Genotype-Phenotype Correlation in 2 Indian Families With Severe Granular Corneal Dystrophy

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Objectives: To determine genotypes in 2 Indian families with severe granular corneal dystrophy, to document clinical and histopathologic features, and to attempt a genotype-phenotype correlation.

Methods: Mutation analysis of exon 12 of the TGFBI gene was carried out in 9 individuals from 2 families.

Results: A C→T mutation at residue 1710 of TGFBI complementary DNA, corresponding to an Arg555Trp mutation in keratoepithelin, was found in affected members of both families. In 5 patients, this mutation was homozygous, and it was heterozygous in the other 4. Clinical examination revealed a severe form of granular corneal dystrophy with early onset and superficial lesions in the homozygous individuals and a milder phenotype in the heterozygous individuals. Histopathologic evaluation of corneal specimens from 2 homozygous patients confirmed the presence of superficial granular deposits.

Conclusions: To our knowledge, this is the first molecular and clinical characterization of severe granular corneal dystrophy in India. Genotype-phenotype correlation and comparison with earlier reports on this entity highlight the uniform expressivity of the Arg555Trp allele in homozygous individuals.

Clinical Relevance: Homozygous granular corneal dystrophy has a severe phenotype and can be recognized based on clinical and histopathologic features, especially in association with consanguinity or inbreeding.

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Granular corneal dystrophy (Online Mendelian Inheritance in Man 121900) is an autosomal dominant disorder characterized by small white, sharply demarcated spots that resemble bread crumbs in the cornea beneath the Bowman layer. The opacities vary in shape but usually fall into 3 basic morphologic types of drop, crumb, and ring shapes. The overall pattern of deposition is radial or disk shaped, or it may be in the form of a Christmas tree. Initially, the region of the corneal stroma between the opacities remains clear. Vision is usually not affected in the early stage of the disease, but some patients may have mild photophobia from light scattering by the corneal lesions. In a subset of patients, erosive episodes are more common. As the condition advances, individual lesions increase in size and number and may coalesce. They frequently extend into deeper and more peripheral stroma. However, 2 to 3 mm of the peripheral cornea usually remains free of deposits. With more advanced disease, the intervening cornea develops a diffuse hazy appearance. Although the lesions can involve the Bowman layer and result in superficial irregularities, recurrent erosions are unusual. Visual impairment is rare before the fifth decade of life and usually develops secondary to the opacification of the intervening stroma. Corneal sensation is variably affected.

Atypical more severe and early-onset forms of granular corneal dystrophy have been reported, in which onset of visual loss occurs within the first to second decades of life and repeated corneal grafts are often necessary. These phenotypes have been linked to homozygous mutations in the dominant disease gene.

Granular corneal dystrophy arises as a result of mutations in the TGFBI (transforming growth factor β induced) gene on chromosome 5q31, the protein product of which is known as keratoepithelin. Keratoepithelin is expressed in the corneal epithelium.
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 APPLICATIONS

Figure 1. Pedigrees of the families studied. Clear circles and squares indicate unaffected women and men, respectively; dark symbols, severely affected; shaded symbols, mildly affected; asterisk, disease status unknown; double line, consanguineous spouses; M/M, homozygous mutant allele (Arg555Trp); and M/+ , heterozygous mutant allele. The number “3” in the first symbol in generation II designates 3 male siblings.

eal and many other tissues, is up-regulated by transforming growth factor β, and is an extracellular matrix protein, possibly mediating cell-cell adhesion.8-10 Specific mutations in the TGFBI gene are responsible for different types of granular and lattice types of corneal dystrophies.7 These mutations lead to the formation of insoluble deposits within the cornea that are recognized by their characteristic histopathologic staining properties, which are distinctive for granular and lattice types of dystrophies. In granular corneal dystrophy, the cornea shows hyaline deposits that are nonamylolysed in na-
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A 40-year-old woman (the proband) was an offspring of a consanguineous marriage. She had had complaints of defective vision since childhood. Her vision had wors-
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ties spared the limbus but were close to it (Figure 2A). Details of the anterior chamber and lens were not visu-
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Patient IV:3

A 37-year-old man, the brother of the proband, had had white opacities in both eyes since childhood. He com-
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Patient IV:3

A 37-year-old man, the brother of the proband, had had white opacities in both eyes since childhood. He com-
plained that the opacities were gradually increasing. He gave a history suggestive of having undergone lamellar corneal grafting in the left eye 17 years earlier. On examination, he had a visual acuity of counting fingers at 1 m OD and at 2 m OS. Corneal examination revealed coarse reticulate subepithelial opacities and some midstromal granular opacities. He underwent superficial keratectomy in the left eye and debridement in the right eye. The keratectomy specimen was submitted for histopathologic examination. One week after surgery, his visual acuity was 20/80 OD and 20/40 OS, and there were midstromal granular opacities and scarring.

