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Leber Hereditary Optic Neuropathy State of the Science

Contents

Background

Leber hereditary optic neuropathy (LHON) is a rare inherited mitochondrial disease that primarily affects the optic nerve leading to sudden and severe vision loss. LHON is most often caused by disease specific mutations in mitochondrial DNA (mtDNA), though other genetic variants have also been associated with LHON pathology. LHON mutations disrupt energy production of the cell, increasing production of reactive oxygen species and compromising the function and survival of retinal ganglion cells (RGCs), the cells whose axons form the optic nerve. Complex I of the electron transport chain (ETC) is specifically disrupted in LHON patients.

When RGCs are lost, the optic nerve atrophies, breaking connections between retinal input and the visual processing centers in the brain and causing vision to deteriorate. Most LHON patients will become legally blind, but few will have a complete loss of vision. The degree of retained light perception and central, peripheral, and color vision will vary significantly from patient to patient and influence the patient's ability to independently navigate the world and the types of accommodation needed to support activities of daily living.

While optic neuropathy and vision loss are the predominant manifestations and hallmarks of the disease, LHON patients can have symptoms outside the ocular system. Reported non-ocular symptoms include cardiac arrhythmias, nonspecific myopathy, and neurological abnormalities such as postural tremor, peripheral neuropathy, movement disorders, and in a small subset of patients, a multiple sclerosis-like disease (LHON-MS) (Patrick Yu-Wai-Man and Chinnery 2021). LHON involving non-ocular symptoms, with or without vision loss, is referred to as *LHON plus*.

Currently, there is no cure for LHON and there are no FDA-approved therapies for use in the United States. Effort has been put into clinical trials that include small molecule therapies that compensate for mitochondrial dysfunction and gene therapies that replace the mutated gene, but effect sizes have been small, and results are inconclusive.

The Milken Institute conducted a comprehensive scientific landscape of LHON, inclusive of emerging trends in research and clinical applications, to inform areas of opportunity for philanthropic support. This landscaping was informed by relevant academic literature and conversations with over 70 key opinion leaders spanning multiple sectors, including academia, industry, non-profit, and government entities, and across the research pipeline, from basic science to clinical care.

The Electron Transport Chain

The electron transport chain (ETC) is a series of protein complexes that transfer electrons and couple electron transfer with the movement of protons across a membrane. The flow of electrons through the ETC is an energy-releasing process. The energy produced creates a proton gradient that drives the synthesis of adenosine triphosphate (ATP), the energy currency of the cell.

The ETC is composed of multiprotein complexes embedded in the inner mitochondrial membrane:

Complex I pumps four hydrogen ions across the membrane to establish the hydrogen ion gradient.

Complex II is involved in the tricarboxylic acid cycle and has no proton pumping capacity. It is a key link between the tricarboxylic acid cycle and oxidative phosphorylation.

Complex III accepts electrons from Complexes I and II. It uses energy released in downhill electron transfers to pump protons across the inner mitochondrial membrane. Complex III then sends its electrons to Complex IV.

Complex IV delivers the electrons that traveled through the other ETC complexes to dioxygen, producing water. The free energy from the electron transfer causes four protons to move across the membrane, contributing to the proton gradient.

Complex V uses the proton gradient generated by the ETC to drive the movement of Complex V, which helps bring an adenosine diphosphate (ADP) and a phosphate together to form ATP.

This document provides an overview of the state of the LHON field within foundational biology research, translational research, and clinical applications. These findings include information publicly released through March 2024.

Etiology and pathophysiology

LHON is a mitochondrial disease usually inherited through maternal lines. Over 90% of LHON cases harbor one of three missense point mutations in mtDNA (Fiorini et al. 2023; Poincenot, Pearson, and Karanjia 2020; Rocatcher et al. 2023; Patrick Yu-Wai-Man and Chinnery 2021) (Table 1). The other 10% are attributed to ultra-rare mtDNA mutations, specific combinations of otherwise non-pathogenic mtDNA variants, or mutations in nuclear DNA that modify mitochondrial function giving rise to an autosomal recessive subtype of LHON (arLHON) (Lenaers et al. 2023; Caporali et al. 2018).

Table 1. Common LHON mutation types and prevalence

Mutation	Gene	Protein	Prevalence estimates*
m.11778G>A	MT-ND4	NADH-ubiquinone oxidoreductase chain 4	59-69%
m.14484T>C	MT-ND6	NADH-ubiquinone oxidoreductase chain 6	14-21%
m.3460G>A	MT-ND1	NADH-ubiquinone oxidoreductase chain 1	13-18%

*Ranges are reported from publications from 2020-2023 (Fiorini et al. 2023; Poincenot, Pearson, and Karanjia 2020; Rocatcher et al. 2023; Patrick Yu-Wai-Man et al. 2022)

LHON mutations affect Complex I subunits of the mitochondrial electron transport chain (Baracca et al. 2005). Functional deficiencies in electron transport cause a bioenergetic crisis and accumulation of reactive oxygen species (ROS) (Fuller et al. 2023) leading to RGC damage and the release of programmed cell death signaling cascades (Patrick Yu-Wai-Man et al. 2016). RGC axons, or nerve fibers, make up the optic nerve which connects the retina at the back of the eye to the vision centers in the brain. When RGC cells and their axons are lost, it causes progressive degeneration of the optic nerve and acute or subacute loss of central vision.

> *Complex I is the entry point for most electrons in the ETC. It is the largest collection of proteins in the ETC and accepts high-energy electrons from molecules produced when glucose is broken down. These electrons are shuttled through Complex I in a series of redox reactions which releases energy at each electron transfer. This energy is used to pump protons from the mitochondrial matrix and into the intermembrane space forming a proton gradient across the membrane. Complexes III and IV also contribute to the proton gradient which then is used by Complex V to produce adenosine triphosphate (ATP), the cell's source for energy use and storage. Because Complex I is the major entry point for electrons to the respiratory chain, it plays a central role in energy metabolism. Importantly, in terms of disease pathology, Complex I is considered a main site of production of reactive oxygen species (ROS). ROS have been demonstrated to contribute to activation of various pathways, including protein kinase C, mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), Akt, and p38 MAPK, as well as Ca2+ signaling. Thus, disruption of Complex I activity will not only alter energy production, but also key cellular signaling pathways, including the programmed cell death pathway.*

Epidemiology

Having a LHON mutation alone is not sufficient for clinical expression of the disease. In the general population, some LHON mutations are relatively common (Elliott et al. 2008). Australian cohort studies and UK Biobank analyses estimate that between 1 in 800 (Watson et al. 2023) and 1 in 1000 (David A. Mackey et al. 2023) people carry the m.11778G>A or m.14484T>C variants. Using the UK estimate and the reported prevalence of 1 in 31,000 LHON patients with vision loss in the UK (Man et al. 2003), the population-wide disease penetrance is roughly 2.5%. (David A. Mackey et al. 2023). It is important to note, though, that disease penetrance can be variable among families and according to mutation type (Watson et al. 2023) and may differ geographically as certain mutations are more common in different regions of the world (Poincenot, Pearson, and Karanjia 2020) which may impact penetrance within different populations. In LHON pedigrees, distribution of mtDNA mutations varies geographically, but the m.11778G>A variant is largely predominant, accounting for between 67-79% of cases across populations where data are available (Yuan et al. 2023). Quebec, Canada is an exception where the predominant LHON mutation is m.14484T>C due to a founder effect dating back to 1660s (Laberge et al. 2005).

