Congenital Stromal Dystrophy of the Cornea Caused by a Mutation in the Decorin Gene

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Purpose. To describe the clinical and pathologic characteristics of a family with a congenital stromal dystrophy of the cornea and to identify the genetic basis for this disorder.

Methods. All family members in three generations underwent ophthalmic examination. Stored corneal buttons were examined by transmission electron microscopy. Molecular genetic studies, including a genome-wide scan with microsatellite markers, linkage analysis, and DNA sequencing, were performed.

Results. The dystrophy was inherited in an autosomal dominant pattern and was seen as clouded corneas shortly after birth. No associated systemic abnormalities or congenital diseases were present. After penetrating keratoplasty (PK), the grafts remained clear in 56% of the eyes with a mean (range) observation period of 19.5 years (3–36). Transmission electron microscopy of corneal buttons revealed lamellae with normal arrangement of collagen fibrils separated by abnormal fibrillar layers. Genome-wide screening revealed linkage to chromosome 12q22, with a maximum LOD score of 4.68 at D12S351. Subsequent sequencing of candidate genes revealed a frameshift mutation in the DCN gene (c.967delT) that encodes for decorin, predicting a C-terminal truncation of the decorin protein (p.S523fsX5).

Conclusions. The authors hypothesize that truncated decorin binds to collagen in a suboptimal way, disturbing the regularity of corneal collagen fibril formation and thereby causing corneal opacities. To the best of the authors’ knowledge, this is the first description of a disorder associated with an inherited alteration in the decorin gene in humans. (Invest Ophthalmol Vis Sci. 2005;46:420–426) DOI:10.1167/iovs.04-0804

The corneal dystrophies are inherited, bilateral, primary alterations of the cornea that are not associated with prior inflammation or secondary to systemic disease. Most show autosomal dominant inheritance and appear in the first decades of life. The corneal dystrophies that present at birth. No associated systemic abnormalities or congenital diseases are present. After penetrating keratoplasty (PK), the grafts remained clear in 56% of the eyes with a mean (range) observation period of 19.5 years (3–36). Transmission electron microscopy of corneal buttons revealed lamellae with normal arrangement of collagen fibrils separated by abnormal fibrillar layers. Genome-wide screening revealed linkage to chromosome 12q22, with a maximum LOD score of 4.68 at D12S351. Subsequent sequencing of candidate genes revealed a frameshift mutation in the DCN gene (c.967delT) that encodes for decorin, predicting a C-terminal truncation of the decorin protein (p.S523fsX5).

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pachymetry (Pachymeter Echograph; Quantel Medical, Clermont-Ferrand, France) and keratometry (Auto Keratometer KM-500; Nidek, Gamagori, Japan). Two eyes that had not been subjected to keratoplasty and four eyes in three patients showing corneal opacities in the corneal graft were examined by confocal microscopy (ConfoScan 3; Nidek). Slit lamp photographs were taken of all eyes.

Transmission Electron Microscopy

Corneal buttons obtained from affected individuals after corneal transplantation had been fixed and stored in 2% glutaraldehyde in 0.2 M cacodylate buffer. For this study, they were osmicated and embedded in epoxy resin. Ultrathin sections were stained with 2% uranyl acetate and Reynold’s lead citrate and examined by transmission electron microscopy (TEM) (JEM 1230; JEOL, Tokyo, Japan).

Genotyping

Genomic DNA was isolated from whole blood using a nucleic acid extractor (model 341; Applied Biosystems, Foster City, CA). Five loci (CHST6, KRT3, KRT12, TGFBI, and COL8A2), in which mutations have been reported in patients with corneal dystrophy, were excluded as candidates in preliminary studies by marker analysis (results not shown). The candidate regions CDPD1 and CHED1 on chromosome 20 (loci not known) were also excluded by marker analysis.

