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Safety and durability of effect of contralateral-eye administration of AAV2 gene therapy in patients with childhood-onset blindness caused by *RPE65* mutatons: a follow-on phase 1 trial

Prof Jean Bennett, MD, **Jennifer Wellman, MSc**, **Kathleen A Marshall, COT**, **Sarah McCague, BA**, **Manzar Ashtari, PhD**, **Julie DiStefano-Pappas, BA**, **Okan U Elci, PhD**, **Daniel C Chung, DO**, **Junwei Sun, MSc**, **J Fraser Wright, PhD**, **Dominique R Cross, MPH**, **Puya Aravand, BA**, **Laura L Cyckowski, BA**, **Jeannette L Bennicelli, PhD**, **Federico Mingozzi, PhD**, **Alberto Auricchio, MD**, **Eric A Pierce, MD**, **Jason Ruggiero, MD**, **Prof Bart P Leroy, MD**, **Francesca Simonelli, MD**, **Katherine A High, MD**, and **Prof Albert M Maguire, MD**

Center for Advanced Retinal and Ocular Therapeutics (Prof J Bennett MD, M Ashtari PhD, D C Chung DO, J Sun MSc, P Aravand BA, J L Bennicelli PhD, Prof A M Maguire MD) and F M Kirby Center for Molecular Ophthalmology (Prof J Bennett, M Ashtari, D C Chung, J L Bennicelli, E A Pierce MD, Prof A M Maguire), Department of Ophthalmology (E A Pierce, J Ruggiero MD, Prof A M Maguire), Scheie Eye Institute, University of Pennsylvania, Philadelphia, PA, USA; Center for Cellular and Molecular Therapeutics (Prof J Bennett, J Wellman MSc, K A Marshall COT, S McCague BA, J Sun, J F Wright PhD, D R Cross MPH, F Mingozzi PhD, Prof B P Leroy MD, K A High MD, Prof A M Maguire), Department of Radiology (M Ashtari, L L Cyckowski BA), Westat Biostatistics and Data Management Core (J DiStefano-Pappas BA, O U Elci PhD), and Department of Ophthalmology (E A Pierce, Prof B P Leroy, Prof A M Maguire), The Children's Hospital of Philadelphia, Philadelphia, PA, USA; Spark Therapeutics, Philadelphia, PA, USA (J Wellman, D C Chung, J F Wright, K A High); Immunology and Liver Gene Therapy, Généthon, Èvry, France (F Mingozzi); Telethon Institute of Genetics and Medicine, Naples, Italy (A Auricchio MD); Medical Genetics, Department of Pediatrics, University of Naples Federico II, Naples, Italy (A Auricchio); Ocular Genomics Institute, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA (E A Pierce); Department of Ophthalmology and Center for Medical

Correspondence to: Prof Jean Bennett, Center for Advanced Retinal and Ocular Therapeutics, University of Pennsylvania, 309C Stellar-Chance Labs, 422 Curie Blvd, Philadelphia, PA 19104, USA, jebennet@mail.med.upenn.edu.

See **Online** for appendix

Contributors

JB, JW, KAM, SM, DCC, JS, FM, KAH, and AMM designed the study. JB, KAM, SM, MA, DCC, DRC, LLC, EAP, and AMM collected the data. JB, JW, JD-P, OUE, MA, PA, LLC, FM, KAH, and AMM analysed the data. JLB and JFW generated reagents used in the study. AA, FS, BPL, and AMM referred patients to the study. AMM, EAP, and JR carried out the surgical procedures. JB wrote the manuscript. All authors contributed to the revision of the manuscript, and saw and approved the final version.

Declaration of interests

AMM and JB are co-inventors on a patent for "a method of treating or retarding the development of blindness" (US Patent number 8147823) but waived any potential financial interest in this technology in 2002. KAH, JFW, DCC, and JW are now employed by and have equity in Spark Therapeutics, a company that was formed after the participants had received intervention to the second eye and that is developing this technology. JB also reports having served on a scientific advisory board for Avalanche Technologies and is a founder of Gensight Biologics. JB, DCC, AMM, KAH, JW, KAM, SM, and JS are coauthors of a provisional patent describing the mobility test used in this study. FM and KAH hold a patent on methods for detection and modulation of T-cell responses to gene therapy vectors. JFW is an inventor on patents relative to adeno-associated vector development. All other authors declare no competing interests.

For more on the **phase 3 study** see https://www.med.upenn.edu/carot/research/clinical-trials/rpe65/

Genetics, Ghent University Hospital and Ghent University, Ghent, Belgium (Prof B P Leroy); Eye Clinic, Multidisciplinary Department of Medical, Surgical and Dental Sciences, Second University of Naples, Naples, Italy (Prof F Simonelli MD); and Howard Hughes Medical Institute, Philadelphia, PA, USA (K A High)

Summary

Background—Safety and efficacy have been shown in a phase 1 dose-escalation study involving a unilateral subretinal injection of a recombinant adeno-associated virus (AAV) vector containing the *RPE65* gene (AAV2-hRPE65v2) in individuals with inherited retinal dystrophy caused by *RPE65* mutations. This finding, along with the bilateral nature of the disease and intended use in treatment, prompted us to determine the safety of administration of AAV2-hRPE65v2 to the contralateral eye in patients enrolled in the phase 1 study.

Methods—In this follow-on phase 1 trial, one dose of AAV2-hRPE65v2 ($1{\cdot}5 \times 10^{11}$ vector genomes) in a total volume of 300 μL was subretinally injected into the contralateral, previously uninjected, eyes of 11 children and adults (aged 11–46 years at second administration) with inherited retinal dystrophy caused by *RPE65* mutations, 1·71–4·58 years after the initial subretinal injection. We assessed safety, immune response, retinal and visual function, functional vision, and activation of the visual cortex from baseline until 3 year follow-up, with observations ongoing. This study is registered with ClinicalTrials.gov, number NCT01208389.

Findings—No adverse events related to the AAV were reported, and those related to the procedure were mostly mild (dellen formation in three patients and cataracts in two). One patient developed bacterial endophthalmitis and was excluded from analyses. We noted improvements in efficacy outcomes in most patients without significant immunogenicity. Compared with baseline, pooled analysis of ten participants showed improvements in mean mobility and full-field light sensitivity in the injected eye by day 30 that persisted to year 3 (mobility p=0·0003, white light full-field sensitivity p<0·0001), but no significant change was seen in the previously injected eyes over the same time period (mobility p=0·7398, white light full-field sensitivity p=0·6709). Changes in visual acuity from baseline to year 3 were not significant in pooled analysis in the second eyes or the previously injected eyes (p>0·49 for all time-points compared with baseline).

Interpretation—To our knowledge, AAV2-hRPE65v2 is the first successful gene therapy administered to the contralateral eye. The results highlight the use of several outcome measures and help to delineate the variables that contribute to maximal benefit from gene augmentation therapy in this disease.

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Introduction

Biallelic mutations in the *RPE65* gene—which encodes all-trans retinyl ester isomerase, an enzyme crucial in the retinoid cycle—cause Leber’s congenital amaurosis type 2 and other

forms of severe retinal degenerative disease.[1–4] Proof of principle of gene augmentation therapy with a recombinant adeno-associated virus (rAAV) for diseases caused by *RPE65* mutations was established in canine and murine models, and results from these studies[5–8] showed that the biochemical blockade of the visual cycle caused by RPE65 deficiency could be overcome. Clinical trials[9–11] were initiated after additional efficacy, safety, and dosing studies were done in large animals.

