Survey of Patients With Granular, Lattice, Avellino, and Reis-Bücklers Corneal Dystrophies for Mutations in the BIGH3 and Gelsolin Genes

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**Objectives:** To search for novel mutations that cause corneal stromal dystrophies and to confirm or revise the clinical diagnosis of patients with these mutations.

**Patients:** Through review of the records of the Cogan Eye Pathology Laboratory at the Massachusetts Eye and Ear Infirmary, Boston, and of clinical records, we ascertained 14 unrelated patients with the clinical or histopathologic diagnosis of granular (3 cases), Avellino (5 cases), lattice (5 cases), or Reis-Bücklers (1 case) corneal dystrophy.

**Methods:** Clinical records and histopathologic findings of the index patients and their relatives were reviewed. Patients and selected relatives donated a blood sample from which leukocyte DNA was purified and assayed for mutations in the BIGH3 gene and, in 2 patients, the gelsolin gene, using the polymerase chain reaction and direct genomic sequencing.

**Results:** All index patients with the diagnosis of granular dystrophy or Avellino dystrophy had the missense mutation Arg555Trp or Arg124His, respectively, previously reported in the BIGH3 gene. Of the 5 index patients with a prior diagnosis of lattice dystrophy, 2 had the originally reported lattice mutation (Arg124Cys) in the BIGH3 gene, 1 had a more recently reported missense mutation (His626Arg) in the same gene, 1 had the missense mutation Asp187Asn in the gelsolin gene, and 1 had no detected mutation in either gene. Affected members of the family with Reis-Bücklers dystrophy did not carry the previously reported mutations Arg555Gln or Arg124Leu but instead carried a novel missense mutation Gly623Asp in the BIGH3 gene.

**Conclusions:** Molecular genetic analysis can improve the accuracy of diagnosis of patients with corneal dystrophies. Two patients with a prior diagnosis of lattice corneal dystrophy had their diagnosis changed to gelsinol-related amyloidosis (1 case) or secondary, nonhereditary localized amyloidosis (1 case). A novel mutation in the BIGH3 gene that causes Reis-Bücklers dystrophy was uncovered through this analysis, and another recently reported novel mutation was encountered. These findings serve to expand our knowledge of the spectrum of pathogenic mutations in BIGH3.


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**The Study** by Jones and Zimmerman in 1961 provided a framework for the classification of granular, lattice, and macular dystrophies of the corneal stroma according to their clinical appearance and, in particular, their histopathologic staining patterns. Specifically, the dominantly inherited granular and lattice dystrophies featured stromal deposits of hyaline material that was red with the Masson trichrome stain and amyloid material that was highlighted with the Congo red stain, respectively. Corneas with macular dystrophy (recessively inherited) had stromas with an abundance of mucopolysaccharides that stained with Alcian blue. Subsequent refinement of the diagnostic classification added the categories Avellino dystrophy (a dominantly inherited disease that shares features of both lattice and granular dystrophy), lattice type II (a dominantly inherited systemic form of amyloidosis involving the cornea, skin, kidney, and other tissues; also known as Meretoja syndrome), lattice type III (a late-onset dystrophy that features amyloid deposits in the stroma and is usually inherited as a recessive trait; it is termed lattice type IIIA when it is inherited as a dominant trait).

By 1996, genetic linkage studies had shown that the mutations responsible for granular, lattice, and Avellino stromal dystrophies were all within the same region of chromosome 5q, as was the gene for the subepithelial dystrophies called Reis-Bücklers corneal dystrophy or Thiel-Behnke corneal dystrophy. The gene that causes macular corneal dystrophy was mapped to chromosome 16q. Subsequent studies showed that specific mutations in the BIGH3 gene cause the stro-
METHODS

This study conformed to the Declaration of Helsinki regarding the enrollment of human subjects. Phlebotomy was performed according to standard methods to obtain 10 to 30 mL of venous blood from participating subjects. Leukocyte DNA was purified using proteinase K digestion followed by chloroform and phenol extractions and ethanol precipitation.

