Epidemiology of the Mitochondrial Disease Leber Hereditary Optic Neuropathy and Current, Emerging Treatments.

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Abstract

Mitochondrial diseases are genetic disorders that are characterized by dysfunction in the mitochondria and its genes. Such diseases are often caused by genetic mutations in mitochondrial DNA genes that specialize in encoding mitochondrial proteins, or proteins that are involved with/work with the mitochondria. Most mitochondrial diseases affect metabolic processes and multiple body systems. Leber Hereditary Optic Neuropathy disease (LHON) affects the central nervous system, and more specifically, the retinal ganglion cells (RGCs) located in the retina by degrading those specific cells. The mutations in the mitochondrial genes ND1, ND4, and ND6 of LHON subjects are found to increase the oxidative stress level produced by the optic nerve cells which in turn leads to rapid aging of the cells, degrading vision. These damaged and degraded retinal ganglion cells are then unable to send visual signals to the brain, hindering vision or even causing blindness in extreme cases. In this research, the details of specific genetic mutations, symptoms, outcomes related to vision, and possible and approved treatments are established. Specifically, idebenone, an approved drug in some countries, has shown data related to statistically significant recovery. Viral vector gene therapy, an upcoming treatment that is currently being clinically tested for LHON and more, shows promise in clinical trial results and may pose to be a viable treatment option in the near future. Through the research of LHON, stem cell therapy is further being studied as another treatment option that may propose a curative treatment option. Information in this research will be gathered from many recent sources focusing on clinical trials working with LHON disease and potential treatments/cures. Data and statistics along with trial analysis will be gathered and used as evidence in this research to support and fortify findings and information regarding potential treatments. LHON and other mitochondrial disease causing mutations should be thoroughly analyzed more, as specific mutations discovered may support treatments such as gene therapy, a genetic tool useful for the future.

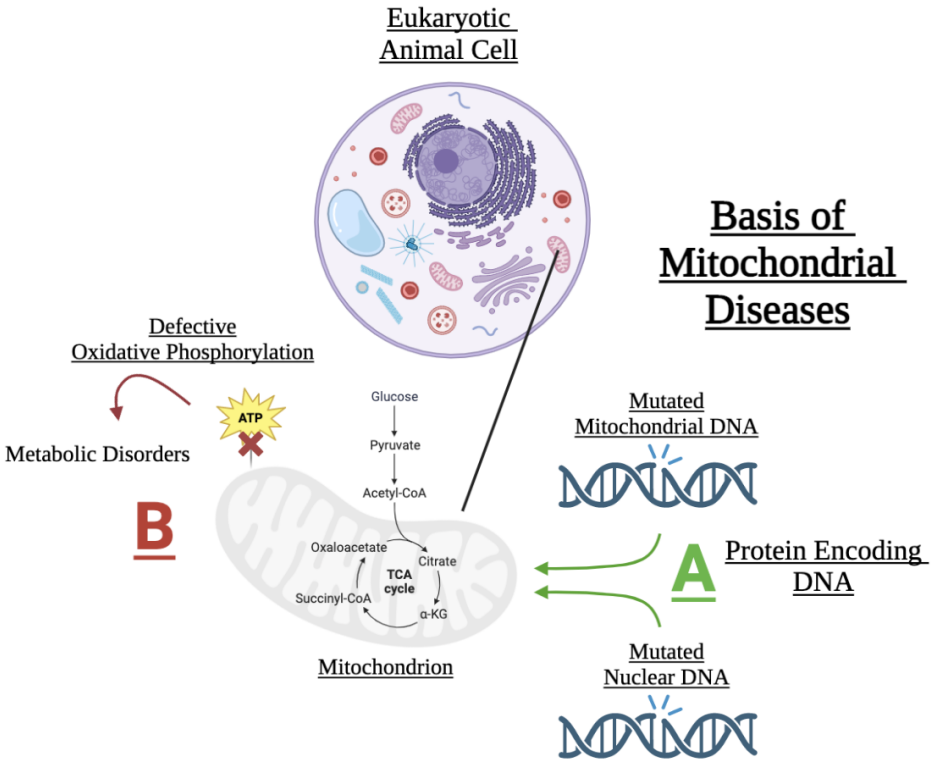
Introduction: Mitochondrial diseases and LHON genetics/epidemiology

A large number of people in Europe, Asia, North, and South America have been getting cases of unknown blindness and severe vision loss (1). Is this a mere curse of an unknown superstition or genetic science? The answer lies in mutations in the genome of mitochondria, some of the most important organelles in the cell. Mitochondria likely evolved from prokaryotes around 2 billion years ago and were engulfed by eukaryotic cells, becoming a functional

part of eukaryotic cells due to their survival advantage and harboring their own unique genome.