Results from our phase 1 study[10,12–14] at The Children's Hospital of Philadelphia (Philadelphia, PA, USA) showed safe and stable improvement in retinal and visual function in all 12 patients. These individuals had been injected unilaterally and subretinally in their worseseeing, non-preferred eyes in a dose-escalation study with doses ranging from $1{\cdot}5 \times 10^{10}$ to $1{\cdot}5 \times 10^{11}$ vector genomes of AAV2-hRPE65v2, an AAV vector carrying the wild-type *RPE65* cDNA.[10,12] Most participants showed improved light sensitivity, increased navigational abilities, improved visual acuity, increased activation of the visual cortex, and evidence of improved function and structure of the visual pathways in their injected eye.[15] The patients also had an acquired afferent pupillary defect in the uninjected eye, suggesting that the injected (but not the uninjected) retina had been corrected by gene therapy.[10,12–15]

Research in context

Evidence before this study

On March 24, 2016, we searched PubMed for English language publications between Jan 1, 1996, and Jan 1, 2016, using the following terms alone and in combination: "retina", "gene therapy", "AAV", "adeno-associated virus", "RPE65", "clinical trial", "readministration", "two administrations", "re-administration", "vaccine", and "immune response". Several dose-escalation studies have tested gene augmentation therapy for retinal dystrophy caused by *RPE65* mutations. Although improvements in various measures (visual acuity, visual fields, pupillary light reflex, full-field light sensitivity, and navigation) have been reported, none of the trials assessed the safety of administration of adeno-associated virus (AAV) vectors to the contralateral eye, even though *RPE65* mutations cause bilateral disease. Furthermore, the variables affecting improvements in retinal and visual function have not been clearly defined. Few, if any, successes have been reported after repeat administration of AAV vectors in human beings in any organ because of immune clearance. Thus, the safety and efficacy of a second administration need to be further tested.

Added value of this study

To our knowledge, our study is the first to successfully administer gene therapy to the contralateral eye. We showed that administration to the second eye is safe after previous exposure to AAV2-hRPE65v2 in individuals with *RPE65* retinal disease, even in those with pre-existing immunity to AAV2. Repeat administration led to durable improvement in functional vision, retinal and visual function, and cortical responses. The ability to safely and effectively readminister AAV to the contralateral retina provides an opportunity to further increase visual function in individuals with vision impairment.

Implications of all the available evidence

Our results support the conduct of phase 3 AAV2 gene therapy studies involving treatment of both eyes (using the reagent, dose, and volume in our study), as well as the use of several different outcome measures. The results also pave the way for cautious administration to the second eye in other ocular gene therapy studies, although in some contexts it might be advisable to maintain the untreated second eye as an internal control.

After the success of the unilateral injections, we hypothesised that functional vision and retinal and visual function could be further improved by delivering AAV2-hRPE65v2 to the contralateral, originally better-seeing eye. A chief concern was that humoral responses to AAV2 or the RPE65 protein generated after the initial AAV2 exposure could have prevented further benefit. More concerning, a cell-mediated immune response might cause inflammation and subsequent destruction of the terminally differentiated retinal cells, thus permanently removing any pre-existing vision. However, in experiments with large-animal models, repeat administrations resulted in efficacy with a high degree of safety.[16] These encouraging pre-clinical results prompted us to do a follow-on study of the dose-escalation trial,[12] in which we aimed to assess the safety of second-eye administration, determine whether retinal and visual function can be further improved after a unilateral injection, and delineate the variables affecting efficacy.

Methods

Patients

We invited 12 patients who had previously participated in the phase 1 dose-escalation study[12] to take part in this follow-on phase 1 study. Enrolment criteria have been described elsewhere;[17] briefly, we enrolled 11 of 12 patients from the original study. All surgery and follow-up tests were done at The Children's Hospital of Philadelphia.

The clinical study had been reviewed and approved by the Institutional Review Board of and the Institutional Biological Safety Committee at The Children's Hospital of Philadelphia (CHOP). All participants gave written informed consent or, in the case of children, assent or parental consent (appendix p 1).

Procedures

Patients received a subretinal administration of AAV2-hRPE65v2 ($1{\cdot}5 \times 10^{11}$ viral genomes) to the contralateral, previously uninjected, eye (ie, the second eye) in a total subretinal volume of 300 μL;[17] the procedure for subretinal injections has been described previously.[18–20] The dose and volume corresponded to the highest dose assessed in the dose-escalation study,[12] which was well tolerated and resulted in improvement in several parameters of retinal and visual function.[20] The targeted region for AAV delivery was the central superior retina or macula (figure 1), after verification of the presence of sufficient retinal cells through ophthalmoscopy, fundus photographs, and optical coherence tomography measurements. Surgical risks were lessened by introducing steps to minimise mechanical stress to the fovea.[18]

Patients were assessed at designated timepoints: at baseline (≤90 days before day 0 [day of surgical AAV delivery]) and on days 1, 3, 14, 30, 90, and 180, then once yearly during years

1–5. Adverse events and immune response were monitored, and efficacy data for all evaluable patients were analysed both individually and in a pooled manner. We used Goldmann (light-adapted) visual field tests to measure retinal and visual function, and full-field light sensitivity threshold testing to measure sensitivity to light over a range greater than five log units. Rod photoreceptor sensitivity was measured through the use of white and blue stimuli, and cone photoreceptor sensitivity was measured through the use of red stimuli. Changes in visual acuity, measured with Holladay off-chart assignments[21] in logarithm of the minimum angle of resolution (logMAR), were also monitored. Retinal-CNS pathways were assessed with a qualitative pupillary light reflex test, and functional vision was assessed with a mobility test.[18–20] Mobility test videos were graded as pass or fail on the basis of predesignated weighting of accuracy and speed data; grading was done at an independent reading centre, and the readers were masked to study details, including treatment regimen (ie, which eye received the second administration) and light levels. Pupillary light reflex assessment was done by the study team, without prior masking of participant or visit details, from light-adapted (photopic) tests of alternating stimuli. Additionally, improvement in visual function was assessed by the change in activation in the visual cortex, measured with functional MRI (fMRI), in response to a contrast-reversing checkerboard stimuli, before and after subretinal injections. Acquisition and analyses of the fMRI data were done as a separate study, with separate consent and assent required, and involved eight participants in this study.[17] Short-term results for three of these participants were previously reported.[17] Additional details are provided in the appendix pp 6–8.

Statistical analysis

For eligible participants, we pooled data and assessed changes in the main efficacy outcomes (mobility testing and full-field light sensitivity) and an additional efficacy outcome (visual acuity) over time (ie, from baseline to day 30, day 90, day 180, year 1, year 2, and year 3) using mixed-effects linear regression models with random intercepts implemented via maximum likelihood. Results from these models were reported as changes with 95% CIs and corresponding p values. Stata 13.1 (Stata Corporation, College Station, TX) was used for statistical analyses, and the significance level was set at 0·05 for all tests. See appendix pp 6, 22 for full details. This study is registered with ClinicalTrials.gov, number NCT01208389.

