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Peripapillary retinal nerve fibre layer thinning, perfusion changes and optic neuropathy in carriers of Leber hereditary optic neuropathy-associated mitochondrial variants

Clare Quigley , Kirk A J Stephenson , Paul F Kenna, Lorraine Cassidy

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Department of Ophthalmology, Royal Victoria Eye and Ear Hospital Dublin, Dublin, Ireland

Correspondence to
Dr Clare Quigley; quigleyclare@gmail.com

ABSTRACT

Background We investigated Leber hereditary optic neuropathy (LHON) families for variation in peripapillary retinal nerve fibre layer thickness and perfusion, and associated optic nerve dysfunction.

Method A group of LHON-affected patients (n=12) and their asymptomatic maternal relatives (n=16) underwent examination including visual acuity (VA), visual-evoked-potential and optic nerve imaging including optical coherence tomography (OCT) and OCT angiography of the peripapillary retinal nerve fibre layer (RNFL). A control sample was also examined (n=10). The software imageJ was used to measure perfusion by assessing vessel density (VD), and statistical software 'R' was used to analyse data.

Results The LHON-affected group (n=12) had significantly reduced peripapillary VD (median 7.9%, p=0.046). Overall, the LHON asymptomatic relatives (n=16) had no significant change in peripapillary VD (p=0.166), though three eyes had VD which fell below the derived normal range at 6% each, with variable VA from normal to blindness; LogMAR median 0, range 0–2.4. In contrast, RNFL thickness was significantly reduced in the LHON-affected group (median 51 µm, p=0.003), and in asymptomatic relatives (median 90 µm, p=0.01), compared with controls (median 101 µm). RNFL thinning had greater specificity compared with reduced perfusion for optic nerve dysfunction in asymptomatic carriers (92% vs 66%).

Conclusion Overall, reduced peripapillary retinal nerve fibre layer perfusion was observed in those affected by LHON but was not reduced in their asymptomatic relatives, unlike RNFL thinning which was significantly reduced in both groups versus controls. The presence of RNFL changes was associated with signs of optic neuropathy in asymptomatic relatives.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Carriers of Leber hereditary optic neuropathy (LHON)-associated mitochondrial variants exhibit variable optic nerve findings and level of visual acuity (VA) (ie, normal to blindness).

WHAT THIS STUDY ADDS

⇒ Optic nerve perfusion changes are observed in LHON families, less often than retinal nerve fibre layer thinning, and may be associated with normal VA, potential preclinical or overt optic neuropathy.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study revealed that retinal nerve fibre layer perfusion abnormalities are less prevalent than thinning and may not provide clinical utility at present, though longitudinal studies of perfusion on larger cohorts are indicated.

INTRODUCTION

Leber hereditary optic neuropathy (LHON), first described by Leber in 1871, is an inherited optic neuropathy that classically causes acute onset of vision loss that usually progresses to blindness and is more common in males.[1] LHON is caused by mitochondrial dysfunction, with one retrospective study demonstrating that over 95% of LHON pedigrees are associated with one of three mitochondrial DNA (mtDNA) mutations, namely mt.11778G>A, mt.3460G>A and mt.14484T>C,[2] which affect complex I of the mitochondrial respiratory chain. A number of other rare LHON-associated mtDNA and nuclear DNA mutations have been described.[3–9]

Important unknowns in LHON include the cause of variable penetrance in disease expression and the mechanism underlying the disease's sex bias, whereby vision loss occurs more commonly in males.[10] In asymptomatic individuals who carry LHON-associated mtDNA mutations, subclinical signs of optic neuropathy may be found.[11 12]

A hallmark sign of LHON at the disease's earliest symptomatic stage is the appearance of peripapillary telangiectatic capillaries, with subtle nerve fibre layer swelling, visible

on slit lamp biomicroscopic examination.[13 14] Over time, this swelling resolves and optic nerve atrophy develops, accompanied by vision loss.[15] Histopathological studies in humans have demonstrated mitochondrial proliferation (ie, increased biomass) within capillaries of the optic nerve head (ONH),[16] which may occur due to a compensatory growth stimulus as mitochondrial function declines. This may underlie the appearance of mild optic nerve swelling at the start of the disease process.

