Vision Research 111 (2015) 124–133

Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

Promising and delivering gene therapies for vision loss

Livia S. Carvalho, Luk H. Vandenberghe *

Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Harvard University, 20 Staniford Street, Boston, MA 02114, USA

ARTICLE INFO

Article history:
Received 16 May 2014
Received in revised form 25 July 2014
Available online 2 August 2014

Keywords:
Retina
Gene therapy
AAV
Lentiviral vector
LCA
Clinical trial

ABSTRACT

The maturity in our understanding of the genetics and the pathogenesis of disease in degenerative retinal disorders has intersected in past years with a novel treatment paradigm in which a genetic intervention may lead to sustained therapeutic benefit, and in some cases even restoration of vision. Here, we review this prospect of retinal gene therapy, discuss the enabling technologies that have led to first-in-human demonstrations of efficacy and safety, and the road that led to this exciting point in time.

1. Clinical success, in triplicate

1.1. Clinical success in Leber Congenital Amaurosis due to mutations in RPE65

Three independent clinical trials for retinal pigment epithelium-specific 65 kDa protein (RPE65) deficiency in 2008 (Bainbridge et al., 2008; Cideciyan et al., 2008; Hauswirth et al., 2008; Maguire et al., 2008) have led to genuine excitement and anticipation from both the scientific and lay communities towards the treatment of recessive monogenetic disorders that cause vision loss. Mutations in RPE65 leads to early onset vision loss within the disease spectrum referred to as Leber Congenital Amaurosis (LCA). LCA-RPE65 patients generally present with significantly decreased vision in the first year of life, nystagmus, and fundus changes consistent atrophy of the pigment epithelium. The *RPE65* gene encodes an isomerase protein that is expressed in the retinal pigment epithelium (RPE) and is an essential player in the recycling pathway of 11-*cis*-retinal in the visual cycle. Briefly, light activation of the visual pigments (opsins) present in the outer segments of photoreceptors occurs after photon capture by the 11-*cis*-retinal chromophore triggering an isomerization event that converts it to all-*trans*-retinal and releases it from the visual pigment (reviewed in Ebrey & Koutalos, 2001). Recovery of the visual cycle after light stimulation is therefore dependent on the conversion, in the RPE, of the chromophore from all-*trans*-retinal to 11-*cis*-retinal by the RPE65 protein (Ebrey & Koutalos, 2001). The re-converted 11-*cis*-retinal chromophore will now travel back to the photoreceptors outer segments and re-attached itself to the visual pigments (Ebrey & Koutalos, 2001).

The first effective intervention using adeno-associated virus (AAV)-based gene therapy in an animal model of retinal dystrophy caused by an RPE defect was done in the Briard dog model which has a naturally occurring mutation in the *RPE65* gene. AAV2/2 mediated gene transfer after subretinal injections shown significant morphological and functional rescue of photoreceptors and therefore recovery of 11-*cis*-retinal recycling by the RPE cells (Acland et al., 2001; Le Meur et al., 2007; Narfstrom et al., 2003; Rolling et al., 2006). These studies showed functional ERG improvement of around 20–30% of wildtype levels and significant improvements in behavioral-based vision tests, especially under photopic conditions (Acland et al., 2001; Le Meur et al., 2007; Rolling et al., 2006). They were also able to demonstrate stable and long-term restoration of vision up to 4 years follow-up post-treatment (Acland et al., 2005; Narfstrom et al., 2008). These initial studies in a large animal model of RPE65 deficiency that mimicked the human LCA condition so well provided great encouragement and an ideal candidate to move a gene therapy platform for inherited retinal dystrophies towards the clinic.

The reports of the early stage clinical trials for RPE65 deficiency were encouraging and attested for both safety and efficacy of the transgene and the selected AAV2/2 vector delivery agent (reviewed in MacLaren, 2009). Some of the differences between the three trials include vector sequence and design, dose (ranging from 1.5×10^{10} to 1.5×10^{11} viral particles) and injected volume (ranging from

* Corresponding author.
E-mail address: luk_vandenberghe@meei.harvard.edu (L.H. Vandenberghe).

http://dx.doi.org/10.1016/j.visres.2014.07.013
0042-6989/

0.15 to 1 ml). Vector production methods also varied between the trials but it is unclear if and how this affects the outcome (see Hauswirth et al., 2008 for summary table of differences between trials).

With over a hundred disease-causing mutations identified so far in RPE65 (source: www.retina-international.org), it was unsurprising that all the initial and subsequent patients selected for the trials presented a diverse group of mutations (Hauswirth et al., 2008; Testa et al., 2013). Identical homozygous mutations was only seen in two patients of the Maguire et al. (2008) trial (E102K) and between one patient in the Hauswirth et al. (2008) and Bainbridge et al. (2008) trials (Y368H). Even after these initial studies were expanded (Jacobson et al., 2012; Maguire et al., 2009), the diversity of both homozygote and compound mutations in the recruited patients remained high. This has made it difficult so far to correlate specific mutations with visual improvement outcomes, therefore studying the effects each mutation has on RPE65 function needs to be an ongoing effort and run in parallel to clinical trial data.

