The Genetics of Leber's Hereditary Optic Neuropathy: A Literature Review

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ABSTRACT

Leber's hereditary optic neuropathy (LHON) is a mitochondrial disease characterized by a painless, acute loss of central vision, with 95% of affected individuals harbouring one of three pathogenic mutations (G11778GA, G3460A, or T14484C). The purpose of this review is to highlight the distinguishing clinical presentations of the disease with respect to the mutation subtype and present our recent understanding of two unique features of the disease: male predominance and incomplete penetrance. We also review recent advancements made in the diagnosis and treatment of this rare mitochondrial disease and their implications for genetic counselling.

RÉSUMÉ

La neuropathie optique de Leber est une maladie mitochondriale caractérisée par une perte aiguë, et indolore de vision centrale, avec 95 % des individus affectés possédant une de trois mutations pathogéniques (G11778GA, G3460A, ou T14484C). Le but de cette revue est de surligner les aspects distincts de la présentation clinique de la maladie selon le sous-type de la mutation et de présenter sur nos compréhensions récentes de deux traits uniques de la maladie : la prédominance mâle et la pénétrance incomplète. Nous allons également revoir les avancements récents dans le diagnostic et traitement de cette maladie mitochondriale rare et leurs implications pour le conseil génétique.

Leber's hereditary optic neuropathy (LHON) is a rare mitochondrial disease with a male predominance that results in painless central vision loss (1,2). More than 90% of patients with LHON carry one of three primary mutations affecting complex I (NADH dehydrogenase) of the mitochondrial electron transport chain (ETC). These mutations are G11778A, G3460A, and T14484C (3). Affected individuals typically experience significantly reduced visual acuity, blurring, central or cecocentral scotoma, and diminished colour vision (2). Visual deficits have been associated with dysfunction of retinal ganglion cells (RGCs) and their axonal transport system (4). RGCs, particularly the papillomacular bundle, are vulnerable to disruptions of the mitochondrial bioenergetics as a result of their structural and energy constraints (4). Moreover, histopathological studies of individuals with LHON show progressive degeneration of myelin and axoplasms at the level of the optic nerve, but early changes can be noted on fundoscopy and include peripapillary retinal nerve fiber layer (RNFL) edema and telangiectasia of the retinal vessels (3).

LHON has a disease prevalence of approximately 1 in 50 000, making it one of the most commonly inherited optic neuropathies (5). The majority of patients are affected prior to the age of 60, with an average age of onset ranging from 15–35 years (5). During the acute phase of the disease, patients present with a painless blurring of vision. Vision loss is bilateral in 25% of cases and sequential in 75% of cases, with an inter-eye delay interval of approximately eight weeks (3). A nadir in visual acuity is often reached by 4–6 weeks following onset of disease and can worsen to less than 6/60 (3). In the acute phase, fundoscopic examination is typically unremarkable; however, it may also demonstrate disc pseudoedema, tortuous vessels, and peripapillary telangiectasias (5). The characteristic visual field finding of central or cecocentral scotoma may be observed on Goldmann perimetry (6). In the more chronic phase of the disease, optic atrophy can be noted in the form of optic nerve head pallor as a result of selective degeneration of the RGCs (6).

MOLECULAR MECHANISM

The causative factor for disease in LHON is a missense mutation in NADH dehydrogenase, a co-enzyme responsible for the production of ATP and cellular energy (7). This results in decreased ATP synthesis, elevated oxidative stress, and disruptions to the transport of glutamate, ultimately leading to

