

Increased vulnerability of photoreceptors to aberrant splicing highlight the utility of AON-based therapy for *CEP290*-LCA

Shannon E. Boye

Department of Ophthalmology, College of Medicine, University of Florida, Gainesville, FL, USA

Correspondence to: Shannon E. Boye, Department of Ophthalmology, College of Medicine, University of Florida, Gainesville, FL, USA.

Email: shaire@ufl.edu.

Provenance: This is an invited Commentary commissioned by Editor-in-Chief Zhizhuang Joe Zhao (Pathology Graduate Program, University of Oklahoma Health Sciences Center, Oklahoma City, USA).

Comment on: Parfitt DA, Lane A, Ramsden CM, *et al.* Identification and Correction of Mechanisms Underlying Inherited Blindness in Human iPSC-Derived Optic Cups. *Cell Stem Cell* 2016;18:769-81.

Received: 01 September 2016; Accepted: 15 December 2016

doi: 10.21037/sci.2016.12.01

View this article at: <http://dx.doi.org/10.21037/sci.2016.12.01>

Mutations in *CEP290* account for the most common form of Leber congenital amaurosis (LCA), a severe, early onset retinal disorder, and are associated with numerous other ciliopathies such as Joubert, Meckel-Gruber, Senior-Loken, and Bardet Biedl syndromes. Disease-causing mutations occur throughout the length of *CEP290* and it is thought that resulting phenotypes vary in severity and organ involvement depending on the amount of residual *CEP290* function. The most common LCA-causing mutation in *CEP290* (c.2991+1655A→G) is found in approximately 86% of patients, and causes aberrant splicing and introduction of a cryptic exon containing a premature stop codon. Alternative splicing occurs in some transcripts, but not all, and thus a fraction of full length *CEP290* is still produced at levels that are seemingly sufficient to maintain function of other organ systems. This study, from the Cheetham lab asks the question—why are only photoreceptors, but not other cells in the kidney or CNS, affected by this mutation? (1). Using human iPSC-derived optic cups, Parfitt *et al.* determined that the basis for the retina's increased sensitivity to the c.2991+1655A→G mutation is increased aberrant splicing in developed photoreceptors. In addition, they validated, using antisense morpholinos, the ability to block this aberrant splicing and restore photoreceptor function (1).

Several clinical trials for Leber congenital amaurosis-2 (LCA2) have provided exciting evidence of biological activity resulting from Adeno associated virus (AAV)-

mediated expression of functional RPE65 in affected patients (2-4). This inspired the research community to evaluate whether gene augmentation approaches could confer therapy in other inherited retinal diseases, including other forms of LCA. While proof of concept and a path to clinical application have been established for *GUCY2D*-LCA1 (5), for instance, a therapeutic approach for *CEP290* LCA has proven more elusive due to the large size of the therapeutic gene (too large to fit within a conventional AAV vector). Clinical characterization reveals that rod photoreceptors develop but degenerate rapidly in these patients. In contrast to early rod losses, *CEP290* LCA patients maintain foveal cone cell bodies, with some abnormalities outer segments (OS) and inner segments (IS), at all ages examined (6). Retention of cone photoreceptors over time suggests these patients may benefit from a corrective therapy targeted to the central, cone-rich retina. Because it is the most common form of LCA and because patients maintain central retinal structure, establishing a therapeutic approach for *CEP290* LCA remains a high priority.

As a first step to establishing a therapy for *CEP290*-LCA, various *in vivo* and *in vitro* studies have been conducted to determine the pathological basis of the disease. *CEP290* localizes to the photoreceptor connecting cilium, in the region of the Y-linkers between the central ring of microtubules and the plasma membrane. It interacts with various other ciliary proteins including, but not limited

to, NPHP5, RAB8A, RPGR, RPGRIP1, and various members of the Bardet Biedl syndrome protein complex (“BBSome”). Studies conducted in serum-starved fibroblasts from CEP290-LCA patients reveal a reduced overall number of ciliated cells and a shorter average length of cilium relative to that seen in cells from unaffected patients (7,8). Full length CEP290 is required for photoreceptor cilia formation and OS biogenesis, as neither occur in the retinas of *Cep290^{ko/ko}* mouse (9). This is in contrast to the *Cep290^{rd16}* mouse retina in which low levels of truncated CEP290 are expressed, and connecting cilia and rudimentary photoreceptor OSs are formed. Despite this, as in CEP290 LCA patients, *Cep290^{rd16}* retinas ultimately exhibit early-onset dysfunction and degeneration (10). In addition to its role in ciliogenesis, seminal work in *Chlamydomonas* revealed an additional role for CEP290 in protein flow through the connecting cilium (11).

Fibroblasts from CEP290 LCA patients express up to 50% of WT CEP290 (12). If this value directly correlates to protein content in photoreceptors, the question becomes—why don't patients heterozygous for c.2991+1655A→G allele have a phenotype? Parfitt *et al.* attempted to answer this question by comparing levels of WT and incorrectly spliced *CEP290* transcript in patient fibroblasts, iPSC-derived ocular cells derived from patient fibroblasts, and in three-dimensional optic cups (1). Interestingly, the authors found that the amount of normal *CEP290* transcript was inversely proportional to how ‘retina-like’ the tissue is, with fibroblasts, iPSC-derived retinal pigment epithelial (RPE) cells, and 3D optic cups expressing ~40%, ~50%, and ~15%, respectively (1). 3D optic cups derived from patient fibroblasts had severe *CEP290* missplicing and levels of incorrectly spliced transcript increased concomitantly with the photoreceptor differentiation (1). These results suggest that, due to higher aberrant splicing in retina, the photoreceptors of c.2991+1655A→G *CEP290* LCA patients have a greater deficit of CEP290 protein than other cells and thus a retina-only phenotype.

