Short term morphological rescue of the fovea after gene therapy with voretigene neparvovec

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ABSTRACT.
Purpose: Leber congenital amaurosis type 2 (LCA2) and early-onset severe retinal dystrophy (EOSRD) are linked to visual impairment with nyctalopia and visual acuity reduction in early childhood. In 2017, the first gene therapy voretigene neparvovec (Luxturna™) for patients with LCA and EOSRD caused by bi-allelic mutations in the RPE65 gene has been approved. Here we report on an example of short-term change in the foveal morphology after functionally successful gene therapy with voretigene neparvovec in a 15-year-old patient.

Methods: The clinical examinations included best corrected visual acuity (BCVA), spectral domain optical coherence tomography (OCT) and adaptive optics retinal imaging.

Results: During follow-up over a period of 3 months after the treatment, an improvement of the central foveal morphology could be observed in OCT, with a clear demarcation of the external limiting membrane and changes in the photoreceptor mosaic on adaptive optics retinal imaging. These morphological rescue parameters correlated in part with the improvement in foveal-mediated vision after the treatment and adaptive optics imaging. Although the visual acuity improved only slightly at month 3, objective central cone evaluation with chromatic pupil campimetry showed an increase in the central sensitivity. In daily life, the patient reported her visual experience after the treatment as ‘brighter’.

Conclusion: Rapid changes in the correlates of photoreceptor morphology after successful gene therapy in patients with LCA/EORD can be quantifiable on individual level.

Key words: case report – gene therapy – OCT – retinitis pigmentosa – RPE65 – voretigene neparvovec

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Background

One of the genes associated with Leber congenital amaurosis (LCA) or early-onset severe retinal dystrophy (EOSRD) is RPE65, causing disease in cases of bi-allelic pathogenic variants. Mutations in RPE65 are thought to be responsible for 5–6% of all cases of EOSRD (Cideciyan, 2010). A recent study by Hanany et al. predicts almost 16 000 patients worldwide with bi-allelic RPE65 mutations. Of these, 60% are estimated to be from the African population, while only 9% have a European heritage (Hanany et al., 2020).

The typical visual impairment normally presents between birth and the age of 5 years with nyctalopia, visual acuity and visual field reduction, and sometimes nystagmus. During adolescence and the following decades the visual acuity tends to decline further and by the fourth decade all patients are normally considered legally blind (Chao et al., 2019).

In December 2017, the U.S. Food and Drug Administration approved the first gene therapy product, voretigene neparvovec-rzyl (Luxturna™) for inherited retinal diseases (IRD), an adeno-associated virus type 2 vector designed to deliver a normal copy of the gene for treating with confirmed bi-allelic RPE65-mediated LCA (Hussain et al., 2019). In Europe, the medication was approved for treatment in November 2018. Clinical trials before and after approval show that the
degeneration process can be slowed down by this gene supplementation therapy in treated patients, but also a functional improvement especially in young patients is possible (Jacobson et al., 2012; Testa et al., 2013; Stingl et al., 2021).

Here we report on the short-term morphological changes in the fovea demonstrated on high-resolution imaging over 3 months after intervention in a 15-year-old patient who was treated on the right eye with voretigene neparvovec. This subject is shown as an example as she showed an impressive functional improvement in the rod and cone function. The aim of this manuscript was to explore morphological changes in the photoreceptors and dynamics of these changes in the short period after intervention.

**Patient and Methods**

A female 15-year-old patient with EOSRD due to bi-allelic RPE65 mutations was treated on her right eye by subretinal application of voretigene neparvovec. She first presented in the Center for Ophthalmology, University of Tübingen at the age of 6 years with low vision and with an older sister affected by the same clinical diagnosis. Genetic testing identified a pathogenic homozygous c.1102T>C mutation (p.Tyr368His) in the RPE65 gene in both sisters. Both parents were shown to be heterozygous carriers, confirming an autosomal recessive mode of inheritance and the true homozygous bi-allelic nature of the mutation in the siblings.

The treatment with subretinal application of voretigene neparvovec on her right eye was performed via 23-gauge pars plana vitrectomy (vitrectomy device: Qube® pro, Ruck Ophthalmologische Systeme GmbH, Eschweiler, Germany, microscope: Zeiss, Jena, Germany). First a core vitrectomy was performed and the hyaloid was lifted. A canula was inserted temporal superiorly to the fovea. Due to a non-elevation of the macula another retinotomy was performed nasal superiorly of the fovea and 0.3 ml of voretigene neparvovec was injected subretinally. Then the resulting bleb elevated the macula which was monitored by simultaneous intraoperative OCT scanning.