A genome-wide scan was performed using a set of 400 microsatellite markers with an average spacing of 10 cM (PRISM Linkage Mapping Set MD, Ver. 2; ABI). PCR and pipetting were then performed (Catalyst 800 Turbo Laboratory workstation; ABI). The PCR products were analyzed with a genetic analyzer (model 3100; ABI) and the GeneScan Analysis software (ABI). High-density mapping was performed with markers identified in the National Center for Biotechnology Information (NCBI) database (available at http://www.ncbi.nih.gov). Their genomic sequences, and all markers used, are found in a single megacotig [accession number NT_019546].

Linkage Analysis

Genotyping data were screened for errors using the PedCheck program. Markers exhibiting Mendelian inconsistencies were assigned an “unknown” genotype. Two point and multipoint linkage analyses, along with determination of the most likely haplotypes, were performed using the Allegro program. The analysis was performed under the assumption of a dominant mode of inheritance with 99% penetrance, 1% phenocopy rate, and 0.001 frequency of the disease allele. Marker allele frequencies were estimated from the family used in this study using the program PedManager (Mapmaker/PedManager).

DNA Sequencing and Mutation Detection

PCR primers for amplification of exons and flanking intron sequences in the lumican, keratocan, and decorin genes were designed on computer (Oligo 6.3 software; National Bioscience, Plymouth, MN) and sequence information are available on request. PCR amplification was performed at standard procedures using DNA polymerase (AmpliTaq Gold; ABI, or Taq; Qiagen, Hilden, Germany). After amplification, the PCR products were treated with SAP/exonuclease I (Amersham Bioscience, Uppsala, Sweden) and sequenced using a dye termination chemistry sequencing kit (Prism BigDye terminator, ver. 1.1; ABI), and analyzed on the genetic analyzer (model 3100; ABI). DNA sequences were analyzed on computer (Staden software).

A simple test for the decorin c.967delT mutation was designed. The pertinent sequence was PCR amplified using the forward (5’TTC TAT CGT TTC ATG TTG TAG T-3’) and reverse (5’GGG CTT TCT TGA GAA TTA CAT AT-3’) primers, followed by size determination of the fluorescence-labeled PCR product on a genetic analyzer (model 3100; ABI). DNA samples from 200 healthy local blood donors were used as control samples.
RESULTS

Medical History

The family members reported that corneal changes could be observed in affected individuals during the first months after birth. The patients described progressive deterioration of visual function over time. The patients did not have any other symptoms from the eyes, especially no symptoms of corneal erosions or photophobia. No other systemic abnormalities or malformations were recorded. Specifically, there were no recognized problems related to skin, teeth, joints, or bones. Bone density measurements were within normal range (data not shown).

The two affected women in generation II died of breast cancer at the age of 46 years and a brain tumor at the age of 58 years. One affected member in the third generation was 70 years old when he died of lymphoma/leukemia. Another individual with the corneal disease had breast cancer when she was 43 years old and colon cancer at the age of 70 years. The unaffected II-3 died young in an accident. None of the unaffected family members in the pedigree have had malignant disease. One affected member of the family have had complicated juvenile diabetes mellitus from the age of 12 years, but this did not seem to have any influence on her corneal disease. Her unaffected sister also has complicated juvenile diabetes mellitus.

Clinical Examination

Only four eyes had not been subjected to PK. Slit lamp examination of these eyes showed a slightly irregular surface. Throughout the stroma a large number of small opacities were seen (Fig. 2a). This was equally pronounced in all areas of the cornea. The endothelium could not be studied in detail due to these opacities. There was no sign of vascularization or staining with fluorescein. The corneal sensitivity was normal or slightly reduced.

Individual III-6 (age 73) had additional band-shaped keratopathy and III-1 (age 79) had arcus senilis in both eyes. Three eyes had primary open-angle glaucoma (III-6 and both eyes of IV-9), two of these eyes had not been subjected to keratoplasty. An iris cyst has been observed unchanged for many years in IV-2. Due to the opacities, gonioscopy was not possible. Bilateral senile cataract was observed in the older women (III-1 and III-6) as well as in IV-4 who also had diabetic retinopathy. Examination of the fundus after corneal transplantation did not reveal any abnormalities.

