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Supplementary webappendix

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Age-dependent effects of RPE65 gene therapy for Leber congenital amaurosis: a phase 1 dose-escalation trial

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Supplementary Text

METHODS

Subjects, the Study Design, the Vector and its Surgical Delivery

Subjects underwent molecular genetic testing in CLIA laboratories prior to enrollment. Patients suffering from RPE65-related Leber congenital amaurosis (also known as *LCA2*) are generally at the better end of this genetically heterogeneous spectrum of congenital retinal dystrophies. Hence, LCA-*RPE65* is sometimes considered as early-onset retinal dystrophy rather than LCA. This ongoing semantic and nosological discussion aside, patients included in this study all suffered from congenital blindness as defined by total night blindness from birth, a best-corrected visual acuity worse than 1.0 on a LogMAR scale (Snellen visual acuity equivalent of 20/200) and/or concentric visual field constriction to a remaining central field of 5o or less in the treated eye. Nevertheless, best-corrected visual acuities were a bit better than 1.0 on a LogMAR scale in the untreated eyes of 5 subjects at presentation, but all were still severely subnormal. The pretreatment visual limitations of the treated eyes represent generally accepted preconditions for legal blindness throughout the USA and Europe. In addition all subjects had totally absent gross retinal function, both under dark- and light-adapted conditions, as evidenced by full-field electroretinography. This indicates that the remaining gross retinal function is below the threshold of what is measurable with this standard test.

The study design is a Phase 1, open-label dose escalation safety study of gene transfer in subjects with LCA-*RPE65*. The test procedures and the schedule of tests are listed in Table 1.

The original plan was to use three dose cohorts and 3 subjects in each dose cohort. After preliminary evaluations of the first six subjects injected with AAV2-hRPE65v2, (three at the low dose of 1.5E10vg and three at a middle dose of 4.8E10vg), the investigators decided to remain at the middle dose of 4.8E10vg for three additional subjects. By expanding the middle dose cohort to six subjects, it may have been possible to determine if there was any evidence of a dose response effect of AAV2-hRPE65v2. The primary objective of this phase 1 trial was to determine the overall safety and tolerability of subretinal administration of AAV2-hRPE65v2; however secondary clinical measures have demonstrated objective improvement in visual parameters following administration in all subjects evaluated suggesting that even the lowest dose evaluated is within the therapeutic window. Therefore, the middle dose group was expanded to include a total of 6 subjects and the entire trial included 12 subjects.

For vector production, orthogonal purification using both ion exchange chromatography and gradient ultracentrifugation yielded a product in which empty capsid constituted <2% of the final product.[1] Excipient was supplemented with a surfactant to prevent losses of vector to contact surfaces.[2, 3]

Analysis of Effects of Test Article on Vision

For statistical analyses, the average of ≥4 pre-operative measurements were compared with the average of ≥4 measurements taken at least 1 month after injection. For NP01, NP02, and NP03, statistical analyses assigned hand motion visual acuity as LogMAR = 2.0. [3]

Mobility testing was carried out as described by Maguire et al. [3] In order to minimize a potential "learning effect, we: 1) changed the course for each eye/test; 2) keep the subject in a separate room every time a new course was set up; 3) did not provide verbal clues to the subjects and only re-directed the subjects if they walked off the course or if they were in danger of colliding with an obstacle. Subjects did not use their hands to try to feel and prevent collisions with obstacles. Subjects were told ahead of time (and shown with practice runs) not to extend their foot to feel for obstacles. If this happened, they usually bumped into an obstacle and this was counted as a collision. In addition to counting collisions, navigation through each obstacle

course was timed. Statistical analysis of mobility test results compared numbers of collisions and time to get through the course (with both eyes and/or with either eye alone) before and after injection.

Safety and Efficacy Assessments

Subjects were evaluated prior to and at designated timepoints following surgery by complete ophthalmic examination, a general physical exam, and clinical and laboratory tests including assessment of vector biodistribution and immune response. [2, 3]

Efficacy for each subject was monitored with objective and subjective measures of vision.

Objective measures included pupillary light reflex (PLR) responses, nystagmus testing, multi-focal ERG (mfERG).[2, 3] Subjective measures included standard LogMAR visual acuity tests, Goldmann visual fields, full-field dark adaptometry sensitivity threshold (FST) testing, and mobility testing to assess differences in the subjects' ability to navigate a standardized obstacle course.[2, 3]

RESULTS

Characteristics of the Subjects

Twelve consecutive patients with LCA-*RPE65* were enrolled and received vector between October 2007 and June 2009. Seven adults provided informed consent while parental permission was obtained from the parents of 5 children with the children providing assent, or affirmative agreement (Table 1). There were no two individuals who had the same disease-causing *RPE65* mutations except for NP01 and NP02.[2, 3] Baseline fundus examination and OCTs documented the extent of retinal degeneration in the subjects.

