Gene therapy in children with *AIPL1*-associated severe retinal dystrophy: an open-label, first-in-human interventional study

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Lancet 2025; 405: 648–57

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Summary

Background Retinal dystrophy caused by genetic deficiency of *AIPL1* causes severe and rapidly progressive impairment of sight from birth. We sought to evaluate whether early intervention by gene supplementation therapy was safe and could improve outcomes in children with this condition.

Methods This non-randomised, single-arm, clinical study conducted in the UK involved four children aged 1·0–2·8 years with severe retinal dystrophy associated with biallelic disease-causing sequence variants in *AIPL1*. We designed a recombinant adeno-associated viral vector comprising the human *AIPL1* coding sequence driven by a human rhodopsin kinase promoter region (rAAV8.*hRKp.AIPL1*). The product was manufactured under a Specials Licence from the Medicines and Health products Regulatory Authority (UK) and made available to affected children with local ethics approval. We administered the product to one eye of each child by subretinal injection. The children were prescribed oral prednisolone to protect against harm from inflammation. Outcome measures included visual acuity (as assessed with a novel touchscreen test), functional vision (assessed by observing and recording the children's visual behaviour and their ability to perform simple vision-guided tasks), visual evoked potentials (assessed by recording cortical electrophysiological responses to full-screen black-and-white flickering stimuli), and retinal structure (assessed with handheld optical coherence tomography [OCT] and widefield fundus imaging). To identify adverse effects, including inflammation and retinal detachment, we conducted ocular examinations using slit-lamp biomicroscopy and dilated fundoscopy. Safety was further assessed by testing of visual acuity, ophthalmoscopy, handheld OCT and widefield fundus imaging.

Findings Patients were selected for treatment between July 12, 2019, and March 16, 2020. Before intervention, the children's binocular visual acuities were limited to perception of light. At a mean of 3·5 years (range 3·0–4·1) after intervention, the visual acuities of the children's treated eyes had improved to a mean of 0·9 logarithm of the minimal angle of the minimum angle of resolution ([logMAR] range 0·8–1·0); visual acuities before intervention were equivalent to 2·7 logMAR. In contrast, the visual acuities of the children's untreated eyes became unmeasurable at the final follow-up. In the two children able to comply with testing, an objective test of visual acuity confirmed improvements in visual function, and measurement of visual evoked potentials showed enhanced activity of the visual cortex, specific to the treated eyes. In three of the children, structural lamination of the outer retina was better preserved in the treated eye than in the untreated eye, and, for all four children, retinal thickness appeared better preserved in the treated eye than in the untreated eye. The treated eye of one child developed cystoid macular oedema. No other safety concerns were identified.

Interpretation Our findings indicate that young children with *AIPL1*-related retinal dystrophy benefited substantially from subretinal administration of rAAV8.*hRKp.AIPL1*, with improved visual acuity and functional vision and evidence of some protection against progressive retinal degeneration, without serious adverse effects.

Funding UK National Institute for Health Research and Moorfields Eye Charity.

Introduction

Early-onset inherited retinal dystrophies cause severe sight impairment in infants, with congenital nystagmus, impaired pupil responses, and severely reduced responses on electroretinography.[1,2] The prevalence of early-onset rod-cone dystrophies is estimated to be one to three in 100 000.[3,4] At least 26 different genes have been implicated to date,[5] with mutations in *CEP290*, *GUCY2D*, *CRB1*, and *RPE65* among the most common causes.[5] With the exception

Research in context

Evidence before this study

We searched PubMed and MEDLINE for studies published between Jan 1, 2000, and June 30, 2019, that addressed *AIPL1*-associated severe retinal dystrophy, using search terms "*AIPL1*", "Leber congenital amaurosis", "retinal degeneration", and "gene therapy". There were no language restrictions. Preclinical studies of gene therapy in *Aipl1*-deficient mouse models reported partial restoration of retinal structure and function. Gene therapy in affected children had not been reported.

Added value of this study

This first-in-human interventional study of gene therapy for children with *AIPL1*-associated severe retinal dystrophy showed that early intervention with gene supplementation can substantially improve visual acuity and functional vision outcomes. Structural preservation of the retina in the treated eyes suggests protection against progressive retinal degeneration.

Implications of all the available evidence

AIPL1-associated severe retinal dystrophy is an ultra-rare cause of severe sight impairment from birth with no existing treatment. Adeno-associated viral-mediated gene supplementation therapy at an early age can markedly improve visual acuity and functional vision and preserve retinal structure. Gene therapy to address severe impairment of sight in early childhood promises lasting benefit for neurodevelopment. The positive outcomes of gene therapy in young children with *AIPL1*-associated disease imply that early intervention in other genetic retinal diseases might provide the greatest potential for benefit.

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For more on **genes implicated in inherited retinal dystrophies** see https://web.sph.uth.edu/RetNet/

of *RPE65*-related disease, for which adeno-associated virus (AAV)-mediated gene therapy (with voretigene neparvovec) can improve vision-guided mobility in low luminance conditions,[6] no specific treatment is available.[5]

Variants in the gene encoding AIPL1 account for up to 5% of infants affected by early-onset rod-cone dystrophy. *AIPL1* is expressed in rod and cone photoreceptor cells during development, and the encoded protein plays a crucial role in phototransduction.[7–9] AIPL1 is a specialised molecular co-chaperone for cGMP-specific PDE6, supporting the stability, assembly and catalytic activity of PDE6 in cones and rods.[9] In *Aipl1*$^{-/-}$ mice, the absence of Aipl1 is associated with reduced concentrations of PDE6, elevated concentrations of cGMP, and rapid degeneration of photoreceptor cells.[10,11] Similarly, *AIPL1*-knockout and patient-derived human retinal organoid models are characterised by a reduction in PDE6 and elevation in cGMP.[12–14]

