SANJAY PATEL: Hello, I'm Dr. Sanjay Patel. I'm a corneal transplant surgeon at Mayo Clinic in Rochester, Minnesota. We have an active research program in Fuchs endothelial dystrophy that spans the genetic basis of disease through the corneal clinical imaging and corneal transplant outcomes.

In this video, I'd like to tell you a little bit about our research related to early corneal abnormalities in Fuchs endothelial dystrophy. This work was supported by Mayo Foundation and by Research to Prevent Blindness. Ernst Fuchs described the dystrophy that has become named after him back in 1910, so over 100 years ago. And he did not have a slit lamp at that time, and therefore termed this disease dystrophia epithelialis corneae. We've come to learn that this disease is actually endothelial in origin, and that the epithelial changes, or the anterior corneal changes, tend to be late in the manifestation of this disease. Specifically, corneal scarring and corneal neovascularization are considered to be the very end stages of disease. In fact, stages that we do not allow patients to reach in the current era.

With confocal microscopy, we've learned a lot about corneas that require transplantation for Fuchs dystrophy. Specifically, we've seen abnormalities in the anterior cornea related to increased corneal haze, shown here, compared to normal, which is the small white line. The haze is a result of increased basal epithelial cell reflectivity, the presence of abnormal subepithelial cells, and increased extracellular matrix in the anterior stromal, with dropout of stromal cell nuclei.

In previous studies, we've shown specific abnormalities of the corneal nerves. These are subbasal corneal nerves as seen by confocal microscopy. In a normal subject, we can see the fine nerves traversing in a superior to inferior direction. In Fuchs dystrophy, the nerves tend to be attenuated, abnormal with their branching patterns. And these persist even after endothelial keratoplasty when endothelial function has been restored. As we can see, there is still fine attenuated nerves here and abnormal branching patterns over here. We've found that decreased nerve density after endothelial keratoplasty is also associated with decreased corneal sensitivity in this disease.

These are images of corneal stromal nerves. In the normal cornea, you can see a well-defined branching pattern here. In Fuchs dystrophy, we tend to see loops of these stromal nerves, often associated with brightly reflecting keratocyte nuclei. These are apparent in other corneas with Fuchs dystrophy, and the pattern persists after endothelial keratoplasty at one and two years. The nerves are abnormal in many corneas with Fuchs dystrophy. As we look at the corneal keratocytes in the anterior stroma, here's a normal 65-year-old patient with many cell nuclei present. In Fuchs dystrophy, we see drop out of keratocytes and also significant increase in reflectivity from the extracellular matrix. Even after restoring endothelial function by endothelial keratoplasty at one, two, and three years after keratoplasty, this pattern does not change.

When we actually quantify the keratocytes in the cornea, in a normal cornea, you can see that the keratocytes have the highest density in the anterior-most stroma, with tapering of density to a more uniform level through the rest of the depth of the corneal stroma. If we look at corneas with Fuchs dystrophy and follow them through three years after keratoplasty, this is the pattern that emerges. The most striking pattern is that keratocyte density is significantly reduced in the anterior cornea, similar to normal in the rest of the cornea, though we do see that several years after the endothelial keratoplasty, there appears to be drop out of keratocytes posterially in the host stroma. When we first discovered this, we weren't sure if this was an artifact of our imaging with confocal microscopy, and therefore we did look at histology of Fuchs versus normal corneas, and we saw the same keratocyte dropout in the anterior stroma.

So if these abnormalities we were interested in went in the course of the disease that they occurred, all the corneas we had examined had been prior to endothelial keratoplasty, and clearly, there were significant abnormalities, suggesting that potentially these abnormalities begin much sooner in the disease course than we actually can see visibly at the slit lamp, or even with confocal microscopy. So we specifically looked at anterior corneal backscatter and keratocyte loss, and also the appearance of subepithelial cells. So for this study, this was a cross-sectional design. We recruited patients from our corneal service with Fuchs dystrophy, and they were defined as having central or paracentral guttata. The cornea was otherwise normal, with the exception of haze related to Fuchs dystrophy. These patients were either phakic or had a posterior chamber intraocular lens. We also recruited an age-matched normal group, who were completely devoid of guttata, they had normal corneas, and were phakic.

All subjects were examined at the slit lamp to determine the grade of disease, and confocal microscopy was performed with a ConfoScan 4 confocal microscope with z-ring adapter. The primary outcome was corneal backscatter, or haze, but we also examined keratocyte density, the absolute number of keratocytes in the presence of abnormal subepithelial cells. So clinical grading of Fuchs dystrophy is performed at the slit lamp. For the sake of this study, we used the modified Krachmer grade, where grade zero would actually be normal, completely devoid of guttae, grade one would be scattered, a few scattered guttae, grade two more than 12 scattered guttae, and then grades three to six would be varying degrees of confluent guttae. The difference between grade five and grade six is the presence of clinically detectable corneal edema.

