LHON Scientific Retreat

A Convening Report

This scientific retreat was jointly hosted by the Milken Institute Science Philanthropy Accelerator for Research and Collaboration (SPARC) and LHON Collective.

February 13-14, 2024 | Lisbon, Portugal

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Executive Summary

Leber hereditary optic neuropathy (LHON) is a rare, inherited mitochondrial disease that primarily affects the optic nerve and can lead to sudden and severe vision loss. Currently, there is no cure for LHON, and there are no FDA-approved therapies for use in the United States. The EMA has approved a single drug, idebenone (Raxone), since 2015. The LHON Scientific Retreat convened to identify and prioritize the most promising opportunities to accelerate the delivery of therapeutic options to LHON patients.

On February 13 and 14, we brought together subject matter experts from nine countries, representing foundational research in LHON and aligned diseases, therapeutic development, novel research tools and technologies, and clinical care, for a two-day retreat. Participants actively engaged in cross-disciplinary dialogue and shared practical experiences in collecting patient data, developing model systems, and moving experimental therapeutics from the laboratory to the clinic. In addition to providing valuable insights and lessons learned, this group of experts demonstrated that there is a motivated contingent of scientists and clinicians ready to work together to change the current scientific offerings for LHON patients and their families. The overarching theme emerging from these discussions was that natural history studies and therapeutic development must proceed hand in hand. The conversation converged on the following focus areas:

- **Natural history and patient stratification**. A need exists for a concerted effort to collect comprehensive, longitudinal patient data to study the natural course of disease and identify the factors that determine which carriers will convert to and/or be protected (or recover) from vision loss. For a natural history study to be comprehensive and most valuable, the information should be actively collected at the point of care using common data collection protocols and standards worldwide. As disease penetrance is incomplete, asymptomatic mutation carriers, especially from families with potential protective genetic backgrounds, where few members are affected, should also be included.

- **Model systems to accelerate therapeutic discovery and development**. A need exists for more and better disease models to support the research pipeline from discovery to target validation, including models that recapitulate the complex interactions of human retinal ganglion cells in their native environment.

- **Global research networks and interdisciplinary research ecosystems**. A need exists for support of global research and collaboration networks to gather rare patient data and create diverse research communities that drive innovation and foster reciprocal learning and progress across disease areas.

Critical insights and information collected from this event will inform the next step: developing a strategic direction for LHON Collective.

Background

LHON Collective engaged the Milken Institute's Science Philanthropy Accelerator for Research and Collaboration (SPARC) in 2023 to evaluate the strengths, resources, and needs in the LHON field to ultimately identify opportunities where investment in research and development can transform the therapeutic landscape.

As part of a comprehensive, unbiased due diligence process, SPARC convened a select group of scientists and clinicians with complementary expertise in LHON and/or aligned diseases or biological mechanisms, model systems, and artificial intelligence and big data, to gather insights that will inform priority targets for philanthropic investment. The aim of these efforts is to identify mechanisms to catalyze innovation, build critical tools, attract new talent, and improve the efficiency of the R&D ecosystem to yield more and better treatment options in LHON. A few topic areas were used to seed discussions including:

- Exploring LHON biology and mechanisms of disease
- Understanding research readiness for translation in LHON
- Identifying cutting-edge advances in genomic and computational technology that can be leveraged to move the field forward

The purpose of this two-day retreat was to invite subject matter experts from across the globe to participate in the exchange of ideas, knowledge, and practical experience. This group was able to identify tangible gaps in knowledge, research, and resources that can be bridged with the help of philanthropic investment to drive innovative translational research toward new diagnostics and therapeutics for LHON patients.