In vivo analysis of ONH perfusion is possible with optical coherence tomography angiography (OCTA). Evaluation of ONH perfusion at different stages of LHON has shown that lower vessel density (VD) correlates with vision loss in established LHON, and the superficial capillary layers may show reduced density in asymptomatic individuals who carry LHON-associated mutations.[17] Further evaluation of peripapillary retinal nerve fibre layer (RNFL) perfusion in LHON may be helpful in detecting patterns of variation that segregate with disease expression or severity, potentially leading to a better understanding of the underlying pathophysiology. Given the large numbers of carriers of LHON-associated mutations who do not develop disease, biomarkers that could identify those at higher risk of losing vision would be helpful. At the least, those at risk could be advised to further minimise exposure to environmental risk factors such as smoking and alcohol.[18 19]

We aimed to measure peripapillary nerve fibre layer thickness and perfusion and assess for association of abnormal findings with LHON expression and severity, in patients with established LHON and in their asymptomatic maternal relatives.

MATERIALS AND METHODS

Attendees with LHON in the neuro-ophthalmology clinic which is led by the only clinician in Ireland with access to state funding for idebenone prescribing (LC) were invited to participate, along with their mother or another maternal relative, and informed consent was given. Study methodology was reported previously.[12] Patients were not involved in the study design.

Controls were also recruited who had no history of diabetes, ocular or neurological disease and who had refractive error <±3 dioptres, and normal visual acuity (VA) and intraocular pressure. This resulted in a mixed study sample of LHON-affected patients, asymptomatic maternal relatives and controls. For each study participant, demographic data, including age and sex, and clinical data, including ophthalmic and medical history, were collected.

OCTA and thickness assessment of the peripapillary RNFL were carried out (Heidelberg Spectralis OCT Heidelberg Engineering GmbH, Heidelberg, Germany). Peripapillary RNFL thickness measurements were derived from the SPECTRALIS Glaucoma Module, and VD values were obtained from the OCTA scans using imageJ software (National Institutes of Health, Bethesda, Maryland, USA).[20] The RNFL segment of a 15×15 degree scan of the ONH (4.4×4.4 mm) was extracted and this square image was analysed with the Vessel Analysis Image J plugin.[21] Briefly, the segment image was binarised and VD was calculated as the proportion of white to total pixels.

Data analysis was performed using R statistical software (V.4.1.2; R Core Team 2021),[22] with Wilcoxon rank sum test carried out to analyse non-parametric data, and median and IQR described. A normal range for peripapillary VD was derived from the control eyes, using mean±1.96×SD.

RESULTS

More detailed demographic, clinical, visual function and genetic data of this LHON cohort, using the same study IDs, are available elsewhere.[12] Of the cohort of 12 LHON-affected patients and 16 asymptomatic LHON relatives, OCTA image capture was not possible in eight patients and five relatives due to fixation difficulties but was measured in 4 patients (4 males, 3 with chronic LHON >12 months duration and 1 with early LHON <3 months duration, median age 21, IQR 17–31 years) and 11 asymptomatic relatives (10 females, median age 56, IQR 45–63 years), respectively, and was also performed in 10 controls with normal eyes (7 females, median age 48, IQR 37–56, see figure 1). Overall, the LHON-affected were significantly younger than the asymptomatic relatives (p=0.047), and the controls were not statistically significantly different in age from either the LHON-affected or the asymptomatic relatives (p=0.08 and p=0.2, respectively).