Mitochondria are membrane bound cell organelles that generate most of the chemical energy needed to power the cell's biochemical reactions. The role of mitochondria is so much more than just producing adenosine triphosphate through oxidative phosphorylation, a process where energy is farmed/harnessed from complex proteins located in their inner membrane. Other roles of the mitochondria include cell growth, cell death, calcium signaling, and cell cycle control. But what makes mitochondria stand out from other organelles is their own genome, mitochondrial DNA (mtDNA), which contains an extra 37 genes and include many vital proteins, 13 of them necessary for respiratory chain function. Unlike nuclear DNA (nDNA), mtDNA is only passed down maternally.

Like genes in nDNA, genes in mtDNA can acquire harmful mutations, too. Mutations in mitochondrial genes that encode for functional mitochondrial proteins can cause mitochondrial diseases (Figure 1). The onset of mitochondrial diseases has a bimodal distribution with the first peak occurring usually in the first three years of life and the second peak occurring around the third or fourth decade (adult onset diseases). Another big factor in mitochondrial diseases is heteroplasmy. Heteroplasmy plays an important role in the emergence of mitochondrial diseases and determines the success of the mitochondrial replacement therapy. Heteroplasmy is the presence of wild type and mutated genomes which are often a result of multiple mtDNA genomes in an individual cell. Heteroplasmy causes complications and is dangerous as it affects the severity of cellular dysfunction. The higher level of heteroplasmy one has, the more cells with high mutant mtDNA and lower number of cells with wild-type DNA. The make up of heteroplasmy determines the cellular phenotype.



A: Mutations in mitochondrial genes that encode for functional mitochondrial proteins can cause mitochondrial diseases.
B: mitochondrial diseases lead to a primary defect in oxidative phosphorylation, the process of adenosine triphosphate production.

Figure 1

The issue with mitochondrial diseases is that they are often sporadic and difficult to diagnose. Mitochondrial diseases typically affect more than one organ system and are relatively new to the world of medication, making it difficult for new, tested and proven therapies to work and be available to patients.

A common, yet advanced mitochondrial disease is Leber Hereditary Optic Neuropathy (LHON). LHON is a mitochondrial disease that is peculiar and different from other mitochondrial diseases. Although many mutations in the mtDNA cause LHON, three mtDNA point mutations; m.3460G>A, m.11778G>A, and m.14484T>C account for over 90% of LHON cases in most populations (2). These genetic mutations in the mitochondrial genes, ND1, ND4, and ND6 directly affect oxidative phosphorylation in the mitochondria

of LHON subjects, preventing the production of adenosine triphosphates to power RGCs. Although most mitochondrial diseases affect the immune system, LHON affects the central nervous system, visual system, and specifically retinal ganglion cells (RGCs) due to their exceedingly active metabolism and particular vulnerability to energy insufficiency (3). Retinal ganglion cells bridge neurons located in the retina of the eye. They collectively transmit image-forming and non-image-forming visual information from the retina in the form of action potential to several regions of the brain responsible for visual processing; RGCs bear the sole responsibility of propagating visual stimuli to the brain (4).

