Supplementary appendix

Supplement to: Xue K, Jolly JK, Barnard AR, et al. Beneficial effects on vision in patients undergoing retinal gene therapy for choroideremia

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Supplementary methods

SURGERY

In choroideremia, unlike many other retinal degenerations, loss of the retinal pigment epithelium leads to a scarring reaction of the underlying choroid which then becomes firmly adherent to the residual retina. The central island of functional retina however remains intact, leaving a tissue plane for vector administration underneath the retina. If however fluid is injected into this plane too quickly, then the developing fluid bleb will form a tight bubble and stretch the central retina, because the peripheral scar tissue will prevent subretinal fluid from propagating outwards into the retinal periphery. This retinal stretch reaction has two negative consequences. First it may directly damage the neurosensory retina – in C1 the papillomacular bundle was subject to stretch as reported previously, because the outer nuclear layer was deficient in this area making it thinner than the central retina and subject to more stretch at a given pressure according to Hooke's Law.[37] Second, a higher fluid pressure in the bleb will result in a tendency for reflux of vector suspension back through the widened retinotomy into the vitreous, thereby reducing the therapeutic dose delivered and increasing the risk of post-operative inflammation. Hence following the complication in C1 in which the vector was injected manually from a 1 mL syringe, a new vector administration device was designed and tested, which allowed a precise foot-pedal controlled slow infusion of the vector.[34,35] This was used successfully in H3—7 in whom vector administration was uncomplicated. A bubble of heavy liquid, perfluoro-n-octane (Bausch & Lomb, Rochester, New York, USA) was used to stabilise focal areas of retinal thinning in H3 and H4 during vector infusion, as has previously been reported.[38] Static intraoperative OCT imaging was also used to identify the extent of retinal detachment in patients H3—7. This comprised a vertically aligned OCT (Spectralis, Heidelberg Engineering, Heidelberg, Germany) that was moved in and out of the operating field at various stages to assess the plane of subretinal injection and degree of retinal stretch. This greatly facilitated the surgical phase and has since been superseded with a dedicated intraoperative OCT system embedded within the operating microscope, which can also assess retinal stretch dynamically during vector injection.[35] Although 0.1 ml was injected in all but C1, it is not possible to see the exact location of the subretinal vector without help from the intraoperative OCT scan which indirectly shows the extent of the retinal detachment enlarging as the vector is infused. While the 41 gauge cannula used to inject the vector creates a self-sealing retinotomy, additional steps taken to minimise vector reflux from the subretinal bleb included positioning the injection sites superior to the macula when possible, avoiding the use of intraocular air or gas tamponade at the end of surgery (which might displace the vector suspension to the inferior retina), and supine posturing of the patients for 1 hour after surgery.

VISUAL FUNCTION

Visual function was assessed with BCVA according to the ETDRS protocol. Microperimetry testing using the MAIA microperimeter (CenterVue, Padova, Italy) followed the protocol described previously,[16] except in H3—7, standard non-customised 20° (38 stimuli) central grids were used. For participants with profound visual field restriction who could achieve barely detectable or 0 decibels (dB) mean threshold retinal sensitivities on the 20° grids (H4—6), a 10° central grid was used instead. Following subjective reports of improved colour vision in L3, colour vision assessment using the Farnsworth-Munsell 100 hue (FM100) test was added in the protocol change and included for H1—7. While further subjective descriptions of improved colour perception in the treated eye were reported by H2, H5, H6 and H7, the test proved difficult to perform in patients with advanced visual field loss. Anatomical assessments included spectral domain OCT and fundus autofluorescence imaging (BluePeak, Heidelberg Engineering). Due to the delay midway through the trial to optimize the surgical technique, the first 5 patients (L1—5) have 5 years of follow-up whereas the last 5 (H3—7) have only recently reached 2-year follow-up, with intermediate follow-up periods in between. The data are therefore presented in two ways: the first is across all 12 patients at 2 years, as per the original end-point defined in the protocol and the second is the last point of follow-up, for which L1—5 and H1—2 extend beyond 2 years. Due to the end-stage nature of the disease in this cohort, reliable electrophysiology responses could not be recorded and no measurable change could be detected. Details of the electrophysiological tests in the first 6 patients are detailed elsewhere.[17]

Figure S1: **Complete gel images for Fig.1b**
Complete images of the western blots from Fig.1b prior to cropping of 3 middle lanes unrelated to this study.

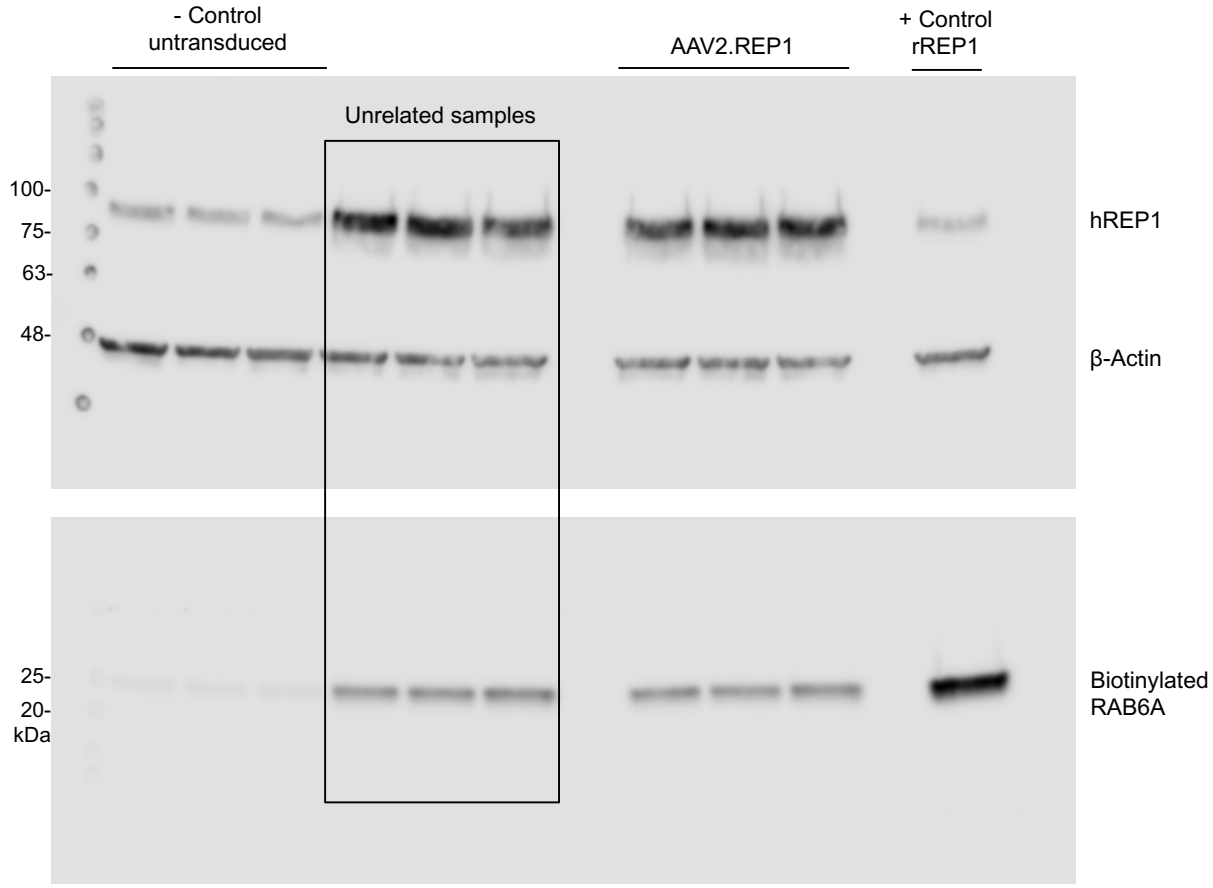
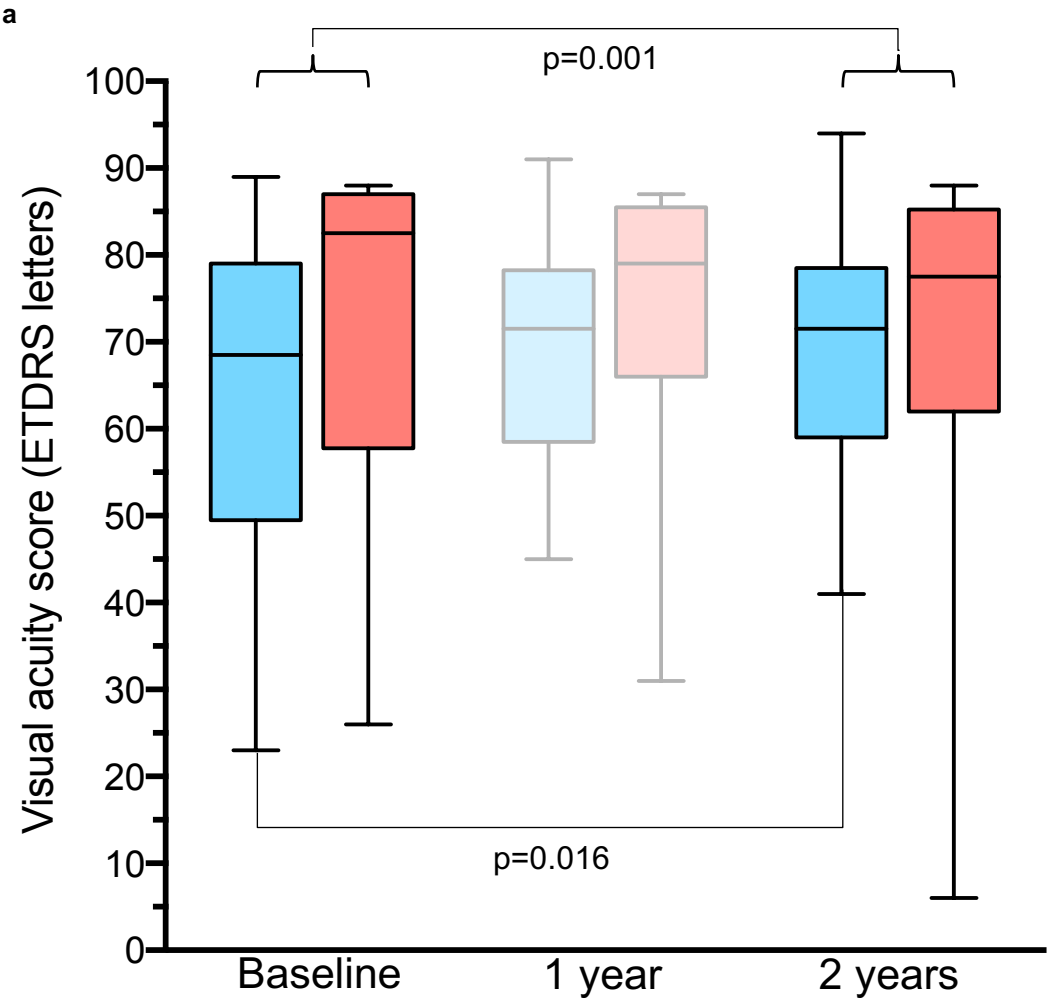