In the control group (20 eyes), the median retinal nerve fibre layer peripapillary VD was 10.9% (IQR 10.2%–12.6%), with a normal 95% CI of 6.7% to 16.7%. Peripapillary VD was significantly lower in the LHON-affected group (n=6 eyes), median 7.9% (IQR 7.1%–10.6%, p=0.046). In asymptomatic LHON maternal relatives (16 eyes), all of whom carried pathogenic LHON-associated variants with varying levels of heteroplasmy, the median VD was not significantly different to that of controls, at 13.7% (IQR 10%–14.7%, p=0.2), see table 1. Relative to the normal range derived from the controls, one eye of the LHON-affected group had reduced VD at 4.1%, and of the asymptomatic LHON relatives, three eyes (19%) of three subjects had unilateral reduced VD at 6%. Raised VD was found unilaterally in two asymptomatic relatives, at 17%, one of whom also had reduced VD in the contralateral eye.

Peripapillary retinal nerve fibre layer thickness was successfully measured in more study subjects, see table 1, median 101 µm (IQR 95–106 µm) in the control group (n=20 eyes), with a normal 95% CI of 86–115 µm. RNFL thickness was significantly lower in the LHON-affected group (n=16 eyes), median 51 µm (IQR 37–77 µm, p=0.003). In asymptomatic carrier relatives (n=19 eyes), median RNFL thickness was also significantly reduced versus controls at 90 µm (IQR 84–98 µm, p=0.01). In the asymptomatic maternal relatives, RNFL thinning below the derived normal range was found in 7 of 19 eyes (37%) and the difference in peripapillary RNFL versus

