

# The Elusive Pathophysiology of Leber's Hereditary Optic Neuropathy

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**Funding:** None  
**Proprietary/financial interest:** None

**Date of submission:** 18/07/2016    **Date of approval:** 01/09/2016

## Abstract

Leber's Hereditary Optic Neuropathy (LHON) is a mitochondrially inherited disorder characterized by rapid, subacute vision loss. LHON has historically been a difficult disease to study due to its low incidence. Many questions concerning its pathophysiology have remained unanswered. A significant enigma concerns LHON's gender bias as represented by the male-to-female ratio of about 4:1. Another source of confusion includes the variable penetrance, since the mitochondrial mutation is necessary, but not sufficient, to cause conversion. A third challenge involves the tissue specificity, since typically only the optic nerve is involved with a specific pattern of optic atrophy due to primary loss of the papillomacular bundle (PMB) in affected individuals. The fourth remaining complexity is the pattern of rapid, subacute vision loss observed in nearly all affected individuals. Several clinical genetic studies, cybrid experiments, and histochemical studies have been conducted to address the three enigmas of gender bias, variable penetrance, and tissue specificity. Despite elucidation of these three aspects of the pathophysiology, the mechanism behind the rapid, significant vision loss remains a mystery.

**Keywords:** Leber's Hereditary Optic Neuropathy; Mitochondrial Disorder; Estrogen; Reactive Oxygen Species; Papillomacular Bundle

## Introduction

Leber's Hereditary Optic Neuropathy (LHON) is a devastating genetic disorder that often results in rapid and significant bilateral vision loss. It was first characterized by Albrecht von Graefe in 1858, but the disorder received its name in 1871 when Theodor Leber described four families with optic atrophy.<sup>1</sup> LHON follows a mitochondrial inheritance pattern; only female carriers pass on the defective gene and it is to all children.<sup>1,2</sup> Mitochondria contain their own genome comprised of only 13 genes which code for oxidative phosphorylation proteins.<sup>3</sup>

In 1988, Wallace identified a mutation at the mtDNA 11778 nucleotide position responsible for many cases of LHON.<sup>2</sup> In addition, two other mitochondrial mutations at nucleotide positions 3460 and 14484 have been discovered as primary pathogenic mutations for LHON.<sup>4</sup> These three mutations account for 95% of LHON cases.<sup>5</sup> These findings provide an explanation for why only the maternal line is affected, but the reasoning for the higher prevalence in men remains unclear.<sup>5</sup>

Mitochondria are the powerhouses of the cell and responsible for the production of ATP.<sup>1,3</sup> The primary pathologic LHON mutations are located in genes that code for proteins which are part of complex I in the electron transport chain.<sup>3</sup> This alteration in complex I causes a decrease in ATP production, but more significantly, contributes to a buildup of reactive oxygen species (ROS). The increased ROS can lead to a cascade resulting in cell death. Given that all patients who carry the mutation have an increase in ROS production, it remains uncertain as to why some patients are affected while others only remain carriers of the disease.

Affected individuals present with severely reduced visual acuity, dyschromatopsia, and a central scotoma. These features are commonly seen in optic neuropathies involving the P-cell pathway that preferentially affects the papillomacular bundle (PMB) of the retinal nerve fiber layer (RNFL). Fundus examinations of affected LHON individuals reveal preferential loss of the PMB and temporal pallor of the optic disc.<sup>6,7</sup> The evidence suggests that tissue specificity is involved in the pathophysiology of LHON, and the reasoning for this preferential destruction has been a source of confusion.

Since LHON is a rare disorder, it is difficult to study and some questions concerning its pathophysiology remain unanswered. Recent advances in our

understanding of LHON have provided explanations for three defining characteristics of the disease. In this paper, we will address the three enigmas by discussing genetic, cybrid, and histochemical studies that provide insight to pathophysiological mechanisms.