Infants with disease-causing variants in *AIPL1* are affected by severe and rapidly progressive impairment of sight from birth. In a cross-sectional survey including 42 individuals aged between 6 months and 43 years with *AIPL1*-related retinal dystrophy, the sight of the affected individuals was limited to perception of light;[15] only exceptionally was visual acuity better than 1·5 logarithm of the minimal angle of the minimum angle of resolution (logMAR). Optical coherence tomography (OCT) imaging identified relative preservation of outer retinal structure at the fovea only in children younger than 4 years. This preservation of viable foveal photoreceptor cells in early life indicates a window of opportunity for potential benefit by gene supplementation therapy.[15] In *Aipl1*$^{-/-}$ mice, gene supplementation with human or mouse cDNA by subretinal injection of recombinant AAV (rAAV) vectors (rAAV2-CMV-*AIPL1*, rAAV2-CMV-*Aipl1*, and rAAV8-CMV-*Aipl1*) improved retinal function as measured by scotopic and photopic electroretinography and preserved thickness of the retinal outer nuclear layer.[16] Furthermore, treatment of *AIPL1* gene knockout and patient-derived retinal organoids with rAAV expressing *AIPL1* cDNA under the control of the human *GRK1* promoter rescued expression levels of PDE6 and cGMP.[12] Here, we describe the outcomes following rAAV-mediated gene supplementation therapy in four young children with *AIPL1*-deficiency.

Methods

Study design

rAAV8.*hRKp.AIPL1* is a recombinant AAV vector, comprising a human *GRK1* promoter region driving the human *AIPL1* coding sequence. In the absence of a clinical trial, we made this innovative experimental product available to children with confirmed mutations in *AIPL1* under a Specials Licence with the approval of the Paediatric Bioethics Service at Great Ormond Street Hospital for Children (GOSH GMOSC #7).[17,18,19] This non-randomised, single-arm, open-label, first-in-human interventional study was done at Moorfields Eye Hospital (London, UK) and Great Ormond Street Hospital for Children (London, UK).

Participants

Children aged 1–3 years with congenital severe retinal dystrophy, biallelic disease-causing variants in *AIPL1*, and relative preservation of outer retinal structure at the central macula on OCT were considered for treatment. As the ethics approval was limited to four children, we offered treatment to the first four children who satisfied the eligibility criteria. If a difference in visual function between the patients' eyes could be identified, the better-seeing eye was selected for treatment. The contralateral eye remained untreated for safety. The parents of each child provided fully informed written consent for treatment.

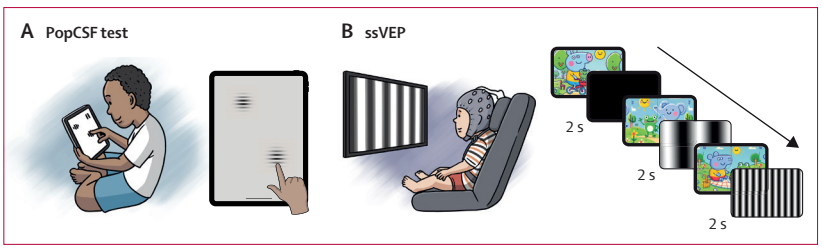

Figure 1: **Methods to measure visual acuity and visual evoked potentials**
(A) The PopCSF test for visual acuity involves searching and touching (popping) moving Gabor patches (bubbles) with varying spatial frequencies, which can appear at any location on a tablet display.[21] The physical spatial frequency of the gratings is adjusted dynamically for viewing distance through real-time head-tracking with the tablet's front-facing TrueDepth camera. (B) ssVEP data were collected by seating the participant in a comfortable chair at 55 cm from a 65° wide screen and presenting 2 s segments of time-varying (flickering) stimuli embedded in child-friendly cartoons. Our analysis focused on ssVEP responses to full-screen black-and-white flicker. ssVEP responses to grating stimuli with various spatial frequencies are in the appendix (pp 8–9). CSF=contrast sensitivity function. ssVEP=steady-state visual evoked potential.