Nystagmus Testing

Nine of the twelve subjects had significant nystagmus (frequency >1 Hz) at baseline. After administration of AAV2.hRPE65v2, these nine of the 12 subjects with significant

nystagmus had a decrease in monocular and binocular amplitude and frequency of nystagmus in primary position, eccentric gaze, and with monocular cover (Table 1 and data not shown).[4]

Safety of Subretinal Injection of Vector

A macular hole was identified in NP02's injected eye at d14, as previously described.[3] Thereafter, procedures were instituted to minimize the chance that such a complication would occur. These included removal of epiretinal membranes, and selection of a retinotomy site a minimum of 2 disc diameters from the fovea. An epiretinal membrane noted during baseline studies in CH10's injected eye was removed prior to injection.

There were no serious adverse events in any of the subjects. Similar to results for NP01 in the low dose group,[3] tear samples were positive at low levels for vector DNA sequences in two of the six middle dose subjects (CH09 and CH11) on day 1 after surgery and were positive in another middle dose subject (NP04) through day 4 after surgery (Table S1). Tear samples were also low level positive in the early post-surgical period in two of the 3 high dose subjects (CH13 and NP15). All tear samples were negative thereafter. There was no evidence of systemic dissemination of vector sequences in any of the low or middle dose subjects at any time. However, peripheral blood mononuclear cells (PBMC's) were mildly positive in the immediate post-operative time period in two of the 3 high dose subjects (CH12 and CH13) and serum was also positive in this early post-operative period in CH13 (Table S1). There were minimal changes in neutralizing antibodies (NAbs) directed against AAV2 after vector delivery (Table S2). An increase in neutralizing antibody (NAb) titer to AAV2 compared to baseline was observed during the first four weeks after gene transfer in the following subjects: NP-02, Day 14; CH-06, Day 14; CH-13, Day 30; NP-15, Day 14. An small increase in NAb titer was observed at Day 90 in subjects NP-01, CH-06, and CH-10 (1/2 log; Table S2). In subject CH06, the Nab titer remained at the d90 level through the d365 visit (Table S2).

There was no evidence of T cell responses to the AAV capsid as measured by IFN-□ ELISPOT in any subject at any time (Table S2). Two of the high dose subjects had a detectable response to the RPE65 transgene product – one at baseline (NP-15) and the other at d30 (CH13; Table S2); however, there was a high level of background in these tests.

Ambulatory Behavior

Post-injection mobility testing in NP15, the other subject under twelve years of age, is pending. The subject's parents report marked improvement in ambulation in daily and nightly activities.

References

1. Wright JF. Manufacturing and characterizing AAV-based vectors for use in clinical studies. Gene Ther 2008;15(11):840-8.

2. Bennicelli J, Wright J, Komaromy A, et al. Reversal of Visual Deficits in Animal Models of Leber congenital amaurosis within Weeks after Treatment using Optimized AAV2-mediated Gene Transfer. Mol Ther 2008:16(3):458-65.

3. Maguire AM, Simonelli S, Pierce EA, et al. Safety and Efficacy of Gene Transfer for Leber congenital amaurosis. New England J Medicine 2008;358(21):2240-8.

4. Simonelli F, Maguire A, Testa F, et al. Gene therapy for Leber's congenital amaurosis is safe and effective through one year after vector administration. Mol Ther, in press.

Table 1. Schedule of Subject Evaluations – Table of Clinical Assessments.

Days are listed relative to the day of injection (day 0) through d365 (year 1). After the year 1 timepoint, follow-up is scheduled at half year and then yearly intervals. After year 5, subjects are contacted yearly through 15 years. B1, B2 = Baseline 1, Baseline 2.

	B1	**B2**	**-3**	**0**	**1**	**2**	**3**	**14**	**30**	**60**	**90**	**180**	**270**	**365**	**1.5**	**2-5**
Informed consent	X															
History/PE	X				X				X		X			X		
Pregnancy test (blood test)	X			X												
Begin prednisone			X													
Discontinue prednisone								X								
Vital signs	X	X		X	X	X	X	X	X					X		
Hematology	X				X		X	X	X		X			X		
Chemistry	X			X	X		X	X	X		X			X		
PBMC collection	X								X							
Urinalysis	X			X	X		X	X	X		X			X		
AAV Ab	X							X	X		X			X		
RPE65 Ab	X							X	X		X			X		
Peripheral blood/Tear PCR	X			X	X	X*										
Ophthalmic exam†	X	X			X	X	X^	X	X	X	X	X	X	X	X	X
OCT	X									X		X		X	X	X
Acuity	X	X			X	X	X	X	X	X	X	X	X	X	X	X
Color vision	X	X							X	X	X	X	X	X	X	X
Contrast sensitivity		X										X		X	X	X
ERG	X									X				X	X	X