Participants

Key Experts

Petr Baranov, MD, PhD
Valerio Carelli, MD, PhD
Anne Chiaramello, PhD
Marni Falk, MD
Sidney Gospe, MD, PhD
Johan Hedström, MS
Rustum Karanjia, MD, PhD (virtual)
Thomas Klopstock, MD (virtual)
Bon-Kyoung Koo, PhD
Chiara La Morgia, MD, PhD
Guy Lenaers, PhD
Joyce Liao, MD, PhD (virtual)
Isabel Lopez Sanchez, PhD
David Mackey, MD
Michal Minczuk, PhD
Vamsi Mootha, MD (virtual)
Nancy Newman, MD
Martin Picard, PhD
Alessandro Prigione, MD, PhD
Holger Prokisch, PhD
Botond Roska, MD, PhD
Fred Ross-Cisneros (virtual)
Alfredo Sadun, MD, PhD (virtual)
Catherine Vignal-Clermont, MD (virtual)
Marcela Votruba, MD, PhD
Douglas Wallace, PhD (virtual)
Phillip West, PhD
Raymond Wong, PhD
Patrick Yu-Wai-Man, MD, PhD
Marinka Zitnik, PhD (virtual)

LHON Collective

Chris Marsh
Malinda Marsh
Lissa Poincenot
Fred Diehl (virtual)
Kevin McGrath (virtual)
Russell Wheeler (virtual)

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Cara Altimus
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Scope

This retreat focused specifically on identifying where investments in scientific research today can catalyze a productive and diversified therapeutic pipeline that offers more and better treatment options to prevent, halt, and reverse vision loss in LHON patients. Discussions briefly addressed the ongoing clinical trials for gene therapies and idebenone, but focus was placed on looking beyond the current options undergoing clinical assessment at other potential interventions. Notably, the retreat organizers recognize that patient care goes beyond pharmaceuticals to include community outreach, social services and supports, orientation and mobility training, vision rehabilitation, assistive technologies, and mental health care, but the focus of this meeting was on the research and drug development efforts in the LHON field.

Summary

SPARC and LHON Collective invited subject matter experts in LHON and adjacent fields to gather and collectively discuss how to chart a path forward to accelerate the delivery of more and better therapeutic options to LHON patients. This two-day retreat was designed to be a discussion-based forum for cross-disciplinary exchange, featuring short presentations and lightning talks with an emphasis on facilitated group discussions and breakout group activities. A summary of discussion points and ideas offered by participants, organized into focus areas, follows. Detailed notes, organized by session, are provided in the Appendix of this document.

Opportunities to Drive Leber Hereditary Optic Neuropathy Research

Natural history and patient stratification

Most cases of LHON are associated with a few known point mutations in mitochondrial DNA (mtDNA) that disrupt normal Complex I activity. However, these mitochondrial mutations are not the only disease-causing mechanisms, and a mutation alone is not sufficient to cause vision loss. Penetrance of the disease is low outside of known LHON pedigrees, and the course and severity of vision loss are variable and can be influenced by age, sex, and environmental exposures. Patient data collected in the clinic can be a valuable source of information to begin to unravel the variability in both penetrance and vision loss, but much of the existing data have been collected for patient care—not research—limiting their use for large-scale, causal studies. Although challenging, a concerted effort to collect comprehensive, longitudinal patient data to study the natural course of disease, (i.e., a natural history study), is needed to improve diagnosis, prognosis, and treatment.

DNA data: The known genetic landscape of LHON is evolving; in addition to the known mtDNA mutations, nuclear DNA (nDNA) mutations, nDNA modifiers, digenic mutations, and combinations of polygenic variants have all been implicated in the disease. Collecting DNA sequencing data on more patients and asymptomatic mutation carriers will likely reveal more of the genetic underpinnings of LHON and provide a path to connect genetic factors with outcomes, prognosis, and variable clinical features of the disease in men, women, and children as well as the more complex multisystem manifestations of LHON, referred to as LHON-Plus and LHON MS.

Clinical and omics data: Participants supported collecting a variety of longitudinal data types, including standard clinical, anatomic, and functional visual outcome measures combined with advanced imaging techniques, genomic, transcriptomic, proteomic, and metabolomic data, collected from LHON patients and families. Subsequent multi-omic analyses are key to deciphering the pathogenic mechanisms of LHON. They can be used to unmask the factors (risk or protective), or combinations of factors, that stratify patients into early or late-onset disease manifestation and further into subgroups: unaffected carriers, carriers with high probability to convert, and of those affected, those with the potential to recover, or those who have recovered. Analysis within subgroups can point to relevant biomarkers, provide insights into disease etiology and progression, and inform evidence-based strategies for personalized medicine by tailoring treatments to individual patients. This is keenly relevant to the LHON spectrum given the clinical heterogeneity among patients in terms of onset of disease manifestations and the ocular and extra-ocular symptoms.