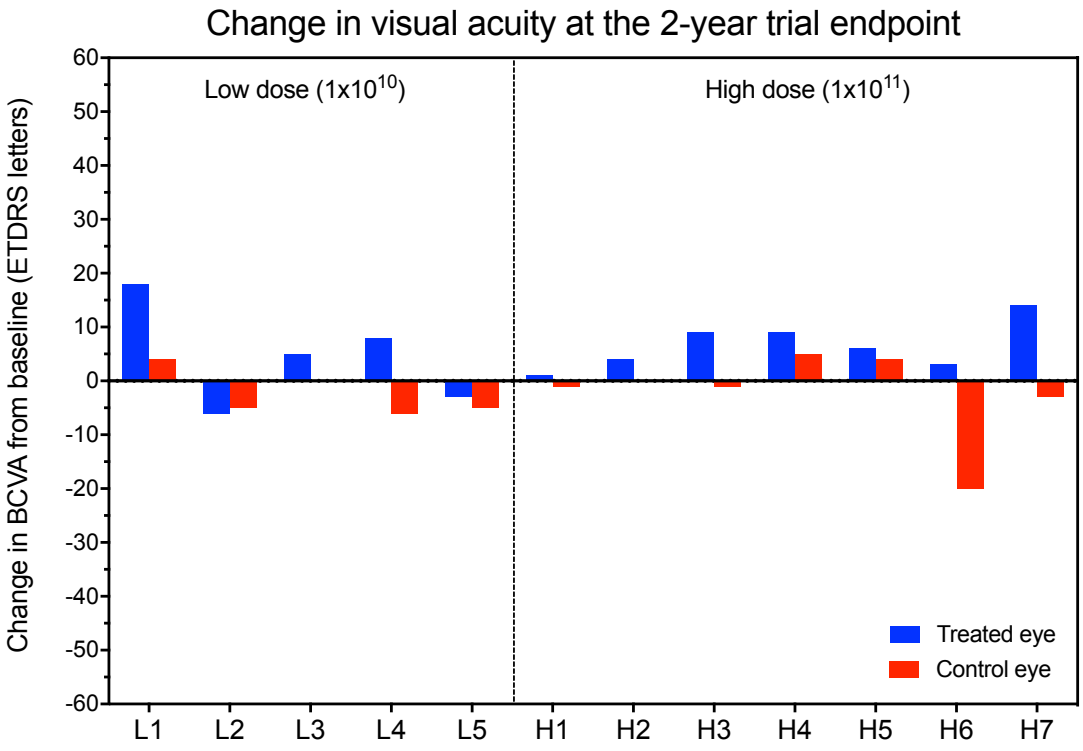
Figure S2: **Visual acuity at the 2-year trial endpoint and last follow-up**
(**a**) Best-corrected visual acuities (BCVA) in the treated eyes (blue) and untreated eyes (red) at 2 years after gene therapy, measured with the ETDRS chart in the 12 choroideremia patients who received gene therapy per protocol without complications. Horizontal line represents median, box plot represents interquartile range, and whiskers represent range of all data points. The interim data at 1 year are also shown. Despite undergoing retinal detachment, which ordinarily reduces visual acuity, the median visual acuity improved by 5.5 letters (IQR: 2.5 to 9.0) above baseline levels in the treated eyes at 2 years (two-tailed Wilcoxon test: W=60, p=0.016). This represented a median relative gain of 4.5 letters (IQR: 2.0 to 14.0) over the untreated fellow eyes (Wilcoxon test: W=76, p=0.001). Approximate Snellen visual acuity equivalents: 6/6=85 letters, 6/12=70 letters, 6/24=55 letters, 6/48=40 letters.

a



Graphic representation of the absolute BCVA change (number of ETDRS letters) compared to baseline in the treated (blue) and untreated control (red) eyes at the 2-year trial endpoint (**b**) and at the last follow-up (up to 5 years) (see **Fig. 2** for length of follow-up for each individual) (**c**). Upward deviations indicate visual acuity gains while downward deviations indicate losses.

b



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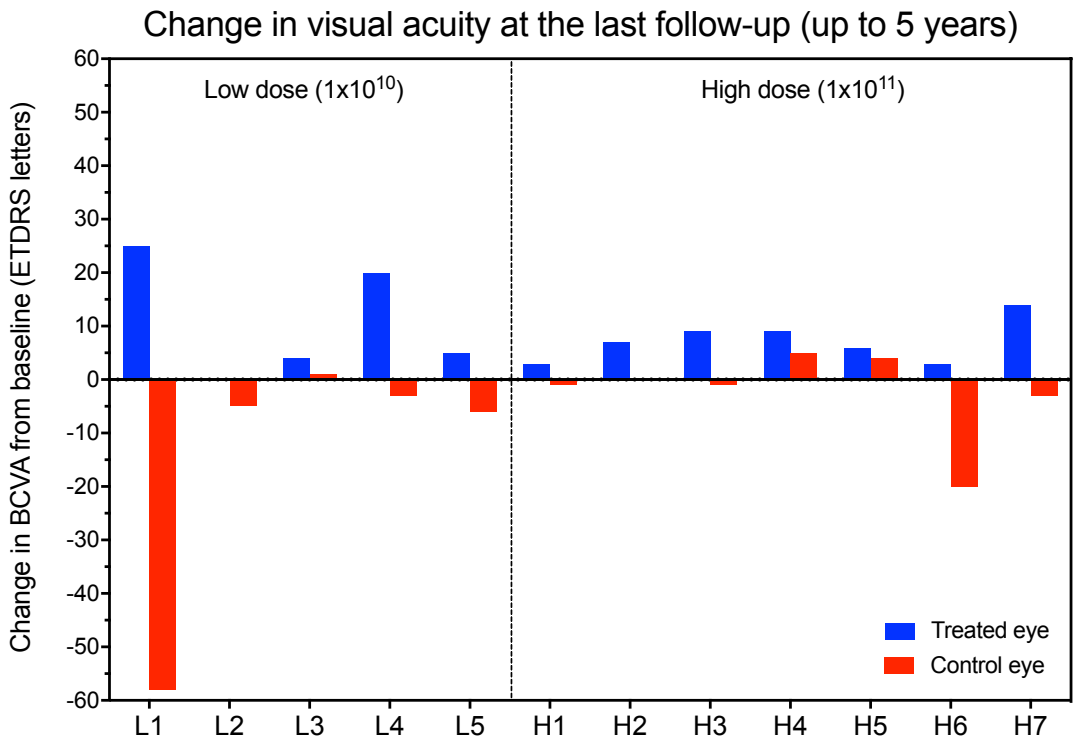
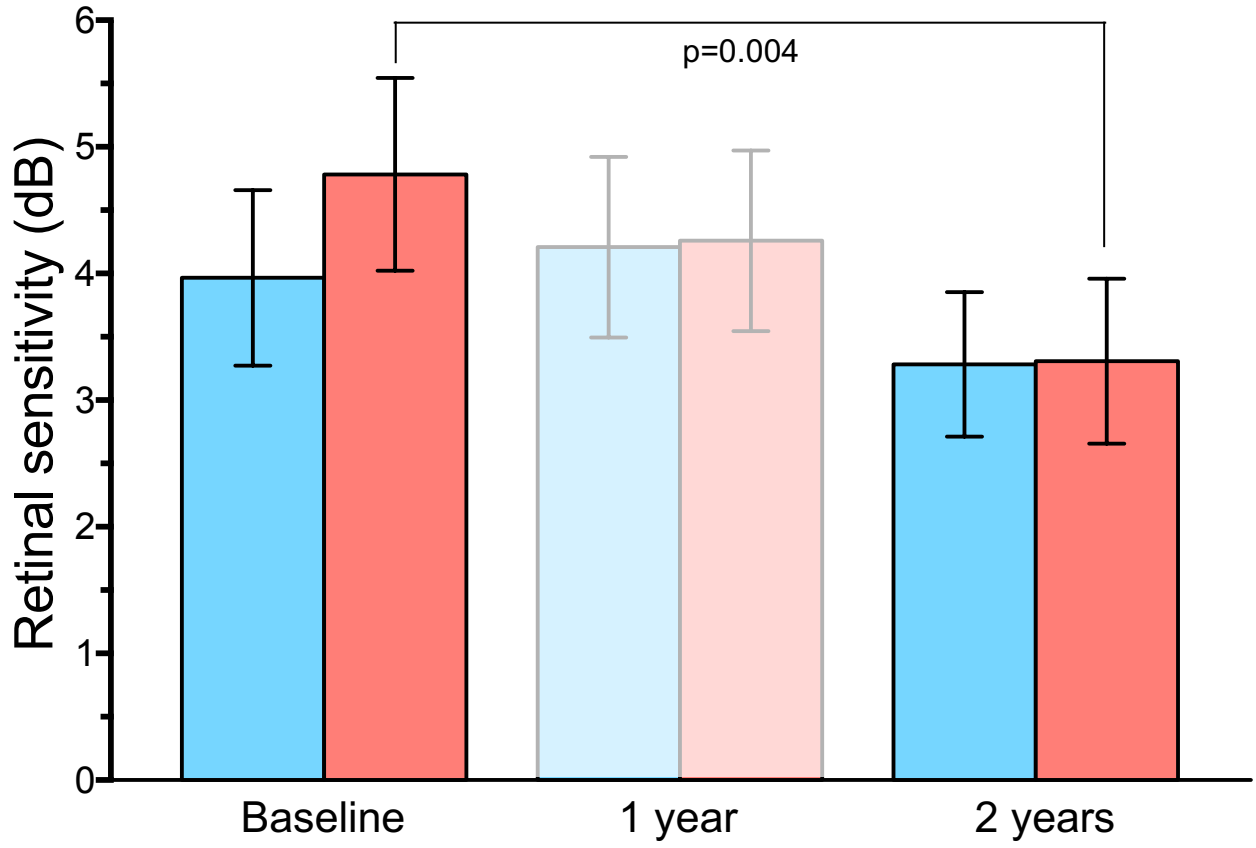