Procedures

rAAV8.*hRKp.AIPL1* was produced at University College London's Wolfson Gene Therapy Unit using three-plasmid transfection in HEK293T cells according to Good Manufacturing Practice guidelines, under a Manufacturer Specials Licence from the UK Medicines and Health products Regulatory Authority. MeiraGTx (holder of a Specials Licence) supported production, storage, quality assurance, and dispensing. 0·1–0·4 mL AAV2/8.*hRKp.AIPL1* vector suspension was administered at a titre of 1×10^{11} vector genomes per mL during vitrectomy surgery under general anaesthesia (appendix pp 1–2). The suspension was delivered subretinally to establish a bleb extending across the posterior pole of the retina from the superotemporal vascular arcade to the inferotemporal arcade, encompassing the surviving central macula. To protect against harm from inflammation, children were prescribed oral prednisolone at a dose of 1·0 mg/kg bodyweight daily for 5 days before surgery and 1 mg/kg for the first week after surgery, with tapering of the dose for a further 4 weeks (appendix p 1). We evaluated the children's functional vision, visual acuity, and retinal structure before intervention and twice subsequently. Functional vision was tested by observing and recording the children's visual behaviour and their ability to do simple vision-guided tasks. For example, the children were tasked with locating by sight white objects of a range of sizes in turn, against a dark background under normal office illumination, and with moving crayons between cups; they were also invited to draw on paper. When possible, treated and untreated eyes were tested independently in the same way. Children were also asked to mobilise along a normally lit corridor with the use of both eyes together and to identify doorways. Visual acuity tests were selected according to the age and ability of each child at the time of testing. We assessed visual acuity subjectively with standard age-appropriate recognition tasks (including Cardiff acuity cards, Kay pictures, and the Sonksen logMAR test using Sheridan Gardiner letters); when visual acuity could not be measured using these methods, we tested the children's ability to perceive and follow a pen torch light source at distances ranging from 5 cm to 50 cm. Near visual acuity was also measured in one child with single optotypes of the Sonksen logMAR test. logMAR equivalent values were obtained from the individual tests (eg, Cardiff acuity cards have a logMAR equivalent value, and the Kay picture test is developed using a logMAR scoring system). A logMAR score of 0·0 indicates perfect acuity; 1·3 indicates severe sight impairment; and 2·7 indicates perception of light only. Visual acuity was assessed objectively with a novel contrast sensitivity function (CSF) touchscreen test, the PopCSF test (appendix p 3; figure 1).[20,21] For the PopCSF test, 100% contrast targets were categorised on the basis of their spatial frequency: low (1·5–2·5 cycles per degree [cpd]), medium (2·5–3·5 cpd), and high (>3·5 cpd). Six additional children with *AIPL1*-associated severe retinal dystrophy (aged 2·9–3·9 years) were recruited and tested monocularly or binocularly with the popCSF test at Moorfields Eye Hospital, offering a benchmark for untreated performance. We evaluated visual signal detection in the visual cortex by recording cortical electrophysiological responses to full-screen black-and-white flickering stimuli and flickering (contrast-reversing) gratings across a range of spatial frequencies (steady-state visually evoked potential [ssVEP] technique; appendix pp 3–5; figure 1). The children's parents were also asked to monitor for signs of discomfort and any changes in the children's functional vision as they occurred; their observations were recorded in the clinical notes. Retinal imaging was done with handheld OCT and widefield fundus imaging (appendix p 5). Ocular examinations, including slit-lamp biomicroscopy and dilated fundoscopy, were done to identify adverse effects such as inflammation and retinal detachment. Safety was assessed by testing of visual acuity (as unexpected deterioration of visual acuity can indicate an adverse event or in itself be an adverse event), ophthalmoscopy, handheld OCT, and widefield fundus imaging.

See Online for appendix

Outcomes

Outcome measures were visual acuity, functional vision, ssVEPs, and retinal structure (qualitative assessment of outer retinal lamination and apparent thickness). Visual acuity after intervention was compared with visual acuity before intervention and with the visual acuity of the untreated contralateral eye. Visual acuity, functional vision, and retinal structure were assessed in child 1 at 3·4 years and 4·1 years after intervention; in child 2 at 2·3 years and 3·4 years after intervention; in child 3 at 2·3 years and 3·5 years after intervention; and in child 4 at 2·1 years and 2·9 years after intervention. ssVEPs were assessed in child 1 at 4·1 years after intervention and in child 2 at 3·4 years after intervention. The regularity and frequency of follow-up assessments were limited by COVID-19-related restrictions to international travel.

	AIPL1 genotype	Age (years) at intervention	Age (years) at final follow-up	Duration of follow-up (years)	Pre-intervention visual acuity, both eyes	Visual acuity, right eye (logMAR)	Visual acuity, left eye (logMAR)
Child 1	c465+1G>C homozygous	2·6	6·6	4·1	Followed light at 10 cm	0·8*	No perception of light†
Child 2	c834G>A p(Trp278Ter) homozygous	2·8	6·2	3·4	Followed light at 50 cm	No perception of light†	0·8*
Child 3	c834G>A p(Trp278Ter) homozygous	1·0	4·5	3·5	Followed light at 20 cm	Unmeasurable†	0·9*
Child 4	c618_619dupCT p(Cys207Serfs*3), c265T>C p(Cys89Arg)	2·1	5·0	2·9	Followed light at 50 cm	Unmeasurable†‡	1·0*

logMAR=logarithm of the minimum angle of resolution. *Treated eye. †Untreated eye. ‡Resisted occlusion of the contralateral eye.

Table: **Characteristics of the patients, with visual acuity before intervention and at most recent assessment**

Statistical analysis

For assessment of visual acuity with the PopCSF touchscreen test, we used logistic regression comparing the relationship between hit rate and target spatial frequency across the two eyes in each child tested. Grating acuity limits were evaluated by comparing performance for low, mid, and high spatial frequencies to a 10% chance level using exact binomial tests (appendix p 3);[20,21] a p value of less than 0·05 was considered significant. We used permutation tests (conducted with Python version 3.12) to compare ssVEP signals induced by presentation of full-screen black-and-white flicker to treated and untreated eyes (appendix pp 3–5). This approach involved randomly resampling the repetitions within each condition and repeating the analysis steps 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.

Role of the funding source

The funders of the study had no role in data collection, analysis, interpretation, writing of the report, or the decision to submit for publication.

Results

Four children aged 1·0–2·8 years with biallelic disease-causing sequence variants in *AIPL1* were treated with rAAV8.*hRKp.AIPL1*. No difference in visual function between eyes was measurable in any of the children before intervention. PopCSF and ssVEP tests after intervention were completed by the two older children; the younger children were unable to comply with these tests.

