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The Effect of Age on Gene Therapy Efficacy for RPE65 Leber's Congenital Amaurosis: A Pooled Analysis

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Abstract - Leber's congenital amaurosis (LCA) is an inherited retinal disease that leads to severe vision loss from birth. Gene therapy for LCA caused by mutations in the RPE65 gene has been discovered to be a promising treatment, and one treatment (Luxturna) has received FDA approval. Within the current body of clinical trials, there is little consensus on whether age has any effect on treatment efficacy and outcomes. This pooled analysis aims to discern the effect of age on changes in visual function in patients undergoing adeno-associated virus mediated gene therapy for RPE65-LCA. The PubMed database was searched for papers describing the results of RPE65 gene therapy on patients with LCA, and data were pooled from 5 selected studies. Linear regression of age on percent change from baseline visual acuity in treated eyes was conducted on the pooled data from one, two, and three years post-treatment. Linear regression of age on change from baseline retinal thickness in treated eyes was conducted on the pooled data at one year post-treatment. At 1 year, a statistically significant linear regression equation of age versus visual acuity was found ($\alpha = 0.05$, $n = 78$, $F(1, 76) = 7.3152$, $p = 0.0084$). At the same time point, a statistically significant linear regression equation of age versus change in retinal thickness was not found ($\alpha = 0.05$, $n = 50$, $F(1, 48) = 0.07756$, $p = 0.7818$). Age appears to be a statistically significant predictor of improvements in visual acuity, but not of retinal degeneration. Younger age appears to be associated with greater improvements in vision. However, the age predictive effect is only visible in the first year after treatment, and may be attributed to the decline in long-term data availability. Future analysis using a larger sample size is needed to evaluate the effect after one year.

Keywords– *Leber's congenital amaurosis, adeno-associated virus, gene therapy, RPE65, pooled analysis*

1. Introduction

LCA is usually inherited as an autosomal recessive genetic condition characterized by nystagmus and rapid vision loss starting from birth. It is the leading cause of inherited blindness in children, accounting for 10-18% of cases of blindness [1] or severe vision reduction. RPE65-LCA is caused by mutations in the RPE65 gene, which encodes an isomerase protein that converts all-trans-retinyl ester to 11-cis-retinol [2]. Without this protein, all-trans-retinyl ester accumulates in the eye, blocking the visual cycle. RPE65-LCA is a popular candidate for gene therapy, whereby a wild-type, functional copy of RPE65 is inserted nonspecifically via a viral vector. While various vectors are currently being tested and optimized, the earliest approaches utilized adeno-associated viral (AAV) vectors, and AAV remains a popular vector today. Several clinical trials report that RPE65 gene therapy improved visual function, though different studies saw varying levels of improvement corresponding to patients of different ages. For example, Jacobson*et al.* [3] and Bainbridge*et al.* [4] found that older patients saw greater improvement, while Weleber *et al.* [5] reported greater improvement in younger patients. Previous meta-analyses have found that improvements in BCVA are modest [6] or not durable after 1 year [7]. This pooled analysis aims to expand on these results and offer additional insights on the effect of age on gene therapy outcomes.To the authors' knowledge, no other studies have been performed that analyze the relationship between patient age and treatment efficacy.