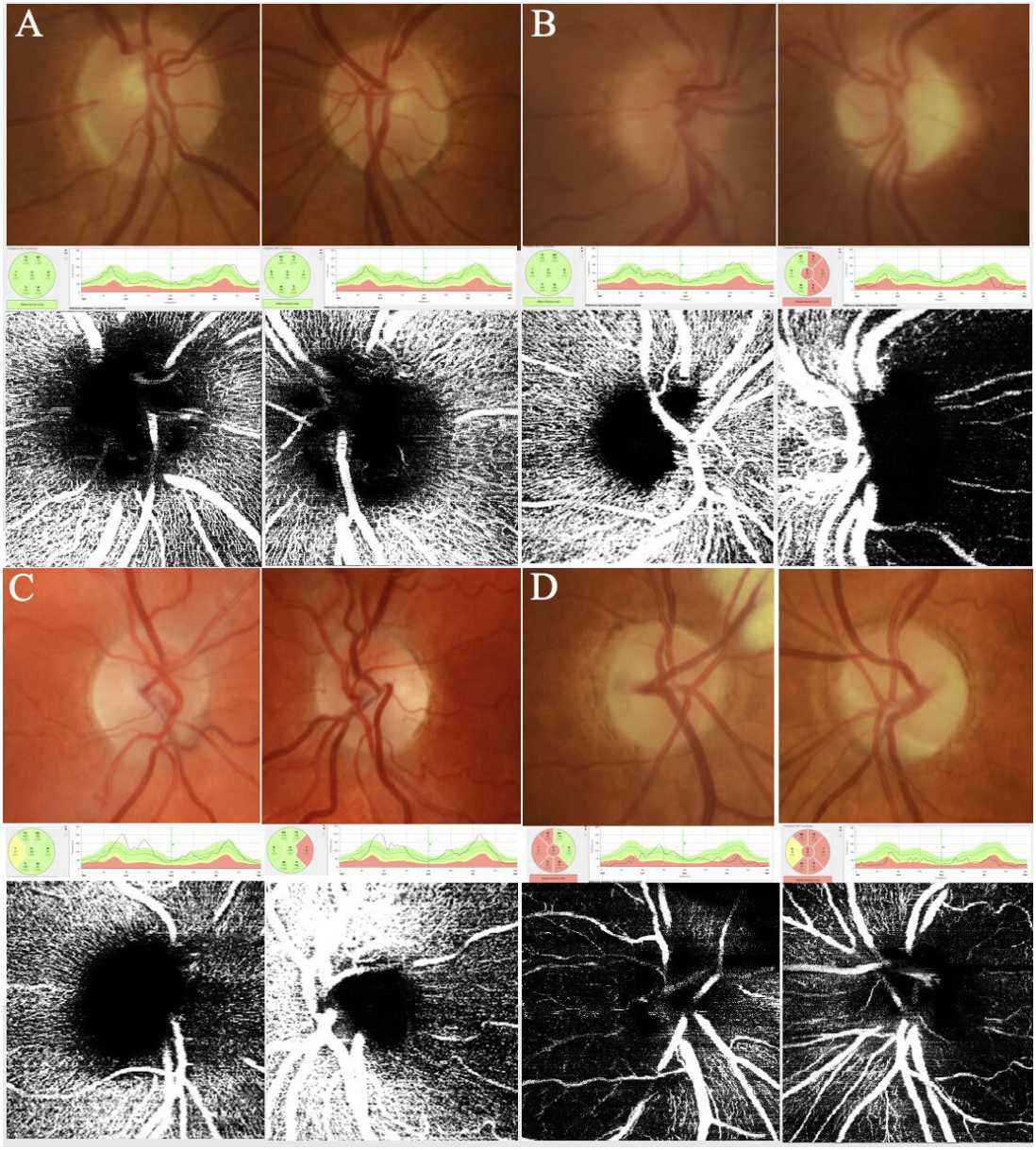

Figure 1 Optic nerve head (ONH) photo, corresponding retinal nerve fibre layer (RNFL) thickness, and peripapillary nerve fibre layer perfusion. (A) U9 asymptomatic Leber hereditary optic neuropathy (LHON) relative with normal visual acuity (VA) and RNFL thickness, and normal perfusion on optical coherence tomography angiography (OCTA). (B) U8 asymptomatic LHON relative with unilateral increased perfusion in the right eye (vessel density (VD) RNFL 17.1%) with normal VA and significantly shortened P100 latency, and reduced perfusion in left eye (6.2%), with corresponding low RNFL thickness (70 µm), with poor vision LogMAR VA 2.4 and delayed P100 latency. (C) E2 LHON-affected patient of duration <3 months with normal RNFL perfusion, reduced vision in both eyes (LogMAR 1.5) and thinning of RNFL left eye. (D) C6 chronic LHON-affected patient with RNFL thinning, normal perfusion and reduced LogMAR VA 1.0 in both eyes and myelinated nerve fibre layer adjacent to right ONH.

controls was statistically significant (p=0.01). There were no subjects in the asymptomatic or LHON-affected group who had raised RNFL thickness above the derived normal range.

Reduced peripapillary VD occurred in association with reduced RNFL thickness, in the LHON-affected patient who showed reduced perfusion (C9) and also in the asymptomatic relatives who showed reduced VD (n=3 eyes). The eyes that showed raised VD (n=2 eyes, U1 and U8) in the asymptomatic relative group did not have raised RNFL. Reduced RNFL thickness was present in each of the included LHON patients, apart from one eye of E2, who had <3 months duration of LHON and was present in 6 of 19 eyes measured of the asymptomatic carrier relatives.

In those eyes with reduced peripapillary VD, VA was variable, see table 2. In the LHON-affected individual (C9) and one of the asymptomatic carriers (U8), VA was reduced to 1.6 LogMAR and 2.4 LogMAR, respectively. The contralateral eye of U8 had increased peripapillary

Table 1 Demographic characteristics, peripapillary vessel density (VD) and retinal nerve fibre layer (RNFL) thickness in LHON-affected (7.9%), asymptomatic LHON relatives (13.7%) and controls (10.9%)

	LHON-affected	Asymptomatic LHON relatives	Controls
Age: years, median (IQR)	27 (19–33) (n=12 subjects)	56 (42–61) (n=16 subjects)	48 (37–56) (n=10 subjects)
Sex (number of males/number of females)	9 M/ 3 F	1 M / 15 F	3 M/ 7 F
Peripapillary vessel density median (IQR)	7.9% (7.1%–10.6%) n=6 eyes, p=0.046	13.7% (10.0%–14.7%) n=16 eyes, p=0.166	10.9% (10.2%–12.6%) n=20 eyes

P value for comparison to control group.
F, female; LHON, Leber hereditary optic neuropathy; M, male.

