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Authors

Ku, Cristy
Igelman, Austin
Huang, Samuel
et al.

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Perimacular Atrophy Following Voretigene Neparvovec-Rzyl Treatment in the Setting of Previous Contralateral Eye Treatment With a Different Viral Vector

Cristy A. Ku[1], Austin D. Igelman[2], Samuel J. Huang[2], Steven T. Bailey[2], Andreas K. Lauer[2], Jacque L. Duncan[3], Richard G. Weleber[2], Paul Yang[2], and Mark E. Pennesi[2]

[1] Department of Ophthalmology, Baylor College of Medicine, Houston, TX, USA
[2] Casey Eye Institute, Oregon Health & Science University, Portland, OR, USA
[3] Department of Ophthalmology, University of California San Francisco, San Francisco, CA, USA

Correspondence: Mark E. Pennesi, Casey Eye Institute, Oregon Health & Science University, 545 SW Campus Drive, Portland, OR 97239, USA.
e-mail: pennesi@retinafoundation.org

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Purpose: To report on cases of unilateral perimacular atrophy after treatment with voretigene neparvovec-rzyl, in the setting of previous contralateral eye treatment with a different viral vector.

Design: Single-center, retrospective chart review.

Methods: In this case series, four patients between the ages of six and 11 years old with *RPE65*-related retinopathy were treated unilaterally with rAAV2-CB-hRPE65 as part of a gene augmentation clinical trial (NCT00749957). Six to 10 years later the contralateral eyes were treated with the Food and Drug Administration–approved drug, voretigene neparvovec-rzyl. Best-corrected visual acuity (BCVA), fundus photos, ocular coherence tomography, two-color dark-adapted perimetry, full field stimulus threshold testing (FST), and location of subretinal bleb and chorioretinal atrophy were evaluated.

Results: Three out of four patients showed unilateral perimacular atrophy after treatment with voretigene, ranging from five to 22 months after treatment. Areas of robust visual field improvement were followed by areas of chorioretinal atrophy. Despite perimacular changes, BCVA, FST, and subjective improvements in vision and nyctalopia were maintained. Perimacular atrophy was not observed in the first eye treated with the previous viral vector.

Conclusions: We observed areas of robust visual field improvement followed by perimacular atrophy in voretigene treated eyes, as compared to the initially treated contralateral eyes.

Translational Relevance: Caution is advised when using two different viral vectors between eyes in gene therapy. This may become an important issue in the future with increasing gene therapy clinical trials for inherited retinal dystrophies.

Introduction

RPE65 encodes for an isomerase involved in regeneration of the essential visual pigment, 11-*cis*-retinal.[1] Defects in RPE65 function result in *RPE65*-related retinal dystrophy, which presents with a spectrum of severity. The most severe cases, which present in the first year of life with nystagmus, sluggish pupils, nyctalopia and decreased vision, are characterized as Leber congenital amaurosis. Less severe cases presenting from one to five years are often termed *early severe-onset retinal dystrophy*, whereas milder hypomorphic mutations can present as retinitis pigmentosa. Autosomal dominant retinitis pigmentosa with choroidal involvement (RP87, OMIM 618697) has been observed in patients heterozygous for a c.1430G-A transition (NM_000329) in the RPE65 gene.

RPE65 gene augmentation clinical trials culminated in the Food and Drug Administration (FDA) approval of voretigene neparvovec-rzyl (Luxturna; Spark Therapeutics, Philadelphia, PA, USA) as the first ocular gene therapy to treat *RPE65*-related retinopathy. As more patients have now been treated, reports of perifoveal chorioretinal atrophy have emerged.[2–4] The incidence of chorioretinal atrophy has been estimated to occur in 13% of patients in one post-authorization safety study,[5] whereas another reported it to be as high as 28%.[6] Mechanisms of chorioretinal atrophy have been potentially attributed to factors including vector toxicity, protein overexpression, inflammatory or immune response to treatment, or surgical related factors such as needle touchdown site, high delivery injection pressure or rate, and mechanical trauma from retinal detachment.[2–4]

Before the FDA approval of voretigene neparvovec (VN), there were three independent clinical trials in the United States and the United Kingdom that evaluated the efficacy of *RPE65* gene augmentation with independently designed and produced viral vectors (NCT#00749957, NCT#00481546, NCT#00516477, NCT#00643747).[7–10] Although a few incidents of chorioretinal atrophy were reported in clinical trials, increasing post-market reports of this ocular adverse event have continued to emerge.[3–6,11,12] We sought to assess a unique cohort of four patients who had previously received subretinal gene augmentation with *RPE65* as a part of a clinical trial (NCT#00749957), followed by treatment of the contralateral eye with the FDA-approved voretigene. We observed chorioretinal atrophy specifically in voretigene-treated eyes five to 22 months after treatment in all four patients, whereas atrophy was not observed in the previously treated eyes since treatment six to 11 years ago.

Interestingly, we observed atrophy in areas that showed significantly improved photoreceptor function on static perimetry, which was followed by a focal area of decreased photoreceptor sensitivity. Similar to previous reports, eyes with chorioretinal atrophy did not show any decrement to visual acuity, and all eyes showed improvements in full field stimulus threshold (FST) and perimetry as compared to baseline.

Methods

Subjects and Viral Vectors

A single-center retrospective chart review was performed on patients who had first received *RPE65*-gene augmentation as part of a clinical trial (NCT#00749957), followed by treatment with VN after its FDA approval. This study was approved by the Institutional Review Board of Oregon Health & Science University and met the tenets of the Declaration of Helsinki. All subjects provided written informed consent prior to completing any study procedures.

Patients received the viral vector, rAAV2-CB-hRPE65, between the ages of six to 11 years old (yo) as a part of a Phase I/II study (NCT#00749957) with dosing and delivery as previously described.[10,13] In brief, patients received either 1.8×10^{11} or 6×10^{11} vector genomes in a volume of 450 uL into their worst-seeing eye. This viral vector was developed by the company Applied Genetic Technologies Corporation (AGTC; Alachua, FL, USA) at that time and may be referred to as the AGTC vector.