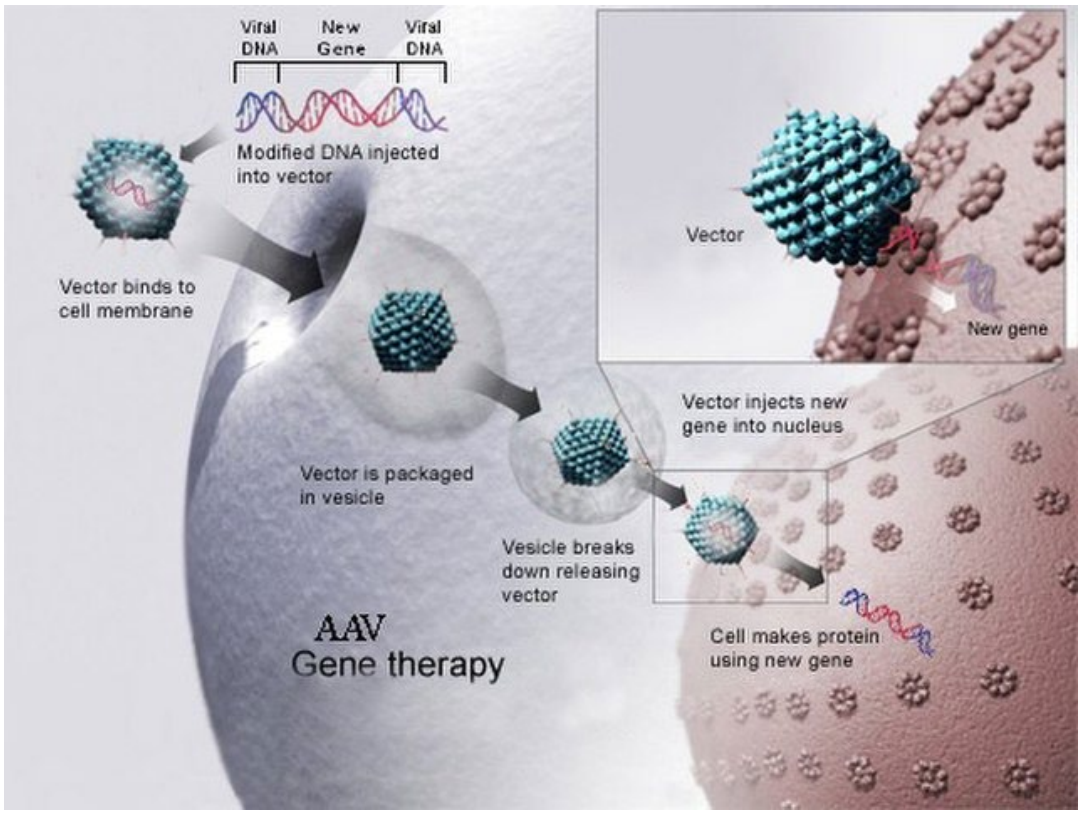

Fig. 1: AAV-mediated gene therapy (George Church, Creative Commons Attribution-Share Alike 3.0)

2. Methods

Search Strategy

The online electronic database PubMed was searched for "gene therapy leber congenital amaurosis RPE65" in October 2021 with no restrictions on date, region, language, or publication type. All articles that were indexed on PubMed on or prior to October 26th 2021 were included in the screening. When multiple published studies described the same intervention on the same group of patients, the most recent or complete report was used. No automation tools were used.

Inclusion-Exclusion Criteria

Papers describing clinical trials (both randomized control trials and observational studies) that reported the effects of human gene therapy for RPE65-LCA using an AAV vector, and had at least one quantitative outcome of visual function reported for each individual patient, were included. Abstracts, conference reports, reviews, and case reports were not included.

Study Selection

Figure 2 shows a flow chart of the screening and selection process. All articles underwent an initial title and abstract screen. Papers that met the inclusion criteria after the first screening underwent a second full-text review for data completeness and recentness (in the case of multiple publications at different time points following a study on the same cohort of patients). Full-text screening was complemented by cross-referencing the articles with publications indexed on ClinicalTrials.gov for the given trial number.

