First-in-Human Gene Therapy Trial of AAV8-hCARp.h*CNGB3* in Adults and Children With *CNGB3*-associated Achromatopsia

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• PURPOSE: To assess the safety and efficacy of AAV8-hCARp.hCNGB3 in participants with CNGB3-associated achromatopsia (ACHM).

• DESIGN: Prospective, phase 1/2 (NCT03001310), open-label, nonrandomized clinical trial.

• METHODS: The study enrolled 23 adults and children with CNGB3-associated ACHM. In the dose-escalation phase, adult participants were administered 1 of 3 AAV8-hCARp.hCNGB3 dose levels in the worse-seeing eye (up to 0.5 mL). After a maximum tolerated dose was established in adults, an expansion phase was conducted in children ≥3 years old. All participants received topical and oral corticosteroids. Safety and efficacy parameters, including treatment-related adverse events and visual acuity, retinal sensitivity, color vision, and light sensitivity, were assessed for 6 months.

• RESULTS: AAV8-hCARp.hCNGB3 (11 adults, 12 children) was safe and generally well tolerated. Intraocular inflammation occurred in 9 of 23 participants and was mainly mild or moderate in severity. Severe cases occurred primarily at the highest dose. Two events were considered serious and dose limiting. All intraocular inflammation resolved following topical and systemic steroids. There was no consistent pattern of change from baseline to week 24 for any efficacy assessment. However, favorable changes were observed for individual participants across several assessments, including color vision (n = 6/23), photoaversion (n = 11/20), and vision-related quality-of-life questionnaires (n = 21/23).

• CONCLUSIONS: AAV8-hCARp.hCNGB3 for CNGB3-associated ACHM demonstrated an acceptable safety and tolerability profile. Improvements in several efficacy parameters indicate that AAV8-hCARp.hCNGB3 gene therapy may provide benefit. These findings, with the development of additional sensitive and quantitative end points, support continued investigation. (Am J Ophthalmol 2023;253: 243–251. © 2023 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/))

A CHROMATOPSIA (ACHM) IS A RARE, AUTOSOMAL, recessive, predominantly stationary cone dysfunction syndrome affecting an estimated 1 in 30 000 births worldwide.[1–3] This disabling congenital disorder is characterized by absent or markedly impaired color discrimination, reduced visual acuity (VA; usually 20/200), central scotoma, eccentric fixation, disabling sensitivity to light (ie, photoaversion [PA]), and pendular nystagmus.[2–4] It is primarily caused by sequence variants in genes that are critical to phototransduction in cone photoreceptors; most cases are associated with changes in either *CNGA3* or *CNGB3*, which encode the α- and β-subunits of the cyclic nucleotide-gated channels, respectively.[2,5] Variants in *CNGB3* account for approximately half of all cases of ACHM.[4] Other genes associated with ACHM include *GNAT2*, *PDE6C*, *PDE6H*, and *ATF6*.[3,5–7]

The majority of those affected have *complete* ACHM, with no function in all 3 classes of cone photorecep-

tor (long-, middle-, and short-wavelength sensitive),[1,4,7,8] which is associated with marked PA and nystagmus, typically from birth, and VA of ≤20/200. *Incomplete* ACHM, in which there is residual function in ≥1 cone subtype, occurs far less frequently and is associated with residual color discrimination, less severe impairment of VA (≥20/40 to ≤20/120), and milder or absent PA and nystagmus.[3,7,8]

There is currently no effective treatment available for ACHM; management strategies aim to mitigate the adverse impact on quality of life (eg, from PA and reduced VA).[3,9] The marked PA, being both uncomfortable and further degrading VA/contrast sensitivity (CS), can result in significant limitations in the ability to perform activities of daily living.[10] In a survey of people with ACHM, 38% named PA as the symptom they would most want to improve above all other aspects of visual impairment, and 73% stated that it affected their employment prospects.[11] These limitations are incompletely mitigated by countermeasures such as sunglasses and tinted contact lenses.[11]

