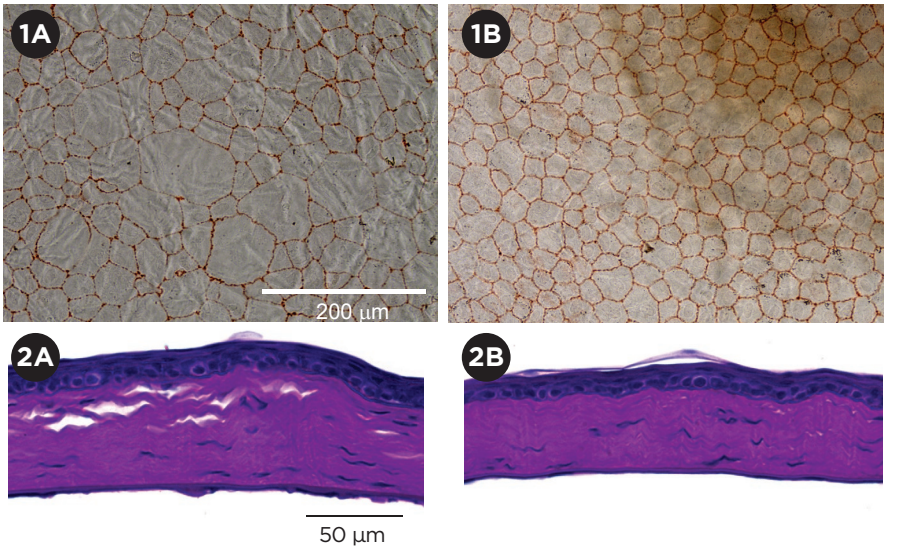
CORNEA

Gene Editing for Fuchs



ENDOTHELIAL RESCUE. *Comparison of the (1A) nontreated and (1B) treated endothelial cells in mice. Representative corneal endothelium in each group stained with alizarin red. The intraocular injection of an adenovirus encoding both the Cas9 gene and guide RNA also reduced guttae-like structures on the corneal endothelium, as shown in the periodic acid-Schiff–stained corneas of (2A) nontreated and (2B) treated mice.*

USING A MURINE MODEL OF EARLY-onset Fuchs corneal dystrophy, researchers found that a single instance of CRISPR/Cas9 gene editing can protect endothelial cells indefinitely from the degeneration that would otherwise cloud the cornea.[1]

Novel approach. The scientists accomplished this feat by taking a different approach to ocular gene therapy, said study coauthor Balamurali K. Ambati, MD, PhD, at the University of Oregon in Eugene.

Their target: *COL8A2*. Normally, this collagen gene is not essential to healthy endothelial cell function—but in its mutated form, the gene leads to the cellular dysfunction and death found in Fuchs, Dr. Ambati noted.

At that point, because the endothelial cells are postmitotic, editing the mutated gene sequence with CRISPR is not an option, he said. Instead of trying to edit out the single point mutation, Dr. Ambati said, "What we did with CRISPR was to use a technique called insertion/deletion to disrupt the start codon of the mutant protein. And by knocking down the synthesis of this mutant protein, we were able to ameliorate all the different adverse effects of it, restoring endothelial cell density and function."

Study in mice. Dr. Ambati and his colleagues worked with gene knockout mice that develop a disease which closely resembles early-onset Fuchs.

The mice were given a single anterior chamber injection of a recombinant adenovirus, which carried an RNA snippet designed to inactivate the faulty gene's "start" codon. This prevented production of the mutant protein, and the therapeutic effect lasted at least 10 months after injection, the researchers said.

"Once you disrupt the gene in these postmitotic cells, you've fixed the problem, because there will be no new cells, since endothelial cells do not reproduce," Dr. Ambati said.

With regard to safety, because adenoviruses can induce inflammation and cell toxicity, the researchers tested a range of titers of the adenoviral vector. Corneal transparency, corneal thickness, and histopathology were normal at low titers, they reported. They also confirmed that the vector did not suppress retinal function or damage the retinal structure, nor did it induce liver or kidney damage or inflammation.

Looking ahead. If this CRISPR-based therapy works in humans, it would reduce or perhaps eliminate the need for corneal transplants in patients diagnosed with Fuchs, Dr. Ambati said. Start codon disruption also might be applicable to other adult-onset diseases caused by missense mutations, he added.

In the next year, the researchers plan to begin testing the therapeutic adenovirus in donor cadaver corneas with and without guttae (a hallmark of Fuchs). If those studies show that the technique works and is not toxic to human cells, tests in larger animals will follow, Dr. Ambati said.

—Linda Roach

1 Uehara H et al. *eLife*. 2021;10:e55637.

Relevant financial disclosures: Dr. Ambati—NEI: S; Research to Prevent Blindness: S.