The patient was examined before the surgery and at follow-up exams after 2 weeks (W2), 5 weeks (W5) and 3 months (M3). The patient was an excellent responder to the gene therapy showing a clear rescue of rod function as measured by dark adapted perimetry with an average dark adapted improvement of the macular sensitivity of 28 dB since 1 month after treatment.

The clinical examinations presented in this manuscript included best corrected visual acuity (BCVA) testing using ETDRS charts, spectral domain optical coherence tomography (OCT) with the Spectralis® OCT (Heidelberg Engineering GmbH, Heidelberg, Germany) and contrast sensitivity was assessed with Pelli-Robson charts.

For the OCT examinations, foveal horizontal and vertical B-scans as well as volume scans (15 × 15 degrees, ART 30, 145 scans) were recorded at baseline and at the follow-up visits.

To test the objective cone function in the macula, chromatic pupil campimetry (CPC) with a defined cone specific protocol was performed (Kelbsch et al., 2019).

High-resolution retinal imaging was performed using the adaptive optics flood illumination retinal camera rtx1™ system (Imagine Eyes, Orsay, France). A protocol of 17 single images (each 4 degrees × 4 degrees) were collected and merged into a montage that covered the central retinal pole (approximately 13 degrees × 13 degrees). Furthermore, four areas of the central fundus were selected and analyzed. The locations are depicted in Fig. 1: (1) a frame closest to the injection site (white square), (2) an adjacent location (light blue square), (3) the central area including the fovea green square) and a juxtafoveal central area that was not treated by the injected fluid intraoperatively (red square).

**Results**

The morphological findings on OCT showed ring-shaped pericentral atrophy around the fovea at baseline. The overall retinal thickness was decreased compared to healthy individuals of the same age. After the treatment the central retinal thickness did not change significantly (central ring in OCT thickness map Baseline: 171 μm; W2: 164 μm; W5: 164 μm; M3: 165 μm).

The central fovea had an altered layering with hyperreflective material at baseline. Centrally, the external limiting membrane (ELM) was not definable and the ellipsoid zone as the representative junction of the inner and outer segments of the photoreceptors was disrupted. The myoid zone was not identifiable nor was a clear distinction possible between the ganglion cell layer, inner nuclear layer (INL) and outer plexiform layer (OPL). In contrary, the parafoveal retina showed clear layering with conservation of the outer retina and photoreceptors at baseline (see Fig. 1).

Already 2 weeks after the treatment the forming of the foveal layering was apparent; the external limiting membrane (ELM) was visible in the fovea with a length of about 880 μm. The overall appearance ameliorated. Five weeks after surgery the layering of the central fovea improved further. The ELM was 1100 μm, after 3 months 1050 μm (see Fig. 1 red line in OCT images). The more homogenous layering was conserved until month three. For the other layers mentioned above no clear change over time could be measured or established.

Adaptive optics (AO) imaging allowed a visualization of the cone photoreceptor mosaic at the central macula to address the changes over time. Unsteady fixation limited the image quality. The fovea was identified by overlay of the AO images and infrared images and OCT scans, marked by the white asterix in the Fig. 1. At the baseline examination all AO frames showed an altered, ‘disrupted’ mosaic of cone photoreceptor cells which is typical for IRD. All frames displayed a different pattern depending on the localization. Dark patchy areas of atrophic retina were apparent in all frames.

The white frame (Fig. 1), which is located the closest to the injection site, had an indistinguishable photoreceptor mosaic at baseline, without obvious cones. After the treatment in the upper nasal part the image seemed to be partly more distinguishable. Single hyperreflective points representing cones could be identified (white frames W2, W5 and M3) and patchy dark areas seemed to be decreased or more defined. Adjected to the white frame, the light blue frame covers the upper part of the central macula. Here, over time the appearance did not seem to have changed much. The green frame...
shows the central macula where the OCT scan is crossing. At baseline an indistinguishable image was visible. At W2 no remarkable changes were visible. However, at W5 there seemed to be a more defined, beginning pattern of the cone mosaic in the central area. Single white points representing cones were detectable (Fig 1, green frames in W2, W5, M3). This correlated with the improvement of the cone morphology in OCT images at this area, although at M3 the image was indistinguishable again.

