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Supplemental Information

Safety and Long-Term Efficacy of AAV4 Gene Therapy in Patients with *RPE65* Leber Congenital Amaurosis

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Supplementary Table 1.

	DNA allele 1	Protein 1	DNA allele 2	Protein 2
CG01	c.700C>T	p.Arg234X	c.1067delA	Asn356Methfs*17
BJ03	c.544C>G	p.His182Asp	c.726-2A>G	fs*
MM04	c.444G>C	p.Glu148Asp	c.1451G>A	p.Gly484Asp
MR05	c.74C>T	p.Pro25Leu	c.1301C>A	p.Ala434Glu
HM06	c.843_858+7del23	p.Asn282fs*	c.843_858+7del23	p.Asn282fs*
HT07	440_441delCA	p.Thr147Argfs*9	c.1448_1450delATG	p.Asp483del
AM08	246-11A>G	fs*	c.615_616delCA	p.Ile206Cysfs*27
HM09	c.989G>A	p.Cys330Tyr	c.843_858+7del23	p.Cys330Tyr
LC10	c.11+5G>A	fs*	c.1039C>T	p.Arg347Cys

Supplementary Table 2.

		D+1	D+2	D+3
BJ03	**lachrymal fluid**	4 (<LOD)	62 (<LOQ)	12 (<LOD)
HM06	**lachrymal fluid**	18 (<LOD)	7 (<LOD)	4 (<LOD)
HT07	**lachrymal fluid**	0	6 (<LOD)	0
HM09	**lachrymal fluid**	0	7 (<LOD)	16 (<LOD)
	blood	38 (<LOQ)	40 (<LOQ)	32 (<LOQ)
LC10	**lachrymal fluid**	6 (<LOD)	201	184

Supplementary Table 3.

		Anti-AAV 4 response			Anti-RPE65 response	
Patient	Day	Humoral (ELISA IgG)	Humoral (NF)	Cellular	Humoral	Cellular
CG01	BI	negative	negative	negative	non relevant results	negative
	D14					
	D30					
	D60					
	D120					
	D180					
BJ03	BI	negative	negative	negative		negative
	D14					
	D30					
	D60					
	D120					
	D180					
MM04	BI	negative	negative	positive (pools 1&2)		negative
	D14	1/10240	1/500			positive
	D30	1/10240	1/1000			positive
	D60	1/10240	1/1000			positive
	D120	1/10240	1/1000			negative
	D180	1/10240	1/1000			negative
MR05	BI	1/10240	1/50	negative		negative
	D14	1/10240	1/50			
	D30	1/2560	1/50			
	D60	1/10240	1/50			
	D120	1/10240	1/50			
	D180	1/10240	1/50			
HM06	BI	negative	negative	negative		negative
	D14					
	D30					
	D60					
	D120					
	D180					
HT07	BI	negative	negative	negative		negative
	D14					
	D30					
	D60					
	D120					
	D180					
AM08	BI	negative	negative	negative		negative
	D14					
	D30					
	D60					
	D120					
	D180					
HM09	BI	negative	negative	negative		negative
	D14	1/10240	1/1000			
	D30	1/10240	1/2500			
	D60	1/10240	1/2500			
	D120	1/10240	1/2500			
	D180	1/10240	1/2500			
LC10	BI	negative	negative	negative		negative
	D14					
	D30					
	D60					
	D120					
	D180					

Supplementary Table 4.

	treated eye	untreated eye
C1	0	0
C2	0	0
C3	- cerebellum	- thalamus (ipsilateral) - occipital lobe (contralateral)
C4	- precuneus (contralateral) - thalamus (ipsilateral) - corpus callosum - cerebellum	0
C5	- thalamus (ipsilateral)	0
C6	- thalamus (ipsilateral) - cerebellum	- frontal sup (contralateral)

Supplementary Table 1. Demographic and genetic characteristics of patients.

Supplementary Table 2. Vector genome values, as detected by qPCR in 5 patients during the biodistribution study (lachrymal fluid and blood samples).

Supplementary Table 3. Summary of the immunogical data collected during follow-up from the 9 patients. BI, baseline.

Supplementary Table 4. Clusters with a significantly higher activation after treatment according to light intensity and the side stimulated.

Supplementary Materials:

Functional MRI:

Each patient underwent an MRI before and 6 months after treatment. Visual stimulations were generated with the help of a specific software to monitor the lighting of images (Cogent, Matlab, The Mathworks, Inc., Natick, MA, USA). The images were projected (Epson EMP-8300 1024 x 768 pixels) onto a transparent stimulation screen (20.5° x 15°) placed 85 cm from the patient's eyes. We used a paradigm with a block design, alternating between different light stimulations (L) and rest in darkness (N). For each condition, monocular stimulation was performed by projecting onto the screen a homogeneous background

