In Vivo Laser Confocal Microscopic Findings of Corneal Stromal Dystrophies

Akira Kobayashi, MD, PhD; Keiko Fujiki, PhD; Takuro Fujimaki, MD, PhD; Akira Murakami, MD, PhD; Kazuha Sugiya, MD, PhD

Objective: To investigate in vivo laser confocal microscopic findings of genetically mapped corneal stromal dystrophies and their relationship to histopathologic findings.

Methods: Seven patients with Avellino corneal dystrophy, 2 patients with lattice corneal dystrophy, and 2 patients with macular corneal dystrophy were examined genetically and using slitlamp biomicroscopy and in vivo laser confocal microscopy. Corneal specimens obtained after surgery in selected patients were histopathologically studied.

Results: In Avellino corneal dystrophy (Arg124His mutation of human transforming growth factor β–induced gene [TGFBI]), highly reflective granular materials with irregular edges were observed in the superficial stroma. In lattice corneal dystrophy (Arg124Cys and Leu527Arg mutations of TGFBI), highly reflective branching filaments of variable width were observed in the stroma. In macular corneal dystrophy (Ala217Thr mutation of the carbohydrate sulfotransferase gene [CHST6]), homogeneous reflective materials with dark stria-like images were observed throughout the stroma. All confocal findings correlated well with histopathologic findings.

Conclusions: In vivo laser confocal microscopy is capable of high-resolution visualization of characteristic corneal microstructural changes related to 3 types of genetically mapped corneal stromal dystrophies. The use of laser confocal microscopy may be valuable in the differential diagnosis of corneal stromal dystrophies, especially when diagnosis is otherwise uncertain.

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During the past 2 decades, in vivo white-light confocal microscopy has been a valuable noninvasive technique for the observation of living corneal microstructures at the cellular level. Its clinical usefulness has been documented in studies of healthy and diseased human corneas, including granular,2 lattice,2,3 Reis-Bücklers, and Thiel-Behnke' corneal dystrophies.

Recently, in vivo laser confocal microscopy (Heidelberg Retina Tomograph 2 Rostock Cornea Module; Heidelberg Engineering GmbH, Dossenheim, Germany) has become available.3,6 This device permits more detailed layer-by-layer observations of the corneal microstructure with an axial resolution of approximately 4 µm,7 better than that obtained using conventional white-light confocal microscopes (eg, 10-µm axial optical resolution with the ConfoScan 2 [Nidek Technologies, Vigonza, Italy]).8 In this study, we report in vivo microstructural characteristics of 3 genetically mapped corneal stromal dystrophies using in vivo laser confocal microscopy. We also report relationships between in vivo microscopic images and subsequent histopathologic section findings.

METHODS

The study was approved by the Ethics Committee of Kanazawa University Graduate School of Medical Science and followed the tenets of the Declaration of Helsinki. Before enrollment, written informed consent was obtained from all subjects. The Table summarizes demographic data of the 11 participants.

GENETIC ANALYSIS

Peripheral blood samples were obtained from the patients. Genomic DNA was extracted from peripheral leukocytes. Patients clinically diagnosed as having Avellino or lattice corneal dystrophy underwent genetic analysis of exons 4 and 12 of the human transforming growth fac-

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tor β–induced gene (TGFBI) as described previously. Patients clinically diagnosed as having macular corneal dystrophy had all exons of the carbohydrate sulfotransferase gene (CHST6) analyzed as described previously.

IN VIVO LASER CONFOCAL MICROSCOPY

After applying a large drop of contact gel (Comfort Gel ophthalmic ointment; Bausch & Lomb, Berlin, Germany) on the front surface of the microscope lens, a sterile cap (TomoCap; Heidelberg Engineering GmbH) was mounted on the holder to cover the microscope lens. Then the centers of the cornea of both eyes were examined layer by layer. The in vivo laser confocal microscopy module uses a ×60 water immersion objective lens (Olympus Europa, Hamburg, Germany) and a 670-nm diode laser as the light source (area of observation, 400 μm × 400 μm). Two examinations per eye were performed.

