



Leber hereditary optic neuropathy: bridging the translational gap

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Purpose of review

Leber hereditary optic neuropathy (LHON) is the most common primary mitochondrial DNA (mtDNA) genetic disorder in the population. We address the clinical evolution of the disease, the secondary etiological factors that could contribute to visual loss, and the challenging task of developing effective treatments.

Recent findings

LHON is characterized by a preclinical phase that reflects retinal ganglion cell (RGC) dysfunction before rapid visual deterioration ensues. Children can present atypically with slowly progressive visual loss or an insidious/subclinical onset that frequently results in considerable diagnostic delays. The LHON mtDNA mutation is not sufficient on its own to precipitate RGC loss and the current body of evidence supports a role for smoking and estrogen levels influencing disease conversion. Clinical trials are currently investigating the efficacy of adeno-associated viral vectors-based gene therapy approaches for patients carrying the m.11778G>A mutation. Mitochondrial replacement therapy is being developed as a reproductive option to prevent the maternal transmission of pathogenic mtDNA mutations.

Summary

LHON is phenotypically more heterogeneous than previously considered and a complex interplay of genetic, environmental and hormonal factors modulates the risk of a LHON carrier losing vision. Advances in disease modelling, drug screening and genetic engineering offer promising avenues for therapeutic breakthroughs in LHON.

Keywords

gene therapy, Leber hereditary optic neuropathy, mitochondrial diseases, mitochondrial replacement, optical coherence tomography

INTRODUCTION

Inherited optic neuropathies are an important cause of blindness in children and young adults [1]. The advent of next-generation sequencing technology has led to a rapidly expanding list of disease causing genes and rather strikingly, all of them encode for structural or functional proteins that contribute to mitochondrial homeostasis. Leber hereditary optic neuropathy (LHON) is the classical paradigm of a mitochondrial optic neuropathy and historically, it is the first disorder for which a pathogenic point mutation was ascribed to the mitochondrial genome, which is strictly maternally inherited [2]. Much progress has been achieved since 1988 when Wallace *et al.* [3] identified the m.11778G>A mitochondrial DNA (mtDNA) mutation in the *MT-ND4* gene as the most common cause of LHON (~60%). The m.3460G>A (*MTND1*) and m.14484T>C (*MTND6*) mutations account for ~15% of cases each, with the remainder harbouring rarer mtDNA mutations [1].

The prevalence of LHON has been well established in Northern European populations with figures ranging from one in 30 000 to one in 50 000 [4–6]. The incidence has proven more

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KEY POINTS

- The onset of visual loss in LHON may be preceded by a prodromal phase characterized by structural and functional evidence of RGC dysfunction.
- Childhood LHON is a distinct subgroup that carries a relatively more favourable prognosis compared with the adult form of the disease.
- Environmental and hormonal factors are likely to influence the overall risk of visual loss in LHON.
- Clinical trials are currently evaluating the therapeutic benefit of gene therapy for patients carrying the m.11778G>A mutation.

difficult to establish in specific defined geographical regions, but an estimate of one in 1000 000 has recently been reported in the Japanese population [7[■]].

Visual recovery in LHON is influenced by the underlying mtDNA mutation, but overall, the visual prognosis is poor and the majority of patients will remain significantly visually impaired for the rest of their lives [8]. Less than 20% of patients harbouring the m.11778G>A mutation will recover 15 letters of vision from the nadir compared with more favourable recovery rates of up to 60% for those with the less deleterious m.14484T>C mutation [9,10[■]]. Treatment options remain limited and this unsatisfactory situation is further compounded by the challenging nature of genetic counselling and our incomplete understanding of the triggers for visual loss in LHON [1,2].

