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New avenues for therapy in mitochondrial optic neuropathies

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Abstract

Mitochondrial optic neuropathies are a group of optic nerve atrophies exemplified by the two commonest conditions in this group, autosomal dominant optic atrophy (ADOA) and Leber's hereditary optic neuropathy (LHON). Their clinical features comprise reduced visual acuity, colour vision deficits, centro-caecal scotomas and optic disc pallor with thinning of the retinal nerve fibre layer. The primary aetiology is genetic, with underlying nuclear or mitochondrial gene mutations. The primary pathology is owing to retinal ganglion cell dysfunction and degeneration. There is currently only one approved treatment and no curative therapy is available. In this review we summarise the genetic and clinical features of ADOA and LHON and then examine what new avenues there may be for therapeutic intervention. The therapeutic strategies to manage LHON and ADOA can be split into four categories: prevention, compensation, replacement and repair. Prevention is technically an option by modifying risk factors such as smoking cessation, or by utilising pre-implantation genetic diagnosis, although this is unlikely to be applied in mitochondrial optic neuropathies due to the non-life threatening and variable nature of these conditions. Compensation involves pharmacological interventions that ameliorate the mitochondrial dysfunction at a cellular and tissue level. Replacement and repair are exciting new emerging areas. Clinical trials, both published and underway, in this area are likely to reveal future potential benefits, since new therapies are desperately needed.

Plain language summary

Optic nerve damage leading to loss of vision can be caused by a variety of insults. One group of conditions leading to optic nerve damage is caused by defects in genes that are essential for cells to make energy in small organelles called mitochondria. These conditions are known as mitochondrial optic neuropathies and two predominant examples are called autosomal dominant optic atrophy and Leber's hereditary optic neuropathy. Both conditions are caused by problems with the energy powerhouse of cells: mitochondria. The cells that are most vulnerable to this mitochondrial malfunction are called retinal ganglion cells, otherwise collectively known as the optic nerve, and they take the electrical impulse from the retina in the eye to the brain. The malfunction leads to death of some of the optic nerve cells, the degree of vision loss being linked to the number of those cells which are impacted in this way. Patients will lose visual acuity and colour vision and develop a central blind spot in their field of vision. There is currently no cure and very few treatment options. New treatments are desperately needed for patients affected by these devastating diseases. New treatments can potentially arise in four ways: prevention, compensation, replacement and repair of the defects. Here we explore how present and possible future treatments might provide hope for those suffering from these conditions.

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Introduction

Mitochondria are present in all of the cells in our body. They are tubular-shaped organelles consisting of an outer and an inner membrane separating the organelle into two compartments. They contain their own DNA known as mitochondrial DNA (mtDNA), with only 37 genes. Thus, the majority of gene products that are needed in mitochondria are encoded by nuclear DNA and imported from the cytoplasm.[1] This means that both mtDNA and nuclear DNA mutations can play a role in causing a mitochondrial disorder, in this case mitochondrial optic neuropathy. Mitochondria arose from a eubacterial ancestor two billion years ago, which may help explain the presence of mtDNA.[2] Mitochondria occupy up to 20% of the cytoplasmic volume of a eukaryotic cell.[3] Moreover, mitochondria form networks by fusing their inner and outer membranes together; consequently, any defects in this process will lead to dysfunction.[4] They play an essential cellular role in adenosine triphosphate (ATP) production through the Krebs cycle (also known as citric acid cycle) occurring in the matrix, and oxidative phosphorylation occurring at the inner membrane of the mitochondria.[5]

The key structural components in oxidative phosphorylation form the electron transport chain, which is made up of complexes I, II, III, IV, and ATP synthase (also known as complex V) present in the inner membrane. Reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide are the two important products from the Krebs cycle. They are used in oxidative phosphorylation by complex I and II respectively,[6] where they undergo oxidation by donating electrons to their respective complexes, releasing large amounts of energy. This allows protons to be pumped into the intermembrane space from the matrix, creating an electrochemical gradient, which is used to convert adenosine diphosphate and inorganic phosphate to ATP. Complexes I and II pass electrons to ubiquinone, also known as coenzyme Q10, which then, in its reduced form, becomes an electron donor for complex III. Complex III transfers electrons to the oxidised form of cytochrome c. The reduced form of cytochrome c is a substrate for complex IV, which oxidises it while reducing molecular oxygen to water. Therefore, ubiquinone and cytochrome c can be seen as electron shuttles.[3,7,8] As a result, any problems with any of the four complexes forming the electron transport chain or the electron shuttles may possibly contribute to the development of mitochondrial disease.

Optic neuropathy involves the hallmark symptoms of visual acuity loss, visual field defect, dyschromatopsia and abnormal pupillary response and can have many aetiologies, for example demyelinating, inflammatory, ischaemic, traumatic, compressive, toxic/nutritional and hereditary causes.[9] The most common types of hereditary optic neuropathies are autosomal dominant optic atrophy (ADOA) and Leber's hereditary optic neuropathy (LHON).[10] However, all types of inheritance are observed in hereditary optic neuropathies, mitochondrial, autosomal dominant, autosomal recessive and possibly X-linked. Coincidentally, both ADOA and LHON result in mitochondrial alteration which leads to the dysfunction and loss of the retinal ganglion cell (RGC) population, ultimately leading to bilateral visual loss.[11] Despite sharing similar putative mechanisms, these two conditions have different genetic aetiologies. A prominent subset of ADOA is caused by mutations in the nuclear gene *OPA1*, which plays an important role in mitochondrial fusion, and accounts for over 50–75% of ADOA.[12] In LHON, the top three most common mitochondrial DNA mutations are m.3460G>A, m.11778G>A and m.14484T>C.[13] In addition, the nuclear gene *DNAJC30* has recently been found to cause recessive LHON.[14] Environmental factors can also play an important role in the development of LHON and ADOA, leading to variable or incomplete penetrance.[15]

The underlying aim for current therapies for both ADOA and LHON is to improve general mitochondrial function and thus preserve RGCs. Current and possible future treatments for ADOA and LHON can be split into four types, namely prevention, compensation, replacement and

repair. Finding effective therapies for ADOA and LHON would not only improve the lives of those suffering from these conditions but would shine light onto potential new therapies for glaucoma, as research suggests a possible role for mitochondrial dysfunction in this disease too.[16] This review sets out to highlight areas of novel potential therapeutic intervention, where there is some theoretical basis for the therapy. We reviewed the literature published in English, including all sources without time limits, with a targeted review approach.

