Review Article

Mitochondrial genetics and therapeutic overview of Leber's hereditary optic neuropathy

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Leber's hereditary optic neuropathy (LHON) is a common inherited mitochondrial disorder that is characterized by the degeneration of the optic nerves, leading to vision loss. The major mutations in the mitochondrial genes *ND1, ND4,* and *ND6* of LHON subjects are found to increase the oxidative stress experienced by the optic nerve cell, thereby leading to nerve cell damage. Accurate treatments are not available and drugs that are commercially available like Idebenone, EPI-743, and Bendavia with their antioxidant role help in reducing the oxidative stress experienced by the cell thereby preventing the progression of the disease. Genetic counseling plays an effective role in making the family members aware of the inheritance pattern of the disease. Gene therapy is an alternative for curing the disease but is still under study. This review focuses on the role of mitochondrial genes in causing LHON and therapeutics available for treating the disease. A systematic search has been adopted in various databases using the keywords "LHON," "mitochondria," "*ND1*," "*ND4*," "*ND6*," and "therapy" and the following review on mitochondrial genetics and therapeutics of LHON has been developed with obtained articles from 1988 to 2017.

Key words: Leber's hereditary optic neuropathy, mitochondria, *ND1, ND4, ND6*

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The blinding disease with maternal mode of inheritance is Leber's hereditary optic neuropathy (LHON). This condition was first reported in 1871 by Theodore Leber, an ophthalmologist from Germany, who described this as a distinctive clinical unit.[1] Mutations in the genes of mitochondria induce apoptosis of retinal ganglion cells (RGCs) by decreasing the production of adenosine triphosphates (ATPs) and elevating oxidative stress.[2] The dead RGCs are then unable to send vision signals to the brain thereby the output is improperly processed causing blindness and extreme visual damage. Usually, this vision loss progresses from a few days to weeks and is painless, but in rare cases cause discomfort due to inflammation of the optic nerves. Loss of RGC happens in around 50% male and about 10%–15% female patients. Recovering the lost vision is possible depending on the mutation present.[3] LHON is not age dependent as it affects people in all age groups, but men around the age of 20 and 30 are reported to be the most affected. At first, one eye is found to be affected but after a gap of a few months to years, the second eye also develops the symptom.[4]

The mutation in the mitochondrial genome (mtDNA) takes place in the subunit which encodes for the complex-I (CI) of the electron transport chain which is NADH:ubiquinone oxidoreductase and the mutation usually involve a single amino acid exchange. This in turn leads to depletion of energy in the neuron cells that cause death of the neurons.[5] Apart from the CI dysfunction, the impairment of glutamate transport system and increased levels of oxidative stress also lead to dysfunction and death of RGC.[6] Although mtDNA mutation is the major cause of LHON, the environmental factors tend to worsen the symptoms.

There is no proper treatment to completely eradicate LHON, but medicines are available to promote ATP synthesis and to decrease oxidative stress. A permanent cure must be found to protect the mutations happening in the mtDNA which is tough as we are not sure about the mutagens that cause these mutations. Various studies are now under process for validating the use of gene therapy for treating LHON. Till date, promising results are obtained which showed improvement in vision of patients suffering from LHON. In this review, the mitochondrial genetics of LHON and treatments available are discussed.

Methods

Articles for writing this review were collected using various search engines such as PubMed, Google Scholar, Science Direct, and Elsevier, Boolean search strategy has been followed using the keywords such as "LHON," "mitochondria," "*ND1*," "*ND4*," "*ND6*," and "therapy." Out of all the articles obtained, 62 articles from 1988 to 2017, that exactly match this study, have been chosen and the references mentioned were cross-checked for perfection.