Patient IV:5

Patient IV:5, a sister of the proband who was 31 years old, complained of having had white opacities in both eyes since she was 11 years old, which were progressively increasing, with occasional pain and watering. She had a best-corrected visual acuity of 20/120 OU. Corneal examination revealed coarse reticulate subepithelial lesions. The cornea between the lesions showed deep stromal granular opacities. She underwent superficial keratectomy in the right eye and debridement in the left eye. The keratectomy specimen was submitted for histopathologic examination. Following surgery, her visual acuity was 20/60 OD and 20/125 OS, and deep stromal opacities remained.

Patient IV:7

Another sister of the proband (patient IV:7), age 25 years, had had diminution of vision in both eyes since childhood. She had undergone surgery in the right eye 10 years previously. On examination, her visual acuity was 20/100 OD and 20/125 OS, the right eye had a failed graft, with reticulate subepithelial opacities. In the left eye, similar opacities were seen that were subepithelial and involving the anterior stroma. These opacities spared the peripheral 1 mm of the limbus. She underwent debridement of the lesions in the left eye. Her visual acuity improved to 20/60 OS. There was anterior stromal haze, with granular deposits in the stroma.

Patients V:1, V:2, and V:3

A 21-year-old man (patient V:1) complained of occasional eye watering. He had a visual acuity of 20/20 to 20/25 OU. On examination, anterior to midstromal discrete granular opacities were seen (Figure 2B), with a clear limbus.

An 18-year-old man (patient V:2) had a visual acuity of 20/20 OU. Anterior to midstromal discrete opacities were seen (Figure 2C). The limbus was free of opacities.

A 14-year-old girl (patient V:3) had a visual acuity of 20/20 OU, with anterior to midstromal opacities. The limbus was clear.

FAMILY B

Patient V:1

A 23-year-old woman, an offspring of a consanguineous marriage, had had complaints of diminution of vision in both eyes since she was 8 years of age. She also had complaints of light sensitivity. She gave a history of having undergone corneal grafting in both eyes about 10 years earlier. Her medical history was unremarkable. She had a visual acuity of counting fingers at 1.5 m OU, which could not be improved further. Anterior segment evaluation in both eyes showed confluent subepithelial opacities involving the entire graft, extending into the posterior cornea (Figure 3A). She underwent penetrating keratoplasty in the right eye. Five months after surgery, she had a visual acuity of 20/40 OD. At her recent follow-up, the graft was clear, and she had an intraocular pressure of 14 mm Hg (Figure 3B). The corneal button was subjected to routine histopathologic evaluation.
A 41-year-old woman had had complaints of diminution of vision in both eyes since age 1 ½ years. Her visual acuity was 20/25 OU. Anterior segment evaluation revealed discrete opacities involving the anterior to mid-stroma in both eyes. The results of the rest of the ocular examination were normal.

RESULTS

Because the Arg555Trp mutation is a predominant cause of granular corneal dystrophy, we screened for the presence of this mutation in 9 members of the 2 families (Figure 1). All individuals were tested by digestion of the PCR-amplified product of exon 12 with BstXI as described in the “Methods” section. A homozygous C→T transition (at residue 1710 of TGFBI complementary DNA) corresponding to the Arg555Trp mutation was found in patients IV:2, IV:3, IV:5, and IV:7 from family A and in patient V:1 from family B. This change was heterozygous in patients V:1, V:2, and V:3 from family A and in patient IV:3 from family B. To confirm that the observed patterns of BstXI restriction digests corresponded to homozygous and heterozygous mutations, a representative sample of each of these patterns was subjected to direct sequencing (data not shown).

