Multimodal Retinal Imaging in the Era of Ocular Gene Therapy

New imaging modalities have shed light on how different eye diseases may benefit from gene therapy.

By Szilárd Kiss, MD

After 2 decades of modest progress, gene therapy appears to be gaining traction by focusing on localized anatomic areas, such as the eye or the brain. Ocular gene therapy is particularly promising because the eye is highly accessible and 1 of the few immunologically privileged sites in the body. With ongoing advances in ocular imaging, the eye can be better observed and monitored than ever before.

The Current State of Ocular Gene Therapy

Retinal degeneration due to gene mutations and alterations is not surprising given the complexity of processes involved in detecting light and transferring this information into sight. To date, over 200 genes have been mapped to cause retinal diseases that can impair vision in myriad ways. Retinal diseases can be generally divided into 2 categories: (1) hereditary retinal dystrophies that are strictly genetic and have been linked to a specific gene, and (2) retinal degenerations that are heavily influenced by environmental conditions but also have a genetic element.

Although hereditary retinal dystrophies, such as retinitis pigmentosa (RP), Leber congenital amaurosis, and Stargardt disease, are relatively rare, retinal degenerations such as age-related macular degeneration (AMD), glaucoma, and diabetic retinopathy affect over 135 million individuals worldwide. Fortunately for those that are personally affected, researchers are making progress to transfect the appropriate cells with a gene that expresses an antiangiogenic protein. Transfection of retinal pigment epithelium (RPE) cells with pigment epithelium-derived factor and other growth factors has potential to prevent or slow degeneration in these retinal diseases, perhaps replacing ongoing anti-VEGF injections with a single vector that will sustain drug delivery within the eye and eliminate the need for repeated intravitreal injections.

Optical Coherence Tomography Imaging

One of the advantages of studying gene therapy in the eye as compared to other areas of the body is the ability to noninvasively observe the structures and monitor the state of disease and results of treatment. Optical coherence tomography (OCT) has greatly influenced the way we diagnose and manage ocular diseases. Continuing advances in high-resolution OCT imaging, such as enhanced depth imaging and active eye tracking, have significantly contributed to our understanding of the relationship between the microstructure of the macular photoreceptor layer and visual function, aiding ocular gene therapy.

In RP and other retinal diseases, examination of the retinal architecture in situ is essential for evaluating the state of the disease. Recently, the ellipsoid zone, formerly known as the inner/outer photoreceptor junction, has been highly correlated with normal visual function and the recovery of good vision after intraocular surgery for macular hole or epiretinal membrane. A continuous ellipsoidal line indicates normal alignment of membranous discs in the photoreceptor outer segments, allowing us to assess the integrity of the photoreceptor cells as a means of monitoring patients with RP. As the disease progresses, the length of the ellipsoidal line shortens, retinal sensitivity decreases, and visual acuity worsens.

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**Case No. 1.** Color fundus photography of a 43-year-old man with an inherited bilateral macular dystrophy showed central atrophy and pigmentary changes (Figure 1A). Fundus autofluorescence (FAF) of the same patient revealed a large area of hypofluorescence with a surrounding region of hyperfluorescence. There remains a central area of nearly normal FAF. Despite this large area of atrophy, the patient’s vision in both eyes was 20/80 (Figure 1B). OCT imaging showed extensive retinal and choroidal atrophy corresponding to the hypofluorescence regions. Note the relative normal outer retina beyond the central macula (Figure 1C).

**Case No. 2.** Despite a significantly decreased visual field, a patient with advanced RP maintained 20/20 central vision. The remaining functional central retina was seen on the infrared (IR) image (outlined by the red arrows; Figure 2A). OCT images correlate by showing a relatively normal central retina (along yellow line) which corresponds to the green line in the IR image. Outside this central region, there was considerable outer retinal atrophy, providing an anatomical explanation for the constricted peripheral visual field (Figure 2B).

**Case No. 3.** Heidelberg Spectralis FAF of a patient with genetically confirmed Stargardt disease. Hyper- and hypofluorescence areas were noted in the macula, as well as multiple, discrete hyperfluorescence lesions outside this central area (Figure 3A). The yellow line in this OCT image corresponds to the green line in the FAF image of this patient, showing an abnormal retina with atrophy in the central macula. Outside of this central region, the retina appeared relatively intact. The hyperfluorescence areas noted on the FAF image correspond to the outer retinal lesions noted on the OCT just at the junction of normal and abnormal retina (red arrows; Figure 3B).

**Case No. 4.** OCT images of both eyes of a 54-year-old man with RP and no visual complaints. The retinas appeared relatively normal at first glance. However, on close inspection, the OCT images revealed much more extensive degeneration.
inspection, outer retinal abnormalities at the level of the retinal pigment epithelium were seen in both eyes (red arrows, Figure 4A). FAF imaging of both eyes of the same patient showed extensive areas of hypo- and hyperfluorescence in both eyes. The extent of retinal degeneration was much more evident in the FAF images compared to the OCT. Some of the abnormal FAF did, however, correspond to the subtle changes noted in the retina on OCT. Assessment of visual function, as well as the changes noted on OCT and FAF, could be important outcomes in any treatment for this patient's degeneration (Figure 4B).

**Case No. 5.** An IR image of a patient with genetically confirmed Stargardt disease shows a central area of atrophy with some macular pigmentary changes (Figure 5A). OCT images showed extensive outer retinal atrophy (yellow line), especially in the fovea, where nearly complete retina atrophy was noted (red arrow, Figure 5B). Given the extent of atrophy on the OCT, significant improvement in vision was unlikely with any treatment. However, the area of retinal atrophy may serve as a surrogate for evaluation of response to gene therapy. FAF images of both eyes of the same patient show hypofluorescence in both maculas. A small area of hyperfluorescence remained in each eye in the central fovea. Outside the central hypofluorescence region, there was a ring of hyperfluorescence with numerous hyper- and hypo-fluorescence disciform lesions. As is typical in Stargardt disease, these lesions spared the peripapillary area. The extent of retinal degeneration is much better appreciated on the FAF images compared to the IR or even dilated fundus exam. The progression of the central atrophic zone, as delineated by the hypofluorescence, may serve as a surrogate endpoint for treatment trials (Figure 5C).

**Fluorescein Angiography and Fundus Autofluorescence**

Other ocular imaging tools are also contributing to the overall understanding of diseases that may benefit from gene therapy. Recent technology has doubled the 45° field of view provided by traditional fundus cameras, allowing imaging of the peripheral retina; this will likely play an important role in research and clinical management of these diseases in the future. Fundus autofluorescence (FAF) imaging is another noninvasive modality essential to understanding the pathophysiology of several diseases that may benefit from gene therapy. Excessive lipofuscin, which manifests with areas of hyperfluorescence in FAF images, is a common symptom in several disease processes, such as AMD, inherited retinal dystrophies, and Stargardt disease. Hypofluorescence is also a valuable indicator of disease state. The information FAF provides regarding the metabolic changes in the eye is distinct from the data provided by OCT and fluorescein angiography, adding an additional layer of understanding.

**Conclusion**

Different imaging modalities highlight distinct pathological details, making it highly useful to employ as many as possible. The ability to see detailed images of the anatomy and impact of treatment will undoubtedly play a large role in the future of gene therapy for the eye.

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