After FDA approval of VN, patients subsequently received VN (AAV2-hRPE65v2) with 1.5×10^{11} vector genomes in a volume of up to 300 uL in their contralateral eye, between the ages of 12–21 years old or six to 10 years after their first treatment with the AGTC vector. Patients were counseled about the unknown risk of treating a second eye with a different vector and agreed to proceed. For the delivery of VN, a standard vitrectomy was performed and a 41 gauge needle connected to the MedOne Microdose syringe (MedOne Surgical, Inc, Sarasota, FL, USA) was used first to create a saline pre-bleb and then deliver voretigene subretinally. Live intraoperative OCT (ReSCAN 700; Carl Zeiss Meditec, Jena, Germany) was used to observe bleb formation and ensure injection into the proper space. Methodology and important considerations in subretinal injections are described in greater detail in Scruggs et al.[14] Patients were assessed with best-corrected visual acuity (BCVA), fundus photography, FST, and two-color dark-adapted static perimetry.

Visual Fields

Two-color dark-adapted perimetry was performed using a modified Octopus 900 Perimeter (Haag-Streit, Köniz, Switzerland) using 500 nm (cyan) and 650 nm (red) filters. Light baffling was used on the perimeter and in the testing room to prevent ambient light escape. Stray light was measured with a IL-1700 that measured ambient light at 1.54^{-4} cd/m^2. We used a 78-point grid that was evenly spaced with 11 points horizontal and eight points vertical at the meridians with size V Goldmann targets and a 4-2-1 testing strategy. The maximum luminance (i.e., the 0 dB luminance) values were 174 cd/m^2 and 63.5 cd/m^2 for the cyan and red stimuli, respectively. Patients underwent a 60-minute dark adaptation, and testing was performed

with the cyan followed by the red stimulus. The mean sensitivity represents an average of the 78 points and recorded in decibels of attenuation (dB). Hill of vision analysis was performed using Visual Field Modeling and Analysis (VFMA) software developed by Weleber et al.[15] Hill of vision volumetric analyses determined for the central 30° (V_{30}) and were reported in units of decibel-steradians (dB-sr).

FST

FST was performed using a Diagnosys Epsion system with the ColorDome stimulator (Diagnosys LLC, Lowell, MA, USA). Patients' eyes were dilated by topical tropicamide 1% and phenylephrine hydrochloride 2.5% and dark-adapted for 45 minutes. Eyes were tested monocularly with patching of the other eye during testing. The starting 0 dB luminance was 0.1 cd · s/m^2 (25 cd/m^2 presented for 4 ms) with 2500 ms response time. The range of luminance available for the test ranged from −75 dB to 15 dB. The tester defined a starting value to initiate testing, and the proprietary program used a forced-choice testing strategy to test within a range of 10 dB around the starting value. Testing was performed with blue (448 nm), red (627 nm), and white stimuli. A meaningful change in FST has been reported as 10 db or 1 log cd/m^2, with test-retest variability as 0.3 log cd/m^2.[16,17]

