Two Cases of Reis-Bücklers Corneal Dystrophy (Granular Corneal Dystrophy Type III) Caused by Spontaneous Mutations in the TGFBI Gene

Reis-Bücklers corneal dystrophy (RBCD) is an inherited corneal disorder that was first described by Reis in 1917 and later by Bücklers in 1949. Affected individuals have an onset early in life and have frequently recurring, painful corneal erosions, superficial corneal opacities, and significant visual impairment. The literature on this entity, which has several synonyms (granular corneal dystrophy [GCD] type III, superficial variant of GCD, corneal dystrophy of Bowman layer type I), is bewildering not only because of the nomenclature but also because this genetically determined disorder has been confused with a different, Thiel-Behnke corneal dystrophy (TBD). Although RBCD and TBD are now considered distinct clinicopathologic disorders, a precise diagnosis of these corneal disorders was difficult until recently because it relied only on the clinical and histopathologic features.

Both RBCD and TBD are autosomal dominant disorders of the superficial corneal stroma that manifest as recurrent corneal erosions in early childhood. Reis-Bücklers corneal dystrophy tends to cause more extensive corneal opacities, more severe visual impairment, and a higher frequency of recurrence compared with TBD. Because the clinical phenotypes of RBCD and TBD are similar (especially in young individuals), an accurate distinction between the 2 disorders necessitates either a microscopical examination of corneal tissue or a molecular genetic analysis. Microscopically, RBCD is characterized by confluent opacities in the Bowman layer and the subepithelium that result from extracellular bodies that stain red with Masson trichrome stain and appear as crystalloid, rod-shaped bodies by transmission electron microscopy. On the other hand, TBD exhibits honeycomb-shaped opacities in the Bowman layer and “curly” fibers by transmission electron microscopy. Thus, RBCD and TBD may be accurately diagnosed if an excised corneal specimen displays these microscopic characteristics.

In addition to examining tissue samples, the identification of specific mutations in the transforming growth factor β-induced (TGFBI) gene on chromosome 5q31 has led to another diagnostic technique. In 1997, Muñier et al established that different mutations in this gene cause several distinct inherited corneal disorders. This landmark publication reported an arginine-to-glutamine mutation at codon 555 (R555Q) in a patient diagnosed with RBCD, but Muñier and colleagues did not document the clinical, light microscopic, or electron microscopic findings in this individual. Soon thereafter, Okada et al documented the R555Q mutation and the slitlamp photographs of a patient with honeycomb opacities diagnosed clinically with the Thiel-Behnke form of GCD. They also identified a novel TGFBI mutation, R124L, in a proband and his mother with the geographic corneal opacities of RBCD. The phenotypes associated with these 2 mutations were not documented histopathologically by light or electron microscopy. Subsequent studies confirmed that RBCD is associated with at least the R124L mutation whereas TBD stems from the R555Q mutation. The discovery that distinct genetic mutations in the TGFBI gene cause RBCD and TBD further defines these disorders as separate clinicopathologic entities. Most importantly, these disorders differ in prognosis, and therapy may be inappropriate if the disease is misdiagnosed.

Methods. Patients. Two unrelated children were diagnosed with Reis-Bücklers corneal dystrophy by ophthalmologists at different major medical institutions. Both affected individuals were invited to participate in a molecular genetic evaluation of their corneal disorder at Duke University Medical Center, Durham, NC. The institutional review board at Duke University approved this research project. After written informed consent was obtained from the parents of the 2 affected individuals, all of the available clinical records and photographs were reviewed. Family histories and pedigrees were compiled. The parents and other family members were examined clinically.

Histologic Examination. Tissue from a superficial diagnostic corneal biopsy of proband A was placed in formalin, embedded in paraffin, then sectioned and stained with hematoxylin-eosin and Masson trichrome. Corneal tissue was not obtained from proband B.

