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Introduction

Albrecht von Graefe, a Prussian ophthalmologist, noted in 1858 that an adolescent male patient of his developed unusual acute central vision loss in both eyes over the course of several months *[1]*. Nearly fifteen years passed before another ophthalmologist by the name of Theodor Leber began to take serious note of this anomaly and characterized the disorder *[2]*. Leber noted that the condition strongly favored males and seemed to be heritable, indicating a genetic basis for the disease. Due to the work of Thomas Morgan several years earlier, Leber determined that the inheritance patterns of the disease must be due to a sex-linked recessive trait. Unfortunately the inheritance patterns were not as clear-cut as the experiments Morgan conducted because males peculiarly failed to pass the disease to their offspring. Nearly a hundred years later additional researchers realized that the disease followed the inheritance patterns of maternal cytosolic inheritance, not of genomic DNA *[3]*. This evidence strongly suggested that the disease takes root in an abnormality in the mitochondrial DNA (mtDNA). The disorder eventually became known as Leber's hereditary optic neuropathy (LHON) and by 1988 the mtDNA-basis for the disease was elucidated. Currently there are no established therapies to treat LHON and much of the treatment is aimed at prolonging eyesight in those who have the disorder. However, there are a number of contemporary therapies that have been shown to improve patient eyesight *[4]*. As a result of its highly structured nature, seemingly insignificant adjustments to the mtDNA result in the molecular basis of LHON.

Molecular Basis of LHON

There are three primary mutations that result in the development of LHON. Surprisingly, each of these defects derives from a single nucleotide point mutation resulting in a dysfunctional

protein subunit associated with complex I of the electron transport chain (ETC). The first mutation to be identified was found in the gene that codes for NADH dehydrogenase 4 (ND4), commonly known as ubiquinone. ND4 is an important and heavily conserved polypeptide subunit of NADH dehydrogenase that is responsible for the transfer of electrons between NADH to coenzyme Q in the inner membrane of the mitochondria *[5]*. As indicated previously, proper expression of the ND4 gene permits the successful flow of electrons down the ETC, ultimately providing the means by which adenosine triphosphate (ATP) synthesis may occur. A mutation in this gene will have a deleterious effect, inhibiting the efficiency of ATP formation. One such mutation involves a simple nucleotide exchange at position 11778 in the mitochondrial genome. For reasons not yet fully understood, a guanine (G) is substituted for an adenine (A), leading to a subtle, yet potent change in the primary structure of the protein *[6]*. The particular mutation in ND4 mentioned above is responsible for nearly 70% of the reported cases of LHON *[6]*. Furthermore, the remaining cases largely stem from mutations in related polypeptide subunits of ubiquinone—ND1 and ND6, specifically. Like ND4, ND1 and ND6 are also involved in the successful transmission of electrons between the NADH and coenzyme Q. Thus, mutations in these subunits are also attributed to the LHON condition. As in the case of ND4, the mutation in ND1 is caused by a G to A substitution at position 3460 in the mitochondrial genome. A mutation in ND6, on the other hand, derives from the substitution of a thymine (T) for a cytosine (C) at point 14484 *[6]*. Together, these three mutations account for approximately 90-95% of all LHON cases. The remaining 5-10% are not fully understood, but are expected to also be associated with abnormalities in complex I of the ETC *[7]*.

The ETC is a collection of enzymes responsible for the energy-yielding reactions of the cell. These enzymes provide a means by which electron transporters such as NADH and flavin

adenine dinucleotide ($FADH_2$) can donate their electrons to release their potential energy in a controlled stepwise process, making the harnessing of ATP possible. The association of the ETC with the inner mitochondrial membrane is vital for its energy-yielding function. Like chloroplasts, mitochondria are double membrane organelles. This provides mitochondria with the unique ability to differentially compartmentalize their membranes to allow for selective molecular transporting that ultimately produces an electrochemical gradient across the inner membrane. This gradient is necessary for the conversion of kinetic energy into potential energy in the form of ATP.

The electron transporters donate electrons to complex I, which passes them to coenzyme Q, a hydrophobic electron carrier that resides in the inner membrane space. Coenzyme Q then diffuses through the membrane until it reaches complex III. Electrons are transferred to complex III, which passes these electrons to cytochrome c, a peripheral protein that resides on the inner membrane space side of the membrane. Cytochrome c donates these electrons to complex IV that then passes them on to the terminal electron acceptor, oxygen, leading to the formation of metabolic water. Throughout this process each complex (with the exception of complex IV) effectively pumps hydrogen ions from inside of the mitochondrial matrix to the inner membrane space. Via diffusion down the concentration gradient, hydrogen ions pass through the final complex and back into the mitochondrial matrix. This complex is known as ATP synthase, whose function is often likened to that of a water mill. As hydrogen ions flow down the concentration gradient the ATP synthase spins, harnessing the kinetic energy and focusing it on the formation of ATP from adenosine diphosphate (ADP) and inorganic phosphate.