Child 1, a female patient aged 2·6 years, homozygous for the variant c465+1G>C in *AIPL1*, received treatment in her right eye in July, 2019. Before intervention, her binocular visual acuity was limited to following a light source at 10 cm. She had shown no interest in Cardiff acuity cards. Her right eye was selected for treatment because a small left esotropia was apparent. 3·4 years after intervention, when she was aged 5·9 years, the acuity of the treated right eye, assessed with Cardiff acuity cards, was 1·1 logMAR, and 4·1 years after intervention, it was 0·8 logMAR; her binocular near visual acuity (assessed with the Sonksen logMAR test) 3·4 years after intervention was N48 at 5 cm. In contrast, she consistently reported no perception of light when using her untreated (left) eye (table). 4·1 years after intervention, objective measurement of visual acuity with the touchscreen (PopCSF) test confirmed the higher performance of her treated eye compared with her untreated eye (β_{eye}=2·55, SE 1·08; p_{eye}=0·018). In the low spatial frequency range (1·5–2·5 cpd), the 24% success rate for the treated eye was significantly higher than the 10% chance level (ten correct responses in 42 trials; p=0·0061), whereas the 2·4% success rate for the untreated eye was no higher than the level attributable to chance (one correct response in 42 trials; p=0·12), indicating low vision or random guessing. Child 1 did not successfully touch any target in the medium or high spatial frequency range (figure 2). Measurement of cortical (ssVEP) responses to

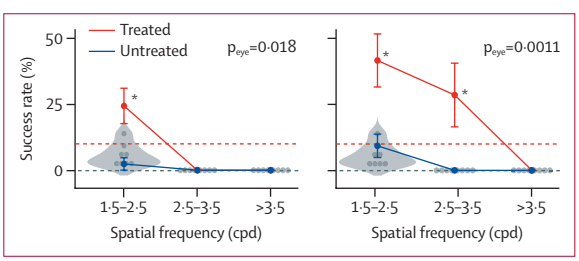

Figure 2: **Visual acuities measured by PopCSF**

Findings for 100% contrast are shown. Child 1 (4·1 years after intervention) and child 2 (3·4 years after intervention) were able to successfully complete the PopCSF test. p_{eye} indicates ocular difference across grating frequencies as tested by logistic regression. Whiskers indicate binomial SEs. Red dashed lines indicate 10% chance level. Grey data points and violin plots represent a reference distribution of untreated performance from eight participants with *AIPL1*-associated severe retinal dystrophy (aged 2·4–3·9 years), including the untreated eyes of child 1 and 2, for comparison. cpd=cycles per degree. *Significant difference from the percentage hit rate expected by chance (dashed red line) at p<0·05, as shown by exact binomial tests.

