THE LANCET

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Michaelides M, Laich Y, Wong SC, et al. Gene therapy in children with AIPL1-associated severe retinal dystrophy: an open-label, first-in-human interventional study. *Lancet* 2025; **405:** 648–57.

Appendix

Table of Contents

Supplementary Methods

Surgical administration of AAV8.hRKp.AIPL1

Three-port 23-gauge pars plana vitrectomy was performed by a single surgeon (CW). Vitrectomy surgery included induction of posterior hyaloid separation from the posterior pole using the vitrectomy cutter. AAV2/8.hRKp.AIPL1 vector suspension was administered at a titer of 1 x 10^{11} vg/mL into the subretinal space, using a 41-gauge subretinal cannula via a single retinotomy positioned adjacent to the superotemporal vascular arcade. The aim was to generate a bleb involving the posterior pole and extending from the superior retinal vascular arcade to the inferior arcade. Intraoperative OCT was used to confirm the anatomical location of the bleb. The vitreous cavity was washed out using infusion fluid for 2 minutes to remove vector that may have refluxed from the bleb. The sclerotomies were closed using 8/0 polyglactin sutures (Vicryl; Ethicon Inc). Dexamethasone and cefuroxime were administered subconjunctivally. Postoperatively, eye drops containing a combination of 1 mg dexamethasone, 6000 IU polymyxin B sulphate and 3500 IU neomycin sulphate (Maxitrol, Novartis UK) were prescribed to be used four times daily for four weeks. To protect against intraocular inflammation, children were prescribed oral prednisone, at a dose of 1·0 mg/Kg bodyweight daily for 5 days before surgery, 1 mg/Kg for the first week after surgery, 0·8 mg/Kg for the second week, 0·6 mg/Kg for the third week, 0·4 mg/Kg for the fourth week and 0·2 mg/Kg for the fifth week. Ranitidine 2·0 mg/Kg bodyweight or omeprazole 10 mg once daily were prescribed for the duration of the steroid regimen to protect against harm from gastric adverse effects.

Measurement of visual acuity by Cardiff Cards

There are 13 visual acuity levels in a full set of Cardiff Cards with 3 cards at each level. Before starting the examiner ensures that the cards are in order and all facing downwards. The position of the optotype, top or bottom, should be unknown to the examiner to avoid examiner bias. The cards are presented vertically at either 50cm or 1m depending on the patient's cooperation and visual potential. Starting at 1m, the distance is reduced if unable to get a response at the largest size. 3 cards are presented at each acuity, to confirm acuity achieved. If the examiner is struggling to confirm a seeing response the card can be rotated to a horizontal position. If the child does not see 2 out of 3 cards on an acuity level, the previous acuity level is repeated to confirm that this is their level of VA.

Measurement of visual acuity by Kays Pictures

The child is asked to name all pictures on the matching card. If limited confidence or delayed speech, the child is allowed to match the optotypes. One eye is occluded with glasses if no prescription glasses worn. At 3 metres distance the book is opened a large size picture is presented to the child. The child is asked to name the first (or last) picture in the row, and indicate that picture by briefly pointing to it. This is repeated with smaller sizes until a size is reached where they cannot see either the first or the last picture in the row. Then the other pictures in that row are presented. If two or more pictures in that row can be seen, testing is continued at the next size smaller. If none or only one picture can be seen, the next size larger is used, checking all four pictures. The second eye is similarly tested by occluding the first eye.

Measurement of visual acuity by Sonksen LogMAR test

The child is familiarised with all the letters within the test; O, X, H, Y, U and V. Sheridan Gardiner letters can be used. If unable to name all the letters, a matching card is used. One eye is occluded with prescription glasses or occlusion glasses if prescription glasses are not worn. The child is be presented with a flipbook, 4 letters within a box. Standing 3m away the child is presented with the largest letter to identify. The child is asked to identify each of the letters in the booklet of starting with the 0.7 letter until a letter is not seen correctly. If a child is unable to see some of the letters at the starting level, the tester presents the preceding larger displays until a full line is identified; the child is then shown each letter in turn until three consecutive letters are failed. Every letter correctly identified after the last whole line achieved contributes to the score. The test is continued until 3 out of the 4 letters are incorrectly named and record. The test is repeated with the second eye.

