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ARTICLE

Retinal gene therapy in RPE-65 gene mediated inherited retinal dystrophy

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BACKGROUND: Voretigene neparvovec (VN) is a gene therapeutic agent for treatment of retinal dystrophies caused by bi-allelic *RPE65* mutations. We illustrate, both the benefits and pitfalls associated with ocular gene therapy in the same patient.
METHODS: Two eyes of one patient with bi-allelic *RPE65* mutations have been treated with VN. The clinical examinations included visual acuity (VA, in normal and low luminance), colour vision, contrast sensitivity, International Society for Clinical Electrophysiology of Vision (ISCEV) standard retinal electrophysiology and dark-adapted full-field stimulus threshold (FST), Goldmann VF analysis and imaging studies, including optical coherence tomography (OCT) and autofluorescence. These were performed at baseline, 2-weeks, 3 and 6-months, 1 and 2-years follow-up.
RESULTS: The first eye showed improvement in rod photoreceptor function with increased peripheral and low luminance vision (baseline VA: 0.9 logMAR and 2-years post-operative VA: 0.7 logMAR). The second eye, whilst showing increased light sensitivity, suffered a drop in central vision (at 2-weeks) with loss of foveal photoreceptors as shown by the loss of ellipsoid zone on OCT scan (baseline VA: 0.6, 2-year post-operative VA: 1.2). FST improvements were maintained in both eyes indicating a sustained efficacy of VN with little waning of its effect.
CONCLUSIONS: We present a previously unreported adverse complication of subretinal VN therapy in bi-allelic RPE65, indicating a probable immune response in treatment of the second eye, resulting in loss of foveal photoreceptors. This case-series highlights the potential and pitfalls of retinal gene therapy in the same patient. The immune responses of the body to a 'foreign vector', remains a challenge.

Eye (2023) 37:1874–1877; https://doi.org/10.1038/s41433-022-02262-5

INTRODUCTION

Inherited retinal dystrophies (IRD) are a group of rare conditions characterised by severe vision loss that result from pathogenic variation in any one of more than 220 different genes [1]. One such gene is *RPE65* which encodes *all-trans* retinyl ester isomerase, an enzyme critical to the visual cycle. Biallelic pathogenic variants in *RPE65* cause a spectrum of severe rod-mediated IRDs, including Leber congenital amaurosis (LCA) type 2 and retinitis pigmentosa (RP) type 20 [2]. Commonly, patients experience nyctalopia from early childhood, and progressive loss of visual field (VF) and visual acuity (VA). LCA type 2 is more severe, patients presenting in infancy with absent fixation, nystagmus, nyctalopia and progressive severe visual loss [3]. RP type 20 is variable, usually starting later in childhood with peripheral visual loss that eventually progresses to involve central vision. Over time, all patients with RPE65 mutation-induced IRD have severe loss of light perception at any intensity along with profound reduction in navigational vision [4].

The role of gene augmentation therapy in *RPE65* mediated disease was studied in two open-label phase one studies, and one randomised controlled phase three trial [5–7]. Overall, the trials reported a consistent improvement in navigational vision, light sensitivity and visual fields. Consequently, the treatment of IRDs reached an inflection point in December 2017, when the US Food and Drug Administration (FDA) approved Voretigene Neparvovec (VN, Luxturna®, Spark Therapeutics, Philadelphia, PA) for patients with IRD caused by biallelic RPE65 mutation, the first ever FDA-approved gene therapy. VN treatment is now offered worldwide in selected ocular gene therapy centres. The real-world data that are being gathers increase our understanding of the vast potential of gene therapy whilst highlighting the associated risks in some cases. The case presented here illustrates both the benefits and pitfalls associated with ocular gene therapy in the same patient.

METHODS

Following a search on our genetic database, a 39-year-old Caucasian male with a pathogenic biallelic RPE65 mutation (Fig. 1) was identified and invited for a clinic visit to discuss the new treatment option of VN. A comprehensive ophthalmic examination was carried out and patient underwent a battery of base line investigations. Other than IRD, he had no ocular or medical history to note.