Multiple therapeutic approaches are being considered to correct this *CEP290* LCA phenotype. Parfitt *et al.* use a short, modified antisense oligonucleotide (AON) to interfere with splicing (1). Indeed, treatment of patient derived fibroblasts, iPSC-derived RPE, and optic cups with AON led to increased levels of WT *CEP290* transcript. AON treatment was especially potent in photoreceptors of optic cups with 50–70% of transcripts reverting to normal. Importantly, AON treatment significantly increased the amount of ciliary CEP290 protein expression, the

number and length of cilia emerging from photoreceptor progenitors, and rescued RPGR targeting to the cilium (1). Taken together with recent results showing that AAV-packaged AONs significantly reduced the amount of misspliced transcript in a humanized mutant *Cep290* mouse model (8), these findings suggest that AONs are a very promising therapeutic approach for correcting the c.2991+1655A→G form of *CEP290* LCA. Alternative therapeutic strategies being considered include a CRISPR-based gene editing approach whereby Cas9 and a guide RNA directed against this *CEP290* mutation will be delivered by AAV vectors. In addition, efforts are still underway to develop a gene replacement approach using AAV. While the full length CEP290 cDNA is too large to fit inside an individual AAV capsid, a dual AAV vector approach whereby the front half of CEP290 is placed in one capsid, the back half is placed in a second capsid (with a region of overlap shared between them) is under investigation. Upon co-infection with both vectors, the two gene halves can recombine to form full length transgene. For example, this technique has proven useful to correct the retinal phenotype in a mouse model of Usher syndrome which is associated with mutations in another large gene, *MYO7A* (13). The existence of multiple strategies by which to tackle this severe, early onset retinal dystrophy greatly increases the chance that a treatment for CEP290 LCA is on the horizon. This study by Parfitt *et al.* has provided new insight in the importance of the specialized splicing in photoreceptors and provides guidance regarding appropriate methods by which to validate potential therapies prior to clinical application.

Acknowledgements

None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Parfitt DA, Lane A, Ramsden CM, et al. Identification and Correction of Mechanisms Underlying Inherited Blindness in Human iPSC-Derived Optic Cups. *Cell Stem Cell* 2016;18:769-81.
2. Hauswirth WW, Aleman TS, Kaushal S, et al. Treatment

- of leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results of a phase I trial. *Hum Gene Ther* 2008;19:979-90.
3. Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med* 2008;358:2240-8.
 4. Bainbridge JW, Smith AJ, Barker SS, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med* 2008;358:2231-9.
 5. Boye SL, Peterson JJ, Choudhury S, et al. Gene Therapy Fully Restores Vision to the All-Cone *Nrl(-/-) Gucy2e(-/-)* Mouse Model of Leber Congenital Amaurosis-1. *Hum Gene Ther* 2015;26:575-92.
 6. Cideciyan AV, Rachel RA, Aleman TS, et al. Cone photoreceptors are the main targets for gene therapy of NPHP5 (IQCB1) or NPHP6 (CEP290) blindness: generation of an all-cone *Nphp6* hypomorph mouse that mimics the human retinal ciliopathy. *Hum Mol Genet* 2011;20:1411-23.
 7. Burnight ER, Wiley LA, Drack AV, et al. CEP290 gene transfer rescues Leber congenital amaurosis cellular phenotype. *Gene Ther* 2014;21:662-72.
 8. Garanto A, Chung DC, Duijkers L, et al. In vitro and in vivo rescue of aberrant splicing in CEP290-associated LCA by antisense oligonucleotide delivery. *Hum Mol Genet* 2016;25:2552-63.
 9. Rachel RA, Yamamoto EA, Dewanjee MK, et al. CEP290 alleles in mice disrupt tissue-specific cilia biogenesis and recapitulate features of syndromic ciliopathies. *Hum Mol Genet* 2015;24:3775-91.
 10. Chang B, Khanna H, Hawes N, et al. In-frame deletion in a novel centrosomal/ciliary protein CEP290/NPHP6 perturbs its interaction with RPGR and results in early-onset retinal degeneration in the rd16 mouse. *Hum Mol Genet* 2006;15:1847-57.
 11. Craige B, Tsao CC, Diener DR, et al. CEP290 tethers flagellar transition zone microtubules to the membrane and regulates flagellar protein content. *J Cell Biol* 2010;190:927-40.
 12. Akimoto M, Cheng H, Zhu D, et al. Targeting of GFP to newborn rods by *Nrl* promoter and temporal expression profiling of flow-sorted photoreceptors. *Proc Natl Acad Sci U S A* 2006;103:3890-5.
 13. Lopes VS, Boye SE, Louie CM, et al. Retinal gene therapy with a large MYO7A cDNA using adeno-associated virus. *Gene Ther* 2013;20:824-33.

doi: 10.21037/sci.2016.12.01

Cite this article as: Boye SE. Increased vulnerability of photoreceptors to aberrant splicing highlight the utility of AON-based therapy for *CEP290*-LCA. *Stem Cell Investig* 2016;3