Measurement of visual acuity by PopCSF

In addition to conventional testing of visual acuity by identification of letters and shapes, we also measured visual acuity objectively using a novel gamified touchscreen-based test named the PopCSF test[1] designed to address the challenges of measuring visual function in young children with severe sight impairment. In this test, children are presented with localised drifting grating patterns ('bubbles') and asked to touch ('pop') them on the screen. An adaptive algorithm[2] adjusts the spatial frequency and contrast of these gratings, based on the participant's performance. To maintain constant spatial frequencies across viewing distance, real-time head-tracking with the tablet's front-facing camera is used to adjust physical spatial frequencies. The likelihood of randomly touching a grating on the screen is very low, with a ~1% chance of any touched pixel falling inside a grating. Conservatively, we define chance-level performance as success rates of 10% or below. We used logistic regression to compare the relationship between hit rate and target spatial frequency across the two eyes. Additionally, to assess grating acuity limits, we categorized the targets into three categories based on their spatial frequency: low (1·5-2·5 cycles per degree (cpd)), medium (2·5-3·5 cpd), and high (>3·5 cpd), and compared the hit rates in each category to a 10% chance level using exact binomial tests. Since these measures are based on drifting gratings, they are not directly comparable to standard acuity tests. However, converted to LogMAR acuity they correspond approximately to 1·10-1·30, 0·95-1·10, and < 0·95 logMAR. For Child 2 we excluded three invalid grating targets from the analysis because they had been viewed binocularly.

Measurement of cortical responses to visual stimuli by steady-state visually evoked potentials