RESULTS

GENETIC ANALYSIS

All 7 patients with Avellino corneal dystrophy had an Arg124His (R124H) heterozygous missense mutation of TGFBI, confirming clinical diagnosis. The 2 patients with lattice corneal dystrophy had an Arg124Cys (R124C) [lattice corneal dystrophy type I] and a Leu527Arg (L527R) [lattice corneal dystrophy type IV] heterozygous missense mutation of TGFBI, confirming clinical diagnosis. The 2 patients with macular corneal dystrophy had an Ala217Thr (A217T) homozygous missense mutation of CHST6, confirming clinical diagnosis.

Table. Eleven Patients From 9 Families With 1 of 3 Corneal Stromal Dystrophies

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Clinical Diagnosis of Corneal Dystrophy</th>
<th>Eye</th>
<th>Previous Surgery</th>
<th>Gene Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/83</td>
<td>Avellino</td>
<td>R</td>
<td>NA</td>
<td>TGFBI Arg124His (heterozygous)</td>
</tr>
<tr>
<td>2/F/66</td>
<td>Avellino</td>
<td>L</td>
<td>NA</td>
<td>TGFBI Arg124His (heterozygous)</td>
</tr>
<tr>
<td>3/M/59</td>
<td>Avellino</td>
<td>L</td>
<td>NA</td>
<td>TGFBI Arg124His (heterozygous)</td>
</tr>
<tr>
<td>4/F/49</td>
<td>Avellino</td>
<td>R</td>
<td>NA</td>
<td>TGFBI Arg124His (heterozygous)</td>
</tr>
<tr>
<td>5 (Son of patient 1)/M/61</td>
<td>Avellino</td>
<td>R</td>
<td>NA</td>
<td>TGFBI Arg124His (heterozygous)</td>
</tr>
<tr>
<td>6/F/73</td>
<td>Avellino</td>
<td>L</td>
<td>NA</td>
<td>TGFBI Arg124His (heterozygous)</td>
</tr>
<tr>
<td>7/M/51</td>
<td>Avellino</td>
<td>R</td>
<td>NA</td>
<td>TGFBI Arg124His (heterozygous)</td>
</tr>
<tr>
<td>8/F/59</td>
<td>Lattice type I</td>
<td>L</td>
<td>Deep lamellar keratoplasty</td>
<td>TGFBI Arg124Cys (heterozygous)</td>
</tr>
<tr>
<td>9/M/82</td>
<td>Lattice type IV</td>
<td>R</td>
<td>Deep lamellar keratoplasty</td>
<td>TGFBI Leu527Arg (heterozygous)</td>
</tr>
<tr>
<td>10/M/65</td>
<td>Macular</td>
<td>L</td>
<td>Penetrating keratoplasty</td>
<td>CHST6 Ala217Thr (homozygous)</td>
</tr>
<tr>
<td>11 (Brother of patient 10)/M/59</td>
<td>Macular</td>
<td>R</td>
<td>Deep lamellar keratoplasty</td>
<td>CHST6 Ala217Thr (homozygous)</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not applicable.

SLITLAMP EXAMINATION

All 7 patients with Avellino corneal dystrophy (patients 1-7) had multiple, discrete, round, sharply demarcated gray-white deposits, as well as scattered stellate opacities (Figure 1A). However, latticelike lines are subtle, and they are not discernible by slitlamp examination in most cases. Areas between dense opacities were clear in all patients. The Descemet membrane and endothelium appeared normal in all patients.

In the patient with lattice corneal dystrophy type I (patient 8), slitlamp biomicroscopy showed numerous thread-like, radially oriented fine spicules throughout the stroma (Figure 2A). The central anterior stroma of both eyes showed dense opacification. In the patient with lattice corneal dystrophy type IV (patient 9), slitlamp biomicroscopy showed typical thick lattice lines with radial orientation (Figure 3A).

In the patients with macular corneal dystrophy (patients 10 and 11), slitlamp biomicroscopy showed ground-glass–like haze with indistinct borders throughout the thickness of the cornea. Scattered gray-white lesions were also seen (Figure 4A).

IN VIVO LASER CONFOCAL MICROSCOPY

All 7 patients with Avellino corneal dystrophy had similar images. In the basal epithelial layer, local deposition of highly reflective granular materials without dark shadows was observed (Figure 1D). At the level of the superficial and middle stroma, clusters of highly reflective granular materials with irregular edges were observed (Figure 1F-H). However, we could not find any confo-
cal images that would correspond to the latticelike lesions because lattice lines are not discernible in patients with Avellino corneal dystrophy. The surrounding stroma and keratocyte nuclei (Figure 1E), as well as the endothelial layer, appeared normal.