For the red frame, the structure remained similar over time. Dark patchy areas dominated all frames at all time points with some cone mosaic visible. Overall, postoperatively on the reconstructed AO montage, a slightly more defined mosaic was gradually visible. As postoperative inflammations can occur after gene therapy with adeno-associated virus (AAV) vectors it is important to mention that there were no giant cells which would represent possible signs of inflammation evident in the presented images.

The functionality of the partially restored cones improved to a certain extent. Already after 2 weeks the patient reported to see brighter in her daily life, which she reconfirmed 5 weeks after surgery. After 3 months the subjective changes persisted. Visual acuity on the right eye was 20/63 before surgery and remained stable 2 and 5 weeks after treatment. After 3 months the visual acuity had improved by one line to 20/50.

No difference was found for contrast sensitivity in the Pelli-Robson chart before and after surgery. At baseline examination the patient exhibited a discrete horizontal nystagmus, that was present also after surgery at W2, W5 and M3.

The CPC readouts showed a relative pupil response to the cone stimuli of 5.5% at baseline (averaged value of the macular area), with 4.3% at W2, and with an improvement up to 9.6% at W5 and 8.9% at M3. The functional maps of the macula (15 degrees of visual field) are shown in the Fig. 2.

**Discussion**

This manuscript demonstrates an example of short-term morphological changes at the fovea after treatment with voretigene neaparvovec in a 15-year-old patient. This case demonstrates a remarkable fast restoration of the central photoreceptors within only 5 weeks after treatment as documented by high-resolution imaging. The effect was still visible after 3 months.

The morphological improvement of the degenerated central photoreceptors was accompanied with some improvements of the cone function measured...
with static perimetry as well as CPC with a cone specific protocol. After 3 months our patient’s visual acuity improved by one line. Contrary to this, the contrast sensitivity remained stable over the observed time period. These results indicate a beginning improvement of the foveal photoreceptor function following short-term morphological rescue in the fovea within 2 weeks after therapy. As can be seen in the OCT images, a full recovery of the foveal cones was not observed over this short time period. Whether in a long-term the inner and outer segment restoration will continue, enabling an even bigger functional improvement remains to be elucidated. Such a foveal recovery over few weeks has not yet been published in the literature so far yet. Still, it is possible, that the functional improvement may follow the morphological rescue with some time delay, as might be indicated by the visual acuity rise only at month three.

Functional improvements after RPE65 gene supplementation therapy were reported frequently before the commercial approval, but also thereafter (Stingl et al., 2021). The phase-3 trial by Russell et al. showed improvements in both navigational abilities and light sensitivity within the first 30 days after subretinal delivery and they remained stable for 1 year [Russell, 2017 #33]. In a 3-years follow-up study of treatment Testa et al concluded that an enlargement of visual field areas was only observed starting from day 60. Yet, there was also some inter-visit variability detected (Testa et al., 2013). Pennesi et al reported that after 5 years pediatric subjects had improvement in visual acuity and static perimetry in the treated eye during the first 2 years of the study. The improvement persisted during the 3–5 years investigated (Pennesi et al., 2018). Of importance are also results of short-term functional improvements of the cone and rod system, measurable in a retinotopic way in patients after therapy as reported recently (Stingl et al., 2021).

Optical coherence tomography (OCT) findings in RPE65-associated EOSRD vary considerably. Until today, although many pathogenic variants have described, no clear genotype-phenotype correlations could be demonstrated so far (Chung et al., 2019). Moreover, the severity of RPE65-associated LCA is not dependent on a certain location or types of RPE65 pathogenic variants (Katagiri et al., 2016). Sometimes a preserved central foveal region with thinning of the ONL that surrounds the fovea can be observed (Jacobson et al., 2009). In general, structural changes in RP patients normally affect the inner segment ellipsoid, OPL and ONL (Hamada et al., 2000; Hood et al., 2009). At the age of 15, our patient had pronounced changes in the central macula. Optical coherence tomography (OCT) scans before treatment showed central disruption of the ellipsoid layer. In autofluorescence a ring-shaped atrophy was visible corresponding to the findings described by Jacobson et al. (2009). After surgery, the ring-shaped appearance showed no considerable change over 5 weeks. Postoperatively, already 2 weeks and even more pronounced 5 weeks after the intervention with subretinal treatment with voretigene neparvovec, there was a considerable amelioration of the layering adjacent to the outer nuclear membrane in the OCT scan. We concluded that the regeneration of photoreceptors led the restoring of inner and outer photoreceptor segments and therefore a visible external limiting membrane in the OCT. The reflectivity of this layer can be well compared to the neighboring healthy parts. Furthermore, one can hypnotize if the photoreceptors were restored as in a normalization of the still existing photoreceptors or if there was a re-growth of photoreceptors.