Penetrance is likely higher in known patient populations relative to the general population due to genetic modifiers within family lineages, for example, haplogroups. Haplogroups are defined by markers in the genome that help trace ancestry and genetic relationships. Specific mtDNA haplogroups have been associated with risk of vision loss in LHON patients (Hudson et al. 2007; Brown et al. 2002; Lopez Sanchez et al. 2021). Other genetic factors likely play a role including combinations of genetic variants in the background that collectively modify risk of penetrance and nuclear genes that modify the function of mitochondrial genes (David A. Mackey et al. 2023; Chen et al. 2023).

Sex also plays a fundamental role in LHON disease penetrance (Lopez Sanchez et al. 2021). Analysis of real-world data shows a 4:1 or 3:1 male to female ratio for most age groups with a 1:1 male to female ratio in patients under 5 years of age and over 45 years of age (Poincenot, Pearson, and Karanjia 2020; Patrick Yu-Wai-Man et al. 2022). Vision loss peaks sharply for males between the ages of 14 and 26 years (median age 20 years) while showing a more even age distribution for females with most onset happening between the ages of 19 and 45 years (median age 30 years), though vision loss can happen at any age in both sexes. Age of onset is not influenced by specific LHON mutations in males or females (the frequency of each mutation is similar between the sexes). Mechanisms of testosterone as a risk factor and estrogens as protective factors have been explored in cell models, but the reason for higher disease penetrance in males remains unknown (Jankauskaitė et al. 2020; Pisano et al. 2015; C. Giordano et al. 2011).

Risk factors

Genetic variation, modifier genes, environmental factors, and specific gene-environment interactions are all implicated as risk factors when there is variability in the penetrance and/or clinical expression of any disease; all apply in LHON (Valerio Carelli et al. 2016). More than 1000 genes encoded in nuclear DNA regulate mitochondrial gene expression, maintenance, and function and may be context-dependent modifiers of LHON (Rath et al. 2021). Among environmental factors, tobacco smoking and heavy alcohol consumption can increase LHON penetrance and vision loss, in some instances, may be reversed with cessation (L. Giordano et al. 2015; Kirkman et al. 2009; Tsao, Aitken, and Johns 1999; Valerio Carelli et al. 2016). Pharmaceuticals can be toxic to mitochondria and some widely prescribed drugs may be LHON risk factors, as evidenced by a handful of case reports with experimental validation and the expert opinion of a panel of mitochondrial medicine professionals

(Reinert et al. 2021; Luca 2004; D A Mackey et al. 2003). And finally, a gene-environment interaction discovered in Asia shows that the m.3394T>C mutation is associated with reduced Complex I activity and LHON in a Chinese population at low altitude while also being associated with increased Complex I activity and no LHON in Tibetan and Indian populations at high altitude, demonstrating that a pathogenic variant in one environment can provide a health or survival advantage in another (Ji et al. 2012).

Clinical presentation and diagnosis

LHON typically presents as a sequential bilateral blurring of central vision with vision changes in the second eye appearing 6 to 8 weeks after the first, though simultaneous loss of vision is reported in 25% of cases (P. Yu-Wai-Man et al. 2009). Changes in color perception are common and can be severe. Generally, there is no pain associated with eye movement or vision loss.

Clinical diagnosis involves collecting family history and evaluating visual acuity or the sharpness of vision, visual fields to identify size and location of scotomas, or blind spots, and color vision. Physical examination of the eye includes a fundus exam to look for abnormalities in the optic disc and blood vessels, and optical coherence tomography, an imaging method used to assess thickness and degeneration of the retinal nerve fiber layer (RNFL) (Shemesh, Sood, and Margolin 2023; Valerio Carelli et al. 2017).

The clinical course of LHON varies by patient, but generally follows four stages (Shemesh, Sood, and Margolin 2023; Patrick Yu-Wai-Man and Chinnery 2021; Newman et al. 2020; Valerio Carelli et al. 2017):

- Stage 0. Asymptomatic (mutation carriers). Functional changes may be happening at the subclinical level, but patients remain asymptomatic.
- Stage 1. Subacute (0-6 months from onset of clinical symptoms). Subacute changes in visual acuity happen over days, weeks, or months; most patients seek care at this stage. Visual acuity in each eye usually stabilizes within 4-6 months of symptom onset at the lowest point, or nadir.
- Stage 2. Dynamic (6-12 months). Visual fields and OCT measures will continue to fluctuate until plateauing around one year after symptom onset. Visual acuity may improve from nadir but recovery of complete vision is exceptionally rare.
- Stage 3. Chronic (beyond 1 year). All measures stabilize with optic nerve atrophy. Most patients have severe and permanent vision loss.

Patterns of vision loss and disease progression in children may differ from adults. A consensus definition of childhood onset LHON has not been published, but most published studies include patients affected at or before age 12. In addition to the classical stages of progression above, approximately one-third of childhood onset cases occur as a slowly progressive disease with continual vision loss for more than six months or an insidious or subclinical disease where the patient is incidentally diagnosed with optic atrophy or subnormal vision but remains asymptomatic for an extended period of time without changes in visual acuity (Majander et al. 2017; Barboni et al. 2023).

Molecular diagnosis confirms a patient has LHON by identifying the underlying DNA mutation. Testing can be narrowly targeted to capture the three primary LHON mutations (m.11778G>A, m.14484T>C, and m.3460G>A) or more broadly targeted to capture a panel of genes that cover all subunits of Complex I or DNA mutations such as *DNAJC30* (Major et al. 2023). If a pathogenic mutation is not found using a targeted approach, whole mtDNA sequencing may be used to look for other rare LHON mutations in the mitochondrial genome (Patrick Yu-Wai-Man and Chinnery 2021). There are rare instances when a known causal mutation cannot be identified and investigation continues to identify all genetic causes of LHON.

