**Title:** Relative preservation of foveal outer retinal structure in infants with *AIPL1* associated Leber's Congenital Amaurosis: implications for gene therapy

**Authors:** Jonathan Aboshiha, *FRCOphth*,[1,2] Adam M. Dubis, *PhD*,[1,2] Jacqueline van der Spuy, *PhD*,[1] Koji Nishiguchi, *MD, PhD*,[1] Edward W Cheeseman, *MD, MBA*,[3] Carmen Ayuso, *MD, PhD*,[4] Miriam Ehrenberg, *MD*, [5] Francesca Simonelli, *MD*, [6] James W. Bainbridge, *PhD, FRCOphth*,[1,2] and Michel Michaelides, *MD, FRCOphth*[1,2]

The corresponding author is Michel Michaelides, UCL Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL, UK. E-mail: michel.michaelides@ucl.ac.uk.

1 UCL Institute of Ophthalmology, University College London, London, UK
2 Moorfields Eye Hospital, London, UK
3 Medical University of South Carolina Storm Eye Institute, SC
4 University Hospital Fundación Jiménez Díaz IIS-FJD, CIBERER, Madrid, Spain
5 Schneider's Children Medical Center, Petah Tikva, Israel
6 Second University of Naples and Telethon Institute of Genetics and Medicine, Naples, Italy

**Running head:** Preserved outer retina in *AIPL1* LCA

**Word count:** 995 words

**Address for reprints:** Michel Michaelides, Department of Genetics (1st Floor), UCL Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL, UK

Leber's congenital amaurosis (LCA) is a group of heterogeneous disorders characterized by an early-onset rod-cone dystrophy and severe visual loss. The estimated incidence of LCA is 2 - 3 per 100,000 births, making it the commonest cause of inherited blindness in childhood.[1] There have been several human clinical trials that have demonstrated the safety and efficacy of gene therapy in LCA type 2 (caused by *RPE65* variants).[2] One of the principal criteria for successful gene therapy of inherited retinal disorders is a degree of cellular preservation of the diseased retina, in order to allow for the potential of functional restoration. In this regard, LCA type 4 is of particular interest. LCA type 4 is caused by variants in the gene Aryl-hydrocarbon-interacting protein-like 1 (*AIPL1*), which plays a role in molecular chaperoning within photoreceptors.[3] Although *AIPL1* sequence variants are associated with a relatively severe LCA phenotype, recent electrophysiological findings suggest that there may be some preservation of retinal function in young children with *AIPL1* mutations.[4] Encouragingly, there have now been successful gene replacement interventions in *AIPL1* animal models,[5] thus lending support for a human clinical trial to be considered in *AIPL1* LCA. Given the very poor prognosis of severe and rapidly progressive visual loss of *AIPL1* LCA, and, at least early in the disease, the possibility of preserved retinal function, we aimed to determine if there was an early window of opportunity during which patients might be suitable for a human treatment trial, by means of assessing high resolution retinal imaging and clinical data, in order to identify young patients who might have relatively preserved outer retinal structure that would potentially be amenable to intervention.

**Methods**

Given that *AIPL1* mutations are estimated to account for only about 5% of LCA, this would equate to an approximate incidence of 1 in a million births.  With such a rare disease, it was necessary to draw from an international pool of potential patients.  We collected data on molecularly confirmed *AIPL1* patients including demographics, visual acuity, fundus examination findings, optical coherence tomography (OCT) findings and genotype, and analyzed this database to give us an impression of the known pool of *AIPL1* patients and their defining characteristics.  In young patients where standard OCT imaging proved difficult or unsatisfactory, attempts were made where possible to obtain high resolution OCT images using hand-held OCT probes.