The integrity of the photoreceptor inner/outer segment junction was identified in studies as an important indicator of visual outcomes such as in macular hole and membrane surgery. The photoreceptor inner/outer segment junction is therefore a representative of the health of the outer retina (Baba et al., 2008; Mitamura et al., 2009; Tao et al., 2016). The visual improvement after 3 months can be probably attributed to this gain of photoreceptor integrity.

The adaptive optics technique allows researchers to obtain microstructural images of the central retina in with specifics in various eye diseases. Adaptive optics (AO) has been investigated for retinitis pigmentosa. Cone photoreceptors and cone density are known to be diminished for patients with IRD but normal central visual acuity and preserved interdigitation zone (EZ/IZ) (Nakatake et al., 2019). Moreover, the regularity of the cone mosaic spatial arrangement is normally disrupted even if the foveal sensitivity is still good. Therefore, it is a sensitive modality to detect photoreceptor changes over time even if visual acuity.

| Visual acuity | Baseline | W2 | W5 | M3 |
|---------------|----------|----|----|----|
| 20/63         | 20/63    | 20/63 | 20/50 |
| Pelli-Robson  | 1.05     | 1.05 | 1.05 | 1.05 |

Fig. 2. Visual acuity, Pelli-Robson contrast sensitivity and chromatic pupil campimetry (CPC) with the cone protocol examination at baseline, week 2 (W2), week 5 (W5) and month 3 (M3).
does not change much as it did in our patient (Makiyama et al., 2013).

The images can be interpreted by overlaying of the obtained equivalents of different imaging modalities to correlate the ellipsoid zone to photoreceptor integrity. Makiyama et al. found that a greater decrease in cone density was related to a disruption of the photoreceptor inner segment (IS) ellipsoid band on SD-OCT images. Decreased cone density correlated to thinner outer nuclear layer and thinner inner segment and outer segment thickness on SD-OCT (Makiyama et al., 2013). In our patient at baseline the outer segment layers were not really distinguishable. The thickness was reduced compared to healthy individuals of the same age. However, over time the overall thickness had not changed significantly. As displayed, in the very bold blue frame (Fig. 1) cone photoreceptors seemed to reappear in the central foveal area as did the ellipsoid layer on OCT imaging.

Over time there was a change of the cone mosaic in the white, light and bold blue frames of the central macular region. Overall, the macula seemed a bit more ‘organized’ and the darker areas seemed to retrace a bit in the white frame. It is known that the dark patchy areas correlate with missing cones and thus are a sign for atrophy.

Unfortunately, adaptive optics is prone to blurred imaging due to media opacities, eccentric or malfixation and eye movements. We observed a change in fixation while constructing the AO montage. This limits the interpretation of the gained images mainly in month three.

Chromatic pupil campimetry (CPC) can be considered a rather objective examination and showed a considerable improvement postoperatively for the cone function. We show an enhanced sensitivity centrally. These findings were in keeping with the overall macula improvement after surgery and correspond to the results gained in perimetry, OCT and adaptive optics imaging. A current report from the authors group reported recently on this objective functional diagnostic results in seven eyes after therapy with voretigene neparvovec at 1 month and 3 months (Stingl et al., 2021). Long-term results for these functional tests are not yet available.

The possibility of treatment with voretigene neparvovec is a substantial advance in gene therapy in inherited eye diseases. This case report demonstrates that after the therapy with this agent, young patients can show morphological rescue; already at 2 weeks foveal photoreceptor layers start to reappear, and at 5 weeks the patient showed improvement in multiple outcome measurements. As age-related photoreceptor rescue has already been reported for RPE65 gene therapy (Maguire et al., 2008) (Stingl et al., 2021), we conclude that the age of just 15 years and residual central functioning photoreceptor cells was a major criterion for the reported treatment success. This is in keeping with observational studies of IRD with RPE65 alterations, that demonstrated a significant relationship between age and worsening of visual acuity and Goldmann visual fields (Chung et al., 2019) and the treatment outcomes of pediatric individuals by Pennesi after 5 years. Most adult patients had no steady changes in visual acuity or static perimetry. Pennesi et al. reported on only three adults that had improvement during the first 1–2 years after treatment due to markedly abnormal baseline kinetic visual field area. Yet, the improvement did not last to successive follow-up visits (Pennesi et al., 2018). There is still some uncertainty on whether effects can improve or persist long term.

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