Treatment and prognosis

In September 2015, the drug Raxone (idebenone 150 mg) received marketing authorization from the European Commission for LHON treatment as an orphan medicine under exceptional circumstances. Idebenone is a synthetic analogue of coenzyme Q10 (CoQ10). CoQ10 carries electrons along the electron transport chain, from Complexes I and II to Complex III. It is also an antioxidant. CoQ10 treatment has demonstrated benefit in mitochondrial respiratory chain disorders not associated with CoQ10 deficiency such as Kearns-Sayre syndrome (Hargreaves 2014), but administration hasn't been demonstrated to be beneficial in LHON, presumably due to an inability to cross the blood-brain barrier. Idebenone, which is hydrosoluble, was developed to enable blood-brain-barrier penetration for use in Alzheimer's disease and other cognitive defects. Due to its shuttling capacity, it was hypothesized that idebenone would transfer electrons directly to Complex III, bypassing the dysfunctional Complex I (Giorgio et al. 2012). The Rescue of Hereditary Optic Disease Outpatient Study (RHODOS) trial assessed oral idebenone in LHON patients with less than 5 years of visual loss and did not demonstrate improvement based on best corrected visual acuity (BCVA) (Thomas Klopstock et al. 2011) but did show signals of potential improvement in post-hoc subgroup analysis (Borg and Laslop 2015).

In a follow-up study (RHODOS-OFU), participants were assessed a median of 30 months after discontinuation of idebenone and placebo (T. Klopstock et al. 2013). A significant improvement in visual acuity was detected in participants treated with idebenone, and the effect was greater in patients carrying either the m.11778G > A or m.3460G > A mutation. The seeming contradiction in study results from the RHODOS and RHODOS-OFU studies may be explained by patient stratification, treatment time, or time post-treatment. Retrospective analysis demonstrated that patients were more likely to recover vision if treatment was initiated early and was maintained for longer than 24 weeks, supporting the hypothesis that idebenone has the greatest potential to benefit patients when administered early in disease when RGC loss is incomplete (V. Carelli et al. 2011). The most recent LEROS trial assesses administration either in the subacute/dynamic phase of LHON or in the chronic phase. Results from the trial indicate that early administration of idebenone in patients with the m.3460G>A mutation may be detrimental (Patrick Yu-Wai-Man et al. 2024). Notably, and worth continued investigation, genetic variants in the cytosolic NAD(P)H oxidoreductase I (NQO1) gene impact the effectiveness of idebenone in treating LHON, particularly for individuals with the m.3460G>A mutation (Aleo et al. 2024). An International Consensus statement on LHON management suggests treating LHON patients at onset with 900 mg/day idebenone until one year after visual acuity plateaus, and then to continue for one year beyond the plateau (Valerio Carelli et al. 2017). Based on clinical trials that have been performed since publication of the Consensus Statement, there would be benefit to revisiting LHON management.

Currently, there are no approved therapeutic interventions for LHON in the United States (US). It is common for LHON patients in the US to self-source and administer idebenone but data on its use is not available (Newman et al. 2020).

Patient age at onset and LHON mutation are the best predictors of visual outcomes. Younger patients tend to have less severe vision loss and are more likely to have partial vision recovery (Newman et al. 2020; Patrick Yu-Wai-Man et al. 2022b). Patients with the m.11778G>A mutation have the worst prognosis with more severe disease at onset, a long-term gradual decline in visual acuity, and almost no propensity for vision recovery (Patrick Yu-Wai-Man et al. 2022b; Newman et al. 2020; Guo et al. 2016; Lam et al. 2014). Between the three primary LHON mutations, patients carrying the m.14484T>C variant tend to have the best vision outcomes based on visual acuity scores and the m.3460G>A is in the middle (Patrick Yu-Wai-Man et al. 2022b; Guo et al. 2016).

Regardless of mutation, most affected patients will have profound and permanent vision loss with visual acuities that meet or surpass the threshold for legal blindness (20/200). (Legal blindness is not a medical diagnosis, it is a governmental definition used to determine eligibility for government support services.)

Clinical trials

Several therapeutic strategies are under clinical assessment to manage LHON, including gene therapies and therapies developed to address mechanisms of disease such as antioxidant therapies. These therapeutic strategies aim to address the deficits resulting from impaired Complex I activity by either supplementing the defective gene with a normal gene (gene therapy) or managing pathogenic mechanisms of disease such as oxidative stress. Unfortunately, limited patient numbers, an incomplete understanding of natural history and disease biology, and a potential need to select additional, disease-specific endpoints have limited progress in LHON clinical trials.

Gene therapy

Due to the relative accessibility of the RGC layer of the retina, gene therapy for LHON is an attractive treatment option. Allotopic gene expression is a process that enables the transport of molecules into the mitochondria, a barrier due to the mitochondrial double membrane (Shamsnajafabadi, MacLaren, and Cehajic-Kapetanovic 2023). This technique leverages a mitochondrial targeting sequence to enable the transport of ND4 mRNA or protein into the mitochondria.

scAAV2-P1ND4v2

Safety Study of an Adeno-associated Virus Vector for Gene Therapy of Leber's Hereditary Optic Neuropathy (NCT02161380) was an open-label dose escalation study sponsored by the National Institutes of Health and initiated in 2014 at the Bascom Palmer Eye Institute, Miller School of Medicine, University of Miami. This gene therapy leverages an adeno-associated virus vector (scAAV2-P1ND4v2). The drug was injected intravitreally into one eye in five participants with vision loss. The initial phase I study revealed no serious safety concerns (Feuer et al. 2016). This trial was continued with intravitreal injection of vector into one eye of participants within three different groups: (1) chronic bilateral visual loss greater than 12 months, (2) bilateral visual loss less than 12 months, and (3) unilateral visual loss. As a result of this trial, the investigators concluded that treatment efficacy, if any, is limited and dose-independent (Lam et al. 2022).

LUMEVOQ

Safety Evaluation of Gene Therapy in Leber Hereditary Optic Neuropathy (LHON) Patients (NCT02064569) is a study sponsored by Gensight Biologics that was launched in 2014 to evaluate the safety of AAV2.2-COX10ND4 (GS010, LUMEVOQ). A single intravitreal injection of the vector was given to the worse-seeing eye of LHON patients with the m.11778G>A *ND4* variant. The phase I trial demonstrated no serious adverse events related to the treatment (Vignal et al. 2018). While initial assessment two and five years after injection revealed improvement in BCVA (Vignal-Clermont et al. 2021), further phase III RESCUE (NCT02652767) and REVERSE (NCT02652780) trials demonstrated unexpected improvement in vision in both treated and sham treated eyes of participants (Newman, Yu-Wai-Man, Carelli, Moster, et al. 2021; Patrick Yu-Wai-Man et al. 2020). In the RESCUE trial, subjects were treated an average of 16 weeks after the onset of vision loss while, in the REVERSE trial, subjects were treated over 20 weeks later, an average of 39 weeks after onset of vision loss. While it would be anticipated that treating patients sooner after vision loss, when RGCs would theoretically be less

damaged, visual outcomes from REVERSE, where treatment was later, were favorable (Newman, Yu-Wai-Man, Carelli, Biousse, et al. 2021).