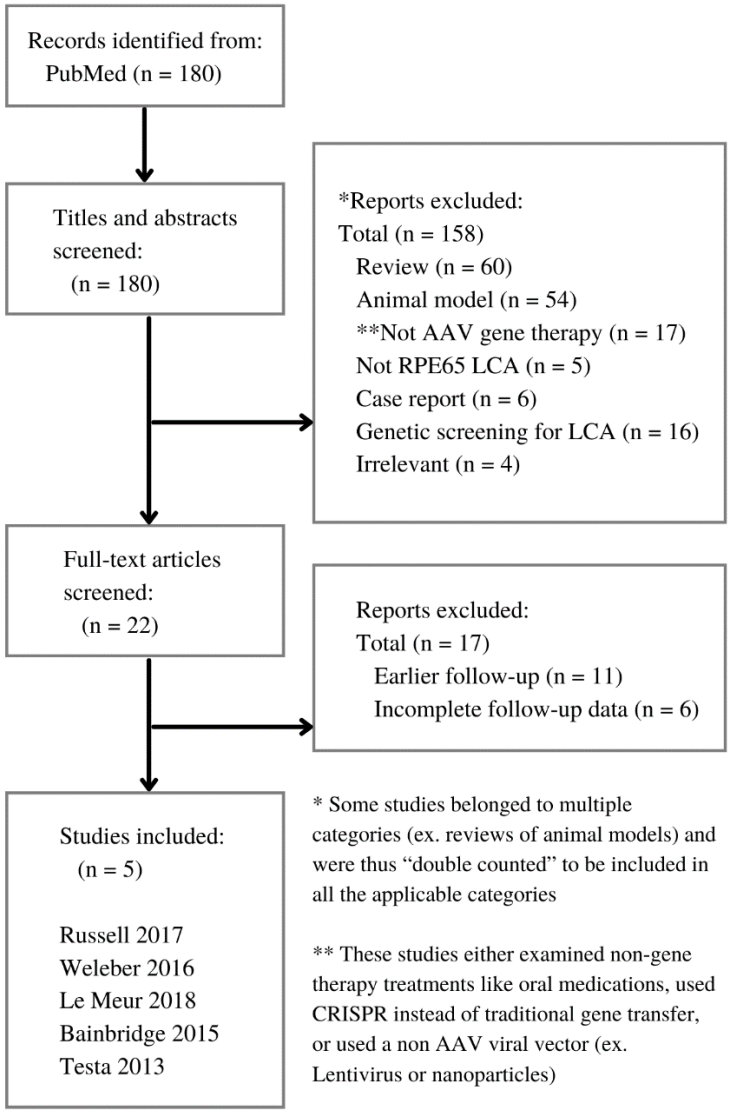

Fig. 2: Database search and study selection flowchart

Data Collection

The main clinical outcome was best-corrected visual acuity (BCVA), measured as the Logarithm of the Minimum Angle of Resolution (logMAR), where higher logMAR corresponded to worse vision. The secondary outcome was retinal thinningmeasured by optical coherence topography (OCT). For each outcome, individual patient-level data were extracted for both the treated and untreated eyes. Each patient's age was also extracted. Russell *et al.* [8], the sole randomized control trial, performed bilateral injections in ⅔ of all enrolled patients. All injected eyes were considered treatment units, while all the eyes of patients who received no injections were considered control units, so that each patient provided two data points. In the case of Weleber *et al.* [5], Le Meur *et al.* [9], Bainbridge *et al.* [4], and Testa *et al.* [10], the worse-performing eye at baseline screening received an injection while the contralateral, uninjected eye served as the control.

BCVA and retinal thickness data from the Bainbridge *et al.* [4] paper were extracted from graphs provided in the Supplemental Data file using the free version of the web-based app PlotDigitizer. PlotDigitizer was also used to extract BCVA data from the Weleber *et al.* [5] paper.

While the Jacobson *et al.* [3] paper met all inclusion criteria and contained patient-level data for BCVA and retinal thickness, the data from this paper were not included for two reasons. On the BCVA data, the graphs were missing segments where data were not reported. Due to a combination of missing segments and parts of the graph where it was difficult to discern what data points belonged to which patient, and at what time points the data was collected, the authors did not feel confident digitizing the graphs to extract data. Graphs displaying data on retinal thickness were also not digitized due to inconclusiveness about the exact timing of what was considered "short-term" follow-up.