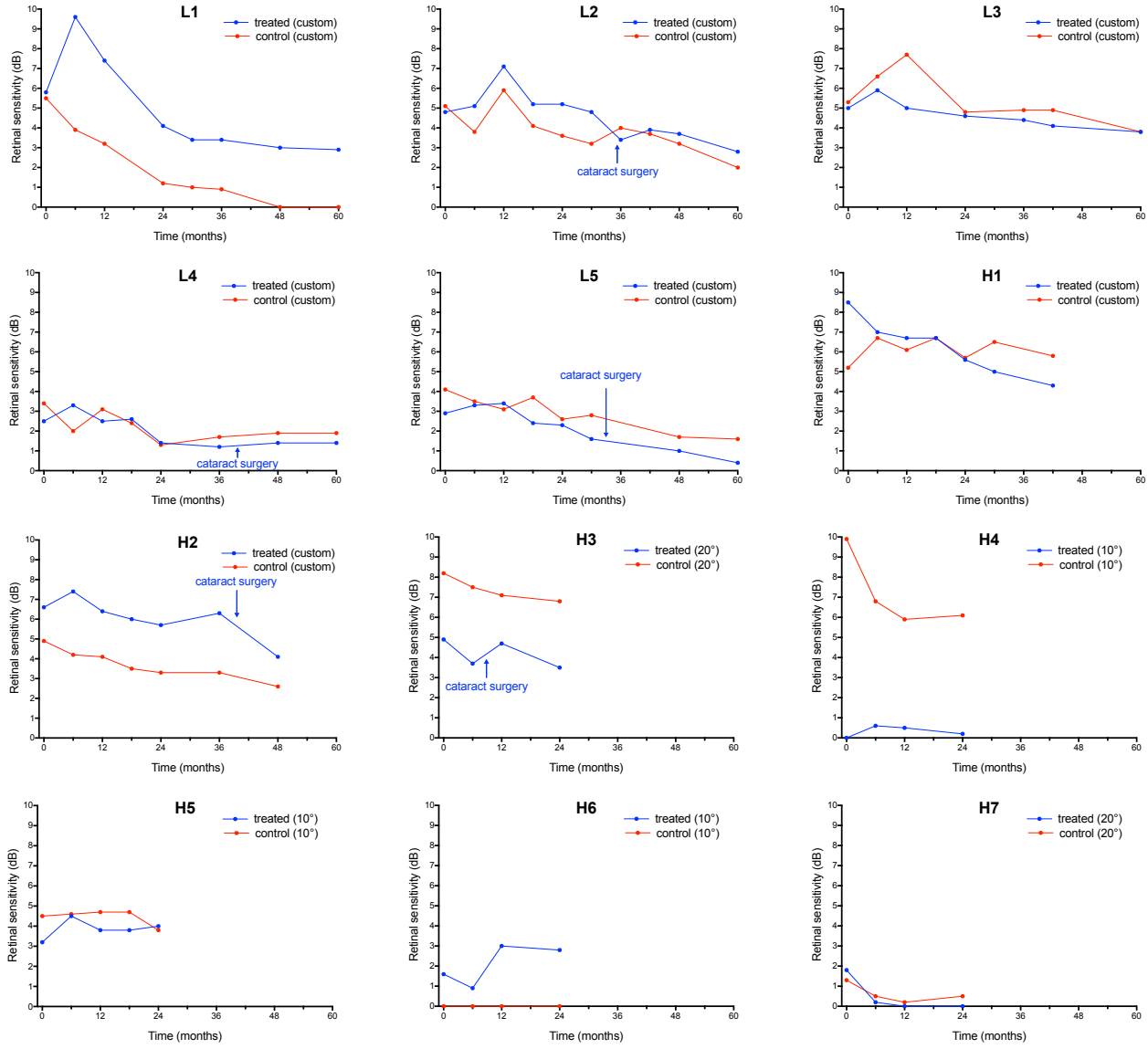
***Figure S3*: Retinal sensitivity changes following gene therapy**
(**a**) Changes in mean threshold retinal sensitivity (decibels, dB) in 12 protocol-treated (blue) and control (red) eyes 2 years after gene therapy. Retinal sensitivity over the central macula was measured using microperimetry (MAIA, CenterVue, Padova, Italy) and increases with improved retinal function. There was a significant decline in retinal sensitivity in the untreated eyes from baseline by 2 years (two-tailed paired t-test: t=3.63, df=11, p=0.004), but no significant change in the treated eyes over this period (t-test: t=1.98, df=11, p=0.07). The relative gain of treated over untreated eyes did not reach statistical significance (t-test: p=0.17, 95% CI=-1.96 to 0.38). The interim data at 1 year are also shown to demonstrate trend but not included in the statistical analysis. Error bars represent ±SEM.

a



(**b**) Individual changes in mean threshold retinal sensitivity up to the last follow-up time point in the treated (blue) and control (red) eyes of patients who received either low-dose (L1-5) or high-dose (H1-7) gene therapy without complications. Note the plateau effect emerging over the last 3 years in the treated eye of L1, consistent with preserved retinal function.

b



(**c**) Retinal sensitivity trends in the treated (blue) versus control (red) eyes of the five low-dose patients (L1-5) who have reached 5-year follow-up. Means (dots) ± SEM (whiskers) are shown. Microperimetry measures a mean of retinal sensitivity from all areas of the macula which will include peripheral areas in which the degeneration may be too advanced to be rescued by gene therapy. In contrast, visual acuity readings generally arise from a single point that is centrally located and targeted by the subretinal injection. Hence it is expected to see a decline in overall retinal sensitivity despite a gain in visual acuity. Although both treated and untreated eyes showed a decline, the mean sensitivity loss from baseline in the treated eyes was less than the untreated eyes by 0.9±0.3 dB (95% CI: 0.3 to 1.5 dB).

c

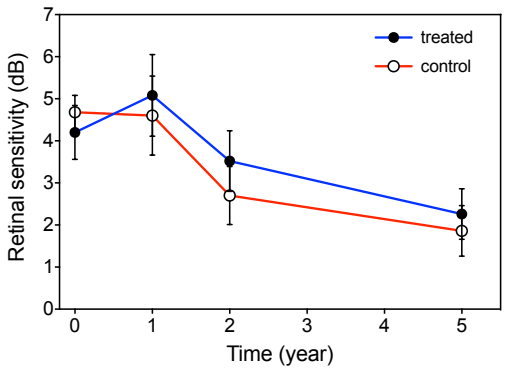


***Figure S4*: Retinal thickness changes following gene therapy**

Retinal thickness (μm) at the point of fixation was measured on serial optical coherence tomography (OCT) of the treated (solid line) and control (dotted line) eyes of all 12 patients who received gene therapy without complications. In the 7 patients who received subretinal injection of AAV vector manually (L1—5 & H1—2), a small amount of retinal thinning was detected in the treated eyes at the 2-year trial endpoint (p=0.02). No significant retinal thinning was detected in the 5 eyes that received automated vector injection (H3—7) after the protocol amendment over the same period (p=0.36).