Efficacy & Safety Study of Bilateral IVT Injection of GS010 in LHON Subjects Due to the ND4 Mutation for up to 1 Year (REFLECT, NCT03293524) was conducted to assess the efficacy and safety of bilateral intravitreal injection as the therapy was unilaterally injected in prior studies. Notably, bilaterally treated patients demonstrated improved BCVA compared to unilaterally treated patients. Additionally, patient reported outcome measures, indicate a treatment benefit. Overall, at 1.5 years post-treatment with LUMEVOQ, there was an improvement in LHON eyes carrying the m.11778G>A mtDNA mutation to a greater degree than that seen in natural history studies (Newman et al. 2023).

Due to the unexpected bilateral improvement with unilateral injection, in September of 2023, GenSight Biologics announced a new study, RECOVER. RECOVER will be a randomized controlled trial with a two-arm design: (1) a sham control arm and (2) a treatment arm consisting of bilateral intravitreal injections of LUMEVOQ.

rAAV2-ND4

Safety and Efficacy Study of rAAV2-ND4 Treatment of Leber Hereditary Optic Neuropathy (rAAV2-ND4, NCT01267422) was initiated to assess the safety of rAAV2-ND4. At three-year follow-up, safety of intravitreal injection of rAAV-ND4 was confirmed and significant improvement in BCVA, visual field, and visual evoked potential was observed in treated eyes (Wan et al. 2016). In 2017, a phase II/III clinical trial (NCT03153293) assessed patients treated intravitreally with a single injection of rAAV2-ND4. Long-term follow-up of these patients demonstrated that age, the period between onset and treatment, and pretreatment baseline BCVA may be predictors of improvement in visual acuity in response to gene therapy (H. Liu et al. 2020). A subsequent clinical trial (NCT03428178) was initiated in 2018 to assess efficacy of rAAV-ND4 in patients with LHON symptom onset within three months. Results from this study have not yet been published. This group is now conducting a study (NCT05293626) in the US.

rAAV2-ND1

Gene Therapy Clinical Trial for the Treatment of Leber's Hereditary Optic Neuropathy Associated With ND1 Mutations (NCT05820152), enrolling in China and the United States, was initiated to evaluate the safety, tolerability, and preliminary efficacy of NFS-02 (rAAV2-ND1) in the treatment of LHON caused by mitochondrial ND1 gene mutation. This study is enrolling subjects aged 18 to 75 years old to receive a unilateral intravitreal injection of NFS-02. The Investigational New Drug (IND) application of NFS-02 was approved by the US FDA in December 2022 and by China National Medical Products Administration (NMPA) on April 17, 2023. The clinical trial was opened, and the first patient was treated in August of 2023.

Therapies addressing mechanisms of disease

EPI-743

EPI-743, or vatiquinone, is a member of a class of drugs known as digital biochemical information transfer and sensing compounds (D-BITS) (Sadun 2012). EPI-743 is a stable, orally bioavailable small molecule that regulates metabolic control and programmed cell death and has been shown to replenish reduced glutathione pools, critical to cellular antioxidant defense systems (Floreani et al. 2005; Ghelli et al. 2008). EPI-743 was initially developed for the treatment of Leigh syndrome and has been investigated in a range of mitochondrial and

neurodegenerative diseases, including Friedreich ataxia (Enns and Cohen 2017). The efficacy of EPI-743 has only been reported in small case series, with reports of improved vision in patients treated within 90 days of acute vision loss (Sadun 2012; Chicani et al. 2013). A clinical trial for emergency administration (NCT02300753) was opened in 2014, but an adequately powered clinical trial is needed to provide true evidence of efficacy.

Curcumin

Curcumin is a dietary polyphenol derived from turmeric that has demonstrated mitochondrial biogenesis *in vivo* (Eckert et al. 2013) and antioxidant effects (Kalpravidh et al. 2010). A phase III randomized, double-blind, placebo-controlled trial of curcumin was performed in LHON (NCT00528151). Investigators planned to assess visual acuity, computerized visual field, electrophysiologic parameters, and oxidative stress enzymes in plasma before and after treatment at 3-, 6-, and 12-month intervals. Although the study was completed in 2007, the results were never published.

Elamipretide

Elamipretide (MTP-131) colocalizes with cardiolipin in the inner mitochondrial membrane and is thought to improve mitochondrial bioenergetics by increasing ATP synthesis and reducing ROS production (Amore et al. 2021). Sadun *et al*. conducted a Phase II Clinical Study (NCT02693119), sponsored by Stealth Biotherapeutics, to evaluate the safety and efficacy of topical Elamipretide in patients with an confirmed m.11778G>A mutation. Results of the trial showed that elamipretide is well tolerated, but no significant differences in BCVA were observed between the eyes that received elamipretide and those that received the vehicle control. During an open label extension, six of 12 patients had a clinically relevant benefit based on visual acuity, however, a larger trial for longer duration would be required to establish benefit (Karanjia and Sadun 2023).

Brimonidine Purite

Brimonidine purite is a topical agent with purported antiapoptotic properties (Newman et al. 2005). A open-labeled, nonrandomized prospective pilot study assessing brimonidine purite prophylactic treatment after first eye involvement in LHON was published in 2005 (Newman et al. 2005). At the dosage at which it was tested, topical brimonidine purite was unsuccessful at preventing second eye involvement in patients that had recently become symptomatic in the first eye.

Béfizal

Opened in 2019, NCT04561466 is a trial of Befizal® 200 mg for the treatment of Leber hereditary optic neuropathy. This phase II/III trial is assessing Béfizal in 3460 or 11778 mtDNA mutant LHON patients that have been affected for fewer than 5 years. Béfizal (bezafibrate) is a lipid-modifying agent. More specifically, it is a pan-PPAR agonist that is used routinely for treating hyperlipidemia. It has been preliminarily assessed for use in patients with mitochondrial myopathy and has been tested in models of mitochondrial dysfunction (Russell et al. 2020). Results for this study have not been published.

Translational and preclinical research

LHON research is expanding in scope from the identification and characterization of individual mutations toward holistically understanding the molecular basis of disease and optimal points for targeted therapeutic interventions. Recent progress in related fields has contributed to our understanding of mitochondrial function, RGC biology, and the importance of the microenvironment in the clinical expression of disease and treatment

response. Applying this knowledge to LHON and leveraging advanced tools and technologies may provide an opportunity to create better model systems, identify biomarkers, and develop targeted therapeutics.