COMPREHENSIVE

Ocular Syphilis Is Back in the Picture

AN OLD FOE—SYPHILIS—HAS RE-emerged. And as a Canadian team of ophthalmologists recently found, there's no such thing as a "typical" patient with ocular syphilis.[1]

"The big surprise was the variety in affected patients—male/female, young/old, single/married," said Lulu Bursztyn, MD, MSc, FRCSC, at Western University in London, Ontario, Canada. "There is no reason to only test certain populations, as anyone can be infected."

What sparked the study? "I had seen a few referrals from experienced ophthalmologists who were stumped," Dr. Bursztyn said. Before too long, she realized that other clinicians were no longer thinking about syphilis, "despite the fact that it has clearly been on the rise over the past decade."

In Canada, syphilis rates more than doubled from 2008 to 2017.[1] And in the United States, the numbers of cases reported to the CDC increased by 81% from 2014 to 2018.[2] "After an outbreak was officially declared in our region (London-Middlesex) in 2019, it was clear that more and more cases were going to surface, so we wanted to use these cases to help educate others," Dr. Bursztyn said.

Signs and symptoms. Dr. Bursztyn and her coauthors assembled a series of 26 cases of ocular syphilis, discussing

GLAUCOMA

POAG Linked to Common Autoimmune Diseases

RESEARCHERS AT MASSACHUSETTS EYE AND EAR (MEE) in Boston have found clinical evidence to support earlier laboratory findings of a link between primary open-angle glaucoma (POAG) and autoimmune diseases.[1]

In a group of patients undergoing cataract and/or glaucoma surgery, those with POAG were significantly more likely to have an underlying autoimmune condition such as rheumatoid arthritis (RA), psoriasis, Graves disease, and/or Raynaud syndrome.

Sources of inspiration. The glaucoma patients of Lucy Q. Shen, MD, at MEE, provided the original impetus for this study. In 2018, several of them flagged an earlier study on an autoimmune mechanism underlying neuronal loss in glaucoma[2]—and asked about alternative therapy for their glaucoma.

Next, she said, "Dr. Maltish Lorenzo, a medical student at the time, was motivated to start a study on immunologic biomarkers in patients undergoing glaucoma surgery. To prevent potential interference with other autoimmune conditions in our study population, we excluded those patients with inflammatory and autoimmune diseases." But after a few months of screening and recruiting, Dr. Lorenzo told Dr. Shen that this would necessitate ruling out a significant number of glaucoma patients, as many had autoimmune diseases. "Based on his observation, we decided to examine the prevalence of autoimmune diseases in patients with POAG."

Study specifics. The researchers evaluated 172 patients with POAG and 179 controls. Results showed that 17.4% of patients with POAG and 10.1% of controls ($p = .044$) had an autoimmune disease, with RA and psoriasis the most common in both groups. In addition, 6.4% of POAG patients and 3.4% of controls had more than one autoimmune condition.

Clues and surprises. The team's investigation provided three important clues about the link between POAG and autoimmunity, Dr. Shen said:

1. Agreement. While the researchers naturally hoped that their clinical findings would line up with those from the earlier laboratory study, "it still surprised us, in a good way, that they agreed," Dr. Shen said. In particular, the finding that T-cell–mediated autoimmune diseases were more prevalent in POAG patients than controls is consistent with the earlier results, which showed a key role of T-cells in mediating glaucomatous neurodegeneration.[2]

2. Role of steroids. A subgroup analysis of POAG patients with and without autoimmune disease did not show a difference in glaucoma severity. When the researchers analyzed this finding, they found that the POAG patients with autoimmune diseases were more likely to use steroids. This may have modulated disease severity, they hypothesized.

3. A role for CD4? An unexpected clue came from a separate study on HIV patients and retinal nerve fiber layer (RNFL) thickness: In this investigation, patients with HIV and low CD4 counts had a thicker RNFL.[3] "This is consistent with our theory that autoimmunity, in particular T-cell–mediated mechanism, is involved in glaucomatous optic nerve damage," Dr. Shen said. "Low CD4 counts may have protected these HIV patients from losing RNFL."

Looking ahead. The MEE team has an ongoing prospective study to assess various immunologic biomarkers in glaucoma, Dr. Shen said, in hopes of further validating the link between autoimmunity and glaucoma. "One day, I do hope that I will be able to tell my patients—especially those who have inspired me on this journey—that we have new treatment options for their glaucoma."

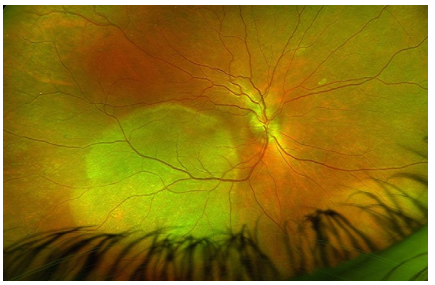
—Jean Shaw

1 Lorenzo MM et al. *Ophthalmol Glau.* Published online Aug. 18, 2021.

2 Chen H et al. *Nat Commun.* 2018;9(1):3209.

3 Van Tassel SH et al. *Int J Ophthalmol.* 2019;12(5):789-794.

Relevant financial disclosures—Dr. Shen: American Glaucoma Society: S; Topcon: S.