DNA Analyses. Blood samples or buccal scrapings from the unaffected parents as well as from affected and unaffected siblings were gathered. Leukocyte DNA was extracted with the Puregene Blood Kit or the Puregene Buccal Cell Kit (Gentra Systems, Minneapolis, Minn). All of the exons of the TGFBI
gene were analyzed by polymerase chain reaction amplification of forward and reverse primers as documented elsewhere: polymerase chain reaction products were purified with the QIAquick PCR Purification Kit (Qiagen, Valencia, Calif) and were sequenced on both strands through the BigDye Terminator Cycle Sequencer (Applied Biosystems, Foster City, Calif) and the ABI PRISM 377 DNA Sequencer (Applied Biosystems). The resulting DNA sequencing gel was analyzed by means of the ABI PRISM DNA Sequence Analysis software program (Applied Biosystems). The resulting DNA sequencing gel was analyzed by means of the ABI PRISM DNA Sequence Analysis software program (Applied Biosystems). The sequences were then aligned with an established TGFBI gene complementary DNA sequence by way of the SeqWeb Sequence Analysis Web-based program (Accelrys, San Diego, Calif). Identification of mutations and amino acid changes in the DNA sequences was confirmed by personal inspection.

To investigate the maternity and paternity of affected individuals, DNA samples from the probands and their parents were analyzed for short tandem repeats by polymerase chain reaction amplification and fluorescent quantization technology (Genetic Identity/STR System, Eugene, Ore). Short tandem repeats in 15 alleles from the parents and affected children were compared to ascertain the probability of paternity and maternity. All of the methods and statistical analyses were performed according to the standards of the American Association of Blood Banks, Bethesda, Md.

Results. Clinical Findings. Proband A was referred to a tertiary care center for recurrent corneal erosions at age 2 years (Figure 1). From birth, she was noted to have a history of recurrent “infections” and multiple abrasions of the cornea that were treated from age 2 years onward. Her symptoms included edematous eyelids, photophobia, and ocular pain. At age 3.5 years, slit-lamp examination revealed a decreased tear film, epithelial haze, and geographic opacities bilaterally. The parents reported a negative family history of any eye disease, and slit-lamp biomicroscopy of their eyes revealed normal corneas.

Proband B experienced recurrent, painful corneal erosions since age 6 months and was referred to a major eye center for evaluation at age 2 years. At that time, slit-lamp biomicroscopy revealed abnormalities in the superficial corneal stroma with sparing of the peripheral cornea and no evidence of neovascularization. A diagnosis of RBCD was suspected, but the family history was negative for corneal disease and slit-lamp examinations of the parents disclosed normal corneas. Proband B later underwent multiple successful phototherapeutic keratectomies on both eyes but had recurrences bilaterally (Figure 2).

Histological Examination. The diagnostic corneal biopsy of proband A disclosed a portion of unremarkable corneal epithelium resting on a thin layer of fibrocollagenous tissue (Figure 3). No other abnormalities were found, and particularly noteworthy was the absence of fuchsinophilic accumulations characteristic of the various types of GCD, perhaps reflecting a small biopsy with insufficient tissue.

DNA Analyses. Mutational analyses of DNA specimens from both probands revealed a single heterozygous guanine-to-thymine substitution at nucleotide position 418 in exon 4 of the TGFBI gene (Figure 4), resulting in a predicted R124L mutation in each affected individual. The parents of both probands lacked this mutation. In addition, proband A was found to have 3 heterozygous nucleotide substitutions that led to silent polymorphisms: exon 6 (651 cytosine to guanine [L217L]) (also in both parents), exon 8 (981 adenine to guanine [V327V]) (inherited from mother), and exon 12 (1620 thymine to cytosine [F540F]) (inherited from father).

The DNA analyses for paternity and maternity of the probands confirmed that the mother and father of each affected individual were the biological parents. The probability of paternity and maternity was greater than 99.999% for both proband A and proband B (Table).