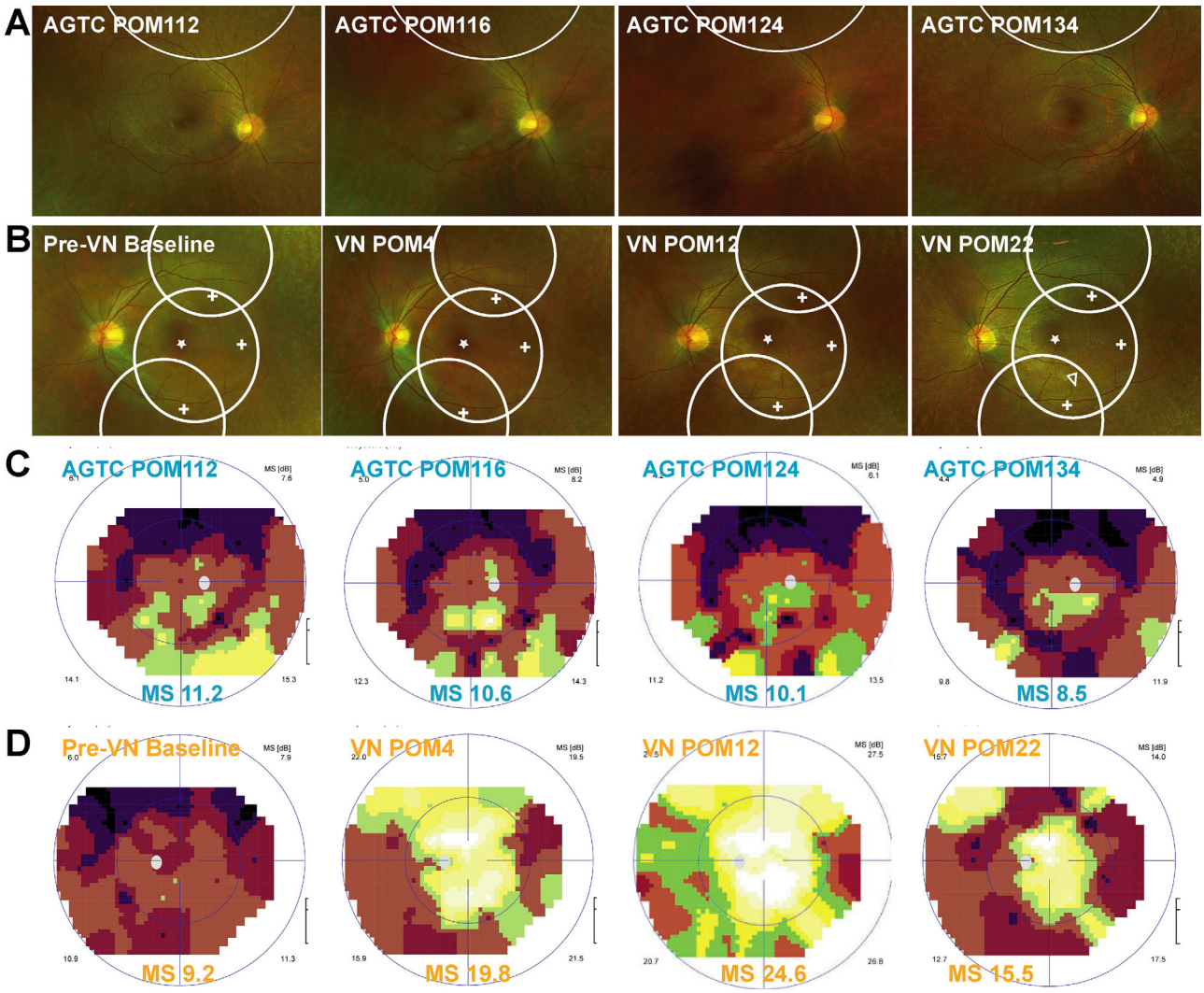

Figure 1. Fundus photos and dark-adapted perimetry of patient 1. (**A**, **B**) Widefield fundus photos of both eyes taken before voretigene treatment and at four, 12, and 22 months after voretigene treatment in the left eye. In the right eye, this was over nine years, or 112, 116, 124, and 134 months after treatment as part of a clinical trial (AGTC). *Arrowheads* show areas of chorioretinal atrophy, *star* indicates foveal center, and *plus signs* indicates the three retinotomy sites. (**C**, **D**) Dark-adapted perimetry to the cyan (500 nm) stimuli in the (**C**) right, and (**D**) left eye are shown.

Results

Three out of four eyes treated with VN developed chorioretinal atrophy. The mean age was 7.25 yo (range 6–16 yo) at time of initial treatment in a clinical trial, and 16.5 yo (range 12–21 yo) at time of VN treatment. Mean logarithm of the minimum angle of resolution at baseline was 0.5 (range 0.3–0.69), and improved to 0.37 at last visit (range 0.1–0.5). Chorioretinal atrophy was identified on average 14.25 months after VN treatment (range 5–22 mos).

In patient 1, the right eye was treated at 6 yo with the AGTC vector. The subretinal bleb was created superiorly and did not involve the macula. There were no areas of chorioretinal atrophy believed to be related to the subretinal bleb, although the patient had a small area of chorioretinal atrophy in the inferotemporal midperiphery that was likely related to disease progression (not shown). Ten years later at age 16 yo, the patient returned for consideration of treatment with VN in the left eye. Baseline visual fields at that time showed that the previously treated right eye had greater sensitivity than the left eye (Figs. 1C, 1D). The left eye was then treated with VN with three subretinal blebs involving the macula and fovea. At postoperative month 4, the VN-treated eye improved to double the sensitivity compared to its baseline and now showed greater visual field response compared to the previously treated right eye. At last follow-up 22 months after surgery, there was a subtle area of perimacular atrophy along the inferotemporal arcade (Fig. 1, *arrowhead*) that was seen best on OCT (see Fig. 2). When visual fields are oriented to the fundus view, the patch of atrophy is noted to occur in an area of great improvement in photoreceptor sensitivity at postoperative month 12, which subsequently declined at postoperative month 22 and stabilized at postoperative month 34 (see Fig. 2).

At 11 yo, the left eye of patient 2 was treated with the AGTC vector in the superotemporal periphery not involving the macula. At 21 yo, the patient received subretinal VN in the right eye in the inferotemporal macula involving the fovea. At baseline prior to VN treatment, the right eye demonstrated a few patches of chorioretinal atrophy in the temporal midperiphery likely related to disease progression. At 18 months after VN treatment, there were increasing patches of atrophy in the temporal midperiphery and numerous patches along the inferotemporal midperiphery of the VN treated eye, but little change in the chorioretinal atrophy of the previously treated left eye. (Fig. 3, *arrowheads*). Additionally, a large patch of atrophy developed at POM18 along the inferior

