

Chapter XX

Gene and Cell Therapy for AIPL1-associated Leber Congenital Amaurosis: Challenges and Prospects

Pedro R. L. Perdigao and Jacqueline van der Spuy

UCL Institute of Ophthalmology, 11-43 Bath Street, London EC1V 9EL, UK

Corresponding Author: Jacqueline van der Spuy, UCL Institute of Ophthalmology, 11-43 Bath Street, London EC1V 9EL, UK, E-mail: j.spuy@ucl.ac.uk

Abstract Leber congenital amaurosis (LCA) caused by *AIPL1* mutations is one of the most severe forms of inherited retinal degeneration (IRD). The rapid and extensive photoreceptor degeneration challenges the development of potential treatments. Nevertheless, preclinical studies show that both gene augmentation and photoreceptor transplantation can regenerate and restore retinal function in animal models of *AIPL1*-associated LCA. However, questions regarding long-term benefit and safety still remain as these therapies advance towards clinical application. Ground-breaking advances in stem cell technology and genome editing are examples of alternative therapeutic approaches and address some of the limitations associated with previous methods. The continuous development of these cutting-edge biotechnologies paves the way towards a bright future not only for *AIPL1*-associated LCA patients but also other forms of IRD.

XX.1 Introduction

LCA, the most rapid and severe form of IRD, is commonly inherited in an autosomal recessive manner and is characterized by the early loss of vision, nystagmus and an abolished or profoundly abnormal electroretinogram (ERG) (den Hollander et al., 2008). To date, mutations in 26 different genes encoding proteins with a critical role in retinal development and physiological function cause clinically distinctive types of LCA (RetNet <https://sph.uth.edu/retnet>). Among these, mutations in the *aryl hydrocarbon receptor-interacting protein-like 1 (AIPL1)* gene are associated with LCA type IV (LCA4). Despite accounting for only 5-10% of LCA cases, the clinical phenotype caused by *AIPL1* deficiency is at the severe end of the spectrum of LCA. Such severe symptoms are caused by the extensive and irreversible degeneration of rod and cone photoreceptors, critical for visual phototransduction in which *AIPL1* plays an indirect but essential role to maintain functional

integrity (Yadav and Artemyev, 2017). Currently, there is no cure or treatment for LCA4. Here, we present a brief review of current progress in the field of gene and cell-based therapies and discuss their potential to treat LCA4 patients.

XX.2 The role of AIPL1 in LCA4 mechanisms of disease

Expression of AIPL1 is exclusive to retinal photoreceptors and the pineal gland (van der Spuy et al., 2002). In the retina, AIPL1 acts as a specialized co-chaperone of cyclic nucleotide phosphodiesterase of the sixth family (PDE6), an essential enzyme effector in the phototransduction pathway (Sacristan-Reviriego and van der Spuy, 2018). Following light stimulation, activated PDE6 hydrolyses cyclic GMP (cGMP), triggering the closure of cGMP-dependent Ca^{2+} ion channels and propagation of the “light” electrical signal through hyperpolarization of the plasma membrane (Yadav and Artemyev, 2017). Stabilization of PDE6 is ensured by a chaperone heterocomplex comprising HSP90 and its cognate PDE6-specific co-chaperone AIPL1 (Hidalgo-de-Quintana et al., 2008), which are required for the stable assembly of the PDE6 holoenzyme (Kolandaivelu et al., 2009).

AIPL1 mutations impact functional domains of the translated protein to disrupt AIPL1 interaction with isoprenylated PDE6 or HSP90 consequently preventing assembly of the PDE6 chaperone heterocomplex (Sacristan-Reviriego and van der Spuy, 2018). In *Aipl1* knockout and hypomorphic mice, loss of AIPL1 function causes misassembly of the PDE6 holoenzyme, causing the destabilization and rapid proteasomal degradation of the PDE6 subunits. Consequently, rapid degeneration of rod photoreceptors occur due to intracellular cGMP increases that leads to prolonged opening of the cyclic nucleotide gated channels and excessive influx of Ca^{2+} (Wang et al., 2017). In contrast, cone cell death is triggered by a

downregulation of cGMP metabolism, caused by a reduction of the Retinal guanylate cyclase-1 (RetGC1) enzyme necessary for cGMP synthesis (Kolandaivelu et al., 2014).

