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Case Report

Long-Term Follow-Up of a Family with Retinal Dystrophy Caused by RPE65 Mutation

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Keywords
Hereditary retinal dystrophy · Leber's congenital amaurosis · Genetic treatment · Electrophysiology · Optical coherence tomography

Abstract
We present here the case histories of two siblings, a boy and a girl, with Leber's congenital amaurosis (LCA). The diagnosis was based on non-recordable full-field electroretinogram (ffERG). The long-term ophthalmologic follow-up included kinetic perimetry (Goldmann), visual evoked potentials with flash stimulation, optical coherence tomography (OCT: B-scan images at the area of fovea), and multifocal ERG. The boy (sibling 1, born in 1986) was sent for electrophysiological examination at the age of four because he had nystagmus from birth. The diagnosis would be LCA based on non-recordable ffERG. Four years later, his visual acuity decreased rapidly due to vitreous opacification, caused by the autoimmune reaction of the retinal pigment epithelial cells. This was treated successfully with steroid injections, administered parabulbarly. Retinal autoimmune panel was not performed. Genetic testing became available only in 2019, and it revealed a RPE65 gene mutation: (NM_000329.2) c.{442G>A};{442G>A} (p.{Glu148Lys}; {Glu148Lys}). His sister (sibling 2, born in 1993) showed similar symptoms, caused by the same genetic mutation. Even though their parents were free of symptoms, it appeared that they were heterozygous carriers of the same mutation. Research of the family tree revealed a consanguineous marriage four generations before. Both siblings received successful gene therapy relatively late in their age: sibling 1 was 35 and sibling 2 was 28 years old, meaning that they were at an advanced stage of the disease. Nevertheless, follow-up examinations showed measurable improvements in their retinal function. The study shows that electrophysiological

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examinations, including flash-evoked responses, are useful in the objective evaluation of the progression in the central photoreceptor loss during the follow-up of LCA. The results also show that gene therapy can have beneficial effects even at an advanced stage of the disease.

Introduction

Leber's congenital amaurosis (LCA) is one of the most severe forms of early-onset retina dystrophies, responsible for blindness at birth or at early childhood. It was described by Theodor Leber in 1869 [1]. The early symptoms include nystagmus, photophobia, sluggish papillary reactions, and nyctalopia (night blindness). Retinal ophthalmoscopic alterations are not pathognomonic in the earliest stage, but full-field electroretinogram (ffERG) can already be abnormal or extinguished. As time passes, pigment epithelial degeneration continues to progress, it will be thinner and thinner, and bone cell formation appeared. The consecutive photoreceptor cell damage spreads toward the central parts of the retina. Vascular alterations and optic disc abnormalities may also occur [2]. Non-recordable ffERG is considered an essential diagnostic feature [3].

The genetic background of early-onset retinal dystrophies is rather varied [4]. Genetic testing for this purpose has become available only recently, in the 1990s. In this report, we present a 32-year-long case history of two siblings, including the results of their genetic treatment.

Case Presentation

All procedures described in this report conformed to the Declaration of Helsinki in all respects. Written informed consent was obtained from the patients. The electrophysiological diagnostic procedures were performed with Roland equipment (Wiesbaden, Germany) according to the ISCEV recommendations and standards for the evaluation of the rod, the cone, and the inner retinal cell function [5]. As the ffERG was extinguished, for the follow-up, flash visual evoked potentials (FVEPs) were recorded with 3 cd/m^2 flash stimulation at 1.4 Hz. One hundred responses were averaged. Because of the patients' poor visual acuity and nystagmus, pattern visual evoked potentials (PVEPs) were not informative. Multifocal electroretinography (mfERG) according to the ISCEV standard [5] and optical coherence tomography (OCT: B-scan images) were performed occasionally with a focus on the foveal area (Heildelberg Spectralis, Heidelberg Engineering GmbH, Germany).

In 1990, sibling 1, a four-year-old boy was sent for electrophysiological examination to our laboratory because of nystagmus and photophobia. LCA was diagnosed based on the non-recordable ffERG. Four years later, in 1994, we could not perform the follow-up examinations, as the vitreous was hazy in both eyes, which also deteriorated visual acuity to a serious extent. Steroid therapy was initiated with parabulbarly administered betamethasone. Three weeks later, after the third injection, the vitreous was clearer, and the visual acuity improved from 1-m finger counting (in both eyes) to 3-m finger counting in the right eye and to 0.1 in the left eye.