CLINICAL EVOLUTION

The acute stage of LHON is characterized by a rapidly evolving dense central or cecentral scotoma. In ~25% of cases, both eyes are affected simultaneously [1,2]. In cases with unilateral disease onset, the fellow eye is affected within 3–6 months. Although the fundus can look entirely normal, the optic disc is more commonly hyperaemic with peripapillary telangiectasias and vascular tortuosity of the central retinal vessels. There is pseudoedema secondary to swelling of the retinal nerve fibre layer (RNFL) and these morphological changes have been documented more closely with the help of optical coherence tomography (OCT) imaging. Disease conversion in LHON is heralded by swelling of the inferotemporal RNFL, which then propagates circumferentially to involve the other sectors around the optic disc [11,12]. The swelling gradually subsides and the development of optic atrophy

coincides with progressive generalized RNFL thinning over the subsequent 6–12 months. The chronology and pathophysiological relevance of these retinal ganglion cell (RGC) axonal changes have been further clarified with high-resolution spectral-domain OCT imaging. It is now apparent that changes occurring at the macula, as measured by the ganglion cell and inner plexiform layer (GC-IPL) thickness, precede the wave of peripapillary RNFL swelling in the acute stage of LHON [13[■]]. In patients presenting with unilateral optic nerve involvement, reduced GC-IPL thickness was noted in the fellow (unaffected) eye 6 weeks before the actual onset of visual loss and maximal thinning at the macula had already occurred within 6 months. Further work is needed to demonstrate whether GC-IPL measurements could prove a more sensitive biomarker for monitoring disease progression or detecting a therapeutic response in clinical trials.

The preclinical stage has also been defined in more detail based on the observations made in a group of six LHON carriers from a large Brazilian family carrying the m.11778A>G mutation who developed visual loss whilst being followed up as part of a prospective natural history study [14[■]]. An increase in peripapillary RNFL thickness and a decrease in mean deviation of up to 5 dB on Humphrey visual field perimetry were observed 4–6 months before the patient-reported subjective visual loss. A combination of OCT and visual field changes may therefore help identify LHON carriers at high-risk for impending visual deterioration and perhaps, most likely to benefit from early intervention when the means to stop or slow RGC loss eventually becomes available.

The vascular changes in the acute stage of LHON and the transient peripapillary telangiectasias seen in asymptomatic mutation carriers are suggestive clues pointing towards a possible vasculopathy contributing to the complex pathophysiology of this disorder. Thickening of the macular and peripapillary choroidal layer has been reported in the acute stage, followed by thinning in the chronic stage [15]. The RNFL changes preceded the choroidal changes, and it remains to be determined whether the latter represents a compensatory response secondary to a state of 'pseudo-hypoxia'. OCT angiography (OCT-A) is a promising development in ocular imaging that is being used to delineate the retinal vasculature in various pathological states, including age-related macular degeneration and diabetic retinopathy. Three case reports of OCT-A in patients with acute LHON confirm the marked vascular dilatation and tortuosity that is evident clinically [16–18]. Importantly, there is no leakage on fluorescein angiography, which differentiates LHON from other inflammatory optic neuropathies

or true papilledema in the context of raised intracranial pressure. In the chronic stage of LHON, OCT-A shows extensive capillary drop out, in particular in the region of the papillomacular bundle.

CHILDHOOD LEBER HEREDITARY OPTIC NEUROPATHY

LHON is predominantly a disease of young adults and the peak age of onset is in the second and third decades of life. Less than 10% of patients are 12 years old or younger at the time of diagnosis and previous reports have described atypical patterns of visual loss in this subgroup [19,20]. Childhood LHON seems to carry a better visual prognosis compared with the adult form of the disease. A recent meta-analysis combined a British pediatric cohort of 27 patients with 69 additional cases that were identified from a systematic review of the literature [10¹¹]. The inclusion criteria were onset of visual loss at 12 years old or younger and molecular confirmation of one of the three primary mtDNA mutations (m.3460G>A, m.11778G>A or m.14484T>C). The phenotype in childhood LHON could be classified into three distinct subtypes: first, classical acute, which was the most common, being seen in about two-thirds of all cases; second, slowly progressive, if visual deterioration occurred over a period exceeding 6 months and third, insidious or subclinical for those individuals who were clinically asymptomatic at the time that a diagnosis of optic atrophy or subnormal vision was made, and in whom no further decline in visual acuity occurred during subsequent follow-ups. This latter subgroup accounted for ~20% of cases and diagnostic delays of up to 15 years were encountered. A significant proportion of patients (~40%) achieved a final best-corrected visual acuity (BCVA) at least 6/12 Snellen in at least one eye, but ~20% of patients did poorly with a final BCVA less than 3/60 in their better seeing eye. Although childhood onset is a positive prognostic factor in LHON, a degree of caution is warranted as part of counselling given that one in five patients will remain within the visual acuity criteria for legal blindness.

