In Vivo Confocal Microscopy of Fuchs Endothelial Dystrophy Before and After Endothelial Keratoplasty

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Importance: This study reveals significant changes of the anterior cornea in Fuchs endothelial dystrophy that probably affect the visual outcomes of endothelial keratoplasty for the disease.

Objective: To determine whether abnormalities of corneal stromal and subepithelial cells in Fuchs endothelial dystrophy resolve after Descemet stripping endothelial keratoplasty (DSEK).

Design: Prospective observational study of 49 corneas of 42 patients with Fuchs dystrophy before DSEK and during 3 years of postoperative follow-up. None of the preoperative corneas were vascularized or had pronounced subepithelial fibrosis on results of slitlamp examination. Corneas were examined using in vivo confocal microscopy to determine stromal cell density and the presence of abnormal subepithelial cells (presumed fibroblasts).

Setting: The cornea service at Mayo Clinic, Rochester, Minnesota.

Participants: Forty-nine corneas of 42 patients.

Intervention: Descemet stripping endothelial keratoplasty.

Main Outcome Measures: Stromal cell density and presence of subepithelial cells.

Results: Subnormal cell density in the most anterior 10% of the host stroma in Fuchs dystrophy before DSEK (mean [SD], 22,030 [6,479] cells/mm³ [n=41]) remained unchanged at 2 (20,433 [4,993] cells/mm³ [n=35]; P=0.36) and 3 years (20,925 [5,333] cells/mm³ [n=23]; P=0.99) after DSEK. Abnormal subepithelial cells, which formed reticular networks deep to the basal epithelial cells, were visible in 33 eyes (67%) and remained present at 3 years after DSEK. Mean preoperative central corneal thicknesses when these subepithelial cells were and were not visible were 652 (45) and 668 (56) μm, respectively (P=0.75).

Conclusions and Relevance: The reduced cellularity of the anterior stroma in Fuchs dystrophy does not recover 3 years after restoring endothelial function. Abnormal subepithelial cells, presumably fibroblasts, are present in most corneas with Fuchs dystrophy requiring DSEK, even in cases with mild edema and in the absence of clinically obvious preoperative subepithelial fibrosis. Anterior corneal structural abnormalities might be related to visual outcomes after DSEK.


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Corneal pathology in vivo can be quantitatively and qualitatively examined using in vivo confocal microscopy,12 and, although some studies have described changes qualitatively in corneas with Fuchs dystrophy,13-15 few studies16,17 have performed quantitative analyses or described changes after AF.

The primary goal of this study was to determine whether restoration of endothelial function in corneas with Fuchs dystrophy promoted repair of the anterior cornea. Previous research showed that keratocytes were depleted in the anterior stroma in Fuchs dystrophy4; in this study, we hypothesized that restoring endothelial function would stimulate repopulation of anterior stromal cells. We examined corneas with Fuchs dystrophy by using in vivo confocal microscopy and determined stromal cell density before and after Descemet stripping EK (DSEK). We also described qualitative changes in corneal cells and structure before and after DSEK for Fuchs dystrophy.

METHODS

SUBJECTS

Subjects requiring DSEK for Fuchs dystrophy were recruited from the cornea service at the Mayo Clinic and were enrolled in a prospective observational study. The cohort and eligibility criteria have been previously described.17,18 Briefly, all subjects had visually significant central guttae with or without edema, as determined using slitlamp biomicroscopy, but subjects were excluded if they had evidence of corneal changes unrelated to Fuchs dystrophy, if the corneas were vascularized, or if they had pronounced subepithelial fibrosis. All eyes were pseudophakic owing to previous small-incision cataract surgery (phacoemulsification) or had lenticular changes requiring cataract extraction.

This study was approved prospectively by the institutional review board, Mayo Clinic, Rochester, Minnesota. Informed consent was obtained from all subjects after discussion of the risks of the study.