The exons of the BIGH3 gene and in 2 patients the gelsolin gene were amplified from leukocyte DNA using the polymerase chain reaction. We evaluated first the exons containing codons 124 and 555, where the originally reported BIGH3 mutations are located. If no mutations were found in these regions, the remainder of the coding sequence was evaluated by direct sequencing. The oligonucleotide primers used for the polymerase chain reaction were those reported by Munier et al, with the exception of exons 1, 2, 8, 13, and 17. The primer pairs used to amplify these exons were, respectively (sense/antisense; 5’-3’):

1. GCGCTCTCATTCCCTGGAG/GACCTCT-TGACCTCCCGAC, GGTGCAGTGCTGTATCCTAGCCAGCGTATCATCGT, CTTGACCTGAGTCGTTTGG/GAAGTGCGCCAAAGATCTCT, GGGATTAATCTACTTCCATT/TTGATATAATTCCATCCCTGG, and GGGATCATGTACCTATTTTG/TGGTGCAATCTCTGTCGAG. The primer pair used to amplify nucleotides 565 through 680 of the gelsolin gene was reported by de la Chapelle et al. Amplified DNA fragments were sequenced directly according to the protocols accompanying the Thermo Sequenase cycle sequencing kit (United States Biochemical, Cleveland, Ohio) and using dyeoxy-nucleotide triphosphates that were α-labeled with phosphorus 33.

Tables 1

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Visual Acuity</th>
<th>Mutation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCD1/F/51</td>
<td>20/200 OD</td>
<td>Gelsolin, Aep187Aen</td>
</tr>
<tr>
<td>LCD2/F/45</td>
<td>20/70 OD</td>
<td>His628Arg</td>
</tr>
<tr>
<td>LCD3/M/38</td>
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<td>Arg1240ys</td>
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<tr>
<td>LCD4/F/40</td>
<td>20/100 OD</td>
<td>Arg1240ys</td>
</tr>
<tr>
<td>LCD5/F/63</td>
<td>20/200 OD</td>
<td>No mutation found</td>
</tr>
<tr>
<td>RBCD1/F/64</td>
<td>20/200 OD</td>
<td>Gly623Aep</td>
</tr>
</tbody>
</table>

*Mutation in BIGH3 gene unless otherwise specified.
†Plus sign indicates no penetrating keratoplasty has been performed in these patients.

REPORT OF CASES

Eleven of the 14 index patients were identified through the archives of the Cogan Eye Pathology Laboratory at the Massachusetts Eye and Ear Infirmary, where specimens obtained during corneal transplantation are processed. Specimens with the diagnosis of lattice, granular, or Avellino dystrophy were reviewed. The corresponding surgeons and subsequently the index patients were contacted and asked to participate in the study by providing a blood sample for DNA analysis. Three index patients ascertained through the clinical practices of the authors had corneal dystrophies not yet treated with a transplant. Affected and unaffected relatives of some patients were contacted and also asked to participate in the study. Table 1 lists the sex, age at the time of the initial penetrating keratoplasty, and the visual acuity in that eye at the time of penetrating keratoplasty. Brief descriptions of the clinical and histologic features of these cases follow.

GRANULAR CORNEAL DYSTROPHY

Three index patients had granular corneal dystrophy. Two patients, GCD1 and GCD2 (pathology numbers E91-4450 and E86-177, respectively), came from families with an inheritance pattern consistent with dominant inheritance. GCD3 has an unknown family history and has not yet undergone penetrating keratoplasty. On slitlamp examination, index case patients had features typical of granular dystrophy (Figure 1A). Histopathologically, the corneas had hyaline deposits in the stroma that stained red with the Masson trichrome stain (Figure 1B).