LHON

The prevalence of LHON is estimated to be around 1 in 31,000–1 in 50,000.[17–19] The prevalence in adult males is significantly higher than that in women, which increases to around 1 in 14,000 when considered separately.[17] The three most common mitochondrial DNA mutations are m.3460G>A, m.11778G>A and m.14484T>C.[13] They code for different polypeptides in complex I, namely ND1, ND4 and ND6 respectively, and are responsible for 90–95% of all cases of LHON.[13] The remaining 5–10% are due to rare genetic variations affecting complex 1 subunits (ND1)[20] and other rare genetic causes such as mutations in the nuclear encoded mitochondrial gene *DNAJC30*. DNAJC30 interacts with the mitochondrial ATP synthase machinery and facilitates ATP synthesis as well as acting as a chaperone in exchanging defective complex 1.[14,21]

Studies have shown that the gender difference could be attributed to the metabolic regulation of oestrogens that directly or indirectly regulate mitochondrial metabolism.[22,23] Furthermore, recent studies have documented that X-linked nuclear modifiers (PRICKLE3, a mitochondrial protein linked to biogenesis of ATPase) modify the phenotypic manifestation of LHON.[24]

A number of factors are thought to play a role in terms of penetrance of LHON. Families with LHON tend towards homoplasmic mutations over the generations, but penetrance can still vary. The respective mutations show incomplete penetrance; disease manifestation may be triggered by environmental factors and disease course may be modified by environmental factors. For example, alcohol and smoking, especially high consumption, have been associated with the onset of vision loss in some LHON carriers.[25,26] In addition, some individuals who do not develop visual loss are effectively 'LHON carriers'. There may be genetic compensation at play, with the mtDNA copy number in key tissues upregulated by a range of mechanisms as yet not completely understood, although this remains controversial.[27,28] In addition to the penetrance of the mutations the synergistic effects of other rarer additional gene mutations[27,29,30] as well as environmental factors may all be contributors to the severity of LHON.

The clinical presentation of LHON can be separated into acute and chronic phases, whereby the acute phase is defined as within 6 months of the start of the disease and the chronic phase is longer than 6 months. In addition to the classic optic neuropathy symptoms described above, in the acute phase, patients most commonly present with acute painless loss of central vision. In 25% of patients, the loss of central vision will be bilateral at first presentation and the remaining patients will have loss of central vision in the other eye on average within 6–8 weeks of the vision loss in the first eye.[31] Visual field defects are seen in the central field with colour vision also severely affected but there are relatively well preserved pupillary light responses.[32] The majority of patients will present with symptoms in their 20s and 30s, with a mean age of 22 years, and the vast majority (>95%) of the carriers of LHON who go on to lose vision will experience a visual defect before 50 years of age. However, approximately 50% of males and 80% of females may never lose vision.[17]

The primary hallmark of LHON is preferential degeneration of papillomacular RGCs associated with impaired colour perception and central scotoma revealed by visual field testing and fundoscopy. The relative sparing of melanopsin RGCs, which contribute to the circadian photo-entrainment through retinal projections to the suprachiasmatic nucleus and to the pupillary light reflex through projections to the olivary pretectal nucleus, means that these functions remain.[33] Up to 50% of acute LHON patients have a normal optic disc while others may manifest circumpapillary telangiectatic microangiopathy, and elevation/swelling of the retinal nerve fibre layer (RNFL) around the disc (pseudo-oedema) confirmed by the absence of leakage from the disc or papillary region on fluorescein angiography and tortuosity of retinal vasculature.[32,34] While in the acute phase the RNFL thickens, it eventually

becomes thinner in the chronic phase.[35] Other changes, including optic nerve pallor and pathological cupping of the optic disc due to extensive loss of RGCs axons, will be observed in the chronic phase.[36]

LHON generally carries a very poor visual prognosis, with permanent vision loss. Only a small number of patients recover any vision, with a limited improvement of central visual acuity and colour vision, which can take many years.[36] The age of onset plays a role in prognosis.[37] It is more favourable for earlier age of onset, with the T14484/ND6 mutation having the highest rate of spontaneous visual recovery when onset is before 20 years of age.[37] More importantly, the genetic defect itself also plays an important role in prognosis, with the highest rate of spontaneous visual recovery in patients with T14484C mutation and the least in patients with G11778A mutation.[38]

ADOA

The prevalence of ADOA is estimated to be around 1 in 10,000–1 in 50,000, with a higher prevalence in the Netherlands of 1 in 12,000 and 1 in 10,000 in Denmark due to a founder effect. This makes it the leading cause of inherited optic neuropathy, for which there is presently no treatment available. Unlike LHON, there is no gender bias.[38–40]

About 57–75% of ADOA patients carry a mutation in the *OPA1* gene. It is estimated that 1% of ADOA patients carry an *OPA3* gene mutation and the remainder of the patients are accounted for by other genes, thus making *OPA1* gene mutation the most common mitochondrial gene mutation for ADOA.[35,36] Other loci which are identified and known to be causative in ADOA include OPA4, OPA5 and OPA8. *OPA1* is responsible for mitochondrial fusion/fission, energy metabolism, control of apoptosis, removal of calcium and maintenance of mitochondrial DNA, while *OPA3* is responsible for energy metabolism and control of apoptosis.[40] Genetic deficiency of Opa1 is associated with mitochondrial fragmentation and impaired respiratory capacity, as reported in primary cell cultures of human fibroblasts and murine RGCs.[41,42] Analysis of the B6;C3-Opa1Q285STOP mouse model reported progressive pruning of predominantly ON-centre RGC dendrites alongside deterioration of visual acuity beginning at 10–12 months in heterozygous Opa1$^{+/-}$ mice.[43,44] Opa1 mutants exhibit selective atrophy of high energy consumption glutamatergic synapses, while OFF-centre RGCs with GABAergic synapses, which have a lower metabolic demand, are relatively unaffected.[44,45] The fact that Opa1 deficiency seems to preferentially target cells with higher metabolic demand may explain why the mutation is associated with optic atrophy, as RGCs have one of the highest metabolic demands of any cell in the body.[46]

The clinical presentation of ADOA is a slowly or insidiously progressive, bilateral loss in visual acuity, normally beginning in early childhood with a mean age of onset of 6–10 years.[36,47] Patients typically present with a progressive symmetrical bilateral visual loss, temporal optic nerve pallor, central, centrocaecal and paracentral scotoma and colour vision deficit.[40,48] ADOA can present with a range of severity from being asymptomatic to being legally blind. Despite these changes, as in LHON, pupillary light reflexes are relatively well preserved.[49] These clinical symptoms are associated with a loss of RGCs and subsequent atrophy of the optic nerve.[48]

The main difference between ADOA and LHON is that the onset of symptoms is not acute and overall patients with ADOA have a relatively better prognosis. Moreover, ADOA has a nuclear gene origin affecting mitochondrial function while LHON usually has a mtDNA origin. Thinning of the RNFL can be observed due to the loss of retinal nerve fibres and is a significant clinical feature.[50]

Therapeutic interventions

At the moment, there are no curative therapies in approved use for either LHON or ADOA. However, there is one approved and reimbursed disease modifying therapy: idebenone. Novel pharmacological and non-pharmacological therapies are under investigation, and some are in development. The therapeutic strategies to manage LHON and ADOA can be split into four broad categories: prevention, compensation, replacement and repair.