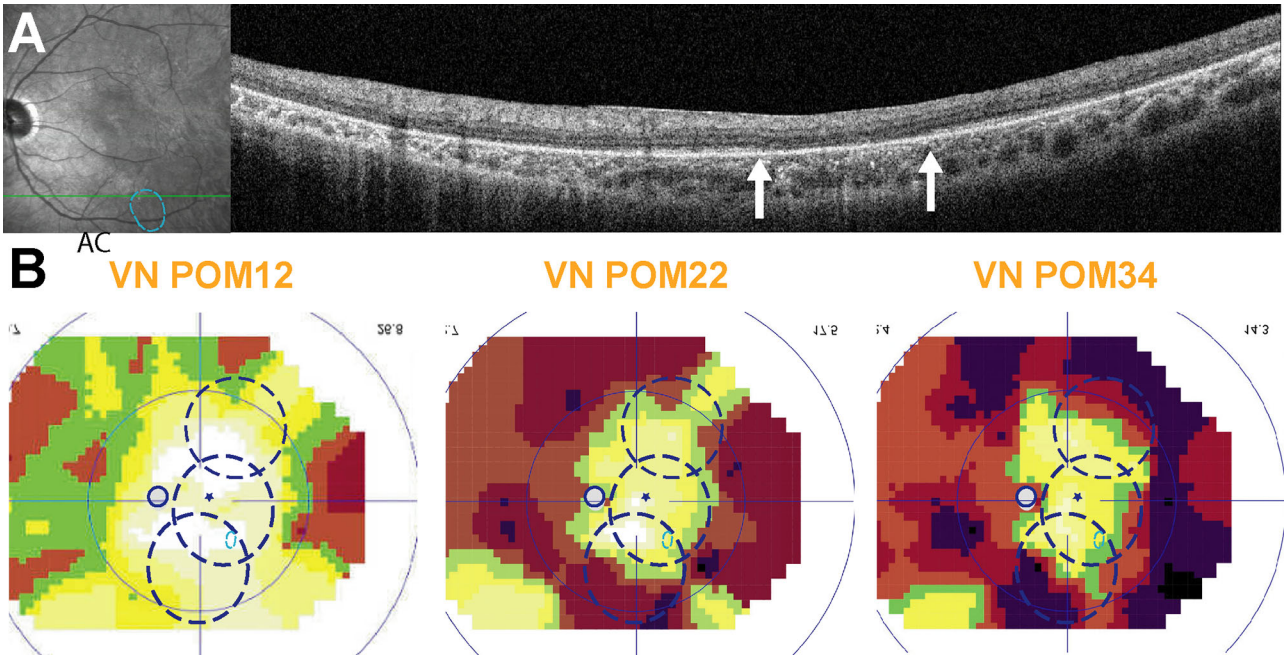

Figure 2. (**A**) An area of atrophy was first observed in patient 1 at POM22 (*light blue shape with dashed lines*) near the inferior temporal arcade on B-scan OCT. (**B**) Visual fields of patient 1 (AC) are shown in the orientation of the fundus photo at POM12, 22, and 34, demonstrating that the area of atrophy corresponded to a focal area of improved sensitivity at POM12, followed by a decrease in sensitivity at POM22 there. The area stabilized at POM34. Subretinal blebs are noted with *navy blue circles with dashed lines*, foveal center as *blue stars*, and the optic nerve with a *solid navy blue circle*.

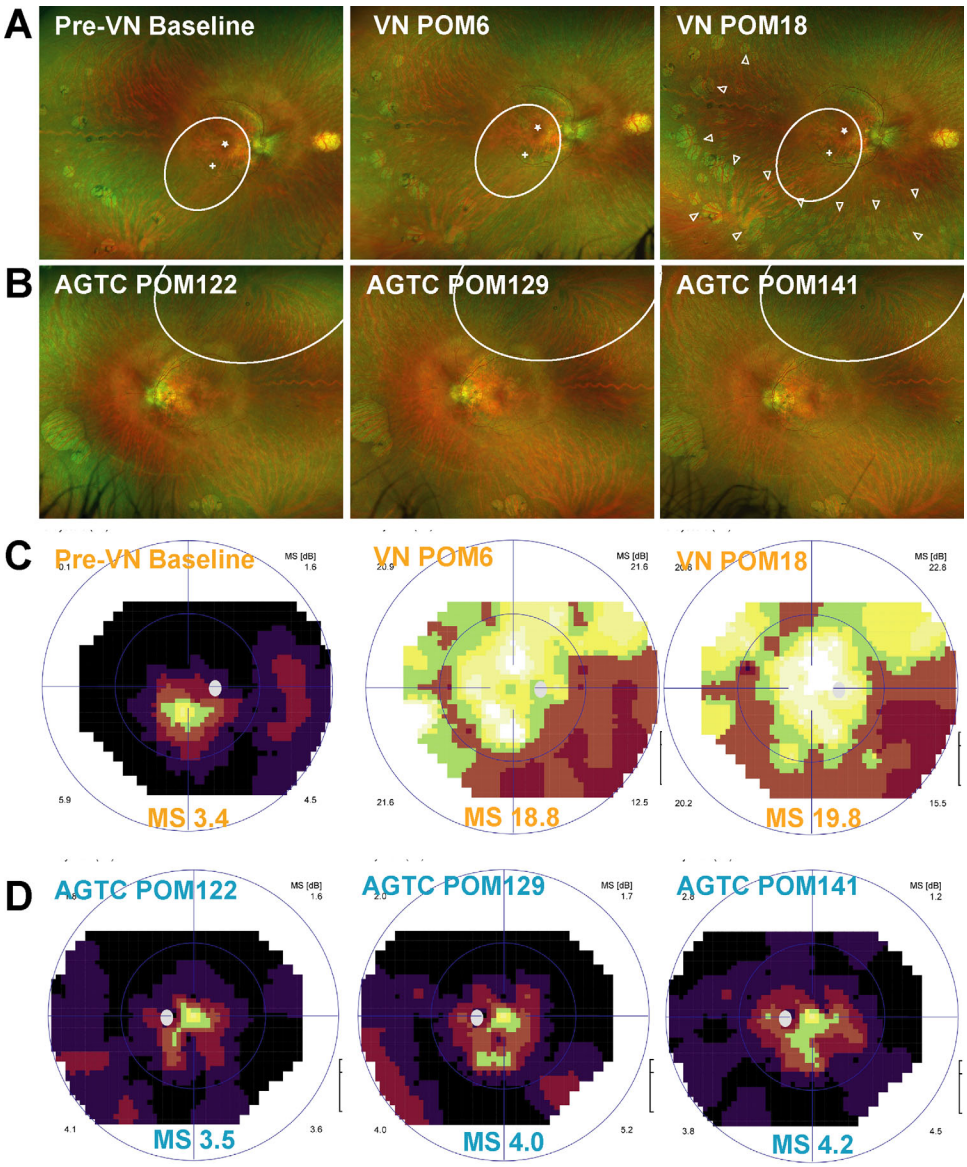

Figure 3. Fundus photos and dark-adapted perimetry of patient 2. (**A**, **B**) Wide-field fundus photos of both eyes taken at screening before voretigene treatment, and at six and 18 months after voretigene treatment. In the left eye, this was over 10 years, or 122, 129 and 141 months after treatment as part of a clinical trial (AGTC). Before VN treatment, there were a few areas of chorioretinal atrophy in the temporal midperiphery. However, at POM18, significantly more areas developed in the inferior and inferotemporal midperiphery. *Arrowheads* show areas of chorioretinal atrophy, *star* indicates foveal center, and the *plus sign* indicates the retinotomy site. (**C**, **D**) Dark-adapted perimetry to the cyan (500 nm) stimuli in the (**C**) right, and (**D**) left eye are shown.

macula, best observed on fundus autofluorescence (Fig. 4A). Despite patches of emerging atrophy, visual field sensitivity overall continued to improve at POM18 from POM6, particularly in the inferior macula (see Fig. 4). Unfortunately, the patient was lost to follow-up, and the visual field sensitivity in these areas of atrophy could not be further monitored.