From a clinical perspective, LHON can have an insidious manifestation in the paediatric population and mtDNA genetic testing should be considered when faced with a child with unexplained subnormal vision and optic disc pallor to avoid potentially prolonged diagnostic delays.

INCOMPLETE PENETRANCE AND MALE BIAS

LHON shows variable penetrance both between families and within branches of the same family.

The lifetime risk of a male carrier losing vision is ~50% compared with ~10% for a female LHON carrier. The secondary factors that underlie this incomplete penetrance and the strong male bias for visual loss have been the subject of intense investigation over the past 3 decades. Genetic modelling initially pointed towards a nuclear modifier gene on the X chromosome, but linkage studies have been inconclusive with different risk loci having been mapped and failure so far to identify a causative gene that segregates with affected disease status in LHON [21–23]. The current body of evidence points towards LHON being a complex disease with multifactorial genetic and environmental triggers interacting with the LHON mtDNA mutation [24]. Uncovering the nature of these modulatory factors has been difficult for several reasons, in particular, the relatively rarity of LHON for conducting adequately powered, large-scale genomic studies and the difficulty in collecting accurate environmental exposure data.

ENVIRONMENTAL TRIGGERS

A number of retrospective epidemiological studies have provided convincing evidence that heavy smoking, and to a lesser extent excessive alcohol consumption, is a major risk factor for visual loss in LHON [25–27]. It is biologically plausible that these environmental triggers play a more prominent role for mutation carriers who experience visual loss in later life, presumably because they have a lower *a priori* nuclear genetic predisposition [28,29]. There are potential confounding factors inherent in the retrospective recall and analysis of environmental exposure, but the deleterious impact of smoking on mitochondrial function has been confirmed *in vitro* with the use of primary LHON fibroblasts established from affected and unaffected mutation carriers [30¹¹]. Cells that were cultured under the influence of cigarette smoke demonstrated a significant reduction in ATP synthesis, which is to be expected given that LHON mtDNA mutations affect critical subunits of the mitochondrial respiratory chain complexes. More prominent oxidative damage were also found in fibroblasts from affected mutation carriers implying a reduced capacity to mitigate the effect of impaired mitochondrial electron flux and the consequent toxic elevation in reactive oxygen species (ROS) levels. LHON mutation carriers should therefore be strongly advised not to smoke and to moderate their drinking habits as these are the only modifiable risk factors over which they have a personal degree of control.

HORMONAL INFLUENCES

A longstanding hypothesis is whether the striking sex bias in LHON could arise because of hormonal differences, especially the higher levels of circulating estrogens in women. This would imply a second peak of female LHON carriers converting in the perimenopausal or menopausal period, but there is no robust data yet to suggest that this is the case. RGCs express high levels of the estrogen beta receptor and estrogen derivatives are known to exert a neuroprotective effect under conditions of heightened cellular stress [31,32]. The work of Giordano *et al.* have provided convincing experimental proof that estrogen derivatives were effective in partially rescuing the downstream consequences of LHON mtDNA mutations on mitochondrial function in a well characterized LHON cybrid model [33,34[■]]. When treated with a specific combination of phytoestrogens, which are natural estrogen-like compounds, LHON cybrids showed improved mitochondrial respiratory chain function, lower ROS levels and a reduced sensitivity to undergo apoptosis. These promising in-vitro results will need to be pursued further and if confirmed, the next step would be to screen for estrogen derivatives that are mitochondrial protective, whilst not having any of the adverse side effects of sustained estrogen use, especially feminising effects and an increased risk of breast and gynaecological cancers if the treatment is to be given prophylactically to male and female LHON carriers, respectively.

FROM BENCH TO BEDSIDE

LHON is a frustrating condition for patients, their families and the physicians looking after them. The stark reality is that there is currently no treatment that will reverse the catastrophic loss of RGCs that occurs rapidly in the first few weeks of disease onset [35]. The development of treatment paradigms for LHON and other mitochondrial optic neuropathies has been severely hampered by the inability to study disease mechanisms directly in RGCs and the lack of animal models that faithfully replicate the optic neuropathy and clinical manifestations.