Role of the funding source

The funders of the study (initially predominantly the Center for Cellular and Molecular Therapeutics at The Children’s Hospital of Philadelphia and then Spark Therapeutics) and personnel working for the funders were involved in study design, data collection, analysis, and interpretation, and writing of the report. None of the other funding sources had any direct role with respect to the design or execution of the study, data collection, analysis, and interpretation, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Between Nov 18, 2010, and Oct 8, 2012, 11 of 12 patients in the original study received the injection. One patient (CH13) was not eligible for the follow-on intervention because of glaucoma in the second (ie, uninjected) eye. Bennett and colleagues[17] have described the 6 month results from the first three patients (all of whom were adults; table 1) to receive injection in the contralateral eye. Ten of 11 patients who received the intervention were included in the analysis (table 1). Results from NP04 were excluded in the analysis because he had culturepositive (*Staphylococcus epidermidis*) endophthalmitis after surgery. The diagnosis of bacterial endophthalmitis was made on the basis of recovery of pathogenic organisms from a vitreous sample obtained under aseptic conditions, an immediate response to the application of intravitreal antibiotics despite the discontinuation of systemic corticosteroids, and the absence of evidence for immunological response to the AAV2 capsid and RPE65 protein (appendix pp 2–4). Although we could not pinpoint the contamination with *S epidermidis* with absolute certainty, procedures were modified to minimise the chance of reoccurrence. Importantly, we have not seen this reaction in any of the other 22 injected eyes in this and the dose-escalation study[12] (including ten contralateral eyes, of which six received second administration after NP04 did; table 1) or in any of the 58 eyes receiving the same investigational agent at the same dose in a phase 3 study.[18,19] In all evaluable patients, the left retina was injected, except for CH09, who had the right retina injected (figure 1).

No adverse events related to the AAV vector were observed. Adverse events related to the procedure were mostly mild and included dellen formation (ie, uneven surface of the cornea) adjacent to the suture in the early postoperative period in three patients (NP02, CH09, and CH10) and cataracts in two patients (CH06 and CH12). The dellens resolved with topical treatment, and the cataracts were successfully treated with standard cataract surgery. The treatment for bacterial endophthalmitis in NP04 led to elevated intraocular pressure and subsequent optic atrophy, which was the only serious adverse event attributed to participation in this trial. CH09 has severe myopia (requiring a correction of more than –10 dioptres) and retinal thinning was noted after injection in the second eye. Compared with other patients, CH09 had lower macular volume and thinner fovea at baseline. In general, no changes in macular volume or retinal thickness were associated with the administration of AAV2-hRPE65v2, even when the injection area included the fovea (data not shown).

The humoral and cell-mediated responses to the AAV2 capsid and the *RPE65* transgene were benign in all patients, with only one patient (CH10) developing a (low) positive cell-mediated response to AAV at follow-up week 4 (appendix p 17). CH09 and NP04 had low positive results at baseline (ie, before vector injection), but not thereafter. Thus, the low positive responses at week 8 (CH10) and week 2 (CH09, NP04) did not occur in response to injection. CH10 had no inflammation, and the importance of this isolated event is unknown. Two individuals (CH06 and CH10) had high baseline neutralising antibodies to AAV2 (appendix pp 11, 12, 18, 19) but no inflammation. Systemic exposure to the vector was limited (appendix pp 13, 20, 21). In summary, administration of AAV2-hRPE65v2 ($1{\cdot}5 \times 10^{11}$ viral genomes) to the contralateral retina appeared safe, irrespective of baseline immune status.

Goldmann visual field tests at baseline revealed islands of responding retina, some without foveal fixation (figure 1). Visual fields of one patient (CH11) showed inter-test variability that was possibly caused by the perception of phosphenes,[22] so that any changes resulting from the administration of AAV2-hRPE65v2 were difficult to interpret. In four patients (NP02, CH08, CH09, and CH10), qualitative analysis showed expansions of the visual field by day 30 that corresponded generally with the area of subretinal injection. In one participant (CH10), vision was restored in areas where large scotomas had been present. Total visual field area decreased in NP15 over time, but vision in the area generally corresponding to the injected region persisted over the 3 year period.

For all other outcomes, results for the second injected eye were compared with results over the same follow-up period for the first injected eye—ie, the eye studied in the dose-escalation study;[12] most patients received a lower dose to the first eye than to the second eye.

Results from full-field light sensitivity threshold testing show robust improvements in both rod and cone function by day 30 in the contralateral eyes, and improvements from baseline persisted until year 3 in pooled analyses ($p<0{\cdot}0001$ for all timepoints; figure 2A–C; appendix pp 22–24). Eight of ten patients showed improvement in sensitivity (figures 3–5; table 2). In six patients (NP01, NP02, CH08, CH10, CH11, and NP15), sensitivity to white light increased by at least 10 dB in the second eye (CH08's improvement was close to this level); smaller increases in sensitivity to white light were noted in two patients (NP03 and CH09; figures 3–5). Sensitivity to blue light increased by at least 10 dB for six patients (NP01, NP02, CH08, CH09, CH10, and NP15); smaller increases were noted in two patients (NP03 and CH11, figures 3–5). There was no change in sensitivity in CH06 or CH12 (figures 4, 5). CH11 had a high degree of inter-test variability (possibly because of her perception of phosphenes[22]). Sensitivity to red light increased by more than 10 dB in the second eyes of three patients (NP01, NP02, and CH10) and by a smaller extent in five patients (NP03, CH08, CH09, CH11, and NP15). Sensitivity to red light increased in the initially injected eye of NP02, but the increase was not as much as that in the second eye. Improvements in rod photoreceptor responses (ie, responses to white and blue light) were larger than those in cone responses (ie, responses to red light; mean changes were white light 17·9 dB [SD 1·33] *vs* blue light 17·6 dB [1·41] *vs* red light 10·8 dB [1·17]). No significant changes were seen in rod or cone function in the initially injected eyes during this study period (full-field sensitivity to white light $p=0{\cdot}6709$, blue light $p=0{\cdot}3112$, red light $p=0{\cdot}8277$; figures 2A–C, 3–5; appendix pp 22–24). In two (NP01 and NP02) of three participants who had received low-dose AAV2-hRPE65v2 in their first eyes, large improvement was seen after second eye (high-dose) administration, and their first eyes had less robust responses (but improved compared with baseline function of their second eyes; figure 3), suggesting a dose-response effect.

Changes in visual acuity from baseline to year 3 were not significant in pooled analysis of logMAR scores in the second eyes or the previously injected eyes ($p>0{\cdot}49$ for all timepoints compared with baseline; figure 2D; appendix pp 24–25). Changes were significant (change in logMAR $\geq 0{\cdot}3$ or a halving of the visual angle) in only two patients (CH12 and CH09). CH12 showed an improvement of 0·15–0·50 logMAR, depending on the off-chart

assignments used (appendix p 27).[21,23] CH09 showed an initial decline in acuity that remained stable for the duration of follow-up; the deterioration in visual acuity was due to myopia-related macular thinning, exacerbated by subfoveal injection. Overall, changes in visual acuity were not associated with the involvement of the fovea in the subretinal injection.

Results of the mobility test showed an improvement in the second eyes by day 30 (p=0·0011) that persisted through year 3 (p=0·0002 compared with baseline; figures 2E and 6; appendix p 22) with observation ongoing, suggesting that patients could complete the mobility test more accurately and quickly under lower illuminances than at baseline. No significant difference was seen in mobility between baseline of the follow-on study and post-intervention timepoints in the previously injected eye (p=0·7398). Most patients had improved mobility after administration to the contralateral eye (figure 6), with the exception of CH06 and CH12, who showed no change in mobility.