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Mitochondrial Genetics of Leber's Hereditary Optic Neuropathy

Present almost in all eukaryotic cells that aid in cellular respiration by a process called oxidative phosphorylation (OXPHOS) is the mitochondria. Being a specialized organelle, the mitochondrion is involved in the regulation of cellular metabolism, synthesis of steroids, and haem, signaling of calcium and apoptosis.[7] Usually, the term "gene" reminds us of nuclear DNA. However, recent research is mainly focused on the genes present in the mitochondria because many hereditary diseases are found to have mutation in the mtDNA which are passed down through maternal inheritance. Mutations in the mitochondria can cause two types of reaction; it can either alter the biosynthesis of mitochondrial proteins, or it can disrupt the OXPHOS by amino acid substitutions.[8]

mtDNA of humans has a special property of replicating ceaselessly in cells without undergoing cell cycle resulting in thousands of identical mtDNA copies, thereby indicating the homoplasmic condition of the cell and heteroplasmic condition results due to mutation in any of the existing mtDNA, resulting in the presence of both mutated and normal mtDNA in a cell.[9] The proportion of normal and mutant mtDNA in the daughter cell varies depending on the process of replicative segregation and in cells, only a definite amount of normal mtDNA can neutralize the effect of mutant mtDNA. When the amount of mutant mtDNA exceeds the normal mtDNA diseases associated with the type of mutation involved prevails in the subject.[10] This property of homoplasmy and heteroplasmy plays a major role in the severity of LHON. The discussed genes have both homoplasmic and heteroplasmic factors that determine the penetrance of the disease that is discussed in the corresponding gene section.

LHON is one of the diseases that have a maternal mode of inheritance. Douglas C. Wallace and colleagues were the first to discover the mtDNA mutations in LHON, which was in the *ND4* gene.[11] Three major genes in mitochondria when mutated and found to cause 90%–95% cases of LHON are MT-*ND1*, MT-*ND4*, and MT-*ND6*.[11-13] Mutations such as 3460A, 11778A and 14484C of *ND1*, *ND4*, and *ND6*, respectively, are most common among the LHON subjects, as it is found in 90% of cases.[14-16] Apart from these genes, various others are also involved but these three have the major prevalence, and the mutation usually involves the exchange of a single amino acid in the CI of mitochondria which is NADH: ubiquinone oxidoreductase.

MT-*ND1*

MT-*ND1*, also known as mitochondrial encoded NADH ubiquinone oxidoreductase core subunit 1 plays a major role in creating NADH dehydrogenase 1, a protein of CI. This gene has 956 bases and the location of the gene starts from 3307 bp from pter and ends at 4262 bp from pter in the mitochondrial genome. Its protein product has 318 amino acids with molecular weight of 35661 Da. Mutation in this gene causes dysfunction of CI, thereby causing LHON.

About 60%–80% of CI activity is reduced due to mutations in 3460 position of *ND1* gene which without affecting the activity of proximal NADH dehydrogenase of CI, reduces the sensitivity of rotenone and ubiquinone dependent electron transfer activity.[17,18] Thus, there is an abundant amount of roteonone which in turn affects the CI activity since rotenone is a potent inhibitor of the CI of mitochondria.[19] Because of this property, rotenone has been used in model studies involving rats to stimulate LHON symptoms by injecting rotenone which causes ganglion cell layers and nerve fiber thinning, loss of RGCs, and thinning of inner plexiform layer, thereby mimicking the human LHON condition.[20] Mutation in the G3640A leads to substitution of the alanine amino acid by threonine in *ND1* gene.[21] and LHON due to this mutation shows gradual visual improvement.[22] However, the homoplasmic 3640 mutation of the *ND1* in LHON subjects is shown to have resistance to rotenone, resulting in the decreased activity of CI.[23] Another study on the M9a mitochondrial haplogroup shows that the penetrance of LHON is more in Chinese families with *ND1* T3394C mutation when present along with G11778A of *ND4*.[24]