CNGB3-associated ACHM has several characteristics that make it an attractive candidate for gene therapy. Effective improvement in cone photoreceptor function would provide a clear and reliable outcome measure. Also, as ACHM is largely nonprogressive, the extended survival of nonfunctional cones presents a wide window of opportunity during which gene supplementation could provide benefit.[12–14] Moreover, in preclinical models of *CNGB3* deficiency, gene supplementation improved cone function and morphology.[5,15]

There are no peer-reviewed publications of clinical trials of gene therapy for the treatment of adults or children with ACHM associated with *CNGB3*; however, a phase 1/2 clinical trial investigating gene therapy for ACHM associated with *CNGA3* has demonstrated early promise.[16]

The phase 1/2 clinical trial described herein was conducted to assess the safety and efficacy of AAV8-hCARp.hCNGB3 gene replacement in adults and children with *CNGB3*-associated ACHM during a 6-month follow-up.

METHODS

This open-label, nonrandomized, phase 1/2 trial (ClinicalTrials.gov Identifier: NCT03001310) was conducted at one site in the United Kingdom (Moorfields Eye Hospital NHS Foundation Trust, London, United Kingdom) and one site in the United States (University of Michigan Kellogg Eye Center, Ann Arbor, Michigan, USA). The study protocol was reviewed by an independent ethics committee or institutional review board, as appropriate, and the study was conducted in accordance with the principles of the Declaration of Helsinki. All participants or their legal representatives provided consent, or parental permission and assent were obtained.

Eligible participants were ≥3 years of age with a diagnosis of ACHM, confirmed by a study-associated retina specialist, and had accredited laboratory-confirmed homozygous or compound heterozygous missense or null disease–causing variants in *CNGB3*. The study timeline is presented in Supplementary Figure S1. Children were enrolled only after determination of the maximum tolerated dose in adults during the dose-escalation phase. Children were defined as ≥3 years of age and <16 years in the United Kingdom or <18 years in the United States, as per medical legislation. Evidence of relative photoreceptor preservation at the macula, as assessed by spectral-domain optical coherence tomography (SD-OCT) and/or adaptive optics scanning light ophthalmoscopy, was required. Exclusion criteria are presented in the Supplementary Methods. All participants were followed for 6 months after surgery before entering a separate long-term follow-up study (ClinicalTrials.gov Identifier: NCT03278873).

- STUDY TREATMENT: AAV8-hCARp.hCNGB3 is a recombinant serotype 2/8 adeno-associated viral vector containing a human *CNGB3* complementary DNA driven by a 0.4-kb fragment of the human promoter. To determine the maximum tolerated dose of AAV8-hCARp.hCNGB3, adult participants were enrolled in a dose-escalation protocol (Supplementary Figure S2) and administered 1 of 3 doses of AAV8-hCARp.hCNGB3 in a total volume of up to 0.5 mL: low dose, 0.1×10^{12} vg/mL; intermediate dose, 0.3×10^{12} vg/mL; or high dose, 1.0×10^{12} vg/mL. Once the maximum tolerated dose was established, 12 additional participants (children) were enrolled in a confirmatory safety phase. If a dose-limiting event (DLE; Table 1) occurred in the first child within the first 6 weeks, the independent data monitoring committee convened to confirm the dose levels administered to future children.

Efficient transduction of the cone photoreceptor cells required AAV8-hCARp.hCNGB3 to be administered to the subretinal space. The recombinant vector was delivered under general anesthesia to the worse-seeing eye (as determined by the participant and investigator using ocular dominance or VA) as a suspension of viral vector particles injected subretinally under direct observation using an operating microscope. This procedure involved 3-port pars plana vitrectomy followed by injection of the viral vector suspension using a 41-gauge subretinal cannula through ≥1 retinotomies into the subretinal space, with the first retinotomy superior to the macula, close to the superotemporal vascular arcade, with the aim of including the central macula in the bleb created. Tapered oral and topical steroids were prescribed pre- and postoperatively to protect against intraocular inflammatory responses. See the Supplementary Methods for more information.

