“WHAT’S THE GENE?”

An update on gene therapy from a choroideremia perspective.

BY THOMAS L. EDWARDS, PhD, FRANZCO

“WHAT’S THE GENE?” is unvaryingly the first question posed to trainees and vitreoretinal fellows after an anonymous color fundus photograph is presented for their scrutiny at the weekly professorial teaching session at the Oxford Eye Hospital. The case, which shows strikingly pale fundi with extensive chorioretinal atrophy and an island of relative sparing at the posterior pole, concerns that of a 28-year-old man with 6/6 vision. A trainee mumbles, “Choroideremia?” prompting the follow up question, “So, how big is the gene?”

That genetics (and choroideremia in particular) is the topic of these vitreoretinal tutorials should come as no surprise; a number of clinical trials of gene therapy for inherited retinal degenerations (IRDs) are under way around the world, including one for choroideremia being conducted here in Oxford.1 Gene therapy for RPE652-4 and MERTK5 (both for retinitis pigmentosa) are in clinical trials at other centers, and additional genes—such as RS1 (X-linked juvenile retinoschisis), CNGB3 (a cause of achromatopsia) and RPGR (the most common cause of X-linked retinitis pigmentosa)—will likely be targeted in clinical trials in the near future. A summary of some of these trials was provided by Boye et al in their comprehensive review of retinal gene therapy.6

WHY CHOROIDEREMIA?

Although choroideremia is relatively uncommon, affecting approximately 1 in 50,000 people, it is well suited to a study of gene therapy for three main reasons. First, because a patient’s central retina is usually preserved until late in the disease course, visual acuity and macular sensitivity remain reliable outcome measures. Second, relative preservation of central retinal thickness may safeguard the target region during subretinal injection. Third, the choroideremia gene CHM has a relatively short coding sequence (1962 base pairs), which is less than the maximum capacity for recombinant adeno-associated virus serotype 2 (AAV2); hence the follow-up question in the above tutorial concerning gene size. This virus is able to transduce all layers of the neurosensory retina,7 making it ideally suited for targeting the affected cells in choroideremia.

PHASE 1/2 TRIAL

Given that the majority of CHM pathologic variants are predicted to result in loss of gene function, gene replacement is a sound strategy for this X-linked IRD. An ongoing phase 1/2 trial demonstrated sustained improvement of central visual acuity 3.5 years after administration of AAV2 containing the nonmutated CHM transgene.1 This was an encouraging finding, given that two recent reports of AAV usage for Leber congenital amaurosis found that patients’ early gains in visual function subsequently declined.4,8 Moreover, the study dose (1 x 1010 viral genome particles) in our choroideremia trial was expected to be at the lower end of the therapeutic range because the primary outcome was safety. A subsequent clinical trial using a higher gene dose is under way to further resolve the optimal treatment dose.

DETERMINING EFFICACY

Determining the best outcome measures for assessing gene therapy efficacy in slowly progressive IRDs is a significant challenge. In choroideremia, central visual acuity is often spared until middle age. Consider this article’s opening tutorial case as an example: If this patient received gene therapy, researchers might need to wait years before observing a rescue effect relative to the untreated fellow eye.

An array of tests—including visual acuity, contrast
sensitivity, color vision, autofluorescence, and micropereimetry—are deployed throughout the follow-up period to detect changes in visual function. These tests are also performed in untreated individuals to help build a better understanding of the disease’s natural history. For example, our laboratory recently developed an index that predicts the rate at which clinicians should expect to observe contraction of the residual area of autofluorescence in choroideremia. One can see how this could become a useful clinical tool for counseling patients and monitoring treatment responses in trial participants. Furthermore, it could be used to identify female carriers with a progressive male-pattern phenotype in whom gene therapy would likely be beneficial.

**DELIVERING A VIRAL VECTOR**

While the phase 1/2 study established the safety of our AAV2 vector in the eye, the emerging challenge for vitreoretinal surgeons will be how to consistently administer a precise volume of viral vector safely into the subretinal space, particularly in eyes with fragile degenerate retinas. The technically simpler alternative—intravitreal administration—requires a larger dose of virus to produce an equivalent multiplicity of infection, increasing the risks both of retinal toxicity and of an adverse immune reaction to the larger viral load.

In the ongoing Oxford trial, the vector is delivered to the subretinal space using the viscous fluid injection mode on the vitrectomy machine and a custom 41-gauge Teflon-tipped cannula held by the surgeon in the subretinal space. Inevitably, improvements in safety and reliability will be driven by technological innovation. Already, operating microscopes equipped with optical coherence tomography devices are being used by some centers to observe in real time the extent and location of the subretinal bleb during gene therapy procedures. In the near future, robot-assisted instrument manipulation will enable precision placement of a cannula under the retina. The cannula tip could be held perfectly still in the subretinal space by the robot, enabling an injection to take place over several minutes or even longer, reducing the risk to the retina from damaging stretch forces or surgeon tremor.

**THE FUTURE OF GENE THERAPY**

A number of IRDs may soon become amenable to subretinal injection of a viral vector capable of slowing or halting retinal degeneration. Identifying the causative gene will become increasingly important for genetic counseling and predicting the expected rate of progression, based on a growing body of gene-specific natural history data. The surgical challenges associated with subretinal delivery will be overcome with the aid of technological innovations, such as robot-assisted surgery, that facilitate micron-level precision and steadiness of intraocular instruments during gene therapy procedures.

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