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We used visual evoked potentials with flash stimulation (FVEPs) to monitor the changes in the patient's visual function objectively. Pattern stimulation VEP (PVEP) was not performed as it would have required better visual acuity. As expected, the FVEPs were subnormal and hardly recordable in the right eye but fairly good and reproducible in the left eye.

We started Etaretin (0.5 mg/1 mL, intramuscular injection; Etapharm GmbH) treatment, containing vitamin A, retinal phosphatides, and sodium bicarbonate. Etaretin was administered [6] for five consecutive days twice a year until 2005. The therapy appeared to be effective: nystagmus was decreased, and the visual acuity of the patient improved. Etaretin was not available after 2005; thus, we had to switch to Difrarel E (50 mg anthocyanin extract from *Vaccinium myrtillus* and 50 mg tocopherolum aceticum tablet, Leurquin Mediolanum Laboratoires, France) [7]. The same transient beneficial effects were seen as with Etaretin. However, in 2007, at the age of 21, the visual acuity of his right eye started to decrease rapidly, and by 2009, visual acuity was not measurable anymore in this eye. Light perception was maintained. Visual acuity in the left eye started to deteriorate as well (Fig. 1a), and, by 2015, only the ability to detect hand movements remained, and visual field was not detectable. The course of worsening was objectively detected by FVEP: at his age of 16, FVEP was nonrecordable on his right eye, and only some residual signal was found in his left eye (Fig. 2a).

This patient's sister (sibling 2) was born in 1993. She also started to exhibit the same visual symptoms as her brother. Her first examination occurred in 1999, at her age of 6. At that time, her visual acuity was 0.15 (right eye) and 0.08 (left eye) with correction (−1.5 D). The FVEP was recordable, but its amplitude decreased progressively (Fig. 2b). ffERG proved the inheritance of LCA. At the age of 10, kinetic perimetry indicated a narrowing of the visual field to 10° in the right eye and to less than 15° in the left eye. The mfERG revealed seriously affected central cone function, reflecting a "patchy" pattern of degeneration, which means that there were some rather good kernels (responses), and also completely extinguished ones in the central 30° of the retina (Fig. 3). Her visual acuity gradually worsened to 0.01 (right eye) and to 0.02 (left eye) by the age of 23 years (Fig. 1b).

Genetic testing of the family became possible in 2019. A new variant (NM_000329.2) c.{442G>A};{442G>A} (p.{Glu148Lys}; {Glu148Lys}) of the RPE65 gene was identified by exome sequencing. It was found that the same mutation of the RPE65 gene was present not only in the siblings in homozygous form but also in the parents in heterozygous form, suggesting that this was a biallelic mutation. Family tree research revealed that four generations earlier, a consanguineous marriage occurred in the family.

Genetic treatment was performed on both patients in the form of submacular injection of 0.3 mL Luxturna, 1.5×10^{11} vector genome at the Sveti Duh Hospital in Zagreb, Croatia, by Mirjana Bjeloš in October 2021. For sibling 1, the treatment was restricted to the left eye since the right eye showed only light perception. His left eye could detect hand movements; therefore, improvement in visual acuity could be expected. Unfortunately, the patient was already 35 years old at that time, but genetic became had not been a possibility earlier. Two weeks after the treatment, his visual acuity was already 0.08, and vision-guided ambulatory navigation became possible for him. The FVEP also showed a marked positive component that emerged from the noise level. The N1–P1 amplitude showed a marked increase from 0.08 μV to 1.88 μV (Fig. 2c).

Sibling 2 was 28 years old when she received genetic treatment, and her visual acuity was only 0.025 before the treatment. Two weeks after the treatment, her visual acuity improved to 0.14. The improvement was detectable also in the FVEP: the N1–P1 amplitude increased from 0.39 μV to 4.02 μV (Fig. 2d). However, OCT did not show any change. The CARE checklist has been completed by the authors for this case report, attached as online supplementary material (for all online suppl. material, see https://doi.org/10.1159/000530086).

