Arg124Cys Mutation of the TGFBI Gene in 2 Chinese Families With Thiel-Behnke Corneal Dystrophy

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Objective: To analyze transforming growth factor β–induced (TGFBI) gene mutations in 2 Chinese families with Thiel-Behnke corneal dystrophy (TBCD).

Methods: Forty-five individuals in 2 Chinese families with TBCD were examined using slitlamp biomicroscopy. Genomic DNA was extracted from peripheral leukocytes of affected and unaffected family members. Molecular genetic analysis of the TGFBI gene was performed using polymerase chain reaction and standard automated sequencing methods.

Results: In 17 family members with TBCD, an Arg124Cys (R124C) mutation of the TGFBI gene was identified, whereas the Arg555Gln (R555Q) mutation was absent. The Arg124Cys mutation was absent in all unaffected individuals.

Conclusions: The Arg124Cys mutation was associated with TBCD in 2 Chinese families. This mutation in the TGFBI gene may induce different phenotypes of corneal dystrophy.

Clinical Relevance: Thiel-Behnke corneal dystrophy may be caused by an Arg124Cys mutation of the TGFBI gene.


Thiel-Behnke corneal dystrophy (TBCD), previously known as honeycomb corneal dystrophy (or Reis-Bücklers corneal dystrophy type 2 [RBGCDII]), is inherited in an autosomal dominant fashion. In 1917, Reis1 first described RBGD as an annular corneal dystrophy. Bücklers2 reported the same pedigree in more detail in 1949. In 1967, Thiel and Behnke3 reported a corneal dystrophy different from the condition described by Reis and Bücklers. The corneal dystrophy described by Thiel and Behnke was characterized by honeycomb-shaped opacities at the level of the Bowman layer with recurrent erosions, moderately decreased visual acuity, and autosomal dominant inheritance.

Thiel-Behnke corneal dystrophy has been mapped to chromosome 5q31 of the transforming growth factor β–induced (TGFBI) gene (OMIM 601692). Bowman layer corneal dystrophy has been associated with 3 mutational sites, including an exon 4 Arg124Leu mutation,4,6 exon 14 Gly623Asp mutation,7,8 and exon 12 Arg555Gln mutation.9 Arg124Leu and Gly623Asp were correlated with Reis-Bücklers corneal dystrophy. Arg555Gln was correlated with TBCD.

In this study, 18 patients in 2 Chinese families with the honeycomb phenotype of corneal dystrophy were clinically diagnosed as having TBCD. The 2 families were screened for the presence of the TGFBI gene.

METHODS

PATIENTS

The patients with TBCD were probands and affected members of 2 Chinese families. The pedigrees are shown in Figure 1.

Family A

In family A, 12 of 37 individuals were affected with TBCD. At age 54 years (March 2007), the proband was examined in our clinic because of progressive visual impairment and recurring intermittent photophobia in both eyes. Onset of ocular irritation began at age 30 years. Best-corrected visual acuity was finger counting at 1 ft (0.3 m) OU. Slitlamp examination revealed bilateral fine corneal opacities in a honeycomb-shaped pattern at the level of the Bowman layer, and the anterior stromal layer was involved (Figure 2A).
The proband’s deceased mother had had a history of intermittent ocular irritation for many years. Three of his siblings and his 2 children were also affected with TBCD (Figure 2B).

**Family B**

In family B, 6 of 11 individuals were affected with TBCD. At age 41 years (October 2007), the proband was examined in our clinic. He had experienced reduced visual acuity and recurrent corneal epithelial erosions for more than 20 years. His visual acuity was 20/200 OU. Bilateral honeycomb-shaped corneal opacities in the Bowman layer were observed by slitlamp examination. He was diagnosed as having TBCD (Figure 2C).

The proband’s mother, 2 brothers, and son (Figure 2D) were also affected with TBCD. No other members of family B exhibited signs of corneal abnormalities.

**SPECIMEN COLLECTION**

In accord with the tenets of the Declaration of Helsinki, the 2 families with TBCD were included in the study after providing informed consent for clinical and molecular investigations. Venous blood samples were collected from family members for genetic analysis and were anticoagulated using EDTA. Genomic DNA was extracted from peripheral blood lymphocytes using an extraction kit (Tiangen Biotech Co, Beijing, China).