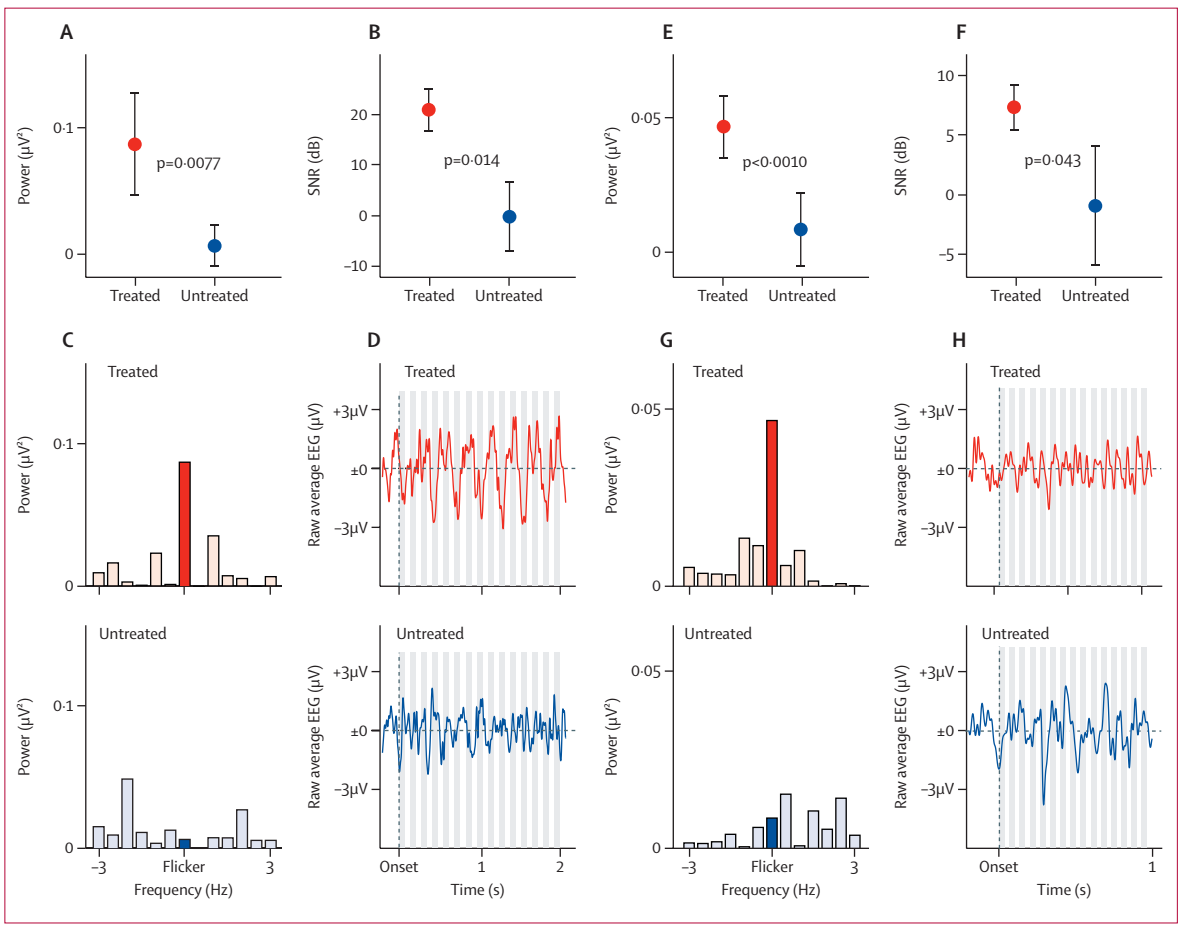

Figure 3: **Cortical responses to visual stimuli measured by ssVEPs for child 1 and child 2**
Data shown are for full-screen black-and-white flickering stimuli. Occipital EEG data for child 1 (left-hand panels), measured 4·1 years after intervention, and child 2 (right-hand panels), measured 3·4 years after intervention, were recorded in 2 s segments and averaged across multiple repeats per condition (n=9–12). A discrete Fourier transform was used to compute signal power at and around the flicker frequency. (A, E) Power of the ssVEPs at the stimulus flicker frequency for the treated and untreated eyes. Whiskers represent bootstrapped SEs. (B, F) SNR comparing power at the stimulus frequency to that at neighbouring frequencies of non-interest. Whiskers represent bootstrapped SEs. (C, G) Power spectra for treated and untreated eyes. Darkly shaded bars indicate the stimulus flicker frequency (set to zero for visualisation). Lightly shaded bars indicate neighbouring temporal frequencies of non-interest. (D, H) Raw average recorded EEG traces. Grey bars represent stimulation cycles. Equal numbers of black-white reversals of the screen are shown for child 1 and child 2 (see appendix pp 3–4 for the stimulus parameters for each child). As no noise filtering or epoch rejection was applied to these traces, the Fourier analyses in panels A–C and D–F are necessary to distinguish the recovering visual signal at the stimulus flicker frequency from noise. SNR=signal-to-noise ratio. ssVEP=steady-state visual evoked potential.

full-screen flickering gratings showed higher response amplitudes at the stimulus flicker frequency for her treated eye (0·087 μV²) than for her untreated eye (0·006 μV², p=0·0077; figure 3A). The power spectrum showed a distinct peak at the flicker frequency only for stimuli presented to her treated eye (figure 3C). In addition, the signal-to-noise ratio (SNR)—comparing power at the stimulus frequency relative to neighbouring frequencies of non-interest (appendix p 4)—was higher for child 1's treated eye (21·03 dB) than for the untreated eye (–0·08 dB; p=0·014; figure 3B). This result shows that the heightened cortical response in the treated eye was driven by the visual stimulus. With her treated eye uncovered, child 1 could reach out to a 5 mm object at 30 cm, a 3 mm object at 15–20 cm, and a 1 mm object at 10 cm. With her treated eye covered, however, she was unable to locate even the 5 mm object (video 1). Child 1's parents reported improvements in her visual behaviour from 6 months after treatment. They described improved fixing and following, reaching, and mobility, with reduced light-staring, eye-poking, and nystagmus (videos 2, 3, and 4). In child 1's treated right eye, focal retinal atrophy was evident at the retinotomy site (colour fundus and fundus autofluorescence images for all children are in the appendix [pp 6–7]). OCT imaging at 3·4 years and 4·1 years after surgery showed no clear preservation of outer retinal lamination (ellipsoid zone) in either of child 1's eyes. However, retinal thickness appeared better preserved in the treated right eye than in the untreated left eye (figure 4A–C).

See Online for videos 2–4

See Online for video 1

Child 2, a female patient aged 2·8 years, homozygous for c834G>A p(Trp278Ter) in *AIPL1,* received treatment in her left eye in March, 2020. Before intervention her binocular visual acuity was limited to following a light