### 1. Gender Bias

The pathophysiology of LHON allows for more likely conversion in men compared to women with a male-to-female ratio of about 4:1.<sup>5</sup> This varies by mutation with the mildest mtDNA type (14484) having the highest gender ratio.<sup>5</sup> There appears to be a protective mechanism in women that reduces the likelihood of conversion. Studies have tested the theory that an X-linked modifying gene contributes to the increased probability of conversion in men or skewed X-inactivation acts as a protective mechanism in women.<sup>8</sup> However, the evidence has failed to support such hypotheses, and identifying the mechanism behind the gender bias has proven elusive until now.

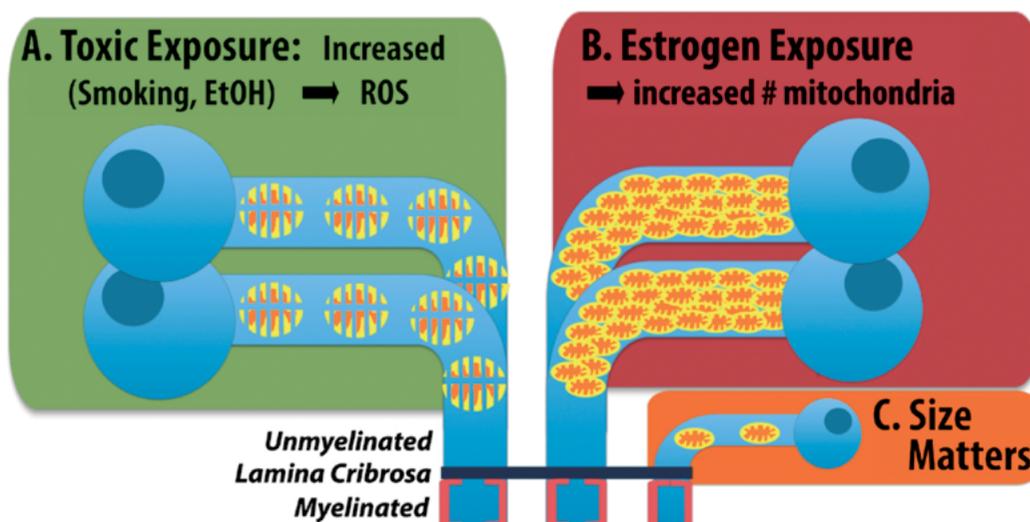
Cybrid lines have provided better understanding of the gender bias. These cybrid experiments involve the fusion of whole cells with enucleated cells to form a hybrid that incorporates the nuclear DNA from one cell with the mitochondrial DNA from another. Experiments conducted by Giordano et al. provide convincing evidence that estrogens are responsible for the decreased rate of conversion in females.<sup>8</sup> The importance of mtDNA copy number was also shown.<sup>8</sup> The mtDNA copy number is normal in LHON affected individuals, but is increased in carriers.<sup>8</sup> Laser capture experiments in regions of the optic nerve head show that carriers have the most upregulated mtDNA copy number. The LHON mutations may signal the need for increased mitochondrial biogenesis which allows

for successful compensation for the defective gene in some carriers, especially women.<sup>8,9</sup>

In cybrids, LHON mutants and controls exposed to estrogen revealed 2.5 times more mitochondria than those without estrogen exposure.<sup>8</sup> Along with the increase in mtDNA copy number, increased mRNA copies of nuclear and mitochondrial genes, including cytochrome c oxidase (COX) subunit I and NADH dehydrogenase subunit 5, were also observed.<sup>8</sup> The increase in mitochondrial number was not observed when the estrogen receptor was antagonized. These findings suggest that mitochondrial numbers can increase with greater estrogen and estrogen is protective to mitochondria.<sup>8</sup>

Giordano et al. also revealed that estrogens appear to induce the activity of superoxide dismutase 2 (SOD2), an essential mitochondrial antioxidant enzyme, by upregulating transcription and non-genomic action.<sup>8</sup> LHON and control cybrids with estrogen supplementation showed increased SOD2 activity, which remained stable for 24 hours. The increase was more prominent in LHON cybrids, and elevated SOD2 gene expression was evident when increased SOD2 protein was noted after 24 hours.<sup>8</sup> Therefore, we can infer that some LHON males are naturally deficient in estrogen and others have less SOD2 than LHON females, providing an explanation for the LHON gender bias.