Model systems

Advances in biotechnology are enabling researchers to develop novel patient-derived models of LHON that better recapitulate the variability in clinical expression and specific cellular context of the human disease as tools to study mechanisms of disease, to identify potential drug targets, and to assess efficacy of therapeutic interventions de-risking the translation to human clinical trials.

Cell models

RGCs cannot be sampled directly from patients, but a variety of cell types that can are used as RGC surrogates in LHON research because the basic biochemistry of mitochondrial function is the same across cell types. Lymphoblasts, fibroblasts, and cybrids (cytoplasmic hybrids) are the most commonly used cell-based LHON model systems. Lymphoblasts are derived from peripheral blood, fibroblasts are derived from skin biopsies, and cybrids are created by fusing the cytoplasm and mtDNA of one cell with the nuclear DNA of another cell. These cell models are used for functional studies of the electron transport chain, cellular respiration, and ATP production, and to quantify mtDNA copy numbers. Cybrids can also be used to study a single mtDNA mutation in the context of many nuclear backgrounds or many mtDNA mutations in the context of a single nuclear background (King and Attardi 1989; Trounce and Pinkert 2007; Wilkins, Carl, and Swerdlow 2014). Lymphoblasts and cybrids were used in the first study to directly compare and show differences in oxidative phosphorylation defects between the three primary LHON mutations (Brown et al. 2000). More recently, lymphoblasts and fibroblasts from affected patients and unaffected carriers with the m.11778G>A mutation were used to show that differences in oxidative phosphorylation between the two does not explain disease penetrance (Lopez Sanchez et al. 2020).

Some processes must be studied in RGCs because of their unique properties. For example, RGCs have largely unmyelinated axons that contain an increased number of mitochondria to match the increased energetic cost of signaling across an uninsulated region (Bahr et al. 2020; Ito and Di Polo 2017). These cellular characteristics become important for investigating questions like why RGCs are selectively lost in LHON. Induced pluripotent stem cell (iPSC) technology enables researchers to develop patient-specific RGCs by reprogramming peripheral blood cells or fibroblasts to behave like embryonic stem cells, and then go through stepwise differentiation to become RGCs. iPSC-derived RGCs are being used to study LHON-specific mechanisms of pathogenesis and have been central to studies that suggest nuclear modifier genes and mitophagy are involved in RGC death, but superoxide production is not (Danese et al. 2022; Chen et al. 2023; Wong et al. 2017).

In vivo, cells live in a three-dimensional environment where their behavior is determined by complex, cell type-specific interactions with neighboring cells, physical and mechanical forces, and circulating chemical messengers. The next generation of cell models will capture these interactions *in vitro* with organoids, or small organ-like structures developed from iPSCs that grow in three-dimensions recapitulating complex tissues with multiple cell types (Cowan et al. 2020; Clevers 2016). Organoid models of RGC development are able to generate a RGC layer and can be used to modulate axonal outgrowth which has implications for cell replacement therapies that would require long-distance extension of axons to make new connections in the brain (Fligor et al. 2018). One of the challenges of using organoids to study RGCs is that this cell type is often too short-lived to reach maturity. This degeneration of RGCs has been partly explained by a recent study that created a digital retinal multimodal map using spatial and temporal data from developing retinal organoids that

revealed it is the local microenvironment during development that determines whether RGCs survive or receive signals that induce programmed cell death (Wahle et al. 2023).

Animal models

In 2010, Marella *et al*. used intraocular injection of rotenone, a Complex I inhibitor, to model optic neuropathy comparable to LHON (Marella et al. 2010). While the relative simplicity of this model makes it attractive for studies assessing Complex I dysfunction, it does not recapitulate mtDNA mutation as is seen in the human condition. Developing a genetic animal model of disease has challenged the LHON field. It is difficult to modify mitochondrial DNA and, while many genetic elements and biological processes are conserved between humans and mice, our retinas are not the same, and there is not a one-to-one mapping between genes and cell types. Nevertheless, a range of different approaches have produced mouse lines that present some characteristic features of LHON and demonstrate Complex I deficiencies.

The first transgenic mouse model demonstrates severe Complex I deficiency (Kruse et al. 2008). This mouse harbors a double knockout of the nuclear encoded *Ndufs4* gene. NDUFS4 is necessary for stability of Complex I. The severity of disease limits the model's utility as it not only causes vision loss, but also leads to encephalomyopathy and death at around 7 weeks of age. Along with additional symptoms, this model is more consistent with Leigh syndrome than LHON. Another mouse model contains the *Nd6* F13997A mutation — equivalent to the rare G14600A mutation in humans — in mouse germline cells (Fan et al. 2008). A limitation of this model is an additional mutation in cytochrome c oxidase subunit I which reduces oxidative phosphorylation by Complex IV in homoplasmic cells by approximately 50%. Characterization of these animals demonstrated reduced retinal function, axonal swelling, demyelination, and accumulation of abnormal mitochondria (Lin et al. 2012).

More recent mouse models express human pathologic Complex I subunits. One mouse was created through intraocular injection of adeno-associated virus to introduce the human *ND4* gene with an m.11778G>A mutation (Yu et al. 2012). These mice demonstrate a significant decrement in RGC function at 1 month and 6 months after AAV injection. A whole-mouse version of this model was created through the introduction of mutant human *ND4* DNA directly into mitochondria of mouse zygotes (Yu et al. 2015). The result of expression of mutant ND4 was a decrease in respiratory chain function and increased oxidative stress. The optic nerve head swelled followed by progressive loss of RGC axons. Researchers have also developed a conditional knockout that inactivates the Complex I accessory subunit *Ndufs4* gene specifically in RGCs. In these mice, RGC loss begins at 45 days after birth with quick progression to a loss of two-thirds of RGCs by day 90 (Wang et al. 2020).

Animal models that closely recapitulate human disease are one of the most useful preclinical tools for evaluating therapeutic safety and efficacy and to help prioritize therapies for translation to human studies. While each of the models add to our understanding of the disease, there are limitations that make preclinical assessment challenging. For one, there are limited models that include the exact LHON *ND1* and *ND6* mtDNA mutations. As noted prior, Fan et al. produced a mouse model containing an *Nd6* F13997A mutation in mouse germline cells (Fan et al. 2008) and mouse models developed through AAV intravitreal injection to deliver mutant human m.3460G>A (Y. Liu et al. 2022) and human m.14484T>C (Yu et al. 2020) have been produced. Nonetheless, there are opportunities for additional models, perhaps in different animals. In addition, current mouse models are on inbred backgrounds such as C57BL6, which do not recapitulate genetic diversity in the human population and preclude exploration of the role of nuclear modifier alleles in shaping LHON. Finally, the mouse

and human retinas differ significantly. Appreciating the limitations of each model and selecting a combination of models that complement one another can advance the pace of translational and preclinical research.

