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

Chitra Kannabiran, PhD; Mittanamalli S. Sridhar, MD; S. Kalyana Chakravarthi, MSc; Geeta K. Vemuganti, MD; Meena Lakshmipathi, MD

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.

Arch Ophthalmol. 2005;123:1127-1133

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 cornea.
trophies. These mutations lead to the formation of different types of granular and lattice types of corneal dys- 
crystals. Three subtypes of granular corneal dystrophy are distinguished based on clinical and histopathologic 
properties, which are distinctive for granular and lattice types of dystrophies. 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 nonamyloid in nature and stain positively with Masson trichrome. Ultrastructurally, the deposits appear as rhomboid-shaped crystals. Three subtypes of granular corneal dystrophy are distinguished based on clinical and histopathologic features. Granular corneal dystrophy type 1, originally described by Groenouw, is predominantly due to a mutation that results in an Arg555Trp substitution. We present herein the clinical and genetic analyses of individuals from 2 Indian families with multiple affected members having granular corneal dystrophy. Consanguinity was present in both families. There were 2 severities of manifestation in the families. Individuals with the more severe phenotype were homozygous for the Arg555Trp mutation, while those with a milder phenotype resembling the “typical” form of granular corneal dystrophy were heterozygous for the Arg555Trp mutation.

Molecular genetic analyses were performed in 7 individuals (patients IV-2, IV-3, IV-5, IV-7, V-1, V-2, and V-3) from family A and in 2 individuals (patients V-1 and IV-3) from family B (Figure 1). The study was approved by the institutional review board, and blood samples were collected from subjects after obtaining informed consent. Genomic DNA was isolated from leukocytes by standard procedures. Exon 12 of the TGFBI gene was amplified from 50 ng of genomic DNA by polymerase chain reaction (PCR) using specific primers (thermal cycler PTC200; MJ Research, Inc, Watertown, Mass). Sequences of the primers were 5’TACGCGTGGTACGATTAATTAAGG 3’ (forward) and 5’GGGCCCTGTGAAGGATC1ACACTAC 3’ (reverse).

Cycling conditions included an initial denaturation at 94°C for 1 minute, followed by 34 cycles of denaturation at 94°C for 45 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds. The amplified products were purified on Amicon columns (Millipore, Billerica, Mass) and directly sequenced on the ABI310 genetic analyzer (Applied Biosystems, Foster City, Calif) using PCR primers. Sequences were compared with those of healthy control samples and with the genomic sequence of TGFBI (GenBank accession No. AY149344, version AY149344.1).

The presence of the Arg555Trp mutation, located in exon 12, was tested by digestion with the enzyme BstXI. Polymerase chain reaction products of exon 12 were digested with BstXI, and the digested fragments were resolved on agarose gels. DNA from healthy individuals was not cut by the enzyme and showed a band corresponding to a fragment of 259 base pairs (bp), which was the full-length PCR product. In addition to the 259-bp fragment, the presence of a heterozygous mutation yielded 2 fragments of 182 and 77 bp, while the homozygous mutation resulted in 2 fragments of 182 and 77 bp. In addition, 100 healthy unrelated control subjects were screened for the sequence changes identified in patients by digestion with BstXI.

REPORT OF CASES

FAMILY A

Patient IV:2

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 worsened in the last 4 years. She had recurrent episodes of watering and photophobia in both eyes. On examination, she had a visual acuity of counting fingers in both the eyes. Corneal examination revealed coarse reticulate subepithelial opacities in both eyes, with spheroidal degeneration involving the central cornea. The opacities spared the limbus but were close to it (Figure 2 A). Details of the anterior chamber and lens were not visualized. She underwent phototherapeutic keratectomy in the right eye (Zyoptic; Bausch & Lomb, Rochester, NY). After the epithelial defect healed, she had a visual acuity of 20/125 OU and midstromal granular opacities and haze. She underwent debridement of the lesions in the left eye.

Patient IV:3

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

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.
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 reticular 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 reticular 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 reticular 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 clear 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 1½ years. Her visual acuity was 20/25 OU. Anterior segment evaluation revealed discrete opacities involving the anterior to midstroma 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 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.

Homozygosity for dominant disease genes is a rare occurrence, found in some communities where inbreeding is prevalent. Most dominant disease genes show partial dominance, resulting in an increased severity of disease manifestation in homozygotes, sometimes affecting other tissues or organs. There are few examples of true dominance, in which there are no differences in phenotype between homozygotes and heterozygotes for the disease gene. A prominent example of true dominance is Huntington disease. A similar phenomenon has been observed in vitreous amyloidosis, arising from a muta-
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 homozygotes has been attributed to a dominant negative effect of the heterozygous mutant allele.