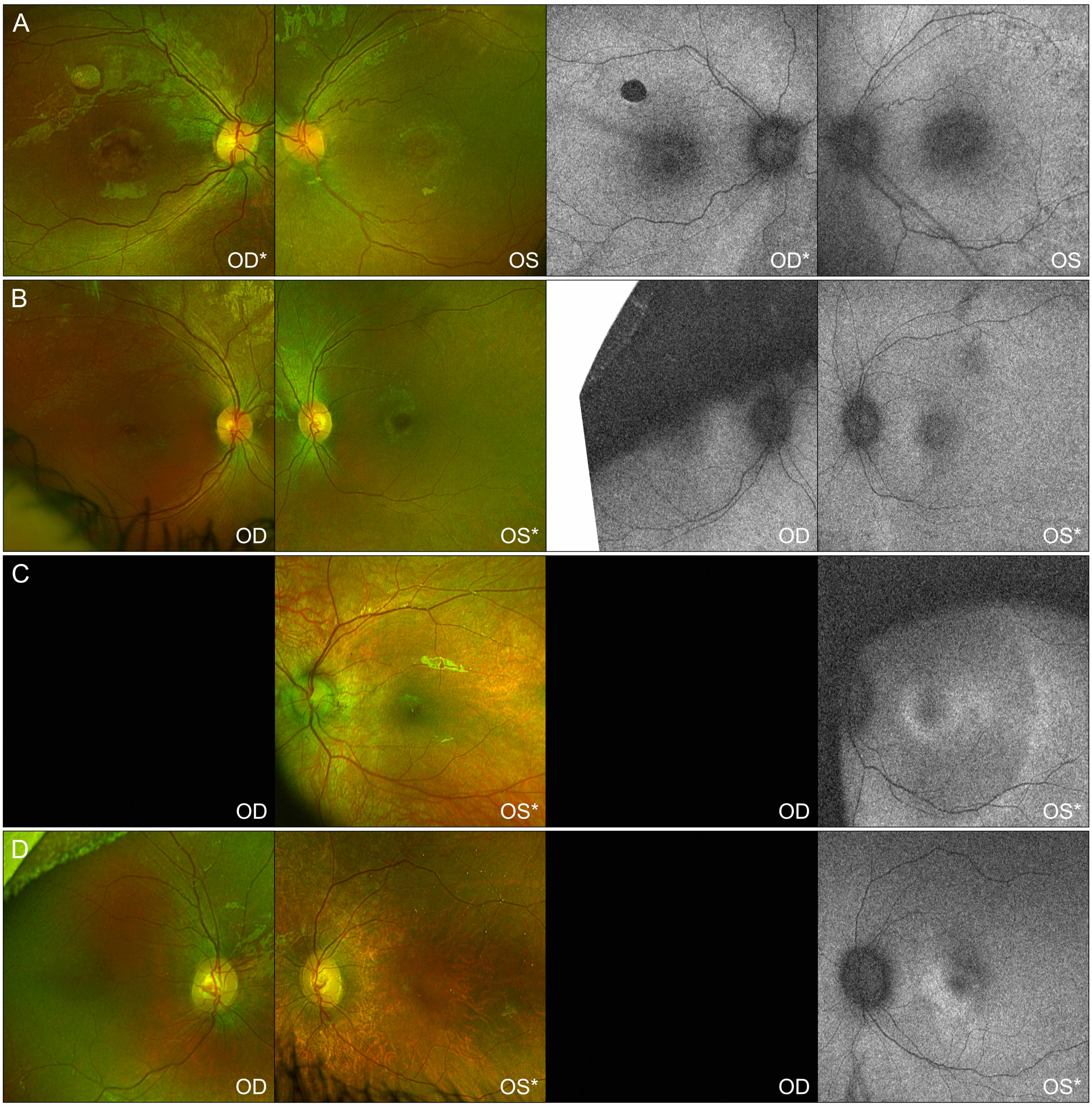
We measured visual signal detection in the visual cortex using the steady-state visually evoked potential (ssVEP) technique[3–5] by recording cortical electrophysiological responses to full-screen black/white flickering stimuli and flickering (contrast-reversing) gratings across a range of spatial frequencies. Main analyses focus on the full-screen stimuli, data for gratings are in Supplemental Figure 2. Testing was conducted with a wide screen setup (65° width, 55 cm viewing distance, 380 cd/m^2 peak luminance) using a Biosemi system.[6] Ambient light levels were mesopic and constant across sessions and patients. Children were not dark-adapted. Individually sized head caps were used to ensure stable and consistent electrode sensor positioning over occipital cortex. Full-field flicker and spatial frequency conditions were administered in 2-second intervals and repeated 9-12 times for each eye. Because a secondary objective of this study was to determine the optimal testing parameters for measuring treatment-related change in ssVEPs in this cohort for future studies, spatial and temporal frequencies of the gratings were varied across participants. Child 1 underwent testing with full-screen black-white flicker and sinusoidal gratings of 0·5, 1, and 3 cpd, each contrast-reversing at a 7·5 reversals per second (rps). Child 2 was tested with a full-screen black-white flicker and checkerboard gratings of 0·3, 1, and 3 cpd, each flickering at 15 rps. Despite these variations in stimulus parameters between individuals, within-subject comparison of treated versus untreated eyes is valid. Retinocortical acuity, reflected in ssVEPs, was assessed by first referencing the electrical signal from a central occipital electrode (Oz in the 10-20 system) over the occipital pole to the average of two adjacent electrodes (O1 and O2). This signal was then averaged across repetitions for each condition and transformed into the frequency domain via a discrete Fourier transform. No offline noise filtering or epoch rejection was applied to the raw EEG signal. Fourier analysis was used to distinguish the recovering visual signal at the stimulus flicker frequency from noise. For a grating that can be detected, visual cortex activity is expected to synchronise with the stimulus flicker frequency, thus increasing signal power at this temporal frequency. Conversely, a grating beyond the acuity limit that this method can detect is not expected to increase power at the stimulus flicker frequency. A significant therapy effect is measured as the relative increase in power between the treated versus untreated eye. To verify that signal enhancement is specific to the visual stimulus frequency, signal-to-noise ratio (SNR) was computed by dividing the power at the target frequency by the average power of the two neighbouring frequency bins of non-interest.[7,8] To determine the statistical significance of ocular differences in ssVEP signal we adopted a permutation-based strategy.[9] This approach involved randomly resampling the repetitions within each condition and repeating the analysis steps as described above 10,000 times to generate a surrogate null distribution of differences between eyes. We determined the statistical significance by comparing the actual observed difference against this generated null distribution.