SURGICAL PROCEDURES

Descemet-stripping endothelial keratoplasty was performed under general or local anesthesia as described previously.19,20 In all cases, the donor tissue was prepared with a mechanical microkeratome (ALTK; Moria) and was inserted in the anterior chamber through a 3- to 6-mm scleral tunnel incision. Phacoemulsification and intraocular lens insertion were performed through the same incision before stripping the Descemet membrane in cases that combined DSEK with cataract surgery.

IN VIVO CONFOCAL MICROSCOPY

Eyes were examined with a slit-scanning confocal microscope (ConfoScan 4; Nidek Technologies) before and at 1, 2, and 3 years after DSEK. The central cornea was examined by using a through-focusing technique,19 with a z-ring adapter to stabilize the cornea and provide accurate depth information.20 Briefly, topical anesthetic (proparacaine hydrochloride, 0.5%) was instilled in the eye, and the objective (original magnification ×40) was coupled to the cornea through the optical medium hydroxypropyl methycellulose, 0.3% (GenTeal Gel; Novartis Ophthalmics). Two to 4 confocal scans of the central cornea were acquired at each examination by using a step distance of 4 μm and 2 passes per scan. The microscope had been calibrated for depth and lateral resolution as previously described.19 Each confocal scan was reviewed by one experienced observer (S.V.P.), and the images that corresponded to the epithelial surface, anterior and posterior boundaries of the stroma (in preoperative corneas), surgical interface (in postoperative corneas), and endothelium were identified.21 Abnormal features at any depth of the cornea were also recorded, including the presence or absence of subepithelial cells, which were presumed to be fibroblasts.

STROMAL CELL DENSITY

Stromal cell density was determined in 5 layers of host stroma (most anterior 10%, 11%-33%, 34%-66%, 67%-90%, and most posterior 10%)19,20 and 2 layers of donor stroma (anterior and posterior 50%). Density in each layer consisted of the mean density of 2 images from each layer. In the most anterior 10% layer, 1 of the 2 images was always the most anterior image with discernible keratocytes, similar to previous studies,21 and density for this image was reported in addition to mean density for the most anterior 10% of the stroma. All selected images were first analyzed by an automated program designed to estimate keratocyte density in normal corneas and corneas after refractive surgery.19 Because the pathological changes in Fuchs dystrophy reduced the accuracy of the automated program for identifying cell nuclei,19 every image analyzed by the automated program was manually reexamined and corrected by one experienced observer (S.V.P.) in a randomized and masked manner. To facilitate the discrimination of cell nuclei from noise, especially in the anterior hazy cornea, the observer viewed consecutive images adjacent to the image being counted. In contrast to noise, cell nuclei were visible through several consecutive images because the depth of field of the confocal microscope was approximately 26 μm and the frame-to-frame step distance was 4 μm.19,20

Before DSEK, stromal cells were distributed in a larger-than-normal volume of tissue because of stromal edema, resulting in decreased cell density. We considered this swelling when comparing preoperative with postoperative cell densities by adjusting preoperative densities for stromal edema and assuming uniform swelling. Adjusted preoperative density was the product of the measured preoperative density and the ratio of preoperative to postoperative (nondenematous) host stromal thickness at 1 year (39 eyes) or 2 years (2 eyes). We measured stromal thickness by using in vivo confocal microscopy and calculated the distance between the most anterior keratocytes and the most posterior keratocytes (preoperative) or surgical interface (postoperative).19,21

STATISTICAL ANALYSIS

The primary outcome was the difference in anterior stromal cell density before and 2 years after DSEK. Assuming a standard deviation of anterior stromal cell density of 5300 cells/mm4,19 a priori analysis indicated that a minimal difference in anterior stromal cell density of 3700 cells/mm4 (equivalent to an anticipated change of 13%) could be detected with a sample of 30 eyes (α = .05/3, where 3 is the number of comparisons; β = .20 [paired analysis]). Although the primary outcome was assessed at 2 years when all possible data were available, data were analyzed for many eyes at 3 years also.