After administration to the second eye, qualitative assessment of pupillary light reflex shows improvement lasting for at least 3 years in all patients (appendix pp 27–28). Whereas the responses were maintained in the initially injected eye, the responses of the second injected eyes, which all received the maximal dose, were generally more robust. For example, before administration to the second eye, CH09 had strong pupillary light reflexes after stimulus of the left (first injected) eye. After injection to the right retina in the follow-on study, additional strong responses were seen in this eye after illumination.

Overall comparison of fMRI results at baseline and at 1 year post-administration showed significant widespread bilateral activation in all areas of the visual cortex, extending from medial to lateral and posterior to anterior aspects of the occipital cortex (figure 7). The pattern of activation varied for each individual depending on their age, extent of disease progression, or other factors (eg, chronic smoking, which is known to abate cortical blood flow and thus the fMRI signal). NP01 had lower cortical activation than other patients, which might be attributable to her being a chronic smoker. In all eight patients who underwent neuroimaging, cortical activation in response to the high-contrast stimuli increased, and responses to medium-contrast and low-contrast stimuli also increased, albeit to a smaller extent. See appendix pp 6–8 for longitudinal analysis.

In summary, delivery of AAV2-hRPE65v2 to the second eye resulted in long-term improvements in multiple measures of rod photoreceptor function and also a measure of cone photoreceptor function in most participants (table 2).

Discussion

Subretinal administration of AAV2-hRPE65v2 to the contralateral eye showed a high degree of safety and stable, persistent improvement (for at least 3 years, with observation ongoing) in retinal and visual function and functional vision in most patients. Parallel improvement in visual cortex responses was seen in all eight individuals who participated in fMRI studies. Improvement in subjective and objective measures of retinal and visual function was observed as early as day 30.

Similar to results from animal studies, participants in our study showed strong and stable improvements in light sensitivity after contralateral-eye administration of AAV2-hRPE65v2, even those with pre-existing systemic antibodies to the AAV2 capsid or the transgenic RPE65 protein. With this particular vector preparation, high-dose AAV2-hRPE65v2 can be safely administered to the subretinal space of the second eye, with evidence of efficacy. Furthermore, no inflammatory response was reported in either eye, and the measured immunological responses were benign.

Previously, following administration of AAV2-hRPE65v2, we noted a strong bias towards improvement in the short-wavelength (ie, blue) spectrum, which is consistent with an improvement in rod photoreceptors.[17] This is analogous to the Purkinje phenomenon, which occurs during dark adaptation in which the peak sensitivity of the retina shifts from the red (cone) to the blue (rod) photoreceptor population.[24] Of note, after administration to the contralateral retina, eight of ten patients had robust and stable improvement in both cone and rod photoreceptor responses; the macula (or a portion thereof) of these patients had been exposed to AAV2-hRPE65v2. The improvement in cone function in most patients suggests that the chromophore generated by the RPE65 isomerase, made from the *RPE65* transgene, might activate cone photoreceptors in addition to rod photoreceptors.

The results of our study help to define clinical variables and procedures that might affect the magnitude of the outcome. Because *RPE65* mutations cause degenerative disease, early intervention is expected to lead to greater potential gain. However, if the number of remaining photoreceptors is sufficient, even patients in the third or fourth decades of life (eg, NP02) could benefit substantially. One young adult (CH06) did not show improvement, possibly because of her history of encephalitis and inner retinal abnormalities caused by optic nerve drusen. CH06 had high-titre neutralising antibodies to AAV2 at baseline; however, another patient (CH10) with high-titre neutralising antibodies did benefit from administration to the second eye. Although CH06 has two disease-causing *RPE65* mutations, her heterozygous *RDH12* mutation might have also affected her outcome. Finally, although retinal thinning associated with high myopia does not prevent benefit (as seen in CH09), this effect might make the macula more vulnerable to mechanical stress during subretinal injection. The risk to pathological myopes might be minimised by limiting the volume of vector injected or by avoiding delivery of the vector in the foveal area.

Six phase 1 dose-escalation trials of interventions targeting *RPE65*-mediated retinal disease have been initiated, three of which have reported on results from the full set of participants.[12,25,26] The vectors used in the trials differed in several crucial parameters, including the method of purification, presence of elements that can affect the level of expression, and composition of the excipient.[9–12] Two of the trials used constitutive promoters;[10,11] the third used a weaker promoter that is active only in the retinal pigment epithelium.[9] Major differences in surgical approaches included delivery volume, regions of retina targeted, and perioperative regimens of steroid use; the ages of the participants and the extent of photoreceptor degeneration also differed. Differences also existed in outcome measures and their analyses.[12,25,26] Nevertheless, all three trials showed a high level of safety and improvement of retinal and visual function, as determined by increased light sensitivity and other parameters.[10–12,26,27] Two of the groups used mobility tests in all

cohorts to assess participants' improved abilities to navigate under dim lighting.[9,10,12,26] Most participants tested had additional improvements in pupillary light reflexes[10,12] and visual cortex responses,[14,15] and in dark-adapted perimetry, microperimetry,[9] and visual acuity.[10,12] Jacobson and colleagues,[28] reporting on three of 15 participants in their trial, described a decrease in light sensitivity after 3 years, although the long-term sensitivity after unilateral injection remained significantly higher than that at baseline. Another concern was that the degenerative process continued, although improvements in visual function again persisted.[29] Bainbridge and colleagues[26] also reported a decrease in sensitivity in six of 12 participants after 6–12 months; however, the initial level of improve ment was not as high as that reported in other studies, perhaps because the weaker RPE-specific promoter was used. Further, the surgical details in this trial[9] differed substantially from those in the other trials.

One question that remains relates to the durability of the functional benefit of a one-time injection. Cideciyan and colleagues[29] have focused on statistical analyses of retinal thickness of participants of gene therapy clinical trials in comparison to that in a normative (so-called natural history) database, but this database might not account for variables that would affect retinal thickness, such as the area of injection or ageing. Jacobson and colleagues[28] have also focused on measures from microperimetry in the injected eyes of three of 15 individuals receiving different doses and volumes of vector and in different retinal locations. Of note, fundamental differences existed in the various trials, including vector design and purification, use of surfactant, systemic immunomodulation, dosing, surgical delivery, and outcome measures used to assess treatment effect. Therefore, to make generalisations from results of all the studies is problematic. Importantly, our study included a clinically meaningful outcome measure—ie, mobility—and has shown durable positive results across multiple measures, with observation ongoing. A second administration to the previously uninjected eye—with the same dose, volume, and general target location in the retina—clearly results in reproducible, large, and persistent improvements in retinal and visual function and in functional vision that is important for vision-dependent activities of daily living. A limitation of our study is that the initially injected eyes were the worse-seeing eyes, which had received different doses previously in the dose-escalation study. Nonetheless, it is now possible to study the durability of effect in a more controlled manner —namely, in a large, randomised controlled phase 3 study (NCT00999609). In this ongoing study, both eyes received the same dose and volume of AAV-hRPE65v2 near simultaneously, which will enable the identification of additional variables affecting outcomes.

In summary, this report is the first to show long-term improved functional vision and retinal and visual function after repeat gene therapy administration. Efficacy after repeat administration of AAVs in human beings has been described in only one other clinical trial,[30] which used AAV to produce an immune response to vaccinate against HIV.[30] The immune-privileged nature of the target tissue is likely to contribute to the strong safety profile of AAV2-hRPE65v2. Other features also contribute to the safety of the administration to the second eye of this particular vector—eg, the relatively low dose of a vector that is largely devoid of empty capsids, thereby resulting in a low antigen load.