Color vision	X	X							X	X	X	X	X	X	X	X
Contrast sensitivity		X										X		X	X	X
ERG	X									X				X	X	X
Visual field test		X							X	X	X	X	X	X	X	X
Pupillometry		X							X	X	X	X	X	X	X	X
Nystagmography (ocular motility)		X									X	X	X	X	X	X
Fundus photography	X			X					X			X		X	X	X
Quality of life	X									X		X		X	X	X
Mobility testing		X									X	X		X	X	X
Adverse event recording	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X

Acceptable Timeframe for Subject Evaluations

Timepoint	± 2 days	± 5 days	± 30 days
Day 14	X		
Days 30, 60, 90		X	
Days 180, 270, 365			X
Years 1.5, 2-5			X

Baseline tests are < 4 weeks before Day 0 (AAV2-hRPE65v2 administration

Supplemental Table 1: Biodistribution (Vector Shedding)

ND, not done; Pos, positive; Neg, negative; NQ, non-quantitative

Low Dose Cohort

	NP01			NP02			NP03	
	Tear	**Blood**		**Tear**	**Blood**		**Tear**	**Blood**
Baseline	Neg	Neg		Neg	Neg		Neg	Neg
Day 0	Neg	Neg		Neg	Neg		Neg	Neg
Day 1	Pos	Neg		Neg	Neg		Neg	Neg
Day 2	Neg	ND		Neg	ND		Neg	ND
Day 3	Neg	ND		Neg	Neg		Neg	Neg

Middle Dose Cohort

	NP04			CH06			CH08	
	Tear	**Blood**		**Tear**	**Blood**		**Tear**	**Blood**
Baseline	Neg	Neg		Neg	Neg		Neg	Neg
Day 0	Neg	Neg		Neg	Neg		Neg	Neg
Day 1	Neg	Neg		Neg	Neg		Neg	Neg
Day 2	Pos	ND		Neg	ND		Neg	ND
Day 3	Pos	Neg		Neg	Neg		Neg	Neg
Day 4	Pos	Neg						
Day 6	Neg	Neg						
Day 8	Neg	Neg						
Day 9	Neg	Neg						

	CH09			CH10			CH11	
	Tear	**Blood**		**Tear**	**Blood**		**Tear**	**Blood**
Baseline	Neg	Neg		Neg	Neg		Neg	Neg
Day 0	Neg	Neg		Neg	Neg		Neg	Neg
Day 1	Pos	Neg		Neg	Neg		Pos	Neg
Day 2	Neg	ND		Neg	ND		Neg	ND
Day 3	Neg	Neg		Neg	Neg		Neg	Neg

High Dose Cohort

	CH12			CH13			NP15	
	Tear	**Blood**		**Tear**	**Blood**		**Tear**	**Blood**
Baseline	Neg	Neg		Neg	Neg		Neg	Neg
Day 0	Neg	Neg		Neg	Neg		Neg	Neg
Day 1	Neg	Neg		Pos	Pos PBMC &		Pos NQ	Neg

					Serum NQ			
Day 2	Pos	ND		Pos Both eyes Uninjected NQ	ND		Pos NQ	ND
Day 3	Pos	Pos PBMC NQ		Neg	Pos Serum NQ		Neg	Neg
Day 8	ND	Neg		ND	ND		Neg	ND
Day 14	ND	Neg		Neg	Neg			
Day 30	ND	Neg		Neg	Neg			

Supplementary Table 2. T Cell Response analysis IFN-γ ELISPOT

- **Poor cell viability; ** high background; BV: baseline visit**

Low Dose Cohort

Subject ID	Visit Name	Capsid IFN-γ ELISPOT	RPE65 IFN-γ ELISPOT
NP01	BV 1	Negative	Negative
	Day 14	Negative	Negative
	Day 30	Negative	Negative
	Day 90	Negative	Negative
NP02	BV 1	Negative	Negative
	Day 14	Negative**	Negative**
	Day 30	Negative	Negative
	Day 90	Negative	Negative
NP03	BV 1	Negative	Negative
	Day 14	Negative	Negative
	Day 30	Negative	Negative
	Day 90	N/A	N/A

Middle Dose Cohort

Subject ID	Visit Name	Capsid IFN-γ ELISPOT	RPE65 IFN-γ ELISPOT
NP04	BV 1	Negative	Negative
	Day 14	Negative	Negative
	Day 30	N/A*	N/A*
	Day 90	Negative	Negative
CH06	BV 1	Negative	Negative
	Day 14	Negative	Negative
	Day 30	Negative**	Negative**
	Day 90	Negative	Negative
CH08	BV 1	Negative	Negative
	Day 14	Negative	Negative
	Day 30	Negative**	Negative**
	Day 90	Negative	Negative

CH09	**BV 1**	**Negative**	**Negative**
	Day 14	**Negative**	**Negative**
	Day 30	**Negative**	**Negative**
	Day 90	**Negative**	**Negative**
CH10	**BV 1**	**Negative**	**Negative**
	Day 14	**Negative**	**Negative**
	Day 30	**Negative**	**Negative**
	Day 90	**Negative****	**Negative****
CH11	**BV 1**	**Negative**	**Negative**
	Day 14	**Negative**	**Negative**
	Day 30	**Negative****	**Negative****
	Day 90	**Negative**	**Negative**