Idebenone is a synthetic, short-chain analogue of ubiquinone, which improves the shuttling of electrons from complexes I and II directly to complex III. Idebenone is partially effective when given in the acute stage of LHON, but only a subgroup of patients experience a clinically significant visual benefit [36–38]. There is clearly the need to develop more targeted treatments that will block one or more of the key pathways that link mitochondrial dysfunction with optic nerve neurodegeneration in

LHON. A combinatorial approach is likely to be necessary and the ultimate objective would be to prevent RGC loss in the first place for those LHON carriers deemed to be at high risk of disease conversion based on preexisting susceptibility factors and/or the presence of ophthalmological features presaging imminent deterioration in visual acuity.

THERAPEUTIC DRUG SCREENING

Mitochondria exist as a highly interconnected network that extends throughout the cell and mtDNA copy number is a surrogate marker of a cell's bioenergetic demand. Significantly, mtDNA copy number was found to be significantly higher in unaffected LHON carriers compared with affected LHON carriers in a range of tissues that were tested, including peripheral blood cells, skeletal muscle biopsies and cells isolated by laser capturing post-mortem specimens of retina and optic nerves [39,40]. Improved mitochondrial biogenesis could therefore be a key compensatory mechanism that protects some LHON carriers from losing vision. This hypothesis is further borne out by the emerging story with regard to tobacco toxins and estrogens, which decrease and increase mtDNA copy number, respectively, potentially accounting for their influence on disease penetrance [30[■],34[■]].

A recent paper highlighted that an imbalance between nicotinamide adenine dinucleotide (NAD⁺) and the reduced form of NAD⁺ (NADH) could be the unifying link through which impaired mitochondrial oxidative phosphorylation (OXPHOS) results in RGC loss in both aged mice and a glaucoma mouse model [41[■],42]. Boosting the level of NAD⁺ by long-term dietary supplementation with the NAD⁺ precursor nicotinamide (vitamin B3) or gene therapy prevented RGC dysfunction and neuronal loss, providing an attractive therapeutic paradigm for glaucoma and, by extrapolation, mitochondrial optic neuropathies.

High-throughput drug screening is another possible way forward to identify molecules with a positive effect on mitochondrial biogenesis and OXPHOS, and these could then be further tested in RGCs, which are the susceptible neuronal population in LHON. Induced pluripotent stem cells can now be routinely produced by reprogramming peripheral blood cells or fibroblasts obtained from a punch skin biopsy [43]. In parallel, differentiation protocols are being refined by a number of research groups worldwide to allow the efficient generation of various retinal cells, including RGCs, providing an ideal 'disease in a dish' model for testing promising drug molecules in a more physiological setting [44–46].

GENE THERAPY

Despite the complexity in unravelling the secondary modifiers that precipitate visual loss, LHON is still amenable to classical gene therapy with replacement of the missing protein product. Mitochondrial gene therapy is a challenging task because of the physical barrier imposed by having two mitochondrial membranes and the need for sustained gene expression following delivery of the gene construct into the mitochondrial matrix compartment [47]. To circumvent these technical difficulties, one ingenious option is allotopic gene expression whereby the gene construct is inserted into the nuclear genome, and the resulting protein has a mitochondrial targeting sequence, which then allows its import into the mitochondrial compartment [48]. Genetically modified adeno-associated viral vectors (AAV2) have been developed to deliver the *MT-ND4* gene construct as a therapeutic strategy to compensate for the m.11778G>A mutation [49,50]. Preclinical data have provided consistent results supporting therapeutic rescue *in vitro* and a reduction in the amount of RGCs lost in the relevant murine models tested, which was associated with an improvement in visual function. Some investigators have expressed legitimate concerns regarding the efficiency with which the imported ND4 subunit will integrate into Complex I and whether this will result in a sufficiently stable complex to promote the efficient flow of electrons along the mitochondrial respiratory chain [47,51]. Preliminary data from early-phase clinical trials support the previously established safety of AAV2-based gene therapy vectors and an encouraging visual trend for the eyes that have been treated with a single intravitreal injection [52–54]. The efficacy of this therapeutic paradigm for patients with LHON still needs to be established and three clinical trials (NCT02161380, NCT02652767 and NCT02652780, <https://clinicaltrials.gov/ct2/home>, accessed on 1 June 2017) are currently recruiting patients in Europe and North America to evaluate the optimal dose and the effects of treatment at different stages of the disease process.