Among the 11 affected family members 4 individuals had manifest strabismus; three had esotropia and one exotropia (III-1, III-6, V-1, and V-8). No strabismus was observed in the eight unaffected family members. There were no other motility problems and no persons with nyctagmus. The unaffected individual IV-5 had diabetic retinopathy and cataract. No other abnormalities were found in the eyes of the unaffected family members.
### Table 2. Results after Penetrating Keratoplasty

<table>
<thead>
<tr>
<th>Eye</th>
<th>Age at PK</th>
<th>Follow-up (y)</th>
<th>VA Pre-PK</th>
<th>Subjective Refraction Pre-PK</th>
<th>Complications</th>
<th>Opacities in Graft</th>
<th>Corneal Thickness (μm)</th>
<th>VA Post-PK</th>
</tr>
</thead>
<tbody>
<tr>
<td>III-1 (r)</td>
<td>42</td>
<td>36</td>
<td>0.3</td>
<td>No benefit</td>
<td>None</td>
<td>+</td>
<td>704</td>
<td>0.32</td>
</tr>
<tr>
<td>III-1 (l)</td>
<td>42</td>
<td>36</td>
<td>0.3</td>
<td>+3.5, sphere</td>
<td>None</td>
<td>647</td>
<td>0.5</td>
<td>0.32</td>
</tr>
<tr>
<td>III-2 (r)</td>
<td>20</td>
<td>31</td>
<td>0.3</td>
<td>−7, −1 × 10</td>
<td>Iris prolapse, postoperative</td>
<td>566</td>
<td>0.5</td>
<td>0.32</td>
</tr>
<tr>
<td>IV-4 (l)</td>
<td>21</td>
<td>28</td>
<td>0.3</td>
<td>−7, −4 × 0</td>
<td>Corneal opacities (not a recurrence); retransplant</td>
<td>600</td>
<td>0.1</td>
<td>0.32</td>
</tr>
<tr>
<td>IV-5 (r)</td>
<td>17</td>
<td>32</td>
<td>0.3</td>
<td>−1, −1 × 20</td>
<td>None</td>
<td>623</td>
<td>0.16</td>
<td>0.32</td>
</tr>
<tr>
<td>IV-4 (l)</td>
<td>21</td>
<td>28</td>
<td>0.3</td>
<td>−1, −1 × 20</td>
<td>None</td>
<td>658</td>
<td>0.16</td>
<td>0.32</td>
</tr>
<tr>
<td>IV-4 (r)</td>
<td>21</td>
<td>28</td>
<td>0.3</td>
<td>+2.5, −2 × 10</td>
<td>None</td>
<td>656</td>
<td>0.16</td>
<td>0.32</td>
</tr>
<tr>
<td>IV-10 (l)</td>
<td>21</td>
<td>23</td>
<td>0.63</td>
<td>+1.5, −2 × 10</td>
<td>None</td>
<td>595</td>
<td>0.63</td>
<td>0.32</td>
</tr>
<tr>
<td>V-1 (r)</td>
<td>16</td>
<td>9</td>
<td>0.1</td>
<td>No benefit</td>
<td>Iris prolapse, traumatic</td>
<td>+</td>
<td>549</td>
<td>0.16</td>
</tr>
<tr>
<td>V-1 (l)</td>
<td>20</td>
<td>5</td>
<td>0.3</td>
<td>No benefit</td>
<td>None</td>
<td>+</td>
<td>530</td>
<td>0.5</td>
</tr>
<tr>
<td>V-4 (l)</td>
<td>16</td>
<td>7</td>
<td>0.12</td>
<td>+1, sphere</td>
<td>Keratitis, perforation; retransplant</td>
<td>532</td>
<td>0.5</td>
<td>0.32</td>
</tr>
<tr>
<td>IV-4 (l)</td>
<td>6</td>
<td>17</td>
<td>0.16</td>
<td>−3, sphere</td>
<td>None</td>
<td>487</td>
<td>0.16</td>
<td>0.32</td>
</tr>
<tr>
<td>V-6 (r)</td>
<td>14</td>
<td>3</td>
<td>0.3</td>
<td>0</td>
<td>None</td>
<td>545</td>
<td>1.0</td>
<td>0.32</td>
</tr>
<tr>
<td>V-6 (l)</td>
<td>14</td>
<td>3</td>
<td>0.63</td>
<td>+1, −0.75 × 25</td>
<td>None</td>
<td>664</td>
<td>0.32</td>
<td>0.32</td>
</tr>
<tr>
<td>V-8 (r)</td>
<td>10</td>
<td>7</td>
<td>0.3</td>
<td>−4.5 × 175</td>
<td>None</td>
<td>519</td>
<td>0.32</td>
<td>0.32</td>
</tr>
<tr>
<td>V-8 (l)</td>
<td>13</td>
<td>4</td>
<td>0.25</td>
<td>+5, −4.5 × 5</td>
<td>Macular edema</td>
<td>513</td>
<td>0.32</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Eyes III-1 (r), III-6 (l), IV-9 (r), V-6 (r) had not been subjected to surgery but are included for completeness. (r), right eye; (l), left eye; Cf, counting fingers; Irrev, irreversible; 0, no opacities; +, minimal opacities; + +, moderate opacities; + + +, severe opacities.