For the sake of this study, we categorized grades one and two as mild, grades three and four as moderate, and grades five and six as advanced. This is the ConfaScan confocal microscope with the z-ring adapter. The z-ring is critical for an accurate measurement of depth in the cornea. The cornea is imaged, and as the focal plane passes through the cornea, we see images of the corneal epithelium, the corneal stroma, and the corneal endothelium. With the same instrument, as we image through the cornea, we can also measure reflected light intensity. And as this progresses through the cornea with depth, we can plot the light intensity. The white line is normal, the green line is after endothelial keratoplasty. And by standardizing this to a reference turbidity standard, we can actually follow the same patients over time in prospective studies.

So what did we find in this study? First of all, the groups, we had approximately 20 eyes in each group. Very similar age distribution in Fuchs dystrophy, and despite age matching the patients, we had a slightly younger cohort for controls. Most subjects in this study were phakic, and just a handful had posterior chamber intraocular lenses in the Fuchs dystrophy group. So here is our backscatter results. What we can see is depth of corneal stroma on the x-axis and corneal haze, or backscatter, on the y-axis. The white line represents normal corneas. And anteriorly, this would be a normal level of anterior corneal haze. Posteriorly, the corneal endothelium reflects brightly in all corneas. The regions of interest for this study were the anterior cornea and the mid-stroma. What we can see in the anterior cornea is that there were increasing degrees of haze from mild to moderate to advanced Fuchs dystrophy compared to normal. The mid-stroma did not show any differences except in the most advanced group, most likely because of corneal edema in that group.

Here we're looking at keratocyte density, again, with depth of stroma along the x-axis and keratocyte density on the y-axis. What we can see in controls is that same high density, and the most anterior stroma becoming more uniform posteriorly. In Fuchs dystrophy, keratocyte density was much lower anteriorly and remained relatively uniform posteriorly. All groups were lower in the deeper levels of the corneal stroma, but that's because of corneal edema distributing cells in a larger tissue volume. When we subanalyze this and actually look at the number of cells present, there is an absolute depletion of the number of cells anteriorly, but not in deeper levels of the stroma in Fuchs dystrophy.

Here are some images of these corneas. We can see here, a cornea with grade two Fuchs dystrophy. Endothelial cells are visible with few guttae, but there is significant dropout of cells in the interior stroma. Another cornea with grade two, with a few more guttae but endothelial cells still visible, also has dropout of cells, but perhaps more cells than the first cornea. In comparison, here's a 76-year-old normal that has a high density of anterior stromal cells. So what we learned from this is that keratocyte depletion was unrelated to stromal edema. It occurs early in the course of disease, but there's probably multiple factors contributing to cell loss that are yet to be determined.

Subepithelial cells should not be present. They are abnormal, and therefore not present in normal corneas. But we did see them in corneas with Fuchs dystrophy, even in mild cases. 10% of mild cases, almost 20% of moderate, and 30% of advanced Fuchs dystrophy. They take on a reticular pattern of varying morphology, and they're closely associated with the basal cells, which is the mosaic that you can see in the background of these images.

Now these subepithelial cells, again, are not present in a normal cornea. So here is Bowman layer with epithelium sitting anterior to Bowman's layer. In Fuchs dystrophy, we can see Bowman's layer is intact, there are no breaks, but there's a layer of subepithelial cells deep to the corneal epithelium anterior to Bowman's layer. And there is connective tissue deposited in association with this. Why might this be important? I liken this to anterior basement membrane dystrophy, which we are more familiar with and we recognize as abberating the front surface of the cornea and affecting vision.

Sometimes, we remove anterior basement membrane dystrophy to improve visual acuity. When we examined anterior corneal aberrations in Fuchs dystrophy, specifically after endothelial keratoplasty, we found that the anterior corneal surface was significantly aberrated. Total high order aberrations, here in yellow, were much higher than a normal control group. And in fact, much higher than what we see after LASIK. So it's quite possible that these subepithelial cells contribute to anterior surface aberrations and poor acuity, and these subepithelial cells do persist after endothelial keratoplasty, and therefore, may affect the outcomes of endothelial keratoplasty for this disease.

So in conclusion, anterior corneal abnormalities occur early in the course of disease, before the onset of clinically-detectable corneal edema. The early onset explains the chronicity of these abnormalities in the incomplete recovery after endothelial keratoplasty. Changes have been associated with visual symptoms in Fuchs dystrophy and outcomes after endothelial keratoplasty. A So what Fuchs described over 100 years ago as an epithelial or anterior corneal dystrophy, and that we have come to recognize as an endothelial dystrophy with secondary anterior changes in the late stages, the study that I've just shown you tells us that, in fact, there are early epithelial or subepithelial changes occurring in this disease. And these changes can impact symptoms in Fuchs dystrophy and also the outcomes of endothelial keratoplasty.

I'd like to acknowledge my contributors, Sejal Amin, who is a resident of Mayo Clinic, Keith Baratz, who is a fellow corneal transplant surgeon here, and Jay McLaren, who helps us with corneal imaging. I'd like to also acknowledge the support of Research to Prevent Blindness, the NIH, and the Mayo Foundation. Again, I'm Dr. Sanjay Patel at the Mayo Clinic in Rochester, Minnesota. Thank you for listening to this presentation.