Personalized models reveal mechanistic and therapeutic insights into CEP290-associated Leber congenital amaurosis

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Lebers congenital amaurosis (LCA) is an early onset form of retinal degeneration, commonly known as childhood blindness, which manifests via mutations in genes required for functional or structural maintenance of photoreceptors. Eighteen genes are currently recognized as causative for disease progression with the largest percentage of confirmed clinical cases occurring due to defects in *CEP290*. This gene encodes the centrosomal protein of 290 kilodaltons (CEP290), which is an evolutionarily conserved component from green algae to modern vertebrates that participates in cellular processes associated with primary cilium assembly/ stability, cell cycle control, and DNA replication (1-3). Photoreceptors are particularly vulnerable to deficits in ciliogenesis as their structure includes a ciliary axoneme that connects the major segments. Loss of CEP290 disrupts several functions of the connecting cilium with respect to intraflagellar trafficking and structural stability, thereby prompting a rapid course of photoreceptor degeneration that culminates in a phenotype of severe vision loss (2,4).

Much of our current understanding encompassing the pathophysiology and mechanisms of inherited ocular disease, including CEP290-associated LCA, is attributed to animal models that firmly replicate the human phenotype. Mouse models are particularly valuable in this regard given their relatively short generation time and ease of manipulation with common noninvasive techniques to monitor visual responses and retinal function. However, animal models do present several limitations. For example, many conventional knockout mouse models for genes implicated in retinal degeneration do not exhibit an appreciable ocular disease phenotype. In addition, the precise genetic deficits in many of these models are not representative of naturally occurring mutations found in the human population. This disconnect between mouse models and clinical phenotypes creates several challenges associated with precise interrogation of disease mechanisms and evaluation of novel therapeutic approaches.

Personalized models generated through pluripotent reprogramming of patient-derived cells provide a powerful means to reveal novel biological insights with respect to human development, disease modeling, and therapeutic intervention (5). In the present report, Parfitt *et al.* generate iPSC-derived models to extend our understanding of the disease process accompanying the most common CEP290 mutation found within the human population (6). The mutation (c.2991 + 1665A>G) creates a cryptic splice donor site within intron 26 of the gene, leading to the inclusion of an additional exon in the mature mRNA sequence. This cryptic exon harbors a premature termination codon that renders the CEP290 protein non-functional. Previous reports characterize the effects of this mutation and others on features of primary cilia formation and CEP290 splicing in patient-derived fibroblasts (7,8). Since this mutation is associated with an ocular-specific disease phenotype, the authors build upon previous investigations by reprogramming dermal fibroblasts derived from a homozygous donor to an induced pluripotent stem cell (iPSC) state followed by differentiation toward a cellular fate representative of retinal precursors within three-dimensional optic cups. Parfitt *et al.* compare CEP290 RNA processing and common markers/measurements of ciliation between wild-type and mutant fibroblasts, iPSC-

derived retinal pigment epithelia, and iPSC-derived optic cups containing photoreceptors. While there is a certain degree of variability in the ciliation assays between models, the most notable finding concerns an aberrant increase in CEP290 splicing deficits upon differentiation to the optic cups containing photoreceptor progenitors. This observation supports the ocular-specific phenotype of LCA patients and highlights the astonishing degree of tissue-specific differences with respect to mRNA processing and protein expression that establish distinct disease phenotypes.

Recent success in gene therapy clinical trials for another form of LCA based on mutations in the *Rpe65* gene provides a template for treating several forms of inherited retinal degeneration (9). However, applying a similar approach to CEP290-associated LCA presents several formidable challenges. Foremost, the large size of the CEP290 cDNA sequence (~7.5 kilobases) exceeds the maximum DNA packaging capacity of adeno-associated virus (AAV), the optimal gene transfer vector for targeting photoreceptors *in vivo*. Therefore, a gene augmentation strategy for CEP290-associated LCA will require an alternative vector platform with perhaps decreased targeting efficiency of photoreceptors and other potentially undesirable features. In addition to the technical barriers of gene augmentation, Burnight *et al.* (7) reported a notable degree of cellular toxicity associated with lentiviral-mediated overexpression of the full length CEP290 sequence within patient-derived fibroblasts. This finding is not surprising given the putative role of CEP290 in processes associated with cell cycle regulation and DNA replication (3). It is possible that CEP290 overexpression may perturb such events in actively dividing cells, thereby expediting their degeneration. While it is unclear how constitutively expressed CEP290 may behave in a quiescent photoreceptor, this finding does warrant caution for future gene augmentation strategies utilizing the complete protein sequence. Alternative approaches that selectively manipulate the endogenous CEP290 DNA using genome editing or mRNA sequence manipulations may provide an attractive means to bypass the drawback of current gene augmentation strategies using AAV gene delivery of large cDNAs. With respect to the c.2991 + 1665A>G mutation, previous reporting highlights the efficacy and potential therapeutic benefit of antisense oligonucleotides (ASOs) in selectively masking this cryptic splice mutation and restoring the correct CEP290 reading frame (8). Using AAV-mediated expression of ASOs, Garanato *et al.* (8) demonstrated functional restoration of CEP290 pre-mRNA splicing, wild-type protein levels, and

ciliogenesis in patient-derived fibroblasts. In the present report, Parfitt *et al.* take this strategy a step further by examining the effects of antisense morpholinos in dermal fibroblasts as well as iPSC-derived retinal pigment epithelia and optic cups harboring the cryptic splice mutation. In the fibroblast and RPE cultures, they show a notable degree of pre-mRNA splicing correction, increased ciliation, and enhanced co-localization of CEP290 with known ciliary and interacting partners. However, the most profound phenotypic correction is displayed following the administration of antisense molecules to the optic cups, further underscoring the utility of an *in vitro* system that closely recapitulates retinogenesis. While these studies demonstrate robust rescue of the disease phenotype in patient samples harboring two copies of the intron 26 splice mutation, it is unclear how efficacious this approach will be when applied to patients possessing heterozygous loss-of-function mutations in CEP290.

Taken together, the evidence presented by Parfitt *et al.* supports iPSC-derived optic cups as a convenient and clinically relevant model system to thoroughly interrogate the mechanisms of inherited retinal degeneration. More specifically, these models will allow us to glean very precise information concerning the downstream effects of patient-specific mutations and perhaps the activity of modifier alleles that may impact a disease processes. Furthermore, these models will be exceptionally valuable for future investigations involving functional testing and evaluation of novel therapeutic strategies aimed to preserve or reverse vision loss.

Acknowledgements

Footnote

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.

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