a degeneration of the RGCs and eventual cell death via apoptosis (7). However, the majority of the proposed mechanisms have been established through mitochondrial cybrid cell lines. One study by Lin et al. employed a mouse model of LHON and introduced the P25L mutation—which results in LHON in humans—into the ND6 subcomponent of the respiratory chain (8). The authors subsequently concluded that oxidative stress is the primary mediator of RGC cell death while ATP production has been relatively spared. Mechanistically, it has been observed that the transport of electrons along the ETC can be disrupted as a result of a mutated complex I, allowing more free electrons to react with molecular oxygen. This process leads to the conversion of superoxide and subsequently increased oxidative stress (9). Interestingly, it is rare for patients to have systemic symptoms. Moreover, no other neuronal cell lines apart from the ganglion cells—including photoreceptors which also require high energy demand and mitochondrial load—appear to be affected (7). A recent study by Levin examined the role of superoxide production in RGC-5 cell line and found superoxide production to occur at significantly reduced levels in RGC compared to mitochondria in neuronal cell lines (10). This has been hypothesized to be due to the regulatory effect of superoxide dismutase (SOD-2), an enzyme that converts the toxic superoxide into nontoxic components (10). Pioneering work by Guy and Qi et al. has also demonstrated that knockdowns of SOD-2 in RGCs result in optic neuropathy, while overexpression of SOD-2 improve optic neuropathy (11, 12). Despite having the SOD-2 system to modulate the amount of superoxide production, mutations in complex I (as observed in LHON) could lead to high levels of superoxide production that may overwhelm this regulatory pathway, manifesting in eventual ROS release and cellular apoptosis (10).

CLINICAL CHARACTERISTICS OF LHON
Features of Pathogenic Mutations

Although there are a multitude of primary mutations responsible for LHON, over 90% of the LHON pedigrees harbour one of three primary mutations in the mitochondrial genome at nucleotide positions 3460, 11778, and 14484 (1). The mutations affect the ETC chain complex I genes at the ND1 (3460), ND4 (11778) and ND6 (14484) subcomponents (1).

There are several distinguishing characteristics based on demographic factors and clinical manifestations across the three mutations. G11778A is the most prevalent mutation subtype, reported to account for approximately 70% of Northern European patients and 90% of Asian patients with LHON (1). T14484C and G3460A are approximately equal in prevalence, but there is a higher representation of the T14484C mutation among French Canadians due to a founder effect (13). With respect to the age of disease onset, the T14484C mutation exhibits the lowest age of onset at approximately 19 years compared to the other primary mutations G11778A (26 years) and G3460A (21 years) (14). Clinically, visual prognosis differs according to the mutation subtype such that the rate of spontaneous visual recovery is highest for the T14484C mutation. Between 37% and 58% of patients with the T14484C mutation experience recovery compared to only 4% of patients with the G11778A mutation and 20% of patients with the G3460A mutation (1). Moreover, the G3460A mutation is associated with more severe disease biochemical phenotype (15).

Though all three mutations present with simultaneous mutations, the percentage of simultaneous versus sequential onset is significantly different (14). There is a greater predilection for simultaneous compared to sequential onset for T14484C. Moreover, as previously observed among LHON mutations with sequential onset, the T14484C mutation subtype also has a more restricted distribution with inter-eye onset occurring at less than 44 weeks; in contrast, the G11778A and G3460A mutations can have onset ranges of up to 2016 weeks and 816 weeks, respectively (14). This is interesting to observe given that T14484C is also the mutation that harbours the mildest defect and greatest potential for recovery. Patient populations affected at a younger age, especially individuals harbouring the T14484C mutation, may be more genetically predetermined to becoming affected and thus demonstrate bilateral involvement at initial presentation.

Male Predominance

One unique characteristic of LHON is its male predominance; previous pedigrees have reported that 80–90% of affected family members are male (1). One hypothesis proposed by Bu et al. has been related to an X-linked recessive susceptibility gene that interacts with the mitochondrial genome which could account for the increased disease prevalence in males (17). Female carriers who become affected are thought to be homozygous at the X-linked locus or display inactivation of the X chromosome (17). Although recent linkage analyses have identified a possible region that may be suggestive of this susceptibility gene in the long arm of the X chromosome, the exact gene remains to be elucidated. Another hypothesis that could account for the disparity in disease representation

between males and females is the influence of hormones and the protective role of estrogen in females. Estrogen has been implicated in modifying the severity of mitochondrial dysfunction including dysregulated ATP synthesis, oxidative stress, and cellular apoptosis. Indeed, estrogen receptors are abundant within the ganglion cells, and their subsequent stimulation has been associated with increased activity of SOD-2 and reduction in ROS (18).