The clinical features of all patients are summarized in the Table. Patients with the homozygous Arg555Trp mutation had early-onset disease that manifested during childhood, severe visual loss, and a dense reticulate pattern of opacities on slitlamp examination that was superficial (Figures 2A and 3A). In family A (Figure 1), this included 4 siblings, who were the offspring of first cousins. Two of these (patients IV:3 and IV:7), who had corneal grafts, had a rapid recurrence of disease, with opacities redeveloping within 2 and 10 years of surgery, respectively. Their mother was reportedly affected, although we did not examine her. No information is available about the father, who was deceased. The children of these homozygous individuals are obligate heterozygotes. Three of the offspring were studied, namely, patients V:1, V:2, and V:3. As shown in the Table, although they had discrete granular opacities on slitlamp examination, they were asymptomatic (the oldest patient examined in this generation was 21 years old) and had visual acuities of 20/20 OU. The opacities were located in the anterior and midstroma, in contrast to the predominantly subepithelial opacities seen in the homozygous parents. In family B (Figure 1), patient V:1 was homozygous; she was the offspring of a consanguineous marriage. Her parents were reportedly affected, although they were not examined at our institution. The features of her disease were similar to those of the homozygous members of family A (Table). Patient IV:3 (Figure 1, family B) displayed a milder phenotype that was consistent with the typical form of granular corneal dystrophy.

Histopathologic examination of the keratectomy specimens from patients IV:3 and IV:5 of family A showed similar features. The epithelium was made up of 2 to 5 layers of cells, with areas of attenuation and absent Bowman layers. There were eosinophilic, patchy to confluent, irregular deposits seen in the subepithelial regions, causing attenuation of the overlying epithelium (Figure 4A). The deposits appeared bright red with Masson trichrome staining (Figure 4B).

The keratoplasty specimen from patient V:1 (family B) showed an intact epithelium with patchy, irregular deposits that were abundant in the subepithelial stroma, with some deposits in the deep stroma. The deposits stained bright red with Masson trichrome, consistent with granular corneal dystrophy (Figure 4C).

Patient IV:3

A 41-year-old woman had had complaints of diminution of vision in both eyes since age 1 ½ years. Her visual acuity was 20/25 OU. Anterior segment evaluation revealed discrete opacities involving the anterior to mid-stroma in both eyes. The results of the rest of the ocular examination were normal.
tion in the transthyretin gene, and in retinitis pigmentosa, caused by a mutation in the rhodopsin gene.16 At the other extreme is an instance of a homozygous missense mutation in the myocilin gene in a family with primary open-angle glaucoma, which resulted in a normal phenotype, whereas heterozygotes for the same mutation were affected.17 The presence of a phenotype only in heterozygotes has subsequently been linked to homozygous mutations in other organs.

Severe forms of granular corneal dystrophy have been documented in previous studies. Before knowledge of the genetic defect underlying granular corneal dystrophy, the severe phenotype was proposed to be due to homozygosity, as suggested by pedigree data.3-5,18,19 Such a phenotype has subsequently been linked to homozygous mutations of TGFBI, including Arg555Trp and Arg124His.20,21 The characteristics of the severe form of disease in all patients described in these earlier reports are early onset (in the first to second decades of life), rapid recurrence after surgery, and multiple surgical procedures.4,6 Notably, deposits are predominantly located in the superficial cornea, with most lesions at the level of Bowman layer,1,6 leading to the term superficial juvenile granular dystrophy.4 These features are similar to those of the homozygous patients in our study (Table and Figure 4). The superficial confluent nature of the deposits may be suggestive of Reis-Bücklers corneal dystrophy,4,22 but the clinical appearance of Reis-Bücklers corneal dystrophy, which consists of fine opacities that show a geographic pattern, is different from that of the patients in our study. Another unusual feature that we observed was that lesions extended close to the limbus in the homozygous individuals (Figure 2A), compared with the typical form of granular corneal dystrophy.

Superficial keratectomy, phototherapeutic keratectomy, and simple debridement are alternative modes of treatment of this condition. In both families, the deposits recurred after penetrating keratoplasty and lamellar keratoplasty (Table). Sajjadi and Javadi4 reported deeper recurrence after penetrating keratoplasty in patients with superficial juvenile granular corneal dystrophy.