Biomarkers

The identification of robust and sensitive biomarkers can help unravel the heterogeneity of the disease and improve diagnostic tools, identify drug targets, track progression and therapeutic response, stratify patients into risk groups, and provide better outcome measures for clinical trials. A biomarker can be any quantitative measure that captures changes in a biological process due to normal cellular activity, a disease state, or a therapeutic intervention. LHON biomarkers may include measures of cellular structure (imaging) and function (electrophysiology) and naturally occurring small molecules (metabolites) required for normal cell maintenance and activity.

Imaging is a non-invasive way to capture structural changes in the RGC complex that correlates with disease stage and progression, and potentially therapeutic response. Optical Coherence Tomography (OCT) is a standard imaging diagnostic technique for LHON that creates highly reproducible, high-resolution 2D and 3D images of the retina and the retinal nerve fiber layer and ganglion cell layer of the RGC complex. OCT measures correlate with changes in visual acuity and field. OCT is being used to measure retinal nerve fiber layer thickness and other changes in the RGC complex throughout the stages of LHON evolution including the key point of pre-symptomatic to symptomatic conversion (Carbonelli et al. 2022; Balducci et al. 2016; Barboni et al. 2010). A variation of the technique, optical coherence tomography angiography (OCT-A) provides complementary information by capturing the evolution of vascular networks in the retina (Balducci et al. 2018). OCT biomarkers are the best structural predictors of visual field and visual acuity and have the potential to help elucidate natural history of the disease and early predictors of risk, treatment response, and optimal therapeutic windows (Zeng, Chou, and Sadun 2023; Borrelli et al. 2023).

Phase contrast Adaptive Optics Scanning Laser Ophthalmoscope (AOSLO) is an imaging technique that can visualize the living retina at single cell resolution and compute quantitative metrics including cell size and density. An early experiment detected inflammatory cells in the RGC layer in the eyes of LHON patients before gene therapy, during gene therapy in the treated, and during gene therapy in the fellow untreated eye (Gofas et al. 2023). Being able to visualize the types and density of cells at the point of degeneration has the potential to reveal underlying biological mechanisms, how the body responds to the disease process, and early signs of risk, targets for intervention, and treatment responses. AOSLO has not yet been adopted for widespread use.

Bioelectric signals of the RGCs are an objective and quantifiable measure of function. Among the signals collected by electroretinograms (ERG), which measure the electrical response of the retina to a light stimulus, are wave forms specific to RGCs called the photopic negative response (PhNR). Amplitude of the PhNR signal is an indicator of RGC function; the signal is diminished in symptomatic LHON patients and in some presymptomatic LHON patients (Miao et al. 2023; Karanjia et al. 2017). PhNR values correlate with OCT measures of retinal nerve fiber layer thickness providing a complementary functional biomarker of RGC activity that can capture dynamic changes in function loss and recovery and may be an early biomarker of risk.

A variation of ERG, the pattern electroretinogram (PERG), uses a pattern reversing stimulus, for example reversing the black and white squares on a checkerboard, to measure function of the central retina and to distinguish optic nerve dysfunction from macular dysfunction. In PERG, two waveforms are linked to RGC activity, and like PhNR, their signals decrease when function is lost. Decreases in PERG signals are detectable in

asymptomatic carriers of the m.11778G>A mutation indicating they may also be able to detect subclinical disease as an early biomarker of risk (Guy et al. 2014).

Small molecule metabolites are the substrates and byproducts of biochemical reactions and circulating messenger molecules that dynamically fluctuate in response to changing cellular conditions. Metabolite signatures can identify cell types or disease states, and changes in a single metabolite or a group of metabolites provide insight into the molecular mechanisms of a disease which can lead to better diagnostics, progression tracking, drug screening, and clinical trial endpoints. Three metabolite signatures have been identified in LHON patient samples using three different study designs. The first, an untargeted screen of 500 metabolites in blood plasma, found 13 metabolites that discriminate LHON patients with severe vision loss in the chronic disease stage from non-LHON controls (Bocca et al. 2021). All 13 have plausible links to known biological mechanisms of disease.

The second study used fibroblasts from patients with LHON mutations and controls with no LHON mutations for a targeted screen of 188 metabolites and identified a signature consistent with endoplasmic reticulum stress, an indication the cells are unhealthy (Chao De La Barca et al. 2016). The third study also used LHON patient and control fibroblasts and identified a signature of increased lipids and decreased amino acids in an untargeted screen consistent with electron transport chain deficiencies and endoplasmic reticulum stress, respectively. In this study, researchers also looked at metabolite signatures after fibroblasts were treated with idebenone or the antioxidant resveratrol and found idebenone reversed the lipid signature and resveratrol partially reversed the lipid signature and increased amino acids, though sample size was limited to 5 patients (Morvan and Demidem 2018). As methods to analyze data of this scale and complexity evolve and metabolomics studies become more routine, metabolite profiling of affected and unaffected LHON mutation carriers and RGCs across all stages of disease has the potential to reveal biomarkers that change the entire LHON research landscape from the laboratory to the clinic.

Experimental therapeutics

As researchers are better understanding the biology of LHON and mitochondrial mechanisms of disease and methods for modifying mitochondrial function, they are exploring novel approaches that are moving along the translational pipeline. While these therapeutic modalities are not yet ready to enter clinical trials, they provide evidence that increased mechanistic understanding has the potential to expand the therapeutic toolbox.

Mitochondrial gene editing

Gene editing techniques for mtDNA are under active development. A variety of gene editing methods have been optimized for use on nuclear DNA, but these methods cannot be used for mtDNA because cells naturally prevent the molecules used in such protocols from entering the mitochondria. It is only recently that a biotechnological breakthrough, the discovery of double-stranded DNA deaminase A (DddA) and DddA-derived cytosine base editors (DdCBEs), has opened the field to include mitochondria (Kar et al. 2023; Mok et al. 2020). DdCBEs introduce single base changes at specific sites in mtDNA and have been used experimentally to introduce pathogenic mutations into human cell lines for research including a putative LHON mutation, m.11696G>A in the *ND4* gene, which demonstrated successful on-target editing in 17-29% of mtDNA across cells and functional changes consistent with a Complex I mutation, including decreased cellular respiration and energy production (Mok et al. 2022). Gene editing is in the earliest phases of development; current research priorities are focused on increasing the efficiency and precision of the techniques to convert more copies of mtDNA and reduce off-target effects (Silva-Pinheiro et al. 2022; Lim, Cho, and Kim 2022). In the shorter term,

gene editing may be used to create mouse models for individual LHON mutations to better understand pathophysiology and to accelerate the development of targeted mutation-dependent and mutation-independent therapeutics (Lee et al. 2021). In the longer term, the goal is to use gene editing to reverse LHON mutations in patients. It may not be feasible to eliminate the mutation with these techniques but there is likely a threshold above which the rescued mitochondria, through gene editing and subsequent replication, will compensate for the mutated mitochondria with the potential to prevent or reverse vision loss.