Future investigations on the therapeutic potential of estrogens in carriers of the LHON mutant mtDNA are expected. The goal of therapy would require limiting the binding of estrogen to cells expressing estrogen beta receptors, which are located on retinal ganglion cells. Studies have shown that phyto-estrogens bind selectively to estrogen beta receptors, making these drugs a potential future therapy to prevent conversion.<sup>8</sup> However, use of these molecules necessitates monitoring of side effects that may accompany exogenous estrogen,



**Figure 1.** A) LHON mutation blockage of complex I contributes to the increase in ROS, which is exacerbated by smoke and alcohol. B) Due to the presence of estrogen, women with the LHON mutation appear more successful with increasing mitochondrial biogenesis to compensate for the defective gene. C) Myelinated neurons have greater protection against ROS. The papillomacular bundle is vulnerable because axons in this region tend to be the smallest and unmyelinated, which makes them most susceptible to ROS injury.

especially in males. There is also a potential limitation: males unable to upregulate their mitochondrial copy number, particularly males with less estrogen, will be more likely to convert and become affected<sup>8,9</sup>

## 2. Variable Penetrance

The mtDNA mutation sets the foundation for LHON, but it is not sufficient to explain conversion. Penetrance requires an additional genetic or experimental factor. Theories have suggested heteroplasmy as an explanation for variable penetrance, but the majority of carriers are homoplasmic, making this unlikely.<sup>10</sup> Besides estrogens, other unknown genetic factors likely contribute to conversion, but in some circumstances, the environment plays a key role.<sup>11</sup> Elucidating the environmental risk factors also provides awareness about what LHON mutant mtDNA carriers should avoid to prevent conversion.

Much of our understanding of environmental triggers comes from the Soave-Brazil (SOA-BR) genealogical tree. Since 2001<sup>10</sup>, we have been studying a very large LHON 11778/ND4 pedigree family that descended from a female carrier who was born in 1861 and emigrated from Italy to Brazil. The pedigree now includes eight generations and 362 members, most living in a rural area between Vitoria, Colatina, and Santa Teresa in Brazil. As they are all maternally related, they have the same LHON mutation and background haplotype. As part of the study, 72 LHON carriers, 20 LHON affected individuals, and 66 age-matched controls without the mtDNA mutation or visual complaints were carefully and systematically observed for 15 years.<sup>7, 10</sup> Smoking and heavy drinking were found to significantly increase the likelihood of conversion probably due to the increase in oxidative stress in the form of ROS.<sup>3, 10, 12</sup> The number of LHON affected individuals exposed to cigarettes was significantly higher than the number of LHON carriers.<sup>10</sup> Additionally, LHON affected individuals had a significantly higher rate of alcohol consumption than carriers.<sup>10</sup> These findings suggest that alcohol and tobacco exposures, which would independently increase ROS, also raise the likelihood of conversion.<sup>10</sup>

These field investigations also suggest that a second genetic factor may contribute to the probability of conversion. A recessive nuclear genetic locus may also in some cases, be involved in modifying the likelihood of conversion.<sup>10</sup> Interestingly, offspring from affected mothers have an increased penetrance of LHON; a son born from an affected mother is more likely to convert. This genetic modifier may be X-linked.<sup>13, 14, 15</sup>

