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INSIDE THIS ISSUE

Sanjay V. Patel, M.D.

Posterior Corneal Profile Changes Impact Fuchs' Endothelial Corneal Dystrophy, Endothelial Keratoplasty and Intraocular Lens Implantation

The role of the posterior corneal surface in the optics of the eye has been poorly understood until recently, when Scheimpflug imaging and anterior segment optical coherence tomography instruments enhanced researchers' understanding of the effect of posterior corneal shape on visual outcomes. "The relationship between the anterior and posterior corneal surface is important; normally the shape of the posterior cornea 'parallels' that of the anterior cornea, compensating for a small but variable percentage of anterior corneal power, astigmatism and high-order aberrations," says Sanjay V. Patel, M.D., Ophthalmology chair at Mayo Clinic's campus in Rochester, Minnesota.

Dr. Patel and a research team used Scheimpflug imaging to assess changes in the profiles of the anterior and posterior corneal surfaces over a range of severity of corneas with Fuchs' endothelial corneal dystrophy (FECD) and normal corneas. The study, which examines the relationship between corneal surface profiles, corneal power and corneal thickness, was published in *Investigative Ophthalmology & Visual Science* in 2015.

"FECD is characterized by progressive central corneal edema because of endothelial dysfunction, with relative bulging of the posterior corneal plane toward the anterior chamber when edema is clinically detectable," notes Dr. Patel. "A change in the relationship between anterior and posterior corneal surfaces may have implications on corneal power, and has been suggested to cause the hyperopic shift after Descemet membrane endothelial keratoplasty. Because corneal changes are known to start earlier in the course of FECD than when keratoplasty is clinically indicated, changes in posterior corneal power and toricity may also be important when considering refractive cataract surgery in these eyes."

The research team acquired Scheimpflug images for 112 corneas from 64 participants with FECD and 101 normal corneas from 54 age-matched participants. Eyes with FECD were categorized as mild, moderate or advanced according to the area and confluence of guttae and the presence of clinical edema. Normal corneas were devoid of guttae. Findings include:

- Normal corneas were 8.6 ± 4.8 µm thicker vertically than horizontally ($P = 0.001$); the steep posterior meridian was vertical in 91 percent of corneas.
- The difference between vertical and horizontal thicknesses decreased to 4.7 ± 7.3 µm in eyes with advanced FECD ($P = 0.008$); only 46 percent had a steep vertical posterior meridian ($P = 0.001$).
- Vertical radius of posterior curvature was flatter than normal in eyes with moderate (by 0.2 millimeters, or mm, $P = 0.011$) and advanced (by 0.4 mm, $P < 0.001$) FECD.
- Mean posterior corneal power was less negative in eyes with moderate (by 0.2 diopters, or D, $P = 0.009$) and advanced (by 0.4 D, $P < 0.001$) FECD compared with normal.

"The normal posterior cornea has an ellipsoid shape that manifests as against-the-rule astigmatism because the cornea is thicker in the vertical than horizontal meridian. Yuta Ueno, M.D., with the University of Tsukuba Hospital, Japan, and others reported the same

findings in *Ophthalmology* in 2015. In contrast, in eyes with FECD, the posterior cornea is flatter and more spherical than normal, resulting in less negative power and loss of normal posterior surface toricity," says Dr. Patel. "These changes might indeed contribute to the hyperopic shift that occurs after Descemet membrane endothelial keratoplasty (DMEK) and have implications for planning refractive outcomes after cataract surgery in eyes with FECD.

"In eyes with FECD, the directional change in peripheral corneal thickness can result in loss of normal posterior corneal toricity, and the axes of anterior and posterior corneal astigmatism can change significantly as FECD progresses. Therefore, toric intraocular lenses and use of limbal relaxing incisions in FECD should be selected cautiously, if at all, considering that both corneal surfaces could change after DMEK. As noted by Katrin Wacker, M.D., with Ophthalmology at Mayo Clinic's campus in Minnesota, and others in *Ophthalmology* in 2015, surgeons should also recognize that corneal high-order aberrations can increase early in the course of FECD, and these can degrade optical quality after cataract surgery or endothelial keratoplasty. Eyes with moderate and advanced FECD should be rendered slightly myopic if DMEK may be required in the future."

For more information

Wacker K, et al. Directional posterior corneal profile changes in Fuchs' endothelial corneal dystrophy. *Investigative Ophthalmology & Visual Science*. 2015;56:5904.

Ueno Y, et al. Corneal thickness profile and posterior corneal astigmatism in normal corneas. *Ophthalmology*. 2015;122:1072.

Wacker K, et al. Corneal high-order aberrations and backscatter in Fuchs' endothelial corneal dystrophy. *Ophthalmology*. 2015;122:1645.