Baseline investigations

At baseline the patient's VA (under normal and low luminance), colour vision, and contrast sensitivity were tested using natural pupils. He

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Received: 11 August 2022 Revised: 13 August 2022 Accepted: 12 September 2022
Published online: 26 September 2022

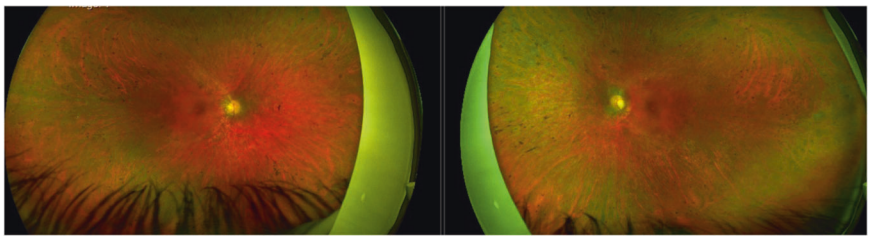

Fig. 1 Fundus picture of the patient. Retinal vascular attenuation and pigmentary changes with bone spicule-like pigment deposition.

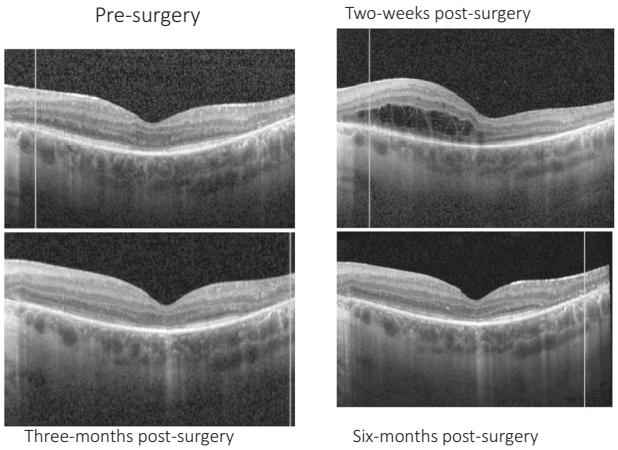

Fig. 2 OCT of the left eye. Marked cystoid macular oedema at 2 weeks which settled with oral steroids, and no change in the central ellipsoid zone as shown by subsequent scans.

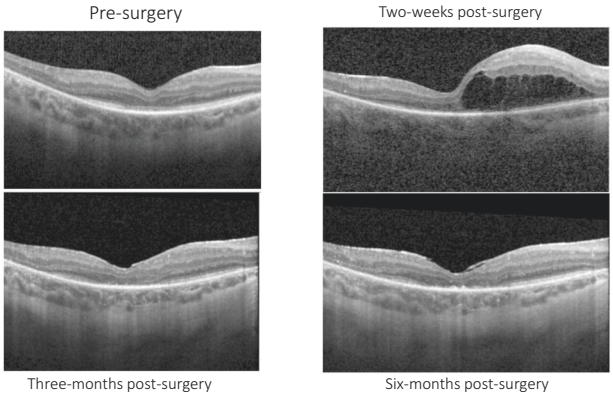

Fig. 3 OCT of the right eye. Marked cystoid macular oedema at 2 weeks which settled with oral steroids, but resulted in permanent loss of central ellipsoid zone as shown by subsequent scans.

underwent International Society for Clinical Electrophysiology of Vision standard retinal electrophysiology and dark-adapted full-field stimulus threshold (FST), Goldmann VF analysis and imaging studies, including optical coherence tomography (OCT) and autofluorescence.

There are three criteria to be met for the patient to be considered for VN treatment in the United Kingdom: [8]

1. Presence of viable retina (At least 100 microns central retinal thickness on OCT scan)
2. Three or more optic disc areas without atrophy or pigmentary degeneration within posterior pole of the retina
3. A remaining VF within 30° of fixation.

The patient met all the criteria and was offered treatment. After a detailed discussion of all the risks and benefits of VN, the patient wished to proceed, and informed consent was taken. It was decided to treat his left eye first, which had the worse VA and VF.

