Chapter 71
Evaluation of Ocular Gene Therapy in an Italian Patient Affected by Congenital Leber Amaurosis Type 2 Treated in Both Eyes

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Abstract Gene therapy clinical trials with gene augmentation therapy for Leber Congenital Amaurosis have shown partial reversal of retinal dysfunction. Most studies described the effect of treatment in a single eye and limited evidence is reported in literature about patients treated in both eyes. In this chapter, we present the findings of a young patient treated in both eyes. Efficacy of the treatment was assessed with Best Corrected Visual Acuity, Goldman Visual Field testing, Esterman computerized binocular visual field and Microperimetric testing. Post-treatment results showed improvement of visual function in both eyes, in particular,

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a strong amelioration was observed after the first injection, by using conventional monocular tests. Moreover, the treatment in the second eye resulted in a further improvement of binocular visual functionality, as easily detected by computerized binocular visual field. In conclusion, our data suggest that gene therapy can inhibit retinal degeneration and can be safe and effective in restoring visual functionality in young subjects treated in both eyes. Finally, new outcome measurements, in particular binocular computerized visual field parameters, can therefore be useful to quantify overall visual gain in patients undergoing gene therapy in both eyes.

Keywords Gene therapy · Leber's Congenital Amaurosis · Optical coherence tomography · Microperimetry · Binocular computerized visual field

71.1 Introduction

In the last decade, gene therapy was explored for the treatment of incurable inherited retinal diseases both in animal models and in human subjects. Particularly, 3 independent clinical trials that began almost contemporaneously in 2007–NCT00481546 (Cideciyan et al. 2009), NCT00516477 (Maguire et al. 2008), NCT00643747 (Bainbridge et al. 2008) - were performed to evaluate safety and efficacy of gene therapy for Leber Congenital Amaurosis type 2 (LCA2), a retinal degeneration resulting from mutations in the RPE65 gene.

In the three initial clinical trials, the patients were treated with a single unilateral subretinal injection of adeno-associated virus 2 (AAV2) carrying the RPE65 gene in the eye with worse vision. A safety assessment showed the presence of minimal systemic immunological response in two trials (Hauswirth et al. 2008; Maguire et al. 2008) and the absence of serious adverse events in all three trials. In particular, in the clinical trial NCT00516477, performed at the Children's Hospital of Philadelphia (CHOP) in conjunction with the Second University of Naples (SUN), 12 patients were treated by subretinal AAV2-hRPE65v2 injection in the worse eye (Maguire et al. 2009). The findings of this clinical trial showed an improvement of visual functionality and a stability over long-term follow-up in most patients (Simonelli et al. 2010; Testa et al. 2013). The promising results obtained motivated a new clinical trial for the re-injection of previously treated patients in the contralateral eye (NCT01208389). Since there is limited evidence in literature about LCA patients treated in both eyes, in this chapter we present our clinical findings in the youngest subject of our cohort of patients treated in both eyes.

71.2 Materials and Methods

All details on design, consent, and vector administration in this clinical trial have previously been reported (Maguire et al. 2009). Briefly, the LCA subject NP15, aged 8 years old was first evaluated at the Second University of Napoli (Napoli,

Italy) and received the diagnosis based on visual and retinal function studies (Simonelli et al. 2007). All patients underwent mutation screening for LCA genes and received molecular diagnosis of LCA2 by the Telethon Institute of Genetics and Medicine. After informed consent and confirmation of trial eligibility criteria, including independent evaluation of the likelihood that the mutations were disease-causing (Carver Lab, Iowa City, IA), the eye with worse visual function was selected for delivery of AAV2-hRPE65v2. The study subject (NP15) underwent an initial AAV2-hRPE65v2 injection in the right eye (at the age of 11 years) and after 3 years in the left eye (at the age of 14 years). NP15 received the same dose/volume (1.5×1011 vg/300 μl) in both eyes. Baseline tests and follow-up visits up to day 30 were performed at both the Children's Hospital of Philadelphia and Second University of Napoli while the follow-up visits were performed at the Second University of Napoli. Follow-up data are available up to 4 years after the initial treatment and 1 year after the treatment in the contralateral eye. In the current study, efficacy of the treatment was assessed with Best Corrected Visual Acuity (BCVA), Goldman Visual Field testing (VF), Esterman computerized binocular visual field and Microperimetric testing (MP).