Table 1: Characteristics of included studies

Study	**Average age**	**Treated Eyes**	**Control Eyes**	**BCVA data at 1 year**	**BCVA data at 2 years**	**BCVA data at 3 years**	**Retinal thickness data at 1 year**
Russell 2017	15	40	18	x			x
Weleber 2016	25	12	12	x	x		
Le Meur 2018	24	9	9	x			
Bainbridge 2015	14	12	12	x	x	x	x
Testa 2013	20	5	5	x	x	x	

Data Synthesis and Analysis

The data from all five studies were combined with simple pooling, without weighting the studies. Linear regressions were performed on age versus percent change from baseline BCVA and on age versus change in retinal thickness from baseline. Both regressions were done on data from the treated eyes only. Data were compiled in Google Sheets and then exported to Microsoft Excel for regression analysis.

When visual acuity was presented as Early Treatment Diabetic Retinopathy Study (ETDRS) letter scores, scores were converted to logMAR with the following formula [11]:

$$\text{logMAR} = -0.02 * (\text{ETDRS letter score}) + 1.7$$

When visual acuity in patients with low vision was presented categorically, conversions to logMAR were accomplished using the values suggested by Schulze-Bonsel *et al.* [12], Bach *et al.* [13], and Lange *et al.* [14]. Finger counting (CF) was approximated to 1.9 logMAR, hand movement (HM) to 2.3 logMAR, and light perception (LP) to 2.7 logMAR. This conversion was applied with some data from the Weleber *et al.* [5] and Le Meur *et al.* [9] studies.

3. Results

Age vs. BCVA

At 1 year post-injection, with a sample size of 78 eyes, a statistically significant linear regression equation was found ($\alpha = 0.05$, $n = 78$, $F(1, 76) = 7.3152$, $p = 0.0084$), with an R2 of 0.0878. At 2 years, only 29 eyes had available data, and a significant regression equation was not found ($\alpha = 0.05$, $n = 29$, $F(1, 27) = 3.1965$, $p = 0.0850$), and R2 equaled 0.1059. At 3 years, there were only 15 available eyes, and similar to year two, a significant regression equation was not found ($\alpha = 0.05$, $n = 15$, $F(1, 14) = 0.3807$, $p = 0.5479$), with an R2 of 0.0285.

One-way ANOVAs were performed to compare the effect of each individual study's design on change in BCVA in the treated eye. At 1 year, ANOVA revealed that there was a statistically significant difference in mean BCVA change between at least two groups ($\alpha = 0.05$, $F(4, 73) = 2.5299$, $p = 0.0476$). At 2 years, ANOVA revealed that there was not a statistically significant difference in mean BCVA change between at least two groups ($\alpha = 0.05$, $F(2, 23) = 1.9863$, $p = 0.16005$). Similar to year 2, at 3 years ANOVA again revealed that there was not a statistically significant difference in mean BCVA change between at least two groups ($\alpha = 0.05$, $F(1, 12) = 0.4099$, $p = 0.5341$).

Age vs. Retinal Thickness

Data on individual patients' change in central retinal thickness (CRT) at one year post-treatment were pooled from two studies, Bainbridge *et al*. [4] and Russell *et al.* [8], resulting in a total sample of 80 treated and untreated eyes from 40 patients. OCT data from patient CH-25 enrolled in the Russell *et al.* [8] study were not included in this analysis due to "quality… [being] highly degraded by the patient's nystagmus", as noted in the supplemental data file. An F-test revealed statistically significant differences in variance between the treatment and control groups ($p = 0.03811$). Based on the results of the F-test, it was desired to test the null hypothesis that mean retinal thinning was not significantly greater in the treated eyes compared to the control eyes at one year. This was accomplished by performing a one-sample T-test assuming unequal variance. There was a significant difference between mean outcomes from the treatment ($n = 50$, $M = -11.908$, $SD = 21.2384$) and control groups ($n = 30$, $M = -0.10656$, $SD = 15.4869$); $\alpha = 0.05$, $p = 0.002798$.