The right eye of patient 3 was treated at 6 yo with rAAV2-CB-hRPE65 in the superotemporal midperiphery with involvement of the macula and fovea. There was no evidence of perifoveal atrophy as late as 12 yo when the patient returned for treatment with VN. At 12 yo, the left eye was treated with VN with two subretinal blebs along the superotemporal and inferotemporal arcades involving the macula and fovea. Two months after treatment with VN, the patient developed frothy vitreous cells (Supplementary Fig. S1) that were treated with topical steroid followed by oral steroids. Little change was observed in the vitreous cells, but subjective and functional vision continued to improve, and

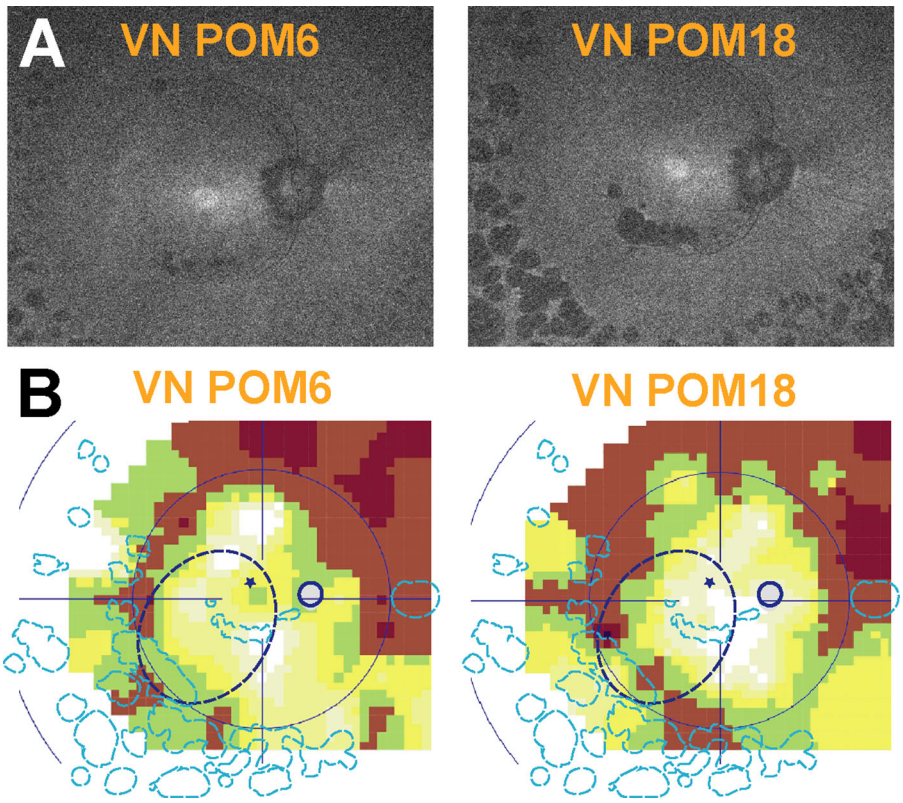

Figure 4. Areas of atrophy in patient 2, in the inferior macula and largely in the inferotemporal midperiphery best observed on (**A**) fundus autofluorescence. (**B**) Areas of atrophy are outlined (*light blue shapes with dashed lines*) and overlaid on the visual field.

steroids were tapered at four months. The patient was lost to follow-up until 22 months after treatment, when chorioretinal atrophy was first noted in the temporal and inferior macula extending past the inferotemporal arcade. The patchy chorioretinal atrophy became more discrete and with pigment clumping by 37 months after treatment. Areas of chorioretinal atrophy corresponded to a decrease in sensitivity from POM3 to POM22 (Figs. 5, 6). In spite of worsening visual field sensitivity, the patient still reported profound subjective improvement in nyctalopia. Although the global mean sensitivity value had decreased due to loss of peripheral vision, there remained a significant improvement centrally compared to baseline (Fig. 5). Quantifying this change demonstrated improvement of visual field sensitivity in the central 30° on hill of vision analysis from 2.15 dB-sr to 4.0 dB-sr at POM3. Although this decreased to 2.61 dB-sr at POM22, it remained an improvement compared to baseline.

Patient 4 was treated at 6 yo in the right eye with the AGTC vector in the superotemporal midperiphery, which did not involve the macula. The left eye was treated with voretigene at age 17 in the temporal macula and splitting the fovea (Fig. 7). At five months after treatment, there was robust improvement in visual field responses, with sensitivity improving more than twofold compared to baseline, particularly centrally and in the area corresponding to the temporal retina (Fig. 7). There was an area of very subtle early chorioretinal atrophy along the superotemporal arcade best observed on fundus autofluorescence (see Fig. 8A) in the area of the retinotomy site. This area of atrophy did correspond to a focal area of very high sensitivity centrally (see Fig. 8B), that is more consistent with chorioretinal atrophy as compared to touchdown atrophy. The patient, however, has been lost to follow-up, therefore, the rate of atrophy growth and subsequent changes in the visual field cannot be ascertained to aid in further differentiation between true chorioretinal atrophy versus touchdown atrophy.

FST was evaluated in patients 1, 2, and 4, with all showing significant improvement in the VN-treated eye. Furthermore, prior to VN treatment (Table, "pre-"), the contralateral previously treated eyes (Table, "CL eye") all showed greater FST responses. However, after VN treatment, FST responses reversed, and VN-treated eyes (Table, "VN eye") showed greater sensitivity than previously treated eyes.