High Dose Cohort

Subject ID	Visit Name	Capsid IFN-γ ELISPOT	RPE65 IFN-γ ELISPOT
CH12	**BV 1**	**Negative**	**Negative**
	Day 14	**Negative**	**Negative**
	Day 30	**Negative**	**Negative**
	Day 90	**Negative***	**Negative***
CH13	**BV 1**	**Negative**	**Negative**
	Day 14	**Negative**	**Negative**
	Day 30	**Negative**	**Positive**
	Day 90	**Pending**	**Pending**
NP15	**BV 1**	**Negative****	**Positive****
	Day 14	**Negative***	**Negative***
	Day 30	**Negative**	**Negative**
	Day 90	**Pending**	**Pending**

Anti-AAV2 Neutralizing Antibody

Low Dose Cohort

Subject ID	Visit Name	Neutralizing Antibody
NP01	BV 1	<1:3.16
	Day 14	<1:3.16
	Day 30	<1:3.16
	Day 90	1:10 – 1:31.6
	Day 365	1:3.16 – 1:10
NP02	BV 1	<1:3.16
	Day 14	1:31.6 – 1:100
	Day 30	1:10 – 1:31.6
	Day 90	1:10 – 1:31.6
	Day 365	Neat – 1:3.1*
NP03	BV 1	1:3.16 – 1:10
	Day 14	1:3.16 – 1:10
	Day 30	1:3.16 – 1:10
	Day 90	1:3.16 – 1:10
	Day 365	1:3.16 – 1:10

Middle Dose Cohort

Subject ID	Visit Name	Neutralizing Antibody
NP04	BV 1	1:3.16 – 1:10
	Day 14	1:3.16 – 1:10
	Day 30	1:3.16 – 1:10
	Day 90	1:3.16 – 1:10
	Day 365	1:3.16 – 1:10
CH06	BV 1	1:31.6 – 1:100
	Day 14	1:316 – 1:1000
	Day 30	1:31.6 – 1:100
	Day 90	1:100 – 1:316
	Day 365	1:100 – 1:316

CH08	**BV 1**	**1:10 – 1:31.6**
	Day 14	**1:10 – 1:31.6**
	Day 30	**1:3.16 – 1:10**
	Day 90	**1:3.16 – 1:10**
	Day 365	**Pending**
CH09	**BV 1**	**1:3.16 – 1:10**
	Day 14	**1:3.16 – 1:10**
	Day 30	**1:3.16 – 1:10**
	Day 90	**1:3.16 – 1:10**
	Day 365	**Pending**
CH10	**BV 1**	**1:300 – 1:350**
	Day 14	**1:300 – 1:350**
	Day 30	**1:300 – 1:350**
	Day 90	**1:1000 – 1:3160**
	Day 365	**pending**
CH11	**BV 1**	**1:3.16 – 1:10**
	Day 14	**1:3.16 – 1:10**
	Day 30	**1:3.16 – 1:10**
	Day 90	**1:3.16 – 1:10**
	Day 365	**Pending**

High Dose Cohort

Subject ID	Visit Name	Neutralizing Antibody
CH12	**BV 1**	**Neat – 1:3.1**
	Day 14	**Neat – 1:3.1**
	Day 30	**Neat – 1:3.1**
	Day 90	**Neat – 1:3.1**
	Day 365	**Pending**
CH13	**BV 1**	**Neat – 1:3.1**
	Day 14	**Neat – 1:3.1**
	Day 30	**1:10 – 1:31.6**
	Day 90	**Pending**
	Day 365	**Pending**

NP15	**BV 1**	**Neat – 1:3.1**
	Day 14	**1:3.1 – 1:10**
	Day 30	**1:3.1 – 1:10**
	Day 90	**Pending**
	Day 365	**Pending**

Anti-RPE65 Antibody ELISA (Humoral Response

#, Minimally positive with one test (shown); negative on repeat test

Subject	Baseline	Day 14	Day 30	Day 90	Day 365
Low dose					
NP01	**ND**	**ND**	**ND**	**ND**	**ND**
NP02	**ND**	**ND**	**ND**	**ND**	**ND**
NP03	**ND**	**ND**	**ND**	**100#/ND**	**ND**
Middle dose					
NP04	**ND**	**ND**	**ND**	**ND**	**ND**
CH06	**ND**	**ND**	**ND**	**ND**	**ND**
CH08	**ND**	**ND**	**ND**	**ND**	**ND**
CH09	**ND**	**ND**	**ND**	**ND**	**Pending**
CH10	**ND**	**ND**	**ND**	**ND**	**Pending**
CH11	**ND**	**ND**	**ND**	**ND**	**Pending**
High dose					
CH12	**ND**	**ND**	**ND**	**ND**	**Pending**
CH13	**ND**	**ND**	**ND**	**ND**	**Pending**
NP15	**ND**	**ND**	**ND**	**Pending**	**Pending**