Patients With Fuchs' Endothelial Corneal Dystrophy Exhibit a Unique Signature of Missplicing Events in *TCF4*

Keith H. Baratz, M.D.

Michael P. Fautsch, Ph.D.

Fuchs' endothelial corneal dystrophy (FECD) is familial, bilateral and progressive — and the most common indication for corneal transplantation in the United States. Studies have linked FECD with an expanded intronic repeat, CTG, in the transcription factor 4 (*TCF4*) gene in most patients in Caucasian cohorts and a smaller proportion in non-Caucasian cohorts. Up to 80 percent of patients with FECD, depending on ethnicity, exhibit trinucleotide repeat expansions.

"FECD tissue harbors focal intranuclear accumulations of the CUG repeat pre-mRNA, termed RNA foci," says Keith H. Baratz, M.D., Ophthalmology, at Mayo Clinic's campus in Rochester, Minnesota. "RNA foci colocalize with and sequester nuclear proteins, most notably splicing factors of the muscleblind (MBNL) family. A pilot study we conducted showed that trinucleotide repeat expansion in the corneal endothelium leads to sequestration of MBNL1 in RNA foci and observable changes in mRNA splicing."

Building on that study, Dr. Baratz, Michael P. Fautsch, Ph.D., with the Ophthalmology Research Unit, Eric D. Wieben, Ph.D., director of the Medical Genome Facility, and a research team at Mayo Clinic's campus in Minnesota have confirmed and validated a larger sample group to identify a core set of splicing events in human corneal endothelium that could be directly associated with FECD through CTG trinucleotide repeat expansion in the *TCF4* gene. Results of their study were published in *Investigative Ophthalmology & Visual Science* in 2017.

RNA analysis

The research team isolated and sequenced total RNA from corneal endothelial tissue obtained during keratoplasty from 12 patients with advanced FECD (modified Krachmer grade 5 or 6) requiring corneal transplantation and four patients undergoing keratoplasty or enucleation for other indications (grade 0).

"The length of the trinucleotide repeat CTG in the *TCF4* gene was determined using leukocyte-derived DNA analyzed by a combination of Southern blotting and GeneScan analysis," says Dr. Fautsch. "Commercial software — along with numerous hours of interpretation and validation by Dr. Wieben — helped to identify expression of a select set of alternatively spliced genes."

Validation of these specific alternative splicing events was performed through the use of reverse transcription polymerase chain reaction with the final gene set undergoing further analysis to identify overrepresentation in functional pathways using the web-based Panther analysis system. Outcomes include the following:

- A unique set of alternatively spliced genes associated with patients with FECD containing a CTG trinucleotide repeat expansion

sequence in the *TCF4* gene was identified.

- Differential splicing of NUMA1, PPFIBP1, MBNL1 and MBNL2 transcripts were identified in all FECD samples containing a trinucleotide repeat expansion.
- Differentially spliced genes identified in patients with FECD were enriched for products that localize to the cell cortex, bind the cytoskeleton and have cell adhesion functions.
- Corneal endothelial tissue from patients with FECD revealed a novel splicing event involving FGFR2.

"Corneal endothelium from patients with FECD harbors a unique signature of missplicing events due to CTG trinucleotide repeat expansion in the *TCF4* gene, consistent with the hypothesis that RNA toxicity contributes to the pathogenesis of FECD," says Dr. Fautsch. "In this study, change to proteins associated with cell adhesion that may interfere with the corneal endothelial barrier function, a known event in the development of FECD, was identified as a key biological process influenced by the missplicing events."

"Divergent genetic variants lead to FECD," says Dr. Baratz. "To date, no unifying pathogenic mechanism has been identified. Nevertheless, the current mechanism involving missplicing of the candidate genes seems to hold true for patients with FECD with trinucleotide repeat expansions. Therefore, changes in gene function through alternate splicing induced by a trinucleotide repeat expansion in the *TCF4* gene can be a valid pathogenic mechanism in most patients with FECD. Development of this genetic signature will be useful for identifying biochemical pathways that may contribute to the pathogenesis of the disease."

Eric D. Wieben, Ph.D.

For more information

Wieben ED, et al. Trinucleotide repeat expansion in the transcription factor 4 (*TCF4*) gene leads to widespread mRNA splicing changes in Fuchs' endothelial corneal dystrophy. *Investigative Ophthalmology & Visual Science*. 2017;58:343.

Intraoperative Use of GenTeal Gel Linked to Subconjunctival Necrotizing Granulomata

A study of 11 patients who developed postoperative subconjunctival necrotizing granulomata after ophthalmologic surgical procedures involving large conjunctival incisions indicates an association between intraoperative use of GenTeal gel and the development of postoperative subconjunctival necrotizing granulomata, potentially related to retained subconjunctival Carbopol 980.