Incomplete Penetrance

A second intriguing characteristic of this mitochondrial disease is its rate of incomplete penetrance, with only 50% of male and 10% of female carriers expressing the disease phenotype during their lifetime (2). Genetic factors cannot completely account for this reduced penetrance. Based on results from five reported monozygotic twin studies (19-24), two pairs had one sibling that did not become affected by the disease during long-term follow-up (21,23). It is, therefore, more likely that LHON is a multifactorial disease whereby the presentation is influenced by the interactions of the primary mutations with environmental influences. Indeed, several prior investigations suggest an increased rate of disease conversion in LHON carriers with increased alcohol and tobacco consumption (25). The effect of smoking has also been examined by Kirkman et al. in a multicentre study of 206 unaffected and 196 affected carriers (25). Their study demonstrated that not only is smoking associated with an increased risk of visual symptoms in carriers of LHON, but that heavy smokers were more likely to manifest the disease phenotype compared to light smokers, lending support for a potential dose-response relationship. Furthermore, it is suggested that cigarette smoke can reduce the efficiency of complex I and, in turn, increase production of ROS. Several other reports have noted other precipitating factors that may contribute to disease conversion including trauma, nutritional deficiencies, toxin exposure, and psychological stress; however, the strength of these relationships require further evaluation.

ADVANCEMENTS IN DIAGONISTICS & MANAGEMENT
Diagnosis

Research in optical coherence tomography (OCT) has also shown changes in the retinal ganglion cell complex (GCC) relative to the RNFL over time. Barboni et al. discovered that there was a thickening of RNFL on OCT in early LHON (<6 months) and disc atrophy in chronic LHON (>6 months). The temporal fibers of the papillomacular bundle were the first and most severely affected areas, whereas there appeared to be nasal fiber sparing of the optic nerve until more advanced stages of the disease (26). Savini et al. investigated RNFL

thickness in unaffected carriers of LHON and found that there was an apparent thickening of temporal fibers in all subgroups of LHON carriers (27).

Additionally, visual electrophysiological recordings including visual evoked potentials (VEPs) and pattern electroretinograms (PERGs), which provides a more objective measure of ganglion cell function, have been employed. For example, it has been documented that LHON patients demonstrate characteristic changes such as increased VEP latencies, waveform distortions, and decreased VEP amplitudes during the acute stage (28,29). Additionally, reductions in PERG amplitude have been noted in LHON carriers despite normal visual acuity, visual field, and RNFL thickness (30). However, VEP elicits potentials from the visual cortex and, as a result, does not directly measure RGC function. While PERGs have the ability to measure RGC activity, their utility is primarily limited to the inner retina and necessitates compensation of the patient's refractive prescription and accurate foveal placement (31).

Recently, it was observed that RGCs generate a slow negative wave signal observable on the electroretinogram (ERG) referred to as the photopic negative response (PhNR). Numerous studies have evaluated the predictability of disease pathology such as glaucoma, various optic neuropathies, retinal vascular diseases, and idiopathic intracranial hypertension using the PhNR (32-37). In full-field ERG, the PhNR amplitude reflects cone-related RGC function and is significantly reduced with advancing visual field loss and reduced RNFL thickness as seen on OCT (38). The thinning of the RNFL typically follows the decreased PhNR amplitude, suggesting that functional changes precede structural abnormalities in the ganglions cells in LHON (38). In summary, there is good evidence to suggest the use of clinical ERG including the PhNR and ocular imaging such as OCT in aiding the diagnosis and monitoring the severity of disease in patients with LHON.