We compared variables using generalized estimating equation models to account for possible correlation between fellow eyes of the same subject.22 Variables that were nonparametric were assessed by applying generalized estimating equation models to a rank transformation of the data. We adjusted P values for multiple comparisons using the Bonferroni method, and P ≤ .05 was considered statistically significant. For post hoc...
analyses, minimal detectable differences were calculated for non-significant comparisons ($\alpha = .05/n$, where $n$ was the number of comparisons; $\beta = .20$). The magnitude of the correlation between stromal cell density and preoperative central corneal thickness, measured by using an ultrasonic pachometer (DGH 1000; DGH Technologies, Inc), was illustrated by the Pearson correlation coefficient, and the significance of the correlation was determined by using generalized estimating equation models.

RESULTS

SUBJECTS

Forty-nine eyes of 42 subjects with Fuchs dystrophy were enrolled in the study; mean (SD) recipient age was 67 (10) (range, 41-87) years. By 12 months, 2 subjects (2 eyes) had withdrawn from the study, 1 subject (1 eye) had died, 1 graft had failed, and 1 subject (1 eye) did not attend for examination, leaving 44 eyes (in 37 subjects) available for analysis. By 24 months, 6 subjects (6 eyes) had withdrawn from the study, 1 subject (1 eye) had died, 2 grafts had failed, and 3 subjects (3 eyes) did not attend for examination, leaving 37 eyes (in 32 subjects) available for analysis. By 36 months, 10 subjects (10 eyes) had withdrawn from the study, 1 subject (1 eye) had died, 2 grafts had failed, 3 subjects (3 eyes) did not attend for examination, and 7 eyes (7 subjects) had not yet reached the 36-month examination, leaving 26 eyes (in 23 subjects) available for analysis.

Preoperatively, confocal data were available for 47 eyes. A quantitative analysis was possible in only 41 eyes owing to significant ocular movement (1 eye) or indiscernible cell nuclei (3 eyes) or because a lack of postoperative confocal data to determine true host stromal thickness prevented the adjustment of preoperative density for stromal reflectivity from the basal epithelial and anterior stromal layers. The subepithelial cells were visible in 16 eyes (33%), but in 9 eyes (19%) they were abnormal with visible cell bodies (Figure 2). In 6 corneas (13%), multiple punctate (2-3 µm), bright reflectivity of the cell borders (Figure 2). Descemet membrane exhibited a honeycomb appearance in 2 corneas (4%), and linear “fibers” within the membrane were noted in oblique sections in 3 corneas (Figure 2). The true frequency of these fibers could not be determined because oblique sections were not obtained in all corneas.

After DSEK, the basal epithelial cells remained visible but were less reflective compared with before DSEK. The subepithelial cells were visible in 16 eyes (41%) at 12 months, 18 eyes (51%) at 24 months, and 8 eyes (35%) at 36 months (Figure 3). Subepithelial cells were visible in 33 of the 49 eyes (67%) in at least 1 examination during the 3-year study. Anterior stromal cells were visible sparse in 15 eyes (38%) at 12 months, 20 eyes (57%) at 24 months, and 11 eyes (48%) at 36 months (Figure 3). The middle and posterior stromal cells had normal morphology in all eyes at 12 months, and, although the stromal punctate opacities were

Figure 1. In vivo confocal microscopic images of anterior corneal pathology in Fuchs endothelial dystrophy before endothelial keratoplasty demonstrate various changes contributing to increased anterior reflectivity (haze). A, The basal epithelial cells were easily visible in all corneas, with brighter than normal cell borders. C and E, Brightly reflective reticular networks of cells, presumably subepithelial fibroblasts, were often visible deep in the basal epithelium; occasionally, the nuclei of these cells were visible and appeared oval and distinct from keratoocyte nuclei (Figure 3). B and D, The most anterior discernible stromal cell nuclei in Fuchs dystrophy were frequently sparse, brightly reflective, fragmented, and associated with increased background (extracellular matrix) reflectivity. F. The most anterior stromal cells of a normal cornea in a 65-year-old patient show the normal morphology and high density of nuclei in this region. All images represent areas with dimensions of 428 × 325 µm.
Anterior stromal cell density in Fuchs dystrophy was markedly lower than in normal corneas and cannot be explained by age-related physiological cell loss. This reduced anterior cell density in Fuchs dystrophy has been confirmed by histologic findings, and the present study did not change between 1 and 3 years after DSEK (Table).