However, the evidence for the X-linked modifier gene is not compelling. Hudson et al. identified two major high-risk haplotypes, DXS8090 and DXS1068, that are associated with increased penetrance in European LHON women and their offspring.<sup>13</sup> This was supported by the finding that 42% of affected men carried the DXS8090/DXS1068 haplotype, while only 5% of unaffected male carriers had the same haplotype.<sup>13</sup> This, however, may only be true in a European population, as Ji et al. revealed conflicting findings in a Chinese

population.<sup>15</sup> They reported no significant difference regarding presence of the DXS8090/DXS1068 haplotype in LHON patients with the 11778 mutation compared to age-matched controls.<sup>15</sup>

In summary, the challenge of variable penetrance has been addressed in numerous studies that attempt to identify environmental and genetic factors. Environmental influences that cause an increase in ROS and subsequently contribute to conversion include smoke and alcohol.<sup>3,10,12</sup> Several studies have also suggested that an additional genetic modifier may be located on the X chromosome.<sup>13, 14, 15</sup> However, there may be two forms of conversion in LHON (Type I and Type II) reflecting modifying genetics and environment respectively, and this confounder may account for the differing results regarding interacting modifier genes.<sup>16</sup>

## 3. Tissue Specificity

Although the brain only accounts for 2% of body weight, it consumes more than 20% of the body's oxygen and energy supplies. The retinal ganglion cells (RGC) may require particularly high maintenance since their axons, that run a long course in the retina, are unmyelinated. This likely makes them especially vulnerable to ROS accumulation in mitochondrial dysfunction.<sup>3</sup> Why is it that a clear pattern of PMB loss and temporal atrophy is observed in nearly all patients? Recently, the factors behind this tissue specificity for the optic nerve and especially the preference for PMB loss that accounts for the correlating clinical symptoms of central scotoma, dyschromatopsia, and diminished contrast sensitivities have been elucidated.<sup>17,18</sup>

The key to this preference for the PMB appears to be in the energetics imparted by axon caliber and myelin. Myelin allows for faster conduction and provides a more energy-efficient method for re-establishing membrane potential after an action potential, hence myelinated neurons use less ATP and produce less ROS.<sup>3</sup> Andrews et al. and Bristow et al. conducted histological studies that revealed high concentrations of COX staining in the optic nerve anterior to the lamina cribosa, suggesting that these anterior regions have increased mitochondria.<sup>19,20</sup> COX distribution is minimal in myelinated sections. As myelination begins, a sharp reduction in COX staining occurs, indicating a drastic decrease in mitochondrial number. This study showed that the unmyelinated axon regions of the optic nerves have higher oxidative phosphorylation activity, suggesting that mitochondrial dysfunction has the highest impact on unmyelinated axons.<sup>3</sup>

In 2012, we described the Nerve Fiber Layer Stress (NFL-S') index more formally to assess the extent of metabolic stress that an axon can endure in relation to its size.<sup>17</sup> The equation involves the ratio of the axon's energy demands to its supply. The prelaminar unmyelinated axons represent energy demand as the sodium and potassium gradients need to be re-established after action potential propagation over the entire length of the prelaminar unmyelinated axon. Myelinated axons only need to reconstitute the sodium-potassium gradients at the small nodes of Ranvier. The energy supply is associated with the number of mitochondrial units, and since mitochondrial number is directly correlated with volume, the axonal volume represents supply. The following equation resulted, with L representing axon length and R representing axon radius:  $NFL-S' = A^S/A^V = L^2\pi R/L\pi R^2 = 2/R$ . Since NFL-S' is inversely proportional to axon radius, this mathematical equation shows that theoretically smaller axons are more susceptible to stress and preferentially destroyed.<sup>17, 18</sup>

The PMB is indeed most vulnerable in LHON especially at the inferior temporal region of the optic disc, where the unmyelinated axons are the smallest. These properties make them most susceptible to injury.<sup>6</sup> The

small size of the axons also presents an anatomical constraint for transporting the mitochondria generated in the RGC cell body further along the axons. Regions that generally have higher mitochondrial concentrations include the varicosities of the RNFL, laminar and prelaminar regions of the optic nerve head, the nodes of Ranvier in the post-laminar portion, and synaptic terminals. Thus, RGCs with smaller axons are predicted to have decreased ability to transport mitochondria to these areas and are more susceptible to metabolic and functional defects.<sup>3</sup>