- ASSESSMENT SCHEDULE: Detailed baseline functional and structural assessments of both eyes were performed, including ocular examination, retinal imaging, psychophys-

TABLE 1. DLEs[a]

Reduction in VA by $\geq$15 ETDRS letters[45,46]
Severe unresponsive inflammation[b]
Infective endophthalmitis
Ocular malignancy
Grade $\geq$3 nonocular suspected unexpected serious adverse reaction

DLE = dose-limiting event, ETDRS = Early Treatment Diabetic Retinopathy Study, VA = visual acuity.
[a]Occurring during the 6 weeks following administration, at least possibly related to AAV8-hCARp.h*CNGB3*, not surgery alone.
[b]Severe unresponsive inflammation was defined according to the Standardization of Uveitis Nomenclature Working Group grading system: anterior chamber cells 3+ (26–50 cells in a field size of 1 × 1-mm slit beam), anterior chamber flare 3+ (marked, iris and lens details hazy), or vitreous haze 3+ that failed to improve by 2 steps (or to grade 0) during a 6-week period.[47]

ical testing, and participant-related outcome assessments. Baseline serology, blood pressure, hematology, and biochemistry were also undertaken. Body mass index, height, weight, pulse, respiratory rate, oxygen saturation, and temperature were measured at baseline; day 2; and weeks 1, 2, and 4. A full clinical ocular examination was conducted on day 1, including VA and intraocular pressure. Color fundus photography, fundus autofluorescence imaging, SD-OCT, and ocular examination were performed at baseline and every postoperative visit (including days 1 and 3).

Measurements of CS, reading, static perimetry, microperimetry, color vision, PA, full-field stimulus testing, and vision-related quality of life (VRQoL) were taken at baseline and weeks 12 and 24. Adaptive optics scanning light ophthalmoscopy was completed for a defined subset of participants (n = 8) in both eyes at baseline and week 24. Blood samples were collected to measure immune response at baseline and then 4 weeks, 3 months, and 6 months after surgery.

• OUTCOME MEASURES: The primary end point was safety and tolerability of subretinal administration of AAV8-hCARp.h*CNGB3*, defined as the absence of a DLE within 6 weeks of administration. Clinical assessment of intraocular inflammation and anatomic integrity was conducted using slitlamp biomicroscopy and retinal imaging, including SD-OCT.

Efficacy was the secondary objective and was explored (1) on an individual basis, (2) by dose, and (3) by adult or child cohort, examining any change from baseline in assessments of visual and retinal function and functional vision.

Best-corrected Early Treatment Diabetic Retinopathy Study VA was measured both monocularly and binocularly, and CS was measured using the Pelli-Robson chart and reported as logarithm of CS (logCS), on which higher logCS values denote better contrast sensitivity.[17–19] Reading ability, including reading acuity, maximum reading rate, and critical print size, was assessed with MNREAD cards[20] and International Reading Speed Texts.[21]

Color vision was assessed using the American Optical Hardy-Rand-Rittler (HRR) plates test.[22] This evaluation was performed at baseline and postsurgery at weeks 12 and 24. The direction of improvement would be an increase in the number of plates correctly identified by the participant. Computerized color vision tests were performed with the Metropsis system (Cambridge Research Systems Ltd, Rochester, United Kingdom) using the low-vision version of the Cambridge Colour Test (lvvCCT) and the Universal Colour Discrimination Test (UCDT), specifically the trivector versions that measure the chromatic discrimination along the protan, deutan, and tritan axes.[23–25] These tests measure the amount of saturation required to discriminate a colored target from a series of gray distractors, with a reduction in needed saturation as a marker of color-sensitivity improvement.

Retinal sensitivity was determined by static perimetry using a custom 185-point grid (Octopus 900; Haag-Streit AG) and full-field stimulus testing, a psychophysical threshold method to evaluate light sensitivity.[26] The retinal locus of fixation and bivariate contour ellipse area were also determined using microperimetry, as captured by the Macular Integrity Assessment device (CenterVue). Full-field electroretinogram (ERG) and pattern ERG were performed according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards[27–29] to assess both generalized retinal (rod and cone systems) and isolated macular function. Modified ISCEV protocols were used as necessary in younger children using internationally recognized protocols.