RELATIONSHIPS AMONG STROMAL CELL DENSITY, SUBEPITHELIAL CELLS, AND CORNEAL THICKNESS

Stromal cell density in the most anterior 10% of the stroma in Fuchs dystrophy (before DSEK) did not correlate with preoperative central corneal thickness ($r = -0.01; P = .93$ [n = 41]). Similarly, mean (SD) preoperative central thickness of corneas in which subepithelial cells were visible (652 [45] µm) did not differ from that when subepithelial cells were not visible (668 [56] µm; $P = .75$; minimal detectable difference, 42 µm). Although we found a trend toward decreased stromal cell density in the most anterior confocal image of stroma when subepithelial cells were visible (22 595 [7078] cells/mm$^3$) compared with when subepithelial cells were not visible (26 435 [9954] cells/mm$^3$), this was not a statistically significant difference ($P = .09$; minimal detectable difference, 7815 cells/mm$^3$).

COMMENT

Corneas with Fuchs dystrophy manifest significant anterior corneal abnormalities, even if the anterior cornea appears uncompromised on results of slitlamp examination, and restoring endothelial function promotes minimal repair of the anterior cornea at 3 years after EK. The depletion of anterior stromal cells and presence of abnormal subepithelial cells might contribute to increased anterior haze and increased anterior corneal aberrations, which are known to affect vision after EK.

Fuchs dystrophy is well characterized by posterior corneal guttae and progressive corneal edema, but this study also found anterior stromal cell loss and the presence of abnormal subepithelial cells despite the unaffected appearance of the anterior cornea during the clinical examination. Although advanced cases of Fuchs dystrophy and other causes of chronic corneal edema are associated with significant anterior corneal pathology, such as neovascularization and visible scarring, none of the cases in this study had neovascularization or pronounced subepithelial fibrosis on slitlamp examination findings. Furthermore, because EK has enabled earlier surgical intervention compared with penetrating keratoplasty, many of the corneas in this study were at an earlier stage in the course of Fuchs dystrophy than those typically examined after penetrating keratoplasty. The lack of relationships between anterior corneal abnormalities and preoperative corneal thickness in this study also suggests that the anterior changes might appear earlier in the course of disease than is assumed to be typical.

Anterior stromal cell density in Fuchs dystrophy was markedly lower than in normal corneas and cannot be explained by age-related physiological cell loss. This reduced anterior cell density in Fuchs dystrophy has been confirmed by histologic findings, and the present study...
found that anterior cell repopulation did not occur during the 3-year follow-up after restoring endothelial function. In normal eyes, cell density is highest in a thin anterior layer of the stroma and lower and more uniform in the remainder of the stroma; the anterior cells have been suggested to be a subpopulation of stromal cells with a specific function. Although the exact function of the anterior stromal cells is unknown, they are known to maintain the architecture of the anterior stroma, which is important for maintaining a regular anterior corneal surface and presumably for maintaining transparency. Thus, anterior cell depletion might contribute to
of cell density. Thus, other mechanisms of fibroblast formation should not be disregarded.4,31,32 We did not find a relationship between the visibility of subepithelial cells and preoperative cell density. Nevertheless, this trend was not statistically significant and might be explained by the large variability of the stroma.

Abnormal subepithelial cells, presumably fibroblasts, were observed in most corneas, and these cells were still visible at 3 years after EK. With in vivo confocal microscopy, Mustonen et al14 found fibrous septae in the basal and subepithelial regions in Fuchs dystrophy; with improvements in confocal imaging, the morphologic characteristics and location of this subepithelial fibrosis have become better defined.15,30 We found that subepithelial cells were easily distinguished from stromal cells by their morphology and by their location between the Bowman layer and the basal epithelium, which corresponds to the location of subepithelial fibroblasts.7,8 The origin of subepithelial cells could be keratocytes migrating through normal channels in the Bowman layer, which remains intact until later in the course of Fuchs dystrophy,7 and the trend toward decreased anterior stromal cell density when subepithelial cells were visible might support this possibility. Nevertheless, this trend was not statistically significant and might be explained by the large variability of cell density. Thus, other mechanisms of fibroblast formation and anterior stromal cell depletion should not be disregarded.4,31,32 We did not find a relationship between the visibility of subepithelial cells and preoperative corneal thickness, indicating that these cells could be present in cases with mild edema or early in the disease course.