Supplementary Table 3 The association of age with VA outcome (in logMar) in the injected eye

	Correlation with age			age 8-11 yrs group (n=4)	age 19-44 yrs group (n=8)		
VA Outcome	Pearson Correlation Coefficient	P-value		Mean (SE)	Mean (SE)		P-value*
Baseline VA of injected eye	0.72	0.009		1.12 (0.11)	1.81 (0.39)		0.04
Final VA	0.65	0.02		1.09 (0.25)	1.45 (0.30)		0.46
Change of final VA from baseline	-0.24	0.45		-0.03 (0.24)	-0.35 (0.13)		0.22
Mean Post-injection VA	0.73	0.007		1.00 (0.18)	1.50 (0.29)		0.28
Mean change of post-injection VA from baseline	-0.20	0.53		-0.12 (0.14)	-0.30 (0.19)		0.34

* From the comparison of means using two group t-test.

Supplementary Table 4 VA at baseline and at post-injection for each of 12 patients

ID	Age (yrs)	Days of last visit	Baseline VA	Final visit VA	Mean of Post-injection VA	Mean Change of post-injection VA from baseline	P-value for test the significant change in post-injection VA
CH06	20	365	1.35	1.83	1.78	0.43	0.04
CH08	9	365	1.03	1.62	1.20	0.17	0.60
CH09	8	365	1.03	1.04	1.03	-0.003	0.91
CH10	10	270	1.45	1.29	1.29	-0.16	0.002
CH11	24	270	1.02	0.60	0.60	-0.42	0.0004
CH12	44	180	3.42	3.25	3.25	-0.17	NA*
CH13	35	90	1.91	1.60	1.63	-0.28	0.19
NP01	26	577	2.00	1.47	1.56	-0.44	0.02
NP02	26	577	2.00	1.41	1.44	-0.56	<0.0001
NP03	19	545	1.50	0.96	1.06	-0.45	0.004
NP04	17	365	1.27	0.54	0.71	-0.56	0.006
NP15	11	90	0.96	0.42	0.49	-0.47	0.03
All patients combined			1.58	1.34	1.35	-0.24	0.02

P-value could not be calculated as there is no variation in the post-injection VA (ie, all post-injection VA are the same).

Supplementary Table 5. Representative Pupillary Light Reflex.Result Summary.

Table 5. Analyses compare baseline values with post-injection values in the treated eye using Test 1 (alternating brief (0.2 sec) flashes of light). Post-injection values, Mean(SD), were measured at 2-3 months after injection except for NP01, NP03, and NP04, where measurements were made at 5 months (for NP01) and at 7.5 or 10 months after injection (for NP03 and NP04;respectively, due to equipment availability). Illumination in all cases was ≥0.4 Lux except for CH08 and CH09 where illumination was 0.04 Lux..
Significant improvements in the PLR were observed in all subjects except NP04 (in whom detailed testing was not performed) and CH11.

p ≤ 0.004
p ≤ 0.09

Subject	Baseline Amplitude	Post-Rx Amplitude	*p*-value	Baseline Velocity	Post-Rx Velocity	*p*-value
NP01*	0.39 (0.07)	0.64 (0.14)	**p=0.003**	-0.76 (0.14)	-1.21 (0.19)	**p=0.0004**
NP02	-0.01 (0.03)	0.46 (0.07)	**p<0.001**	-0.02 (0.06)	-1.5 (0.24)	**p<0.0001**
NP03**	0.48 (0.03)	0.80 (0.07)	**p = 0.03**	-1.7 (0.27)	-2.3 (0.12)	**p = 0.09**
NP04***	0.51 (0.07)	0.35 (0.06)	p=0.16	-1.0 (0.12)	-0.91 (0.17)	p=0.6
CH06^	0.27 (0.42)	0.67 (0.01)	**p =0.03**	-0.26 (0.40)	-2.0 (0.20)	**P=0.001**
CH08	-0.14 (0.11)	0.82 (0.13)	**p = 0.0002**	-0.26 (0.4)	-2.0 (0.2)	**p = 0.004**
CH09	0.16 (0.28)	1.20 (0.20)	**P=0.04**	-0.07 (0.28)	-2.58 (0.22)	**P=0.001**
CH10	0.65 (0.03)	1.83 (0.69)	**p = 0.07**	-2.13 (0.13)	-3.97 (0.54)	p=0.002
CH11	0.2 (0.17)	0.32 (0.07)	p=0.46	-0.61 (0.09)	-.97 (0.18)	p=0.39
CH12^	0.24 (0.08)	0.96 (0.21)	**P=0.001**	-0.001 (0.46)	-1.84 (0.76)	**p=0.01**
CH13^	0.009 (0.05)	1.04 (0.73)	**P=0.06**	-0.04 (0.05)	-1.96 (1.29)	p=0.16
NP15^	0.15 (0.84)	0.43 (0.16)	P=0.33	-0.54 (0.21)	-1.4 (0.28)	**P=0.03**