Following a second administration of this vector, eight of ten participants included in the analysis can now navigate under dimmer lighting conditions with their second injected eyes.

This clinically meaningful test of functional vision was corroborated by additional measures of retinal sensitivity.

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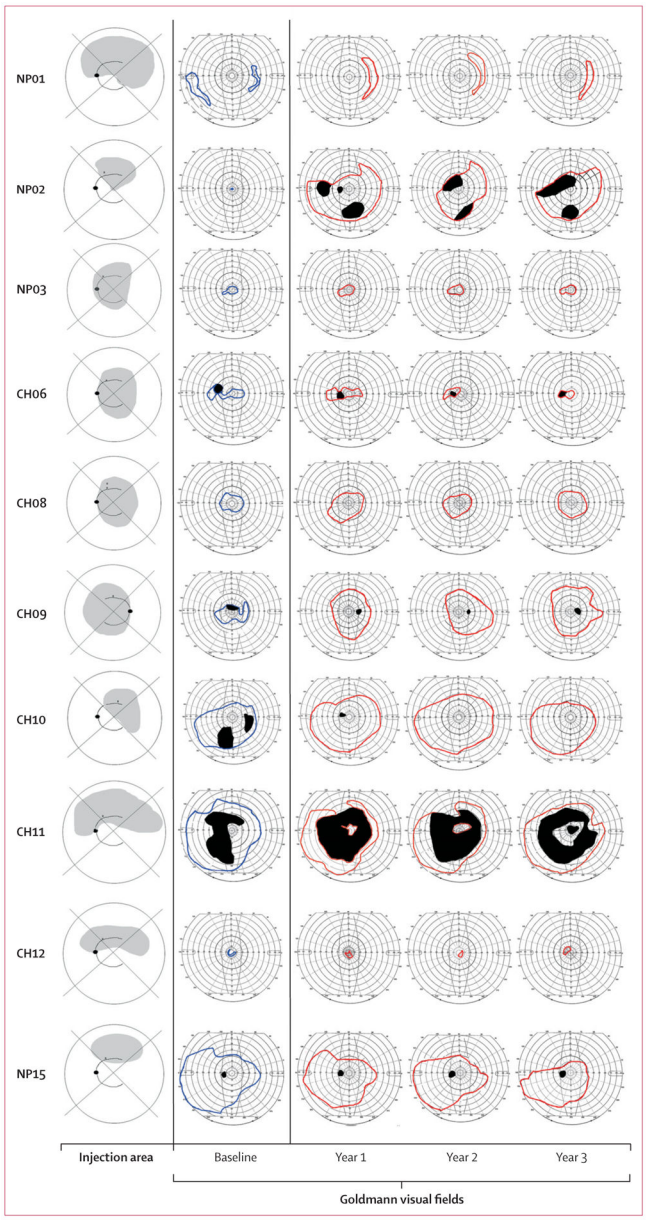

Figure 1. Subretinal areas of second-eye injection and Goldmann visual fields
Scotomas and the natural blind spot are shown in black.

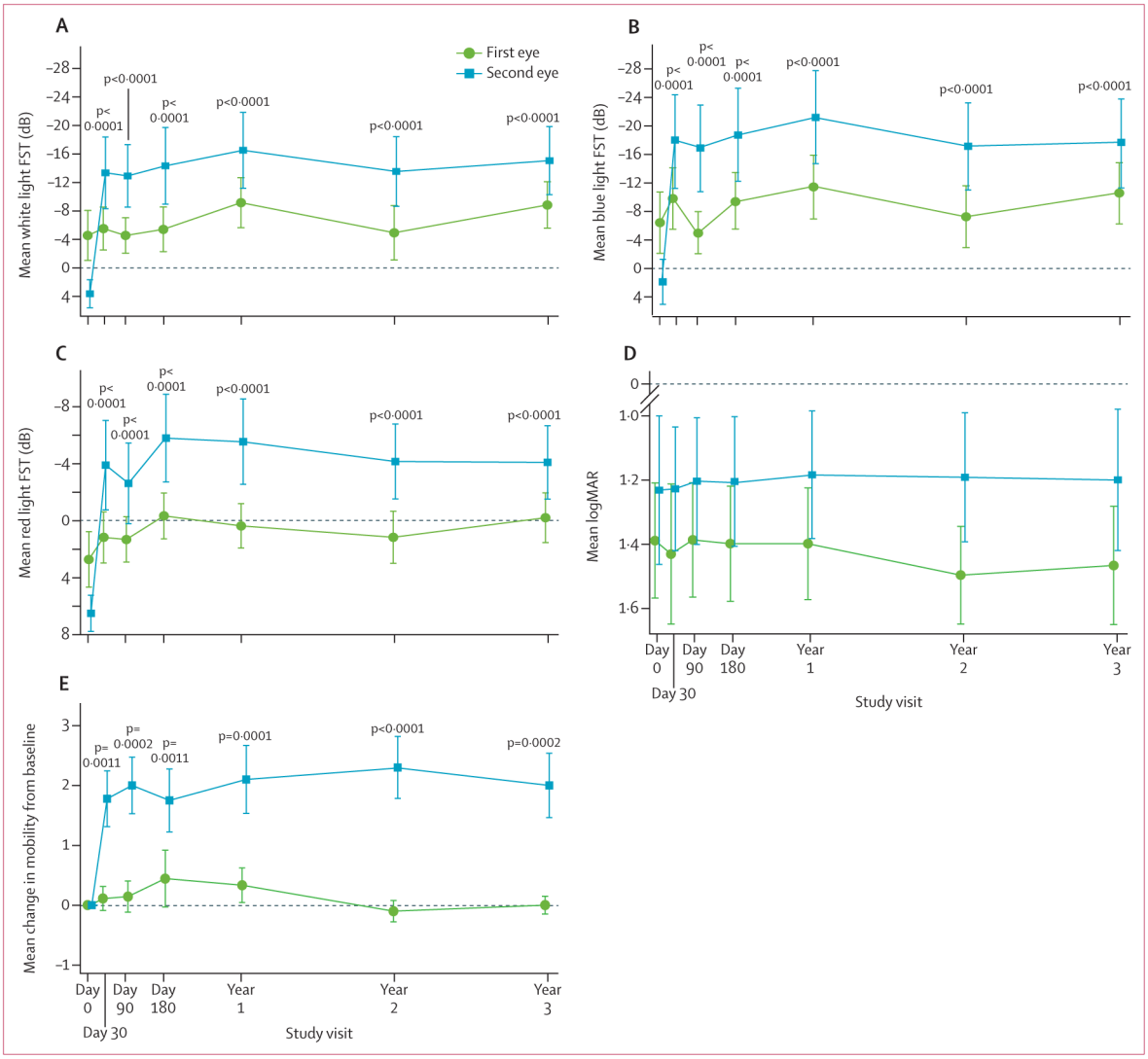

Figure 2. FST with (A) white, (B) blue, and (C) red stimuli, (D) visual acuity measurements, and (E) mobility change score

(A–C) Note that the scales of the *y* axes are different. The more negative the threshold, the higher the sensitivity. (D) Visual acuity measurements are shown as mean logMAR scores for each visit. (E) The mobility change score reflects the change in the ability of the patients to pass the mobility test under lower illuminance than at baseline. Error bars represent 1 SE. Only significant p values (ie, p<0·05) are shown. See appendix p 6 for detailed statistical analyses. FST=full-field light sensitivity thresholds.