Mitochondrial regulation

Mitochondria are highly dynamic organelles. They undergo coordinated cycles of fission and fusion in order to maintain their shape, distribution, and size, and they communicate with the cell to modulate and contribute to the cell cycle, immunity, apoptosis, and cellular health (Tilokani et al. 2018). As mitochondria become dysfunctional in response to the mtDNA mutations seen in LHON, pharmacological enhancement of mitochondrial dynamics, including biogenesis, fission and fusion, mitophagy, and degree of heteroplasmy may prove promising for the treatment of LHON (Bahr et al. 2020). It is possible that increased mitophagy could help clear damaged mitochondria, those harboring Complex I mutations, more efficiently. Activation of mitophagy in cybrids using rapamycin led to improved cell survival through selective targeting and destruction of damaged mitochondria (Sharma et al. 2019). Similarly, mitoTALENs, mitochondrial zinc finger nucleases, and mitochondrially targeted nucleases have been used in cybrids and mice to selectively lesion mutant mtDNA and promote destruction of mitochondria harboring mutant mtDNA through the cell's mitophagy process (Hashimoto et al. 2015; Reddy et al. 2015; Minczuk et al. 2008; Gammage et al. 2014; Bacman et al. 2012).

The fission/fusion balance may also influence LHON pathogenesis. Mitochondrial depletion can result from excessive fission. The re-purposed drug hexestrol has been shown to rescue mitochondrial fragmentation and both hexestrol and clomiphene have been able to ameliorate mtDNA depletion in a yeast-based screen (Delerue et al. 2019).

Mitochondrial replacement and transfusion

Mitochondrial replacement is a therapeutic method aiming to prevent the passing of mtDNA mutations from the mother to her child. This process involves replacing the cytoplasm – which contains mitochondria – of an egg or embryo with the cytoplasm from another cell donor containing normal mitochondria (Tachibana, Kuno, and Yaegashi 2018). Mitochondrial replacement has been performed in primate models. As mtDNA is not always completely replaced in the recipient cell, there is not a guarantee that mitochondrial disease will be fully prevented by mitochondrial replacement, but there is significant potential for it to reduce disease burden in the population (Hyslop et al. 2016). This was seen when mitochondrial replacement was performed in human oocytes (Kang et al. 2016). Though donor mtDNA was stably maintained in embryonic stem cells derived from embryos produced, some cell lines demonstrated gradual loss of donor mtDNA and a return to the maternal haplotype. In addition to the aforementioned limitations to this approach, it is considered controversial due to germline modification of a child that inherits genetic material from a third person (Patrick Yu-Wai-Man 2016). Recently, a heavily regulated mitochondria donation treatment has been initiated at the Wellcome Centre for Mitochondrial Research (WCMR) at Newcastle University alongside the Newcastle Fertility Clinic at Life (Newcastle Hospitals NHS Foundation Trust). This is the only regulated service in the world that is licensed to perform the technique. As of 10 May 2023, 32 patients have been given approval for mitochondrial donation treatment by the HFEA Statutory Approvals Committee. The team at Newcastle has not yet published information of their mitochondrial treatment program in a peer reviewed journal. A newer program, the mitoHOPE (Healthy Outcomes Pilot and Evaluation) Program out of Monash University, is a pilot introduction

of mitochondrial donation into Australian clinical practice. Further learnings about the feasibility and efficacy of this process should arise from these programs.

Perhaps less controversial, albeit a less well-understood method, mitochondrial transfusion is the administration of mitochondria from healthy tissue into the bloodstream or into tissues affected by injury, disease, or aging (Adlimoghaddam, Benson, and Albensi 2022). Aged mice that receive mitochondria from the livers of young mice via intravenous administration demonstrated improved mitochondrial function within the hippocampus. Moreover, cells incubated with isolated mitochondria demonstrate improved ATP production, respiratory function, and cell proliferation (Pacak et al. 2015; Katrangi et al. 2007) and mitochondrial injections have been shown to rescue motor dysfunction in a Parkinson's disease animal model (Chang et al. 2016). As a hallmark of Parkinson's disease is loss of functional mitochondrial Complex I in the dopaminergic neurons of the substantia nigra (Surmeier, Obeso, and Halliday 2017), this rescue is especially interesting in light of Complex I deficits in LHON.

Modified metabolism and respiration

Mitochondria not only influence energy production, they are also influenced by the intake of nutrients. It has been hypothesized that caloric restriction or ketogenic diet which can produce drastic alterations in metabolism and/or respiration may have the potential to treat mitochondrial dysfunction in LHON. Dietary changes are thought to have neuroprotective effects, increase mitochondrial biogenesis and antioxidant capacity, boost ATP levels, and enhance Complex I function (Storoni, Robert, and Plant 2019). Similarly, exercise and intermittent fasting demonstrate protective effects in neurodegenerative disease, promoting mitochondrial biogenesis and neurogenesis (Van Praag et al. 2014). Respiration changes, like those seen in the case of chronic hypoxia, have also been linked to prevention of the onset of neurodegenerative disease and even reversal of disease symptoms in the setting of Complex I deficiency (Jain et al. 2016; Ferrari et al. 2017). The potential for hypoxia to be protective in the context of LHON has been supported *in vivo* using the RGC-specific *Ndufs4* deletion mouse model. In this model, continuous hypoxia was demonstrated to markedly reduce RGC degeneration (Warwick et al. 2022). The relevant protective factors induced by hypoxia and the feasibility of targeting them therapeutically in humans remain to be determined in future research.

Cell-based therapy and retinal ganglion cell regeneration

Regenerative medicine aims to replace damaged cells. The RGC Repopulation, Stem Cell Transplantation, and Optic Nerve Regeneration (RReSTORe) Consortium has recently developed a roadmap for RGC repopulation for vision restoration in optic neuropathy (Soucy et al. 2023). This roadmap has been built around five critical areas of focus:

1. RGC development and differentiation: Advances in stem cell biology have yielded approaches for differentiating RGCs from pluripotent cells. Notably, a second approach relies on the induced transdifferentiation of RGCs from endogenous Müller glia through the administration of transcription factors that promote neuron reprogramming which may make transplantation moot.
2. Transplantation methods and models: The introduction of exogenous RGCs into the diseased eye can pose challenges and a complete understanding of transplantation timing, location and technique, survival and integration, and glia and immune system responses must all be assessed in animal models to avoid mistakes when moving into humans.

