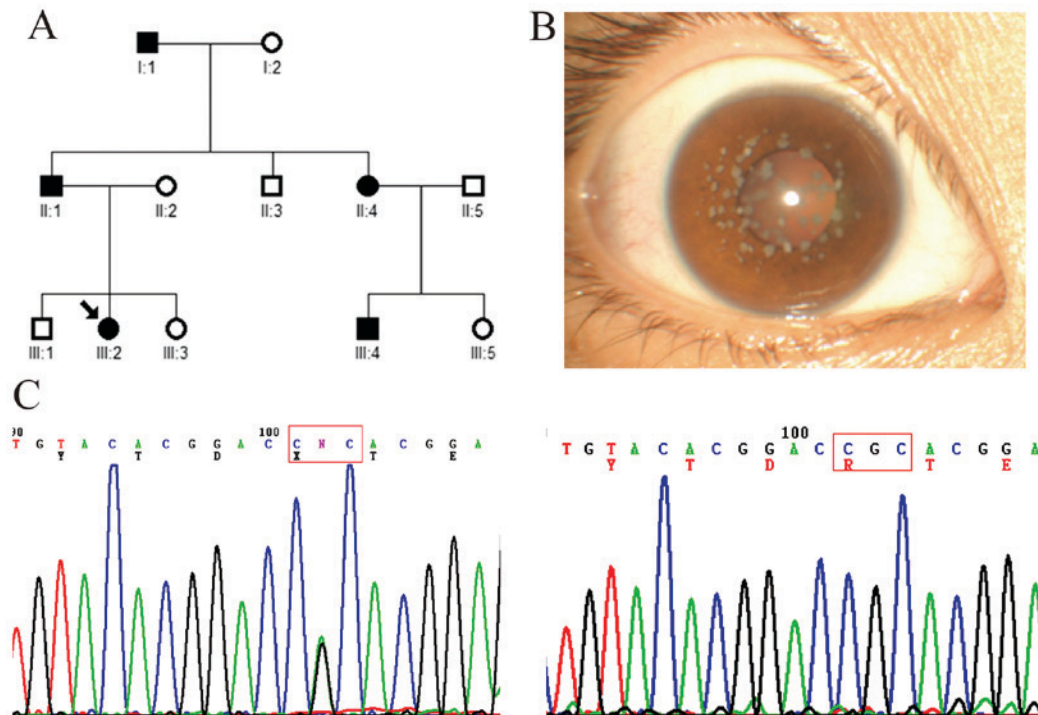


Figure 3. (A) Pedigree of family affected with the GCD2. Open symbols indicate unaffected individuals; solid symbols indicate affected members. Arrows indicate the proband. (B) Corneal images of the proband show scattered grayish small dot, annular and snowflake-like opacities in the superficial mid-stroma in the central cornea. (C) Sequence around codon 626 within exon 4 in the patients with GCD2 shows a nucleotide alteration at codon 124 (CGC→CAC), replacing an arginine with a histidine (R124H). GCD, granular corneal dystrophy type 2.

Similar to the previous studies, the present study found the p.H626R mutation in the family affected by LCDIIB.

The onset of GCD2, previously termed avellino dystrophy, often occurs in the second decade and demonstrates fewer opacities, compared with GCD1, exhibiting granular, branching deposits in a clear superficial mid-stroma, with lattice lines sometimes in deeper corneas (21). This disease has clinical and histologic features of granular and lattice dystrophy. To date, the molecular genetic techniques used for the majority of cases of GCD2 have revealed the R124H mutation in *TGFBI*, however, the phenotype of the affected individuals vary markedly in severity between families (22). Direct sequencing in the present study revealed that the c.371 G>A mutation of *TGFBI* resulting in Arg124His was responsible for GCD2 in the pedigree, which was consistent with the results of a previous study (21). These results further support the importance of *TGFBI* in maintaining corneal transparency.

In conclusion, although no novel mutations were found in the three pedigrees with CD in the present study, the previously reported phenotype-genotype correlations were confirmed in all patients with *TGFBI*-linked CDs. As the mutation at *TGFBI* can be readily, rapidly and cost-effectively detected via PCR sequencing or using PCR-RFLP, the identification of these mutations enables Chinese patients to benefit from the timely and accurate molecular diagnosis of CDs.

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