Prevention

Between 90% and 95% of all LHON cases are owing to m.3460G>A, m.11778G>A or m.14484T>C mtDNA mutations. Of ADOA patients, 57–75% carry a mutation in the *OPA1*

gene. However, it is worth noting that one study shows that at least 8% of all LHON cases are sporadic while another study suggests that sporadic cases with *de novo* mutation and unknown familial history account for up to 50% of all patients presenting with ADOA.[40,51]

In those who give birth using *in vitro* fertilisation (IVF), embryo screening and selection could be a theoretical option. The strategy involves identifying high risk genes and mutations, alongside other rarer genes, and other mitochondrial mutations for LHON in preimplantation genetic testing (PGE) during IVF. Screening and selection of embryos based on the aforementioned mutations may reduce the risk of developing these neuropathies later in life.[13,39,52] The main advantage of this approach is that this would be highly effective. On the other hand, it is unlikely to be widely acceptable and applicable, due to the need for IVF with this approach. In addition, due to the highly variable clinical phenotypes of mitochondrial optic neuropathies (MONs) and the non-life-threatening nature of the conditions, it is not clear whether it is warranted.[53] Another strategy is the use of mitochondrial donation by using a donated egg or zygote with healthy mitochondria, removing the nucleus and replacing it with the nucleus from the egg or zygote from the affected mother.[54] There are two main methods being used in the UK, namely maternal spindle transfer (ST), which involves transferring spindle-chromosome complexes from donors, and pronuclear transfer (PNT), which involves transferring pronuclei from donors.[55] MtDNA carryover in PNT blastocytes is less than 2%[56] while the carryover in ST is less than 1%.[57]

Aside from ethical issues, inheriting disease-associated mutated DNA does not mean that the individual will develop vision loss in LHON or ADOA, due to incomplete penetrance. For example, the estimated penetrance in *OPA1* can be as low as 43%.[58] Moreover, parents and doctors need to assess the risk–benefit of PGE, as the rate of a successful pregnancy is significantly decreased by 15.6% for women who are aged 38 years and above when compared with those in a control group without PGE.[59] Moreover, the high cost and the difficulty in mitochondrial transfer are two major limitations for mitochondria donation.[60]

In terms of preventing clinical disease, it is worth considering environmental factors, which have been shown to play an important role in the triggering or evolution of vision loss. Smoking, excessive use of alcohol and living in an environment with high pollution will increase the risk of oxidative stress induced damage to the body and mitochondrial function of the RGCs. Phenocopies of mitochondrial optic neuropathy such as the Cuban Epidemic Optic Neuropathy, and 'tobacco–alcohol amblyopia', have been reported in the literature, caused by alcohol and tobacco consumption leading to folate and vitamin B12 deficiency, leading to optic neuropathy.[61–64] Furthermore, epigenetic factors such as exposure to toxins (n-hexane and other organic solvents), various forms of smoke (including rubber tyre fires), drugs [erythromycin, ethambutol (antituberculosis medication)] and nucleoside analogues (antiretroviral therapy) are known to cause optic neuropathy leading to bilateral vision loss.[26,61,65–69] In particular, multiple studies have shown that alcohol consumption at high levels, especially binge-drinking, and smoking tobacco have a strong association with more severe symptoms and prognosis in both LHON carriers and ADOA patients with *OPA1* mutation.[25,70,71] Both tobacco and ethanol can lead to a thiamine (vitamin B1) deficiency, which is essential for metabolism.[68] Vitamin B1 also prevents glyoxal toxicity, which causes oxidative stress and mitochondrial toxicity.[72]

Compensation

Quenching of reactive oxygen species (ROS) that accumulate owing to respiratory chain dysfunction in mitochondrial optic neuropathy is one potential therapeutic strategy. Small molecules that possess antioxidant properties, such as coenzyme Q10 (CoQ10), MitoQ (a quinone analogue targeted to mitochondria), cysteine, EPI-743 (hydrolysed form of vitamin E, alpha-tocotrienol), JP4-039 (mitochondria-targeted nitroxide) or a mitochondrial cocktail including cyanocobalamin, folic acid, ascorbic acid, alpha-lipoic acid, acetyl-L-carnitine, creatine monohydrate, riboflavin, alone or in combination, have been widely tested for therapy in mitochondrial disorders, with limited success.[73–77] However, other small molecules that protect mitochondrial cristae and oxidative phosphorylation function by enhanced ATP synthesis, such as MTP-131 (elamipretide), have also been tested in mitochondrial disorders.[78,79]

Historically the lack of effective treatments has led to a range of symptomatic therapies aimed at delaying the progression of the disease.[80] One such approach has been to use a high dose of a variety of vitamins and co-factors, called a 'mitochondrial cocktail', comprising variably CoQ10, vitamin E, folic acid, L-carnitine, creatine and vitamins B2 and B1. This approach has not given rise to significant clinical benefit.[81,82] More recently a range of new small molecules are under clinical investigation.[83] These compounds can be classified by their targets and pharmacological activity into the following groups:

- Compounds which increase the rate of oxidative phosphorylation, thereby increasing ATP production;
- Antioxidants that reduce ROS levels;
- Compounds that influence mitochondrial biogenesis.

One such example is L-carnitine, which is an amino acid. It has a role in transporting long-chain fatty acids across the inner mitochondrial membrane.[84] L-carnitine is a Food and Drug Administration (FDA) approved compound; however, little direct evidence supports its use in LHON or ADOA.

