

EDITORIAL

Developing Gene Therapy for CEP290-Associated Retinal Ciliopathies



“Developing a mutation-independent approach that will deliver even low levels of the functional CEP290 could be sufficient to mitigate not only retinal degeneration, but also other ciliopathy-associated phenotypes in syndromic diseases.”

Hemant Khanna

Photoreceptors are the neurons of the central nervous system that help mediate the bulk of our light responses. They form large part of our light-sensitive tissue called the retina, in the back of the eye. There are two major types of photoreceptors: rods and cones. Rods respond

to dim light stimuli (starlight from a dark sky) but get bleached at higher light intensities. Cones, on the other hand, are less light sensitive and have the ability to respond to a wider range of light stimuli. They are, therefore, responsible for much of our vision during the

day as well as with artificial bright lights during the nighttime. Given that photoreceptors are the major components of our vision, retinal degeneration due to photoreceptor dysfunction is the largest cause of inherited blindness in the world [1].

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Photoreceptor degeneration is highly genetically heterogeneous with nearly 200 cloned genes [2]. These numbers seem to account for a majority of monogenic forms of retinal degenerative diseases. Among these, retinitis pigmentosa (RP) is the most common form of inherited retinal degeneration, with a prevalence of approximately 1 in 3000 people worldwide [3]. RP patients show a wide range of age of onset (15–40 years of age) and typically exhibit dysfunction of rod photoreceptors, due to which they start complaining of night blindness. This disorder then progresses to complete blindness due to the subsequent loss of cone photoreceptors. Hence, RP is also termed rod–cone degeneration. Other forms of inherited photoreceptor degenerative diseases show both rod and cone dysfunction at the same time or exhibit a predominant involvement of only cones or of cones followed by rods (cone–rod degeneration). RP is also commonly observed in syndromic diseases with extraocular manifestations, the majority of which are classified as ciliopathies. These include Joubert Syndrome (cerebellar defects), Bardet–Biedl Syndrome (polydactyly and obesity), Senior–Loken Syndrome (renal defects), and Usher Syndrome (hearing disorders) [4–6].

A relatively earlier onset form of photoreceptor degeneration is Leber congenital amaurosis (LCA) [7]. It is a rare inherited blindness disorder that usually occurs in early childhood. There are different forms of LCA, which can occur due to defects in either the photoreceptors or the retinal pigment epithelium (RPE). Recent reports on the success of clinical trials of gene therapy for RPE65-LCA and choroideremia have generated considerable excitement in the field of AAV-mediated gene delivery into the diseased retina [8–10]. Recently, the Food and Drug Administration (FDA) approved Luxturna™ for adeno-associated virus (AAV)-mediated gene delivery of RPE65 in patients with LCA type 2. The RPE65 gene is expressed specifically in the RPE and encodes for an enzyme that participates in the visual cycle. This gene therapy approach delivers the correct copy of RPE65 into the RPE.

Recent investigations have now focused on another frequent form of LCA (LCA10) that is caused by mutations in CEP290, a cilia-centrosomal protein involved in maintaining photoreceptor and RPE health [11–15]. The CEP290 gene encodes 55 exons and ~7.4 kb cDNA. As this size exceeds the packaging limit of the conventional AAV vectors, it poses a challenge for gene delivery and replacement strategies. The majority of efforts to design a rational therapeutic strategy for CEP290-LCA are directed towards a commonly occurring deep intronic mutation (c.2991+1665A>G; pCys998X). Elegant studies have shown the use of antisense oligonucleotides (AONs) to induce either normal splicing or exon skipping in human fibroblasts [16–18]. Parfitt *et al.*

demonstrated the use of antisense morpholinos to target CEP290 aberrant splicing in induced pluripotent stem cell-derived RPE cells and 3D optic cups [19]. In fact, ProQR therapeutics is testing QR-110, a therapeutic product designed to repair the defective CEP290 mRNA, as part of a Phase I clinical trial. In addition, Editas Medicine Inc. is carrying out a preclinical study that utilizes CRISPR/Cas9 mediated genome editing approach to delete the mutated region carrying the deep intronic mutation.