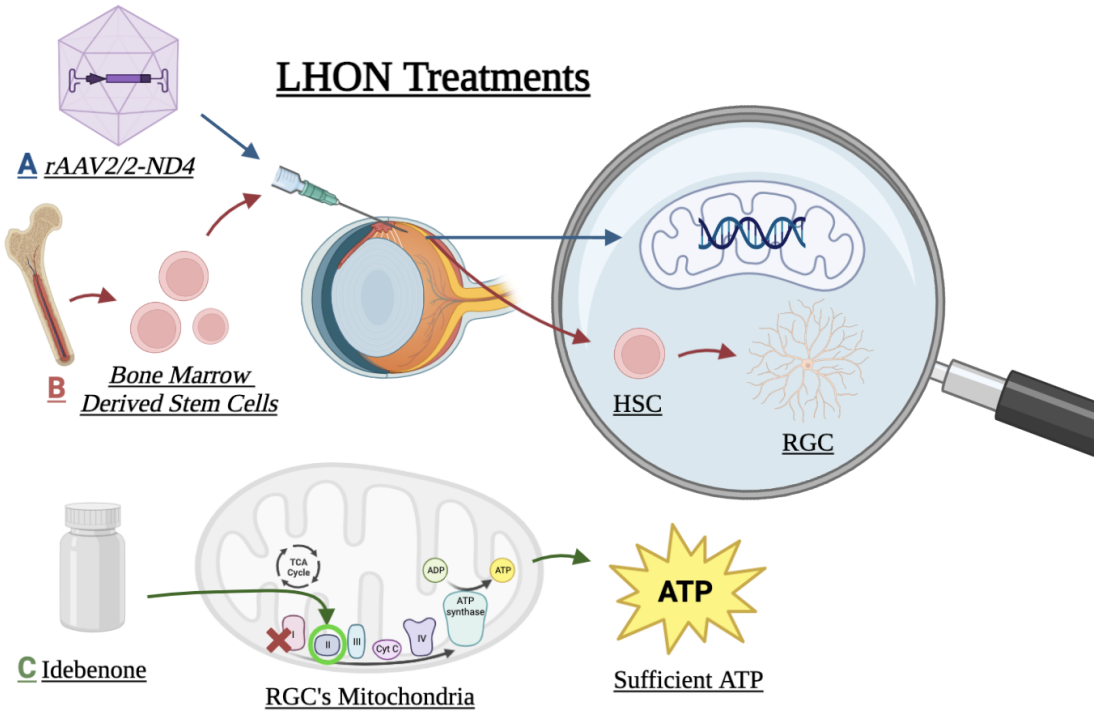
Although diseases usually involve pain and discomfort, LHON takes on its patients painlessly. Mutations in these genes are found to increase the oxidative stress level produced by the optic nerve cells (5) which in turn leads to rapid aging of the cells, nerve damage, and eventually apoptosis. Due to the dysfunction in the mitochondria of these RGCs, the RGC become damaged and send degenerated visual signals to the brain, causing vision loss or in severe cases, blindness. When it comes to the bimodal onset of mitochondrial diseases, LHON usually occurs during the second peak of the distribution in the third to fourth decade of life. Another unique feature of LHON is its sporadic case rate. Sporadic cases in diseases are cases when the mother is not affected by the disease, but the child gets LHON via randomized, haphazard mutations. In many clinical trials, the sporadic rate was as high as 44.8% (6). One striking feature of LHON is the unknown, marked incomplete penetration and sex-specific bias. Only ~50% of the males and ~10% of the females who harbor one of these mutations actually develop optic neuropathy/temporary or permanent vision loss in one eye.

LHON may be overlooked for its painless symptoms, but its effects are devastating. There have been many scientists looking for possible therapies and treatments for LHON, but since its discovery in 1871 (7), and difficult characteristics, finding an effective solution is far from today.

Current and Upcoming LHON Therapies

When it comes to treatment of mitochondrial diseases, including LHON, treatment and therapies are developing and forthcoming. As mitochondrial diseases have proven relatively difficult to treat in patients, there are currently few therapeutic options for treatment. The treatment of patients with mitochondrial diseases remains a challenge, but guidelines are available to manage the complications

of disease. Moreover, an increasing number of therapeutic options are being considered and several clinical trials are in progress (8). That being said, there are currently no cures to LHON, but there are therapies and treatments for LHON.



A: Adeno-viral vectors; viral vector *rAAV2/2-ND4* encodes the ND4 gene that is associated with oxidative phosphorylation back, restoring the production of ATP in the RGC's mitochondria.
B: Stem cell therapy; extracting stem cells out of the patient's bone marrow and inserting them into the retina to differentiate into RGCs
C: Idebenone; Idebenone bypasses complex I in the mitochondria electron transport chain and maintain ATP production Figure 2

Drug Therapies: Idebenone

A clinically tested drug and the only disease-specific drug approved to treat visual impairment in adolescents and adults with LHON is idebenone. Idebenone is an antioxidant drug that functions as a mitochondrial electron carrier; idebenone is a short-chain ubiquinone analog that easily enters the brain and reaches mitochondria. Idebenone has been shown to bypass complex I in the mitochondrial electron transport chain and maintain ATP production, and it inhibits lipid peroxidation to protect mitochondria from oxidative damage (Figure 2) (9), ultimately restoring RGCs that were both active or inactive (10). LHON related gene mutations

are found in complex I, specifically the gene ND4, which affects oxidative phosphorylation in the mitochondria, so idebenone was designed to override complex I. Although idebenone comes in many forms such as pills, serums, and creams, clinically tested and approved idebenone were only in the form of pills, specifically 900 mg/day.