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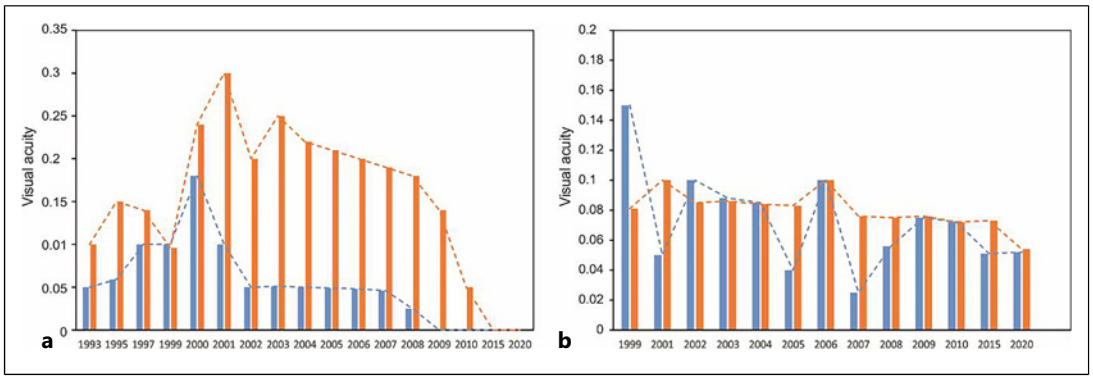

Fig. 1. A timeline representing the changes in the visual acuity of both patients over the course of following up their progression, sibling 1 (**a**), sibling 2 (**b**). Notice the increase in visual acuity after the genetic treatment.

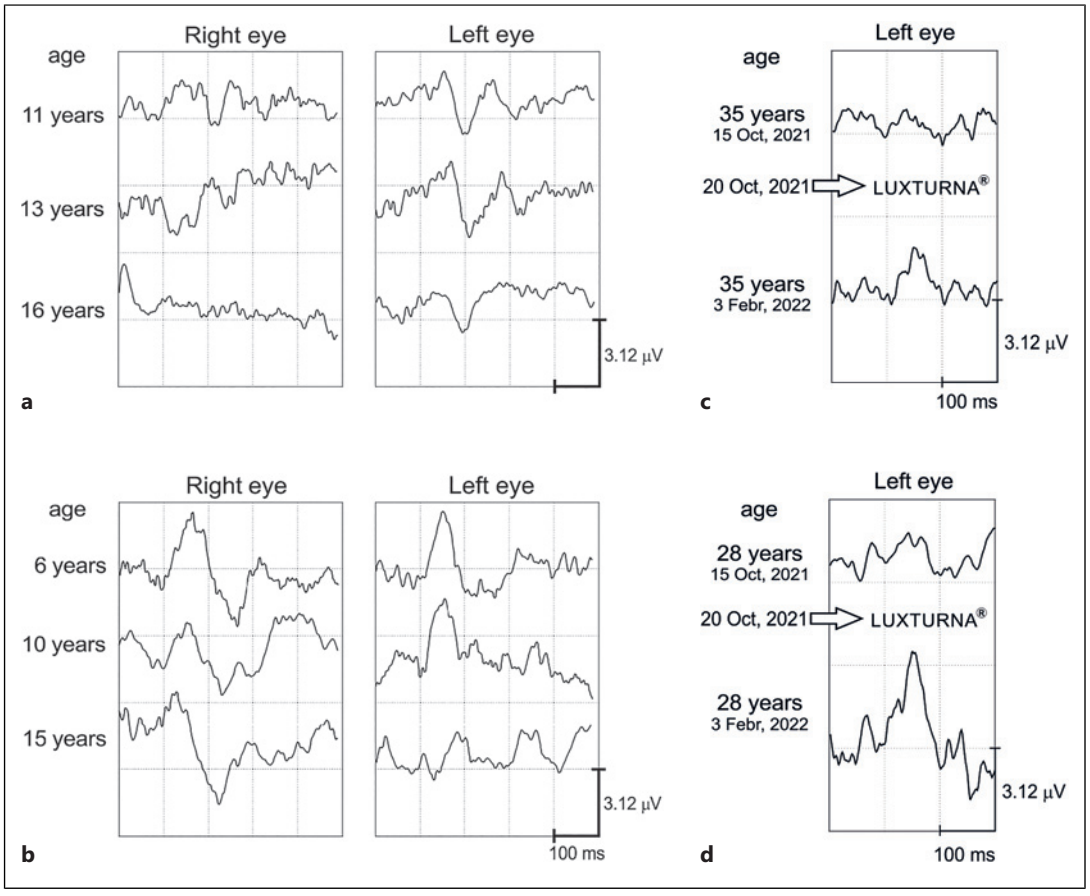

Fig. 2. Flash-evoked visual evoked potentials from the left and right eyes of both siblings before treatment over the follow-up period (**a**, **b**) and from the left eyes of both siblings before and 4 months after the Luxturna treatment (**c**, **d**).