Treatment

Presently, there is no definitive medical treatment for LHON. Nevertheless, there is theoretical benefit in the use of anti-oxidants including Coenzyme-Q10 in mitigating stress from ROS (39). Recent trials have also been conducted on idebenone, a short-chain benzoquinone related to Coenzyme-Q10 which allows electrons to proceed from complex I directly to complex III in the ETC, bypassing complex II (40). The Rescue of Hereditary Optic Disease Outpatient Study (RHOSOS) recruited 84 patients with primary LHON mutations (40). Patients were randomized and either received high dose idebenone for 24 weeks or

placebo. Post-hoc analyses revealed improvement in visual acuity in the treatment group, particularly those with G11778A and G3460A mutations. Investigations are currently being conducted for EPI-743, a more potent para-benzoquinone compared to idebenone, and have shown some encouraging results in smaller cohort studies (41).

Gene therapy has become an exciting avenue for current and continued research in advancing treatments for LHON. Exogenous DNA can be inserted into the nuclear DNA through adenovirus-associated virus (AAV) type 2. Given the defect in LHON is within the mitochondrial genome, allotopic rescue has been employed. In this process, genetic material is incorporated into the nuclear genome and is then targeted to the mitochondria (11). Preliminary results have been demonstrated in a mouse model injected with an AAV vector containing ND4 (the protein affected by the LHON 11778 mutation); it was successful in preventing vision loss, stabilizing ATP synthesis, and rescuing ganglion cells from cellular apoptosis (41). Apart from ND4 viral vectors, the NDI1—a yeast nuclear gene which encodes an equivalent of complex I—can be delivered directly as demonstrated by a study by Marella et al. (42). Through gene therapy, the NDI1 gene was successfully delivered to the optical layer within the superior colliculus in a LHON rat model. Subsequently, rats with previously impaired vision that were treated with the NDI1 gene experienced complete improvement in vision compared to animals injected with green fluorescent protein (GFP) controls.

Additionally, gene therapy holds promising potential and has been part of a Phase 1 and 2 dose-escalation trials of GS010, an intravitreal gene therapy for LHON (43). The early phase recruited 14 patients with the ND4 mutation who received a one-time dose of GS010 in their more severely affected eye. Follow-up at 96 weeks revealed that the eyes treated with AAV improved by an average of 21 ETDRS letters from their initial baseline. The treatment was also found to be most effective in 5 patients who had experienced vision loss within 2 years prior to enrollment. Though these findings are promising, continued follow-up is required to assess the long-term outcome of these patients.

Genetic Counseling

In many inherited diseases, with LHON being no exception, the role of genetic counselling is vital for patients to be informed of the risk of disease and chance of development in their children and relatives. More than 85% of individuals with LHON are homoplasmic, meaning that all of their mitochondria express the pathogenic DNA mutation (2). Due to maternal inheritance, men who are carriers of the mitochondrial mutation cannot pass it on to their offspring, while all children of a female carrier will harbour the mutation. However, as noted above, LHON demonstrates incomplete penetrance with only 50% of male and 10% of female carriers becoming affected; the difficulty in genetic counselling lies in the fact that genetic testing cannot predict the severity or rate of progression of disease carriers. LHON is a disease that can have significant visual consequences and impact quality of life—thus, a referral to a low vision specialist is warranted to educate patients on techniques to help preserve and improve reading and mobility. Lastly, given the multifactorial nature of the disease, it would be beneficial to also counsel on risk factors for disease conversion including tobacco and alcohol use.

CONCLUSION

LHON is a rare but well-studied mitochondrial disease that results in bilateral central vision loss and is a result of one of three pathogenic mutations (G11778A, G3460A, and T14484C). Currently, there is no proven treatment. However, given our growing understanding of the disease mechanism and ongoing research and trials being conducted on idebenone, EPI743, and gene therapy, there remains hope for emerging solutions for this devastating visual disease.

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