In the basal epithelial layer of patient 8 (with lattice corneal dystrophy type I), reticular, highly reflective extracellular deposits were observed (Figure 2E). At the level of the superficial and middle stroma, highly reflective branching filaments were observed (Figure 2G and H).

In patient 9 (with lattice corneal dystrophy type IV), highly reflective deposits were observed in the Bowman layer (Figure 3E). In the superficial and middle stroma, highly reflective lattice-shaped materials were seen (Figure 3F and H). Some stromal images showed highly reflective thick, branching deposits (Figure 3G).

In both patients with macular corneal dystrophy (patients 10 and 11), the epithelial layer appeared normal (Figure 4C). In the basal epithelial layer and superficial stroma, highly reflective deposits without distinct borders were observed (Figure 4D and E). Subepithelial nerves can partly be seen at the level of Bowman layer.
In the superficial and middle stroma, homogeneous reflective materials with dark striated images were observed (Figure 4F). Normal keratocytes were not seen. The endothelial layer appeared normal.

**HISTOPATHOLOGIC FINDINGS**

In the anterior corneal tissue section from patient 1 (with Avellino corneal dystrophy), clusters of deposits with irregular edges were observed using Azan stain (Figure 1B). Some deep stromal deposits were positive for amyloid (direct fast scarlet stain). C, Deposits show apple-green birefringence under polarized light. D, The basal epithelium appears normal. E, In the Bowman layer, highly reflective deposits are observed using in vivo laser confocal microscopy. F, In the superficial and middle stroma, highly reflective lattice-shaped materials are observed. G, Some stromal images show highly reflective, thick branching deposits. H, Oblique section of the superficial layer shows highly reflective lattice-shaped materials just beneath the Bowman layer. b indicates Bowman layer; e, epithelium; and s, anterior stroma. All are confocal images, 400×400 µm (original magnification ×300). Bar indicates 100 µm.

In corneal sections from patients 8 and 9 (with lattice corneal dystrophy), anterior subepithelial deposits were positive for amyloid (Figures 2B and 3B, direct fast scarlet stain). They showed apple-green birefringence under polarized light (Figures 2C and 3C).

In corneal sections from patients 10 and 11 (with macular corneal dystrophy), deposits throughout the anterior stroma were positive for Alcian blue (Figure 4B). This represented the presence of mucopolysaccharide deposits.
In this study, we demonstrate in vivo laser confocal microscopic findings for the first time (to our knowledge) for 3 genetically mapped corneal stromal dystrophies, namely, Avellino, lattice, and macular corneal dystrophies. Characteristic pathologic microstructures were visualized noninvasively and with high resolution. In Avellino corneal dystrophy, highly reflective granular materials with irregular edges were observed in the superficial stroma. In contrast, in lattice corneal dystrophy types I and IV, highly reflective branching filaments were observed in the stroma. In macular corneal dystrophy, homogeneous reflective materials with dark striaelike images were observed throughout the stroma, along with highly reflective deposits in the stroma. Because observations obtained using in vivo laser confocal microscopy are unique to each stromal dystrophy, we conclude that this modality can differentiate these stromal dystrophies in vivo.