The refractive errors in the affected family members are shown in Table 2. The corrected visual acuity (VA) in unoperated eyes varied from counting fingers in individuals III-1 and III-6 to 0.63 in IV-9 and V-6. The VA of the eyes before corneal transplantation was obtained from medical records. Best corrected VA varied from counting fingers to 0.63, with a mean of 0.25. One eye was aphakic myopic, eight emmetropic (or no benefit from corrective glasses) and eight hypermetropic.

The mean (range) corneal diameter in affected eyes was 12.2 mm (11.5–12.5) which was similar to the diameter of 12.5 mm (12.1–13) in the eyes of the unaffected family members. Pachymetry showed a mean corneal thickness in affected eyes of 673 μm (658–704) as opposed to the unaffected eyes, which had a mean corneal thickness of 544 μm (511–620). The mean thickness of eyes with no postoperative opacities was 543 μm (506–623) and in those with minimal clouding, 565 μm (487–647). The eye with the most severe opacity was the eye with the most extensive opacities. Three patients required retransplantation, one (V-4) because of keratitis and perforation 7 months after surgery, another (IV-4) because of superficial corneal opacities that were not related to the stromal dystrophy (Table 2) and patient IV-7, because of corneal clouding (data not shown).

### Penetrating Keratoplasty

Four individuals (III-1, III-6, IV-9, and V-6) had been treated with PK in only one eye. The remaining seven affected individuals had undergone bilateral surgery. Thus, a total of 18 eyes had undergone PK. The mean (range) age at surgery was 20 years (6–44). The mean follow-up time for these eyes was 19.5 years (3–36). Ten transplanted corneas (56%) were without any sign of opacities, whereas six (33%) showed minimal changes. In two (11%) individuals (IV-10 and IV-7) one eye had moderate or severe opacities (Table 2, Fig. 2c). In patient IV-7 corneal clouding was first seen in the left eye 15 months after transplantation. The opacities then developed rapidly and, after 3 years, the graft was described to have similar changes as the remaining host cornea. The graft in his right eye has remained clear for 30 years after surgery (Fig 2b). The right eye of patient IV-10 had chronic iridocyclitis, secondary glaucoma and persistent mydriasis after keratoplasty. The graft was described as almost clear 21 years after the operation with VA 1.0. Now, 27 years after the keratoplasty, moderate clouding was observed with a VA of 0.5. Only minimal opacities were seen in the graft of his left eye with an observation period of 23 years.

The best corrected visual acuity after PK varied from counting fingers to 1.0, with a mean value of 0.35 (Table 2). The eye with the worst visual acuity was the eye with the most extensive opacities. Three patients required retransplantation, one (V-4) because of keratitis and perforation 7 months after surgery, another (IV-4) because of superficial corneal opacities that were not related to the stromal dystrophy (Table 2) and patient IV-7, because of corneal clouding (data not shown).