Patient registry data: Patients can contribute to the collection of real-world data, which may include symptoms, stress levels, quality of life, environmental exposures, diet, and other external factors that may modify disease course and help delineate genetic vs. environmental drivers of vision loss in LHON or identify critical gene-environment interactions. During treatment, quality-of-life surveys and patient-reported outcomes data are valuable inputs to guide clinical trial design and negotiate endpoints and outcomes that are meaningful for patients and compatible with the requirements of regulators and payors.

Noted barriers to progress: Patient cohorts are siloed by country, data are heterogeneous, the quality of clinical data is variable, there are disparities in access to whole exome/genome sequencing (or other advanced technologies), environmental exposures are missing, and consent and privacy laws can be prohibitive to data collection and sharing.

First next step: Identify existing national and international clinician and patient driven registries and local data sets around the world that can be shared and integrated to develop a large, retrospective natural history study.

Goal: Leveraging existing registries, launch a worldwide, prospective study designed with common data and biological sample collection protocols and standards, anchored by foundational data-sharing agreements and systems.

Future promise: Generating large, high-quality, LHON-specific data sets presents opportunities to accelerate learning and discovery. Using AI and machine-learning models in combination with known pathogenic mechanisms, scientists can identify patterns that explain disease heterogeneity, triggers of conversion and drivers of progression, and predict drug targets and drug candidates for repurposing.

Model systems to accelerate therapeutic discovery and development

Model systems are critical to understanding the functional impact of the mutations and other genetic variations that cause LHON. The field has relied heavily on fibroblasts, cybrids, induced pluripotent stem cell-derived (iPSC) models, and a handful of mouse models that each recapitulate some aspect of the disease to study Complex I biochemistry and the effects of select therapeutic interventions. Something can be learned from every model, but the current set of models is not sufficient to move from understanding pathobiology to target discovery to ultimately target validation for safety and efficacy. There are no mouse models with mtDNA mutations analogous to the most common LHON patient mutations, and there is a lack of model systems to study human retinal ganglion cells (RGC) directly, and to begin to understand the complex interactions that support their structure and function *in vivo*. More and better-suited models are needed to accelerate therapeutic discovery and development in LHON. Some considerations include:

Cell type-specificity: RGCs have at least five major subtypes, and iPSC-derived RGCs exist as mixed populations of cells. When working with RGCs, regardless of their origin, it is important to use single-cell genomics to identify the specific subtypes comprising the sample. Similarly, mitochondria are a family of organelles that have tissue-specific signatures, (i.e., mitotypes), and mitochondrial gene expression and bioenergetics will vary accordingly. Understanding the uniqueness of cell types is critical for model selection, experimental design, and interpretation of results.

Recapitulating microenvironments: Cellular microenvironments provide structural and functional support and regulate signaling and communication between cells. The microenvironment and the role of non-neuronal cell types that support RGCs, such as astrocytes, oligodendrocytes, and microglial cells, and the involvement of the surrounding vasculature is largely unknown. These cells may play a definitive role in RGC degeneration and cell death and/or they may serve a protective role by transferring mitochondria to RGCs to compensate for their bioenergetic deficits. Identifying the right targets requires a model that can expose these complex relationships and the processes that regulate them in context.

Human tissues as models: Modeling LHON in human retinas is the most straightforward way to understand LHON pathogenesis and to validate discoveries from other model systems. Human retinas – for the most part collected post-mortem – can currently be kept alive for 14 weeks, used as source material to generate organoids and to create mutation-specific models of LHON using advanced gene editing techniques. Understanding the dynamics of normal and pathological

human retinas requires a large number of eyes, spanning all age groups. In addition, physiological readouts from human retinas can provide a metric for evaluating the similarities and differences between human retinas and other model systems.

