TGFBI gene mutation analysis in a Chinese pedigree of Reis-Bücklers corneal dystrophy

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Purpose: To analyze transforming growth factor beta-induced (TGFBI) gene mutations in a Chinese pedigree with Reis-Bücklers dystrophy (RBCD).

Methods: In a four-generation Chinese family with Reis-Bücklers dystrophy, six members were patients and the rest were unaffected. All members of the family underwent complete ophthalmologic examinations. Exons of TGFBI were amplified by polymerase chain reaction, sequenced, and compared with a reference database. The sequencing results were reconfirmed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: A single heterozygous C>T (R124C) point mutation was found in exon 4 of TGFBI in all six members of the pedigree affected with RBCD, but not in the unaffected members.

Conclusions: Within this pedigree, RBCD segregates with the R124C variance, which is a known mutation for lattice corneal dystrophy type I. Therefore, along with G623D and R124L, the R124C mutation in TGFBI is also found to be responsible for RBCD.

Inherited corneal dystrophy is mainly classified as lattice, granular, Avellino, Thiel-Behnke, and Reis-Bücklers corneal dystrophy (RBCD/CDB1, OMIM 608470). RBCD, a dominantly inherited dystrophy, is characterized by bilateral, progressive, and painful corneal erosions, and significant visual impairment [1]. Histopathologically, the involvement of the Bowman’s layer, the presence of band-shaped granular and subepithelial deposits that stained intensely red with Masson trichrome, and “rod shaped bodies” in cornea were confirmed in this disease [2,3]. The mechanisms remain unclear, but it is generally accepted that transforming growth factor beta-induced (TGFBI) is actively involved in the pathogenesis of RBCD.

Initially known as kerato-epithelin, TGFBI is an extracellular matrix protein induced by transforming growth factor-beta 1 and is highly expressed in the corneal epithelium. It contains a Arg-Gly-Asp (RGD) motif that acts as a ligand recognition sequence for several integrins, and thus is associated with cell-collagen interactions with a role in the regulation of cell-adhesion. Therefore, it is believed that TGFBI plays a role in corneal development and wound healing by mediating cell adhesion via its interaction with collagen, fibronectin, and integrins. The human TGFBI gene encodes a 682 amino acids protein (68 kDa) with four internal homologous repeats. Currently, more than 30 mutations in TGFBI have been demonstrated in four different types of corneal dystrophies [4].

Mutated TGFBI has been linked to the four types of corneal dystrophy, including RBCD [5-11]. Two mutations, R124L [12,13] and G623D [14], have previously been identified in patients with typical RBCD, including Chinese RBCD pedigrees. In this study, R124C mutation, rather than R124L or G623D, was identified—to the best of our knowledge—for the first time in a Chinese family with RBCD.

METHODS

Patient recruitment: A four-generation Chinese family from the Sichuan province with RBCD was included in this study (Figure 1). This study includes six RBCD patients and six unaffected relatives. This study was approved by a local institutional medical ethics committee, and informed consent conforming to the tenets of the Declaration of Helsinki was obtained from each of participants.

Clinical examination: A Snellen best-corrected visual acuity test, a slit-lamp biomicroscopy, and a fundus examination were conducted by an experienced ophthalmologist for all subjects. Laser scanning in vivo confocal microscopy (Heidelberg Retina Tomograph III, Rostock Corneal Module

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DNA extraction and polymerase chain reaction: Peripheral blood samples were drawn from six RBCD patients and six unaffected members. Leukocyte DNA was extracted from 200 μl peripheral blood using a TIANamp Genomic DNA Kit (Tiangen Biotech Co. Ltd, Beijing, China), following the manufacturer’s instructions. DNA integrity was evaluated by 1% agarose gel electrophoresis. The intronic primers flanking the exons were designed based on genomic sequences of TGFBI (Consortium Human Build 37 NC_000005) and synthesized by Invitrogen Company (Carlsbad, CA). The sequences of the primers are listed in Table 1.

RESULTS

Clinical presentation: In the family, six individuals with RBCD and six unaffected individuals were examined. The proband (a 33-year-old male, patient III:3, Figure 1) experienced recurrent photophobia, progressive vision loss, and corneal erosion since the age of 10. At the time of the examination, the best corrected visual acuity was hand motion (OD) and 6/600 (OS). Slit lamp examination showed multiple annular grayish opacities at the subepithelial and anterior stroma of the central cornea of both eyes. Representative clinical photographs of the cornea of an affected family member are shown in Figure 2. Patient IV:3, the 4-year-old son of the proband, presented with no clinical symptoms, but manifested bilateral diffuse and small dot epithelial and subepithelial opacities in the central cornea (Figure 2).