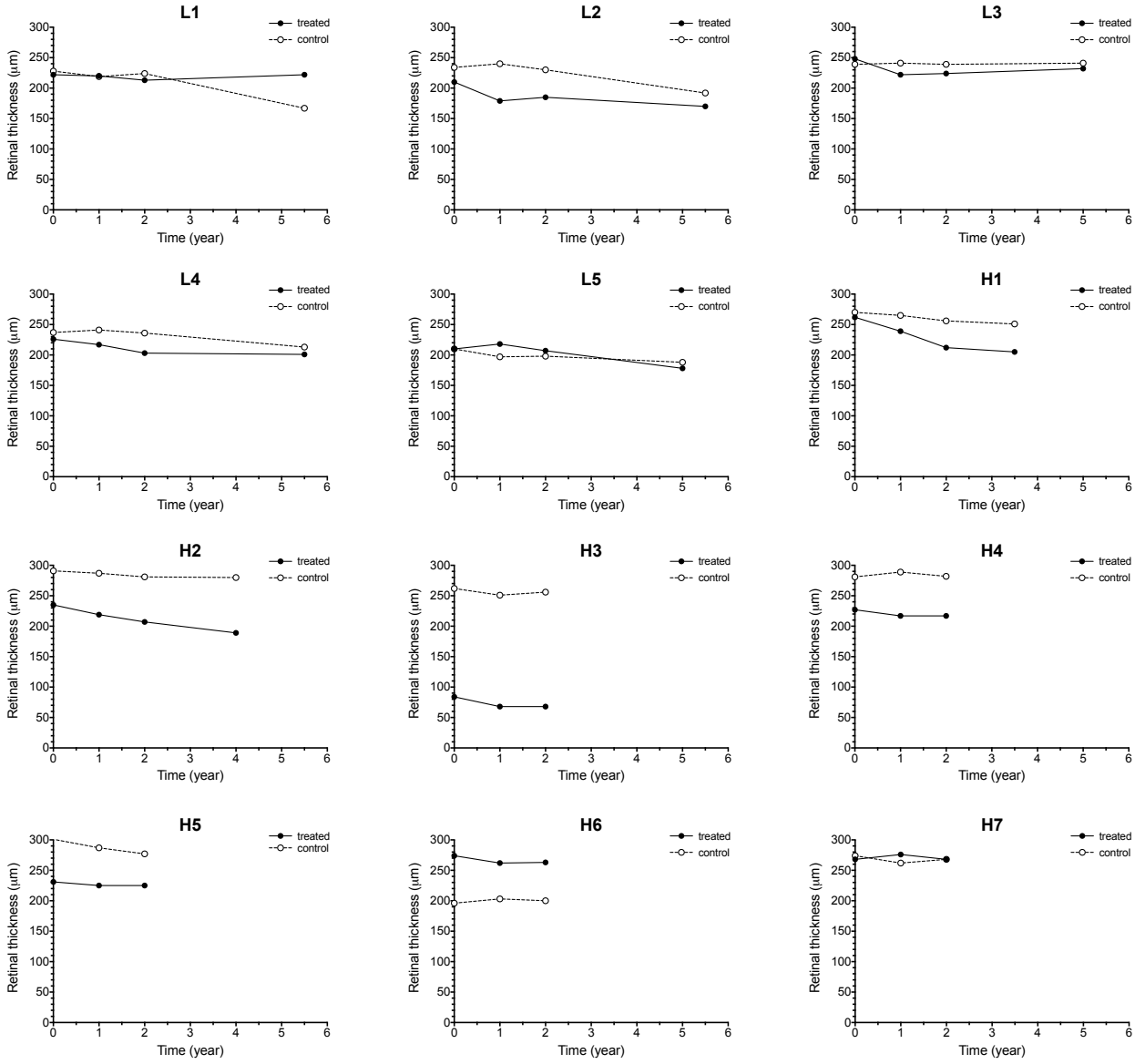
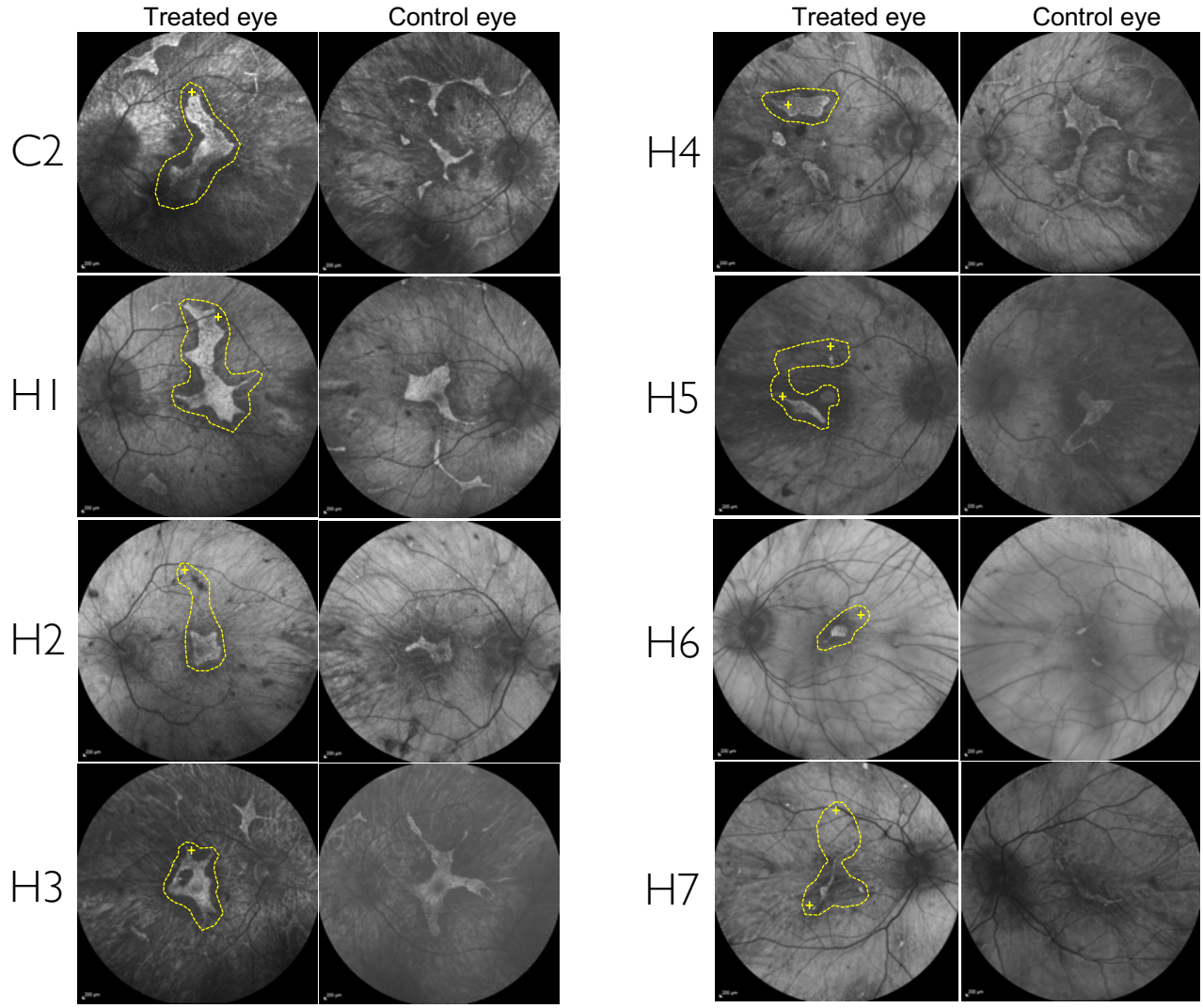


***Figure S5*: Changes in fundus autofluorescence (AF) following gene therapy**
Autofluorescence is a biomarker for surviving retinal anatomy, although the edges represent the leading edge of the degeneration, much like the coast of a melting polar ice cap. Hence shrinkage of the AF area over the long-term may provide information on the rate of degeneration. (**a**) The extent of estimated iatrogenic retinal detachment and AAV vector exposure (yellow dotted lines) shown as overlay on the baseline autofluorescence images of 8 trial participants (C2 & H1—7). Injection site(s) are marked with crosses. Similar images for the remaining 6 participants (L1—5 & C1) have been reported previously.[16]

a



(**b**) Residual AF area expressed as percentage of baseline in the treated (blue) and control (green) eyes of all 12 patients who received gene therapy without complications. This showed a mixed picture, with some patients with large visual acuity gains (L1, L4 and H5) also showing slower loss of AF in the treated eyes. However, across the whole cohort at 2 years, 80.7±3.0% of AF area was preserved in the treated eyes compared with 80.8±2.1% in the control eyes, a difference which was not statistically significant (paired t-test, p=0.975). It should however be noted that the AF changes in choroideremia relate only to the extreme outer rim of surviving retina, which represents the leading edge of the degenerative process. Against the background of improved retinal function in central areas of retina, this might imply that cells at the retinal periphery may be beyond the point of rescue using gene therapy. Alternatively, mechanical effects in relation to detaching the retina up to the edge of the AF area and its subsequent slow reattachment in the reverse direction are likely to leave vector suspension in contact with the central retinal pigment epithelium for far longer than at the periphery. This could result in suboptimal doses of vector to the periphery. Finally, it should be remembered that the AF area measurements can be extremely challenging in end-stage patients in whom the fluorescence signal is almost extinguished. The resulting variability may explain why the % AF change appears non-linear or the AF area appears to increase at some points (e.g. L1 control eye at 6 months; H4 control eye at 1 year). Long-term (5-year) follow-up of the first 5 patients showed 66.1±5.0% remaining autofluorescence in the treated eyes compared with 64.9±3.6% in the untreated eyes (paired t-test, p=0.835) (Supplementary Table S4).

