# Mitochondrial gene therapy

*By Lev Osherovich, Senior Writer*

A **University of Miami** team has used mitochondrion-targeted gene therapy to restore functional NADH dehydrogenase subunit 4 levels in Leber's hereditary optical neuropathy.[1] Next steps include optimizing the approach to cover several variants of the disease and determining the therapy's potential advantages over pharmacological strategies in development.

Leber's hereditary optical neuropathy (LHON) affects about 1 in 30,000–50,000 people and manifests as sudden vision loss in early adulthood. Unlike in most hereditary diseases, the defective genes behind LHON are encoded by DNA in the mitochondria, not in the nucleus. The mitochondria, cellular subcompartments that generate energy for the cell, retain a small circular genome encoding a set of key proteins that cannot be correctly manufactured elsewhere in the cell.

The most common mutations underlying LHON are in a gene encoding NADH dehydrogenase subunit 4 (ND4). As a result of ND4 defects, mitochondria in retinal ganglion cells are unable to make energy and instead undergo apoptosis. Because retinal ganglion cells relay information from the retina's photoreceptors to the brain, their degeneration leads to rapid-onset blindness.

Now, a team led by John Guy, professor of ophthalmology at the Bascom Palmer Eye Institute at the **University of Miami Miller School of Medicine**, has devised an adeno-associated virus (AAV) vector that delivers a wild-type *ND4* gene directly into the mitochondria.

Using this vector, Guy's team restored mitochondrial function and prevented disease progression in cell culture and mouse models of LHON.

"The advance was getting DNA into mitochondria and doing it efficiently. Nobody has been able to do that in the animals" until now, said Guy.

## Mitochondrial insertion

Guy told *SciBX* that getting transgenic DNA or proteins into mitochondria has been a major challenge for gene therapy for LHON. He said a number of research teams, including his own, have tried various strategies over the years, including formulations of DNA and protein designed to enter the cytoplasm and then penetrate the mitochondrion's double membrane layer.

"People have tried to use peptide nucleic acids or chaperone proteins to get DNA into mitochondria, but there were toxicity and efficacy problems with those techniques," said Guy.

While testing various AAV vectors, Guy's team devised a new delivery strategy. The group modified the exterior of the AAV capsid to incorporate a short peptide sequence that acts as a mitochondrial localization signal.

In a cell culture model of LHON, a fluorescently tagged version of the engineered AAV with a human *ND4* gene localized to the mitochondria and increased ATP synthesis compared with no treatment. Guy said with the new construct, his team "saw a 25% increase in mitochondrial efficiency compared to an older technology" involving nuclear delivery of the transgene.

Guy's team then injected the AAV vector into the vitreous body of the eyes of mice with a mutant allele of *Nd4* that causes degeneration and vision loss. The AAV vector led to slower degeneration of retinal ganglion cells than an AAV vector without the mitochondrial localization sequence.

Results were reported in the *Proceedings of the National Academy of Sciences*.

"They are targeting the entire virus into the mitochondria by putting the mitochondrial targeting sequence into the capsid of the virus, which by itself is quite astonishing," said William Beltran, assistant professor of ophthalmology at the **University of Pennsylvania**.

Beltran was part of a team that recently reported a gene therapy strategy for a form of retinitis pigmentosa, another hereditary vision loss disorder that affects a different part of the retina than LHON.[2]

He noted that the mutations behind hereditary vision disorders affect a variety of genes and manifest in different parts of the retina, so the specific details of an AAV-mediated gene delivery strategy will differ with each indication.

Guy's success with mitochondrial targeting "illustrates how the field of AAV vector–mediated gene therapy is going—the key point is tropism, tropism, tropism," said Beltran. "People are now making all kinds of modifications to allow cell- and even organelle-specific targeting."

## Universal vector

Although delivering a transgene to the mitochondria is a major step toward correcting the genetic lesions behind LHON, translation of the approach to the clinic is likely to require years of preclinical optimization.

One challenge is the heterogeneity of genetic etiologies behind LHON. Alfredo Sadun, professor of ophthalmology and neurological surgery at the **University of Southern California**, noted that Guy's strategy addresses only one of the three genetic lesions that cause LHON.

"In LHON, there are three possible mutated genes, all in different parts of the mitochondrial genome," said Sadun. "For a successful gene therapy, you'd have to fix the specific component" that is defective in each patient.

To that end, Guy said his next step is to make an optimized AAV vector construct that would be useful in any patient with LHON.

"I can fit all three LHON genes into one virus," said Guy. "Rather than making separate constructs [for each form of LHON], you would

want to make a virus with a promoter that drives the expression of all three genes."

But Douglas Wallace, director of the Center for Mitochondrial and Epigenomic Medicine at **The Children's Hospital of Philadelphia**, thinks it will be challenging to make a universal vector that corrects mutations in all three LHON genes. He said mitochondrial protein levels are finely balanced, and overexpression of all three genes may throw off this balance.

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***—Alfredo Sadun, University of Southern California***

Combining the multiple genes into one virus "is a good idea, but one of the concerns is that the mitochondrial energy complex is made of 54 polypeptides" that must be kept in proportion, said Wallace.

Wallace discovered the genetic basis for LHON in the 1980s and has since studied the global distribution of the disease and identified biomarkers for at-risk populations.[3]

Another concern is whether the mouse model used by Guy's team truly recapitulates the mitochondrial defects in LHON. The group used transgenic mice expressing a dominant-negative form of *ND4* rather than the loss-of-function mutations that lead to disease in humans.

"We need a preclinical model that accurately models this disease," said Wallace, who noted that his own lab has been working on making such a model.

For now, Wallace and Beltran agreed that further characterization of the effect of transgenic *ND4* expression and activity in mice is needed.

### Drugs vs. genes

Another open question is whether gene therapy will be more effective than the two pharmacological strategies for LHON that are in clinical testing.

Thomas Meier, CEO of **Santhera Pharmaceuticals Holding AG**, said that Guy's study establishes a proof of principle for the genetic correction of a specific LHON mutation but that the translational path for the approach will require overcoming many manufacturing, safety and efficacy hurdles.

"There is still a very long way before this can be considered an emerging therapy for humans," said Meier.

Santhera's Catena idebenone (SNT-MC17), a co-enzyme Q10 derivative, is in registration for LHON in Europe and is marketed for Friedreich's ataxia in Canada.

Meier noted that Catena, which is thought to restore mitochondrial energy flow and reduce apoptosis in retinal ganglion cells, works irrespective of the specific mutations that underpin LHON in any particular patient.

Meanwhile, Sadun is enrolling patients in a Phase I/II LHON trial of EPI-743, a co-enzyme Q10 derivative from **Edison Pharmaceuticals Inc.** Sadun said EPI-743 is "about 500-fold more potent" than Catena.

Guy said he is trying to adapt his AAV vector for use in other mitochondrial diseases outside of the eye, including Leigh's disease, a rare neurological disorder.

The technology described in the *PNAS* paper is patented and available for licensing.

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### COMPANIES AND INSTITUTIONS MENTIONED

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