Studies involving the genetic analysis of LHON revealed many point mutations in the *ND1* gene which are m. 4171C>A/p. L289M, m.3700G>A/p. A132T, and m.3733G>A-C/p. E143K-Q.[25] Other than causing LHON, a mutation of 3697G>A in the *ND1* gene is reported in patient having spastic dystonia along with LHON, and this mutation is also responsible for causing mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS).[26] A novel mutation in the *ND1* gene of homoplasmic cell is reported as m.3635G>A, p.Ser110Asn which is also associated with LHON by impairing the OXPHOS system.[27] Another mutation in the *ND1* gene m.3472T>C was observed in LHON affected patient which changes the amino acid is the position 56, which is phenylalanine to leucine and efforts are taken to determine its role in causing mitochondrial complex dysfunction.[28,29] m. 4171C>A/p.L289M mutation in *ND1* is also found to be associated with MELAS and Leigh syndrome in addition to LHON.[30] Alzheimer's disease and Parkinson disease are due mitochondrial dysfunction associated with NADH dehydrogenase subunits 1, 2, 3, 4, 4L, 5, and 6 as a result of MT-*ND1* gene defect.[31] Among all the mutations mentioned for the *ND1* gene, most of them play a direct role in impairing the CI function thereby causing LHON.

MT-*ND4*

Apart from the MT-*ND1* gene mutations, another most common gene in which most mutations happen that leads to LHON is the mutation in the MT-*ND4* gene. This *ND4* is known as mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 4. This gene produces protein called NADH dehydrogenase 4 which also constitutes the CI of mitochondria. The gene is formed with 1378 bases with its genomic location starting from 10,760 bp from pter and ends at 12,137 bp from pter. The protein product of this gene has molecular weight of 51,581 Da with 459 amino acids. About 70% of LHON patients suffer due to mutation in this gene which impairs the CI of the mitochondria.

About 90% of people affected by LHON in Asian countries like China, Thailand, and Japan have point mutation G11778A in MT-*ND4* gene[32-35] which results in the substitution of arginine by histidine at the 340th position of the amino acid of *ND4*.[11] In a study conducted among the Chinese cohorts, many other mutations in the *ND4* gene like m.11204T>C, m.11430C>G, m.11213T>G, m.11447G>A, and m.10934G>A with the incidence of 0,312%, 0.078%, 0.078%, 0.078%, and 0.078%, respectively, were reported.[36,37] The 11447G>A

mutation is limited to the transmembrane domain whereas 11204T>C, 11430C>G, 11213T>G, and 10934G>A mutations are limited to the intermembrane domain, and all mutations in the *ND4* gene is reported to alter the structure of the polypeptide of that gene and thereby altering its function.[38,39] The penetrance of the LHON is found to be more in case of homoplasmic A4435G mutation in $tRNA^{Met}$ of LHON subjects having homoplasmic *ND4* G11778A mutation reported in the D5 Asian haplogroup.[40] However, the presence of homoplasmic G11778A alone in the LHON subjects has least penetrance level in causing vision loss.[41] Another study reported the presence of mutation A15951G in the $tRNA^{Thr}$ region has a major role to play in the expressivity and penetrance of LHON when present along with the G11778A mutation.[42]

Other diseases associated with mutation in the *ND4* gene are Leigh syndrome, by affecting the CI activity;[43] cardiomyopathy, and MELAS due to mitochondrial dysfunction;[44] sporadic myopathy;[45] Parkinson's disease, has more mutation in the *ND4* gene;[46] gastric cancer, has elevated expression of *ND4*;[47] oral cancer, due to mitochondrial dysfunction by mutation in *ND4*;[48] in acute myeloid leukemia, where the subjects had either germline or somatic *ND4* mutations;[49] in blood and breast cancer, mutation in the protein coding regions of *ND4* and other genes have been identified;[50] studies show that women with the G11778A mutation develop symptoms similar to multiple sclerosis which makes us to differentiate the disease that the patient actually has.