AVELLINO CORNEAL DYSTROPHY

Five patients had Avellino corneal dystrophy. Index patients ACD1 through ACD3 had undergone penetrating keratoplasty (pathology numbers E86-366, S97R44231, and S98A13291, respectively), whereas patients ACD4 and ACD5 had not as yet. All 5 patients with Avellino dystrophy came from families with an apparently dominant in-

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heritance pattern. They all reported ancestors who lived in the area surrounding Avellino, Italy. The index patients had stromal opacities that were larger than those seen in lattice dystrophy and had snowflake shapes (Figure 1C). Histopathologically, the deposits stained with both the Masson trichrome stain and the Congo red stain (Figure 1D-E).

**LATTICE CORNEAL DYSTROPHY**

Five index patients had lattice corneal dystrophy. Patients LCD1 through LCD4 (pathology numbers S97D42704, E90-3246, S97F50454, and E84-999, respectively) came from families with corneal dystrophy transmitted in an apparently dominant inheritance pattern. They all had typical latticelike stromal deposits seen with the slitlamp. The clinical and histopathologic findings of one of these patients (LCD2) are shown in Figure 1F-G. Of these 4 index cases, patient LCD1 was notable because the patient's affected maternal uncle had the diagnosis of Ehlers-Danlos syndrome made at another institution because of lax skin (Figure 1H); molecular genetic analysis indicated that the diagnosis of the Ehlers-Danlos syndrome in this family was incorrect (see below). This relative had peripheral neuropathy, but reportedly a
Peripheral nerve biopsy specimen failed to show amyloid. The patient’s sister and mother had similar skin abnormalities by history. Slitlamp and histopathologic findings of the index patient are shown in Figure 1 I-J.

The fifth case of lattice dystrophy, patient LCD5 (pathology number S97T43634), was a 63-year-old woman with no family history of corneal dystrophy. Bilateral corneal opacities were present since the age of 5 years by history. The stromal deposits were diffuse and without lattice lines (Figure 1K). Histopathologically, the corneal stroma had numerous congophilic deposits that showed birefringence and dichroism and were interpreted as consistent with lattice dystrophy (Figure 1L).

**REIS-BÜCKLERS CORNEAL DYSTROPHY**

One patient had Reis-Bücklers corneal dystrophy. The index patient RBCD1 (pathology number E92-2215) had a history of recurrent painful corneal erosions since childhood. There were subepithelial corneal opacities in a geographic pattern. Histopathologically, Bowman’s layer was disrupted with fibrous tissue and hyaline deposits that did not stain with periodic acid–Schiff stain or with the Masson trichrome or Congo red stains. Slitlamp and histopathologic findings are shown in Figure 1M-N.

**RESULTS**

All 3 index patients with the clinicohistopathologic diagnosis of granular dystrophy, as well as 2 affected relatives of these patients who participated in this study, had the missense mutation Arg555Trp (CGC to TGG) in the BIGH3 gene. None of the unaffected relatives of these patients was analyzed. All 5 index patients with Avellino dystrophy (ACD1-ACD5) and 3 of their affected relatives had the missense mutation Arg124His (CGC to CAC) in the same gene. None of the unaffected relatives of these patients was analyzed.

Of the 5 index patients with a prior diagnosis of lattice dystrophy, patients LCD3 and LCD4 had the missense mutation Arg124Cys (CGC to TGC) in the BIGH3 gene. One affected relative of one of these patients was analyzed and was found also to carry this mutation, whereas one unaffected relative who was analyzed did not.

Patients LCD1, LCD2, and LCD5 with clinically diagnosed lattice dystrophy had none of the reported mutations affecting codons 555 and 124, so the entire coding sequence of the BIGH3 gene was analyzed. Patient LCD2 (Figure 1F-G) heterozygously carried the missense mutation His626Arg (CAT to CGT). A schematic pedigree of the family of this patient is shown in Figure 2. Of 10 affected relatives who were analyzed (individuals II:15, II:17, III:3, III:6, III:7, III:8, III:10, III:11, IV:1, and IV:2), all carried this same mutation heterozygously. Of 2 unaffected relatives who were analyzed (individuals II:19 and IV:4), 1 carried the mutation and 1 did not. The unaffected carrier (individual IV:4) was 34 years old at the time of the last ocular examination. A slitlamp photograph of the cornea of this patient is shown in Figure 10.