The anterior changes in Fuchs dystrophy are important because they persisted at 3 years after EK, and they might help explain visual outcomes after EK. After EK for Fuchs dystrophy, increased anterior corneal backscatter (or haze) has been associated with increased disability glare,3,11 and increased anterior corneal aberrations have been associated with decreased visual acuity.10,31,34 Anterior corneal backscatter is higher than normal before EK and improves after EK but does not return to normal with resolution of corneal edema.3,35 By confocal examination in this study, we found that anterior haze before EK was caused by increased reflectivity from basal epithelial cell borders, the Bowman layer, the anterior stromal extracellular matrix, and subepithelial cells. After EK, the improvement in haze was largely explained by an improvement in basal epithelial reflectivity, presumably because of resolution of subtle, nonbulbous, epithelial edema, whereas reflectivity from the subepithelial cells, Bowman layer, and anterior stroma did not improve as much. Thus, even after resolution of corneal edema, haze remains higher than normal after EK because of persistent tissue abnormalities that are probably caused by chronic edema from early in the course of disease.5,18 One explanation for increased anterior corneal aberrations after DSEK could be the networks of subepithelial cells that, because of their location between the basal cells and Bowman layer, might cause an irregular anterior corneal surface. Indeed, anterior corneal haze correlates with anterior corneal aberrations,10 both of which could be attributed to the abnormal subepithelial cells. Further studies that better characterize the onset of and relationships between the anterior corneal abnormalities could help determine whether an optimum time exists for intervening in Fuchs dystrophy to achieve the best outcomes.

In the posterior cornea, observations of guttae and the lamellar interface after DSEK using in vivo confocal microscopy have been described previously.13,23,24,36 Of interest was that stromal cell density in the most posterior 10% of the host stroma was also decreased at 2 and 3 years after DSEK. This finding suggests that these cells form a subpopulation that normally interacts with the endothelium or aqueous humor25,27 and that their separation affects the endothelial monolayer. In addition, these cells might help explain visual outcomes after EK, and they might help explain visual outcomes after DSEK. Nevertheless, this trend was not statistically significant and might be explained by the large variability of stromal cell density in the most posterior 10% of the host stroma. Therefore, further studies of subepithelial cells in Fuchs dystrophy are needed to better understand their role in the disease process.

### Table. Stromal Cell Density in Eyes Before and After DSEK for Fuchs Dystrophy

<table>
<thead>
<tr>
<th>Layer of Stroma</th>
<th>Before DSEK</th>
<th>12 mo</th>
<th>24 mo</th>
<th>36 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host, %b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10</td>
<td>22,030 (6479)</td>
<td>21,139 (5562)</td>
<td>20,443 (4993)</td>
<td>20,925 (5433)</td>
</tr>
<tr>
<td>11-33</td>
<td>22,485 (5523)</td>
<td>23,270 (3737)</td>
<td>23,599 (3347)</td>
<td>23,216 (4228)</td>
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<tr>
<td>34-66</td>
<td>21,187 (5799)</td>
<td>20,893 (3868)</td>
<td>22,335 (4775)</td>
<td>21,427 (37,993)</td>
</tr>
<tr>
<td>67-90</td>
<td>20,155 (4694)</td>
<td>20,735 (5165)</td>
<td>21,401 (5086)</td>
<td>20,139 (5061)</td>
</tr>
<tr>
<td>91-100</td>
<td>20,967 (5643)</td>
<td>20,202 (6454)</td>
<td>17,191 (6505)c</td>
<td>17,680 (6223) d</td>
</tr>
<tr>
<td>Anterior 50</td>
<td>17,588 (6295)</td>
<td>15,916 (5889)</td>
<td>18,198 (6825)</td>
<td></td>
</tr>
<tr>
<td>Posterior 50</td>
<td>21,188 (5933)</td>
<td>21,159 (4500)</td>
<td>20,289 (5044)</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell Density, Cells/mm², Mean (SD)</th>
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<tr>
<td>Layer of Stroma</td>
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<td>----------------</td>
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<tr>
<td>Host, %b</td>
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<td>0-10</td>
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<td>91-100</td>
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<tr>
<td>Anterior 50</td>
</tr>
<tr>
<td>Posterior 50</td>
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</table>