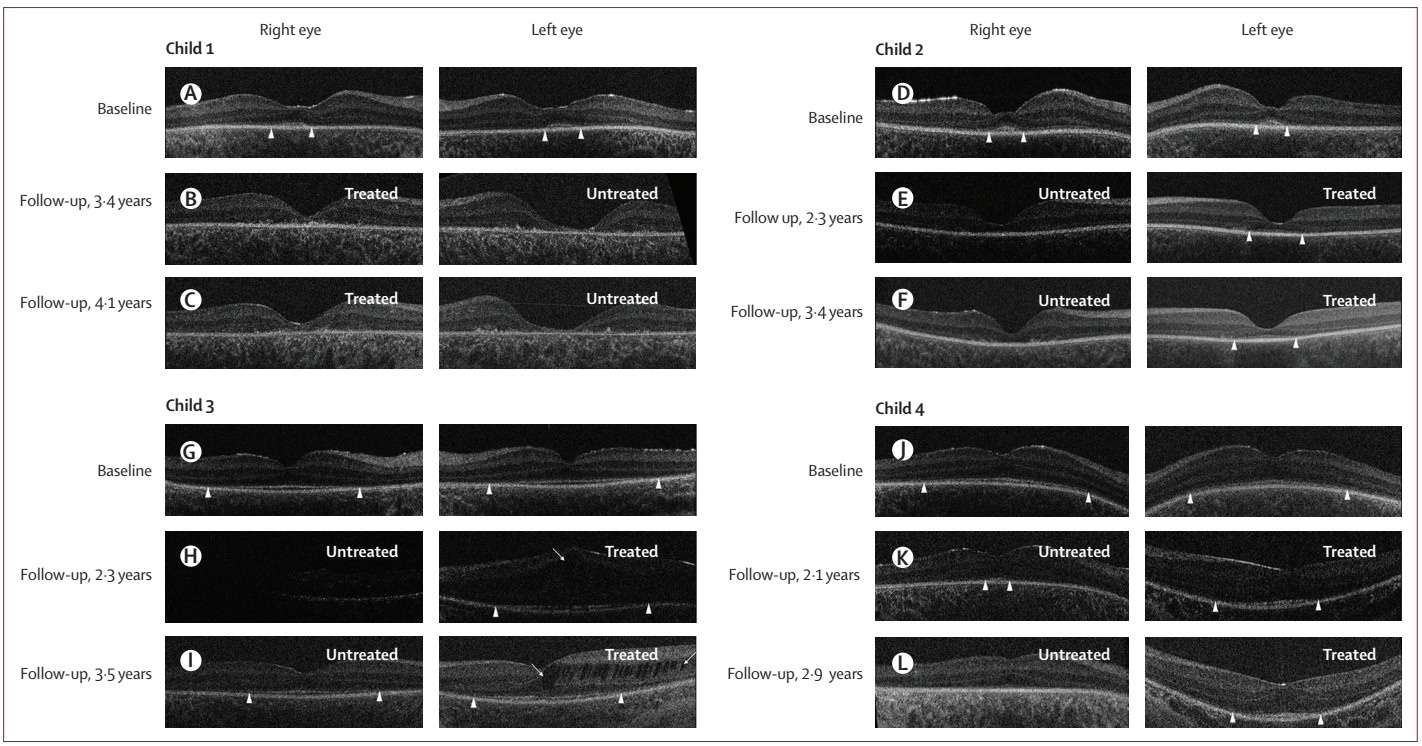

Figure 4: **Retinal structure evaluated by OCT**

Horizontal OCT scans of the central retina for child 1 (A–C), child 2 (D–F), child 3 (G–I; imaging of child 3's untreated [right] eye at 2·3 years after treatment [3·3 years of age] was compromised owing to low compliance with image acquisition), and child 4 (J–L) before intervention and at two timepoints after treatment. The extent of preserved outer retinal structure is labelled with arrowheads. In all children, before intervention, there is evidence of bilateral central preservation of outer retinal layering to some extent (A, D, G, and J). In Child 1, no clear preservation of outer retinal structure was evident in either eye at 3·4 years (B) or 4·1 years (C) after treatment. However, the central retinal thickness in their treated right eye appeared better preserved than that of their untreated left eye. In Child 2, preservation of outer retina was evident in their treated left eye at 2·3 years (E) and 3·4 years (F) after intervention but was not apparent in their untreated right eye. In Child 3, the treated left eye showed preservation of outer retinal structure at 2·3 years (H) and at 3·5 year after treatment (I). Arrows in (H) and (I) show cystoid macular oedema in child 3's treated (left) eye, which had partly regressed by 3·5 years after treatment. At 3·5 years after treatment the outer retinal structure appeared less well preserved than that of their treated left eye (I). In Child 4, areas of outer retinal preservation were evident in both eyes prior to treatment (J). The outer retina appeared better preserved in their treated left eye than in their untreated right eye at 2·1 years (K) and at 2·9 years (L) after treatment. For comparison, OCT images of unaffected infants have been previously published.[2] OCT=optical coherence tomography.

source at 50 cm. She had shown no interest in Cardiff acuity cards. Her left eye was selected for treatment because a small right esotropia was apparent. 2·3 years after intervention, when child 2 was aged 5·1 years, the visual acuity of her treated left eye was 0·70 logMAR (assessed with Cardiff acuity cards). 3·4 years after intervention, the acuity of her treated eye was 0·8 logMAR (assessed with Sheridan Gardiner letters). A binocular letter-based test at 3·4 years after intervention showed near vision of 1·0 logMAR at 40 cm, increasing to 0·4 logMAR at 8 cm. In contrast, she was unable to perceive a light source when using her untreated eye alone (treated eye covered; table). Objective measurement of child 2's visual acuity using the PopCSF test confirmed higher performance for the treated eye than for the untreated eye (β_{eye}=3·69, SE 1·13; p_{eye}=0·0011; figure 2). For child 2's treated eye, we found above-chance performance for both low and medium (2·5–3·5 cpd) spatial frequency targets, with success rates of 41% and 29%, respectively (low frequency targets: ten correct responses in 24 trials, p<0·0001); medium frequency targets: four correct responses in 14 trials, p=0·044). In contrast, the level of performance of child 2's untreated eye was no higher than that attributable to chance, indicating low vision or random guessing, with a 9·3 % success rate for the low spatial frequencies and no other correct responses (low frequency targets: four correct responses in 43 trials, p=1·00). Measurement of cortical (ssVEP) responses to full-screen flicker showed higher response amplitudes at the stimulus flicker frequency for her treated eye (0·0467 μV²) than for her untreated eye (0·008 μV²; p<0.0010; figure 3E). The power spectrum showed a distinct peak at the flicker frequency only for stimuli presented to child 2's treated eye (figure 3G). The SNR was significantly higher for child 2's treated eye (7·34 dB) than for the untreated eye (–0·94 dB; p=0·043; figure 3F), confirming that the heightened cortical response in this eye was driven by the visual stimulus. When using her treated eye alone, child 2 could reach out to objects of 5 mm, 3 mm, and 1 mm from a distance of 30 cm; she was unable to locate any object with her treated eye covered (video 5). Child 2's family reported that before treatment, she had stared at bright lights and played

See **Online** for video 5

alone. They described considerable improvements in her visual behaviour from 4 weeks after intervention—namely, scanning; fixing and following; watching television; and identifying colours, letters, and numbers. She was able to learn handwriting, respond to faces, interact with other children and copy their behaviour, and to mobilise safely (videos 6, 7, 8, and 9). OCT imaging 2·3 years and 3·4 years after surgery showed relative preservation of outer retinal lamination in child 2's treated eye, compared with the untreated eye, and the retinal thickness appeared better preserved in the treated eye (figure 4D–F).