Other examples are the compounds AICAR, bezafibrate and rapamycin, which function to induce mitochondrial biogenesis,[81] and thus increase the mass and number of mitochondria in cells.[85] Mitochondrial biogenesis is regulated by the transcriptional activator PGC-1α[85] Bezafibrate and other mitochondrial biogenesis activators can activate PGC-1α and consequently mitochondrial biogenesis. However, these molecules have not so far been clinically evaluated with robust data for the treatment of mitochondrial optic neuropathies.[85]

Finally, ubiquinone, which participates in aerobic respiration as an electron carrier (electrons from complex I and II to complex III), and is also an anti-oxidant, is another potential therapeutic.[86] It is reported to have been used as a food supplement which may improve the efficiency of electron transfer, ATP rescue and reduce oxidative stress.[87] *In vitro* studies show that ubiquinone in its reduced state could reduce the lipid peroxidation of mitochondrial membranes.[87,88] However, studies show that CoQ10 administration in patients is ineffective owing to its poor bioavailability.[84]

Ubiquinone analogues, for example, MitoQ, EPI-743 and idebenone,[89] have been subject to evaluation in order to overcome the poor pharmacokinetic properties of ubiquinone itself. These analogues may increase ATP production, directly interacting with the electron transport, and/or act as an antioxidant reducing ROS level. Among them, idebenone is the only drug approved by the European Medicines Agency[90] for the treatment of LHON. The RHODOS study[91] found that idebenone was safe and well tolerated. Whilst the primary endpoint of best recovery in visual acuity did not reach statistical significance in the intention to treat population, *post hoc* analysis did show a response in patients with discordant visual acuities at baseline. Recently, real-world evidence[92] has been published from an open-label, multicentre, retrospective, non-controlled analysis of long-term visual acuity and safety in 111 LHON patients treated with idebenone (900 mg/day) in an expanded access programme. This study has shown that the average gain in best-corrected visual acuity for responders is equivalent to more than seven lines on the Early Treatment Diabetic Retinopathy Study chart. Furthermore, 50% of patients who had a visual acuity below 1.0 logMAR in at least one eye maintained their vision.

There is evidence that NAD(P)H:quinone oxidoreductase 1 (NQO1), a cytosolic enzyme, plays a critical role in the pharmacological activation of idebenone.[93,94] However, the lack of this enzyme in target cells has contributed to the limited efficacy of the drug in neurodegenerative disease treatment and might also have an adverse impact on off-target cells with low expression of NQO1, increasing superoxide generation.[94,95] The drug EPI-743 (vatiquinone) targets NQO1, leading to an increase in cellular glutathione concentration and consequently reducing oxidative stress. EPI-743 showed approximately 1000 times greater activity than idebenone in protecting cells from oxidative damage *in vitro*.[96] EPI-743 has shown some promise, albeit in only one open-label trial with five patients.[97]

Another NQO1-dependent prodrug is KL1333, a novel NAD^+ modulator. KL1333 increases the NAD^+/NADH ratio, improving the cellular redox state and increasing the cellular bioenergetics.[98] KL1333 is currently in phase I clinical trial for Mitochondrial Respiratory Chain Deficiencies (NCT03888716). The potential of NQO1 as a key enzyme for the pharmacological activation of

drugs has led to the identification of a series of new redox-active molecules, which show high activity, potentially better than idebenone, *in vitro* and *ex vivo*.[99,100]

A molecule with a different mechanism of action is elamipretide (MTP-131), a mitochondria-targeting peptide.[101] MTP-131 interacts with and stabilises cardiolipin, a physiological component of the inner mitochondrial membrane. Cardiolipin is directly involved in mitochondrial function, regulating metabolism and maintaining the morphology of the mitochondrial membranes.[102] MTP-131 completed a phase II trial on 12 subjects with LHON in 2020, showing no serious adverse events and a trend towards improvement (NCT02693119).

Replacement

Gene therapy providing replacement DNA may hold the key to a possible cure for LHON and ADOA in the future. Gene therapy has often involved the use of adeno-associated viruses (AAVs) as a vector to deliver genes into retinal cells.[103] The issue of safety has been addressed *via* multiple trials around the world and AAV delivery is deemed to be safe for use in patients with LHON.[104–108] GS010, also known as Lumevoq, is currently in its third phase III clinical trial. GS010 is a recombinant AAV2 (adeno-associated virus serotype 2) vector carrying the ND4 gene (Raav2/2-ND4) encoding the wild-type ND4 protein.[107] ND4 is dysfunctional in the m.1178G>A mutation.

The RESCUE phase III randomised, double-blinded, placebo-controlled trial included 39 patients with LHON owing to the m.1178G>A mutation in the ND4 gene. Patients had clinical manifest disease for 6 months or less and were recruited from the US, UK, France, Germany and Italy. One eye of each participant was randomly selected to be injected with GS010 intravitreally while the other was injected with a placebo. The results for the primary outcome, visual acuity, showed a change in the actively treated eye at week 48. The treated eye had a −0.012 LogMAR change compared with the eye without GS010, a 3.16% increase in visual acuity in the GS010 compared with the placebo group. At week 72 and week 96, the improvement was more significant, with a −0.024 LogMAR change, equating to 12.5 %, and −0.029 LogMAR change, equating to 16.3% increased improvement in the eye with GS010 compared with the placebo group for week 72 and week 96 respectively.[109,110]

The REVERSE phase III trial included 37 patients with LHON due to the m.1178G>A mutation in the ND4 gene for 6–12 months. The trial was a randomised, double-blinded placebo study in which GS010 caused a −0.008 LogMAR change compared to the eye without GS010. This represents a 3.65% increase in visual acuity improvement in the GS010 group compared to the placebo group. At week 96, the improvement was more significant with a −0.049 LogMAR change, equating to a 15.91% increase in improvement.[110] Other than visual acuity improvements, contrast sensitivity was also shown to improve in both week 48 and week 96 in the GS010 group compared to the placebo group. However, an interesting point to note is that the eyes given the placebo injection were also shown to have improved visual acuity and contrast sensitivity, thought to be due to viral transport from one eye to the other.[111]

There are currently no human gene therapy clinical trials for OPA1 ADOA. However, the introduction of a wild type *OPA1* gene using AAV2 into a mouse model of ADOA prevented RGC degeneration and dysfunction.[112] Promising effective gene independent therapeutics that focus on transcriptional regulation by targeting micro RNAs are being explored. Indrieri *et al.*[113] have demonstrated that down regulation of miR-181a/b enhances mitochondrial turnover in retina, as they are involved in the downregulation of key regulatory genes implicated in mitochondrial biogenesis and mitophagy.