"Topical ophthalmic lubricants containing hydroxypropyl methylcellulose are commonly used to maintain corneal clarity during vitreoretinal surgical procedures," says Andrew J. Barkmeier, M.D., Ophthalmology, at Mayo Clinic's campus in Rochester, Minnesota. "GenTeal gel has been increasingly used for this purpose because of its ability to maintain corneal clarity."

GenTeal contains both hydroxypropyl methylcellulose, a hydrophilic polymer that forms a viscous hydrogel, and Carbopol 980 as a thickener. "Biocompatibility studies of highly cross-linked, polyacrylic acid polymers have found that these compounds may stimulate chronic inflammation and macrophage infiltration. Intraoperative exposure to a similar high molecular weight polyacrylic acid polymer, Carbopol 934, has been associated with nonhealing, disfiguring lipoplasty wounds characterized by palisading necrotizing granulomata on histopathology," says Dr. Barkmeier.

Dr. Barkmeier and Raymond Iezzi, M.D., Ophthalmology, at Mayo Clinic's campus in Minnesota, performed all vitreoretinal surgeries for patients involved in the study between 2010 and 2015. Clinical and histopathologic features of study participants include:

- 10 of the 11 affected patients had a 360-degree conjunctival peritomy. The remaining patient had a 4 × 4 millimeter (mm) conjunctival cutdown to supplement retrobulbar anesthesia.
- Single surgery anatomic success was achieved for all patients with respect to the

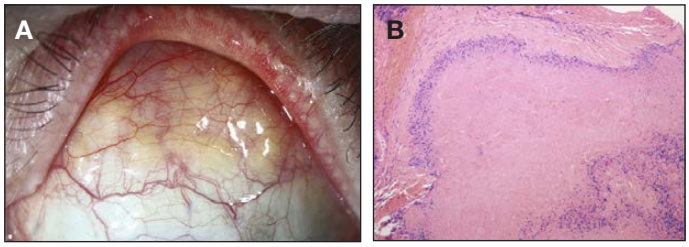

Figure 1. A. Slit lamp photograph of the left eye of a 64-year-old man demonstrates multifocal yellow subconjunctival granulomata after vitrectomy and scleral buckling. B. High-power view shows details of the palisading histiocytic reaction. Stain: hematoxylin and eosin; 100 times original magnification. Image reprinted with permission from *Ophthalmology*.

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initial surgical indication.

- Mean age of the patients at initial surgery was 52 ± 13 years.
- Best-corrected visual acuity was 20/191 preoperatively and 20/60 at last follow-up.
- Diffuse multifocal yellow elevated subconjunctival lesions were noted in all patients who had 360-degree conjunctival peritomy incisions (Figure 1A). The remaining patient had a single large granulomata at the site of a 4-mm conjunctival cutdown incision.
- A total of six lesional biopsies were performed on three patients. One patient required scleral buckling explantation owing to persistent postoperative discomfort.

All biopsy specimens shared similar histopathologic features with palisading histiocytic reactions surrounding areas of necrosis (Figure 1B). Histochemical stains for microorganisms were negative. There was no growth from bacterial, fungal or mycobacterial cultures. The research team performed multiple investigations looking for other potential causes of granulomatous inflammation, including microbial cultures and mass spectrometry studies of surgical gloves, suture materials and scleral buckling components, but results were unrevealing.

No patient experienced significant clinical progression after diagnosis, but persistent granulomata were visible at last follow-up for all patients. Eight patients required topical corticosteroid therapy beyond the standard postoperative regimen to control inflammation and discomfort, and three of the eight required a continued, indefinite course of topical anti-inflammatory management.

"Our research team did not identify any postoperative subconjunctival granulomata over the same time frame after procedures in which Goniosol or Balanced Sterile Saline Solution was used to maintain corneal clarity," says Dr. Barkmeier, "or in patients who underwent transconjunctival microincision vitrectomy surgery procedures without conjunctival cutdown incisions, with or without the use of GenTeal gel. None of the affected patients had a known systemic condition associated with necrotizing granulomatous inflammation. Although direct causality cannot be established by this retrospective investigation alone, ongoing animal studies appear to support the hypothesis."

Recommendations

"When larger conjunctival incisions are required, surgeons should consider either avoiding the use of intraoperative GenTeal gel or performing an extensive ocular surface irrigation before conjunctival closure," says Dr. Barkmeier. "In clinical practice, prior intraoperative exposure to high molecular weight, cross-linked polyacrylic acid polymers should be considered in the differential diagnosis for patients presenting with subconjunctival granulomatous inflammation." Study results were published in *Ophthalmology* in 2016.

For more information

Dalvin LA, et al. Necrotizing subconjunctival granulomata and intraoperative use of topical GenTeal gel. *Ophthalmology*. 2016;123:2262.

Andrew J. Barkmeier, M.D.

Raymond Iezzi, M.D.

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