In vivo laser confocal microscopic characteristics have recently been reported for the following 2 genetically proven Bowman layer dystrophies: Thiel-Behnke corneal dystrophy (dystrophy of Bowman layer and superficial stroma type II [TGFBI R555Q]) and Reis-Bücklers corneal dystrophy (dystrophy of Bowman layer and superficial stroma type I [TGFBI R124L]). In Thiel-Behnke corneal dystrophy, deposits in the epithelial basal cell layer showed homogeneous reflectivity with round-shaped edges accompanying dark shadows. In contrast, deposits in the same cell layer for patients with Reis-Bücklers corneal dystrophy showed high reflectivity from small granular materials without shadows. In each dystrophy, the Bowman layer was totally replaced with pathologic material; reflectivity was much higher in Reis-Bücklers corneal dystrophy than in Thiel-Behnke corneal dystrophy. It was concluded that in vivo laser confocal microscopy can differentiate Thiel-Behnke and Reis-Bücklers corneal dystrophies in vivo; differentiation is impossible using conventional white-light confocal microscopy because of limited resolution. The small granular materials with high reflectivity in the epithelial basal layer in Reis-Bücklers corneal dystrophy are similar to those observed in the present study in Avellino corneal dystrophy in shape and laser light reflectivity. Mashima et al investigated histologic features of genetically proven Reis-Bücklers corneal dystrophy and proposed that it is histologically a “superficial variant of granular corneal dystrophy.” In contrast, Avellino corneal dystrophy, also referred to as combined granular-lattice corneal dystrophy, is a variant of granular corneal dystrophy that has histologic features of lattice and granular corneal dystrophies. Because Avellino and Reis-Bücklers corneal dystrophies have histologic features of granular dystrophy, it is not surprising that they have similar laser confocal images. In vivo laser confocal microscopy is able to differentiate these 2 histologically similar dystrophies because there is no apparent stromal involvement in Reis-Bücklers corneal dystrophy.

In our study, histologic sections from patients with lattice corneal dystrophy showed deposition in the superficial stroma that was positive for amyloid staining and demonstrated apple-green birefringence under polarized light, confirming a previous report. Using in vivo laser confocal microscopy, these lattice lines were observed as highly reflective branching filaments, consistent with slitlamp biomicroscopic and histologic findings. A previous in vivo white-light confocal microscopic study of lattice corneal dystrophy type I showed punctiform structures and highly reflective irregular materials in the epithelial and Bowman layers, with the stroma containing reflective filaments up to 50 µm in diameter with blurred edges and characteristic reflective branching filaments 80 to 100 µm in diameter. Chiu et al reported that white-light confocal microscopy of a cornea with lattice corneal dystrophy revealed linear and branching structures in the stroma measuring approximately 40 to 80 µm in width with changing reflectivity and poorly demarcated margins. These observations are consistent with our findings obtained using in vivo laser confocal microscopy. However, in this study we tested only 1 patient each with lattice corneal dystrophy type I and type IV. Further analysis using multiple patients with lattice corneal dystrophy is required to fully understand the in vivo histologic features of this type of dystrophy, as some corneal dystrophies are known to represent several different phenotypes clinically, even with the same genetic mutation.

Herein, histologic sections from patients with macular corneal dystrophy showed deposits throughout the cornea that were positive for Alcian blue, representing the presence of mucopolysaccharides as previously reported. In vivo laser confocal microscopy showed homogeneous reflective materials throughout the stroma (Figure 4G), which may represent the diffuse stromal opacity of macular corneal dystrophy. In contrast, the highly reflective deposits observed in the epithelial basal cell layer and superficial stroma (Figure 4D and E) may correspond to the scattered gray-white discrete deposits as seen using the slitlamp. Numerous dark striaelike images were observed in the stroma in both patients. These striaelike images are not indentation lines from the flat microscope lens cap (TomoCap), as the images were present without any cap pressure. In vivo white-light confocal microscopic observation of similar dark striae was previously reported among stromal materials with high reflectivity in the posterior stroma adjacent to the endothelium in patients with central cloudy dystrophy of François. The significance of these dark striae remains unclear.

In conclusion, in vivo laser confocal microscopy is capable of visualizing with high resolution the microstructural changes related to 3 types of genetically mapped corneal stromal dystrophy. These results suggest that this technique may be valuable in the differential diagnosis of corneal stromal dystrophies, particularly when diagnosis is uncertain. It may also be useful for further research into corneal dystrophies, especially to follow their natural courses.

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Author Contributions: Dr Kobayashi had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Financial Disclosure: None reported.

REFERENCES


From the Archives of the Archives

There were innumerable articles during the past century warning against probing the nasolacrimal duct in cases of dacryocystitis in infancy.

In the cases of dacryocystitis in which early probing was used recovery was prompt, and no untoward results of injury were experienced. In the American or the foreign literature I could find expressed only fears as to what might happen with early probing, and no evidence to substantiate the belief that injury resulted from early instrumentation. In my own cases, and in those of the ophthalmic literature, probing the duct (like pushing a stylet through an obstructed hypodermic needle) cleared the dacryocystitis in most instances with a single probing.