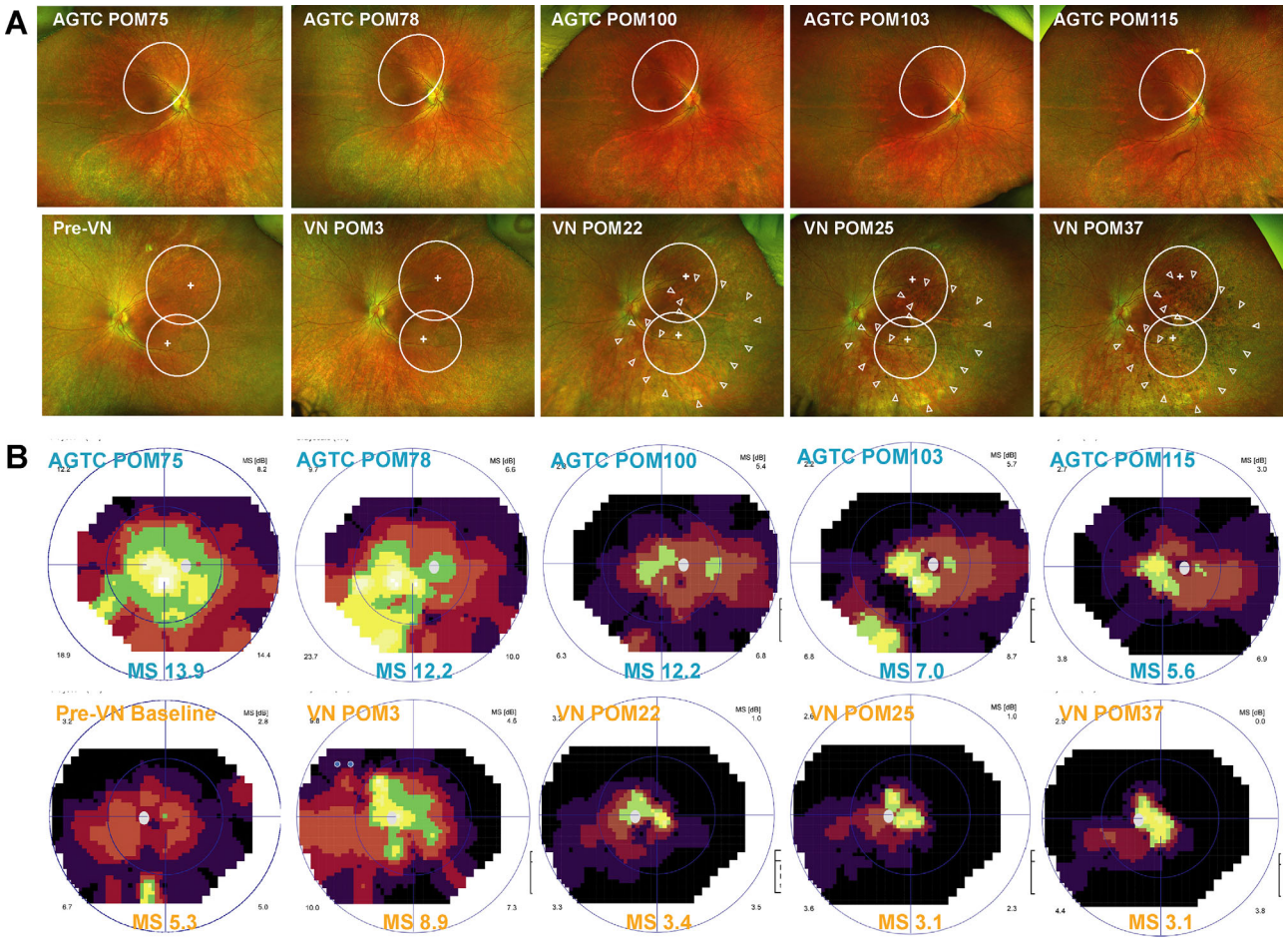

Figure 5. Fundus photos and dark-adapted perimetry of patient 3. (**A**) Wide-field fundus photos of both eyes taken at screening before voretigene treatment and at three, 22, 25, and 37 months after voretigene treatment. In the right eye, it was over six years after treatment as part of a clinical trial (AGTC). *Arrowheads* show areas of chorioretinal atrophy, *star* indicates foveal center, and the two *plus signs* indicates the two retinotomy sites. (**B**) Dark-adapted perimetry to the cyan (500 nm) stimuli in the right and left eye.

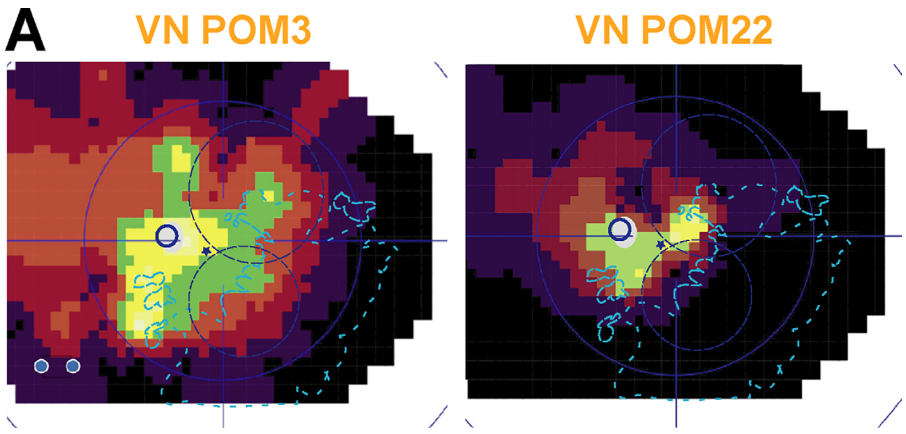

Figure 6. (**A**) Visual fields of patient 3 at POM3 and POM22 with a large area of atrophy perifoveally and extending to the posterior pole (*light blue dashed lines*).