### Confocal Microscopy

When examined with confocal microscopy in vivo the superficial epithelial cells of affected corneas appeared normal. From the anterior stroma, there was an increased reflectivity caused by the stromal opacities. The opacities prevented detailed studies of the individual keratocytes and the endothelium.

When eyes with minimal clouding after keratoplasty were studied, endothelial changes were found with low cell counts (<700 cells/mm²), a high degree of polymegathism and pleomorphism. In one (V-4) of the four eyes studied, reflectivity from the stroma prevented detailed examination of the endothelium. In three eyes (IV-2 and both eyes of V-1) there were changes in the stroma compatible with corneal edema (data not shown).
Transmission Electron Microscopy

When the corneal buttons were examined by TEM (Fig. 3a–c) the corneal epithelium and its thin basement membrane were found to be normal. The posterior part of Bowman’s layer was slightly irregular. In the stroma lamellae of seemingly normal collagen fibrils were separated by abnormal layers that consisted of thin filaments. These filaments were randomly arranged and embedded in an electron-lucent ground substance. The abnormal layers were broader in the posterior than in the anterior stroma. The keratocytes had a normal appearance, without inclusions. Descemet’s membrane (D) was of normal thickness, with a normal banding of the anterior portion. There were no vacuoles or lacunas in Descemet’s membrane, but both the anterior and posterior borders were slightly irregular. The corneal endothelium (E) was of normal thickness and appearance, with interdigitating cell membranes. Original magnification (a) ×8000; (b) ×6000, (c) ×2600.

Mode of Inheritance

The dystrophy was present in both males and in females in approximately equal proportions. Each generation had affected individuals, and the disease was not transmitted through unaffected persons. There were examples of male-to-male transmission (Fig. 1). Thus, the observed pattern was compatible with autosomal dominant inheritance with complete penetrance.

Linkage Analysis

Genome-wide screening was performed and revealed linkage to chromosome 12q22 with a maximum LOD score of 4.68 at the marker D12S351. No other candidate regions with LOD scores >1.5 were detected. High-resolution mapping was subsequently used to narrow down the candidate region to an 8.4-Mb region between the markers D12S1719 and D12S101 (Fig. 4). Within this interval, haplotype analysis indicated that all the affected family members shared a single ancestral haplotype, whereas this haplotype was not present in any of the unaffected individuals.

In our candidate region, we identified three genes known to be relevant for the eye. Lumican, keratocan, and decorin are all expressed in the human cornea. The coding sequences and flanking introns of these genes were sequenced. No sequence variants were detected in the lumican or the keratocan gene. In the decorin gene, DNA sequencing identified a deletion of 1 bp in exon 10 (c.967delT; Fig. 5). PCR amplification followed by capillary electrophoresis verified a 1-bp deletion in all affected family members. The deletion was observed neither in any healthy members of the family nor in 200 control samples. The c.967delT mutation leads to a frame shift and predicts the expression of a truncated decorin protein lacking the 33 C-terminal amino acids (p.S323fsX5).

DISCUSSION

The clinical and pathologic characteristics of a family with an autosomal dominant congenital stromal dystrophy of the cornea are described. Genetic analysis showed that the disorder is cosegregating with a mutation in the decorin gene. Support for the congenital nature of the disease is provided by the parents of affected individuals who have observed the...
corneal changes shortly after birth. Odland,\(^2\) reported the changes in one individual at the age of 1 year. He also noted that the opacities increased with age indicating a progressive nature of the disease.

As is common with diseases with congenital corneal opacities, refractive errors, amblyopia, and strabismus were observed in the affected individuals. The manifestation of the dystrophy in the present family differs in some aspects from that reported in the other known families with congenital stromal dystrophy of the cornea. The two affected patients in the family reported by Van Ginderdeuren et al.,\(^5\) had severe photophobia, and four of five patients described by Witschel et al.,\(^4\) had a searching nystagmus and all presented with an alternating esotropia. Nystagmus and photophobia were not seen in any of the affected individuals in our study. However, visual acuity was better in our patients than in those described by Witschel et al., which could be relevant for the development of nystagmus. Furthermore, the corneas in the other pedigrees are described to be of normal thickness and were measured to be so by Pouliquen et al.,\(^10\) whereas we observed an increased corneal thickness. The different reports describe similar morphologic changes in the stroma, but the status of Descemet’s membrane varied significantly between studies (Table 1). It is not uncommon that the clinical presentation of corneal dystrophies varies from one individual to another, as well as within a family that shares the same genetic defect. DNA analysis would clarify whether the reported families with congenital stromal dystrophy of the cornea represent different clinical manifestations of the same genetic defect or separate diseases.