Surgical procedure and treatment protocol

Three days prior to administration of the gene therapy, the patient was started on oral steroids as per the protocol (prednisone at one mg/kg/day, maximum of 40 mg/day for a total of 7 days, starting 3 days before administration of Voretigene Neparvovec, followed by a tapering dose for 10 days) [7]. The surgery consisted of 25 G pars plana vitrectomy (PPV), subretinal injection of 0.3 ml of VN, fluid air exchange and closure of all surgical ports with 8/0 vicryl. There were no complications during the procedure. Following the operation, the patient was advised to maintain supine position for 24 h and was prescribed topical prednisolone 1% four times daily for 4 weeks; topical cyclopentolate 1% twice daily for 2 weeks and topical chloramphenicol 0.5% four times daily for 2 weeks (standard practise following PPV).

RESULTS

At baseline, we report, logMAR VA was 0.6 (right) and 0.9 (left). Under low photopic illumination, VA was 1.2 (right) and 1.46 (left), and he could not see the chart under mesopic illumination. Ganzfeld electroretinograms (ERGs) were extinguished in both eyes; multifocal ERGs did not show any recordable signal from any location in the central 60°. FST mean thresholds were −2.32 dB for the right and −2.29 dB for the left eye respectively. OCT and VF were also carried out to determine patient eligibility.

Early in the postoperative period, the patient started noticing increased light sensitivity and improved low luminance navigation. Two weeks following surgery, he developed left-sided cystoid macular oedema (Fig. 2), which showed little improvement with steroid eye drops but settled completely on a short 2-week course of oral steroids. VA remained at 0.8 for the left eye a month after surgery. After careful consideration, with the patient reporting marked improvement in light sensitivity and low luminance vision, it was decided to proceed with the surgery of the second eye. The procedure of the right eye was uneventful with no complications and was covered with a 17-day course of perioperative oral steroids as before.

In the early postoperative period following the second surgery, the patient again noted a significant improvement in low luminance and navigational vision. However, at 2 weeks post-surgery, he reported a sharp decrease in central vision. At this point, VA of the right eye had dropped from 0.6 preoperatively to hand movements. OCT showed significant cystoid macular oedema, worse than in the left eye. Oral prednisolone, which would have been stopped at 2 weeks post-surgery, was increased to 40 mg and continued on a tapering dose for another 4 weeks. The macular oedema of the right eye completely resolved by 6 weeks after surgery and the VA, although showing some improvement, remained considerably lower at 1.2 compared to preoperative levels of 0.6. By now, OCT showed complete loss of the ellipsoid zone (EZ) localised to the fovea, and not the entire surgical bleb size (Fig. 3). EZ is linked to photoreceptor layer integrity and is a marker of central visual function. Foveal EZ loss is a previously unreported adverse effect of gene therapy.

At the 3-month follow-up after the second eye's surgery, the patient underwent further tests. Low luminance VA showed