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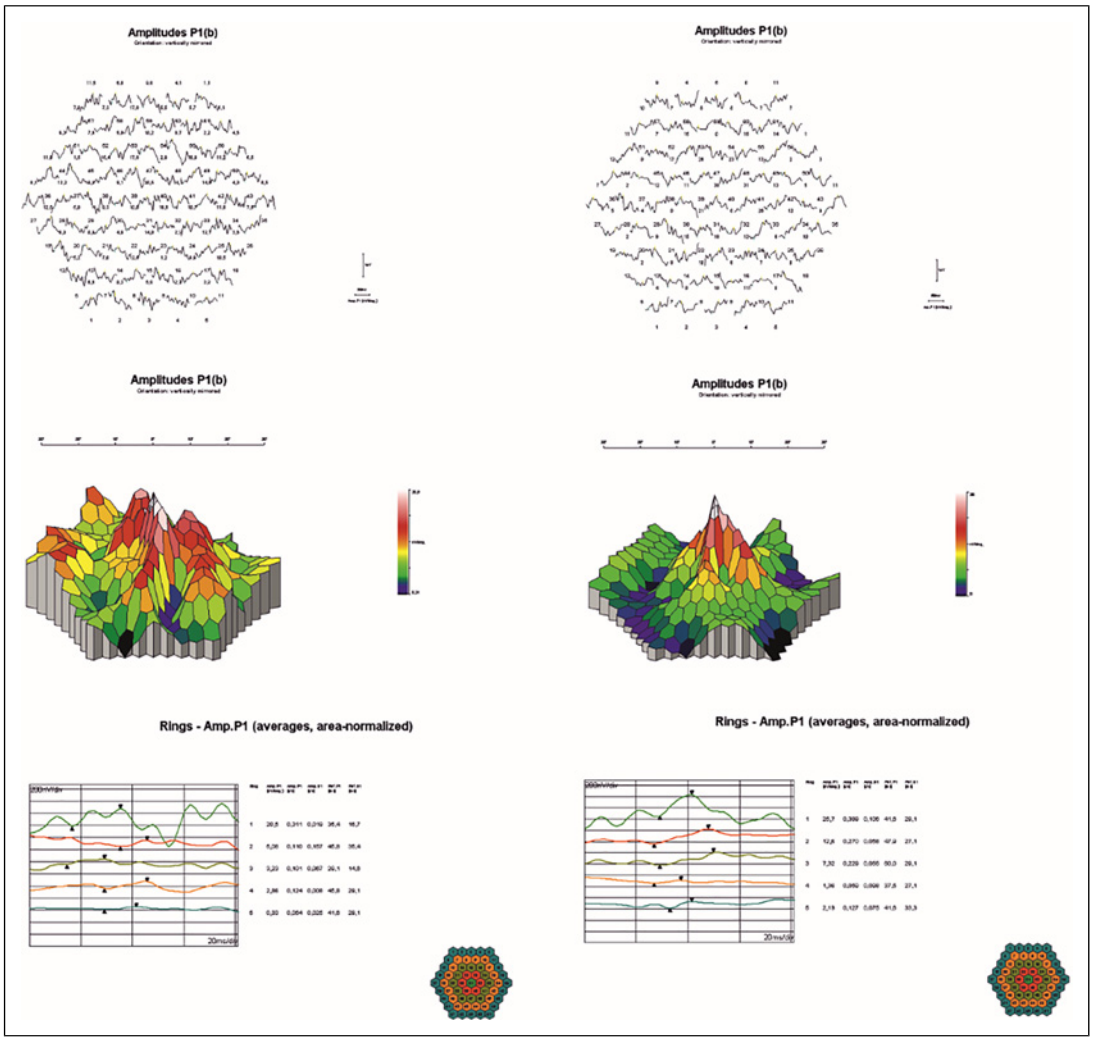

Fig. 3. Multifocal ERG of the sibling 2 at her age of 10 years. Top: trace arrays (plots), middle: three-dimensional representation, bottom: ring analysis of the responses.