In vivo laser scanning confocal microscopy was performed on the proband. Focal depositions of homogeneous reflective materials with rounded and hyporeflective edges were observed (Figure 3).

TGFB1 mutation analysis: A single heterozygous C>T mutation was found (R124C) in exon 4 of TGFB1 in all affected members of this pedigree (Figure 4). This R124C mutation co-segregated with the disorder within the family. This mutation causes an Arginine to Cysteine substitution at the protein level. The results of the sequencing analysis were confirmed by PCR-RFLP analysis (Figure 5). After digestion, wild type alleles were cut into two fragments, 181 bp and 172 bp, whereas the affected patients’ alleles were cut into three fragments, 353 bp, 181 bp, and 172 bp.

DISCUSSION

Corneal dystrophy is a group of diseases with autosomal dominant inheritance. Until now, corneal dystrophies failed...
to be clearly classified because of the variability in phenotypic expression of the diseases. A proposed corneal dystrophy classification system, which is identical or similar to those in the current nomenclature, is anatomically based with dystrophies classified according to the layer mainly involved, such as the epithelial and subepithelial, Bowman’s layer, stroma, Descemet’s membrane, and endothelium [16]. Clinical characteristics such as the depth of the cornea affected, the morphology of the deposits, and the histopathological features are also important for classifying different corneal dystrophies [17]. However, dystrophies with overlapping and atypical characteristics are still too similar to be distinguished from one another. A Chinese family with atypical RBCD was recently reported [14]. This family presented with a unique corneal dystrophy within the Bowman’s layer and the corneal stroma. However, no lattice was noted in the proband or other affected members, and the deposits were located in the mid-stroma of the cornea, which was different from the phenotypes previously reported in lattice corneal dystrophy patients with R124C mutation. Some corneal dystrophies affect multiple corneal layers and therefore cannot be classified as a single type based on morphologic criteria.

Phenotypically, the pedigree we documented here exhibited typical features of RBCD. The affected individuals presented with a gray-white geographic opacity in the anterior to mid-stroma of both eyes. In addition, geographic and round opacities in the subepithelial layers and anterior to mid-stroma were found in all of the affected family members. The clinical features, including recurrent erosion and gradually

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**Table 1. Primers used in polymerase chain reaction for amplification of TGFBI.**

| Exon | Primer direction | Sequence (5′→3′) | Annealing temperature (°C) |
|------|-----------------|-----------------|---------------------------|
| 1    | Forward:        | GCTTGCCCGTCGGTGCCTGA | 62                        |
|      | Reverse:        | TCCGAGCCCGACTACCTGGA |                          |
| 2    | Forward:        | AGGCAAACACGATGGGAAGTCA | 60                        |
|      | Reverse:        | TAGCAGCGAGGGTCCGAGACA |                          |
| 3    | Forward:        | CCAGATGACCTGTGAGAAGTGA | 60                        |
|      | Reverse:        | CTTTTATGTTGGTACTCTCTCT |                          |
| 4    | Forward:        | TCTCAGTCTCTCACCAGCTTGT | 58                        |
|      | Reverse:        | CTCCCATTTCATCGCCCAC |                          |
| 5 and 6 | Forward: | CTTGGGCTACCGAGGGCTGAGAACAT | 64                        |
|      | Reverse:        | GCCCCCTCTTGGGAGGCAATGTGTC |                          |
| 7    | Forward:        | GTAGAGCTTGTTTGGCTTCT | 63                        |
|      | Reverse:        | ACCTCATGGAGGTTGATAG |                          |
| 8    | Forward:        | TGAGTTATCGTGGAGTG | 53                        |
|      | Reverse:        | CACATCACTCTGTCACA |                          |
| 9    | Forward:        | ACTCACAGAGATGACATTCTC | 60                        |
|      | Reverse:        | TCCAGGGACAAATACACAGG |                          |
| 10   | Forward:        | TAGAAGATACAGATGTAAAG | 56                        |
|      | Reverse:        | TGTCAGCAACCAGTTCAT |                          |
| 11   | Forward:        | CCTGCTACATGCTGAGAACA | 58                        |
|      | Reverse:        | GAAATCCCAGAATGAGAAAG |                          |
| 12   | Forward:        | GACTCTACTATCTCTAGTGT | 58                        |
|      | Reverse:        | ATGTGCCAACTGTTGGCT |                          |
| 13   | Forward:        | CATTAGACAGATGTTGGGCTCA | 60                        |
|      | Reverse:        | GGGCTGCAACCTGGAGTT |                          |
| 14   | Forward:        | GCGACAAGATTGAACACTCCAT | 58                        |
|      | Reverse:        | CTCTCCACCAACTGCGACAT |                          |
| 15   | Forward:        | CCCTCAGTCACGGTGTCTT | 58                        |
|      | Reverse:        | GGAGTGCTCCTGGCTTCTTT |                          |
| 16   | Forward:        | CTTCGCAACTAATGCTGCC | 58                        |
|      | Reverse:        | TGCACATGAGTTGCCTAT |                          |
| 17   | Forward:        | AGTGAAATTCATCGAAACCAC | 58                        |
|      | Reverse:        | CCACATTTGGGATAGGTT |                          |