XX.3 Gene-based therapy for AIPL1-associated LCA4

Given the unique features of the eye (accessible location, small and enclosed structure, immune privilege), IRDs are one of the most attractive targets for gene therapy. Over the past years, gene therapy clinical trials for IRD have multiplied, culminating in the approval of the first gene therapy to treat an IRD - voretigene neparvovec-rzyl (Luxturna) - a recombinant adeno-associated virus (AAV) expressing the *RPE65* gene for the treatment of LCA type II (Auricchio et al., 2017). AAVs are the most successful gene delivery vectors and the standard choice for transduction of photoreceptors. Several naturally-occurring or engineered AAV serotypes have been shown to efficiently transduce photoreceptors in animal models (Day et al., 2014).

The encouraging clinical data of retinal gene therapy for several forms of IRD inevitably raises its potential for the treatment LCA4. The *AIPL1* coding sequence is small (~1.2 Kb) and can therefore be efficiently packaged in AAV. The first report of *AIPL1* gene therapy reported the rescue of photoreceptor degeneration in slower degenerating *Aipl1* hypomorphic mice (h/h) following subretinal injection of an AAV encoding murine *Aipl1* (*mAipl1*) driven by the cytomegalovirus (CMV) promoter. PDE6 levels were upregulated and the localization restored to the photoreceptor outer segments (OS) in 4-week-old treated mice, with retinal morphology and organization preserved at 12 months post-injection. More importantly, subretinal injection of AAV2/8-CMV-*mAipl1* was able to rescue photoreceptor degeneration in the most severe *AIPL1*^{-/-} knockout model treated at post-natal day 12 (P12), with preservation of retina outer nuclear layer (ONL) thickness for over 3 months post-injection.

The same group reported similar benefits using a photoreceptor-specific rhodopsin kinase (RK) promoter to drive *AIPL1* expression (Sun et al., 2010). Injection of AAV8-RK-h*AIPL1* into *Aipl1*^{-/-} mice at P9 forced substantial accumulation of rod and cone PDE6 in the OS, delaying photoreceptor degeneration for at least 5 months after treatment.

Subsequently, Ku *et al.* further improved preservation of photoreceptors and retina function in *Aipl1*^{-/-} mice using an engineered AAV2/8-based self-complementary Y733F tyrosine capsid mutant (scAAV-Y733) that triggers earlier onset and increased levels of h*AIPL1* expression (Ku et al., 2011). Subretinal injection of *Aipl1*^{-/-} mice at P2 with scAAV-Y733-RK-h*AIPL1* restored rod and cone PDE6 expression over 2 months post-treatment, while improving photoreceptor ultrastructure. This study supported the use of next-generation enhanced AAV vectors to maximize photoreceptor rescue in advanced retinal degenerations. Interestingly, the same group demonstrated that scAAV-Y733-RK-h*AIPL1* can rescue cone-mediated vision in a mouse model that manifests autosomal dominant cone-rod dystrophy (CORD) caused by a 12-bp deletion at proline 351 of h*AIPL1* (P351Δ12) (Ku et al., 2015). Overall, these studies provide clear evidence that AAV-mediated *AIPL1* gene augmentation is a promising approach for LCA4 patients.

While gene augmentation is a powerful approach to treat monogenic diseases by delivering a normal copy of the faulty gene, the adaptation of the *Streptococcus pyogenes* CRISPR/Cas9 endonuclease to rewrite the human genome has unlocked a range of opportunities to correct genetic disorders *in situ*, permanently restoring endogenous gene function and regulation. CRISPR/Cas9-mediated genome editing has been explored for the treatment of various IRD (Burnight et al., 2018). Off-target activity at genomic sites that share homology to the target site, as well as low levels of precise gene correction via homology directed repair (HDR) are the major bottlenecks of genome editing applications. Evolution of CRISPR/Cas9 platforms and delivery methods have pushed the boundaries of this technology to overcome some of

these critical limitations (Komor et al., 2017). One of the most innovative methods is the development of CRISPR-deaminase fusions, known as base editors, which can induce targeted base-pair conversions without causing disruptive DNA breaks or requiring template-mediated HDR (Rees and Liu, 2018).