To determine the efficacy of idebenone, double blinded, clinical trials were performed on the effects of the drug. With a sample size of 111 patients from a total of 38 different sites in 10 different countries, long term efficacy data was collected in favor of idebenone. The patients that were tested had LHON, previously diagnosed by physicians, and had experienced visual impairment in one eye within one year of enrollment. Given 900 mg of idebenone (pill) a day, patients were followed up every 3 months to test their visual improvement via Best-corrected visual acuity (BCVA) scale and log MAR measures. A BCVA scale refers to the measurement of the best correction of vision that can be achieved with the use of glasses or contact lenses. BCVA is an important predictor of treatment response in patients (11). LogMAR measurement is also used in many retinal diseases. A logMAR measurement is a score from a logMAR chart that provides more accurate results when compared to other visual acuity charts, and the results are more easily used in vision analysis, making it the preferred chart in clinical studies (12). For each participant, visual activity data and adverse events were recorded throughout the entire duration of the clinical trial. In the results, only 87 participants were recorded because they were carriers of the three major LHON mtDNA mutations: m.3460G>A, m.11778G>A, and m.14484T>C; a majority of the participants, specifically 71 out of the 87, were male, further demonstrating the puzzling gender bias of LHON. At time of analysis, all 87 patients carrying one of the three major LHON mutations provide long term data regarding idebenone and out of the 87 patients, 46% of the observed patients showed restoration in vision referred to as clinically relevant recovery (CRR) (13).

In a similar study more focused on a concentrated population, Dutch LHON patients who experienced visual decay in at least one eye and had a follow-up of more than 6 months after their treatment was started, were included in this clinical trial. Unlike many other LHON trials measuring the BCVA and visual improvements from using idebenone, the purpose of this study was to prove the efficacy of idebenone not only through visual function, but also through the thickness of the retinal ganglion cell layer. The treatment duration from different physicians involved in the trial had an average treatment time of 24 months, with some extremes

being 9 months and 38 months. In the results, data from 72 patients was used and CRR or vision stabilization, clinically relevant stabilization (CRS), occurred in 53% and 11% of the patients, respectively (14). Although the uses of idebenone are promising, treatments for mitochondrial diseases, including LHON, are still in clinical trials and are not fully approved yet.

Drug Therapies: Adeno-viral Vectors

Viral vector gene therapy is a common associated and upcoming treatment for genetic diseases, including mitochondrial diseases. Gene therapy uses the understanding of the body's genome, both nDNA and mtDNA. Gene therapy targets these variants/mutations and attempts to add DNA back, which then codes altered proteins or even produces new, modified proteins within the cell. The genetic material gets into the body with the help of a carrier such as a viral vector. In adeno-viral vector gene therapy, a common vector used is a virus (viral vector), as they are efficient in getting into the body's cells. These viral vectors are harmless and only serve the purpose of delivering the new genetic material into the cell. The viral vectors can be injected in two techniques: *in vivo*, by injection directly into the body, and *ex vivo*, taking the cell out and adding new vectors outside the body, then inserting it back intravenously. Although gene therapy is new and not fully tested, it provides promise to LHON due to its ability to repair malfunctioned/degenerated DNA that encodes RGCs.

In terms of LHON, the three main point mutations, m.3460G>A, m.11778G>A, and m.14484T>C affect 90% of patients suffering with the condition; out of these three point mutations, m.11778G>A is the most common mutation worldwide (15), and therefore, more treatments have been clinically tested on the m.11778G>A mutation more than any other mutation in mtDNA. In many clinical trials, gene therapy is delivered using an *in vivo* technique, or intravitreal injection, an injection that places medication or adeno-viral vector into the space at the back of the eye called the vitreous cavity due to the difficult location of RGCs and also difficult to attempt an *in vivo* technique approach.

The adeno-viral vector used in LHON gene therapy is called *rAAV2/2-ND4,* this encodes the ND4 gene that is associated with oxidative phosphorylation/production of adenosine triphosphates in complex 1 of the mitochondria (16). By restoring the ND4 gene, hopes are to recover the respiration process of oxidative phosphorylation, which in turn will generate more ATP to power RGCs in the eye, improving vision (Figure 2) (9).