*Analyses carried out at 5 mos
** Analyses carried out at 10 mos
***Analyses carried out at 7.5 mos
^ Analyses carried out at 2 mos

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Supplementary Table 6 Mobility Course Data

The subjects were tested for their ability to avoid objects on a mobility course and the time that it took them to navigate this course was measured. Both eyes were open except for some cases where either the injected eye or the uninjected eye was occluded. The time to navigate the obstacle course was affected by the individual's ability to see the course and the individual's level of self-confidence. Their ability to see the course was affected by the room lighting – at least prior to injection. Post-injection data is presented for day 90. In some cases after injection, the mobility test was given under dim light in order to probe the limits of the improved navigation.

Several individuals improved their ability to navigate the obstacle course (highlighted in yellow). In some of these situations, it took longer to complete the course, but in others, the completion time was considerably reduced. Three of the subjects (CH08, CH09, CH10) could navigate the course - even under dim illumination- using their injected eye, but not their uninjected eye.

Data for the three low dose subjects NP02 was presented in part by Maguire et al[1] and is listed here for comparison.. NA, not available

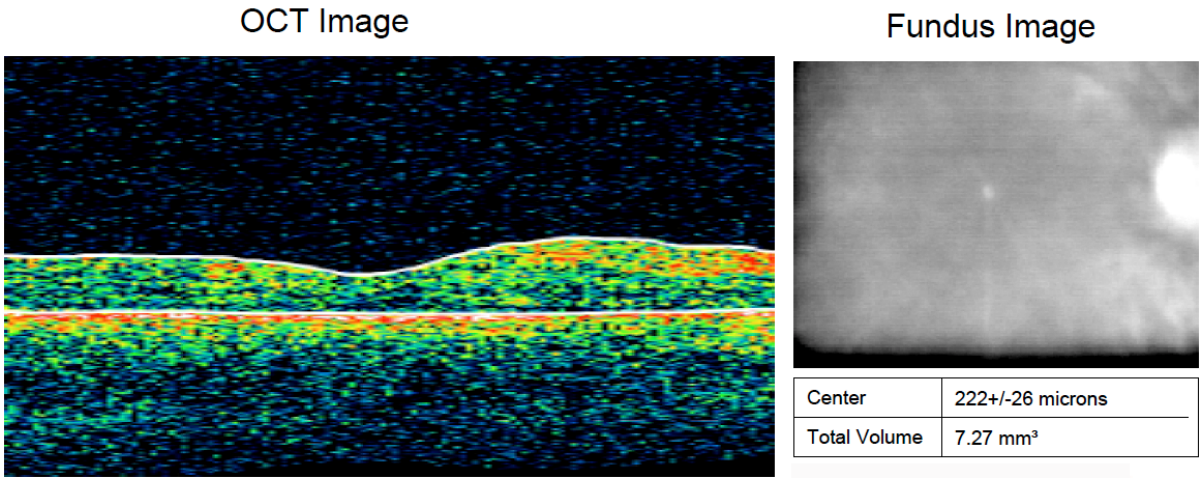
Subject	Baseline Time (sec)	Post-Injection Time (sec)	Baseline Obstacles Avoided	Post-Injection Obstacles Avoided	Dimmest Light Tested (lux)	Other
NP01	152	155	3/13	3/13	250	
NP02	112	177	0/14	12/13	250	
NP03	69	71	11/14	12/13	250	
NP04	30	NA	12/14	NA	250	
CH06	67	66	3/11	6/13	250	
CH08	73	57	2/12	11/12	250	
CH08	69		1/12		50	
CH08		94		11/12	10	Uninjected eye occluded
CH08		355	3/13		50	Injected eye occluded
CH09	46	20	10/13	12/13	250	Uninjected eye occluded
CH09		27		12/13	10	Uninjected eye occluded
CH09		421		0/12	10	Injected eye occluded
CH09		19		12/12	1.0	Uninjected eye

						occluded
CH10	346		10/13		250	Uninjected eye occluded
CH10		37		13/13	150	Uninjected eye occluded
CH10		283		12/13	4	Uninjected eye occluded
CH11	54		9/13		250	Uninjected eye occluded
CH11		84		12/13	150	Uninjected eye occluded
CH12	70	122	3/11	4/12	250	
CH13	60		3/11		250	Uninjected eye occluded
CH13		92		6/12	200	Uninjected eye occluded
NP15	99	10	6/12	14/14	50-60	Uninjected eye occluded

1. Maguire AM, Simonelli S, Pierce EA, et al. Safety and Efficacy of Gene Trnasfer for Leber Congenital Amaurosis. New England j Medicine 2008;358(21):2240-8.