The ETC pathway is conserved and highly regulated in virtually all eukaryotic cells. The life of the cell and the organism itself is at risk if one of these enzymes develops a mutation. As

mentioned earlier, LHON is caused by a mutation in a single subunit belonging to one of the many enzymes described above (viz. complex I). Incredibly, a single nucleotide mismatch in one of the enzyme's many subunits can result in LHON, which speaks to the importance of ensuring that this process is accurately regulated.

A reduction in the cell's ability to produce ATP ultimately leads to cellular apoptosis. However, the question remains: why do these mutations manifest themselves phenotypically as an acute onset of blindness? The answer to this question has yet to be fully understood, but research suggests that LHON leads to a degeneration of retinal ganglion cells (RGC) and their axons due to a deficit in ATP and the formation of a large number of oxygen free radicals. Essentially, RGCs are responsible for relaying visual information from photoreceptors in the eye and propagating these signals down their axons to the brain *[8]*. The RGC's axons compose what is collectively known as the optic nerve (Fig. 1).

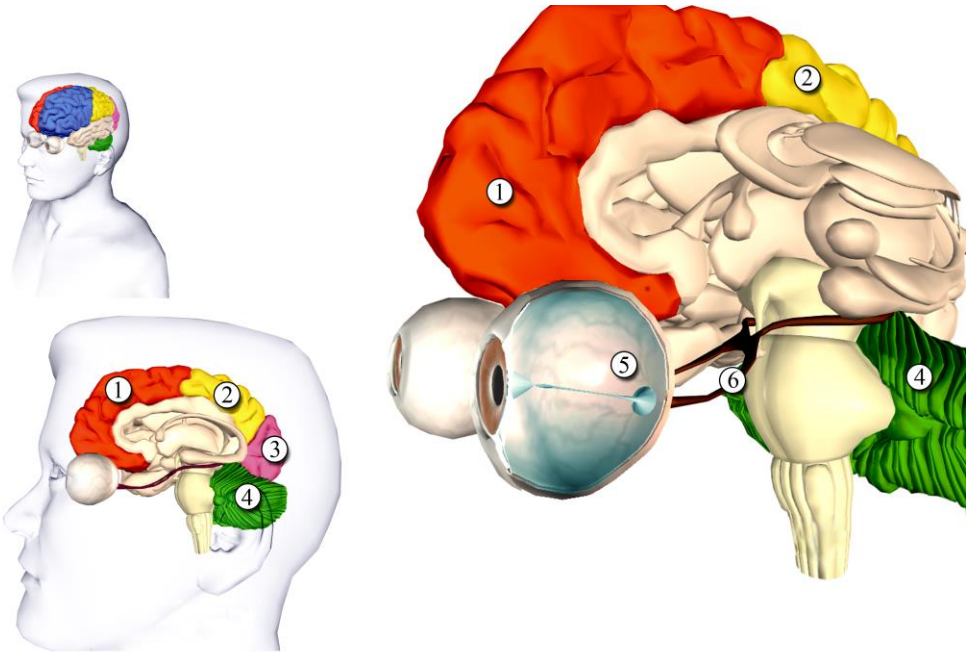

Fig. 1: Optic Nerve and the Retinal Ganglion Cells. LHON is characterized by a sudden decrease in visual activity, which is attributed to the degradation of retinal ganglion cells (RGC) and their associated axons. The RGCs reside within the innermost layer of the eye (5) and feed their axons to a centralized location at the back of the eye. The axons of all the RGCs compose

the optic nerve (6), which relays visual information to the brain. The lobes highlighted above constitute the outer most layer of the brain: (1) cross section of the right frontal lobe, (2) parietal lobe, (3) occipital lobe, (4) cerebellum (temporal lobe not highlighted). Degradation of the optic nerve is responsible for a number of vision-related issues, including LHON. This degradation is typically associated with apoptosis of the RGCs, and in the case of LHON it is believed to result from a dysfunction of complex I in the ETC leading to an increase in free radicals.

These cells are remarkably active, requiring an incredible amount of ATP to function and survive in the extracellular environment *[8]*. Most cells throughout the body have less stringent ATP demands than that of RGCs, which is largely the reason these parts of the body remain unaffected by LHON despite having the same genetic makeup. Although research in this area has made a considerable number of advancements in recent years, a clear molecular pathway still remains to be identified.