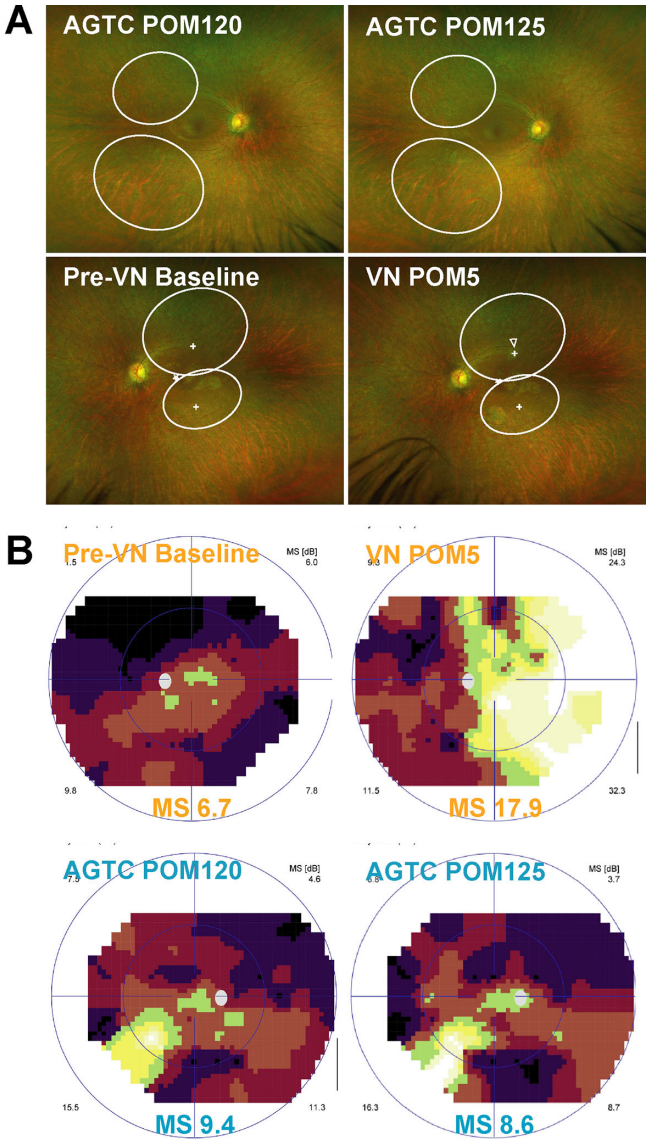

Figure 7. Fundus photos and dark-adapted perimetry of patient 4. (**A**, **B**) Wide-field fundus photos of both eyes taken at screening before voretigene treatment and at five months after voretigene treatment. In the right eye, it was over 10 years after treatment as part of a clinical trial (AGTC). The *arrowhead* shows an area of early chorioretinal atrophy, *star* indicates foveal center, and the two *plus signs* indicates the two retinotomy sites. (**B**) Dark-adapted perimetry to the cyan (500 nm) stimuli.

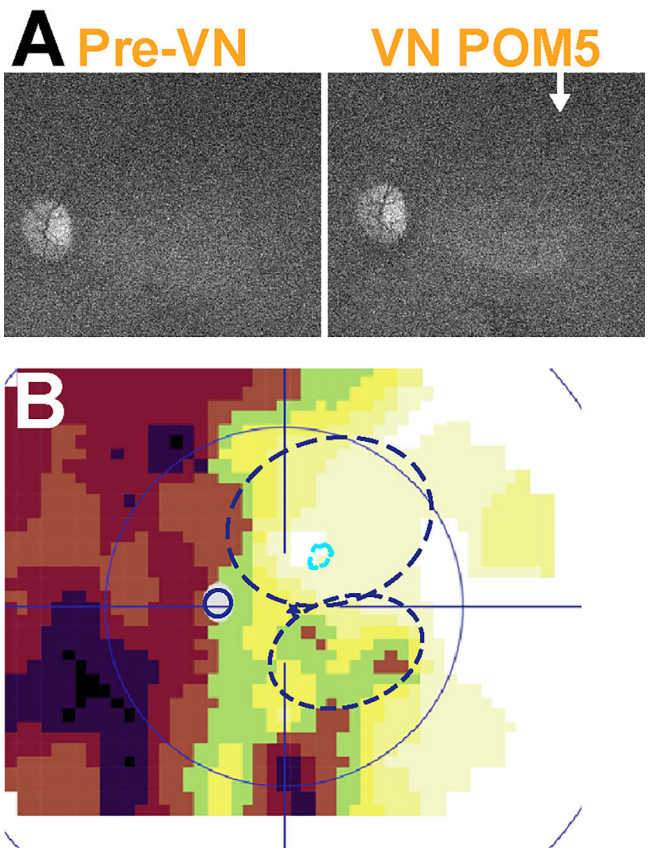

Figure 8. (**A**) Fundus autofluorescence images from patient 4 with subtle early area of hypoautofluorescence emerging at POM5 superiorly along the superotemporal arcade, corresponding to an area of high sensitivity on (**B**) visual fields.

Discussion

Adult patients treated with rAAV2-CB-hRPE65 as part of NCT#00749957 did not show a significant improvement in BCVA and only limited improvements in visual fields that were not sustained after year 2. However, sustained improvements in BCVA and visual fields were observed in four pediatric patients treated between age 6 to 11 yo that were sustained up to five years after treatment.[10,13] These four pediatric patients were subsequently treated with voretigene in their contralateral, better-seeing eye described in this study.[2–4]

It is difficult to compare the efficacy between the two vectors because of differences in outcome measures and the timing of treatments relative to disease stage in each trial. However, the AGTC vector was likely less effective than VN. In this study, eyes previously treated with the prior vector showed at baseline improved FST responses compared to the untreated eye. However, after treatment with VN, the relationship reversed and the VN treated eye demonstrated a much higher sensitivity. This occurred in spite of administration of VN almost six to 10 years later (Table).