In patients LCD1 and LCD5, no sequence changes that would alter the primary structure of BIGH3 were found. Subsequently, the sequence of codon 187 of the gelsolin gene was determined in these 2 patients. In patient LCD1, the missense mutation Asp187Asn (GAC to
AAC) was found in the gelsolin gene, a mutation previously reported to cause gelsolin-related amyloidosis (Meretoja syndrome). Analysis of DNA from the index patient's affected sister showed that she also carried this mutation heterozygously. We did not analyze the DNA from any other relative in this family. The full-face photograph of the index patient's affected maternal uncle is shown in Figure 1H.

Patient LCD5 had no mutation in the coding sequence of the \textit{BIGH3} gene and no mutation of codon 187 in the gelsolin gene. In view of these findings and the negative family history, and because the patient's history and slitlamp findings were atypical for lattice dystrophy, this patient's diagnosis was changed to presumed secondary amyloidosis.

The index patient RBCD1 with Reis-Bücklers corneal dystrophy, a 70-year-old woman, had no defect in the sequence of codons 124 or 555 of the \textit{BIGH3} gene. A subsequent determination of the entire \textit{BIGH3} coding sequence revealed the missense change Gly623Asp (GGC to GAT) (Figure 3). The patient's affected 89-year-old aunt (I:4) and this aunt's affected 54-year-old son (II:10) also carried the same mutation (see Figure 2 for pedigree). The deceased mother of the index patient was affected by history. DNA from 2 unaffected relatives was also evaluated: a 65-year-old sister (individual II:4) who did not carry this mutation and a 64-year-old maternal cousin (individual II:6) of the index patient who carried this missense mutation. This mutation was not found among a screen of 95 unrelated, healthy control individuals.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{pedigree.png}
\caption{Schematic pedigree of the family with lattice corneal dystrophy and the His626Arg missense mutation (top) and the family with Reis-Bücklers dystrophy and the Gly623Asp mutation (bottom). Arrows point to the index cases. Filled symbols indicate affected individuals. Beneath the symbols of individuals whose DNA was analyzed are their \textit{BIGH3} genotypes.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{sequence.png}
\caption{DNA sequence around codon 623 of the \textit{BIGH3} gene in the index patient RBCD1 (E92-2215) with Reis-Bücklers dystrophy and an unaffected control individual. The patient is heterozygous with both the wild-type sequence of codon 623 (GGC), specifying glycine, and a mutant sequence (GAT), specifying aspartic acid.}
\end{figure}

The diagnosis of hereditary corneal dystrophies is customarily based on slitlamp and histopathologic findings. Until the identification of the responsible genes, there was no way to test the accuracy of the clinicopathologic...
diagnosis. Particularly disconcerting were patients with equivocal or ambiguous findings. Examples were cases with clinical and histopathologic features of both granular and lattice dystrophy (cases now categorized as Avellino dystrophy)\(^3\) and cases with features of both granular and Reis-Bucklers dystrophy.\(^20\) The identification of the gene defects responsible for most of the corneal dystrophies provides precision to the diagnosis.

Our study demonstrates the diagnostic value of molecular genetic analysis of patients with corneal dystrophies. In 2 cases, DNA analysis altered the prior diagnosis based on clinical and histopathologic findings. In 1 case (LCD1), the patient and some affected relatives had the clinicopathologic diagnosis of lattice dystrophy type I. Lax skin in some affected relatives prompted the additional diagnosis of Ehlers-Danlos syndrome that was presumed to be fortuitously present. Our identification of a mutation in the gelsolin gene provided the correct diagnosis, Meretoja syndrome, which explained both the ocular and systemic disease in the affected family members. In a second case, DNA analysis failed to uncover a mutation in either the BIGH3 or gelsolin gene. Subsequent review of the clinical findings suggested that the corneal amyloid deposits were secondary to a corneal disease of uncertain etiology that occurred in childhood.