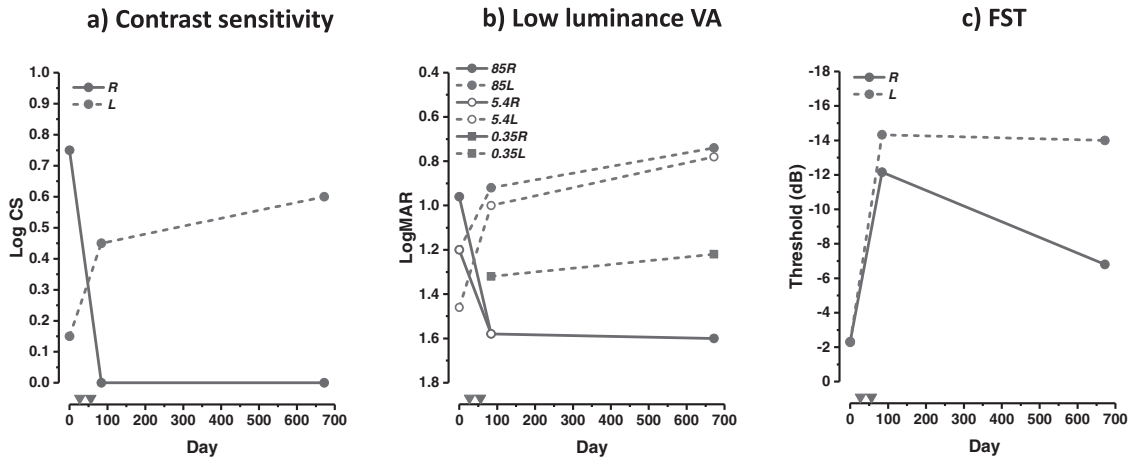

Fig. 4 Baseline and post-surgical measures of visual function. Dates of surgery are indicated with red (right eye) and green (left eye) triangles. **a** Contrast sensitivity measured using a Pelli-Robson chart at 1 m distance under photopic (85 cd m^{-2}) illumination. **b** Distance-corrected LogMAR VA measured at 1 m on a 3 m EDTRS chart under photopic (85 cd m^{-2}), low photopic (5.4 cd m^{-2}) and mesopic (0.35 cd m^{-2}) illumination using neutral density filters. Patient could not see the mesopic chart with the right eye at any time. **c** FST thresholds in dB where 0 dB = 0.01 cd s m^{-2} and 1 dB = 0.1 log units.

significant improvement in the left eye of 0.2 log units at 85 cd m^2 and by 0.45 at 5.4 cd m^2. Furthermore, the patient could now see the mesopic (0.35 cd m^{-2}) chart, with VA of 1.32. The right eye, as expected, showed reduced central acuity. FST showed marked improvement in scotopic sensitivity, by a factor of 10 (one log unit) in the right eye and by a factor of 16 (1.2 log unit) in the left eye (Fig. 2). Hence there was improvement in rod photoreceptor-mediated retinal sensitivity in both eyes, with improvement of VA in the left eye as well, but reduction in central vision in the right eye due to loss of foveal photoreceptors.

Further follow up at 6 months, 1 year and 2 years showed stable vision of 1.2 logMAR in the right and 0.7 logMAR in the left eye. FST improvements were maintained in both eyes indicating a sustained efficacy of VN with little waning of its effect (Fig. 4).

DISCUSSION

In 1972, Theodore Friedman and Richard Roblin laid the foundation of gene therapy in their ground-breaking article in the journal Science, writing: 'In our view, gene therapy may ameliorate some human genetic diseases in the future' [9]. There was slow progress in this field until 2017, when interest in gene therapy suddenly exploded, with US FDA approving three gene therapy products including VN, the first approved gene therapy treatment for patients with IRD caused by biallelic RPE65 mutation.

VN, being a subretinal gene therapy requires a surgical procedure (vitrectomy) and subretinal injection for administration. Safety and efficacy of VN and its route of administration have been studied in 40 patients in the phase 1 follow-on and phase 3 studies [10]. The safety profile of the drug was considered to be consistent with vitrectomy and the subretinal injection procedure. Twenty-seven patients (68%) had ocular treatment-emergent adverse events, mostly mild. Two serious ocular adverse events occurred resulting in irreversible visual loss, both attributable to the surgical procedure: one patient had macular atrophy and the other developed endophthalmitis and increased intraocular pressure leading to optic atrophy. Three patients (7.5%) had ocular inflammation which settled without any sequelae. There were no drug-related serious adverse events or severe immune responses.

Since the original trials, the real-world data on the efficacy and safety of VN is gradually trickling in ref. [11], and a post-authorisation multi-centre study, PERCEIVE, collecting safety data for 5 years after treatment with VN, is currently underway. In this backdrop, we present our unique case which shows the success and risks of retinal gene therapy in the same patient.