Discussion

The first genes to be associated with the LCA phenotype have been identified by Perrault et al. [4]. The RPE65 protein is specific to the retinal pigment epithelium and is implicated in the metabolism of vitamin A, the precursor of the rhodopsin, which is critical for the normal functioning of the visual phototransduction cascade. Knocking out the RPE65 gene in animal models was initiated, and these models showed phenotypes similar to their human counterparts. Proof of concept studies have been carried out in these animal models using subretinal RPE65 gene replacement therapy that resulted in improvements in various visual function markers, including electroretinograms, pupillary light responses, and object avoidance behaviors [8]. Results of the initial human studies have also shown positive impact on visual function with acceptable patient safety levels [9]. Multi-luminance mobility testing has been used to measure the primary efficacy [10]. After the study met its primary endpoint, the Food and Drug Administration (USA) approved voretigene neparvovec (Luxturna®) for use in RPE65-associated inherited retinal diseases in human [11].

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When sibling 1 first visited our electrophysiology laboratory in 1990, genetic testing was not yet possible. Furthermore, it had been widely held that LCA was inherited in an X-linked recessive manner, not in autosomal recessive manner. Therefore, the parents were confident that their daughter would not be affected by the same condition.

The episode of severe vitreous opacification in sibling 1 was probably a genuine LCA-related finding of autoimmune origin. It has been known for relatively long that patients suffering from progressive retinal degeneration may become sensitized to retinal antigens, especially to those found in the rod outer segments [12].

Before the identification of the causative role of the RPE65 gene and specific gene therapy, our therapeutic armamentarium was quite limited. We made pharmacotherapeutic attempts based on the then-current literature [6, 7]. Both Etaretine and Difrarel E had some beneficial effects: the amplitude of the nystagmus decreased, and the treatment resulted in better visual performance. However, apart from slight and temporary improvements, neither of these attempts brought significant change in the retinal condition of the siblings, nor could they stop the progression of the photoreceptor degeneration. They underwent gene therapy as soon as it was established that their condition is associated with a mutation of the RPE65 gene, but they were already adults at that point.

In sibling 2, the mfERG revealed some remaining cones in the central 30° area of the retina. Therefore, it was still possible for the patient to naturally orient her head in a way that enabled the image to fall on the intact cone patches. Even though OCT imaging may provide additional information regarding the thinning of the retina, but excluding cystic macular edema is the most important use of OCT in this condition. However, obtaining high-quality images of the foveal layers may be more difficult due to the nystagmus of the patient.

While ffERG is a good method to set up the clinical diagnosis, it is not a very good approach to the assessment of the actual severity of visual loss. Visual evoked potentials are much better suited for that purpose. Information on the degenerative process in the macular area can be provided by mfERG. In our experience, FVEP is the best method not only to follow up the progression but also to assess the efficacy of gene therapy.

To the best of our knowledge, this is the first report on a long-term electrophysiological follow-up to describe the progression of visual loss due to LCA. The few previous studies that used electrophysiological methods (especially FVEP) for this purpose have not gone beyond early childhood [13].

We claim that the electrophysiological examinations proved to be an appropriate way to objectively monitor the visual functions. Thus, electrophysiology is not only useful for the initial diagnosis, of which it is already considered a key element, but also in the objective monitoring of disease progression in terms of function. We propose that for the follow-up in LCA, a complex approach should be followed, consisting of various electrophysiological tests in conjunction with OCT imaging, in order to enable the assessment of progression both in anatomy and in function. OCT is best used for anatomical assessment, especially to detect cystoid macular edema, while the different electrophysiological methods offer information on the various aspects of function.

Conclusion

The case of the siblings we report here shows that genetic treatment of LCA related to RPE65 mutation may slow the progression of the photoreceptor loss or even improve the visual performance of these patients. For this intervention, it is important that the patients have no cystoid macular edema. The three-decade follow-up of our patients showed that genetic treatment can bring some improvement even after a long disease progression.