SUSPICION. *Posterior placoid chorioretinopathy, seen in a 44-year-old woman who initially denied typical symptoms of syphilis.*

five in detail. Although two of the five had "typical" features of ocular syphilis (e.g., optic neuritis, uveitis), the others did not. "There really were no pathognomonic features, and I think we demonstrated the wide variety of both symptoms and signs in our paper," she said.

Take-home message. "The primary message is that as the prevalence of syphilis continues to increase, ophthalmologists should consider syphilis in the differential diagnosis of disease affecting any ocular structure," Dr. Bursztyn said.

What about adding sexual history screening to the medical history? For instance, of the 26 cases presented in this report, only 12 patients disclosed risk factors for syphilis—and of these, only three disclosed their risk factors before diagnosis. However, as the authors pointed out, "patients may be reluctant to disclose [sexual history] even to focused questioning."[1] A simpler solution, then, would be to add serologic syphilis screening to any atypical case of uveitis or optic neuritis, regardless of any identified risk factors, they said.

And as Dr. Bursztyn noted, "The treatment is cheap, simple, and effective, so there is significant benefit of making the correct diagnosis."

—Jean Shaw

1 Schulz DC et al. *Can J Ophthalmol.* 2021; 56(5):283-293.

2 Ghanem KG et al. *N Engl J Med.* 2020;382(9): 845-854.

Relevant financial disclosures: Dr. Bursztyn—None.

PEDIATRIC OPHTHALMOLOGY

Virtual Tx Effective for Amblyopia

HOW CAN PEDIATRIC OPHTHALMOLOGISTS transform amblyopia therapy into something that children enthusiastically embrace? The answer might lie in replacing patches and eyedrops with videos and TV shows displayed with a dichoptic program on a virtual reality (VR) headset, results of a phase 3 multicenter clinical trial suggest.[1]

Gains in vision. Researchers randomized 4- to 7-year-old children to one of two groups. While all children continued with their vision correction, those in the treatment group (n = 45) viewed dichoptic videos for one hour a day, six days a week, while those in the comparison group (n = 45) wore a placebo headset.

At the end of 12 weeks, the children in the video group had gained a mean of 1.8 lines (95% confidence interval [CI]: 1.4-2.3 lines) of visual acuity in the amblyopic eye. In contrast, those in the control group gained a mean of .8 lines (95% CI: 0.4-1.3 lines). The 1-line difference between groups was statistically significant (p = .0011).

Study surprises. "We weren't really expecting to see results so quickly, but within four weeks we were already seeing statistically significant improvements [in those who watched videos], compared to wearing eyeglasses, and that difference persisted over the full 12 weeks of the trial," said David G. Hunter, MD, PhD, at Harvard Medical School and Boston Children's Hospital. Moreover, he said, "Adherence to prescribed therapy was 86%, far beyond what we can achieve with patching."

How it works. The video system, Luminopia One (Luminopia), consists of an off-the-shelf VR headset (currently the Samsung Gear VR) plus a smartphone and associated app for choosing the video that will be streamed.

To keep children engaged, the system allows them to choose from a cloud-based library of 546 hours of popular commercial programs. (Parents have an online portal to monitor their child's progress and viewing choices.)

The system's treatment algorithm blurs different sections of the images seen by each eye, Dr. Hunter said. "It blurs complementary parts in the amblyopic eye and the better eye, so this requires the brain to put the puzzle pieces together in order to see the full picture," he said.

What's next? Further research will be needed to determine how long gains in vision persist and how well the video system works with older children, Dr. Hunter said. In addition, outcomes achieved with the system need to be directly compared with those achieved with patching and atropine therapy.

On Oct. 20, the FDA granted de novo premarket approval of Luminopia One. The company plans to launch it in the second quarter of 2022. For now, the results are exciting for clinicians who are concerned about noncompliance with the existing therapies, Dr. Hunter said. "It's great to be able to imagine a day when we can tell parents that we can treat their child's amblyopia by having them watch TV for an hour a day through this device."

—Linda Roach

1 Xiao S et al. *Ophthalmology*. Published online Sept. 14, 2021.

Relevant financial disclosures: Dr. Hunter—Luminopia: C,O.

VIDEO TX. *The software used in the system modifies content in real time to encourage binocular fusion.*

See the financial disclosure key, page 10. **For full disclosures,** including category descriptions, view this News in Review at aao.org/eyenet.