Supplementary Figure 1

An optical coherence tomography (OCT) image from CH10 at d8 is shown demonstrating normal foveal anatomy in this child with nystagmus.

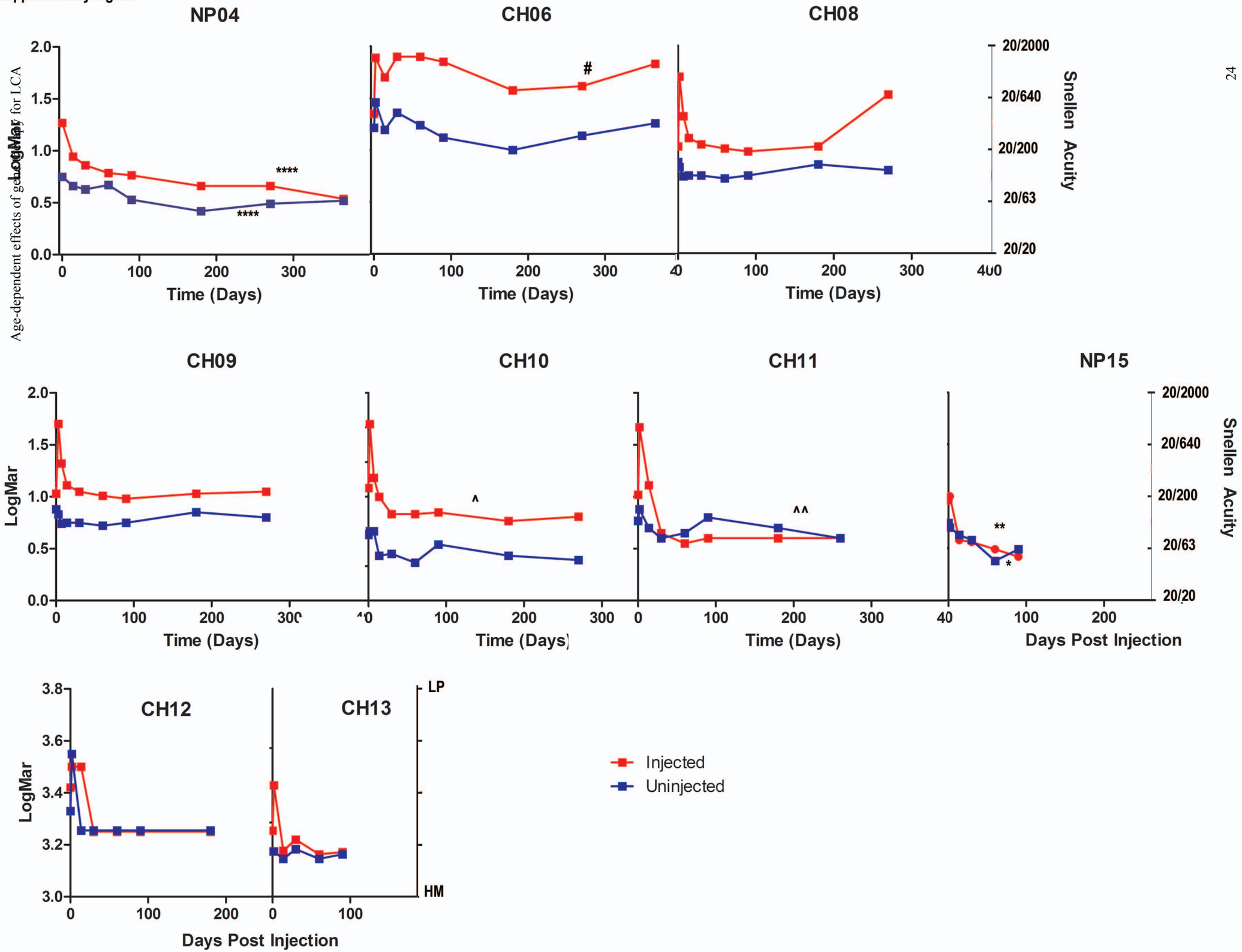


Supplementary Figure 2. Visual Acuity

Testing showed that the postoperative average visual acuity LogMAR and Snellen values in the injected and uninjected eyes. Data for the low dose cohort (NP01 -> NP03) are presented elsewhere (15, 20). Significant improvements in visual acuity were noted in the injected eyes of NP04, CH10, CH11, and NP15. A worsening of visual acuity was noted in CH06.

