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Gene therapy for retinal dystrophy

Counteracting splice defects in the *CEP290* gene using RNA antisense oligonucleotides or Cas9-mediated gene editing is a therapeutic strategy for Leber congenital amaurosis type 10—a severe untreatable retinal dystrophy leading to childhood blindness.

José Alain Sahel and Deniz Dalkara

Type 10 Leber congenital amaurosis (LCA10) is a severe retinal dystrophy leading to vision loss that starts in childhood. Patients affected by this disease require lifetime assistance, and their overall quality of life is poor. The underlying causes of LCA10 are mutations in the ciliopathy gene *CEP290*, which encodes a connecting protein between the cell body and the delicate outer segment of the photoreceptor cells. The lack of functional protein leads to outer segment degeneration and defective phototransduction. A known effective therapeutic approach to treat monogenic recessive diseases of the retina is gene-replacement therapy, whereby cDNA encoding the mutated protein is delivered to affected cells using adeno-associated virus (AAV) as a vector. Indeed, the Food and Drug Administration (FDA) recently approved Luxturna, a gene-replacement therapy medicine for type 2 Leber congenital amaurosis caused by a recessive mutation in *RPE65*—encoding a retinal isomerase enzyme—following successful clinical trials in which this strategy was applied¹. Unfortunately this mode of therapy cannot be applied to LCA10 owing in part to the large size of the *CEP290* cDNA, which exceeds the carrying capacity of AAV—the only effective vector to target photoreceptor cells. In this issue of *Nature Medicine*, two new nucleic-acid-based treatments are proposed to fill the unmet therapeutic need in this disease and potentially other genetic diseases.

LCA10 is typically detected in young patients with a family history of the disease, and the therapeutic window for intervention is long because the cone photoreceptor structure is preserved mainly in the region of the retina providing high acuity vision (fovea). The most prevalent mutation causing LCA10 creates splicing defects, meaning that there are therapeutic opportunities at the mRNA level using antisense oligonucleotides and at the DNA level using gene editing to correct the aberrant splicing. The molecular genetics and pathological mechanisms in LCA10 suggest that removal of the mutation is highly likely to result in normal splicing

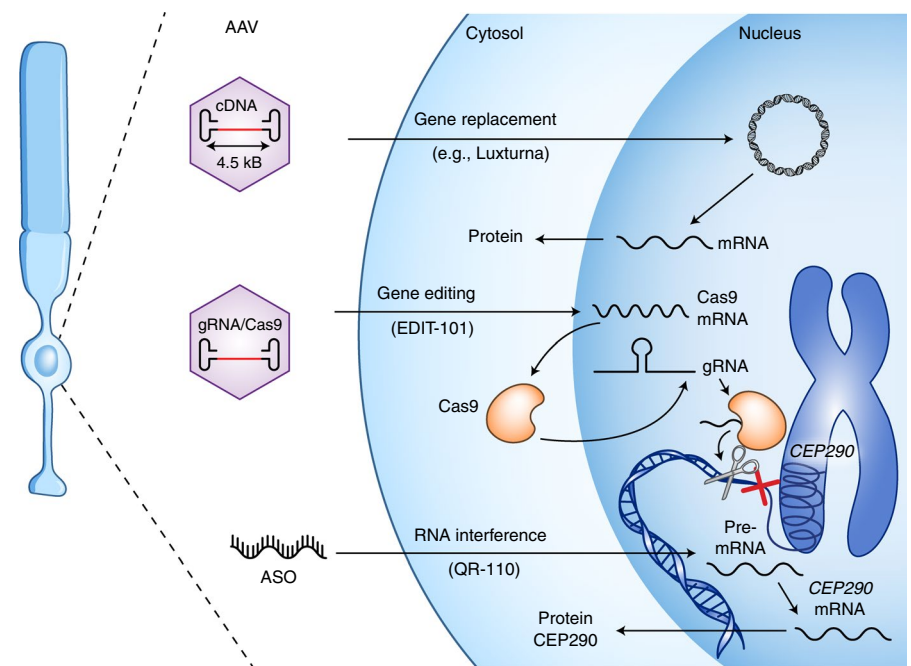


Fig. 1 | Nucleic-acid-based therapies for inherited retinal degenerations. AAV vectors can be used to deliver cDNA encoding replacement genes in diseases caused by monogenic recessive mutations, such as the mutation in the *CEP290* gene represented by a red cross. This vector is limited to a 4.5-kB carrying capacity, which has made it suitable for gene-replacement therapy in some forms of retinal degeneration (top). Maeder et al.³ develop EDIT-101, an approach to treat splicing mutations in which AAV carries DNA encoding saCas9 and its gRNAs for gene editing. Alternatively, antisense oligonucleotides can target mutations on the pre-mRNA and do not require AAVs to penetrate the target cells. Cideciyan et al.² use this approach to correct the *CEP290* splicing.

and subsequent restoration of normal *CEP290* expression. There is thus a clear opportunity to create a noticeable therapeutic benefit in this disease since improvement in only 10% of cone photoreceptors located in the fovea is believed to be sufficient for useful visual acuity.