MT-*ND6*

MT-*ND6*, mitochondrial-encoded NADH:ubiquinone oxidoreductase core subunit 6 that codes for protein called NADH dehydrogenase 6 plays a major role in the CI of the mitochondria. The gene has a size of about 525 bases, and its location starts from 14,149 bp from pter and ends at 14,673 bp from pter. Many point mutations in the *ND6* gene have been reported and out of those T14484C mutation is found frequently in most LHON patients[51] which results in amino acid substitution from methionine to valine.[13] The 14484 mutation is homoplasmic in most LHON cases and found to have low penetrance level.[52] The 14484T>C mutation is found to be homoplasmic in most cases and is found to have an average LHON penetrance rate of 23.8% in Chinese family, and few other secondary mutations reported in the *ND6* are m.3497C>T and m.14502T>C.[53] A study on LHON showed complete penetrance in subjects harboring m.14841A>G of *ND6* in association with m.4171C>A of *ND1*.[54]

Out of the previously discussed mutations of T14484C, G3460A, and G11778A, the T14484C mutation show high-visual recovery and also have numerous epigenetic elements other than the genetic one in causing LHON.[55] Another mutation at the nucleotide pair 14459 in the *ND6* gene leads to the transition of base G to A, thereby replacing alanine by valine at the 72nd residue of the *ND6* which is proved to be the reason behind LHON.[56] This mutation in the *ND6* makes the CI more sensitive to ubiquinone and gets inhibited by it, and its analogs and thereby the neuronal activity gets hindered and cause LHON.[57] Other point mutations in the *ND6* that cause LHON are m.14495A>G, m.14568C>T, m.14482C>G/A.[25]

Mutation in the *ND6* not only causes LHON but other diseases also. Mutation G14569A in *ND6* is found to be the cause for autism subjects.[58] T14487C mutation in the *ND6* gene has been discovered in patient with Leigh syndrome that affects the stability and assembly of CI.[59] 13885insC and G13997A are two mutations in the *ND6* gene which results in high metastasis behavior of the cells resulting in tumor growth by overproduction of reactive oxygen species (ROS) leading to cancer.[60] In colorectal adenocarcinoma and in villous adenomas, the levels of expression of the *ND6* is much higher proving its relatedness with cancer.[61]

Therapeutics of Leber's Hereditary Optic Neuropathy

Therapeutics for any diseases usually involves the use of drugs to reduce the symptoms experienced by the patient. Although LHON is proved to be an untreatable disease with limited rate of success, the drugs that are used for clinical trials which show a potent recovery in patients with LHON, they are now researched further to determine their role in curing the disease. Being an inherited disease, LHON can be prevented from being inherited through genetic counseling to the couples who have a family history of LHON. Gene therapy is also another approach which helps in eliminating the disease-causing gene and replacing with the normal gene thereby preventing the disease.

Drugs for treating Leber's hereditary optic neuropathy

Idebenone

With the trade name Raxone®, Idebenone is a short-chain benzoquinone, which is used to treat visual impairment associated with LHON with its antioxidant nature and ability to carry the mitochondrial electrons to complex III of mitochondria directly thereby promoting the ATP production which in turn activates the ganglion cells of retina leading to vision recovery.[62] Visual recovery was reported in patients in a study with early and prolonged treatment of Idebenone in patients with acute LHON and it was best suggested that treatment with Idebenone should commence in early stages of LHON when there is still some RGC are active to get best vision recovery.[63-65]

EPI-743

EPI-743 is a drug designed to treat mitochondrial diseases that are caused due to defects in respiratory chain by targeting the minimum production of glutathione.[66] In LHON, EPI-743 is used to treat vision loss due to oxidative stress.[67] Use of the EPI-743 drug has proved to improve the vision in 70% patients with LHON without developing any adverse effects on the patients, proving its safe nature.[68] When compared with idebenone, the *in vitro* activity of EPI-743 has proved to be 1000 times more which makes the irreversible vision loss a reversible one.[69]