Improvements in visual acuity in the injected eyes were often accompanied by improvements in the uninjected eye. The LogMAR value for NP04's injected eye improved by 0.56, from a Snellen equivalent of 20/300 to 20/104; ****$p < 0.0001$, Wilcoxon ranksum test). The uninjected eye improved by a smaller increment (by LogMAR 0.21, from a Snellen equivalent of 20/112 to 20/69; $p<0.0001$). The postoperative average visual acuity LogMAR value for NP15 improved by LogMAR 0.44 from a Snellen equivalent of 20/200 (LogMAR 0.96) to 20/61.2 (LogMAR 0.525) (**$p=0.0003$; Figure 2). Visual acuity in the contralateral un-injected eye improved by a smaller extent; from a Snellen equivalent of 20/80 (LogMAR 0.744) to 20/63 (LogMAR 0.48; *$p=0.0002$). Improvements were also noted in CH10 and CH11 (^, ^^: $p =0.002, 0.004$, respectively). A worsening ($p=0.04$) was noted in CH06.

Supplementary Figure 2



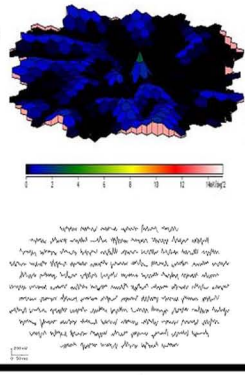
Age-dependent effects of gene therapy for LCA

Supplementary Figure 3. Multifocal ERGs (mfERGs)

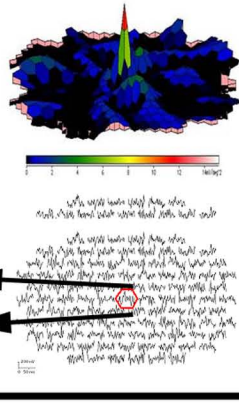
NP15: Baseline, d30, and d60 recordings suggest development of a mfERG signal in the injected region of the retina (macula) in NP15. 3-dimensional graphs of the macular recordings are shown for each timepoint. Underneath are ERG traces in each field. Individual ERG traces are magnified. NP04: Suggestion of a small mfERG signal post-injection (d90) in the left (injected) retina (macula). The mfERG was performed with use of a Veris Science 6.1 system (EDI, Inc, San Mateo, CA) according to ISCEV (International Society for Clinical Electrophysiology of Vision) standards using DTL electrodes and a stimulus array of 103 hexagons (Hood DC ISCEV Guidelines 2007). The number of potential mfERG identified responses, in the mfERG traces array, range from 1 to 3 of the 103 areas stimulated in these two subjects.

NP15 **Right (injected)** **Left**

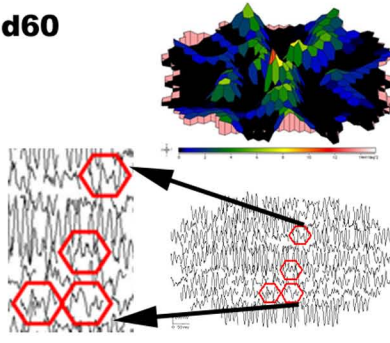
Baseline



d30

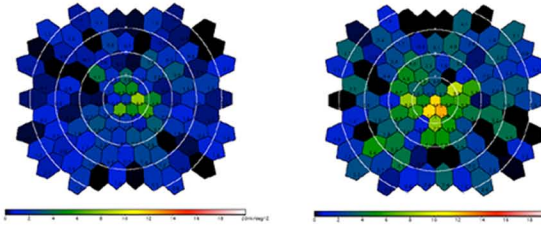


d60



NP04 **Right** **Left (Injected)**

d90



Web Videos

Web Videos 1, 2: Results of mobility course testing in subject CH09 (8yo) 3 months after injection of the left eye. Video 1 shows the subject traversing the obstacle course with his left (injected) eye patched. He has difficulty seeing the course and it takes him a long time to complete the test. Video 2, captured within 10 minutes of Video 1, shows the same subject traversing a reconfigured obstacle course with his contralateral (right, uninjected) eye patched. {He is using his injected eye for navigation.} He has no difficulty completing the test accurately. Room light for both tests is 10 lux. {Movies have been color corrected.}

Web Videos 3, 4: Results of mobility course testing in subject CH08 (9yo) at d270 after injection of the right eye. Video 3 shows the subject traversing the obstacle course with his left (injected) eye patched. He has difficulty seeing the course and tells his father that he cannot see anything except white and black (in Flemish Dutch). He says, “The eye that didn’t have surgery I s not good in the dark. You know that. That eye can’t see anything.” It takes him a long time to complete the test. Video 4 shows the same subject traversing a reconfigured obstacle course with his contralateral (right, uninjected) eye patched. {He is using his injected eye for navigation.} He has no difficulty completing the test accurately. Room light for both tests is 4 lux.

Web Videos 5, 6: Results of mobility course testing in subject CH10 (10yo) at d180 after injection of his right eye. Video 5 shows the subject traversing the course with his left (injected) eye patched. He has difficulty seeing the course, complains to his mother that he cannot see anything (in Flemish Dutch) and it takes him a long time to complete the test. Video 6 shows the same subject traversing the mobility course with his contralateral (right, uninjected) eye patched. {He is using his injected eye for navigation.} He looks carefully at the landmarks but completes the test with good accuracy. Room light for both tests is 4 lux.