Comment. Two unrelated young children were suspected of having RBCD despite the absence of a family history of eye disease. Because a definitive diagnosis could not be made clinically, an analysis of the TGFBI gene was performed. It disclosed an R124L mutation, which is currently considered to be diagnostic for RBCD. This mutation was not identified in any of the biological parents, indicating that each child developed a spontaneous new mutation. To our knowledge, this is the first described instance of sporadic mutations causing RBCD. As shown here, in patients with an uncertain corneal disease, a molecular genetic analysis may become necessary to establish a definitive diagnosis.

Not only are these spontaneous mutations in the TGFBI gene noteworthy, but their finding also has implications for the practicing ophthalmologist who needs to be aware that natural mutations do occur and can cause clinically significant disease such as RBCD. Furthermore, this article emphasizes the importance of genetic testing in the diagnosis of RBCD as well as the importance of accurate diagnoses for the proper selection of treatment.

Figure 1. Cornea of proband A at age 4 years, showing subtle abnormalities.

Figure 2. Cornea of proband B at age 10 years, showing recurrence of opacities 6 years after successful phototherapeutic keratectomy.
The finding of identical spontaneous mutations at codon 124 of TGFBI in 2 independent families is remarkable and warrants an explanation. The occurrence of the same mutation in 2 individuals may have been a chance phenomenon, but this seems unlikely considering that the mammalian DNA mutation rate is estimated to be $10^{-8}$ per nucleotide. A more probable explanation for our finding of 2 identical random mutations in TGFBI is that they occurred at the same site because this part of the gene has an increased mutational frequency, referred to as a “hot spot.” This theory was first postulated with regard to the gene in question, TGFBI, by Korvatska et al15 using evidence from haplotype analyses of families with the R124C, R124H, and R555W mutations and later with the R124L mutation16 in TGFBI. They showed that mutations in codons 124 and 555 of TGFBI represented multiple independent occurrences. We provide direct evidence of sporadic mutations at codon 124 in the 2 children described here, and our laboratory has also documented a similar sporadic mutation involving a different nucleotide change at codon 124 (R124H) leading to GCD type II. Thus, these 3 spontaneous mutations in codon 124 provide additional support that this codon is an unusually frequent site for genetic mutations. It is noteworthy that aside from RBCD,4 other distinct clinical disorders have been attributed to missense mutations of TGFBI at codon 124: GCD type I (R124S),18 GCD type II (R124H),36 GCD type IV (R124L with ΔT125 and ΔE126),19 and lattice corneal dystrophy type I (R124C).6

The silent single-nucleotide polymorphisms found in proband A did not affect the encoded protein, and all 3 polymorphisms have been previously reported: L217L,20,21 V327V,15,20,21 and F540F.15,20-23

For the clinician, it is important to realize that an individual with clinical manifestations of a genetically determined disorder, even in the absence of a positive family history, may truly have this usually inherited disease. When an individual has such atypical clinical findings, a genetic analysis is essential. In particular, RBCD and TBD exhibit such similar clinical manifestations that a differentiation between these 2 inherited disorders necessitates a genetic evaluation or a corneal biopsy. Despite its popularity, an examination of a sample of corneal tissue obtained at biopsy may be insufficient to confer a definitive diagnosis, as found in proband A. For example, in lattice corneal dystrophy, Dighiero et al5 demonstrated that histologic examination and transmission electron microscopy did not reveal obvious specific characteristics that might permit the distinction between 2 variants of that dystrophy. However, genetic analyses provide a noninvasive and virtually fail-safe alternative for establishing a diagnosis.