3. RGC survival, maturation, and host interactions: The mammalian retina is a highly organized tissue structure. In order for new RGCs to survive and mature, appropriate donor neuron function must be supported. While some human cells may successfully migrate following injection into a non-human primate retina, the survival rate is usually below 1% (Chao et al. 2017). Mechanisms to promote survival of transplanted cells must be developed.
4. Inner retinal wiring: Inner retinal wiring is a complex process and there are limitations to our understanding of the process. Researchers need to study donor RGC polarization, neurite outgrowth, and characteristic electrophysiological properties, identify factors for successful RGC integration, establish mechanisms for maintaining dendritic integrity, and factor in the impact of innate immunity.
5. Eye-to-brain connectivity: The process of the axon overcoming mechanical and inflammatory obstacles to regenerate over long distances remains a significant challenge for optic neuropathy therapy development. While feasibility has been demonstrated in adult rats (Benowitz, He, and Goldberg 2017) there are further complexities in humans that will need to be overcome.

The Consortium's white paper emphasizes that *a combinatorial approach to advancing methods of promoting eye-to-brain connectivity must be considered and should combine work investigating signaling pathways necessary for neuronal survival, axon regeneration, and chemotropic guidance of axons* (Soucy et al. 2023).

Challenges and opportunities

LHON is a complex disease with variable penetrance and risk factors that are not fully understood. Gene therapy was pursued under the hypothesis that supplementation of the mutant gene in the mitochondria would reverse disease pathology, but results have not been decisive. For gene therapy, it may be that constructs can improve delivery of the gene to the RGC, intake into the mitochondria, and incorporation of the protein into the appropriate complexes in the ETC. For example, an alternative to allotopic gene therapy under development is mitochondrial-targeted gene therapy. This method leverages the ability of viruses to traverse the mitochondrial double membrane to access the inner matrix and deliver DNA directly into mitochondria (Velmurugan et al. 2023). Researchers fused a mitochondria targeting sequence (MTS) to the capsid of adeno-associated virus (MTS-AAV) to redirect the virus to mitochondria rather than the nucleus. When an MTS-AAV carrying the *ND4* gene was introduced into the LHON cybrids, ATP synthesis was rescued. Moreover, when injected into mice carrying mutant human *ND4* (m.11778G>A), visual loss and optic atrophy improved post gene therapy, even more so than with allotopic expression. There is also indication from research into the mechanisms of disease and all clinical trials performed that there may be a range of genetic and environmental modifiers that impact risk and penetrance that should be better understood in the context of gene therapy administration. Additionally, a heterogeneous clinical presentation and disease presentation that extends beyond the eye emphasizes the complexity of the disease.

It is generally not well-known to what extent, and in which individuals, therapies such as idebenone and gene therapy are effective. It is clear that gene therapy has not been the home run that was anticipated, and it is important to understand why. In terms of clinical trial design, unless the therapeutic benefit is profound, there is a need for more objective outcome measures so that true therapeutic impact can be assessed. Benefit in treatment may come from stratifying patients – better understanding which therapeutics work best in which patients and why. Patient stratification requires a patient registry with comprehensive data including age of onset, family history, mtDNA mutation, additional genetic and other molecular data, markers of disease progression, environmental exposures, and if available, treatment response(s). Biomarkers that can confirm on-target effects of therapeutics, establish risk of developing vision loss, track disease progression, and predict

treatment response would greatly benefit the field. In patients with a known LHON mutation, robust and sensitive biomarkers acting as risk factors may help find the therapeutic window for early intervention to prevent vision loss. Additionally, thoughtful clinical trial design and data collection will be essential for understanding not only which therapeutics work in which patients but also why certain therapeutics do not work in patients, should that be the case. Determination of risk factors will help clinicians determine which patients should be monitored more closely and, potentially, suggest those patients that would benefit from clinical trials or future therapies.

Recognizing that the current therapeutic options will not cure LHON, it is critical that LHON biology and disease mechanisms are well-understood so that future clinical trials in this rare disease population can be undertaken with a higher probability for success and, even if not successful, researchers come away with a better understanding of why. Cell and animal models should be expanded to better represent the disease for the benefit of translational and preclinical studies. Importantly, there are a range of mitochondrial diseases that share mechanisms and some disease characteristics, such as optic neuropathy, with LHON. Discoveries in those diseases should be leveraged to benefit LHON. Collaborative research teams with expertise in a range of LHON-aligned fields such as genetics, immunology, mitochondrial biology, and biochemistry should collaborate to improve the overall biological understanding of LHON. Importantly, researchers with innovative ideas should be supported and mentored in a manner that enables them to move beyond the field-wide paradigms and explore novel modeling, research, and therapeutic development techniques.

While complex, LHON has the potential to serve as a model for other mitochondrial diseases. Its strong impact on a single, contained system, the ease with which treatment can be administered in a confined space and system, and the breadth of understanding of the developmental processes related to RGCs make this an optimal disease for better understanding the interplay of mitochondria and cells and testing potential therapeutics, whether they be small molecules, gene therapies, gene editing, or other techniques.

Notably missing from the above analysis is a thorough assessment of “LHON plus”. LHON plus refers to LHON presentation that includes non-ocular symptoms. These symptoms are not - as the term suggests – only additive to ocular symptoms, they can occur with or without ocular symptoms. As LHON mitochondrial mutations are systemic, one would expect that other tissues with high energy demands or susceptibility to Complex I deficiency might also be impacted. In fact, though limited, there are case studies that report additional symptoms in association with LHON mutations, including neurological abnormalities such as postural tremor, chronic motor tic disorder, parkinsonism with dystonia, peripheral neuropathy, and thoracic kyphosis (Nikoskelainen et al. 1995) and cardiac abnormalities including myocardial hypertrophy and conduction defects (Sorajja 2003; Quigley et al. 2023). It is likely that other symptoms could be related to LHON but have not been assessed in a systemic manner, likely due to a range of factors. For one, carriers are not monitored for the range of potential symptoms in a well-defined manner. Many carriers become patients only when they develop ocular symptoms and their disease-based care is most often carried out by neuro-ophthalmologists. Additionally, comprehensive natural history studies and registries that assess non-ocular symptoms are needed. Finally, symptoms of LHON plus can be mild or attributed to other diseases/disorders or aging processes. When patients do not know the symptoms to look for and are not monitored for additional symptoms, they can go undiscovered, limiting the ability of researchers to know the data that should be collected to better understand the symptoms and their prevalence. It is critical to define and standardize when a carrier of a LHON mutation becomes a patient, how information and symptoms are identified and collected, and which specialists should be regularly involved in their care.

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