VD, which was associated with a short P100 latency of 94 ms and normal VA. In the eyes of the other asymptomatic carriers with reduced peripapillary VD (n=2 eyes), VA was normal. In one asymptomatic carrier (U2) with normal VA but reduced VD, visual evoked potential (VEP) was prolonged in the contralateral eye to 130 ms.

Of the asymptomatic relatives who had a measurement of peripapillary VD, signs of optic neuropathy, including reduced VA worse than 0.2 LogMAR, or prolonged VEP or both, were present in 5 of 19 eyes (26%) in four subjects (bilaterally in U2). Reduced peripapillary VD had a sensitivity of 40%, with a specificity of 66%, and reduced RNFL thickness had a sensitivity of 80% and specificity of 92%, for the presence of optic neuropathy in the asymptomatic relatives.

DISCUSSION

In this cohort, detection of atrophy of the peripapillary nerve fibre layer was more successful and was more closely associated with carriage of LHON-associated pathogenic mitochondrial variants than perfusion measurement, and also more closely correlated with optic neuropathy. The evaluation of peripapillary retinal nerve fibre layer perfusion by OCTA measurement was not possible in the majority of LHON-affected patients due to fixation difficulty, usually associated with poor vision. In contrast, of the patients who could fixate for scanning by OCTA (33%), we found that the LHON-affected individuals had reduced peripapillary VD compared with controls. A greater proportion of the asymptomatic relatives were able to fixate for OCTA measurement (69%), and overall the peripapillary VD was not significantly different to that of controls, of whom all could fixate for perfusion measurement. Of the asymptomatic relatives, 17% were found to have reduced VD, and increased VD was observed in 10%. Abnormal VD had a variable association with visual function and was present with a VA level varying from normal VA through to blindness, and also occurred in a subject with normal vision but subclinical signs of optic neuropathy including prolongation of VEP. Normal nerve fibre layer thickness, with a lack of atrophy, showed greater specificity for signs of normal optic nerve function (92%) versus measurement of normal perfusion (66%) for normal nerve function.

The problem with using OCTA measurement in LHON is obtaining measurements when VA is poor and fixation is difficult, as a scan requires steady fixation for several seconds. Notwithstanding this difficulty, OCTA findings in the peripapillary circulation have been described in LHON, demonstrating variable associations including negative findings of the asymptomatic stage of LHON,[23] and reduced vessel density in established LHON.[17] Furthermore, cohorts of LHON families have been studied, demonstrating reduced vessel density in asymptomatic individuals,[23] or increased vessel density affecting the temporal ONH, after correcting for retinal nerve fibre layer thickness, in asymptomatic male carrier relatives.[24]

Peripapillary RNFL vessel density was lower in LHON than in normal eyes in our study. We suspect that the reduced perfusion is secondary to atrophic changes of the ONH and secondary loss of the RNFL vascular plexus, resulting in relative ischaemia. This ischaemia may be a recovery-limiting factor when considering therapies that promote mitochondrial respiratory chain function (ie, idebenone) and reduced OCTA VD may suggest limited benefit from such treatments. Indeed, each eye that showed reduced RNFL perfusion in the LHON and asymptomatic relative groups was also found to have RNFL thinning relative to controls. In this study, 66% of the LHON group could not fixate adequately for scanning and were therefore excluded from analysis. We suspect these unmeasured individuals may have had low peripapillary VD also based on the clinical characteristics of ONH pallor and peripapillary RNFL thinning.[12] Our control group was older than our LHON group (median age 48 vs 21 years), which may lead to the LHON group having undetected cases of even lower VD, as with advancing age there is a reduction in peripapillary VD.[25] There were sex differences in the comparison groups, with only males included in the measured LHON group. In healthy subjects, VD parameters measured by OCTA do not vary by sex,[25] but LHON shows some sex-dependent penetrance with more vision loss seen in males.[10]