Achieving a precise diagnosis of RBCD by genetic testing is desirable for the parents, the patient, and the clinician. It gives the parents and eventually the patient confidence and an opportunity to be further educated about the natural history of the disease and its response to therapy. For the clinician, diagnostic accuracy is critical for timely and appropriate treatment. When corneal deposits significantly decrease visual acuity and when painful corneal erosions recur despite medical therapies consisting of cycloplegic drops, antibiotic ointment, and bandage contact lens placement, surgical treatment is often indicated. The selection of the appropriate therapeutic procedures for RBCD and other corneal dystrophies varies. While penetrating keratoplasty effectively removes opacities in all of the corneal layers, the less invasive...
phototherapeutic keratectomy, which removes lesions solely in the anterior portion of the cornea, is an optimal surgical treatment for the superficial corneal lesions in RBCD.\textsuperscript{24} It can be performed multiple times on the same cornea, which is ideal considering the high recurrence rate of RBCD.\textsuperscript{10} A penetrating keratoplasty may eventually be indicated, but phototherapeutic keratectomy can successfully delay this invasive procedure. Of note, phototherapeutic keratectomy of a corneal graft has also been shown to be a safe and effective alternative to repeat grafting.\textsuperscript{25} However, in clinically similar disorders such as atypical cases of GCD type I, penetrating keratoplasty may be indicated at an earlier stage of disease because the recurrence rate is lower and because phototherapeutic keratectomy may not satisfactorily improve vision if there are deeper stromal opacities.

Less than a decade has passed since the discovery that mutations in the \textit{TGFBI} gene cause several inherited corneal disorders,\textsuperscript{6} and genetic testing is now essential for the correct diagnosis of these corneal dystrophies and thus for selecting the most appropriate treatment.

**Table. Maternity and Paternity Testing Results for Both Probands by 15 DNA Allelic Markers**

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*The probability that both sets of parents are the biological parents is greater than 99.999%.

Figure 4. Nucleotide sequencing of exon 4 of \textit{TGFBI} for both probands disclosed an arginine-to-leucine mutation at codon 124 (A), but their unaffected parents were identical to controls (B).
deed, descendants of the 2 probands are now at risk for RBCD. For those individuals, genetic testing at an early age may improve clinical outcomes by encouraging frequent ocular examinations as well as treatment at earlier stages of this particular genetically determined disorder. As demonstrated in these cases, genetic testing provides an accurate and noninvasive diagnosis as well as peace of mind for the parents, who may be unaware of this painful inherited eye disorder before its onset in their children, and for the clinician, who can confirm the diagnosis and tailor the therapy appropriately.

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Supporting Information:

Granular corneal dystrophy.

Table 1. Clinical outcomes of four 5q31-linked corneal dystrophies.

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Acknowledgment: Paternity and maternity testing was provided by Genetic Identity, Eugene, Ore.


Corneal Graft Fold: A Complication of Deep Lamellar Endothelial Keratoplasty

Corneal endothelial dysfunction accounted for 36% of the more than 30 000 corneal transplants performed in the United States in 2003.1 Deep lamellar endothelial keratoplasty (DLEK) is an evolving procedure2-4 that, in comparison with full-thickness keratoplasty, may result in a decreased incidence of high or irregular astigmatism and suture-related complications, such as microbial keratitis or sterile suture reactions. Published complications associated with DLEK have included graft rejection, mismatch of donor thickness to recipient bed thickness, and partial dislocation of the graft.3,4 We report another complication encountered in 2 patients undergoing DLEK.

Methods. Two patients were randomly assigned to DLEK as part of an institutional review board–approved, prospective, randomized study comparing DLEK and standard penetrating keratoplasty. Deep lamellar endothelial keratoplasty was performed through a 9-mm limbal incision in the manner described by Terry and Ousley.3 An 8-mm posterior lamellar disc of recipient tissue was excised and replaced with a partial-thickness donor disc 8 mm in diameter. Patient 1 also underwent concomitant phacoemulsification and intraocular lens implantation through a separate temporal, clear corneal wound. Intraoperatively, the donor disc was noted to be very thin. The procedure was otherwise uncomplicated. Patient 2, a 74-year-old woman who was previously pseudophakic, underwent an apparently uncomplicated DLEK for Fuchs dystrophy as well. Postoperatively, potential visual acuity was measured (Guyton/Minkowski PAM; Mentor, Inc, Norwell, Mass) and corneal thickness was calculated using confocal microscopy (Tandem Scanning Microscope, Reston, Va).

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