Repair

Whilst it may seem intuitive that new RGCs would be the best way to repair MON, there are many scientific hurdles and few, if any, clinical trials to currently support this approach. Another possible technological option is the application of photobiomodulation (PBM). This is the use of relatively low-level light (irradiance below 0.5 W/cm^2) in the red to near infrared (NIR) (600–1100 nm wavelength) range of the electromagnetic spectrum as a therapy.[114] The primary site of red/NIR light absorption underpinning PBM is thought to be cytochrome c oxidase (also known as complex IV), the terminal enzyme in the

electron transport chain.[115] Absorption of red/NIR by complex IV triggers a range of signalling pathways by triggering a transient increase in reactive oxidant species without exacerbating oxidative stress, and thus acts as a 'repair'.[116,117] In fact, NIR actually reduced oxidative stress in cortical neurons treated with hydrogen peroxide or rotenone.[118] PBM also initiates the photodissociation of nitric oxide from cytochrome c oxidase, stimulating its signalling cascade, leading to an upregulation of a plethora of genes, including those with a role in the suppression of apoptosis, cell survival and cell proliferation.[119] Furthermore, the absorption of 650–980 nm light by complex IV also has a direct effect on mitochondrial function as it increases the oxidation state of the enzyme and increases mitochondrial membrane potential, resulting in augmented electron transport chain efficiency and ATP production in the retina.[120–122] These molecular events and benefits can therefore potentially be triggered using red and NIR to repair damaged and dysfunctional RGCs as well as prevent cell degeneration.

The therapeutic potential of PBM has been highlighted in a variety of retinal diseases, including age-related macular degeneration,[123,124] retinopathy of prematurity[125] and diabetic retinopathy,[126] and following retinal damage induced by light or toxins.[127–129] PBM with 670 nm light provided neuroprotection of RGC dendrites following axotomy[130] and in an *in vivo* model of mitochondrial optic neuropathy.[131] Therefore, it may be a possible treatment to inhibit the RGC dendropathy seen in mitochondrial optic neuropathies, particularly considering that the mechanism of PBM is thought to augment mitochondrial efficiency.[132] The FDA approved a clinical trial of NIR-LED therapy [Med Light 630 PRO (Medical Devices Inc.)] for LHON patients to determine its effect on retinal functional abnormalities. The outcome was assessed with comprehensive ophthalmic examination comprising visual acuity, optical coherence tomography, pattern electroretinography (PERG N95 RGC peak), flash ERG–Photopic Negative Response and fundus photography. However, this study was terminated at Phase 1 due to the inability to record the N95 PERG peak due to the subjects with LHON not being able to focus on the target (NCT01389817). Future clinical trials are possible. (https://www.clinicaltrials.gov/ct2/show/NCT01389817?term=NCT01389817&draw=1&rank=1).

Conclusion and future directions

Novel therapeutic interventions are needed in rare diseases as there is clear unmet need. Mitochondrial optic neuropathies such as LHON and ADOA are a significant cause of visual impairment with no cure, sharing mitochondrial dysfunction and selective damage and loss of retinal ganglion cells. In clinical trials, idebenone, the only currently licensed therapy, is not able to fully reverse mitochondrial dysfunction, highlighting the urgent need to develop new disease modifying interventions that are either supportive or curative. New avenues for therapy will increasingly include repair, regeneration and genetic therapies.

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Conflict of interest statement

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References

1. DiMauro S and Schon EA. Mechanisms of disease: mitochondrial respiratory-chain diseases. *N Engl J Med* 2003; 348: 2656–2668.
2. Gray MW, Burger G and Franz Lang B. The origin and early evolution of mitochondria. *Genome Biol* 2001; 2: reviews1018.
3. Alberts B, Johnson A, Lewis J, *et al. Molecular biology of the cell*. New York: Garland Science, 2014.
4. Glancy B. Visualizing mitochondrial form and function within the cell. *Trends Mol Med* 2020; 26: 58–70.
5. Friedman JR and Nunnari J. Mitochondrial form and function. *Nature* 2014; 505: 335–343.
6. Martínez-Reyes I and Chandel NS. Mitochondrial TCA cycle metabolites control physiology and disease. *Nat Commun* 2020; 11: 1–11.
7. Yoshida M, Muneyuki E and Hisabori T. ATP synthase – a marvellous rotary engine of the cell. *Nat Rev Mol Cell Biol* 2001; 2: 669–677.
8. Berardo A, Musumeci O and Toscano A. Cardiological manifestations of mitochondrial respiratory chain disorders. *Acta Myol* 2011; 30: 9–15.
9. Behbehani R. Clinical approach to optic neuropathies. *Clin Ophthalmol* 2007; 1: 233–246.
10. Newman NJ and Biousse V. Hereditary optic neuropathies. *Eye* 2004; 18: 1144–1160.
11. Yu-Wai-Man P, Votruba M, Moore AT, *et al.* Treatment strategies for inherited optic neuropathies: past, present and future. *Eye* 2014; 28: 521–537.
12. Zanna C, Ghelli A, Porcelli AM, *et al.* OPA1 mutations associated with dominant optic atrophy impair oxidative phosphorylation and mitochondrial fusion. *Brain* 2008; 131: 352–367.
13. Kirches E. LHON: mitochondrial mutations and more. *Curr Genomics* 2011; 12: 44–54.
14. Stenton SL, Sheremet NL, Catarino CB, *et al.* Impaired complex I repair causes recessive Leber's hereditary optic neuropathy. *J Clin Invest* 2021; 131: e138267.
15. Yu-Wai-Man P, Griffiths PG, Hudson G, *et al.* Inherited mitochondrial optic neuropathies. *J Med Genet* 2009; 46: 145–158.
16. Lee S, Van Bergen NJ, Kong GY, *et al.* Mitochondrial dysfunction in glaucoma and emerging bioenergetic therapies. *Exp Eye Res* 2011; 93: 204–212.
17. Man PYW, Griffiths PG, Brown DT, *et al.* The epidemiology of Leber hereditary optic neuropathy in the North East of England. *Am J Hum Genet* 2003; 72: 333–339.
18. Puomila A, Hämäläinen P, Kivioja S, *et al.* Epidemiology and penetrance of Leber hereditary optic neuropathy in Finland. *Eur J Hum Genet* 2007; 15: 1079–1089.
19. Mascialino B, Leinonen M and Meier T. Meta-analysis of the prevalence of Leber hereditary optic neuropathy mtDNA mutations in Europe. *Eur J Ophthalmol* 2012; 22: 461–465.
20. Achilli A, Iommarini L, Olivieri A, *et al.* Rare primary mitochondrial DNA mutations and probable synergistic variants in Leber's hereditary optic neuropathy. *PLoS One* 2012; 7: e42242.
21. Tebbenkamp ATN, Varela L, Choi J, *et al.* The 7q11.23 protein DNAJC30 interacts with ATP synthase and links mitochondria to brain development. *Cell* 2018; 175: 1088–1104.e23.
22. Giordano C, Montopoli M, Perli E, *et al.* Oestrogens ameliorate mitochondrial dysfunction in Leber's hereditary optic neuropathy. *Brain* 2011; 134: 220–234.
23. Klinge CM. Estrogenic control of mitochondrial function. *Redox Biol* 31: 101435.
24. Yu J, Liang X, Ji Y, *et al.* PRICKLE3 linked to ATPase biogenesis manifested Leber's hereditary optic neuropathy. *J Clin Invest* 2020; 130: 4935–4946.
25. Kirkman MA, Yu-Wai-Man P, Korsten A, *et al.* Gene environment interactions in Leber

hereditary optic neuropathy. *Brain* 2009; 132: 2317–2326.