BCVA was measured by trained vision examiners using a standard protocol involving Early Treatment Diabetic Retinopathy Study (ETDRS) charts and letter counts. Letter scores were converted to the log of the Minimum Angle of Resolution (logMAR), on a scale ranging from 0.00 to 2.00, with higher values indicating poorer vision. Eyes that could detect hand motion were assigned a score that was one line worse than the largest printed line on the chart tested at a standardized distance of 4 m (<20/1600) to provide the most conservative evaluation in terms of underestimating the actual extent of visual impairment.

VF was measured using Goldman perimetry (Haag Streit Perimeter 940; Haag Streit, Mason, OH).(Ross et al. 1984) The visual field isopters were obtained using the V4e test object.

The Esterman binocular visual field test on the field analyzer perimeter uses a grid of 120 test points to examine more than 130° of visual field. It was originally developed for manual perimeters and, similar to its monocular predecessor, gives more weight to the functionally more important parts of the visual field (i.e., central and inferior).(Esterman 1982)

Microperimetry was performed by an automatic fundus-related perimeter (MP1 Microperimeter, Nidek Technologies, Padova, Italy). For the purpose of this study, the following parameters were used: a fixation target of 2° in diameter consisting of a red ring; a white monochromatic background with a luminance of 4 abs; and a Goldman III–size stimulus with a projection time of 200 ms.(Sohn et al. 2010) The stimulus was randomly projected according to a customized radial grid of 61 points covering the central portion of the retina (108 centered onto the fovea; points aligned on the 08, 308, 608, 908, 1208, and 1508 radial axes, 18 apart), and a 4-2-1 double staircase strategy was used with an automatic eye tracker that compensated for eye movements.(Midena et al. 2007)

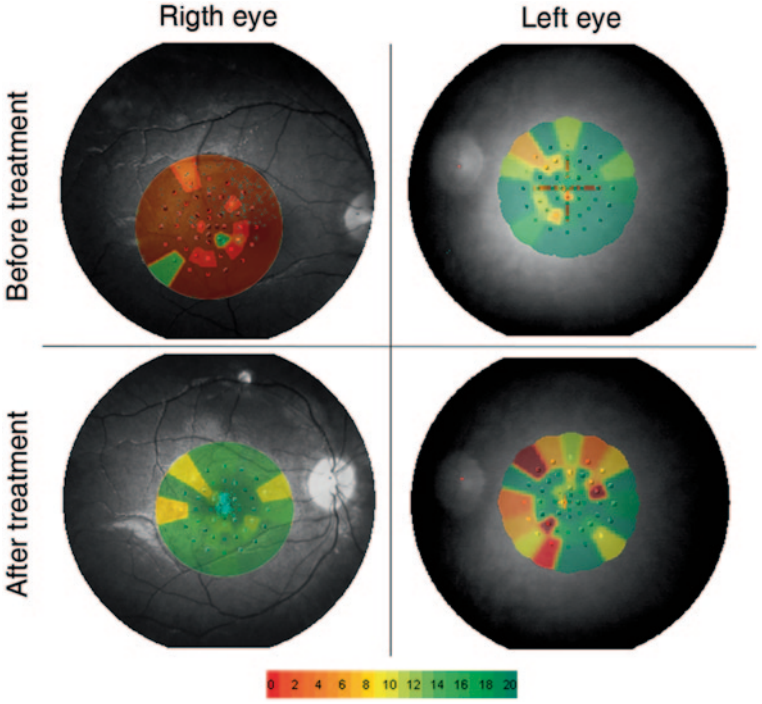

Fig. 71.1 Microperimetry macular sensitivity maps before and after treatment

71.3 Results

Both eyes showed improvement in visual functionality, as evaluated 1 year after treatment.

In particular, in the first treated eye (right eye), BCVA improved from 0.85 to 0.42 logMAR, Mean macular sensitivity increased from 0.8 dB (with unstable fixation) to 17.9 dB (with stable fixation), and central VF radius increased from 44° (area: 6197°^{2}) to 52° (area: 8549°^{2}; $p<0.001$). Figure 71.1 shows the microperimetry macular sensitivity maps before and after treatment.

One year after treatment in the second eye (left eye) BCVA improved from 0.42 to 0.34 logMAR; Mean macular sensitivity remained stable (16.6 vs 14.2 dB with stable fixation); and central VF radius increased from 46° (area: 6659°^{2}) to 50° (area: 7,762°^{2}; $p=0.02$). Moreover, binocular computerized visual field, performed before and after the injection in the contralateral left eye and reported in Fig. 71.2, showed an improvement of Esterman score from 59 to 74%, associated with an increase of mean sensitivity from 2.9 to 8.4 dB.