Arguably the most significant difference between these trials that may have influenced outcome was the choice and design of promoter driving RPE65 expression. Although all three trials used the recombinant AAV2/2 vector (rAAV), Bainbridge et al. (2008) used a human RPE65 promoter while both Maguire et al. (2008) and Hauswirth et al. (2008) used a modified version of the ubiquitous chicken β actin promoter referred to as CAG promoter (Miyazaki et al., 1989). Other vector sequence and design differences include the addition of an optimized Kozak sequence in Maguire et al. (2008) trial. Although the human RPE65 promoter has a weaker expression pattern when compared to the CAG promoter, it was shown to drive enough transgene expression to rescue the phenotype of both younger and older treated Briard dogs (Annear et al., 2013; Le Meur et al., 2007; Rolling et al., 2006). These preclinical studies showed that the human RPE65 promoter was capable of driving RPE-specific expression of the transgene, which was opted to be preferred in terms of safety in this study. In contrast, a ubiquitous promoter like CAG with a non-specific cell expression profile could generate concerns about RPE65 expression in cell types other than RPE and what effect this would have in the recovery of the visual cycle. However, a ubiquitous promoter has its advantages, offering a more robust and stronger expression pattern.

The promoter choice in the RPE65 clinical trials could offer an explanation for the differences seen between the reported outcomes, and indeed, visual improvements were more robust from the two trials that used the CAG promoter (Hauswirth et al., 2008; Jacobson et al., 2012; Maguire et al., 2008, 2009). These included improved visual acuity and pupil response, increased sensitivity and in visual field size and a fixation shift in the extrafoveal treatment in one patient (Hauswirth et al., 2008; Jacobson et al., 2012; Maguire et al., 2008). Long-term follow up of these studies have demonstrated that stability, safety and efficiency of treatment can persist up to at least 3 years post-treatment (Jacobson et al., 2012; Testa et al., 2013). However it remains debated whether these studies have been able to show an age-dependent effect of the treatment since the two studies with larger patient cohorts including younger aged patients have reached contradictory conclusions. In the first study Maguire et al. (2009) concludes that treatment at a younger age does have an overall effect on improved visual function although a later study conducted by Jacobson et al. (2012) shows no correlation between age and treatment effect. The younger patients in the first study (Maguire et al., 2009) do indeed show a more consistent improvement in visual sensitivity when compared to the older group of patients where the results were more variable but this could easily be explained by the heterogeneity of disease severity caused by RPE65 deficiency, generating a complex and individualist relationship between disease progression and age. Indeed, a few of the older patients show a similar increase in sensitivity when compared to the younger ones and the visual acuity measurements do not seem to show an age-related correlation. Our conclusion from these data at this early stage of the field is that too many variables (mutation-dependent or idiosyncratic progression of disease, vector, injection parameters, and endpoint measures) between these studies and subjects are at play. That being said, our understanding of disease pathology and the data from these studies indicate a greater benefit from intervention at an earlier stage of the disease process, which is age-related.

Next, the hypothesis was challenged whether gene augmentation therapy in this form of LCA would stem degenerative processes in the outer retina, and ultimately determine whether the benefit observed in these pivotal trials would be long-lived (Cideciyan et al., 2013). In this study Cideciyan and colleagues extensively and thoroughly analyzed the natural history of the disease using the patients enrolled in one of the initial trials. They concluded that despite the treatment, disease progression and photoreceptor degeneration remained unchanged and followed the expected natural history (further reviewed in Cepko & Vandenberghe, 2013). Surprisingly they also show that the standard binary hallmark of inherited retinal degenerations namely combined dysfunction and degeneration of photoreceptor cells – is different between humans and the main animal model used for the pre-clinical studies of LCA, the Briard dog model. While in humans, dysfunction and photoreceptor degeneration are timely coupled, in dogs impaired visual function occurs well before any degeneration is seen. The authors conclude that the accumulation of certain changes by non-functional RPE65 contributes to the generation of a threshold or window where treatment needs to fall within to have significant impact on photoreceptors degeneration and visual improvement. This indeed aligns well with another study from Cideciyan et al. where they show that despite significant increase in visual sensitivity after *RPE65* gene therapy in humans, the kinetics of rod photoreceptors recovery is still impaired and suboptimal (Cideciyan et al., 2009). While these studies are thorough in their analysis, the bold and disappointing conclusions have been critiqued and challenged (Cepko & Vandenberghe, 2013; Wojno, Pierce, & Bennett, 2013). Indeed, the small group sizes, the multitude of variables delineated above, and the very early assessment of long-term benefit within a slow degenerative process make any definitive conclusion difficult. For these reasons the Cideciyan study (Cideciyan et al., 2013) had to use a novel methodological approach, measuring the thickness of photoreceptors outer nuclear layer (ONL) based on normalized OCT data and age correction between species and degenerative states, to generate a predictive slope of the natural history of RPE65-LCA. However, this approach may have its limitations in accurately modeling long-term progression and treatment effects since only one disease parameter, ONL thickness, was taken into consideration. Patients with RPE65-LCA usually present a highly variable disease progression rate, demonstrated by the weak correlation between age and ONL thickness seen in this study, which only showed a more consistent and stepper correlation when adjusted for age of onset (Cideciyan et al., 2013). More importantly, their analyses did not account for the diversity in *RPE65* genetic lesions present in this patient cohort that could have helped to better understand the effect of different mutations on disease progression.

Nonetheless, these studies do emphasize the point that gene augmentation therapy has an inherently delineated therapeutic window between the earliest time intervention can be considered and the point when degenerative processes cannot be reverted and eventually therapeutic target cells are terminally atrophied.