Cideciyan et al.² describe an approach based on an RNA antisense oligonucleotide referred to as QR-110—an investigational therapeutic that is first in its class—to address *CEP290* mutation at the mRNA level. The targeted mutation creates a splice donor site, and aberrant splicing results in the insertion of a cryptic exon into many *CEP290*

mRNA transcripts. QR-110 promotes the production of a normal *CEP290* protein by binding to the mutated location in the pre-mRNA and thereby allowing normal splicing (Fig. 1). This approach does not rely on gene transfer by AAV. Instead, QR-110 is designed to be administered through intravitreal injections into the eye. QR-110 has been granted orphan drug designation in the United States and the European Union and has received fast-track designation by the FDA. Improvement following QR-110 administration has been perceptible by patients and quantifiable with several outcome measures such as visual

acuity, ocular stability and photoreceptor anatomy. An important question remains regarding the longevity of the efficacy and the effect of repetitive dosing. Indeed, this new therapeutic option requires frequent re-administration of the RNA drug to patients over their lifetime—a concern for an early-onset disease that may require the development of long-term delivery methods.

An attractive alternative therapeutic strategy applicable to LCA10 is the use of gene editing to target the mutations within the genome. Gene editing using CRISPR–Cas9 allows a one-time modification of the genome, which leads to a precise and permanent intervention that can halt disease progression. Moreover, today there are Cas9 orthologues, such as saCas9, which can be packaged into single AAV vectors along with guide RNAs (gRNAs) to direct the Cas9 nuclease to precise genomic locations within the photoreceptor cells. Maeder et al.³ report one such gene-editing approach to remove the major underlying splice defect causing LCA10. With remarkable proof-of-concept studies conducted on disease models from patient fibroblasts to human organotypic retinal cultures to nonhuman primates, they make a convincing case for clinical translation of their gene-editing medicine, which they call EDIT-101.

In their preclinical study, Maeder et al.³ identified Cas9 gRNAs that are highly specific with no verified off-targets during the course of their study. They confirmed that removing the aberrant splice site with this pair of gRNAs in patient fibroblasts allows removal or inversion of the IVS26 mutation, thereby correcting the *CEP290* gene defect. The study examined the efficacy of the approach across dozens of EDIT-101-treated retinal explant punches from a donor, in which they show that the average total

rate of editing was over 40% with an average productive editing rate exceeding 16%, confirming the functionality of EDIT-101 in fully mature human photoreceptors. They also carried out in vivo analysis in a human *CEP290*-IVS26 knock-in mouse model and with a surrogate vector in normal nonhuman primates. The authors examine both the efficacy and off-target effects in a battery of tests, taking into account challenges associated with CRISPR–Cas9 drug development, and propose a generalizable outline for preclinical development of a gene-editing medicines in the eye.

Both the Cideciyan et al.² and Maeder et al.³ studies have implications for the treatment of inherited retinal degenerations involving large genes that cannot benefit from AAV-mediated gene-replacement therapy. Moreover, the study by Maeder et al.³ offers hope regarding the implementation of gene-editing technologies for the treatment of diseases resulting from dominant mutations whose negative effects cannot be compensated by delivering a replacement gene significantly broadening the promise of retinal gene therapy. A lingering concern with the clinical implementation of gene editing is the persistent expression of the Cas9 endonuclease in post-mitotic retinal cells, which may reveal off-target cuts in the long run. Nevertheless, with improvements in technologies for delivering the Cas9 and gRNAs⁴ along with improvements in gene-editing technologies, such as development of base-editing tools⁵, uncertainties for broad application of this therapeutic strategy will be overcome in the future.

After an intense period of gene discovery and identification of mutations causing retinal dystrophies, the field is moving forward using genetic and mechanistic knowledge to design elegant molecular therapies for these

previously incurable forms of blindness. The research reported by Cideciyan et al.² and Maeder et al.³ provides evidence that in a primary photoreceptor ciliopathy improvement in vision can be observed by using an antisense oligonucleotide therapy targeting pre-mRNA splicing or by using gene editing at the DNA level. Elimination of mutations causing life-long blindness in patients carries immense hope for improving the lives of many suffering from genetic diseases of the retina. Today, inherited conditions beyond the retina account for a substantial burden of disease, including up to 70% of all admissions to children's hospitals and one-tenth of admissions to adult hospitals. Application of gene-editing drugs and antisense oligonucleotides at the clinical level opens up a myriad of potential drug targets with the potential to reduce this burden in the years to come. □

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