Bendavia

The ROS created by the altered proteins in LHON patient damages the mitochondria which are blocked by a drug called Bendavia also known as MTP-131. Being a tetrapeptide, this Bendavia is found to restrict the generation of ROS, ATP synthesis,[70] and also prevents the mitochondrial chain from uncoupling which in a study has been reported that the injury due to oxygen-glucose deprivation can be treated by using Bendavia.[71]

Genetic counseling for preventing Leber's hereditary optic neuropathy

After determining the exact mutation responsible for the LHON in affected subjects, the other members in the family

can be checked for the presence of mutation. Since the mutation responsible for LHON resides in the mitochondrial genome, the maternal inheritance pattern can be traced among the affected family members. They are given counseling regarding the inheritance of the mutated gene and are made aware about the problems that their future generation might face when they do not avoid the inheritance of the mutated gene.

Although genetic counseling helps in avoiding the inheritance of the mutated gene, there are two major limitations that are to be overcome to make this genetic counseling a successful one. The primary task is that it is not an easy job to determine a person having the mutated gene to develop the risk of optic atropy, and in secondary, not all the mutated genes in a heteroplasmic female is transferred to the next generation.[72] Also verifying the location of the mutation like homoplasmic or heteroplasmic is important in case of genetic counseling.[73]

Gene therapy for treating Leber's hereditary optic neuropathy

Other than the use of drugs to suppress the progression of the LHON in the affected subjects, an alternative way of treating the disease has been used, which is gene therapy, where the defective gene is replaced with the normal wildtype gene so that the normal gene product is expressed. The limitations of this technique lie in the fact that the introduced normal gene must be successfully integrated into the mitochondrial genome of the affected subject, and no technique is available presently to successfully integrate the normal gene into the mitochondrial genome.[74]

Gene therapy for LHON is still under clinical trials and one such trial (Phase I) involving adeno-associated virus as a vector of different doses showed slight improvement in the visual loss of LHON subjects with G11778A mutation of *ND4*, where no other major adverse events were observed but minor discomforts such as a sore throat, subconjunctival hemorrhage, keratitis due to exposure, and intraocular pressure were observed along with the presence of developed antibodies against the adeno-associated virus vector.[75] When these minor discomforts experienced by the subjects can be reduced, gene therapy would eliminate LHON completely. Another clinical trial for gene therapy in targeting the *ND4* LHON mutations in the retina of mouse and nonhuman primate models reveal that powerful and longer expression interval nature of adeno-associated viral vectors with added advantage of safety over other viral vectors.[76]

Allotropic expression is an advanced method used to overcome the difficulty faced with gene therapy. In allotropic expression, the nuclear version of the mitochondrial gene is constructed, and the proteins are synthesized cytoplasmically by targeting the sequence in the reading frame, and the mutation in the *ND4* has been corrected using this technique which is reported to improve the survival rate.[77] In a study of human therapy for LHON, the mRNAs of *ND1 and ND4* are localized on the membrane of mitochondria.[78] The same method can be used for other genes, thereby the LHON subjects affected with different mutations can be treated effectively.

Conclusion

The vision loss in LHON is mainly due to the oxidative stress experienced by the optic nerves by the impaired OXPHOS of the mitochondria due to mutations in the genes *ND1, ND4,* and *ND6.* The exact pathway in which the oxidative stress cause vision loss is still a debate. The treatments currently available reduce the stress in the optic nerves to some extent and do not completely eradicate the condition. Gene therapy is the major key to completely eradicate LHON. However, gene therapy is yet to be standardized as many limitations are present that prevent the technique from completely curing the disease. At present, many works are being done to improve the gene therapy, and in the future, the LHON will be completely cured without any complications, and other kinds of specified-targeted treatments will be available in curing any mitochondrial gene-related diseases.

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Conflicts of interest

There are no conflicts of interest.

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