MITOCHONDRIAL REPLACEMENT THERAPY

LHON is a disease of young adults and reproductive choice is a topic of discussion that is frequently raised by patients and other maternally related family members during genetic counselling. Some female carriers, most of whom will be unaffected, are keen to have their own biological children, but they are understandably anxious about the future implications for their children who will be inheriting their mtDNA mutation.

Mitochondrial replacement techniques have been developed to prevent the maternal transmission of mtDNA mutations, some of which cause early-onset, severe and invariably fatal encephalomyopathies [47,55,56]. The methods being proposed are based on modification of established IVF techniques. They involve the transfer of parental nuclear material into a mitochondrial donor zygote carrying only wild-type mtDNA and the experimental data obtained so far has been encouraging with minimal carry over of mutant mtDNA [57[■],58[■]]. In a primate model, embryonic development proceeded normally following implantation of the embryos, resulting in the births of healthy offspring [57[■],58[■]]. This reproductive approach remains controversial as it entails germline modification with a child inheriting genetic material from a third person, albeit a limited number of 37 mitochondrial genes compared with the estimated 20 000 genes that constitute the nuclear genome. The concern also relates to the possibility that the adverse effects from this new genetic admixture might not become apparent until later in life [47,55,56]. The scientific and ethical debate as to whether mitochondrial donation should be offered to prospective mothers carrying pathogenic mtDNA mutations has accelerated further when the media released the somewhat unexpected news about the first live birth arising from mitochondrial replacement in September 2016 [59[■],60]. The child's mother is heteroplasmic for the m.8993T>G mtDNA mutation in *MT-ATP6*, which in addition to the syndrome of neuropathy, ataxia and retinitis pigmentosa can also cause Leigh syndrome when present at high mutant levels. Although clinically asymptomatic, she suffered from multiple miscarriages and two of her children died at the age of 8 months and 6 years old from Leigh syndrome with the m.8993T>G mutation detected at levels exceeding 95%. The newly born baby boy was reported to be healthy at 7 months age and relatively low levels of the mutation (2.4–9.2%) were detected in the tissues that were analysed. The child's neurodevelopmental progress will be carefully reviewed and fertility testing will be considered when he reaches the age of 18 years. Following an extensive public consultation exercise and a stringent scientific peer review process, the Human Fertilisation and Embryology Authority in the United Kingdom has granted a licence in March 2017 for the first clinic to be set up in Newcastle upon Tyne, United Kingdom, to provide mitochondrial donation as a reproductive option for eligible female carriers harbouring disease-causing mtDNA mutations (<http://www.hfea.gov.uk/10635.html>, accessed on 1 June 2017).

CONCLUSION

The past 5 years have seen a major shift in LHON research to clinical translation and there is renewed hope that the translational gap can be bridged by bringing together scientific communities with different skill sets and fostering greater collaborations with industry, patient-led organizations and national regulatory agencies, which until recently only had a limited understanding of the unique challenges of research into rare diseases. Experimental therapies by their very nature impose a degree of calculated risks on the participants, and the onus is on the physicians and researchers involved to clearly communicate the nature of the intervention being studied, the benefits that are being contemplated, and crucially, the grey areas that will only be answered with long-term follow-up. LHON causes devastating visual loss and the urgent need to develop and validate new treatment strategies should not cloud our judgement that patient safety should always remain the abiding central factor. There will inevitably be set-backs along the way, but the major breakthroughs being achieved in the fields of ocular phenotyping, disease modelling, drug screening and genomic manipulation all point towards a building momentum that will help drive LHON research forward.

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Conflicts of interest

P.Y.-W.-M. holds a consultancy agreement with GenSight Biologics (Paris, France).

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