In patients with corneal grafts, the thickness of the graft was related to the degree of corneal clouding. When corneas with opacities after grafting were examined by confocal microscopy and TEM, endothelial changes and corneal edema was seen. In addition, when examined by TEM, there were areas with collagen filaments different from the microfibrils typical of the dystrophy itself. Although these changes most likely represent corneal decompensation, a recurrence of the dystrophy in the graft cannot be excluded.

Decorin, a dermatan sulfate proteoglycan, is known to be involved in several important biological processes recently reviewed by Goldoni et al.\(^{11}\) Among these are the ability to modulate growth factor activity, tyrosine kinase receptor activity, cancer growth, angiogenesis, tissue remodeling, bacterial infection, and cardiovascular and periodontal disease.\(^{11}\) Decorin is important in collagen fibrillogenesis and binds to multiple collagen types, including types I,\(^{12}\) II,\(^{13}\) III,\(^{14}\) and VI.\(^{15}\) Homozygous decorin knockout mice do not show any gross anatomic abnormalities. Radiographic studies have not revealed any alterations in bone mass. However, they have fragile skin with dermal thinning and markedly reduced tensile strength. Ultrastructural analyses have revealed abnormal collagen morphology in skin and tendons, with coarser and irregular fiber outlines. The packing of the fibrils was shown to be loose and irregular. No reference was made to any corneal problems. Mice heterozygous for the knockout mutation are normal.\(^{16}\)

Decorin is known to modulate cancer growth.\(^{16}\) Although homozygous decorin-knockout mice do not show increased spontaneous tumorigenesis, Iozzo et al.\(^{17}\) reported that double-knockout mice deficient in both decorin and p53 show a faster rate of lymphoma development than deficiency of p53 alone. There were several cases of malignant disorders among the affected family members. However, due to the limited number of cases, the relationship between decorin and cancer cannot be established.
of cases, it is difficult to ascertain whether this occurs by chance or is related to the mutation in the decorin gene.

In bone, decorin together with biglycan plays an important role in the development of the initial osteoid matrix and also during bone mineralization. The presence of biglycan can compensate for a decorin deficiency, and evidence suggests that these proteins act synergistic during bone formation.

Bone-density measurements were normal in the two family members we tested, and there were no indications of concurrent abnormalities or problems related to skin, bone, teeth, or joints in this family. It has not been determined whether this is because the disturbance of collagen structure caused by the mutation we have detected is insignificant in these tissues, or if the mutated decorin acts differently in the cornea than in other connective tissues.

Corneal transparency requires the regular spacing of collagen fibrils with uniform diameter and a regular interfibrillar space. Experimental evidence suggests that the dermatan sulfate proteoglycans participate both in the lamellar adhesion properties of collagen and in the control of the regular fibril spacing found in the cornea. Decorin knockout mice have clear corneas, indicating that decorin is not essential for the regular collagen pattern needed for corneal transparency. It is likely that other proteoglycans can compensate for the absence of decorin in the cornea. We have found a frame shift mutation that theoretically leads to alteration of four amino acids and loss of the C-terminal of the decorin amino acid sequence formed by the arch shape of decorin and thus coat the collagen fibrils. We postulate that the mutant decorin interact with collagen in a suboptimal way thereby disturbing the regularity of corneal collagen and causing corneal opacities. Thus, a cornea with homozygous decorin deficiency may be clear, whereas a heterozygote with both a normal and a truncated decorin product may have corneal clouding. Possibly, corneal fibroligenesis after birth explains the tendency for these proteins to increase with age.

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References