Retinal imaging

Retinal imaging was performed using handheld spectral-domain optical coherence tomography (OCT; Figure 4) (Envisu C2300; Leica Microsystems, United Kingdom) and wide-field fundus autofluorescence imaging (Supplementary Figure 1; Optos Panoramic 200; Optos PLC, United Kingdom). Rectangular, horizontal OCT volume scans were obtained using the Free Run mode with an 8mm nominal width, 1000 A-scans per B-scan, 75 B-scans and 1 frame per B-scan.

Supplementary Results

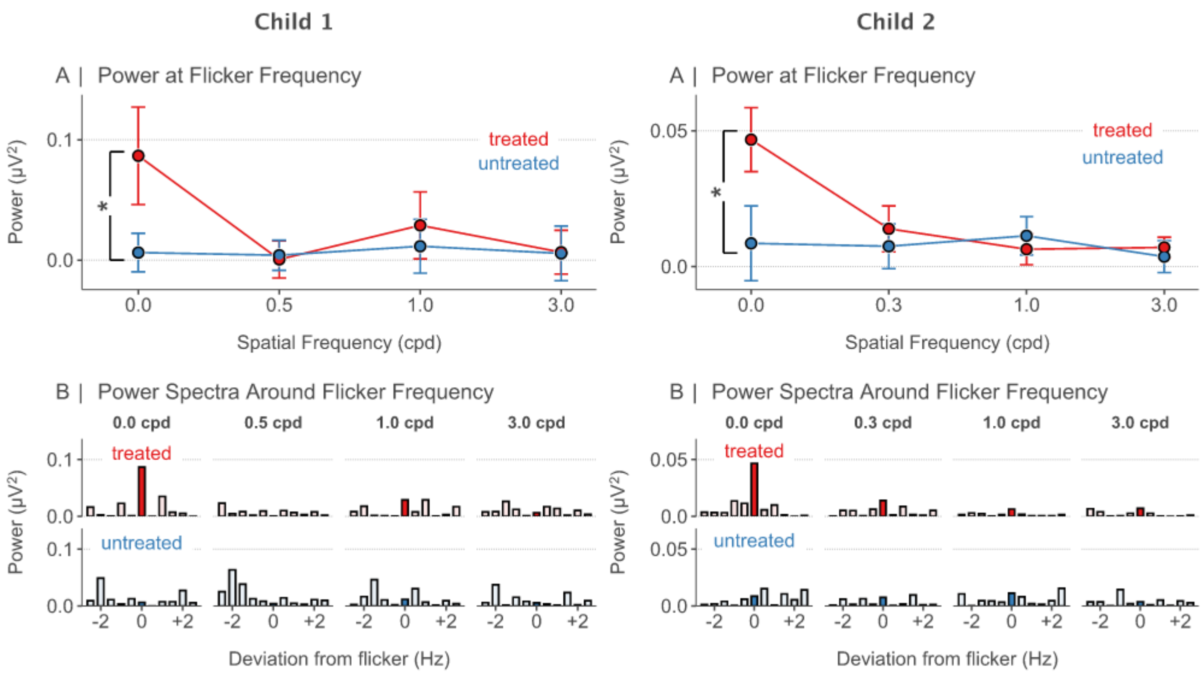
Supplementary Figure 1 Wide-field color fundus imaging and fundus autofluorescence (FAF) captured by Optos Panoramic 200 following treatment



Panel A; Child 1, 6 years old, 3·4 years after treatment; Panel B; Child 2, 5·1 years old, 2·3 years after treatment; Panel C; Child 3, 3·2 years old, 2·3 years after treatment; image acquisition of the untreated eye was not possible owing to limited cooperation; Panel D;

Child 4, 4·2 years old, 2·1 years after treatment; FAF acquisition of the untreated eye was not possible owing to limited cooperation. Asterisks denote the treated eye.