Penetrance: Identifying the genetic and environmental factors impacting penetrance is not only important for understanding natural history but also outcomes of model systems. How reduced penetrance impacts model systems is unknown. We need to learn which factors affect penetrance in model systems and if we can expect that a cellular patient-derived model or a genetic mouse model represents patient pathology.

Noted barriers to progress: Challenges in creating mtDNA mutations, lack of vision/eye phenotypes in single gene nuclear mouse models of LHON (DNAJC30), knowledge gaps around penetrance, lack of availability of eyes/retinas, small size of patient base, large time and cost associated with developing new model systems.

First next steps: Inventory and characterize existing models. Then, develop new models with specific single and even digenic mutations and a variety of genetic backgrounds, and create a plan to standardize the collection and processing of retinas (or eyes) globally.

Goal: Build a repository of diverse, well-characterized LHON models with experimental data tracking to share progress and setbacks in real-time.

Future promise: A shared repository or set of repositories globally of LHON-specific (and relevant) models will accelerate biomarker and therapeutic discovery and development by enabling rapid testing of ideas in a variety of systems. Sharing experimental data and experimental outcomes will enable head-to-head comparisons of treatments to each other and benchmarks established by prior experiments.

Global research networks and interdisciplinary research ecosystems

As a rare disease, LHON research requires inclusive, scientific collaboration on a global scale. At the most basic level, establishing interdisciplinary research networks can expedite the collection of rare patient data and put systems into place for data and model sharing. These networks will also provide a centralized place to develop data standards and benchmarks, access to more resources like infrastructure, technologies, and funding, and a community where research goals can be aligned to minimize fragmentation and duplication of efforts. As a practical matter, collaborative networks with clear goals can attract new talent and funding and provide a vantage point that helps researchers align their career goals and incentives with patient interests and needs.

Reciprocal learning: Developing strong research networks requires fostering interdisciplinary ecosystems by encouraging collaboration and communication between clinicians, scientists, and patient advocates. Clinicians and scientists need shared goals and resources to ensure advances in understanding the disease in the clinic and the laboratory progress in tandem. These collaborators must come from a diversity of fields, including aligned disease areas like dominant optic atrophy and glaucoma, Complex I deficiencies, and the wider group of mitochondrial diseases, such as Leigh syndrome. Every discovery in LHON has implications for other optic neuropathies as well as mitochondrial and neurodegenerative diseases, and the reverse may also be true. The greatest impact will come from reciprocal learning and progress.

Innovation: There are scientists around the world working on high throughput disease models (e.g., zebrafish and patient-derived fibroblasts) and tools and technologies (e.g., gene editing, cellular reprogramming and cell replacement, machine learning and AI) that can be applied to LHON as research tools and/or treatments. Connecting the people who have (or know how to build) tools and technologies with the clinicians and scientists looking for breakthroughs is a fast track to innovation. Moving innovative ideas to the market requires scale, which likely entails industry partnerships; industry is the partner that will complete the research network.

Noted barriers to progress: Lack of infrastructure for and difficulties in managing large-scale, international LHON research collaborations, including ensuring trust and shared attribution between different scientific groups, developing clear objectives and channels of communication, and navigating consent and privacy laws across different legal and health systems.

First next steps: Create an online community to connect the researchers who participated in this retreat to identify potential data and resource-sharing networks (patient data and biological samples, model systems, eyes/retinas) and collaborations.

Goal: Establish an inclusive, global, interdisciplinary LHON research network that serves as a research and resource hub for LHON and aligned diseases and provides opportunities for investigators to cross-train at other institutions to expand their skills.

Future promise: An established global research network will increase knowledge and resource sharing while reducing inefficiencies to accelerate discovery, and this community will increase the visibility of the research and the researchers to attract new talent and funding to the field.