b

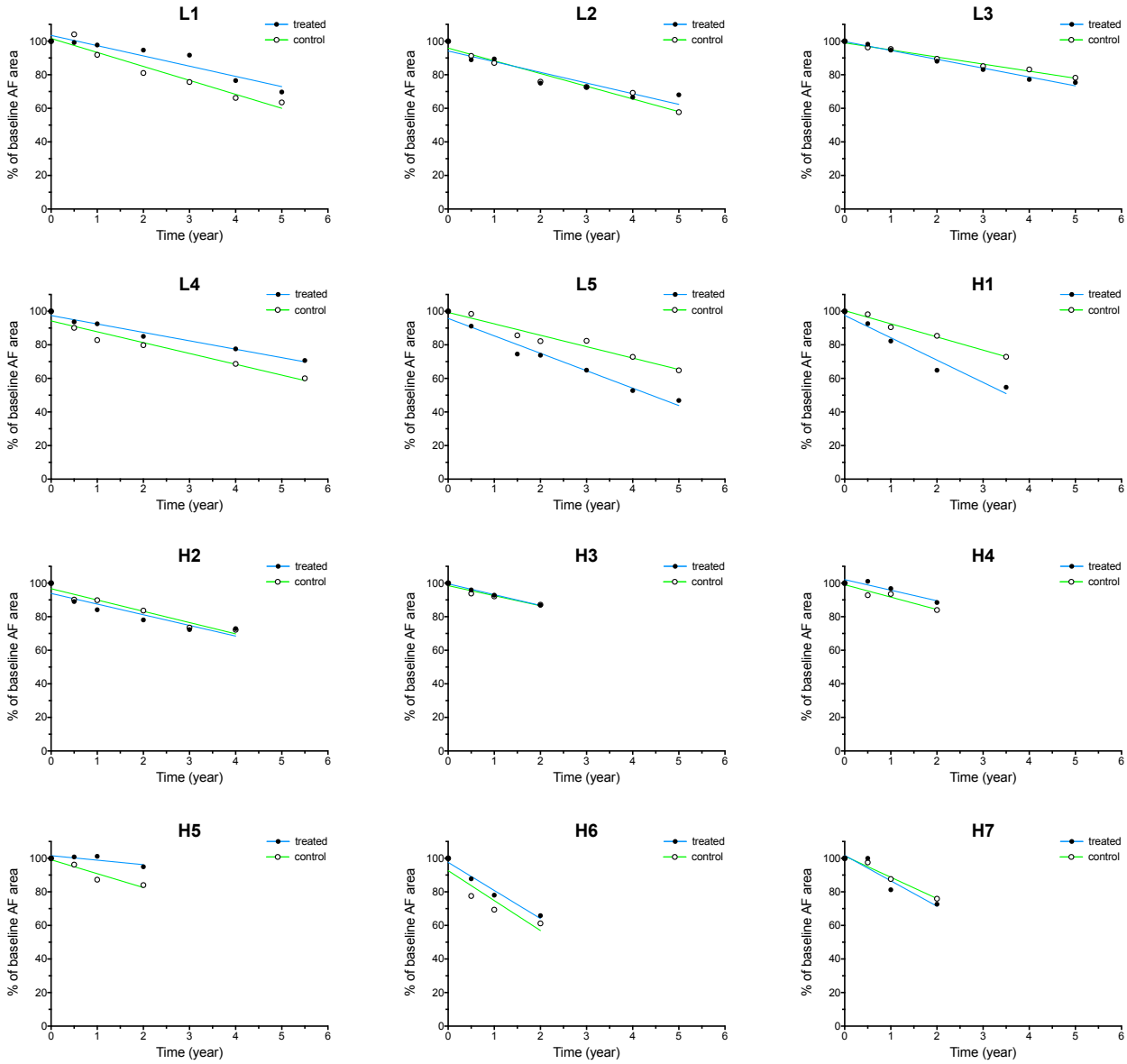
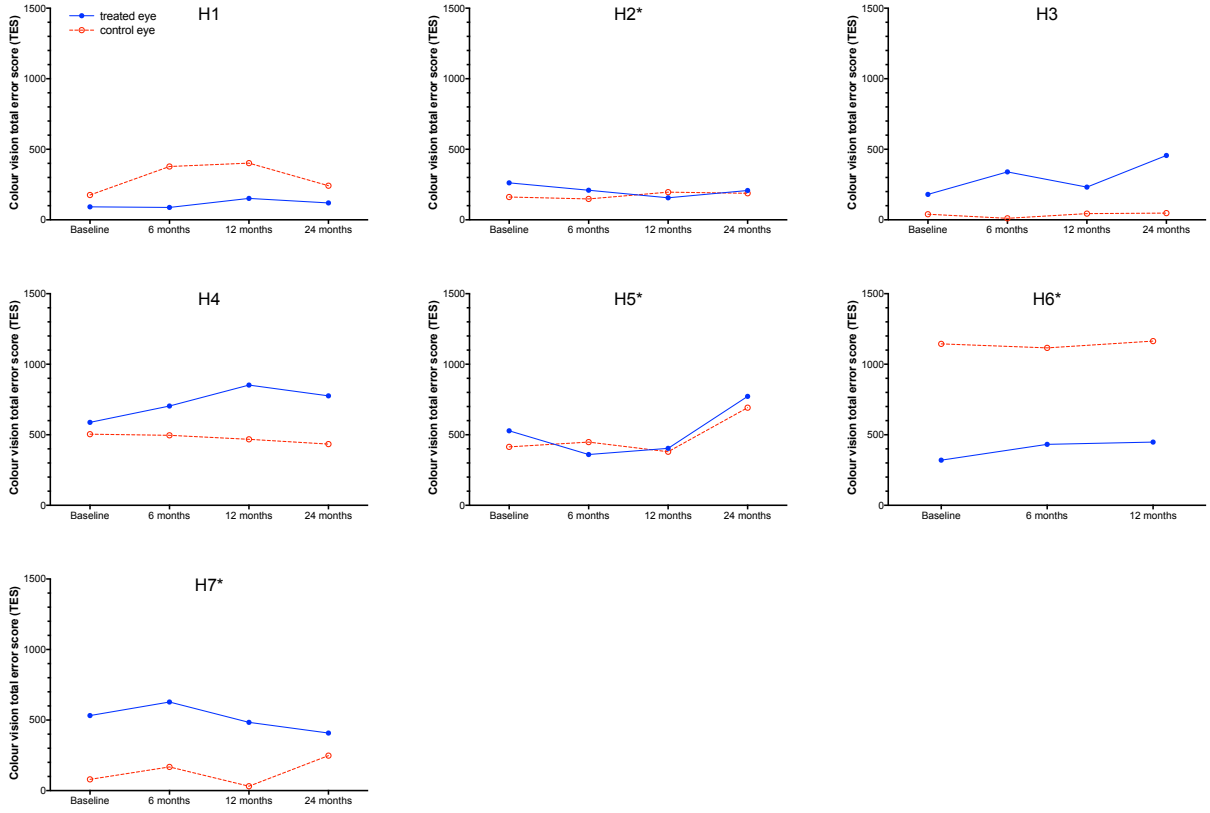


***Figure S6*: Colour vision following gene therapy**

Following subjective improvements in colour vision in L3, the Farnsworth-Munsell 100 hue (FM100) colour test was added to the study protocol and performed at baseline, 6, 12 and 24 months following gene therapy in the 7 high-dose patients (H1—7). The test proved very difficult to perform in patients with advanced visual field constriction as it involved sorting 100 colour tiles in order of hue and some patients could only see one tile at a time. (**a**) While subjective improvements in colour perception of the treated eye (e.g. seeing more shades of green) were reported by H2, H5, H6 and H7 (indicated by *), no significant correlation with reduced overall colour vision error scores was detected. (**b**) Colour wheel analysis of the treated and control eyes indicate a consistent defect in green perception in choroideremia at baseline. No obvious treatment-induced improvement in green perception was however detected using the test, despite this being the colour almost invariably reported by the trial participants as having become clearer.