Following up the results of the T-test, a linear regression of age versus change in CRT was performed with a sample size of 50 treated eyes. A statistically significant linear regression equation was not found ($\alpha = 0.05$, $n = 50$, $F(1, 48) = 0.07756$, $p = 0.7818$), with an R^2 of 0.0016.

Table 2: Results of linear regressions on age vs. BCVA and age vs. CRT in treated eyes

Years post-treatment	Outcome	n =	p =	R^2	Regression Equation
1	*BCVA	78	0.0084	0.0878	y = 0.6555x - 23.658
2	*BCVA	29	0.0850	0.1059	y = 0.8016x - 28.743
3	*BCVA	15	0.5479	0.0111	y = -0.6285x - 11.628
1	+CRT	50	0.7818	0.0016	y = 0.0825x - 12.707

*Percent change in BCVA from baseline, measured in logMAR

+Change in central retinal thickness from baseline, measured in microns

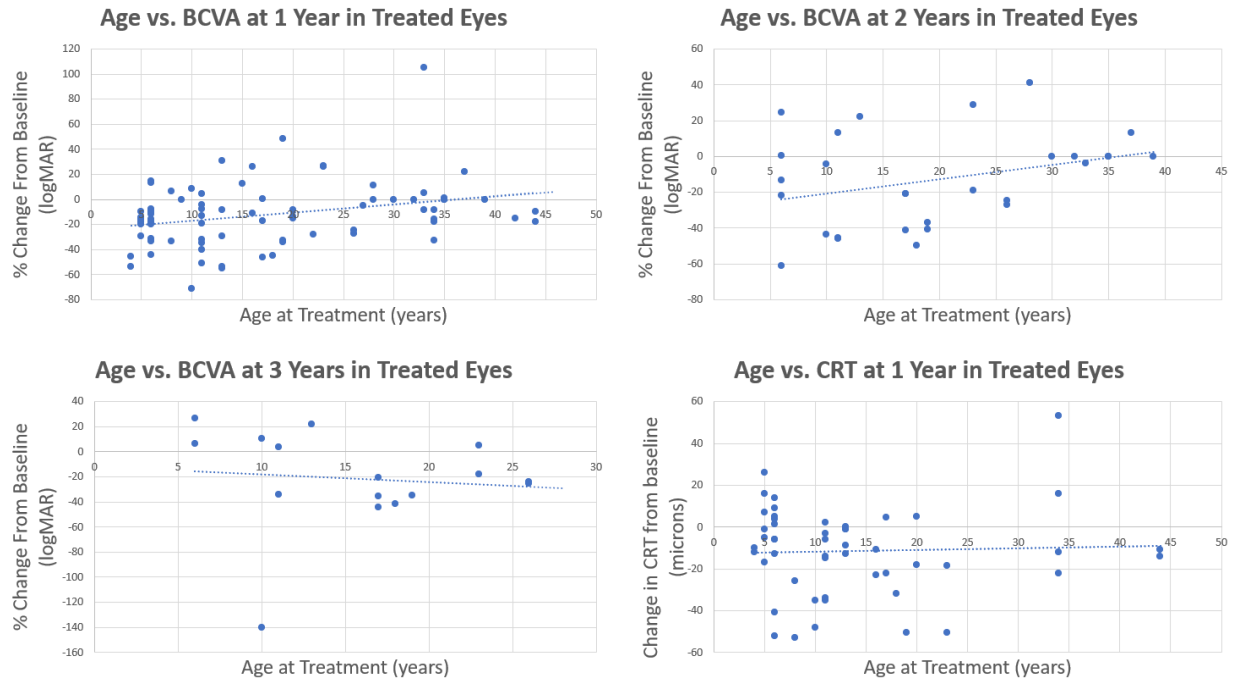

Fig. 3: Scatterplots of age vs. percent change in BCVA from baseline, and age vs. change in CRT from baseline in treated eyes