The degree of light sensitivity (ie, PA) was investigated by video analysis of participant response to a uniform light stimulus (3 study-independent reviewers evaluated the videos to assess monocular palpebral aperture narrowing in response to a light stimulus) and by a participant-reported symptomatology questionnaire (assessing severity of PA with and without aids, and its impact on daily activities on an 11-point [0–10] rating scale).[11] Participant response (ie, no change, improved, or worsened) was determined by agreement of 2 of 3 reviewers. Further details of this assessment, as well as for palpebral aperture measurements, are presented in the Supplementary Methods.

Patient-reported outcome (PRO) assessments included the Impact of Visual Impairment (IVI) questionnaire (IVI for adults, IVI-C for children), which is an assessment of VRQoL.[30–34] General health-related quality of life (HRQoL) was measured with the EuroQol 5 Dimension 5 Level (EQ-5D-5L)[35] for adults and the EuroQol 5 Dimension 3 Level Youth (EQ-5D-Y) for children and adolescents.[36,37] More information can be found in the Supplementary Methods.

• STATISTICAL ANALYSES: Given that the primary outcome of this study was safety and tolerability, and given the exploratory nature of the efficacy assessments, this study was not powered for formal statistical analysis. Because of the rare nature of ACHM and the small study sample, no formal sample size calculations were performed, and no formal hypothesis testing was conducted. Furthermore, the evaluation of response was based on clinical interpretation as standards of clinical meaningfulness have not yet been established for most outcome assessments in ACHM.

RESULTS

AAV8-hCARp.hCNGB3 was administered to 23 participants (20 in the United Kingdom, 3 in the United States). Following completion of dose escalation in 11 adults, 12 children were treated in the confirmatory safety phase. The first child, who received the high dose of 1.0×10^{12} vg/mL, developed a DLE of ocular inflammation. As a result, all subsequent doses were either the intermediate dose (0.3×10^{12} vg/mL) or an additional "other" dose (0.6×10^{12} vg/mL). The additional dose of 0.6×10^{12} vg/mL was selected in consultation with the independent data monitoring committee and administered to maximize therapeutic effect while maintaining the safety profile of AAV8-hCARp.hCNGB3.

In total, 3 participants received 0.1×10^{12} vg/mL (low dose), 12 received 0.3×10^{12} vg/mL (intermediate dose), 3 received 0.6×10^{12} vg/mL ("other" dose), and 5 received 1.0×10^{12} vg/mL (high dose). The majority of participants were female (69.3%), and all were White. Mean (SD) age in years was 24.4 (5.8) for adults (n = 11) and 10.3 (2.7) for children (n = 12). Demographic and baseline characteristics were generally balanced within the adult and child cohorts (Supplementary Table S1).

Twenty-two of the 23 participants had a single retinotomy performed during administration, and 1 participant had 2 retinotomies. Adult and child participants received subretinal injection volumes ranging from 0.2 to 0.5 mL (mean, 0.35 mL); the total number of viral genomes delivered ranged from 0.3 to 4.5×10^{11}. No surgical complications were observed during dose administration.

• SAFETY: AAV8-hCARp.hCNGB3 demonstrated an expected and manageable adverse event (AE) profile. Three (13.0%) participants experienced 1 primary safety outcome event, as defined in Table 1 (all severe inflammation: 1 in the intermediate-dose group, 2 in the high-dose group), that met the Standardization of Uveitis Nomenclature Working Group criteria, also defined in Table 1. One participant experienced severe inflammation on day 19, and the 2 other participants experienced severe inflammation on day 43 after surgery. All events were resolved before the end of the study (6 months posttreatment).