Abbreviation: DSEK, Descemet stripping endothelial keratoplasty.

a Cell densities before DSEK were adjusted for stromal edema by using the nonedematous stromal thickness after DSEK and assuming uniform swelling to enable direct comparison with postoperative cell densities.

b Postoperative cell density did not differ from that before DSEK in any layer of stroma unless stated; the average minimal detectable difference was approximately 4600 cells/mm² (α = .05/3, where 3 is the number of comparisons; β = .20). Percentages indicate the most anterior to the most posterior portions of the stroma.

cP = .01 vs preoperative measurement.
dP = .04 vs preoperative measurement.
eDonor cell density did not change after DSEK; the average minimal detectable difference was approximately 4400 cells/mm² (α = .05/2, where 2 is the number of comparisons; β = .20).
same anatomical relationship with the endothelium and aqueous humor.\textsuperscript{37} will further our understanding of these cellular interactions, although the consequence of this decrease in posterior stromal cells, if any, is unknown. The punctate stromal opacities, which were prominent in some corneas before EK, might correspond to focal lipid accumulations that have been observed by electron microscopy in corneas with Fuchs dystrophy.\textsuperscript{38}

Automated analysis of stromal cell density from confocal images is consistent and valid in normal corneas and corneas after refractive surgery.\textsuperscript{19,21,39} In corneas with Fuchs dystrophy, however, increased image reflectivity and poor contrast associated with corneal haze render this automated analysis inaccurate, as determined in a previous confocal and histologic study of the same corneas.\textsuperscript{4,40} Thus, we modified the method of stromal cell density measurement in the present study. First, we used a slit-scanning confocal microscope (ConfoScan 4) that provided better image contrast than the rotating disc confocal microscope (Tandem Scanning Corporation) used in a previous study.\textsuperscript{2}\textsuperscript{nd, after automated analysis of confocal images, all analyzed images were examined manually by an experienced observer (S.V.P.) to correct inaccuracies in cell identification. This modified method proved valid because preoperative and postoperative cell densities were very consistent, and the decreased anterior cell density was similar to that found by histologic analysis.\textsuperscript{4} In addition, the lower-than-normal estimate of anterior stromal cell density in Fuchs dystrophy could have been lower yet because preoperative density was adjusted for stromal edema by assuming uniform swelling, when in fact the anterior cornea swells less than the posterior cornea.\textsuperscript{41-43} We analyzed all stromal cells, which are predominantly keratocytes but might include other cell types, such as bone marrow-derived cells.\textsuperscript{4,44} Another limitation of this study was that the presence of subepithelial cells might have been underestimated because of the small field of view (0.14 mm\textsuperscript{2}) of the confocal microscope.

In summary, the anterior cornea is abnormal in Fuchs dystrophy, even when results of the slitlamp examination suggest a normal appearance, and it remains abnormal 3 years after EK. The mechanisms of this anterior corneal pathology and the relationships between anterior corneal cells in Fuchs dystrophy are yet to be described. Further studies are required to establish the onset of anterior abnormalities in the disease course and to determine their implications with respect to the timing and outcomes of intervention.

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Author Contributions: Dr Patel 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.

Conflict of Interest Disclosures: None reported.

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Additional Contributions: David O. Hodge, MS, Department of Health Sciences Research, Mayo Clinic, provided statistical expertise. Keith H. Baratz, MD, helped recruit subjects with Fuchs dystrophy and provided a critical review of the manuscript.

REFERENCES


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