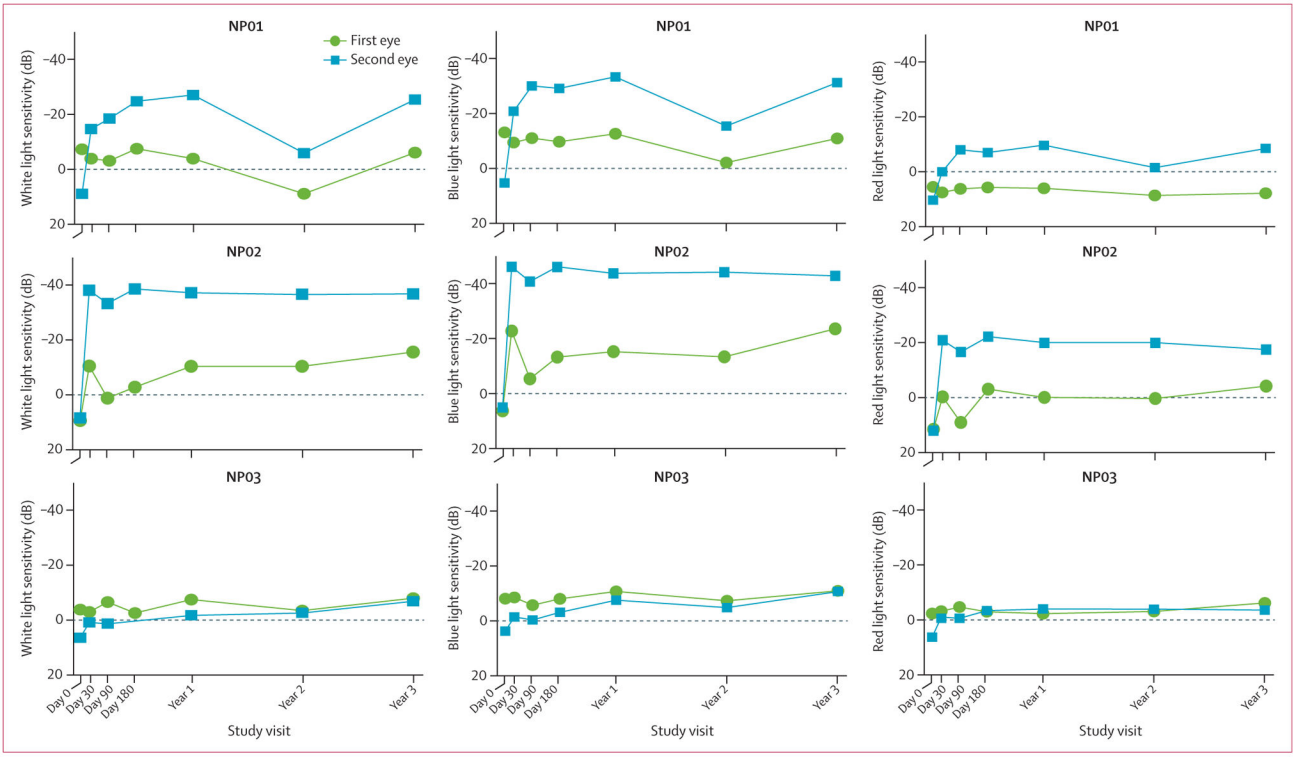

Figure 3.

Full-field light sensitivity threshold results immediately before and after administration of AAV2-hRPE65v2 to the second, contralateral eye in patients given low-dose treatment in their first eye

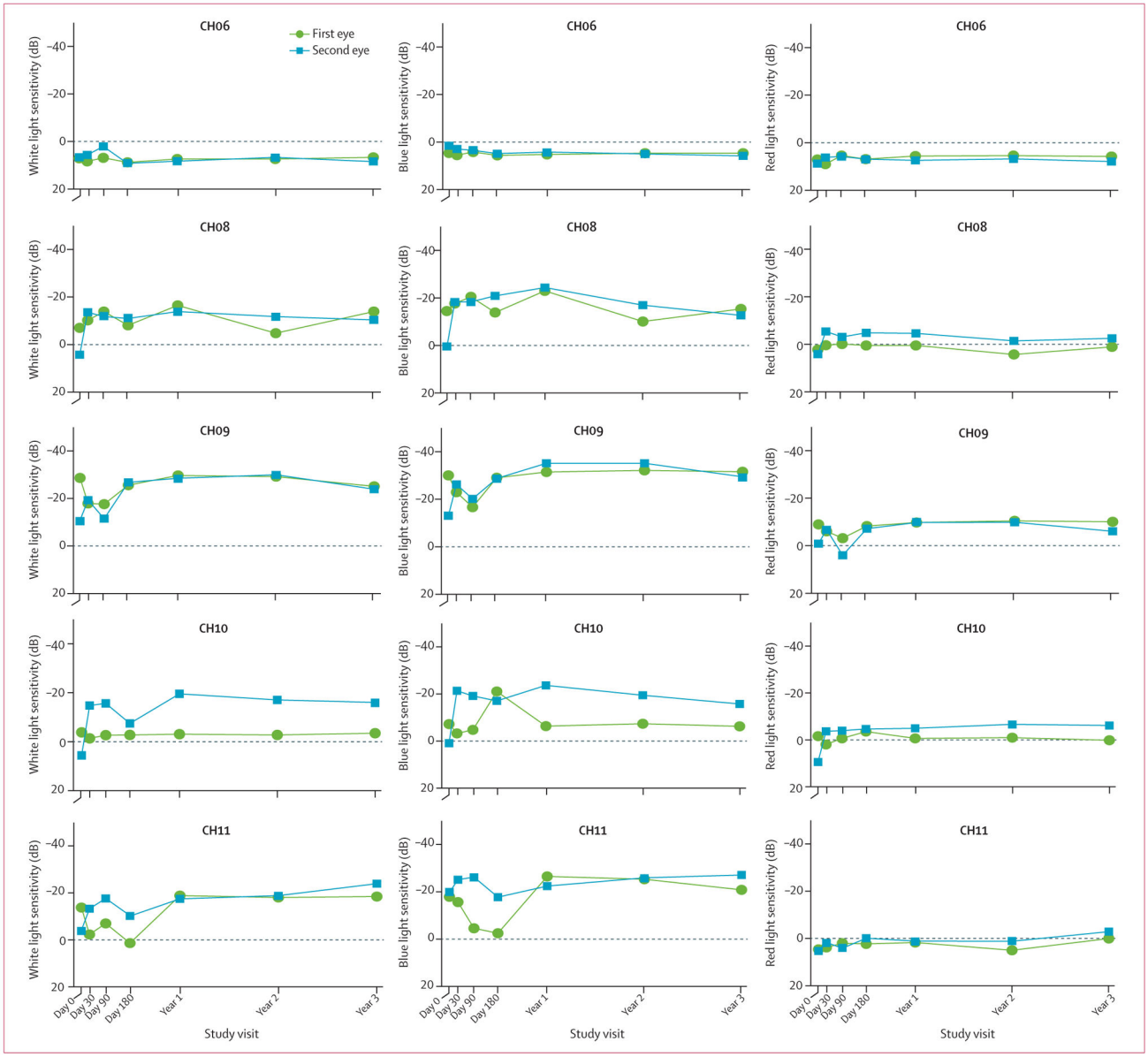

Figure 4.