The first eye of our patient shows an excellent outcome in all visual parameters following treatment with VN. IRD due to RPE65 mutation is rod-mediated, so VA is not a true marker of disease progression. FST is a sensitive global measure of dark-adapted sensitivity to light and hence photoreceptor function. In addition, it correlates well with navigation under dim illumination [10]. There was a significant improvement in FST in the left eye in our patient by a factor of 16, which corresponded with the subjective improvement noted by the patient and was also reflected in low-luminance VA gains. Furthermore, photopic VA, which is a foveal cone-mediated function, improved by 0.2 logMAR units. This may be a secondary consequence of improved cone health due to RPE65 gene augmentation. OCT revealed mild inflammatory macular oedema 2 weeks after surgery; this spared the photoreceptor layer. This could have been a postoperative inflammatory response to the surgery, which normally develops later. As subsequent events showed, it is more likely that this was an immune response to the drug. This is in contrast to the type-1 cell-mediated effector immunity response that has been previously implicated with subretinal gene therapy in murine eyes [12], Either way, a temporary increase in oral steroids immediately settled the macular oedema and the photoreceptor layer at the fovea remained unaffected. The improvement in visual function in this eye was sustained at 2 years of follow-up, an outcome clearly indicating the vast potential of gene therapy in IRD.

Whilst progress was initially similar, the second eye followed a different trajectory. The patient noticed improvement in light sensitivity, which was picked up by the FST, but at 2 weeks post-surgery noticed a significant fall in VA. There was marked macular oedema coming up to the EZ, showing the involvement of photoreceptors. The macular oedema responded to an immediate increase in oral steroids, but by the time the retinal layers settled, there was loss of foveal photoreceptors. The fact that the macular oedema was worse in the second eye indicates previous immune sensitisation, and it was steroid-responsive, both factors pointing to an immune-mediated aetiology. This resulted in loss of central foveal function. Such a complication of VN treatment has not been reported before. Although mechanical damage to the other segment resulting from surgical retinal detachment is well recognised [13], this is typically time dependent [14] and unlikely to cause the structural changes observed on the OCT after such a

short duration of detachment necessary to administer the VN. Additionally, the loss of ellipsoid zone was localised to the fovea, rather than the whole retina that was detached in the surgical bleb.

Severe immune responses have always been a danger with retinal gene therapy and thus the original trials used a perioperative immunomodulatory regimen. The vector was optimised to remove the empty capsids from the final product [7]. The surgical technique which we used was completely according to protocol and had innovations to reduce the risk of vector-related adverse events. VN was administered subretinally through a single 41-gauge retinotomy to reduce the egress of the drug into the vitreous cavity, a further vitrectomy was done after drug administration to remove escaped capsids, and all vitreous fluid was removed at the end by carrying out a fluid-air exchange. Despite all this, loss of foveal photoreceptors due to a presumed immune response was detected. One option in such cases may be the use of increased immunomodulation for the second eye by a higher and more prolonged course of oral steroids if an inflammatory response is seen in the first eye.

Our case is unique in that it highlights the potential and pitfalls of retinal gene therapy in the same patient. The future is promising but challenges remain, especially the immune responses of the body to a 'foreign vector'. As further evidence comes in and more gene-based therapies become available, we will find better and safer ways to deliver gene therapy, whilst minimising the associated risks, in our pursuit of finding the ultimate cure for hereditary diseases.

Summary

What was known before

- Immune response to the body as a 'foreign vector', is a significant challenge in gene therapy.
- Voretigene neparvovec (VN) is a gene therapeutic agent for treatment of retinal dystrophies caused by bi-allelic *RPE65* mutations.

What this study adds

- We present a previously unreported adverse complication of subretinal VN therapy in bi-allelic RPE65, indicating a probable immune response in treatment of the second eye, resulting in loss of foveal photoreceptors.
- This case highlights the benefit and pitfalls of retinal gene therapy in the same patient.

DATA AVAILABILITY

The raw data are available upon reasonable request.

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AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS STATEMENT

As this was a retrospective anonymized study, as per our local protocol from our Clinical Effectiveness Department, and as per national guidelines from the National Code of Clinical Research, and the Health Research Authority (HRA), this study has ethical approval exemption and no patient consent was required for participation. All procedures were completed prior to the design of this study. Patients were diagnosed and treated according to local guidelines and agreements and written consent from patients was acquired prior to all procedures as clinically indicated. This study does not report on the use of new or experimental protocols.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Assad Jalil.