Conclusions and next steps

This convening brought together subject matter experts in LHON and adjacent fields to collectively discuss how to chart a path forward to accelerate the delivery of more and better therapeutic options to LHON patients. Over the course of the two-day retreat, three focus areas emerged (natural history and patient stratification, model system development to accelerate therapeutic discovery and development, and global research networks) as places where investment can have an outsized impact in bringing to fruition a diversified therapeutic pipeline that offers interventions to prevent, halt, and reverse vision loss due to LHON. The group prioritized areas that will lead to better data collection, more reliable models, a better understanding of the genetic causes of disease and mechanisms of pathogenesis, and alignment with other disease areas—all of which are important factors for de-risking translational research and attracting industry partners. The opportunities noted in this meeting summary are those that were prioritized by the attendees, an additional list of opportunities noted over the course of the meeting is included in the Appendix.

The next step is to develop a strategic direction for LHON Collective, informed in part by the LHON Scientific Retreat.

Key Priorities for LHON Research:

(1) Gain a better understanding of the natural history of LHON through prospective large-scale studies

(2) Identify the secondary factors driving disease penetrance and severity through integrative omics studies

(3) Develop better in vitro and in vivo LHON disease models to support mechanistic and translational efforts

(4) Foster a global, multidisciplinary LHON research network

Research Opportunities

This section gathers a list of research opportunities discussed throughout the meeting beyond those prioritized in the summary report. This list is not exhaustive and may not include every opportunity highlighted, but it is an attempt to gather a range of potential research projects that could advance LHON research and therapeutic development. The proposed opportunities have been roughly categorized, though it is important to acknowledge that these categories frequently overlap and success in one category may contribute to or depend on successes in one or more other categories.

Improving Understanding of Disease Biology and Mechanisms

- Identify the drivers of disease that contribute to catastrophic vision loss.
- Determine normal and disease-state RGC structure and function.
- Identify the role of the microenvironment in LHON onset and/or protection.
- Define mechanisms of RGC cell death.
- Define the role of inflammation in LHON RGC loss.
- Understand how each mutation affects RGC loss and biology; identify mutation-dependent mechanisms
- Determine why Complex I deficiency in LHON is more likely to lead to RGC loss than in other diseases with Complex I deficiency.
- Identify whether other cell types are lost or contribute to the loss of RGC function or cells. Is RGC loss primary or secondary cell loss?
- Determine if there is non-canonical cell death (e.g., pyroptosis or necroptosis) that contributes to RGC loss.
- Determine whether hormones are a contributor to LHON-related RGC loss or protection.

Driving Therapeutic Development

- Determine the role of mitobiogenesis and mitophagy (mitochondrial dynamics) in LHON and how their regulation could be potential therapeutic opportunities.
- Identify mechanisms of neuroprotection. What factors can drive recovery in those that “spontaneously” recover?
- Identify biomarkers that are able to inform risk of conversion, disease progression, target discovery, treatment efficacy, and recovery
- Identify the optimal therapeutic window for therapies that are currently available or being assessed in patients.
- Determine whether mitochondrial transfer from other cells might be leveraged as a therapeutic strategy.
- Screen for small molecules, pathways, and/or physiologic processes to move away from gene therapy as a primary therapeutic modality. The important area of need for such screening is the identification of the most appropriate models.
- Address the technical challenges associated with RGC replacement. Continue optimizing RGC replacement therapy for LHON.
- Identify well-proven AAVs for RGC-relevant targeting.
- Gene editing is still promiscuous and inefficient; there is a need for concerted efforts to improve the technology.

Developing Disease Models

- Utilize DddA-TALE fusion deaminases and other emerging technologies to generate LHON mtDNA mutants in C57BL6/J and other inbred strains.
- Use the intraocular rotenone or AAV-mND4 models or create F1 hybrids of mtDNA mutant models crossed onto other inbred strains or diverse mouse panels.
- Modify TET enzyme activity or DNMTN3A (epigenetics) to age RGCs *in vitro*.
- Create a set of standardized models for gene therapy testing, and share data from these models to facilitate comparison and advancement of the field.

Understanding Disease Course and Natural History

- Improve development and sharing of large, prospective, longitudinal cohort studies to better understand disease progression and visual recovery.
- Improve patient diagnosis by providing access to faster genetic testing, improving interpretation of results, and better understanding the significance of different genetic variants.