Intraocular inflammation was the principal AE related to the study therapy. Nine (39.1%) participants (5 adults, 4 children) experienced an intraocular inflammation AE considered related to the gene therapy itself; 2 of these (n = 2/23; 8.7%) were considered serious AEs (SAEs; 1 in the intermediate-dose group, 1 in the high-dose group) and were DLEs. More severe events of inflammation tended to occur at higher dose levels, whereas those at the lowest dose were less severe and of shorter duration. Inflammation was reported both in the anterior chamber and in the intermediate and posterior ocular segments in the form of intermediate uveitis and panuveitis.

All cases resolved with an extended course of topical and/or systemic corticosteroid treatment. No baseline factors appeared to be predictive of ocular inflammation. Both participants with an SAE of inflammation completed the study per protocol and participated in the long-term follow-up study. One of these 2 participants also experienced an SAE of ectopic pregnancy that was managed by emergency surgery; this resolved and was considered unrelated to the gene therapy.

The most common treatment-emergent AEs (Table 2) were eye disorders. All participants reported ≥1 ocular AE, as well as ≥1 AE considered "definitely related" to the surgery, including conjunctival hemorrhage (20 [87.0%]), lenticular opacities (15 [65.2%]; 9 children, 6 adults), and reduced VA (15 [65.2%]). VA reduction was transient and related to the surgical procedure; VA returned to baseline for all affected participants during the first 4 weeks of follow-up.

SD-OCT imaging revealed a minimal mean increase in central retinal thickness (CRT) from baseline to week 24 postintervention at the group level (1.2 μm). This finding was not secondary to edema or fibrosis and is likely not clinically significant. The manufacturer's technical specifications include a 1.8-μm estimated SD of repeatability of CRT volume scans.[38] See Supplementary Figure S3 for line plots of CRT change from baseline over time for individual participants. Additionally, no safety signals were noted from the color fundus photography or fundus autofluorescence imaging.

There were no clinically significant changes in laboratory tests, vital signs, or physical examination. No participants had an AE leading to study discontinuation.

	Low Dose (n = 3)	Intermediate Dose (n = 12)	Other Dose (n = 3)	High Dose (n = 5)	Total (n = 23)
Participants with ≥1:					
AE	3 (100)	12 (100)	3 (100)	5 (100)	23 (100)
AEs related to treatment	1 (33.3)	3 (25.0)	2 (66.7)	3 (60.0)	9 (39.1)
SAE	0	1 (8.3)	0	1 (20.0)	2 (8.7)
SAEs related to treatment	0	1 (8.3)	0	1 (20.0)	2 (8.7)
AE leading to discontinuation from study	0	0	0	0	0
AE of interest related to ocular inflammation	2 (66.7)	2 (16.7)	2 (66.7)	3 (60.0)	9 (39.1)
AE leading to death	0	0	0	0	0
Participants with AEs by severity					
Mild	0	2 (16.7)	1 (33.3)	1 (20.0)	4 (17.4)
Moderate	3 (100)	9 (75.0)	2 (66.7)	2 (40.0)	16 (69.6)
Severe	0	1 (8.3)	0	2 (40.0)	3 (13.0)
Participants with AEs related to surgery					
Possibly related	0	0	0	0	0
Probably related	0	0	0	0	0
Definitely related	3 (100)	12 (100)	3 (100)	5 (100)	23 (100)
Specific AEs					
Eye disorders	3 (100)	12 (100)	3 (100)	5 (100)	23 (100)
Conjunctival hemorrhage	3 (100)	10 (83.3)	2 (66.7)	5 (100)	20 (87.0)
Lenticular opacities	2 (66.7)	7 (58.3)	3 (100)	3 (60.0)	15 (65.2)
VA reduced	2 (66.7)	7 (58.3)	1 (33.3)	5 (100)	15 (65.2)
Subretinal fluid	2 (66.7)	4 (33.3)	0	3 (60.0)	9 (39.1)
Foreign body sensation in eyes	0	7 (58.3)	0	1 (20.0)	8 (34.8)
Ocular discomfort	0	5 (41.7)	0	3 (60.0)	8 (34.8)
Chorioretinal folds	0	4 (33.3)	0	2 (40.0)	6 (26.1)
Retinal hemorrhage	2 (66.7)	2 (16.7)	0	2 (40.0)	6 (26.1)
Nervous system disorders	2 (66.7)	7 (58.3)	1 (33.3)	2 (40.0)	12 (52.2)
Headache	1 (33.3)	3 (25.0)	1 (33.3)	1 (20.0)	6 (26.1)
Visual field defect	0	4 (33.3)	0	1 (20.0)	5 (21.7)
Infections and infestations	1 (33.3)	6 (50.0)	1 (33.3)	1 (20.0)	9 (39.1)
Rhinitis	0	4 (33.3)	0	1 (20.0)	5 (21.7)
Investigations	2 (66.7)	1 (8.3)	1 (33.3)	2 (40.0)	6 (26.1)
Intraocular pressure increased	2 (66.7)	1 (8.3)	1 (33.3)	2 (40.0)	6 (26.1)