26. 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: e17.
27. Caporali L, Maresca A, Capristo M, *et al.* Incomplete penetrance in mitochondrial optic neuropathies. *Mitochondrion* 2017; 36:130–137.
28. Bianco A, Bisceglia L, Russo L, *et al.* High mitochondrial DNA copy number is a protective factor from vision loss in heteroplasmic Leber's hereditary optic neuropathy (LHON). *Invest Ophthalmol Vis Sci* 2017; 58: 2193–2197.
29. Catarino CB, Ahting U, Gusic M, *et al.* Characterization of a Leber's hereditary optic neuropathy (LHON) family harboring two primary LHON mutations m.11778G>A and m.14484T>C of the mitochondrial DNA. *Mitochondrion* 2017; 36: 15–20.
30. Saikia BB, Dubey SK, Shanmugam MK, *et al.* Whole mitochondrial genome analysis in South Indian patients with Leber's hereditary optic neuropathy. *Mitochondrion* 2017; 36: 21–28.
31. Meyerson C, Van Stavern G and McClelland C. Leber hereditary optic neuropathy: current perspectives. *Clin Ophthalmol* 2015; 9: 1165–1176.
32. Newman NJ. Hereditary optic neuropathies: from the mitochondria to the optic nerve. *Am J Ophthalmol* 2005; 140: 517.e1–517.e9.
33. La Morgia C, Carbonelli M, Barboni P, *et al.* Medical management of hereditary optic neuropathies. *Front Neurol* 2014; 5: 141.
34. Fraser JA, Biousse V and Newman NJ. The neuro-ophthalmology of mitochondrial disease. *Surv Ophthalmol* 2010; 55: 299–334.
35. 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: 120–126.
36. Yu-Wai-Man P, Griffiths PG and Chinnery PF. Mitochondrial optic neuropathies - disease mechanisms and therapeutic strategies. *Prog Retin Eye Res* 2011; 30: 81–114.
37. Barboni P, Savini G, Valentino ML, *et al.* Leber's hereditary optic neuropathy with childhood onset. *Investig Ophthalmol Vis Sci* 2006; 47: 5303–5309.
38. Pilz YL, Bass SJ and Sherman J. A review of mitochondrial optic neuropathies: from inherited to acquired forms. *J Optom* 2017; 10: 205–214.
39. Yu-Wai-Man P, Griffiths PG, Burke A, *et al.* The prevalence and natural history of dominant optic atrophy due to OPA1 mutations. *Ophthalmology* 2010; 117: 1538.
40. Lenaers G, Hamel C, Delettre C, *et al.* Dominant optic atrophy. *Orphanet J Rare Dis* 2012; 7: 46.
41. Djp F, Liao C, Ashley N, *et al.* Dysregulated mitophagy and mitochondrial organization in optic atrophy due to OPA1 mutations. *Neurology* 2017; 131–142.
42. Sun S, Erchova I, Sengpiel F, *et al.* Opa1 deficiency leads to diminished mitochondrial bioenergetics with compensatory increased mitochondrial motility. *Investig Ophthalmol Vis Sci* 2020; 61: 42–42.
43. Davies VJ, Hollins AJ, Piechota MJ, *et al.* Opa1 deficiency in a mouse model of autosomal dominant optic atrophy impairs mitochondrial morphology, optic nerve structure and visual function. *Hum Mol Genet* 2007; 16: 1307–1318.
44. Williams PA, Morgan JE and Votruba M. Opa1 deficiency in a mouse model of dominant optic atrophy leads to retinal ganglion cell dendropathy. *Brain* 2010; 133: 2942–2951.
45. Williams PA, Piechota M, Von Ruhland C, *et al.* Opa1 is essential for retinal ganglion cell synaptic architecture and connectivity. *Brain* 2012; 135: 493–505.
46. Wong-Riley M. Energy metabolism of the visual system. *Eye Brain* 2010; 2: 99.
47. Kjer P, Jensen OA and Klinken L. Histopathology of eye, optic nerve and brain in a case of Dominant Optic Atrophy. *Acta Ophthalmol* 1983; 61: 300–312.
48. Votruba M, Moore AT and Bhattacharya SS. Clinical features, molecular genetics, and pathophysiology of dominant optic atrophy. *J Med Genet* 1998; 35: 793–800.
49. Bremner FD, Tomlin EA, Shallo-Hoffmann J, *et al.* The pupil in dominant optic atrophy. *Investig Ophthalmol Vis Sci* 2001; 42: 675–678.
50. Ito Y, Nakamura M, Yamakoshi T, *et al.* Reduction of inner retinal thickness in patients with autosomal dominant optic atrophy associated with OPA1 mutations. *Investig Ophthalmol Vis Sci* 2007; 48: 4079–4086.
51. Chan C, Mackey DA and Byrne E. Sporadic Leber hereditary optic neuropathy in Australia and New Zealand. *Aust N Z J Ophthalmol* 1996; 24: 7–14.