The increased dosage effect due to homozygosity for the mutant TGFBI allele may explain an increased severity of disease, yet it is not clear why the deposits showed an altered distribution. Typical granular corneal dystrophy resulting from a heterozygous Arg555Trp mutation shows granular deposits primarily in the stroma. Keratoepithelin is present mainly in the corneal epithelium and Bowman layer but is also seen in the stromal interlamellar regions and at the Descemet membrane.23,24 However, TGFBI messenger RNA is expressed primarily in the epithelium of the normal adult cornea; during corneal

**Table. Clinical Features and Mutation Status of Patients With Granular Corneal Dystrophy**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age at Onset</th>
<th>Nature of Opacity</th>
<th>Location of Lesions</th>
<th>Prior Surgery*</th>
<th>VA at Presentation</th>
<th>Surgical Procedure</th>
<th>VA After Surgery</th>
<th>Final Clinical Picture</th>
<th>Mutation Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family A</td>
<td></td>
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<tr>
<td>IV:2</td>
<td>First to second decades</td>
<td>Coarse reticulate</td>
<td>Subepithelial</td>
<td>None</td>
<td>CF OU</td>
<td>PTK</td>
<td>20/125 OU</td>
<td>Central corneal haze</td>
<td>Homozygous</td>
</tr>
<tr>
<td>IV:3</td>
<td>First to second decades</td>
<td>Coarse reticulate</td>
<td>Subepithelial, anterior stroma</td>
<td>LK (left eye)</td>
<td>CF OU</td>
<td>Debridement (right eye), SK (left eye)</td>
<td>20/80 OD, 20/40 OS</td>
<td>Corneal haze</td>
<td>Homozygous</td>
</tr>
<tr>
<td>IV:5</td>
<td>First to second decades</td>
<td>Coarse reticulate</td>
<td>Subepithelial, anterior stroma</td>
<td>None</td>
<td>20/120 OU</td>
<td>SK (right eye), debridement (left eye)</td>
<td>20/60 OD, 20/125 OS</td>
<td>Slight corneal haze, midstromal deposits</td>
<td>Homozygous</td>
</tr>
<tr>
<td>IV:7</td>
<td>First to second decades</td>
<td>Coarse reticulate</td>
<td>Subepithelial</td>
<td>PK (right eye)</td>
<td>20/100 OD, 20/125 OS</td>
<td>Debridement (left eye)</td>
<td>20/60 OS</td>
<td>Filled graft (right eye), corneal haze (left eye)</td>
<td>Homozygous</td>
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<tr>
<td>V:1</td>
<td>Asymptomatic at 21 y</td>
<td>Discrete granular</td>
<td>Anterior to midstroma</td>
<td>None</td>
<td>20/20-20/25 OU</td>
<td>None</td>
<td>...</td>
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<td>Heterozygous</td>
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<tr>
<td>V:2</td>
<td>Asymptomatic at 18 y</td>
<td>Discrete granular</td>
<td>Anterior to midstroma</td>
<td>None</td>
<td>20/20 OU</td>
<td>None</td>
<td>...</td>
<td>...</td>
<td>Heterozygous</td>
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<tr>
<td>V:3</td>
<td>Asymptomatic at 14 y</td>
<td>Discrete granular</td>
<td>Anterior to midstroma</td>
<td>None</td>
<td>20/20 OU</td>
<td>None</td>
<td>...</td>
<td>...</td>
<td>Heterozygous</td>
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<tr>
<td>V:1</td>
<td>First to second decades</td>
<td>Confluent</td>
<td>Subepithelial, anterior stroma</td>
<td>PK (both eyes)</td>
<td>CF OU</td>
<td>PK repeated (right eye)</td>
<td>20/40 OD</td>
<td>Clear graft</td>
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<td>40 y</td>
<td>Discrete granular</td>
<td>Anterior to midstroma</td>
<td>None</td>
<td>20/25 OU</td>
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*Abbreviations: CF, counting fingers; LK, lamellar keratoplasty; PK, penetrating keratoplasty; PTK, phototherapeutic keratectomy; SK, superficial keratectomy; VA, visual acuity.

*Before the patient was seen at our institution.
The severe phenotype of the disease described herein is distinguishable from the typical heterozygous form of granular corneal dystrophy. The presence of these clinical features suggests possible underlying homozygosity, especially among patients from communities where inbreeding is prevalent. Our data further establish that there is a consistent genotype-phenotype correlation among homozygous individuals with granular corneal dystrophy across different populations.

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