AE = adverse event, SAE = serious adverse event.

[a] All data are displayed as n (%).

[b] Any AE occurring on or after the initial administration of gene therapy was considered treatment emergent.

[c] Incidence (%) is based on the number of participants in each group, not the number of events.

[d] AEs are coded using the *Medical Dictionary for Regulatory Activities*, version 22.1.

• **EFFICACY:** There was no systematic pattern of change from baseline to week 24 for any specific efficacy assessment between the treated and untreated eye across the entire study population, within either cohort (adult or child), or with respect to dose. However, favorable changes were observed for individual participants across several assessments, including ≥1 domain of color vision (lvvCCT; n = 6/23), PA (investigator assessed; n = 11/20), and the VRQoL questionnaires at week 24 (≥1 domain of the IVI; n = 21/23). See Supplementary Table S2 for a summary of responses for the treated vs untreated eyes at baseline vs week 24 for bivariate contour ellipse area, CRT, microperimetry, HRR, UCDT, and lvvCCT.

Pattern ERGs were unrecordable or undetectable at baseline and follow-up. Additionally, no participant had any significant treatment-related change in full-field ERG measures beyond expected intersession variability.

For color vision, 6 of 23 (26%) participants had improvements in ≥1 axis using the lvvCCT, as assessed by comparison of 24-week measurement with baseline. There were no systematic differences between the treated and untreated eyes across all participants (Table 3; Supplementary Figures S4-S6).

A qualitative assessment of PA by graders blinded to which eye had been treated (video review of palpebral aperture narrowing in response to a light stimulus) detected improvement in comparison to baseline in the treated eye in

| | Measured Value | Change From Baseline | | |
	Mean (SD)	Range	Mean (SD)	Range
Treated (n = 23)				
Baseline				
lvvCCT deutan	104.9 (6.84)	87, 110		
lvvCCT protan	104.9 (6.84)	81, 110		
lvvCCT tritan	107.3 (3.55)	98, 110		
Week 24				
lvvCCT deutan	105.3 (14.48)	44, 110	0.6 (11.47)	−43, 21
lvvCCT protan	103.3 (15.87)	54, 110	−1.6 (11.07)	−40, 8
lvvCCT tritan	102.2 (11.48)	74, 110	−5.1 (11.55)	−33, 10
Untreated (n = 23)				
Baseline				
lvvCCT deutan	104.3 (9.81)	74, 110		
lvvCCT protan	101.7 (11.67)	64, 110		
lvvCCT tritan	105.7 (5.82)	91, 110		
Week 24				
lvvCCT deutan	107.3 (5.42)	88, 110	3.0 (9.77)	−20, 27
lvvCCT protan	106.3 (5.04)	94, 110	4.7 (11.84)	−13, 35
lvvCCT tritan	105.2 (11.60)	56, 110	−0.6 (9.50)	−35, 13
Treated − untreated (n = 23)				
Baseline				
lvvCCT deutan	0.6 (8.26)	−11, 31		
lvvCCT protan	3.2 (9.01)	−12, 35		
lvvCCT tritan	1.5 (4.17)	−10, 11		
Week 24				
lvvCCT deutan	−1.8 (13.68)	−56, 22	−2.3 (15.88)	−63, 20
lvvCCT protan	−3.0 (15.33)	−49, 16	−6.3 (18.41)	−55, 17
lvvCCT tritan	−2.9 (13.49)	−36, 39	−4.5 (12.78)	−33, 28