This work also provides evidence that the optic atrophy spreads as a wavefront across the optic nerve with smaller axons affected first. Our results allowed us to conclude that loss of smaller axons is associated with LHON, but as with any observational study no cause and effect assumptions should be made.<sup>17</sup>

### Remaining Challenge

The initial symptom of LHON conversion involves blurry vision that progressively worsens over the span of a few weeks. The central visual field defect expands to a dense and large central scotoma within this short timeframe.<sup>21,10</sup> The end result involves bilaterally symmetrical visual losses with early and significant color vision loss.<sup>6,10</sup> This rapid loss of vision is a defining characteristic of LHON, and the mechanism is not fully elucidated.

Our comprehensive annual longitudinal study of the SOA-BR genealogical tree with the 11778 mtDNA mutation led to a better understanding of this phenomenon. Some individuals in this pedigree displayed a unique pattern of late and slowly progressive vision loss. Examination of individuals with this unusual clinical picture revealed slowly worsening visual fields, while OCT examination initially revealed dramatic swelling of the temporal RNFL prior to vision loss.<sup>7</sup> Once a threshold was reached, subacute vision loss occurred with subsequent dramatic optic atrophy. The mechanism of swelling may involve a buildup of mitochondria to compensate for the mitochondrial mutation, and once the critical threshold occurred, dramatic decompensation led to atrophy.<sup>7,22</sup>

Savini et al. conducted a study comparing unaffected LHON carriers and healthy controls that further supported this compensatory swelling mechanism. OCT examinations in unaffected males more than females revealed significantly thicker temporal and inferior quadrants.<sup>23</sup> Our study of the Brazilian family revealed similar results with asymptomatic carriers showing mild RNFL sectoral disc edema and telangiectasis.<sup>7</sup> In carriers, OCT demonstrates increased RNFL thickness on the inferior temporal side. Compensatory swelling by mitochondria may account for the swelling in carriers and that precedes vision loss in affected individuals. This aggregation of mitochondria has also been reported in other mitochondrial diseases.<sup>7,23</sup>

### Conclusion

A mtDNA mutation is necessary but not sufficient to produce vision loss in LHON. Other genetic or environmental factors that increase ROS may contribute to the conversion from the asymptomatic carrier to affected state. Common risk factors that increase ROS include smoking especially and high alcohol consumption. Increased mtDNA copy number in carriers, especially women, provides an explanation for the gender bias of LHON. The lack of myelin in the RNFL and the susceptibility of small axons explain the preferential loss of the PMB. Yet the mystery responsible for the rapid, subacute vision loss, remains. ■