4. Discussion

Age vs. BCVA

For AAV-mediated gene therapy of RPE65-LCA, age appears to be a statistically significant predictor of improvements from baseline visual acuity at one year post-treatment ($p = 0.0084$). Younger age appears to be associated with greater decreases in logMAR, which translates to more significant improvements in visual acuity, as higher logMAR indicates worse vision. However, the age predictive effect is only visible in the first year after treatment. This effect was not detected at two and three years post-treatment ($p > 0.3$), and may be attributed to the decline in data reported beyond the first year. Previous meta-analyses have concluded that the effect of gene therapy on visual acuity beyond one year is not statistically significant [7], so the absence of a relationship between age and visual acuity at two and three years is not as meaningful as this study's finding from the first year after treatment. Future analysis using a larger sample size is needed to evaluate the long-term effects.

There were also detectable differences in treatment outcomes between studies at 1 year, when single-factor ANOVA resulted in $p < 0.05$. This indicates that there was a statistically significant difference in mean BCVA change between at least two of the five groups of patients enrolled in different studies. However, this was not the case at 2 and 3 years after treatment, when $p > 0.05$.

Age vs. Retinal Thickness

A one-tailed, two-sample T-test revealed significant impacts of treatment on average change in CRT at 1 year post-treatment ($p = 0.002798$). This finding contrasts with those of Wang *et al.* [7], who concluded that though a tendency for thinning in treated eyes was visible at 1 year, it was not statistically significant. This difference in findings could be attributed to a difference in methods. The data in this analysis was not weighted by study, and Wang *et al.* [7] did not exclude patient CH-25 from the Russell *et al.* [8] study.

Though retinal thinning is a significant effect of the treatment, it does not appear to be correlated with age. This suggests that younger patients could receive greater benefits to visual acuity from treatment, without experiencing risk of more severe retinal thinning and detachment. However, this age-related effect on visual function is only modest and short-term. Additional data on long-term change in retinal thickness is needed to assess potential age-related effects that may appear at a later time.

Limitations

Since RPE65-LCA is a rare disease that affects around 1 in 81,000 people [15], one major limitation of this pooled analysis is the sample size, which decreased quickly with each year after treatment was administered. Additionally, though the studies inserted the same RPE65 gene, used AAV vectors, and utilized subretinal injection with similar surgical procedures, injections varied in vector dose (108 to 1012 vector genomes) and volume (0.15 to 1.0 mL). They also differed in the number of injection sites with varying distance from the fovea. Variability in the surgical procedure could have impacted the amount of retinal thinning, detachment, and other ocular adverse events experienced [3] [10]. However, pooling the data from studies with low sample sizes is important for assessing the potential benefits of treatment.

5. Conclusion

The purpose of this pooled analysis was to evaluate the impact of age on treatment outcomes for patients undergoing AAV-mediated gene therapy for RPE65-LCA. Five studies representing 78 treated eyes were pooled. The outcomes evaluated were change in visual acuity and retinal thickness in the treated eyes. Linear regressions revealed age to have a significant effect on improvements in visual acuity at 1 year post-treatment, but not at years 2 and 3 post-treatment. There was also no significant correlation between age and retinal degeneration. These results suggest that younger children could see greater visual improvements in the short-term due to the therapy, without experiencing significantly greater risk of retinal damage. Additional long-term follow-up data after the first year are needed to better assess overall improvements to visual function and retinal degeneration, and evaluate potential age-related effects on both outcomes. This study and future findings are important for informing policy and treatment recommendations for patients with RPE65-LCA who are eligible for gene therapy.

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