a



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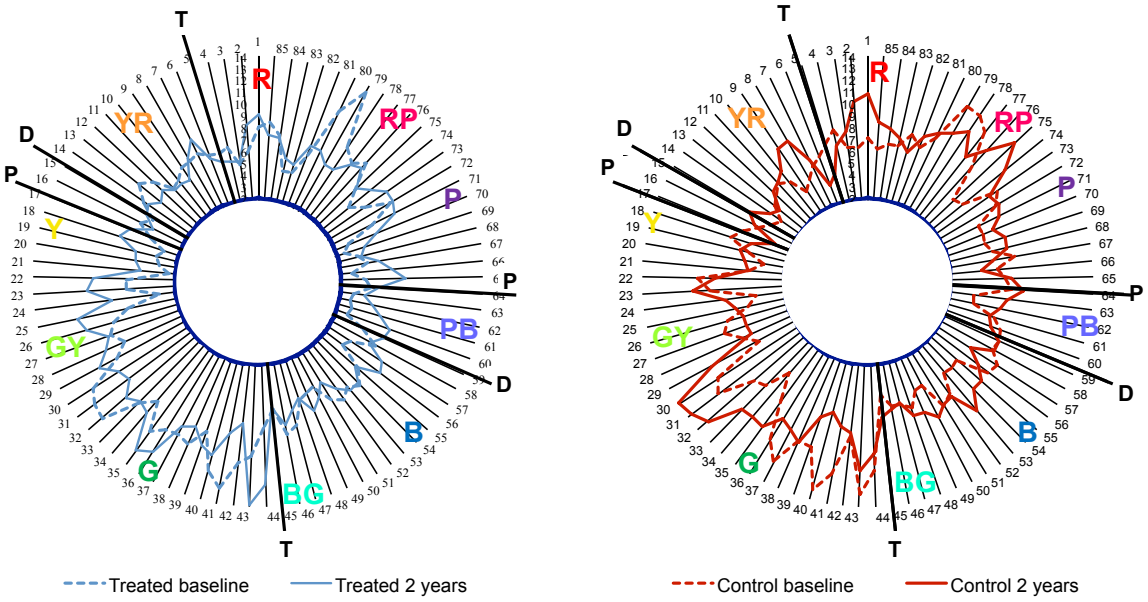

***Figure S7*: Retinal sensitivity, thickness and autofluorescence area changes in participants C1 and C2**
(**a**—**c**) Subretinal injection of AAV vector in participant C1 was complicated by difficulty detaching the retina, which resulted in excessive foveal stretch and under-dosing ($6x10^9$ gp). Foveal thinning was detectable from 1-month post-op onward and was associated with early decline in visual acuity (Fig. 3) and delayed decline in macular sensitivity (**a**). The foveal thinning leveled off by 2 years (**b**), although the overall area of surviving retinal autofluorescence (AF) did not appear to undergo accelerated decline (**c**). Since C1 received the lowest vector dose of the whole cohort — lower than the 5 'low dose' patients — the retinal thinning is unlikely to be related to vector toxicity. (**d**—**f**) Participant C2 was the first to receive the high dose of vector ($1x10^{11}$ gp) under the original 10 days peri-operative systemic corticosteroid regime. Vitritis, retinitis, and choroiditis were observed at 2 weeks post-operatively, associated with an acute decline in visual acuity (Fig. 3) and macular sensitivity (**d**). The inflammation resolved with partial recovery of visual function following an additional 3-week tapering course of oral prednisone. No significant retinal thinning (**e**) or acceleration of autofluorescence (AF) area loss (**f**) was detected. Interestingly, no anti-AAV2 antibodies were detected in the serum of C2 at pre-operative screening or up to 6 months post-op, suggesting that the intraocular inflammation was a dose-related local response (Supplementary Table 6). This case led to a protocol amendment extending the perioperative corticosteroid regime to 21 days, and no significant inflammation was observed in the remainder of the trial.

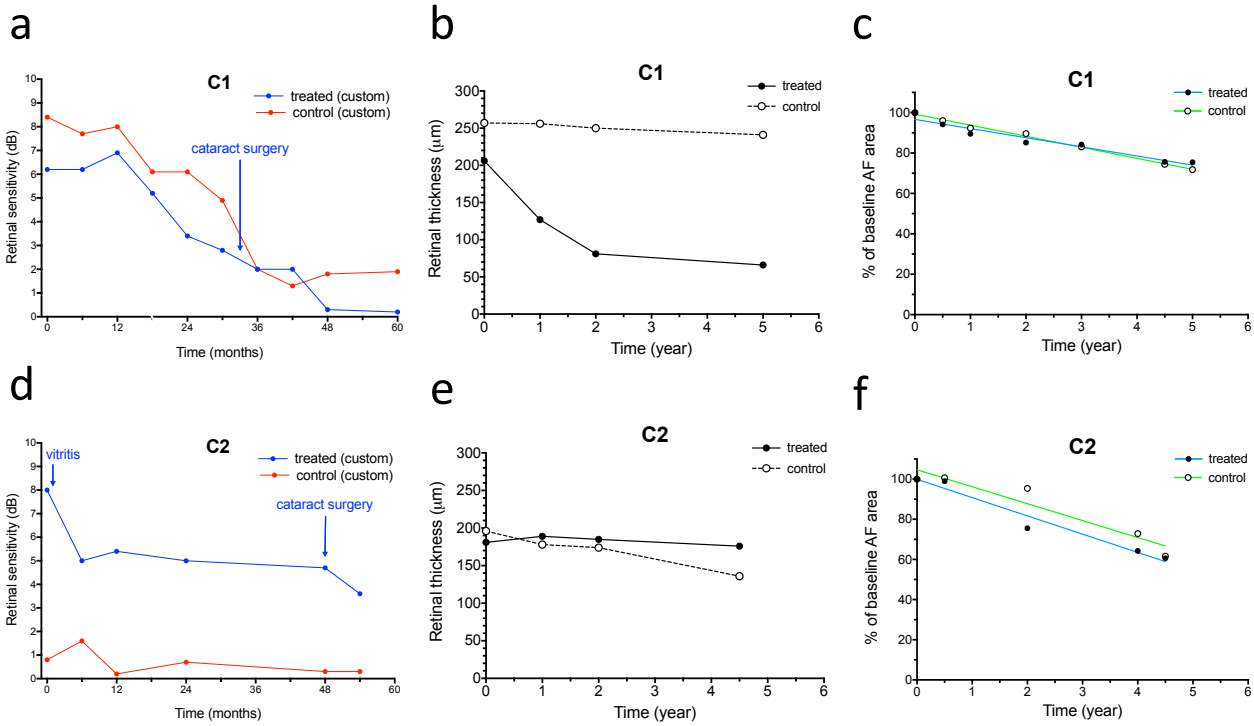

Table S1: **Demographic characteristics of trial participants**

All participants were Caucasian males with genetically confirmed choroideremia. L1—5 and H1—7 underwent uncomplicated gene therapy at the low dose ($1x10^{10}$ gp) and high dose ($1x10^{11}$ gp), respectively. Two patients (C1 & C2) had complications resulting in deviations from the Protocol. The surgery in C1 was complicated by retinal stretch which resulted in under-dosing of the vector and subsequent retinal thinning. C2 developed post-operative intra-ocular inflammation (vitritis and choroiditis), which led to an acute reduction in visual acuity 2—4 weeks after initial recovery from surgery. The inflammation responded to a repeat 3-week course of oral prednisolone.

ID	Age (yr)	*CHM* mutation	Predicted protein sequence	Eye treated	Vector dose (gp)
L1	63	c.940+2T>C	splice donor site mutation – intron 7 (+2)	left	$1x10^{10}$
L2	47	c.189+1G>C	splice donor site mutation – intron 3 (+1)	left	$1x10^{10}$
L3	36	c.492_493delGA	N165Cfs*8 (exon 5)	left	$1x10^{10}$
L4	55	c.535_538delGAAA	E179Tfs*17 (exon 5)	left	$1x10^{10}$
L5	41	c.529delG	E177Kfs*20 (exon 5)	right	$1x10^{10}$
H1	38	c.799C>T	R267* (exon 6)	left	$1x10^{11}$
H2	43	c.877C>T	R293* (exon 7)	left	$1x10^{11}$
H3	41	c.1264C>T	Q422* (exon 10)	right	$1x10^{11}$
H4	59	c.1335_1336insA	R446Tfs*16 (exon 10)	right	$1x10^{11}$
H5	72	c.757C>T	R253* (exon 6)	right	$1x10^{11}$
H6	55	c.799C>T	R267* (exon 6)	left	$1x10^{11}$
H7	24	c.525_526delAG	E177Kfs*6 (exon 5)	right	$1x10^{11}$
C1	57	c.819+1G>T	splice donor site mutation – intron 6 (+1)	left	$6x10^{9}$
C2	44	c.130G>T	G44* (exon 3)	left	$1x10^{11}$

***Table S2*: Visual acuity results at the 2-year primary endpoint for the 12 participants treated as per protocol**

Best-corrected visual acuities are presented as number of letters measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart. One patient (H3) developed a symptomatic cataract that was removed at 8 months whereas another (H5) developed posterior capsule opacification and had YAG laser capsulotomy at 18 months. However the improvement in visual acuity in these two patients (7 letters in H3 and 5 letters in H5) had already occurred by 6 months after gene therapy, which was prior to the media opacity intervention. Although removal of secondary lens opacities caused by the surgery did not deviate from the protocol, a sub-analysis excluding these two eyes in addition to the two with off-protocol treatments showed a median gain in the treated eyes of 4.5 letters (IQR: 1.5 to 8.8) against a loss of -2.0 letters (IQR: -5.0 to 0.0) in the untreated eyes (two-tailed Wilcoxon test: W=53, p=0.008). Hence the clinical trial met its primary endpoint in terms of showing beneficial effects of choroideremia gene therapy on visual acuity compared with the untreated eyes, overcoming the trauma of mechanical detachment of the retina. TE=treated eye; CE=control eye; m=months; yr=years; Diff=difference (relative change) between treated and control eyes; gp=genome particles of AAV2.REP1 vector; Med=median; vision changes of 3 lines (15 letters) or higher are shown in bold; Snellen visual acuity score equivalents: 40 letters=6/48, 55 letters=6/24, 70 letters=6/12, 85 letters=6/6.