The efficacy of *rAAV2/2-ND4* was tested by comparing an experimental group (a group that is receiving the *rAAV2/2-ND4* adeno-viral vector) and a control group which is given a *sham* shot. Both groups are checked up twice during the 96 day clinical trial; check ups occurred on week 48 and 96 and check up progress was measured through log MAR chart and best-corrected visual acuity (BCVA) scale. The patients who were tested had the m.11778G>A mtDNA mutation and vision loss within six months of onset/treatment. In the results, the proportion of eyes with an improvement from onset of the trial to week 96 was 63% for rAAV2/2-*ND4*–treated eyes and 55% for *sham*-treated eyes (percentages in improvement of LogMar scores); improvement was not statistically significant. (17).

The safety of *rAAV2/2-ND4* also comes into suspicion as ethical and safety approval concerns come into the question. In a similar trial, nine patients with the m.11778G>A mutation were tested for the safety of *rAAV2/2-ND4.* This trial also used an *ex vivo* method via intravitreal injection in an eye of the patient to inject the adeno-viral vector into the patient's eye. As the patients were tracked for nine months, check ups included urine and blood sample tests as well as log MAR tests to track visual improvements in the patients (18). The patient's eye that was injected was compared to the uninjected eye for results. At the end of the clinical trial, statistical significance in improvement was shown in six out of the nine patients and in the other three, log MAR scores remained the same. None of the nine patients had local or systemic adverse events related to the vector during the 9-month follow-up period. These findings support the feasible use of gene therapy for LHON and furthermore, supports its safety.

Upcoming Drug Therapy: Stem Cells

Stem cell therapy is an upcoming treatment idea that has not been clinically tested frequently. This field of therapy is the newest and most upcoming treatment method compared to listed or approved medications such as idebenone, and even gene therapy based *rAAV2/2-ND4*. Stem cells are unspecialized cells of the human body. They are able to differentiate into any cell of an organism and have the ability of self-renewal (19). Stem cells have certain characteristics that define the cell type and one commonly studied group of stem cells are multipotent cells: stem cells that can develop into more than one specific cell type. In the case of LHON specific research, autologous bone marrow-derived stem cells (BMSCs) were the target stem cell of approach. BMSCs are stem cells with characteristics that are favorable for regenerative

therapy, which have been widely described including their multipotency, anti-inflammatory and immune-modulatory properties. BMSCs are capable of differentiating into neuron-like cells. They have been demonstrated to provide neurotrophic factors that promote the regeneration of axons and protect retinal ganglion cells and to integrate into existing neural networks thus re-establishing neural connections (Figure 2) (20). Furthermore, the release of paracrine factors by BMSC influences the surrounding microenvironment, which is a characteristic of particular interest for organ repair (21). BMSCs are used in LHON specific treatment because of the "repair" characteristic that these stem cells have.

. The idea of stem cell therapy in LHON or visual improvement can be described as utilizing BMSCs to treat optic nerve and retinal diseases. BMSCs can be delivered into the eye in many ways including intravitreal injections. (20). Once injected, the BMSCs will develop into RGCs and therefore, promote the production of ATP, further improving vision. What's promising about stem cell therapy is not just that it's a new and emerging idea, but that stem cell therapies have already been applied to the treatment of many diseases affecting the ocular surface, leading to preservation of vision .

SCOTS, Stem Cell Ophthalmology Treatment Study, was an open labeled, non-randomized, efficacy study. In this study, there was no placebo or *sham* shot given to patients included in the clinical trial. Three groups of patients were made in order to target observations that were focused on during the trial: the cause of vision loss, associated risk factors for the treatment, and the patient's medical risk status. A total of five patients were observed in the clinical trial. In all five LHON patients, there were improvements in visual acuity and peripheral vision. Several of the eyes experienced dramatic, persistent increases in visual acuity attributable to the BMSC treatment in SCOTS (20). Importantly, visual improvement was due to the revitalization of already existing mitochondria function and the transfer of more viable mitochondria into existing neuron cells. In SCOTS, there were no recorded adverse events, nor any side effects of the therapy.

Discussion

Proposed LHON treatments are all upcoming and have no over the counter, or even prescribed access, treatments with the exception of idebenone. In general, mitochondrial diseases are very difficult to cure due to the vast majority of body systems they target and their genetic aspects.

Mitochondrial diseases are new to epidemiology compared to airborne diseases, respiratory diseases, and autoimmune diseases, therefore, treatment is all upcoming and clinical testing is necessary. The difficulty and even failure of clinical trials in mitochondrial diseases partly relates to the inefficacy of the compounds studied. However, it is also likely to be a consequence of the significant challenges faced by clinicians and researchers when designing trials for these disorders, which have historically been hampered by a lack of natural history data, biomarkers and outcome measures to detect a treatment effect (22).