52. Sallevelt SCEH, Dreesen JCFM, Drüsedau M, *et al.* Preimplantation genetic diagnosis in mitochondrial DNA disorders: challenge and success. *J Med Genet* 2013; 50: 125–132.
53. Ladoukakis ED and Zouros E. Evolution and inheritance of animal mitochondrial DNA: rules and exceptions. *J Biol Res* 2017; 24: 2.
54. Kang E, Wu J, Gutierrez NM, *et al.* Mitochondrial replacement in human oocytes carrying pathogenic mitochondrial DNA mutations. *Nature* 2016; 540: 270–275.
55. Tang M, Guggilla RR, Gansemans Y, *et al.* Comparative analysis of different nuclear transfer techniques to prevent the transmission of mitochondrial DNA variants. *Mol Hum Reprod* 2019; 25: 797–810.
56. Craven L, Tuppen HA, Greggains GD, *et al.* Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. *Nature* 2010; 465: 82–85.
57. Paull D, Emmanuele V, Weiss KA, *et al.* Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants. *Nature* 2013; 493: 632–637.
58. Toomes C, Marchbank NJ, Mackey DA, *et al.* Spectrum, frequency and penetrance of OPA1 mutations in dominant optic atrophy. *Hum Mol Genet* 2001; 10: 1369–1378.
59. Hardarson T, Hanson C, Lundin K, *et al.* Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial. *Hum Reprod* 2008; 23: 2806–2812.
60. Reznichenko AS, Huyser C and Pepper MS. Mitochondrial transfer: implications for assisted reproductive technologies. *Appl Transl Genom* 2016; 11: 40–47.
61. Carelli V, Ross-Cisneros FN and Sadun AA. Optic nerve degeneration and mitochondrial dysfunction: genetic and acquired optic neuropathies. *Neurochem Int* 2002; 40: 573–584.
62. Amaral-Fernandes MS, Marcondes AM, Miranda PM, do AD *et al.* Mutations for Leber hereditary optic neuropathy in patients with alcohol and tobacco optic neuropathy. *Mol Vis* 2011; 17: 3175–3179.
63. 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: e17.
64. Feibel RM and Arch J. Cuban epidemic optic neuropathy (1991–1993) and José Saramago's novel blindness (1995). *Am J Ophthalmol* 2018; 193: xix–xxvii.
65. Mackey DA, Fingert JH, Luzhansky JZ, *et al.* Leber's hereditary optic neuropathy triggered by antiretroviral therapy for human immunodeficiency virus. *Eye* 2003; 17: 312–317.
66. Luca CC, Lam BL and Moraes CT. Erythromycin as a potential precipitating agent in the onset of Leber's hereditary optic neuropathy. *Mitochondrion* 2004; 4: 31–36.
67. Sanchez RN, Smith AJ, Carelli V, *et al.* Leber hereditary optic neuropathy possibly triggered by exposure to tire fire. *J Neuro Ophthalmol* 2006; 26: 268–272.
68. Carelli V, Franceschini F, Venturi S, *et al.* Grand rounds: could occupational exposure to n-hexane and other solvents precipitate visual failure in Leber hereditary optic neuropathy? *Environ Health Perspect* 2007; 115: 113–115.
69. Seo JH, Hwang JM and Park SS. Antituberculosis medication as a possible epigenetic factor of Leber's hereditary optic neuropathy. *Clin Exp Ophthalmol* 2010; 38: 363–366.
70. Tsao K, Aitken PA and Johns DR. Smoking as an aetiological factor in a pedigree with Leber's hereditary optic neuropathy. *Br J Ophthalmol* 1999; 83: 577–581.
71. Mei S, Huang X, Cheng L, *et al.* A missense mutation in OPA1 causes dominant optic atrophy in a Chinese Family. *J Ophthalmol* 2019; 2019: 1424928.
72. Lonsdale D. A review of the biochemistry, metabolism and clinical benefits of thiamin(e) and its derivatives. *Evid Based Complement Alternat Med* 2006; 3: 49–59.
73. Rodriguez MC, MacDonald JR, Mahoney DJ, *et al.* Beneficial effects of creatine, CoQ10, and lipoic acid in mitochondrial disorders. *Muscle Nerve* 2007; 35: 235–242.
74. Tarnopolsky MA. The mitochondrial cocktail: rationale for combined nutraceutical therapy in mitochondrial cytopathies. *Adv Drug Deliv Rev* 2008; 60: 1561–1567.
75. Shrader WD, Amagata A, Barnes A, *et al.* α-Tocotrienol quinone modulates oxidative stress response and the biochemistry of aging. *Bioorganic Med Chem Lett* 2011; 21: 3693–3698.
76. Grings M, Seminotti B, Karunanidhi A, *et al.* ETHE1 and MOCS1 deficiencies: disruption of mitochondrial bioenergetics, dynamics, redox homeostasis and endoplasmic reticulum-mitochondria crosstalk in patient fibroblasts. *Sci Rep* 2019; 9: 12651.

77. Orsucci D, Caldarazzo Ienco E, Siciliano G, *et al.* Mitochondrial disorders and drugs: what every physician should know. *Drugs Context* 2019; 8: 212588.

78. Mileykovskaya E and Dowhan W. Cardiolipin-dependent formation of mitochondrial respiratory supercomplexes. *Chem Phys Lipids* 2014; 179: 42–48.

79. Szeto HH. First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. *Br J Pharmacol* 2014; 171: 2029–2050.

80. Hurko O. Drug development for rare mitochondrial disorders. *Neurotherapeutics* 2013; 10: 286–306.

81. Khan NA, Govindaraj P, Meena AK, *et al.* Mitochondrial disorders: challenges in diagnosis & treatment. *Indian J Med Res* 2015; 141: 13–26.

82. Enns GM. Treatment of mitochondrial disorders: antioxidants and beyond. *J Child Neurol* 2014; 29: 1235–1240.

83. Rai PK, Russell OM, Lightowlers RN, *et al.* Potential compounds for the treatment of mitochondrial disease. *Br Med Bull* 2015; 116: 5–18.

84. Sharma S and Black SM. Carnitine homeostasis, mitochondrial function and cardiovascular disease. *Drug Discov Today Dis Mech* 2009; 6: e31–e39.

85. Komen JC and Thorburn DR. Turn up the power – pharmacological activation of mitochondrial biogenesis in mouse models. *Br J Pharmacol* 2014; 171: 1818–1836.

86. Wang Y and Hekimi S. Understanding Ubiquinone. *Trends Cell Biol* 2016; 26: 367–378.

87. Gueven N, Woolley K and Smith J. Border between natural product and drug: comparison of the related benzoquinones idebenone and coenzyme Q10. *Redox Biol* 2015; 4C: 289–295.

88. Frei B, Kim MC and Ames BN. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proc Natl Acad Sci U S A* 1990; 87: 4879–4883.

89. Kanabus M, Heales SJ and Rahman S. Development of pharmacological strategies for mitochondrial disorders. *Br J Pharmacol* 2014; 171: 1798–1817.

90. European Medicines Agency. Raxone idebenone. EMA/ 480039/2015. https://www.ema.europa.eu/en/medicines/human/EPAR/raxone

91. Klopstock T, Yu-Wai-Man P, Dimitriadis K, *et al.* A randomized placebo-controlled trial of idebenone in Leber's hereditary optic neuropathy. *Brain* 2011; 134: 2677–2686.

92. Catarino CB, von Livonius B, Priglinger C, *et al.* Real-world clinical experience with idebenone in the treatment of Leber hereditary optic neuropathy. *Neuroophthalmol* 2020; 40: 558–565.

93. Haefeli RH, Erb M, Gemperli AC, *et al.* NQo1-dependent redox cycling of idebenone: effects on cellular redox potential and energy levels. *PLoS One* 2011; 6: e17963.

94. Jaber SM, Ge SX, Milstein JL, *et al.* Idebenone has distinct effects on mitochondrial respiration in cortical astrocytes as compared to cortical neurons due to differential NQO1 activity. *J Neurosci* 2020; 40: 4609–4619.