Summary of the primers and annealing temperatures used for the amplification of the 17 exons of TGFBI.
developing opacities of the Bowman’s layer, were consistent with the characteristic of RBCD [1,18] and to those found in the families previously described by Afshari et al. [10] and Aldave et al. [19]. In vivo confocal microscopy images were also consistent with the reported findings in RBCD [20].

*TGFB1* has been closely involved with the inherited corneal dystrophies, as mutations in this gene were identified in at least 5 types of corneal dystrophies, including granular corneal dystrophy (R555W), Avellino corneal dystrophy (R124H), lattice corneal dystrophy type I (R124C), Thiel-Behnke corneal dystrophy (R555L), and RBCD (R124L, G623D) [10,19]. Among those mutations reported, R124 appeared to be a “hot-spot” point mutation in *TGFB1* [21,22] as the R124 mutation has been detected in three types of corneal dystrophies, including Avellino corneal dystrophy, lattice corneal dystrophy type I, and RBCD. In previous
reports, R124C is associated with lattice corneal dystrophy [15,21,23]. Interestingly, R124 in exon 4 of TGFBI is conserved among several species, including Homo sapiens, Mus musculus (R124), Pan troglodytes (R124), Macaca mulatta (R124), and Rattus norvegicus (R124), and is incompletely conserved in Gallus gallus (R117) and Danio rerio (R118). It strongly suggests that this residue is an important—functional and structural—site of the protein.

To date, TGFBI was the only gene found to be associated with RBCD. Here, we demonstrated an unusual R124C mutation in TGFBI associated with the RBCD, which was a mutation known to be responsible for corneal lattice dystrophy type I. Therefore, along with G623D and R124L, the R124C mutation in TGFBI is also found to be responsible for RBCD.

In 1996, Small et al. [24] suggested that Reis-Bücklers, lattice type 1, Avillino, and granular corneal dystrophies are all the same disease as they stem from the same gene products. Recently, it was suggested by the International Committee for Classification of Corneal Dystrophy that the following corneal dystrophies be named as TGFBI corneal dystrophies, including granular corneal dystrophy type 3 (RBCD), Thiel–Behnke corneal dystrophy (TBCD), classic lattice corneal dystrophy (LCD1), Granular corneal dystrophy, type 1 (classic; GCD1), and Granular corneal dystrophy, type 2 (granular-lattice; GCD2) [16]. Corneal dystrophies caused by mutations in TGFBI are characterized by abnormal extracellular deposits of mutated TGFBI protein in the corneal stroma. Most of TGFBI mutations so far reported are located in the fourth Fas1 domain with two mutational hot spots in R124 and R555. There is a strong correlation between phenotype-genotype in most corneal dystrophies caused by TGFBI mutations. The corneal dystrophy with R124L mutation usually has a worse prognosis, whereas the clinical manifestations of corneal dystrophies resulting from R555T or R555C mutations are usually mild. Other mutations such as P501T and N622K are related to various subtypes of lattice-like dystrophies. However, as dystrophies have a known common genetic basis, they may be classified into a single category, TGFBI dystrophy [16].

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