TABLE 3. Summary of Trivector lvvCCT and Change From Baseline Over Time

lvvCCT = low-vision version of the Cambridge Colour Test.

11 of 20 (55.0%) participants. Quantitative palpebral aperture measurements for selected individual participants are shown in Supplementary Figure S7.

Improvements in VRQoL were observed across a range of domains. Ten of 11 (90.9%) adult participants showed improvements in ≥1 IVI domain, and 11 of 12 (91.7%) child participants showed improvement on the IVI-C questionnaire. Overall, 9 of 11 (81.8%) adult participants and 10 of 12 (83.3%) child participants exhibited improvements across ≥2 domains (Supplementary Tables 3 and 4).

Ratings of the more general HRQoL were equivocal. On the EQ-5D-5L and the visual analog scale (EQ-VAS), no changes from baseline were observed at week 24 across the domains of mobility, self-care, and anxiety/depression. Among child participants, a reduction was observed in the percentage of participants reporting problems on the EQ-5D-Y domains of mobility (2/12; 16.7%), usual activities (2/12; 16.7%), and anxiety/depression (2/12; 16.7%). No change was observed in the pain (1/12; 8.3%) dimension, and 1 (8.3%) participant reported problems with self-care at week 24. A small improvement in change from baseline in mean ± SD EQ-VAS score was observed at week 24 (4.2 ± 8.7).

PRO item-level post hoc analyses revealed ceiling effects at baseline in >25% and up to 100% of study participants for 20 of 28 items of the IVI, 17 of 24 items of the IVI-C, and 5 of 5 items of the EQ-5D, an indicator that many items in each instrument may not be relevant to the target population and would inherently limit the ability of the PRO instrument to detect treatment effects. PRO item and domain analyses are shown in Supplementary Tables S3 to S6.

One 21-year-old female participant (01-009) demonstrated improvement at week 24 in color vision and positive changes in functional vision based on observed improvements on the IVI reading and accessing information domain. She had evidence at baseline of residual color vision and better VA than average, suggesting incomplete ACHM. Measures of color vision for this participant are shown in Supplementary Figure S8.

DISCUSSION

In this first-in-human study, subretinal delivery of AAV8-hCARp.hCNGB3 gene therapy in adults and children with

ACHM associated with *CNGB3* was found to have an AE profile that is anticipated and manageable. There were no consistent changes from baseline in any efficacy assessments across the dose or age cohorts at week 24.

As expected, given surgical delivery, ocular AEs were common.[39] Ocular inflammation is an anticipated response to the injection of viral protein and is known to occur in ocular gene therapeutic applications with other similar agents.[40] Most ocular AEs were temporally related to the surgery, were mild in severity, and resolved with minimal intervention. There was a trend toward more severe cases of intraocular inflammation occurring at higher doses. Inflammation appeared to be dose related and was reduced through appropriate dose selection and prophylactic use of corticosteroids. Overall, the safety results support further exploration of efficacy in future studies.

Improvements of varying magnitude were observed in several assessments at the individual participant level in both children and adults (eg, in color vision and PA). Based on a clinical interpretation of change, the assessments that most frequently suggested benefit were PA and color vision testing and the participant-reported scores on the IVI questionnaire. One participant demonstrated a notable improvement in color discrimination.