The original report\(^13\) of mutations in the BIGH3 gene associated each of 4 corneal dystrophies (granular, lattice, Avellino, and Reis-Bucklers) with its own causative mutation. All subsequently described patients with granular or Avellino dystrophy have had the originally reported BIGH3 mutations (Table 2 and Figure 4), suggesting that these clinically defined entities are genetically homogeneous. This is not true for lattice and Reis-Bucklers dystrophies. Although new cases with the originally reported lattice and Reis-Bucklers mutations have been found by other groups, additional mutations in BIGH3 have also been encountered (Table 2). Our analysis adds to the multiplicity of mutations that can cause Reis-Bucklers dystrophy, because our patient carried a novel mutation in the BIGH3 gene. This mutation, Gly623Asp, is 3 codons away from the reported mutation, His626Arg,\(^2\)\(^,3\) causing lattice corneal dystrophy. Gly623Asp is the fourth mutation reported so far to be associated with Reis-Bucklers dystrophy (Table 2).\(^13,30,32\) Although one individual who carries the Gly623Asp mutation has not exhibited disease at the age of 64 years, it is nevertheless likely that the mutation is pathogenic, because no other mutation in the coding region of the BIGH3 gene was found in the index patient and because the identified mutation was present in all affected relatives examined. Furthermore, the Gly623Asp change has never been previously reported among patients or healthy controls, and we did not find it in our screening of 95 healthy controls (190 chromosomes). With regard to the unaffected Gly623Asp carrier, it is possible that this patient will develop subepithelial opacities later in life or he may be an example of reduced penetrance of a BIGH3 mutation. To our knowledge, there are no prior examples of reduced penetrance for dominant BIGH3 mutations.

We found another possible example of reduced severity vs incomplete penetrance in the family of index patient LCD2 with lattice corneal dystrophy and the mutation His626Arg. A 34-year-old woman in this family (relative IV:4 of patient LCD2) carried this mutation but was asymptomatic and had no corneal deposits seen by slitlamp examination. The affected members of 3 previously reported families with His626Arg mutation exhibited a late disease onset, with symptoms in some cases not arising until the fourth or fifth decade of life.\(^28,29\) Considering these reports, one might predict that our 34-year-old asymptomatic carrier will develop lattice dystrophy later in life.

The function of the BIGH3 protein in the cornea is unknown, but its primary sequence (683 amino acids) has features suggesting a role in cell adhesion, perhaps through binding to integrins.\(^33\) It is normally produced by the corneal epithelium\(^35,34\) and resides in the stroma, particularly in Bowman's layer.\(^35\) The protein or degradation products thereof are found in the stromal and subepithelial deposits that characterize granular, lattice, Avellino, and Reis-Bucklers dystrophies.\(^24,30\) To date, all...
reported pathogenic mutations in the BIGH3 gene result in the change or deletion of a single amino acid in the encoded protein (Table 2 and Figure 4). We have only a rudimentary knowledge of the protein product of the BIGH3 gene, so it remains unclear how the structures of the mutant protein products form the corneal deposits of various shapes and histopathologic staining patterns.

Although the BIGH3 mutations causing lattice type I, granular, Avellino, and Reis-Bücklers dystrophies are referred to as dominant alleles, at least 2 of them are actually semidominant. Patients who are homozygous for the Avellino (Arg124His)21,22 or granular (Arg355Trp)24 mutations have been identified, and they are more severely affected than heterozygotes, with visually debilitating corneal deposits appearing within the first decade of life and recurring soon after corneal transplantation. It is not known whether the mutations causing lattice type I and Reis-Bücklers dystrophy are semidominant, since homozygotes have not been reported.

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