The deep intronic mutation in CEP290 accounts for ~15% of non-syndromic CEP290-LCA patients in the European and French-Canadian populations [20]. Many LCA10 patients carry other CEP290 mutations, which are predicted to result in relatively lower amounts of the functional protein [21]. Moreover, CEP290 mutations also result in syndromic ciliopathies, which are likely due to low or non-functional protein. While the focus on the deep intronic mutation is encouraging and will provide new knowledge to develop the specific therapeutics, these approaches will only be suitable for one type of CEP290 mutations. Developing a mutation-independent approach that will deliver even low levels of the functional CEP290 could be sufficient to mitigate not only retinal degeneration but also other ciliopathy-associated phenotypes in syndromic diseases.

As mentioned above, the conventional approach to deliver the full-length CEP290 gene using AAV vectors as vehicles would not be suitable. We and others have shown the involvement of the distinct domains of CEP290 in its interaction with other ciliary proteins and

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localization to cilia [22–25]. The deletion of some CEP290 domains does not significantly alter cilia formation but results in ciliary dysfunction (Cep290rd16 mouse model) [11, 14, 24]. These studies suggested that different domains of CEP290 are involved in distinct steps of cilia formation and modulating its function. Based on this knowledge, we constructed shorter forms of the CEP290 gene (miniCEP290) that are deliverable by AAV and can retain at least partial function as determined in cilia growth assays [26]. A similar approach has been used to mitigate muscular dystrophy due to dystrophin mutations [27]. We identified that miniCEP290s could delay the progression of photoreceptor dysfunction and degeneration when delivered subretinally in a Cep290-LCA mouse model. The treated retinas showed ~30% increase in photoreceptor function, improved photoreceptor morphology and delayed photoreceptor cell death. However, the improvement in the function and morphology progressively declined after 8 weeks of age.

Developing a suitable and longer-lasting strategy for CEP290-gene therapy remains a challenge in the field. One of the most important criteria to evaluate the efficacy of any gene therapy is the use of an appropriate testing platform. Recent studies have reported the development and characterization

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of iPSC-derived RPE and 3D optic cups. Moreover, several vertebrate models of Cep290 mutation have been reported. Mice generated either using gene trap alleles or by creating a null allele within exons 1–4 or exons 36–37 of mouse Cep290 recapitulate the phenotypes associated with syndromic ciliopathies [28–30]. However, they are not ideal model systems to investigate non-syndromic LCA due to CEP290 mutations. Earlier studies also identified a naturally occurring feline model of retinal degeneration due to CEP290 mutation in exon 50. As predicted, this model undergoes relatively delayed and late onset form of retinal degeneration [31]. We had previously reported the identification and characterization of a naturally occurring mutant of Cep290, termed Cep290rd16 [11]. This mutant carries an in-frame deletion within exons 37–41 of mouse Cep290, which encodes a part of the myosintail homology domain. This deletion results in the production of a truncated CEP290 variant, which is only partially functional. The Cep290rd16 mouse exhibits non-syndromic retinal degeneration, which mimics the LCA onset and phenotype observed in patients [11,32]. Moreover, the region of the human CEP290 gene that is homologous to the domain deleted in the Cep290rd16 mouse harbors

LCA-causing mutations. It should be noted that a miniCEP290 that can delay or mitigate retinal degeneration in an LCA model may not be equally effective in a syndromic Cep290 disease model.

Taken together, a considerable amount of work is needed to develop a rational gene therapeutic approach to treating CEP290-associated retinal degeneration. Some of them include:

- ▶ Developing improved miniCEP290s
- ▶ Delivering full-length CEP290 safely using lentiviral vectors (they have larger cargo capacity)
- ▶ Using transsplicing technology with AAV to deliver two different components of CEP290 into the same cells
- ▶ Minimizing off-target effects of AONs and CRISPR/Cas9 components

Nonetheless, the accomplishment of these studies will also benefit patients with retinal degeneration in:

- ▶ CEP290-associated syndromic ciliopathies; and
- ▶ Patients carrying mutations in other large genes, including ABCA4 (Stargardt disease) and Usher Syndrome genes.



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AFFILIATION

Hemant Khanna PhD UMass
 Department of Ophthalmology &
 Visual Sciences, UMass Medical School 368 Plantation St.,
 Worcester, MA 01605
 hemant.khanna@umassmed.edu
 Tel.: 508-856-8991