Furthermore, we followed the patients for 8 further months after the gene therapy, and no progression or further decrease in visual performance was detected. Naturally, it is assumed that the same therapy attempted at a younger age may result in even better recovery.

Our mfERG results suggest that the central cone damage occurs in a "patchy" manner, which can help visual orientation. Of course, this is an isolated observation, and it does not necessarily happen in the same manner in all LCA cases. Further studies are needed to support or refute this idea. Finally, our study suggests that the combination of the electrophysiological tests has a role not only in the clinical diagnosis of LCA, but it may also provide information on the central cone loss, which is important in the monitoring of the disease progression and in the assessment of the improvements elicited by the applied therapy.

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Statement of Ethics

This study protocol was reviewed and approved by the Regional Research Ethics Committee for Medical Research at the University of Szeged, Hungary, approval number: 5075. Written informed consent was obtained from participants for publication of their medical case and the accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.J. (Agnes Janossy) participated in the electrophysiological measurements and in the treatment of the patients. E.V. (Eszter Vizvari) participated in the OCT measurements. M.L. (Mate Lorincz: university student) participated in the electrophysiological measurements and the analysis of the electrophysiological data of the RP patients. S.P. (Szilvia Pal) participated in the electrophysiological measurements. D.N. (Dora Nagy) did the genetic testing of the patients and reviewed the genetics-related content of the manuscript. G.B. (Gyorgy Benedek) participated in the analysis of the electrophysiological data and co-drafted the manuscript. E.T.-M. (Edit Toth-Molnar) participated in the treatment of the patients and in the critical

review of the manuscript. M.J. (Marta Janaky) supervised the follow-up from its beginnings, planned and organized the follow-up methodology, participated in the treatment of the patients and the electrophysiological measurements, and drafted the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included herein. Further inquiries can be directed to the corresponding author.

References

1 Leber T. Über Retinitis pigmentosa und angeborene Amaurose. Arch Ophthalmol. 1869;15(3):1–25. (in German).
2 Chacon-Camacho OF, Zenteno JC. Review and update on the molecular basis of Leber congenital amaurosis. World J Clin Cases. 2015;3(2):112–24.
3 Lambert SR, Kriss A, Taylor D, Coffey R, Pembrey M. Follow-up and diagnostic reappraisal of 75 patients with Leber's congenital amaurosis. Am J Ophthalmol. 1989;107(6):624–31.
4 Perrault I, Rozet JM, Ghazi I, Leowski C, Bonnemaison M, Gerber S, et al. Different functional outcome of RetGC1 and RPE65 gene mutations in Leber congenital amaurosis. Am J Hum Genet. 1999;64(4):1225–8.
5 Robson AG, Nilsson J, Li S, Jalali S, Fulton AB, Tormene AP, et al. ISCEV guide to visual electrodiagnostic procedures. Doc Ophthalmol. 2018;136:1–26.
6 Hruby K. Ist die Retinitis pigmentosa zweifellos unheilbar [Is retinitis pigmentosa absolutely incurable?] [Article in German]. Klin Monbl Augenheilkd. 1988;192(4):358–9.
7 Milbury PE, Graf B, Curran-Celentano JM, Blumberg JB. Bilberry (Vaccinium myrtillus) anthocyanins modulate heme oxygenase-1 and glutathione S-transferase-pi expression in ARPE-19 cells. Invest Ophthalmol Vis Sci. 2007;48(5):2343–9.
8 Gao J, Hussain RM, Weng CY. Voretigene neparvovec in retinal diseases: a review of the current clinical evidence. Clin Ophthalmol. 2020;14:3855–69.
9 Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med. 2008;358(21):2231–9.
10 Russell S, Bennett J, Wellman JA, Chung DC, Yu ZF, Tillman A, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. Lancet. 2017;390(10097):849–60.
11 FDA approves novel gene therapy to treat patients with a rare form of inherited vision loss. Available from: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm589467.htm (accessed December 28, 2017).
12 Brinkman CJ, Pinckers AJ, Broekhuyse RM. Immune reactivity to different retinal antigens in patients suffering from retinitis pigmentosa. Invest Ophthalmol Vis Sci. 1980;19(7):743–50.
13 Brecelj J, Stirn-Kranjc B. ERG and VEP follow-up study in children with Leber's congenital amaurosis. Eye. 1999;13(Pt 1):47–54.