**Results**

We compiled data on 42 patients with molecularly confirmed *AIPL1* mutations and a LCA4 phenotype from 18 countries worldwide.  The age of these patients ranged from 0.5 to 43 yrs old (median age 8 yrs), with 24 patients (57%) aged less than 10 yrs, and 10 patients (24%) aged less than 5 yrs old.  The modal visual acuity was perception of light (PL), which was found in 21 patients (50%), with a range of visual acuities from no perception of light (NPL) to LogMAR 0.90.  Posterior pole examination findings were available in 39 patients (93%).  There was a normal posterior pole appearance in 7 of these 39 patients (18%) (age range 0.5 - 5 yrs), with a further 18 patients (46%) having retinal pigmentary changes without macular atrophy, and 13 patients (33%) exhibiting macular atrophy.  One further patient had an epiretinal membrane (3%). The youngest patient with macular atrophy was 6

yrs old.  The commonest observed sequence variant was p.W278X(c.834G>A), which was

found in at least one allele in 26 patients (62%), and in the homozygous state in 15 patients

(36%) (Table).

OCT images were obtained for 19 patients (45%).  The significant nystagmus and poor vision

observed in this phenotype, along with the young age of some of the patients, posed

considerable challenges to obtaining high quality foveal OCT scans.  Of these 19 patients, six

(32%) had poor quality scans or scans that were not foveal.  Of the remaining 13 patients in

whom good OCT images had been obtained, nine patients (69%) had no evidence of

photoreceptor structure at the macula.  Four of these 13 patients who had good macular

scans, had undergone imaging with hand-held OCT probes.  Promisingly, however, three of

these 13 patients (23%) did demonstrate significant outer retinal structure, with relative

preservation of the inner segment ellipsoid (ISe) layer and outer nuclear layer at the fovea,

with one further patient (patient #41, 3 yrs old) demonstrating qualified evidence of a foveal

ISe layer (Figure).  Three of these four patients (75%) were homozygous for the common

*AIPL1* mutation p.W278X(c.834G>A).  Of note, the three patients with clear evidence of a

foveal ISe layer constituted the youngest in the cohort, each being only one year of age or

younger.  A 4-month follow-up scan carried out on one of the 1-yr-old patients (patient #37)

continued to demonstrate evidence of retained outer retinal structure.

**Discussion**

This study shows that there is promising evidence from high resolution OCT imaging that, in

the very youngest *AIPL1* LCA patients imaged, there is relative preservation of foveal outer

retinal structure.  To the best of these authors' knowledge, the three youngest children in this cohort represent the youngest *AIPL1* LCA patients in the literature to date that demonstrate foveal outer retinal structure, which might be amenable to gene therapy. It is also encouraging that the majority of the patients with preserved foveal outer retinal structure in this study harbored the most commonly observed severe sequence variant found in *AIPL1* LCA; if these findings can be extrapolated, it may be that the majority of LCA *AIPL1* patients still retain preserved foveal outer retinal structure when very young.

Given the very poor visual prognosis of this condition, and the possibility of a therapeutic window which seems to exist both structurally and functionally, along with the success of recent animal and human trials in this and other forms of LCA, a human gene therapy based approach may be worthy of consideration in a small group of selected patients with preserved outer retinal structure in *AIPL1* LCA.  The results of this study suggest that any intervention may need to be initiated in the first few years of life.

**References**

1.      Richard G Weleber, Peter J Francis, Karmen M Trzupek, & Catie Beattie. Leber Congenital Amaurosis. 2004 16/07/2014 [cited 16/07/2014]. In: GeneReviews™ [Internet]. Seattle (WA) University of Washington, Seattle, [cited 16/07/2014]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1298/.

2.      Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. New England Journal of Medicine. 2008;358(21):2231-9.

3.      Liu X, Bulgakov OV, Wen X-H, Woodruff ML, Pawlyk B, Yang J, et al. AIPL1, the protein that is defective in Leber congenital amaurosis, is essential for the biosynthesis of retinal rod cGMP phosphodiesterase. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(38):13903-8.

4.      Pennesi ME, Stover NB, Stone EM, Chiang P-W, Weleber RG. Residual electroretinograms in young Leber congenital amaurosis patients with mutations of AIPL1. Investigative ophthalmology & visual science. 2011;52(11):8166-73.

5.      Tan MH, Smith AJ, Pawlyk B, Xu X, Liu X, Bainbridge JB, et al. Gene therapy for retinitis pigmentosa and Leber congenital amaurosis caused by defects in AIPL1: effective rescue of mouse models of partial and complete Aipl1 deficiency using AAV2/2 and AAV2/8 vectors. Human molecular genetics. 2009;18(12):2099-114.