One of the most interesting characteristics of LHON is its unique pattern of inheritance. When a fetus is formed, half of the genetic material is paternal while the other half is maternal, creating a diploid organism. Essentially all that is contributed paternally is the genomic information in the form of DNA. That said, virtually all of the cellular components of a fertilized egg are donated maternally from the mother's ovum, including the mitochondria and its associated mitochondrial genome. Due to the molecular roots of LHON in the mtDNA, the disease is not associated with the genomic DNA to which, as stated previously, the male does contribute through the nuclear fusion of sperm and ovum. As a result affected males are not able to pass the disorder onto their children (Fig. 2).

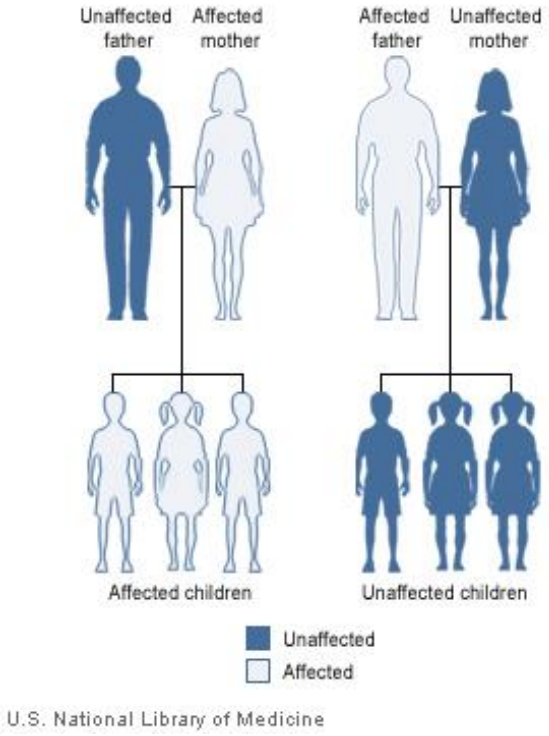

Fig. 2: Inheritance Patterns of LHON and mtDNA-Associated Diseases. LHON pathology follows the inheritance pattern of the maternal contribution to a developing fetus. A point mutation in one of three genes encoded for by the mtDNA has been shown to be the molecular driver of the disorder. As a result, the pathology follows the inheritance of the maternal mitochondria. Mating between an affected mother and unaffected father (left) results in 100% of affected progeny because the disorder is passed maternally. On the other hand, mating between an affected father and unaffected mother (right) would result in 100% unaffected progeny. Due to incomplete penetrance, there is no guarantee that symptoms in each of the progeny will occur, if ever. Adapted from *ghr.nlm.nih.gov*, n.p., Retrieved April 18, 2015, from http://ghr.nlm.nih.gov/handbook/illustrations.

This mode of inheritance baffled early LHON investigators, including Theodor Leber himself. Thomas Morgan unraveled the closest explanation for this pattern of inheritance in the early 1900s, shortly before Leber began to make his initial observations. In his lab at Columbia University, Morgan identified a pattern of recessive sex-linked inheritance in a number of fruit flies (*Drosophila melanogaster*) with peculiar mutations in eye color. Morgan effectively discovered that the chromosomes responsible for male and female differentiation also carry other information, particularly for eye color in his model. This inheritance pattern predicted that if an affected mother carried the mutated gene on one or both of her X chromosomes, then her male

offspring would have an increased risk for the condition over the female siblings. This idea fascinated Leber, and managed to explain many of his results. In the end Leber determined that this sex-linked inheritance pattern must be the way LHON is passed down from generation to generation. Researchers today realize that Leber's conclusion was premature. His incorrect conclusion was largely due to the fact that the field of cellular biology was still in its infancy. Although Morgan's mechanism of inheritance explained why males primarily inherited the disease, it failed to explain why these males were incapable of passing the condition to their progeny. Nearly 60 years would pass until the discovery of mtDNA. Researchers finally had sufficient evidence to theorize that the molecular basis of LHON resides in the mtDNA, not genomic DNA. Douglas C. Wallace discovered a point mutation in the ND4 polypeptide in 1988, almost 130 years after the first documented case of the disorder *[5,6]*.