Comparing the 1 year post-injection time-points, we observed a BCVA improvement of 51% (RE) and 19% (LE), a fixation stability increase of 10 (RE) and 0.02 times (LE), and a VF enlargement of 38% in the right eye and 17% in the left eye.

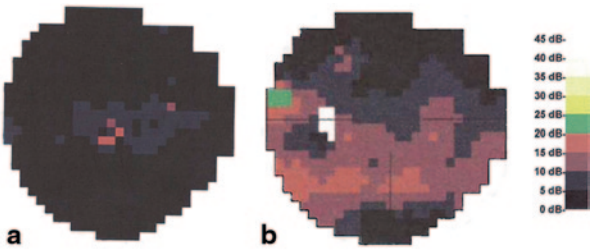

Fig. 71.2 Binocular visual field performed before (**a**), and after (**b**), treatment in the *left* eye

As regards the 4-year follow-up, the right eye showed an improved visual functionality compared to baseline, i.e., improved BCVA (0.56 vs 0.82 logMAR), increased mean macular sensitivity (10.3 vs 0.8 dB), enlarged VF area (8211 vs 6197°^{2}). Moreover, mean Macular Thickness evaluated by Spectral Domain OCT remained stable over the follow-up in both eyes (239±3 μm in the right eye, 239±8 μm in the left eye, see Fig. 71.3).

71.4 Discussion

The results of previous studies on gene therapy for LCA patients with RPE65 mutations support the hypothesis that the greatest improvement in visual function with subretinal gene therapy will occur in young individuals (Simonelli et al. 2010). Although young patients had better visual function at baseline than did older individuals, they also had the greatest overall improvement in vision. However, most previous studies focused on treatment of the first eye, while only one study reported the results of re-injections in the contralateral (untreated) eye in three patients, showing that the gains in retinal and visual function that had resulted from the initial injection were maintained after the second eye was injected (Bennett et al. 2012). In addition, the results of retreatment may reflect an age effect whereby the individuals who were younger (and thus whose retinas had not undergone as much degeneration) showed larger gains than older individuals. Here we reported

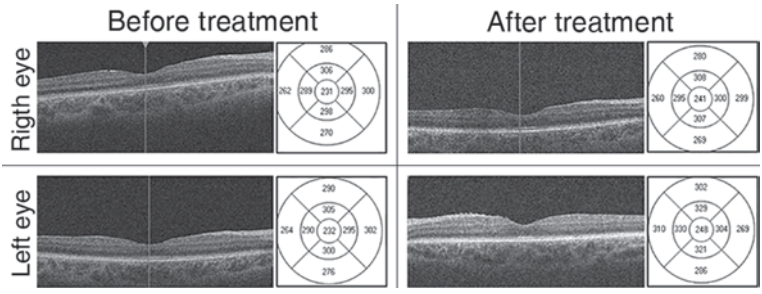

Fig. 71.3 OCT scans performed before and after treatment

the preliminary findings related to a teenager treated in both eyes, who represents the youngest subject among those treated in both eyes and described in literature. The post-treatment results showed improvement of visual function in both eyes, in particular, a strong amelioration was observed after the first injection, by using conventional monocular tests (i.e., BCVA, microperimetry and Goldman visual field). Moreover, the treatment in the second eye did not alter the gain achieved in the first eye and resulted in a further improvement of binocular visual functionality, as easily detected by computerized binocular visual field.

In literature, data on retinal degeneration revealed by OCT scan in patients treated in another clinical trial showed that therapy did not slow retinal degeneration, since a thinning of the outer nuclear layer (ONL) was detected by an ad hoc segmentation algorithm (Cideciyan et al. 2013). Although the comparison was limited by differences in methods (ad hoc developed versus commercial software), in demographic characteristics (i.e. age), and in the vector preparation and surgical approach, our observations showed that the overall macular thickness, including ONL, measured in OCT scans, remained stable over the whole follow-up (4 years), suggesting that gene therapy can slow retinal degeneration. However, further analysis on the overall treated cohort with a similar technique could be useful to confirm this hypothesis.

In conclusion, our data suggest that gene therapy can inhibit retinal degeneration and can be safe and effective in restoring visual functionality in young subjects treated in both eyes. In particular, the treatment in the second eye resulted in a further improvement of binocular visual functionality. Finally, new outcome measurements, in particular binocular computerized visual field parameters, can therefore be useful to quantify overall visual gain in patients undergoing gene therapy in both eyes.

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