The need to treat mitochondrial diseases open many doors to potential discoveries using gene therapy, gene editing, and even stem cells; Although many treatment and therapy trials are currently under testing, it is important to understand that they are far from done, as new information regarding the mitochondrial disease can be discovered. Therefore, treatment for mitochondrial diseases remains largely symptom-based and does not significantly alter the course of the disease, however, clinical research evaluating agents that target different aspects of mitochondrial dysfunction is promising and is expected to generate more therapeutic options for these diseases in the future (23).

Gene therapy, throughout the course of its history in LHON, has only been significantly acknowledged regarding the m.11778G>A mutation, and although this mutation is the most common mutation in LHON, there are two other main point mutations that are heavily present in patients diagnosed with LHON: m.3460G>A and m.14484T>C. In the clinical trial of (17), the viral vector, *rAAV2/2-ND4,* was used to specifically target the m.11778G>A mutation by encoding the ND4 gene that is associated with oxidative phosphorylation, the production of adenosine triphosphates in complex 1 of the mitochondria (16), restoring RGCs' ability to carry out oxidative phosphorylation. However, this treatment idea is very selective. New treatment studies can be done and clinically tested on other common LHON mutations, including m.3460G>A and m.14484T>C. Viral vector based gene therapy is a promise that isn't only restricted to the 11778 mutation, but viral vectors can be made to specifically target other mutations as well. Clinically testing new viral vectors will not only further research findings, but also propose an alternative solution to LHON affected patients, if the efficacy of *rAAV2/2-ND4* is not statistically significant.

Another important aspect of clinical trials is the sample size relevant to the population. In the stem

cell therapy (SCOTS) and viral vector gene therapy related clinical trials, the sample size of the trial was very small. However, when put in context, another reason why mitochondrial diseases are difficult to research and perform successful clinical trials is because of the rarity of the disease and the willingness of subjects diagnosed with mitochondrial diseases to participate due to the unknown risk. While having a low sample size for clinical trials is understandable, discoveries may be more significant and valuable if the sample size was larger, in the SCOTS case study, especially.

Stem cells are like a blank slate, ready to specialize into any cell necessary. The idea of SCOTS was to utilize autologous bone marrow derived stem cells to differentiate into healthy RGCs to increase the number of RGCs, outpopulating the previously existing RGCs that are affected by LHON mutations, further improving vision. Stem cells in SCOTS were derived from the patient's own bone marrow, but stem cells derived from a healthy sibling's bone marrow have not been clinically tested yet. In Sporadic LHON cases, a healthy sibling's bone marrow stem cells can be used, as the chance of mutation can be reduced. The question of using a healthy sibling's bone marrow derived stem cells can be put to use, though there are many conditions involved such as the patient's LHON case must be sporadic and the sibling must be healthy. Sporadic LHON cases are not uncommon especially in certain haplogroups (6), therefore the application of healthy sibling's bone marrow for LHON treatment is a possibility in the future. These healthy stem cells provided from an unaffected sibling could have a promising and more effective result compared to autologous bone marrow. Stem cells can also be derived from the umbilical cord. These hematopoietic stem cells (HSC) for transplantation in many blood-related diseases are mainly obtained from adult bone marrow, umbilical cord blood, and mobilized peripheral blood (24). In the case of LHON, focusing on the umbilical cord HSC would be more appropriate as it is not clinically tested and focused currently. Another issue with HSC is their ability to differentiate into nerve cells (RGCs). Although many studies supported that bone marrow mesenchymal stem cells (BM-MSCs) can differentiate into neural cells, few researchers detected maturity and function of nerve cells, especially in *in vivo* study (25). Although the circumstances may be challenging due to the patient's ability to reject the incoming stem cells and compatibility with the received stem cells, HSC and other stem cells should be looked at more as they pave a way for hopeful scientific breakthrough regarding different therapies and treatments.

Mitochondrial disease treatments are currently being clinically tested by clinicians and doctors, as they may provide hopeful discoveries in treating patients suffering from them. Although many mitochondrial diseases, including LHON, are young to the world of epidemiological studies, many current treatments such as idebenone and gene therapy or stem cells have provided encouraging results (16), not only leading to a new era of curable LHON, but new eras of biotechnology, disease treatments, and understanding mitochondrial diseases and curable aspects of them.

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