Asymmetric changes in VD were observed in some asymptomatic LHON relatives. One case had discordant VD changes across both ONHs. This was associated with optic nerve dysfunction and poor vision of LogMAR 2.4

Table 2 Optic nerve function including LogMAR visual acuity (VA) and P100 latency, peripapillary retinal nerve fibre layer (RNFL) vessel density (VD) and thickness and Leber hereditary optic neuropathy (LHON) status

	ID	Age	Mitochondrial pathogenic variant, % heteroplasmy	Eye	Peripapillary		Best-corrected visual acuity (LogMAR)	Pattern visually evoked response: P100 latency (deviation)*
					Vessel density (%)	Retinal nerve fibre layer thickness (µm)		
LHON chronic	C6	50	11778 G>A, 80%	RE	7.1	54	1	NR
	C6			LE	8.4	46	1	NR
	C9	25	3460 G>A, 100%	RE	4.1*	37*	1.6	NR
	C10	15	*DNAJC30*	RE	7.4	67*	1.4	NR
LHON early	E2	17	14484 T>C, 100%	RE	12.8	108	1.5	NR
	E2			LE	11.3	57*	1.5	NR
Asymptomatic relatives	U1	60	11778 G>A, 65%	RE	17.1*	98	0	118 (6)
	U1			LE	13.8	96	0.2	120 (8)
	U2	36	11778 G>A, 98%	RE	6.0%*	58*	0	126 (14)
	U2			LE	7.4	55*	0.2	130 (18)
	U3	72	11778 G>A, 100%	RE	13.7	88	0.1	112 (0)
	U3			LE	10.8	83*	0.2	111 (–1)
	U4	67	11778 G>A, 100%	RE	14.1	107	0.1	107 (–5)
	U8	56	11778 G>A, 100%	RE	17.1*	104	0.1	94 (–18)
	U8			LE	6.2*	70*	2.4	121 (9)
	U9	55	11778 G>A, 100%	RE	14.9	95	0	107 (–5)
	U9			LE	15.3	98	0	114 (2)
	U11	37	11778 G>A, 100%	RE	14.0	88	0	100 (–12)
	U11			LE	14.5	87	0	106 (–6)
	U12	56	3460 G>A, 10%	RE	15.9	113	0	98 (–14)
	U12			LE	13.2	110	0.5	97 (–15)
	U14	36	11778 G>A, 100%	LE	6.1*	66*	0	120 (+8)
	U15	65	11778 G>A, 100%	RE	9.3	90	0	106 (–6)
	U16	52	14484 T>C, 100%	RE	13.0	85*	0.4	117 (5)
	U16			LE	13.6	93	0	111 (–1)

P100 latency is given in milliseconds (ms), with deviation from the mean normal reference value (112 ms) shown, or result displayed as non-recordable (NR).
*Denotes an abnormal value of vessel density (normal range 6.7%–16.7%) or abnormal retinal nerve fibre layer value (normal range 86–115 µm).
LE, left eye; LHON, C, chronic >1 year duration LHON; LHON, E, early <6 months duration LHON; RE, right eye; U, unaffected relative.

in one eye, which had low VD, and normal vision with an abnormally short VEP of 94 ms in the contralateral eye, which had raised VD. The implications of this are not clear, as our study was not longitudinal. Onset of LHON is usually sequential, with one eye affected within 3 months of the fellow eye, though cases of more than 10 years between eyes losing vision have been reported.[26] A shortened VEP implicit time implies increased conductivity of retinal ganglion cells. This may represent compensatory increased mitochondrial biomass as mitochondrial dysfunction develops, prior to visual loss.