We report herein for the first time, to our knowledge, the clinical, histopathologic, and genetic analyses of a severe form of granular corneal dystrophy in India. Our data from 2 families support the view that there is a “dosage” effect of the mutant protein in homozygotes for the Arg555Trp mutation in keratoepithelin. Compared with heterozygotes from the same family, individuals homozygous for the mutation displayed a severe phenotype. Despite the ubiquitous expression pattern of the TGFBI gene, homozygotes had no obvious manifestations 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,1,5 leading to the term superficial juvenile granular dystrophy.5 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 Javadi6 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>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<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>Failed graft (right eye), corneal haze (left eye)</td>
<td>Homozygous</td>
</tr>
<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>
<td>...</td>
<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>
</tr>
<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>Family B</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>
<td>Homozygous</td>
</tr>
<tr>
<td>IV:3</td>
<td>40 y</td>
<td>Discrete granular</td>
<td>Anterior to midstroma</td>
<td>None</td>
<td>20/25 OU</td>
<td>None</td>
<td>...</td>
<td>...</td>
<td>Heterozygous</td>
</tr>
</tbody>
</table>

*Before the patient was seen at our institution.

Abbreviations: CF, counting fingers; LK, lamellar keratoplasty; PK, penetrating keratoplasty; PTK, phototherapeutic keratectomy; SK, superficial keratectomy; VA, visual acuity.
development and wound healing, it is expressed in all regions of the cornea.23 Mutant keratoepithelin is presumably a component of the corneal deposits found in granular and lattice dystrophies, as indicated by immunohistochemical studies.24,26 Although, to our knowledge, there have been no studies on the Arg555Trp-induced changes in the keratoepithelin protein, it can be speculated from investigation of the Arg124Leu mutation that the nonamyloid types of deposits do not result from abnormal proteolysis of keratoepithelin but may be associated with a change in protein stability or abnormal interactions of keratoepithelin. The predominantly epithelial location of deposits in homozygous individuals may suggest that the wild-type allele of TGFBI has a protective or alleviating effect and that its absence, as in the case of a homozygous Arg555Trp mutant, promotes the deposition of other proteins of epithelial origin along with mutant keratoepithelin.

Whatever the exact pathogenesis of the homozygous form of granular corneal dystrophy, the resulting phenotype in terms of visual prognosis and pattern of corneal deposits appears distinctive in its severity, as observed in our patients and in previously described patients from different populations.4,6 We suggest that 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.

Submitted for Publication: September 29, 2003; final revision received September 28, 2004; accepted September 30, 2004.

Correspondence: Chitra Kannabiran, PhD, Kallam Anji Reddy Molecular Genetics Laboratory, Professor Brien Holden Eye Research Centre, L. V. Prasad Eye Institute, L. V. Prasad Marg, Banjara Hills, Hyderabad 500 034, India (chitra@lpei.org).

Funding/Support: This work was supported by the Hyderabad Eye Research Foundation, L. V. Prasad Eye Institute, and by a fellowship from the Council of Scientific & Industrial Research, New Delhi, India (Mr. Chakravarthi), and by grant BT/PRPR3573/Med/12/157 from the Department of Biotechnology, Government of India (Dr. Kannabiran).

REFERENCES


April 2005 Web Quiz Winner

Congratulations to the winner of our April quiz, Steven M. Friedlander, MD, FACS, Nevada Retina Associates, Reno, Nev. The correct answer to our April challenge was central retinal vein occlusion due to an intraocular nematode. For a complete discussion of this case, see the Photo Essays section in the May ARCHIVES (Greven CM. Central retinal vein occlusion secondary to an intraocular nematode. Arch Ophthalmol. 2005;123:704).

Be sure to visit the Archives of Ophthalmology Web site (http://www.archophthalmol.com) and try your hand at our Clinical Challenge Interactive Quiz. We invite visitors to make a diagnosis based on selected information from a case report or other feature scheduled to be published in the following month’s print edition of the ARCHIVES. The first visitor to e-mail our Web editors with the correct answer will be recognized in the print journal and on our Web site and will also be able to choose one of the following books published by AMA Press: Clinical Eye Atlas, Clinical Retina, or Users’ Guides to the Medical Literature.

Figure 1. Right eye at initial examination showing optic disc edema, hemorrhagic retinopathy, and distended veins.