See Online for video 13

See Online for videos 6–9

Child 3, a male patient aged 1·0 year, homozygous for c.834G>A p.(Trp278Ter) in *AIPL1,* received treatment in his left eye in March, 2020. Before intervention, his sight was limited to following a light source at 20 cm binocularly. His left eye was selected for treatment as mild right cyclotorsion was noted during examination under anaesthesia. 2·3 years after the intervention, when child 3 was aged 3·3 years, he was unable to complete formal acuity testing but was able to identify Kay pictures binocularly. 3·5 years after intervention, he was unable to comply with either PopCSF acuity testing or ssVEP recording. His binocular visual acuity of 0·9 logMAR at 4·5 years of age (assessed with Kay pictures; table) was attributed to his treated eye, as he would not tolerate occlusion of his treated eye. He was able to detect a 1 mm diameter object at 30 cm with both eyes open, pick up small toys, and mobilise safely unaided (videos 10, 11, and 12). Child 3's family reported that from 3 months after treatment they had observed a substantial improvement in his visual behaviour; whereas previously he had only reacted to bright lights, child 3 began to track small objects in ambient lighting, identify colours, and interact better with other children (eg, playing hide and seek). OCT imaging of child 3's treated right eye at 2·3 years and 3·5 years after intervention showed relative preservation of the outer retinal structure compared with the untreated eye, and the retinal thickness appeared better preserved in the treated eye. Cystoid macular oedema was evident on OCT 2·3 years after intervention and had partly regressed at 3·5 years after intervention (figure 4G–I).

See Online for video 14

See Online for videos 10–12

Child 4, a male patient aged 2·1 years, compound heterozygous for c.618_619dupCT p.(Cys207Serfs*3) and c.265T>C p.(Cys89Arg) in *AIPL1,* received treatment in his left eye in September, 2020. His left eye was selected for treatment because, although his esotropia was alternating, a possible preference for his left eye was apparent. During vitrectomy surgery, two possible peripheral retinal breaks were managed by cryoretinopexy and injection of air endotamponade. Before intervention, his binocular visual acuity was limited to following a light source at 50 cm. At 2·1 years and 2·9 years after surgery, the visual acuity of child 4's treated left eye was 1·0 logMAR (assessed with Cardiff acuity cards). At 2·9 years after intervention, he could reach out to a 5 mm object at 30 cm with both eyes open (video 13). The acuity of his untreated right eye was unmeasurable, as he would not tolerate occlusion of his treated left eye. At the age of 5·0 years, child 4 was unable to comply with either PopCSF acuity testing or ssVEP recording. Before treatment, his eyes had symmetrical refractive errors of +6·00 diopters; following treatment, the high hyperopia affecting his treated left eye reduced substantially (to +0·50/–1·75×90), whereas that of his untreated right eye persisted (+5·50/–0·50×90). 2·1 years after intervention, child 4 was able to locate and pick up small objects from the floor and mobilise independently, even in dim light (video 14). Child 4's parents reported that from 2 weeks after surgery, they observed an increase in his light perception, and from 6 months after treatment, they noted improvements in his visual behaviour, with tracking, fixing, and recognition of objects and faces by sight. OCT imaging showed greater preservation in the outer retinal structure of both eyes at baseline than was evident in the other children, with some residual preservation of structure in both eyes at initial follow-up. At 2·9 years after surgery, preservation of the outer retina was evident in the treated eye but not in the untreated eye (figure 4J–L), and retinal thickness appeared better preserved in the treated eye.

The intervention was generally well tolerated with no serious adverse events. Apart from cystoid macular oedema affecting the treated eye of one child, there were no safety concerns.

Discussion

We report the results of the first, to our knowledge, human clinical study to evaluate gene therapy for *AIPL1*-related retinal dystrophy. Subretinal injection of rAAV8.*hRKp.AIPL1* available to four affected children under a Specials Licence from the UK Medicines and Health products Regulatory Authority was safe and improved visual acuity and function.

The safety profile of rAAV8.*hRKp.AIPL1* compared favourably with that of other recombinant AAV vectors for gene supplementation in retinal disease.[6,22,23] Given that young children are more predisposed than adults to postoperative inflammation,[24] they may also be at greater risk following intraocular administration of an AAV vector. However, using perioperative prophylactic steroids, we identified no significant inflammation. We identified cystoid macular oedema in the treated eye of one child (child 3), which we considered attributable to the intervention, but it had partly resolved at their most recent follow-up (3·5 years after intervention) and did not preclude a substantial benefit to visual function. Adverse events were otherwise limited to mild, predictable effects of surgery, with no harm to sight.

Before intervention, the children's visual function was limited to perception of light; all were legally blind from birth. 3–4 years after treatment, the visual acuities of their treated eyes had improved substantially. In contrast,

the visual acuity of the children's untreated contralateral eye showed no improvement. The outcomes observed for the treated eyes would not be expected from the natural history of *AIPL1*-associated severe retinal dystrophy, which is characterised by rapid, inexorable progression, with visual acuity better than 1·5 logMAR being exceptional for individuals with this condition.[15] Spontaneous improvement in visual function has not been reported and would not be expected, given the progressively severe atrophy of the congenital remnant central macula during the first years of life. All four children had symmetrical disease before intervention. Subsequently, the visual acuities of their treated eyes were substantially higher than those of their untreated eyes, which were either unmeasurable in terms of visual acuity or did not have light perception at follow-up. In the two older children, a novel objective touchscreen-based acuity test confirmed striking improvements in visual function specific to their treated eyes. The reduction in hyperopia specific to the treated eye of child 4 indicates emmetropisation consistent with improved macular function. Substantial differences in visual acuity were accompanied by enhanced activity of the visual cortex specific to the treated eyes, as measured by ssVEPs. Acuity estimates based on these data provide objective measures that are both overall consistent with and complementary to standard subjective measures. Together, these results provide objective evidence of a substantial benefit to visual function involving higher visual pathways.