Visual acuity	Eye	Low dose (1 x 10^{10} gp)					High dose (1 x 10^{11} gp)							Med
		L1	L2	L3	L4	L5	H1	H2	H3	H4	H5	H6	H7	
Baseline	TE	23	79	89	53	79	77	76	70	61	67	60	39	68.5
	CE	58	82	85	76	83	87	88	88	57	74	26	87	82.5
1 yr	TE	**45**	79	91	**69**	75	78	74	80	65	70	60	**54**	72.0
	CE	68	77	85	70	84	87	87	88	60	69	31	81	79.0
2 yr	TE	**41**	73	94	61	76	78	80	79	70	73	63	53	73.0
	CE	62	77	85	70	78	86	88	87	62	78	**6**	84	78.0
Change (2 yr)	TE	18	-6	5	8	-3	1	4	9	9	6	3	14	+5.5
	CE	4	-5	0	-6	-5	-1	0	-1	5	4	-20	-3	-1.0
Diff (2 yr)		14	-1	5	14	2	2	4	10	4	2	23	17	+4.5

***Table S3*: Long term sustainability of visual acuity gains following choroideremia gene therapy**

Due to the break midway through the trial, the follow-up for the first 7 patients extends beyond the formal 2-year trial endpoint. By the last follow-up, the treated eyes had improved by a median of 6.5 letters (IQR: 3.8 to 10.3), whereas the untreated control eyes had lost -2.0 letters (IQR: -5.3 to 0.3). This represented a median relative gain of 8.5 letters (IQR: 4.0 to 23.0) in the treated eye over the untreated eye (two-tailed Wilcoxon test, W=78, p=0.0005). By the last follow-up, visual acuity had been maintained or increased in all 12 eyes that received gene therapy per protocol, but 8 of the 12 control eyes had deteriorated by variable amounts during this period. In 7 patients the visual acuity was 70 letters (6/12) or lower in the treated eyes at baseline and these represent the more advanced choroideremia cases. By the last follow-up, 4 (57%) had gained more than three lines (15 letters) relative to their untreated eyes (gains shown in bold). At the last follow-up, the visual acuity in the treated eye obtained relative gain against the untreated eye in every one of the 12 patients treated per protocol with a median of 8.5 letters (IQR: 4.0 to 18.5). Six patients who had secondary cataract extraction or YAG laser (L5 and H5) before their last follow-up are asterisked. The 6 patients who did not have cataract surgery or YAG laser had experienced a mean gain in their treated eyes of +9.7 letters (median 6.5 letters) at the last follow-up. Last FU (yr) – last follow-up time point after gene therapy; TE=treated eye; CE=control eye; gp=genome particles; Med=median. All numbers represent best-corrected visual acuities (BCVA) in number of letters correctly identified from the ETDRS chart tested at a distance of 4 m.

Visual acuity	Eye	Low dose (1 x 10^{10} gp)					High dose (1 x 10^{11} gp)							Med
		L1	L2*	L3	L4*	L5*	H1	H2*	H3*	H4	H5*	H6	H7	
Baseline	TE	23	79	89	53	79	77	76	70	61	67	60	39	68.5
	CE	58	82	85	76	83	87	88	88	57	74	26	87	82.5
Last FU	TE	48	79	93	73	84	80	83	79	70	73	63	53	76.0
	CE	0	77	86	73	77	86	88	87	62	78	6	84	77.5
Change (last FU)	TE	25	0	4	20	5	3	7	9	9	6	3	14	+6.5
	CE	-58	-5	1	-3	-6	-1	0	-1	5	4	-20	-3	-2.0
Diff (last FU)		**83**	5	3	**23**	11	4	7	10	4	2	**23**	**17**	+8.5
Last FU (yr)		5	5	5	5	5	4	4	2	2	2	2	2	4.0

***Table S4*: Autofluorescence retina area changes in trial participants**

(**a**) Autofluorescent area changes in all 12 patients who received gene therapy as per protocol and reached 2 years follow-up. TE=treated eye; CE=control eye; base=baseline measurement. (**b**) Autofluorescent area changes in the 5 patients who have reached 5 years follow-up. The percentage autofluorescence area reductions favored the treated eyes in L1 and L4, who also had 4 lines or greater improvements in visual acuity in the treated eyes.

a

ALL	L1	L2	L3	L4	L5	H1	H2	H3	H4	H5	H6	H7	Mean	SEM
TE base (mm^2)	1.32	4.16	9.96	1.74	2.71	11.95	3.47	6.43	3.73	2.56	0.82	1.28	4.18	1.02
CE base (mm^2)	0.74	2.86	10.51	2.33	5.23	6.23	3.06	11.44	11.04	5.02	0.49	1.62	5.05	1.15
TE 2 yr (mm^2)	1.25	3.12	8.77	1.48	2.00	7.75	2.71	5.59	3.30	2.43	0.54	0.93	3.32	0.77
CE 2 yr (mm^2)	0.60	2.17	9.41	1.86	4.30	5.32	2.56	9.97	9.27	4.22	0.30	1.23	4.27	1.02
% TE 2 yr	95	75	88	85	74	65	78	87	88	95	66	73	80.7	3.0
% CE 2 yr	81	76	90	80	82	85	84	87	84	84	61	76	80.8	2.1

b

> 5 YR	L1	L2	L3	L4	L5	Mean	SEM
TE base (mm^2)	1.32	4.16	9.96	1.74	2.71	3.98	1.57
CE base (mm^2)	0.74	2.86	10.51	2.33	5.23	4.33	1.70
TE 5 yr (mm^2)	0.92	2.83	7.51	1.23	1.27	2.75	1.24
CE 5 yr (mm^2)	0.47	1.65	8.22	1.40	3.39	3.03	1.38
% TE 5 yr	70	68	75	71	47	66.1	5.0
% CE 5 yr	64	58	78	60	65	64.9	3.6

Table S5: **Vector shedding following retinal gene therapy**

Following a mid-trial protocol amendment, as part of safety monitoring, we were able to collect samples of secreta/excreta to assess the levels of AAV vector shedding after retinal gene therapy in the last 5 patients (H3—7). Saliva, tears (each eye independently) and urine were collected at baseline, day 1, day 7, month 1 and month 3 after treatment. Bloods were also collected, although blood is not a true secreta/excreta as it is not normally released into the environment. Sample collection was performed according to recently published methods.[39] Samples were assessed for the presence of AAV2 vector genome (DNA) using a validated qPCR assay with a limit of detection of 50 genome copies in 5 µL, which meets the regulatory requirements for sensitivity. Samples at all time points from all patients tested negative except for a tear sample taken from the treated (right) eye on day 1 post-gene therapy in H4 (shaded grey).

			Sample type (genome copies/5 µL)				
ID	Dosed eye	Time point	Blood	Saliva	Tears left eye (OS)	Tears right eye (OD)	Urine
H3	OD	Pre-dose/screening	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 7	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 3	<50.0	<50.0	<50.0	<50.0	<50.0
H4	OD	Pre-dose/screening	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 1	<50.0	<50.0	<50.0	920.9	<50.0
		Day 7	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 3	<50.0	<50.0	<50.0	<50.0	<50.0
H5	OD	Pre-dose/screening	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 7	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 3	<50.0	<50.0	<50.0	<50.0	<50.0
H6	OS	Pre-dose/screening	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 7	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 3	<50.0	<50.0	<50.0	<50.0	<50.0
H7	OD	Pre-dose/screening	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Day 7	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 1	<50.0	<50.0	<50.0	<50.0	<50.0
		Month 3	<50.0	<50.0	<50.0	<50.0	<50.0

Table S6: **Anti-AAV2 neutralising antibodies (NAb) following retinal gene therapy**

In order to assess the systemic immunological responses to subretinal administration of AAV vector, serum samples were collected at baseline and various time points up to 6 months following retinal gene therapy and tested for the presence of antibodies that neutralise AAV2 using a cell based assay, as described previously.[40] Briefly, the validated assay allows for the quantification of NAb based on the degree of inhibition of transduction of an AAV2.luciferase vector in HeLa cells, with the reported titre values being the reciprocal of the dilution that crosses a cut-point determined in the validation. The majority of patients (13 of 14) had baseline antibody titres that would indicate the absence of any pre-existing anti-AAV2 NAb (titre <10). In all these patients, the anti-AAV2 NAb levels remained at the same, low levels throughout the follow-up period. In one patient (L3), the NAb titre recorded at baseline was over range (>640) on initial analysis, but repeat analysis at a higher starting dilution gave a value of <300, suggesting the presence of pre-existing anti-AAV2 NAb. Importantly, however, there was no detectable increase in his anti-AAV2 NAb titre following gene therapy and the presence of anti-AAV2 NAb had no noticeable effect on his treatment response (shaded grey). Notably, the development of post-operative vitritis, retinitis and choroiditis in C2 was not associated with raised level of anti-AAV2 NAb.

Key: *nd* = not done (according to the original protocol, which stated that serum samples were not collected at these time points unless specifically requested by the chief investigator).