In addition to relative global improvements in sensitivity measured by FST after VN treatment, we also observed spatial correlation of improved sensitivity as measured by two color dark adapted perimetry. It is notable that before treatment with VN, the previously treated eye demonstrated higher sensitivity than the untreated eye. However, once the contralateral

Table. FST Results in Patients 1, 2, and 4 at Last Follow-Up

	White FST		Blue FST		Red FST		Blue-Red	
	VN Eye	CL Eye	VN Eye	CL Eye	VN Eye	CL Eye	VN Eye	CL Eye
Patient 1								
Before	−21.2	−23.9	−15.1	−30	−14.2	−13.6	−0.9	−16.4
After	−18.6	−12	−24.5	−17.2	−20.2	−14.2	−4.3	−3.0
Patient 2								
Before	−8	−14.2	−13.1	−18.5	−14	−12.6	0.9	−5.9
After	−27.2	−17.9	−38.4	−23.2	−23.2	−16.4	−15.2	−6.8
Patient 4								
Before	−13.1	−31.2	−12.4	−41.1	−6.4	−15.1	−6.0	−26.0
After	−26.4	−27.4	−49.7	−33.4	−20.7	−9.3	−29.0	−24.1

CL, contralateral previously treated eyes; VN, voretigene treated eyes.
FST values are from before and after VN treatment, with post-treatment results taken from the last follow-up visit.

eye is treated with VN, there is a dramatic improvement in sensitivity as early as three months in all four patients.

Paramacular chorioretinal atrophy has been reported in eyes with VN ranging from 13% to 28% of treated eyes.[5,6] We observed development of chorioretinal atrophy in three out of four eyes that were treated with VN after treatment with a different vector in the contralateral eye. Furthermore, the fourth patient had early atrophy at three months after surgery that could not be fully characterized as either true perimacular atrophy or touchdown atrophy from the retinotomy site because of subsequent lack of follow-up. Albeit a small sample size, the incidence of chorioretinal atrophy in our entire cohort must raise concern that incidence might be higher when VN is administered to patients who have previously received a different vector for gene augmentation for *RPE65*-related retinopathy.

An overlay of the degree of sensitivity improvement spatially with the areas of atrophy does suggest a correlation. Interestingly, in eyes first treated under clinical trial NCT#0074995, there were no areas of chorioretinal atrophy and these eyes demonstrated lower sensitivities compared the VN treated eyes. Our findings are consistent with a recent report that showed that chorioretinal atrophy was correlated to greater improvements in FST.[6] Recently, Stingl et al.[18] reported a retinopic relationship to growth of chorioretinal atrophy and the improvement in rod function measured by pupillary campimetry but not with two-color dark-adapted perimetry.

Our study has several limitations. First, it is challenging to directly compare results between two trials that were designed with different protocols and outcome measures. Patient follow-up after VN treatment was irregular and limited secondary to constraints from the COVID-19 pandemic. Greater correlation between atrophic areas and visual field sensitivity in patients 2 and 4 may have been seen if there were more frequent follow-ups. However, the results do serve to generate a hypothesis that the etiology of chorioretinal atrophy may stem from metabolic overuse of previously sick and functionally borderline photoreceptors following restoration of RPE65 expression. Atrophy may have been more likely in this situation given that second eyes were treated at a later stage of disease (6–10 years later), at which point cells may have been more vulnerable to insult. Patients with heterozygous RPE65 variants resulting in an Asp477-to-Gly substitution in a highly conserved residue develop retinitis pigmentosa with choroidal atrophy (RP87), demonstrating that expression of abnormal levels of RPE65 are associated with chorioretinal atrophy; perhaps a similar mechanism contributes to chorioretinal atrophy in eyes treated with VN due to toxicity from RPE65 overexpression.[19,20] No eyes treated first as part of the clinical trial NCT#0074995 showed chorioretinal atrophy, which we hypothesize is due to lower expression levels of RPE65 with this vector and treatment at an earlier stage of disease.

Chorioretinal atrophy has also been hypothesized to occur secondary to damage to photoreceptors and the RPE from physical damage associated with subretinal injections themselves. Although not specifically assessed in this study, our site had previously reported on the importance of maintaining low injection pressures and bleb propagation speed to reduce the risk of retinal damage in all gene therapy surgeries.[14]

Finally, immune reactivity and inflammation may play a role in development of chorioretinal atrophy. Although overt inflammatory responses are not always observed, we cannot rule out subclinical immune responses, including responses intrinsic to RPE cells with cytokine production, nitric oxide production, and lymphocyte migration.[21] Young patients such as that of our cohort may also have greater immune reactivity, which has been observed in achromatopsia patients in which three serious adverse events leading to atrophy related to uveitis were observed in children at a dose tolerated by adults.[22]

The exact mechanism of chorioretinal atrophy after VN remains unknown and likely multifactorial, with all of the above mechanisms potentially contributory. Incidence of chorioretinal atrophy appears highly variable between sites; reasons behind this remain unclear and are being actively explored. Reported incidence of chorioretinal atrophy have ranged from 12% in the PERCEIVE post-authorization safety study[5] to as high as between 28% to 50% of patients.[4,6] At the OHSU-Casey Eye Institute, 15 patients received the typical bilateral VN treatment without chorioretinal atrophy, as compared to this specific population in which at least 75% of patients showed chorioretinal atrophy. Overall our findings suggest that overactivity may play a large role in the development of chorioretinal atrophy, but we also propose that previous treatment with a different vector may pose a risk because of immunosensitization with prior treatment that is separated in time, which is not the case with typical VN treatment. Although an overt inflammatory response was only observed in one patient in this study who demonstrated vitreous cell after VN, we cannot rule out subclinical immune responses in the other patients. Caution should be used in future treatments of patients who might have been previously treated with a different vector.

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