The measurement of peripapillary RNFL perfusion did not equate to that of RNFL thickness and each provided different data, and correlated differently with optic nerve function. Unlike RNFL perfusion, where we found no difference between controls and asymptomatic relatives, RNFL thickness was significantly reduced versus controls in both asymptomatic relatives and LHON-affected

individuals. RNFL thinning was present in the chronic cases of LHON who could fixate (two eyes only in each of C9 and C10) and was present in 6 eyes of 19 who had measurement in the asymptomatic relatives sample. The presence of normal RNFL showed greater specificity (92%) for normal optic nerve function, including VA and VEP latency, in the asymptomatic relatives, compared with the specificity of normal vessel density for normal optic nerve function (66%). In this cohort, lower perfusion did not give any additional insights into the aetiology of thinned RNFL. RNFL thinning may occur at an earlier stage of progressing neuropathy than vessel density changes, which may follow later when atrophy is established. Individuals in our asymptomatic relative cohort may represent subclinical cases of LHON.

None of the asymptomatic relatives had raised RNFL thickness above the derived normal range, whereas one of the samples had raised vessel density, that was found in association with shortened P100 latency. These asymptomatic relatives may have early preclinical optic neuropathy due to impaired mitochondrial function. Distinctly, increased perfusion may represent an earlier again stage prior to manifestation of vision loss.

Future studies could investigate correlation of perfusion and thickness with onset of ONH telangiectasia, classically seen at the onset of symptoms of LHON. ONH analysis by quadrant would be useful, and longitudinal studies of changing perfusion over time may give more insight into the significance of our findings. Analysis of these measurements could be done with other tests of visual function, including perimetry.

Limitations of this study include its small size and cross-sectional nature, and that age-matching was limited to the control group comparisons; the LHON-affected group was significantly younger than the asymptomatic relative group. LHON is already a rare condition and fixation difficulties meant that accurate peripapillary OCTA could be captured in only a small subset of affected patients. We evaluated the peripapillary RNFL, as the primary site of pathology, but more information may be derived from also evaluating perfusion changes at the macula and within the papillomacular bundle. Strengths of the study include the evaluation of multiple individuals who have LHON and also a group of asymptomatic maternal relatives when compared with a normative database assessed on the same imaging platform.

Potential utility of OCTA and OCT in LHON could be in monitoring asymptomatic relatives for optic nerve RNFL perfusion and thickness changes over time. Those eligible for monitoring may be as many as 1 in 10 000 based on population studies of LHON prevalence,[27] or as high as 1 in 300 based on prevalence of LHON-associated mitochondrial variants.[28] While historically there was no treatment available for LHON, monitoring and screening may become of more relevance as more treatment options are being investigated and developed, including idebenone and intravitreal gene replacement therapy.[29 30] Longitudinal studies are required to evaluate whether microcirculatory abnormalities may be static or progress over time and how reduction of peripapillary RNFL VD may impact outcomes of these novel treatments. Our finding of raised peripapillary RNFL VD associated with shortened P100 latency is interesting but requires longitudinal studies to determine its significance.

Twitter Clare Quigley @q_clare

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Competing interests None declared.

Patient and public involvement statement Patients were not involved in the planning of this study.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and was approved by the Research Ethics Committee of Royal Victoria Eye and Ear Hospital (no ID was given by the committee—contact person Cathy Fox, cathy.fox@rveeh.ie). Participants gave informed consent to participate in the study before taking part.

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ORCID iDs
Clare Quigley http://orcid.org/0000-0001-6914-7447
Kirk A J Stephenson http://orcid.org/0000-0002-7462-7725

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