Following subretinal injection of rAAV8.*hRKp.AIPL1,* OCT imaging showed relative preservation of outer retinal lamination in the treated eyes of three children at the ages of 4·5 years, 5·0 years, and 6·2 years. This finding is consistent with the improvements in visual function and points to the possibility of sustained benefit from protection against progressive degeneration. Intervention even earlier in infancy might result in greater protection of retinal architecture and greater potential for lasting benefit. The younger the children in our cohort, the wider their ellipsoid zone at baseline and, hence, the most pronounced its preservation over time.

The adverse neurodevelopmental consequences of sight impairment in young children are well recognised (eg, disrupted development of language, communication, and social behaviour,), as vision is a driving factor for many aspects of normal development. Children with the most severe impairment of sight, including those affected by *AIPL1*-related retinal dystrophy, are most profoundly affected.[25–27] In a retrospective comparative case series including 102 children, 31% of those with profound sight impairment (light perception or less) in the second to third year of life were affected by impaired neurodevelopment, whereas none of those with better vision (awareness of large near objects) were affected.[28] Targeted interventions designed to ameliorate these developmental consequences[29] showed that from an early age, specialist intervention in young, visually impaired children was required to support their development.[30] In our cohort, improvement of sight following early intervention by gene therapy was associated with striking benefits to independent vision-guided mobilisation and reports of more effective learning behaviours and social interaction transforming the quality of their lives.

In our small interventional case series, the strength of evidence is limited by the low number of individuals treated, the absence of a contemporaneous control group, and the challenges of measuring visual function reliably in young children with severe sight impairment. Despite these inherent limitations, promising evidence of benefit is provided by the magnitude of improvements in visual function, the specificity of this effect for the treated eye in each child, and the objective evaluation of visual acuity, outer retinal lamination, and cortical responses.

In inherited retinal diseases, gene therapy at an early age is expected to offer the highest likelihood of benefit by taking greatest advantage of retinal viability and neuronal plasticity. The efficacy of gene therapy for *RPE65* deficiency with voretigene neparvovec (Luxturna, Spark Therapeutics; Philadelphia, PA, USA) appears greater in children than in adults.[31,32] Subretinal administration of voretigene neparvovec has been reported in children as young as 2 years of age, with measurable improvements in visual acuity in children aged 3–6 years.[31,33] However, subretinal gene therapy in very young children demands additional considerations. Retinal surgery in young children presents a higher risk of harm from adverse events, including rhegmatogenous retinal detachment, which must be weighed against the potential benefit of gene therapy.[33] Nonetheless, our findings in children with *AIPL1* deficiency show that subretinal gene therapy in children as young as 12 months of age can be well tolerated and can result in substantial sustained benefit.

Measurement of outcomes in young children is less reliable than in older children, given that they are less able to comply with conventional assessments of visual function and retinal imaging. Development of more reliable assessments of visual function in infants is needed to evaluate the effect of early interventions with greater confidence. Age is only a proxy for retinal degeneration, and these results should not be extrapolated directly to gene therapy of other genetic retinal diseases. However, the findings show that subretinal gene therapy in very young children is feasible and can result in highly favourable outcomes. Given the safety profile and the improvement in visual acuity and functionality observed in the four children reported here, we are now administering sequential bilateral gene therapy to affected young children under a Specials Licence and exploring the feasibility of making the product more widely available. This could mean an improvement in neurodevelopment and social behaviour and provide

lifelong psychosocial benefit for children affected by this retinal dystrophy.

Contributors

MM, RRA, and JWBB conceived the study. MM, TMD, RRA, and JWBB designed and acquired funding for the study. YL, SCW, NO, SZ, NK, AK, HP, MG, VT, MP, KS, ROM-M, PRJ, SHS, AG, TMD, ERA, and AJS acquired and curated the data. ERA and RRA directed manufacture of the vector. MM, TMD, and JWBB accessed and verified and analysed the data. SN and AF provided laboratory and regulatory resources. YL prepared the first draft of the manuscript. All authors vouch for the accuracy and completeness of the reported data. MM, RRA, and JWBB were responsible for the decision to submit the manuscript. All authors had access to and interpreted the study results, provided critical review and revision of the article, and approved the decision to submit for publication.

Declaration of interests

MM has received consultancy or advisory board fees from MeiraGTx, Janssen Pharmaceuticals, Saliogen, and Octant; travel grants from MeiraGTx and Janssen Pharmaceuticals; and stock options from MeiraGTx. AG, SN, and AF are employees and stockholders of MeiraGTx. JWBB has received research grants, contracts or consultancy fees from MeiraGTx, Janssen, Astellas, and Novartis outside of the submitted work. All other authors declare no competing interests.

Data sharing

Beginning 3 years after publication, de-identified participant data will be made available on reasonable request to the corresponding authors with a signed data-sharing agreement.

Acknowledgments

The research was supported by Moorfields Eye Charity (through generous donations raised by Michael Wade OBE), the National Institute for Health Research (NIHR) Moorfields Biomedical Research Centre, and the NIHR Moorfields Clinical Research Facility. MM was supported by a Foundation Fighting Blindness Career Development Award and a Department of Health/Higher Education Funding Council for England New Blood Clinical Senior Lectureship Award. KS was supported by a Santen SenSYT PhD studentship. JWBB was supported by a NIHR Research Professorship. TMD was supported by a Wellcome Career Development Award. The authors would like to thank Navjit Singh and Thomas M Kane for their help with handheld OCT and Almudena Sacristan-Reviriego for her help with genetic diagnosis.

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