Gene-specific therapeutic approaches are inevitably associated with huge production costs, limiting their development for every rare form of IRD. Optogenetic methods to stimulate neuroretinal function or delivery of neuroprotective factors to preserve neuronal cell viability are examples of emergent therapies (Auricchio et al., 2017) that act independently of the disease-causing mutation or gene, and may thus offer the possibility of a common treatment for different forms of IRD, greatly reducing the cost of these treatments for rare gene defects.

Importantly, any therapeutic approach for LCA4 is challenged and limited by the early onset and rapid progression of this disease. Clinical studies in LCA4 patients (Pennesi et al., 2011) reported that residual ERG responses were observed within young individuals (≤ 5 years), while foveal retinal structure was exclusively preserved in infants (≤ 1 year) (Aboshiha et al., 2015). These studies demonstrate that the window for therapeutic intervention is narrow for LCA4 patients, and any gene-based treatment will have to target very young patients. Longitudinal studies of human gene therapy clinical trials for RPE65 deficiency – a far less severe form of LCA – demonstrated continued retinal degeneration while functional and morphological benefit was variable and temporary (Auricchio et al., 2017), highlighting that the stable and substantial restoration of retinal function will require the highly efficient transduction of the appropriate cell type at an early stage of disease prior to the progression of retinal degeneration. LCA4 patients with advanced stages of retinal degeneration lack target photoreceptors for gene therapy and must therefore rely on alternative treatments. In these circumstances, therapy by means of retinal cell transplantation is a viable option for repairing the degenerated retina.

XX.4 Cell-based therapy for AIPL1-associated LCA4

Currently, several cell-based therapies have been developed for the treatment of a wide range of ocular disorders, and in some cases ongoing clinical trials have demonstrated its potential to restore vision (Stern et al., 2018). In IRD patients with loss of photoreceptors, cell therapy aims to transplant exogenous photoreceptors that integrate with the retinal neurocircuit and replace the lost cell population.

Selection of the donor cell development stage is critical for successful photoreceptor transplantation. Previously, it was reported that only post-mitotic post-natal photoreceptor precursors are able to efficiently integrate in the degenerated retina of adult mice and form synaptic connections leading to recovery of visual function (Pearson et al., 2012). Moreover, it has been demonstrated that cone photoreceptor precursors derived from mouse embryonic stem cells (ESC) (Kruczek et al., 2017) or human induced pluripotent stem cells (hiPSC) (Gonzalez-Cordero et al., 2017) injected into the retina of 8 to 12 week-old *Aipl1*^{-/-} mice regenerate the photoreceptor layer and form synaptic-like structures by 3 weeks post-transplantation. Nonetheless, future studies will need to evaluate functional integration and long-term photoreceptor survival in this model.

Obtaining large numbers of stage-specific photoreceptors precursors is a critical step to implement cell-based therapies for IRD patients. Based on the breakthrough that 3D retinal organoids can be generated *in vitro* from stem cells, several laboratories have developed protocols to produce and isolate large numbers of stem cell-derived photoreceptors to be used as source for cell transplantation (Stern et al., 2018). Exogenous stem-cell derived photoreceptors may, however, also pose serious risks, such as tumorigenesis or severe immune responses, highlighting the importance of thorough characterization of cell-based products to ensure efficient and safe delivery to patients. The generation of human leukocyte

antigen (HLA)-defined stem cell biobanks (de Rham and Villard, 2014) is of high importance to provide a vast source of universal donor cells for a wide range of HLA-matched patients. Alternatively, gene-corrected patient-specific hiPSC are a potential source for autologous transplantation to avoid immune rejection (Ovando-Roche et al., 2017). However, the generation of a gene-corrected patient-specific source of photoreceptors is not only time-consuming but costly, and might exceed the window of opportunity for intervention, particularly in an aggressive retinopathy such as LCA4. In this regard, the ready availability of clinically manufactured and well-characterized HLA-matched allogenic donor tissue poses a striking advantage over autologous cell transplantation.

XX.5 Conclusion

Blindness caused by *AIP1* mutations is one of the most challenging retinal disorders due to the severe and rapid photoreceptor degeneration observed in LCA4 patients, rendering a minimal period of intervention to rescue this retinopathy. Preclinical work in LCA4 models demonstrated that gene augmentation or photoreceptor transplantation has the potential to restore vision even during late-stages of retinal degeneration. This review brought to light groundbreaking advances in the fields of gene and cell-based therapies and discussed their potential to rescue LCA4.

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