Full-field light sensitivity threshold results immediately before and after administration of AAV2-hRPE65v2 to the second, contralateral eye in patients given medium-dose treatment in their first eye

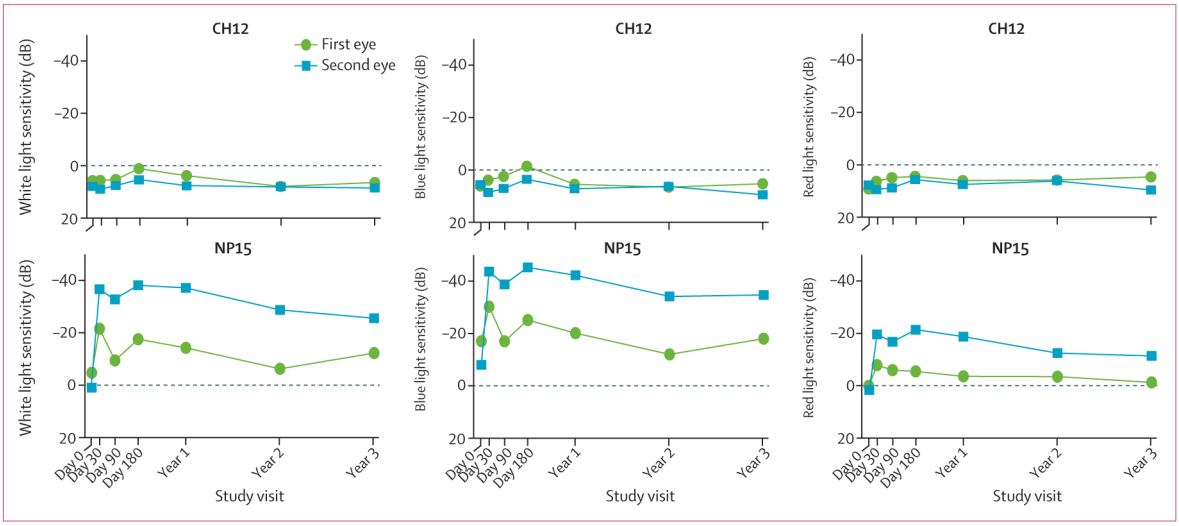

Figure 5.

Full-field light sensitivity threshold results immediately before and after administration of AAV2-hRPE65v2 to the second, contralateral eye in patients given high-dose treatment in their first eye

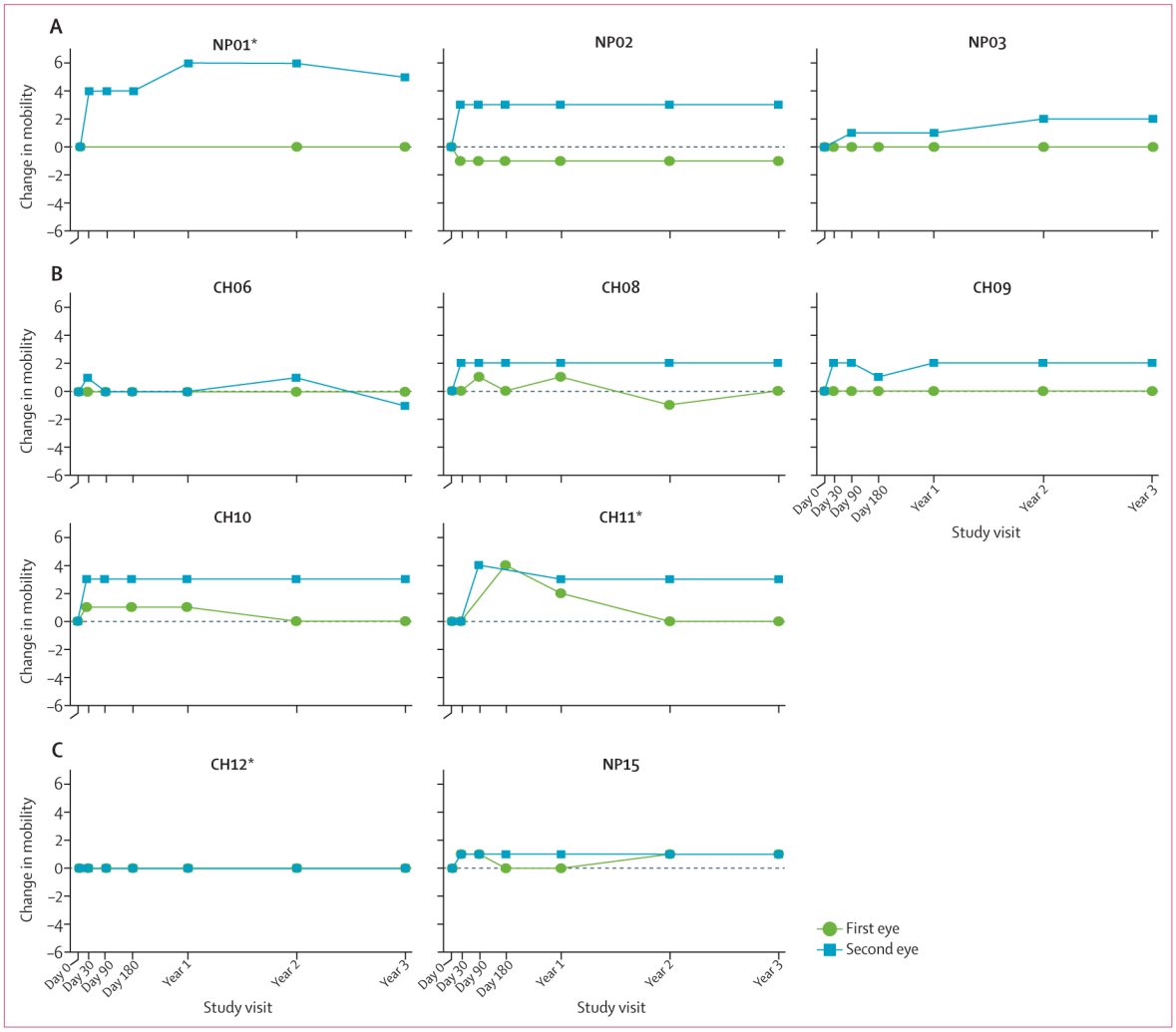

Figure 6. Change in mobility score

Participants are listed according to their original dose assignment when the first eye was injected: (A) low dose, (B) medium dose, and (C) high dose. A positive change score reflects the ability to navigate more accurately and quickly at dimmer light levels than at baseline. See appendix p 6 for detailed statistical analyses. *Mobility testing for NP01, CH11, and CH12 was not standardised until their follow-on visits in year 1. All other participants began the follow-on study after mobility testing standardisation.