**POLYMERASE CHAIN REACTION ANALYSIS**

Based on previous findings,6,9,10 exons 4, 12, and 14 of the *TGFBI* gene were analyzed in this study. Primers were synthesized according to previously reported sequences6,9 (Table). Polymerase chain reaction (PCR) was performed in a 25-µL mixed volume (Tiangen Biotech Co), and the conditions11,12 were as follows: Exon 4 was denatured at 95°C for 5 minutes, then cycled 30 times at 94°C for 30 seconds, 65.5°C for 30 seconds, and 72°C for 30 seconds, and finally extended at 72°C for 7 minutes. Exon 12 was denatured at 96°C for 2 minutes, then cycled 35 times at 96°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds, and finally extended at 72°C for 7 minutes.

**DNA SEQUENCING**

To confirm the PCR product, electrophoresis was performed on a 2% agarose gel (Gene Tech Company Limited, Shanghai, China). The separation result was observed by nucleic acid staining (GoldView; SBS Gene Tech Company Limited, Beijing, China). The PCR products were sent for standard automated sequencing (SinoGenoMax Co, Ltd, Beijing).13

**RESULTS**

In the 2 families in this study, 2 probands and 15 affected siblings demonstrated a typical TBCD phenotype (Figure 2). In addition, the deceased member of family A had exhibited symptoms of TBCD. Genomic DNA sequences for all living affected members revealed a C→T missense mutation (CGC→TGC) in the first nucleotide position of codon 124 (Figure 3) at exon 4, causing a Cys→Arg substitution (Arg124Cys).14,15 The results of forward and reverse sequencing were identical. Unaffected individuals did not have this mutation. Genomic DNA sequencing analysis of exons 12 and 14 in the 2 families revealed no other putative disease-causing mutations (R124H, R124S, R124L, R555W, R555Q, H9004, or Gly623Asp).16-20

**COMMENT**

The *TGFBI* gene is implicated in the pathogenesis of most of the corneal dystrophies, including Avellino, granular,
Reis-Bücklers, Thiel-Behnke, lattice corneal dystrophy, and others. A previous study showed that a single type of 5q31-linked autosomal dominant corneal dystrophy was associated with 1 or more different mutations.

Thiel-Behnke corneal dystrophy is characterized by bilateral honeycomb-shaped corneal opacities in the Bowman layer. The onset of ocular irritation usually occurs between the ages of 10 and 20 years. It runs a progressive course of gradual deterioration of vision, with painful, erosive episodes. Previous literature reported that the Arg555Gln mutation of the \textit{TGFBI} gene is associated with TBCD. In this study, the phenotypes in 2 Chinese families were consistent with clinically typical TBCD, and genomic DNA sequencing results revealed an Arg124Cys mutation. To our knowledge, the Arg124Cys mutation associated with TBCD has not been reported.

Earlier investigators reported that the Arg124Cys mutation of the \textit{TGFBI} gene is responsible for lattice corneal dystrophy. As a group of inherited corneal dystrophies, lattice corneal dystrophies are categorized according to clinical, histologic, and genetic features. Classic lattice corneal dystrophy type I is characterized by childhood onset of central fine lattice lines in the central stromal layer and by progressive visual impairment in early life.

The results of this study suggest that the Arg124Cys mutation in the \textit{TGFBI} gene may induce different types of corneal dystrophies, expanding the heterogeneity of the clinical spectrum. Single gene mutations have been associated with different phenotypes in other studies. For example, RPGR mutations are responsible for up to 70% of X-linked recessive retinitis pigmentosa and for X-
linked cone-rod dystrophy and atrophic macular degeneration. Conversely, 1 phenotype can be caused by different gene mutations. For example, Reis-Bücklers corneal dystrophy is associated with R124L, Gly623Asp, and Δf540. There may be 2 explanations for this phenomenon. One is the interaction between TGFBI and other genes, and the other is the effect of environmental factors on gene presentation.

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REFERENCES


Figure 3. A portion of the first nucleotide position of codon 124 at exon 4 in TGFBI found in all affected members of the 2 families. A. Shown is a heterozygous C→T transition (Arg124Cys) at position 417 in the sense strand. B. The unaffected individuals did not have this mutation.