Supplemental Figure 2 Cortical responses to all visual stimulus conditions (full-field and grating stimuli) measured by visually evoked potentials



Panels A (Child 1) and B (Child 2) show occipital steady-state visually evoked potentials (ssVEP) obtained from EEG, for each child's treated eye (red circles and lines) and untreated eye (blue grey circles and lines). Children viewed flickering contrast reversing stimuli at 55cm on a 65° wide screen, including full-screen flicker (0·0 cycles per degree, cpd) and gratings with a range of spatial frequencies up to 3cpd (Figure 1). Data were recorded in 2s segments and averaged across multiple repeats per condition with no off-line data filtering or epoch rejection (n=9-12). A discrete Fourier transform was used to compute signal power at and around the flicker frequency. Whiskers represent bootstrapped standard errors. Both children showed clear responses at the stimulus flicker frequency for their treated eye (red lines). As reported in Main Figure 3A, permutation tests revealed that both children's treated eyes showed a significantly enhanced response to full-screen flicker compared to the untreated eye. For spatial frequency stimuli, no significant ssVEP amplitude differences were detected before or after treatment, highlighting the full-screen condition as the more robust test of treatment effects. Stars indicate statistically significant differences ($p<0.05$) between the two eyes. Panels C (Child 1) and D (Child 2) show the power spectra at the stimulus flicker frequency (set to zero for visualization, darkly coloured bars) and at neighbouring temporal frequencies of non-interest (lightly coloured bars) for their treated eye (red) and untreated eye (blue).

Videos

Video 1

Child 1, 4·1 years after treatment: assessment of functional vision

Child 1 is asked to locate white objects of various sizes against a black background using each eye in turn, with the other eye occluded. Using the sight of her treated right eye (OD) alone she accurately locates white object of 5 mm, 3 mm, and 1 mm diameter. Using her untreated left eye (OS) alone she is unable to locate even the largest (5 mm) object. Instead, she adjusts the spectacles to unmask her treated right eye.

Video 2

Child 1, approximately 1 year after treatment: vision-guided behaviour

This video was provided by family of Child 1 while she was unable to travel to the UK for formal assessment during the COVID19-pandemic. With both eyes open, she accurately and repeatedly locates a bottle on the floor using her sight.

Video 3

Child 1, 4·1 years after treatment: assessment of functional vision

Child1 is asked to transfer crayons from one cup to another using her treated right eye (OD) alone. She appears to use her sight to locate the missed crayons.

Video 4

Child 1, 4·1 years after treatment: assessment of vision-guided mobility

Child 1 is asked to walk the length of the corridor with the use of both her treated and untreated eyes. She completes the task safely and confidently using her sight alone. When asked to locate the second door she completes the task safely and accurately using her sight alone.

Video 5

Child 2, 3.4 years after treatment: assessment of functional vision

Child 2 is asked to locate white objects of various sizes against a black background using each eye in turn, with the other eye occluded. Using the sight of her treated left eye (OS) alone she accurately locates objects of 5 mm, 3 mm, and 1 mm diameter. Using her untreated right eye (OD) alone she is unable to locate even the largest (5 mm) object; instead, she adjusts the spectacles to unmask the sight of her treated left eye (OS).

Video 6

Child 2, 2.3 years after treatment: vision-guided behaviour

Child 2, with the use of both her treated and untreated eyes, uses her sight to draw using coloured pens.