		Anti-AAV2 neutralising antibody (NAb) titre					
ID	Dose	Baseline	Day 1	Day 7	Month 1	Month 3	Month 6
L1	Low	<10	*nd*	<10	<10	*nd*	*nd*
L2	Low	<10	*nd*	<10	<10	*nd*	*nd*
L3	Low	<300	*nd*	<300	<300	*nd*	*nd*
L4	Low	<10	*nd*	<10	<10	*nd*	*nd*
L5	Low	<10	*nd*	<10	<10	*nd*	*nd*
H1	High	<10	*nd*	<10	<10	<10	*nd*
H2	High	<10	*nd*	<10	<10	*nd*	*nd*
H3	High	<10	<10	<10	<10	<10	<10
H4	High	<10	<10	<10	<10	<10	<10
H5	High	<10	<10	<10	<10	<10	<10
H6	High	<10	<10	<10	<10	<10	<10
H7	High	<10	<10	<10	<10	<10	<10
C1	Low	<10	*nd*	<10	<10	*nd*	*nd*
C2	High	<10	*nd*	<10	<10	<10	<10

***Table S7*: Baseline-emergent adverse events (n=84) and serious adverse event (n=1)**

An adverse event (AE) was defined as any untoward medical occurrence in a participant who has been recruited to the clinical trial, including occurrences that are not necessarily caused by or related to ocular gene therapy. The details of the off-protocol treatments for C1 and C2 are discussed in the main text. In C2, intraocular inflammation in the form of vitreous floaters, outer retinal opacities and choroidal thickening were observed at 2 weeks post-gene therapy, which had an adverse effect on BCVA (Fig. 2). This necessitated restarting systemic prednisolone and almost certainly resulted in loss of available AAV2.REP1 capsids by immune targeting. Although C2 recovered to a loss of 9 letters of VA, subjectively he reports improved night vision in the treated eye compared with the untreated eye. This event led to a change of protocol to extend the post-operative oral prednisolone from 7 to 18 days and no further cases of inflammation occurred in the remaining patients in this study. One serious adverse event (SAE) unrelated to the vector occurred in H3. An air bubble in the injection system expanded into the subretinal space and vector administration was deferred because it was felt the vector would be displaced by the subretinal air. The injection system was amended in order to allow more controlled infusion of vector into the subretinal space by using a foot-pedal to apply continuous and limited pressure. The protocol allowed for deferred vector administration if indicated for surgical reasons: H3 was brought back for retinal gene therapy at a later date when the full dose of vector was administered without complication. This in addition to the adverse event relating to excessive foveal stretch in C1 (Fig. 3 and Supplementary Fig. S7) led to a change in protocol midway through the study. This resulted in the development of an automated injection system and intraoperative OCT guidance, which allowed for a considerably more controlled detachment of the retina in subsequent patients.[25,35] All patients were enrolled without visually significant cataract at baseline because of the need for a clear view of the 41 gauge retinotomy (injection site) during administration of gene therapy, but cataract formation is a well-known and almost inevitable side effect of vitrectomy surgery. One participant (H5) had a pre-existing cataract removed prior to gene therapy and YAG laser was performed at 18 months for significant opacification of the posterior lens capsule, a common occurrence after routine cataract surgery (Fig. 2). One participant (H3) developed visually-significant cataract during the 2-year trial period which was removed at 8 months following gene therapy. These interventions were not prohibited in the protocol and took place after the post-gene therapy visual acuity gains in both patients. Six other patients (L2, L4, L5, H2, C1 and C2) had cataract surgery in the treated eye after completion of 2 years follow-up, whereas cataract surgery has not yet been performed in the remaining 6 patients (L1, L3, H1, H4, H6, and H7).

Adverse events (AE)

ID	System	Adverse event (AE)	Plausible relationship to study	Action taken	Outcome
			1=Drug 2=Procedure 3=Unknown	1=None 2=Vector injection postponed 3=Medication taken 4=Withdrawn	1=Recovered 2=Recovered with sequelae 3=Continuing 4=Patient died 5=Change in AE severity 6=Unknown
L1	Ocular	Raised IOP in right (untreated) eye	No	1	1
	Non-ocular	Herpes on lip	No	1	1
		Cold	No	1	1
L2	Ocular	Subconjunctival haemorrhage left (treated) eye	Yes (2)	1	1
		Slightly painful left (treated) eye movement	Yes (2)	1	1
		Metamorphopsia left (treated) eye	Yes (2)	1	3
		Violet coloured tint to vision in left (treated) eye	Yes (2)	1	3
		Reduced vision in right (untreated) eye	No	1	1

		Dry feeling in right (untreated) eye	No	1	1
	Non-ocular	Fractured vertebra following a fall	No	1	1
		Knee cartilage damage	No	1	5
		Knee cartilage repair	No	1	2
L3	Ocular	Metamorphopsia left (treated) eye	Yes (2)	1	1
		Micropsia left (treated) eye	Yes (2)	1	1
		Colours appear 'washed out' in left (treated) eye	Yes (2)	1	1
		Dimming of vision lasting a few seconds in both eyes	No	1	1
		Raised IOP in left (treated) eye	Yes (2)	3 (eye drops)	2
		Amaurosis fugax with increased IOP following use of hemp hand cream	No	1	1
L4	Ocular	Slight ocular discomfort post-op left (treated) eye	Yes (2)	1	1
		Metamorphopsia left (treated) eye	Yes (2)	1	1
		Development of cataract in left (treated) eye	Yes (2)	Cataract surgery	1
	Non-ocular	One episode of feeling faint	No	1	1
L5	Ocular	Blurred vision right (treated) eye	Yes (2)	1	1
		Flashing lights right (treated) eye	Yes (2)	1	1
		Metamorphopsia right (treated) eye	No	1	1
		Raised intraocular pressure right (treated) eye	No	1	1
		Vision feel 'duller' in both eyes	Yes (2)	1	3
C1	Ocular	Left (treated) eye pain	No	1	1
		Slight blurring of vision in left (treated) eye post-op	Yes (2)	1	1
		Corneal haze	No	1	1
		Development of cataract in left (treated) eye	Yes (2)	Cataract surgery	1
	Non-ocular	Acne rosacea	No	1	1
C2	Ocular	Slight discomfort after baseline tests in left eye	Yes (2)	1	1
		Foreign body sensation left (treated) eye	Yes (2)	1	1
		Reduced vision in left (treated) eye with blurring. Clinically and OCT confirmed vitritis — systemic prednisolone given	Yes (1)	3 (oral prednisone)	1
		Colours appear 'washed out' in left (treated) eye	Yes (2)	1	1

		Occasional flashing lights in left (treated) eye related to AE No.1	Yes (2)	1	1
	Non-ocular	Insomnia	No	1	1
		Insomnia (due to steroids)	Yes (1)	1	1
		Hernia surgery	No	1	1
H1	Ocular	Occasional flashing lights in left (treated) eye	Yes (1)	1	3
		Mild post-op inflammation left (treated) eye	Yes (2)	3 (eye drops)	1
		Blurred vision and distortion in left (treated) eye	No	1	1
		Colours appear ‘washed out’ in left (treated) eye	Yes (2)	1	1
		Conjunctivitis both eyes	No	3	1
	Non-ocular	Mild cold	No	1	1
		Mild tooth ache on left side — intermittent	No	1	1
		Flu like symptom	No	3	1
		Mild cold	No	3	1
		Flu like symptom	No	3	1
H2	Ocular	Occasional flashing light left (treated) eye	Yes (2)	1	1
		Floaters in right eye (untreated) eye	No	1	3
	Non-ocular	Slight insomnia (maybe steroid related)	Yes (1)	1	1
		Upset stomach/Feeling unwell post steroids	Yes (1)	1	1
		Sinusitis	No	3	1
		Mild cold	No	1	1
H3	Ocular	Cataract development in right (treated) eye	Yes (3)	Cataract surgery	1
		IOP rise due to steroid response post right (treated) eye cataract surgery	No	3 (eye drops)	1
	Non-ocular	Blocked Eustachian tube right ear	No	3	1
		Swollen right ankle due to sprain	No	1	1
		Blocked Eustachian tube right ear	No	1	1
		Cold	No	1	1
H4	Ocular	Grittiness due to dissolving sutures right (treated) eye	Yes (2)	1	1
		Intermittent flashing lights right (treated) eye	Yes (3)	1	3
		Post-op photopsia in the dark in right (treated) eye	Yes (3)	1	3

		Intermittent flashing lights in left (untreated) eye	No	1	3
	Non-ocular	Vasovagal episode after taking bloods	No	1	1
H5	Ocular	Right eye blurred vision / clouding intermittently	Yes (3)	1	1
		Occasional foreign body sensation in right (treated) eye	Yes (3)	1	1
		Discomfort cause by loose suture in right (treated) eye	Yes (2)	3 (eye drops)	1
		Suture-related conjunctival inflammation in right (treated) eye – suture removed	Yes (2)	3 (eye drops)	1
		Cataract development in left (untreated) eye	No	Cataract surgery	1
		Posterior capsular opacification in right (treated) eye – treated with YAG laser capsulotomy	No	YAG laser capsulotomy	1
	Non-ocular	Low potassium levels pre & immediately post-op likely due to anti-hypertensive medication and corticosteroid tablets	Yes (3)	3 (Sando-K)	1
		Tooth extraction	No	3	1
H6	Ocular	Flashing lights in left (treated) eye	Yes (3)	1	3
	Non-ocular	Mouth ulcers	No	1	1
		Tooth extraction left side	No	3	1
		Flu	No	1	1
H7	Ocular	Worsening of pre-existing diplopia in right (treated) eye	No	1	1
		Post-op discomfort in right (treated) eye	Yes (3)	3 (eye drops)	1
	Non-ocular	Upset stomach	No	1	1
		Cold	No	1	1
		Cold	No	1	1

Serious adverse event (SAE)

H3	Ocular	Air bubble in BSS injection system during surgery. Vector injection postponed	Yes (2)	2 (vector injection postponed)	2

Supplementary references

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