An analysis of 4 children with *CNGA3*- or *CNGB3*-associated ACHM receiving gene therapy in clinical studies NCT03758404 and NCT03001310 evaluated treatment-related changes in cone function against untreated participants.[40] Two of the 4 demonstrated cone-mediated signals in the visual cortex and significant improvements in psychophysical measures of cone-mediated visual function. These results represent the opportunity for gene therapy to activate dormant cone-signaling pathways in ACHM.[41] Overall, our findings demonstrate short-term safety and tolerability, with the positive trends observed in select participants, meriting further investigation.

There are several limitations and areas for development, some of which are inherent to the small sample sizes of trials in ultra-rare diseases. Additionally, the lack of a concurrent control group limited the generalizability of the findings, notably regarding the interpretations of end points such as PROs. Most of the efficacy assessments performed in this study are neither conducted in routine clinical practice nor fully validated. In addition, international-standard suprathreshold assessments may not be sufficiently sensitive to identify small or close-to threshold responses and do not exclude the possibility of a therapeutic response at week 24. This study attempted to identify sensitive end points, but 24 weeks may be of insufficient duration to determine later-onset benefit. The results of the study should be interpreted in the context of these limitations and considered in the design of forthcoming trials.

Although there are no approved treatments for ACHM, several small-scale gene therapy studies for AAV8-h*CNGA3* and AAV2tYF-PR1.7-h*CNGA3* have demonstrated preliminary safety and efficacy.[16] Assessing novel end points will be important to provide treatment options that address the limitations of VRQoL and visual function assessments for people with ACHM. There are no precedents for clinical development of a therapeutic agent for ACHM or for its regulatory approval. As such, limited information was available to guide optimal trial design, inclusion criteria, or end points.

In this study, concerns that a lack of cortical plasticity might preclude a benefit to vision in older participants led to a decision to enroll younger participants in the safety-confirmation expansion phase.[42] The youngest participant included in this study was 5 years old, which may be beyond the critical period of visual development. Therefore, there may not be significant differences in cortical plasticity between the ages of participants included in this study, and, thus, studying gene therapy in even younger children may identify more meaningful improvements in vision.[41] Nevertheless, age did not appear to be a determinative factor in measurable response in our trial, with arguably the most promising response being in a young adult, likely with incomplete ACHM. Future studies should also work toward a better understanding of factors such as age, baseline severity of illness, phenotype, and genotype.

In addition, it will be necessary to further refine and validate relevant efficacy measures that address clinically meaningful treatment effects. Reduction in PA, a frequent and debilitating symptom, is one of the priorities for this participant population,[11,43] with further investigation needed to better understand how PA can be optimally and objectively quantified and potentially correlated with improvements in visual function and PROs. The data from our trial, along with findings from qualitative and quantitative evaluations of PROs in an ACHM population, indicate that developing or modifying PRO measures specific to ACHM are required to address the ceiling effects and lack of content validity of existing PRO measures.

Initial reports of participant feedback to investigators after the intervention indicated that they were, for example, better able to "see colors," "go outside without having to wear protective eyewear," "distinguish between black and white," and "see more clearly when exposed to high light levels." This was reflected in post hoc analyses of item 3 of the PA questionnaire (ie, 3 fewer participants reported "increased difficulty seeing due to PA" at week 24 compared to baseline).

Similarly, tailoring VA and CS measurements to the specific abilities and deficits of adults or children with ACHM may help capture possible treatment effects. Adults or children with ACHM have greater functional difficulty in photopic conditions.[8] Best-corrected VA (BCVA) in this study was evaluated under standard conditions with room lights turned off and chart lights turned on, with BCVA unchanged over the course of the study. Assessing BCVA at varying levels of ambient luminance merits further exploration.[44] Regarding CS, the Pelli-Robson

test may be insensitive in this population because this test presents only 1 spatial frequency and is limited in terms of contrast resolution. To address these limitations, contrast-detection thresholds across multiple spatial frequencies may indicate improvements with greater precision.

In conclusion, the results of this study demonstrate an acceptable safety profile for AAV8-hCARp.h*CNGB3* in adults and children with ACHM. Indications of benefit in individual participants merit further investigation of efficacy and provide valuable data to optimize the design of future trials.

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