Video 7

Child 2, 3·4 years after treatment; assessment of functional vision

Child 2 is asked to transfer crayons from one cup to another using each eye in turn with the other eye occluded. Using her treated left eye (OS) alone she completes the task confidently, appearing to locate the missed crayons using her sight. In contrast, using the sight of her untreated right eye (OD) alone she appears to rely heavily on tactile cues and her memory of the cup's location. When the cup's location is changed, she fails to identify its new position. Instead, she adjusts the spectacles to unmask the sight of her treated left eye (OS).

Video 8

Child 2, 3·4 years after treatment: writing skills

Child 2, with the use of both her treated and untreated eyes, uses her sight to write letters and draw shapes.

Video 9

Child 2, 3·4 years after treatment: vision-guided mobility

Child 2, with the use of both her treated and untreated eyes, navigates confidently and safely using her sight alone. She runs along a corridor, sits at a table, locates the examination room and steps up to sit in the examination chair.

Video 10

Child 3, 2·3 years after treatment: assessment of functional vision

Child 3, with the use of both his treated and untreated eyes, plays with toys and transfers crayons from one cup to another. He appears to use his sight to locate missed crayons.

Video 11

Child 3, 3·5 years after treatment: assessment of functional vision

Child 3, with the use of both his treated and untreated eyes, removes and replaces a 1 cm diameter white object from a transparent tube. He appears to use his sight to complete the task.

Video 12

Child 3, 3·5 years after treatment: assessment of vision-guided mobility

Child 3, with the use of both his treated and untreated eyes, runs confidently and safely along a corridor.

Video 13

Child 4, 2.9 years after treatment: assessment of functional vision

Child 4, with the use of both his treated and untreated eyes, accurately locates a white object of 5mm diameter against a black background. Neither monocular assessment nor location of smaller objects were possible owing to limited compliance.

Video 14

Child 4, 2.9 years after treatment: assessment of vision-guided navigation

Child 4, with the use of both his treated and untreated eyes, walks comfortably along a corridor. He makes unintended contact with a fire extinguisher but otherwise completes the task without error.

References

1. Elfadaly D, Abdelrazik ST, Thomas PBM, Dekker TM, Dahlmann-Noor A, Jones PR. Can Psychophysics Be Fun? Exploring the Feasibility of a Gamified Contrast Sensitivity Function Measure in Amblyopic Children Aged 4–9 Years. Frontiers in Medicine. 2020;7. Accessed December 17, 2023. https://www.frontiersin.org/articles/10.3389/fmed.2020.00469
2. Farahbakhsh M, Dekker TM, Jones PR. Psychophysics with children: Evaluating the use of maximum likelihood estimators in children aged 4–15 years (QUEST+). Journal of Vision. 2019;19(6):22. doi:10.1167/19.6.22
3. Hamilton R, Bach M, Heinrich SP, et al. VEP estimation of visual acuity: a systematic review. Doc Ophthalmol. 2021;142(1):25-74. doi:10.1007/s10633-020-09770-3
4. Hoffmann MB, Brands J, Behrens-Baumann W, Bach M. VEP-based acuity assessment in low vision. Doc Ophthalmol. 2017;135(3):209-218. doi:10.1007/s10633-017-9613-y
5. Harris L, Atkinson J, Braddick O. Visual contrast sensitivity of a 6-month-old infant measured by the evoked potential. Nature. 1976;264(5586):570-571. doi:10.1038/264570a0
6. Biosemi EEG ECG EMG BSPM NEURO amplifier electrodes. https://www.biosemi.com/.
7. Meigen T, Bach M. On the statistical significance of electrophysiological steady-state responses. Doc Ophthalmol. 1999;98(3):207-232. doi:10.1023/A:1002097208337
8. Norcia AM, Appelbaum LG, Ales JM, Cottereau BR, Rossion B. The steady-state visual evoked potential in vision research: A review. Journal of Vision. 2015;15(6):4. doi:10.1167/15.6.4
9. Westfall PH, Troendle JF. Multiple Testing with Minimal Assumptions. Biom J. 2008;50(5):745-755. doi:10.1002/bimj.200710456