LHON may arise at any age, however the disorder usually affects young males during their second or third decade of life *[7]*.[1] Interestingly, nearly 50% of males and approximately 90% of affected females never develop any signs of vision loss, indicating that the disease has a level of penetrance associated with the condition *[9]*. Despite this, affected individuals who are asymptomatic may still transmit the disorder to their offspring. Symptoms of LHON are characterized by an acute loss of central vision in one eye, followed by the other a few weeks to months later *[7,9]*. Typically, the disorder progresses to the point of severe optic atrophy and permanent impairment of visual activity, and in some cases, a total loss of central vision *[7]*. Affected individuals are born with no distinction between their unaffected peers. Once the onset of the disorder occurs the only distinguishing feature is their loss of visual activity, although

[1] The age of onset is largely a question of penetrance in specific pedigrees. Geographic locations seem to also be a contributing factor because some mutations are more prevalent than in other areas of the globe. The median age of onset for males worldwide is 22, give or take 2 years depending on the specific mutation *[9]*.

some heart-related issues have been reported *[7]*. Apart from this, individuals remain phenotypically indistinguishable from their peers.

Prevention and Therapies

Because of the genetic basis of the disease there are no true preventative measures for LHON, although avoiding excessive alcohol consumption and smoking may prevent early onset of the disease *[9]*. Many of the therapies currently being developed focus on vision management and slowing the progression of the disorder. Several coping methods have been developed for those who suffer from LHON, focusing particularly on occupational rehabilitation. Occupational rehabilitation for LHON is similar to other forms of visual impairment and is thus made possible by the utilization of visual and auditory aids *[9]*. Technologically enhanced assisted living via personal computers and cellphones with text-to-speech capabilities and magnified font sizes is common for occupational rehabilitation. Currently, pharmaceutical interventions are being developed in which administration of particular drugs could prevent additional vision loss in the early stages of LHON *[9]*. For example, idebenone (previously marketed as Raxone), a synthetic analog of coenzyme Q_{10}, has shown some clinical success in treating LHON in its early stages *[9,10]*. Patients have recorded an increase in visual activity shortly after treatment with idebenone *[10]*. Because of this success, authorization for idebenone was submitted to the European Medicines Agency (EMA) in January of 2013. Idebenone is believed to function by reducing the number of free radicals that form as a byproduct of LHON, thus reducing the number of cells that undergo apoptosis. How this works is not entirely understood. As a result of this and the fact that idebenone is effective only in those patients who are in the early stages of LHON, the EMA rejected this proposal for idebenone authorization *[11]*. Today researchers are working to increase treatment efficacy in the later stages of the disease in the hopes of reapplying

for EMA authorization in the future. For individuals who think that they may be at risk for LHON, diagnosis of the disorder is primarily concerned with identifying one of three point mutations in the mtDNA via qualitative RT-PCR. Additional testing is also not uncommon *[12]*.

In some studies, phosphorus magnetic resonance spectroscopy was used to distinguish the various mutations that result in LHON *[9]*. Interestingly this technique has also been successful in identifying asymptomatic carriers of the disorder. Despite having the same genetic defects, these individuals show no signs of the disease. Those who have been diagnosed with LHON may want to consider the possibility of speaking with a genetic counselor, particularly in the event of family planning. Gene therapy, the selective removal and/or addition of particular genes into specific loci, has also been explored as a potential treatment option for LHON. Due to the physical nature of the mtDNA, the method of gene therapy is expected to be significantly more effective than related methods on genomic DNA *[12, 13]*.[2] Implications of this method as a treatment option for LHON seem promising, but require further investigations to assess safety and efficacy *[9]*. In asymptomatic carriers, LHON symptoms may be precipitated by nutritional deprivation, exposure to toxins, antiretroviral drugs, and even excessive stress. These environmental factors have been correlated in individuals who have early onset of the disease, however there is no consensus among experts as to the degree of their significance in accelerating the disease *[9]*.

Conclusion

LHON is a heritable disorder characterized by an acute onset of vision loss that serves as a common model for mtDNA-associated disorders. Since its first documented observation in

[2] One of the major setbacks for gene therapy in treating genetic disorders is that genomic DNA resides on linear chromosomes, preventing the insertion/exertion of specific genes. The circular nature of mtDNA resembles yeast and bacterial DNA, which has shown a significant level of success in gene therapy.

1858, the molecular basis of LHON has been formulated and remodeled many times over, largely due to the gradual scientific revelations over the eras. The exact mutations responsible for approximately 90% of all LHON cases have been identified. However, an explanation of how the disease manifests itself in the degradation of the optic nerve remains theoretical at best. Despite this, researchers are exploring potential therapies for LHON that are proving to be quite promising. Contemporary treatments such as gene therapy may prove to be beneficial for disorders like LHON. With the combination of occupational rehabilitation therapies as well as the potential for pharmaceutical drug intervention, LHON may soon be a manageable disease for many of those affected by it. The ultimate goal of treatment is targeted at the reversal of the disorder, leading to improved visual activity. Although it may seem like this goal is distant in the future, it may actually be closer than it appears.

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