alternating between a grey and black value at a frequency of 5 Hz for 15 seconds. The luminance of the grey background was selected from 6 values (L1, L2, L3, L4, L5, L6), and remained unchanged for the 15-s presentation period, but varied from one value to another between presentations. L1 and L6 corresponded to minimum and maximum lighting values, respectively. Intermediate values (L2, L3, L4, L5) and were distributed homogeneously to cover the entire stimulation spectrum. The rest in darkness condition was set up by projecting a black background without visual or auditory stimulations for 15 seconds. During a run, the six conditions L and condition N were presented twice in a pseudorandom order (duration of a run = 3 minutes 30 seconds). Each patient performed 6 successive runs (3 right and 3 left monocular stimulations) during the acquisition session, with several minutes of rest between each run. The first eye stimulated was selected randomly, and the contralateral eye occluded with an ortopad. The same order of stimulation was always used (2 × eye 1, 2 × eye 2, 1 × eye 1, 1 × eye 2). The cache was changed between 2 runs. Functional acquisitions were performed with a 3-Tesla magnetic resonance system (32-channel Siemens Magnetom TrioTim syngo MR. VB13) and a standard 12-channel antenna placed around the head. During the MRI session, brain acquisition sections were oriented according to the AC-PC line. Functional data were acquired with T2-weighted gradient-EPI sequences (gradient-echo planar image; time repetition, 2800 ms; time echo, 30 ms; flip angle, 90°; matrix size, 80 x 80; field of view, 200 x 200 mm^2; voxel size, 2.5 x 2.5 x 2.5 mm^3; 50 transverse slices; 0.25 gap). Each run lasted 3.5 mins, enabling the acquisition of approximately 75 volumes. Two additional imaging sequences were acquired during the acquisition session. A T1-weighted anatomic acquisition (MP-RAGE) was performed, enabling superimposition of functional data on anatomic data. The acquisition session lasted approximately 1 hour. Functional data were analyzed using the SPM8 software (Wellcome Department of Cognitive Neurology, London, UK). The same pretreatment method was applied to all functional images acquired

(temporary resetting, correction of head movements, joint recording of anatomical and functional data, spatial normalization using the Montreal Neurological Institute (MNI) brain type, and spatial smoothing with the assistance of isotropic Gaussian kernels of 5-mm full-width at half-maximum (FWHM)) (1). In a first-level analysis, individual data were fitted to a general linear model (2). The model was designed to define for each of the 7 conditions (6L and 1N) a regressor describing the theoretic variation of the BOLD (blood oxygen level-dependent) signal during a run. This model was estimated and contrasts were then individually determined. The following contrasts were calculated using SPM8: C1=(L1-N), C2=(L2-N), C3=(L3-N), C4=(L4- N), C5=(L5-N), and C6=(L6-N). Positive effects of each contrast represent voxels significantly activated by luminosity stimulation (increasing intensity from L1 to L6) according to rest in the dark. Statistical parametric maps were thresholded at $P<0.05$ corrected for multiple comparisons using the familywise error (FWE) correction and for cluster extent at $P<0.05$. These analyses were performed separately for the treated eye (TE) and untreated eye (UTE) at each pre-and post-treatment session. For each stimulation condition, we determined for each patient the excess activation observed during the post-treatment session in regard to the pre-treatment session. Fixation in darkness remained the reference condition. Fixation in darkness remained the reference condition. The effect C1 of treatment during stimulation L1 was defined by contrast Cb1=(L1post-Npost) - (L1pre-Npré). Individual responses were variable both in terms of intensity (weak response of patients with the heaviest visual handicap) and topographic locations of activations within the visual cortex (3). We performed one analysis per region of interest. To characterize the functional properties of occipital region, we computed activity profiles plotting percentage signal change relative to “fixation in darkness”, averaged across subjects, under the different conditions. The primary visual cortex was defined as the region of interest (ROI) using a standardized map (WFU PickAtlas, Juelich Histological Atlas, BA17 R and L).

Simultaneously including the right and left parts of the primary visual cortex, the analysis included at the same time ipsilateral voxels for stimulation activated by direct ganglion fibers (temporary retina) and contralateral voxels for stimulations activated by crossed ganglion fibers (nasal retina). Therefore, the same ROI allowed comparison of results depending on whether the treated or contralateral eye was stimulated. The percentage voxel significantly activated for each contrast in ROI was extracted in addition to the average T score for the whole region. The analysis was performed with an initial threshold of $P<0.001$ for the detection of clusters of >10 voxels and confirmed with a second threshold of $P<0.1$ for the detection of clusters of >10 voxels so as not to disregard moderate effects. This has enabled setting out a parametric normogram representing the level of cortical activation (as intensity or as a percentage of voxels activated) according to luminance of light stimulation before and after treatment for the treated and control eye. One-way ANOVA was performed to observe a difference in activation according to stimulations conditions, subjects, treated or control eye (laterality) and timing of data acquisition (pre-or post-treatment). A three-way ANOVA with repeated measures with subject, laterality and timing condition as factors yielded significant main effects for the three factors, significant two-way interactions between each pair of factors, and a significant three-way interaction. To undertake group analyses, all data from patients treated on the left eye were inverted according to the axis xx'. This technique enables artificially defining the right eye as treated eye for all subjects, enabling coherent averaging of cortical activities for all patients. Voxels presenting more significant activation post-treatment in regard to pretreatment were sought in a group study using post-pre-individual results.

A second smoothing with a 5-mm FWHM isotropic Gaussian kernel was applied to the contrast images, and a random effect second-level group analysis was performed to assess the significance of activations at the population level (1). Statistical parametric maps were

thresholded at P <0.001 uncorrected with clusters of >15 voxels.

References:

1. Friston K, Frith C, Poline J-B, Heather J, Frackowiak R. Spatial registration and normalization of images. 1995;165–89.

2. Friston KJ, Holmes AP, Poline JB, Grasby PJ, Williams SC, Frackowiak RS, et al. Analysis of fMRI time-series revisited. NeuroImage. 1995 Mar;2(1):45–53.

3. Ogawa S, Takemura H, Horiguchi H, Terao M, Haji T, Pestilli F, et al. White matter consequences of retinal receptor and ganglion cell damage. Invest Ophthalmol Vis Sci. 2014 Oct;55(10):6976–86.