### References

- Meyerson C, Van Stavern G, McClelland C. Leber hereditary optic neuropathy: current perspectives. *Clin Ophthalmol.* 2015;9:1165-76.
- Wallace DC, Singh G, Lott MT, et al. Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. *Science.* 1988;242(4884):1427-30.
- Carelli V, Ross-Cisneros FN, Sadun AA. Mitochondrial dysfunction as a cause of optic neuropathies. *Prog Retin Eye Res.* 2004;23(1):53-89.
- Mackey DA, Oostra RJ, Rosenberg T, et al. Primary pathogenic mtDNA mutations in multigeneration pedigrees with Leber hereditary optic neuropathy. *Am J Hum Genet.* 1996;59(2):481-5.
- Newman NJ. Hereditary optic neuropathies: from the mitochondria to the optic nerve. *Am J Ophthalmol.* 2005;140(3):517-23.
- Sadun AA, Win PH, Ross-Cisneros FN, Walker SO, Carelli V. Leber's hereditary optic neuropathy differentially affects smaller axons in the optic nerve. *Trans Am Ophthalmol Soc.* 2000;98:223-32; discussion 223-25.
- Sadun AA, Salomao SR, Berezovsky A, et al. Subclinical carriers and conversions in Leber hereditary optic neuropathy: a prospective psychophysical study. *Trans Am Ophthalmol Soc.* 2006;104:51-61.
- Giordano C, Montopoli M, Perli E, et al. Oestrogens ameliorate mitochondrial dysfunction in Leber's hereditary optic neuropathy. *Brain.* 2011;134(Pt 1):220-34.
- Giordano C, Iommarini L, Giordano L, et al. Efficient mitochondrial biogenesis drives incomplete penetrance in Leber's hereditary optic neuropathy. *Brain.* 2014;137(Pt 2):335-53.
- Sadun AA, Carelli V, Salomao SR, et al. Extensive investigation of a large Brazilian pedigree of 11778/haplogroup J Leber hereditary optic neuropathy. *Am J Ophthalmol.* 2003;136(2):231-38.
- Chicani CF, Chu ER, Miller G, Kelman SE, Sadun AA. Comparing EPI-743 treatment in siblings with Leber's hereditary optic neuropathy mt14484 mutation. *Can J Ophthalmol.* 2015;48(5):e150-3.
- Cullom ME, Heher KL, Miller NR, Savino PJ, Johns DR. Leber's hereditary optic neuropathy masquerading as tobacco-alcohol amblyopia. *Arch Ophthalmol.* 1993;111(11):1482-5.
- Hudson G, Keers S, Yu Wai Man P, et al. Identification of an X-chromosomal locus and haplotype modulating the phenotype of a mitochondrial DNA disorder. *Am J Hum Genet.* 2005;77(6):1086-91.
- Shankar SP, Fingert JH, Carelli V, et al. Evidence for a novel x-linked modifier locus for leber hereditary optic neuropathy. *Ophthalmic Genet.* 2008;29(1):17-24.
- Ji Y, Jia X, Li S, Xiao X, Guo X, Zhang Q. Evaluation of the X-linked modifier loci for Leber hereditary optic neuropathy with the G11778A mutation in Chinese. *Mol Vis.* 2010;16:416-24.
- Carelli V, d'Adamo P, Valentino ML, et al. Parsing the differences in affected with LHON: genetic versus environmental triggers of disease conversion. *Brain.* 2016;139(Pt 3):e17.
- Pan BX, Ross-Cisneros FN, Carelli V, et al. Mathematically modeling the involvement of axons in Leber's hereditary optic neuropathy. *Invest Ophthalmol Vis Sci.* 2012;53(12):7608-17.
- Ziccardi L, Parisi V, Giannini D, et al. Multifocal VEP provide electrophysiological evidence of predominant dysfunction of the optic nerve fibers derived from the central retina in Leber's hereditary optic neuropathy. *Graefes Arch Clin Exp Ophthalmol.* 2015;253(9):1591-1600.
- Andrews RM, Griffiths PG, Johnson MA, Turnbull DM. Histochemical localisation of mitochondrial enzyme activity in human optic nerve and retina. *Br J Ophthalmol.* 1999;83(2):231-5.
- Bristow EA, Griffiths PG, Andrews RM, Johnson MA, Turnbull DM. The distribution of mitochondrial activity in relation to optic nerve structure. *Arch Ophthalmol.* 2002;120(6):791-6.
- Howell N. Leber hereditary optic neuropathy: respiratory chain dysfunction and degeneration of the optic nerve. *Vision Res.* 1998;38(10):1495-1504.
- Barboni P, Savini G, Valentino ML, et al. Retinal nerve fiber layer evaluation by optical coherence tomography in Leber's hereditary optic neuropathy. *Ophthalmology.* 2005;112(1):120-6.
- Savini G, Barboni P, Valentino ML, et al. Retinal nerve fiber layer evaluation by optical coherence tomography in unaffected carriers with Leber's hereditary optic neuropathy mutations. *Ophthalmology.* 2005;112(1):127-31.