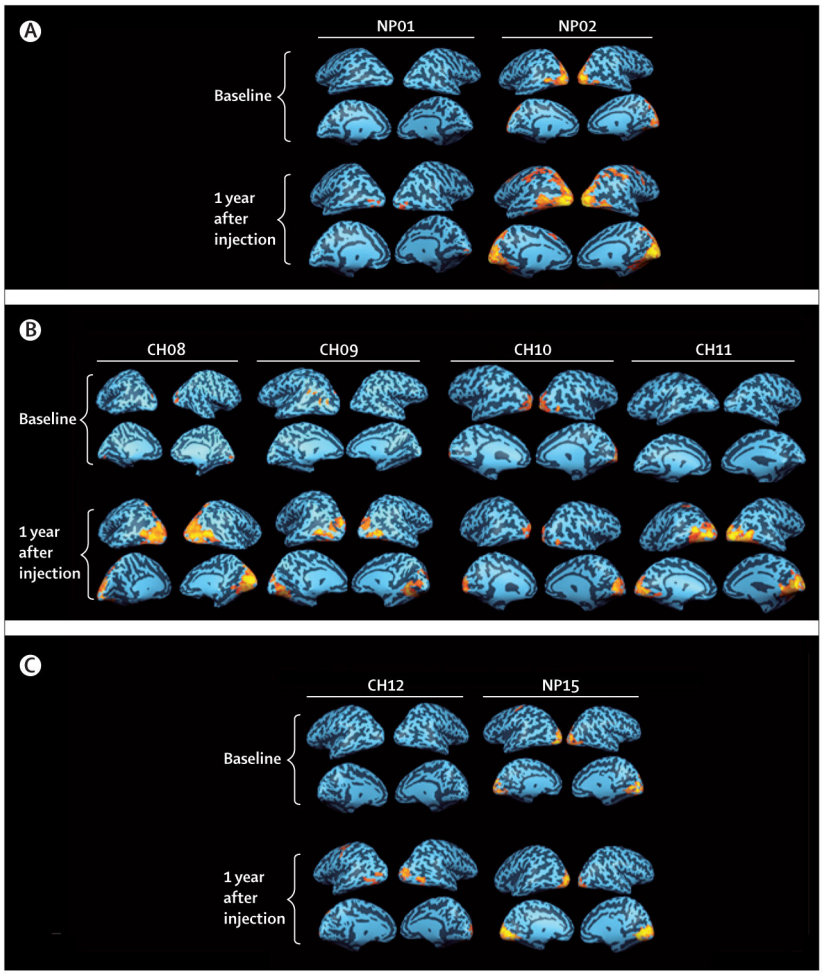

Figure 7. fMRI results at baseline of the follow-on study and at 1 year after administration to the second eye

Participants are listed according to their original dose assignment when the first eye was injected: (A) low dose, (B) medium dose, and (C) high dose. Significant areas of cortical activations are shown as orange clusters overlaid onto the medial and lateral representations of the inflated cortex. Results are presented for the left eye for all but one participant (CH09), who received administration to his right eye in the follow-on study. For follow-up fMRI results, a stringent statistical threshold of false discovery rate <5%, corrected p<0·004, and continuous cluster area ≥100 mm^2 was used for all patients (except NP01).[14,17] For baseline fMRI results, a relaxed threshold of uncorrected p<0·05 and continuous cluster area ≥50 was used for all patients (except NP15 and NP02) to reveal cortical activations.

Table 1

Baseline characteristics and readministration details

	Age at readministration (years)	Retina injected in this study	Time between first and second injection (years)	AAV2-hRPE65v2 dose (viral genomes) and total subretinal volume		*RPE65* mutations	Order of enrolment in this study
				First eye	Second eye		
NP01	29	Left	3·43	$1{\cdot}5 \times 10^{10}$ in 150 μL	$1{\cdot}5 \times 10^{11}$ in 300 μL	Glu102Lys/Glu102Lys	3
NP02	30	Left	4·22	$1{\cdot}5 \times 10^{10}$ in 150 μL	$1{\cdot}5 \times 10^{11}$ in 300 μL	Glu102Lys/Glu102Lys	8
NP03	23	Left	4·58	$1{\cdot}5 \times 10^{10}$ in 150 μL	$1{\cdot}5 \times 10^{11}$ in 300 μL	Arg234X/Arg234X	9
NP04*	21	Right	3·61	$4{\cdot}8 \times 10^{10}$ in 150 μL	$1{\cdot}5 \times 10^{11}$ in 300 μL	Arg91Trp/Thr149Asn	5
CH06	25	Left	4·38	$4{\cdot}8 \times 10^{10}$ in 150 μL	$1{\cdot}5 \times 10^{11}$ in 300 μL	IVS1+5g→a/Leu341Ser†	11
CH08	12	Left	3·41	$4{\cdot}8 \times 10^{10}$ in 150 μL	$1{\cdot}5 \times 10^{11}$ in 300 μL	Phe530fs/Phe530fs	6
CH09	11	Right	3·08	$4{\cdot}8 \times 10^{10}$ in 150 μL	$1{\cdot}5 \times 10^{11}$ in 300 μL	Arg124X/Lys297del1aggA	4
CH10	14	Left	3·21	$4{\cdot}8 \times 10^{10}$ in 100 μL	$1{\cdot}5 \times 10^{11}$ in 300 μL	IVS1+5g→a/Phe530del1ttc	7
CH11	26	Left	2·04	$4{\cdot}8 \times 10^{10}$ in 150 μL	$1{\cdot}5 \times 10^{11}$ in 300 μL	Val473Asp/Val473Asp	2
CH12	46	Left	1·71	$1{\cdot}5 \times 10^{11}$ in 300 μL	$1{\cdot}5 \times 10^{11}$ in 300 μL	Lys303X/Trp431Cys	1
NP15	14	Left	3·28	$1{\cdot}5 \times 10^{11}$ in 300 μL	$1{\cdot}5 \times 10^{11}$ in 300 μL	Asp167Trp/His313Arg	10

Participants are listed in the order in which they were enrolled in the dose-escalation study.[12]

*Enrolled in the follow-on study but not included in the analysis because of bacterial endophthalmitis.

†Also heterozygous for *RDH12* Ser203Arg.

Table 2

Visual and retinal function, functional vision, and activation of the visual cortex of the contralateral eyes, compared with baseline of the follow-on study

	Goldmann visual field*	Change in full-field light sensitivity threshold (dB)†			Visual acuity	Mobility‡	Conversion of rAPD	Cortical activation§	Overall improvement¶
		White	Blue	Red					
NP01	Gain	30	40	20	No change	+++++	Yes	Increased	Yes
NP02	Gain	50	50	30	No change	+++	Yes	Increased	Yes
NP03	Gain	10	10	10	No change	++	Yes	NA	Yes
CH06	Loss	0	0	0	No change	No change	No	NA	No
CH08	Gain	10	10	10	No change	++	Yes	Increased	Yes
CH09	Gain	10	20	10	Worsened by ≥0·3 logMAR	++	Yes	Increased	Yes
CH10	Gain	20	20	20	No change	+++	Yes	Increased	Yes
CH11	Loss	20	10	10	No change	+++	Yes	Increased	Yes
CH12	No change	0	0	0	Improved by ≥0·3 logMAR	No change	Yes	Increased	Yes
NP15	Loss	30	30	10	No change	+	Yes	Increased	Yes

NA=not applicable. logMAR=logarithm of the minimum angle of resolution. rAPD=relative afferent pupillary defects.

*Gain is defined as an increase of ≥20 sum total degrees, and loss is defined as a decrease of ≥20 sum total degrees.

†Values were rounded to the nearest 10.

‡Defined as the ability to navigate at progressively lower light levels (appendix p 6); the number of light levels of improvement (ie, the ability of the participant to pass the test under progressively dimmer illumination) is indicated by the number of plus signs.

§Measured at follow-up year 1.

¶Defined as improvement in at least three measures, including gain of ≥20 degrees on visual fields, gain of ≥10 dB in full-field light sensitivity threshold, improvement by at least one unit on the mobility test, and increased cortical activation.