95. Varricchio C, Beirne K, Heard C, *et al.* The ying and yang of idebenone: not too little, not too much – cell death in NQO1 deficient cells and the mouse retina. *Free Radic Biol Med* 2020; 152: 551–560.

96. Enns GM and Cohen BH. Clinical trials in mitochondrial disease: an update on EPI-743 and RP103. *JIEMS* 2017; 5: 232640981773301.

97. Sadun AA, Chicani CF, Ross-Cisneros FN, *et al.* Effect of EPI-743 on the clinical course of the mitochondrial disease Leber hereditary optic neuropathy. *Arch Neurol* 2012; 69: 331–338.

98. Seo KS, Kim JH, Min KN, *et al.* KL1333, a Novel NAD+ modulator, improves energy metabolism and mitochondrial dysfunction in MELAS fibroblasts. *Front Neurol* 2018; 9: 1–12.

99. Varricchio C, Beirne K, Aeschlimann P, *et al.* Discovery of novel 2-aniline-1,4-naphthoquinones as potential new drug treatment for Leber's hereditary optic neuropathy (LHON). *J Med Chem* 63: 13638–13655.

100. Woolley KL, Nadikudi M, Koupaei MN, *et al.* Amide linked redox-active naphthoquinones for the treatment of mitochondrial dysfunction. *Med Chem Comm* 2019; 10: 399–412.

101. Chen M, Liu B, Ma J, *et al.* Protective effect of mitochondria-targeted peptide MTP-131 against oxidative stress-induced apoptosis in RGC-5 cells. *Mol Med Rep* 2017; 15: 2179–2185.

102. Allen ME, Pennington ER, Perry JB, *et al.* The cardiolipin-binding peptide elamipretide mitigates fragmentation of cristae networks

following cardiac ischemia reperfusion in rats. *Commun Biol* 2020; 3: 389.

103. Vandenberghe LH and Auricchio A. Novel adeno-associated viral vectors for retinal gene therapy. *Gene Ther* 2012; 19: 162–168.

104. Guy J, Feuer WJ, Davis JL, *et al.* Gene therapy for Leber hereditary optic neuropathy: low- and medium-dose visual results. *Ophthalmology* 2017; 124: 1621–1634.

105. Feuer WJ, Schiffman JC, Davis JL, *et al.* Gene therapy for Leber hereditary optic neuropathy initial results. *Ophthalmology* 2016; 123: 558–570.

106. Yang S, Ma S qi, Wan X, *et al.* Long-term outcomes of gene therapy for the treatment of Leber's hereditary optic neuropathy. *EBioMedicine* 2016; 10: 258–268.

107. Bouquet C, Vignal Clermont C, Galy A, *et al.* Immune response and intraocular inflammation in patients with Leber hereditary optic neuropathy treated with intravitreal injection of recombinant adeno-associated virus 2 carrying the ND4 gene: a secondary analysis of a phase 1/2 clinical trial. *JAMA Ophthalmol* 2019; 137: 399–406.

108. Vignal C, Uretsky S, Fitoussi S, *et al.* Safety of rAAV2/2-ND4 gene therapy for Leber hereditary optic neuropathy. *Ophthalmology* 2018; 125: 945–947.

109. Biologics G. Efficacy study of GS010 for the treatment of vision loss up to 6 months from onset in LHON due to the ND4 mutation (RESCUE). *ClinicalTrials.gov*, https://clinicaltrials.gov/ct2/show/results/NCT02652767?view=results (2020, accessed January 20, 2021).

110. Newman NJ, Yu-Wai-Man P, Carelli V, *et al*; LHON Study Group. Efficacy and safety of intravitreal gene therapy for Leber hereditary optic neuropathy treated within 6 months of disease onset. *Ophthalmology* 2021; 128: 649–660.

111. Yu-Wai-Man P, Newman NJ, Carelli V, *et al.* Bilateral visual improvement with unilateral gene therapy injection for Leber hereditary optic neuropathy. *Sci Transl Med* 2020; 12: eaaz7423.

112. Sarzi E, Seveno M, Piro-Mégy C, *et al.* OPA1 gene therapy prevents retinal ganglion cell loss in a dominant optic atrophy mouse model. *Sci Rep* 2018; 8: 2468.

113. Indrieri A, Carrella S, Romano A, *et al.* miR-181a/b downregulation exerts a protective action on mitochondrial disease models. *EMBO Mol Med* 2019; 11: e8734.

114. Ramezani F, Neshasteh-Riz A, Ghadaksaz A, *et al.* Mechanistic aspects of photobiomodulation therapy in the nervous system. *Lasers Med Sci*. Epub ahead of print 2021. DOI: 10.1007/s10103-021-03277-2.

115. Desmet KD, Paz DA, Corry JJ, *et al.* Clinical and experimental applications of NIR-LED photobiomodulation. *Photomed Laser Surg* 2006; 24: 121–128.

116. Tafur J and Mills PJ. Low-intensity light therapy: exploring the role of redox mechanisms. *Photomed Laser Surg* 2008; 26: 323–328.

117. Amaroli A, Ravera S, Baldini F, *et al.* Photobiomodulation with 808-nm diode laser light promotes wound healing of human endothelial cells through increased reactive oxygen species production stimulating mitochondrial oxidative phosphorylation. *Lasers Med Sci* 2019; 34: 495–504.

118. Huang YY, Nagata K, Tedford CE, *et al.* Low-level laser therapy (LLLT) reduces oxidative stress in primary cortical neurons in vitro. *J Biophotonics* 2013; 6: 829–838.

119. Zhang Y, Song S, Fong CC, *et al.* cDNA microarray analysis of gene expression profiles in human fibroblast cells irradiated with red light. *Jf Investig Dermatol* 2003; 120: 849–857.

120. Kaynezhad P, Tachtsidis I and Jeffery G. Optical monitoring of retinal respiration in real time: 670 nm light increases the redox state of mitochondria. *Exp Eye Res* 2016; 152: 88–93.

121. Kokkinopoulos I, Colman A, Hogg C, *et al.* Age-related retinal inflammation is reduced by 670 nm light via increased mitochondrial membrane potential. *Neurobiol Aging* 2013; 34: 602–609.

122. Gkotsi D, Begum R, Salt T, *et al.* Recharging mitochondrial batteries in old eyes. Near infra-red increases ATP. *Exp Eye Res* 2014; 122: 50–53.

123. Begum R, Powner MB, Hudson N, *et al.* Treatment with 670 nm light up regulates cytochrome C oxidase expression and reduces inflammation in an age-related macular degeneration model. *PLoS One* 2013; 8: e57828.

124. Muste JC, Kalur A, Iyer A, *et al.* Photobiomodulation therapy in age-related macular degeneration. *Curr Opin Ophthalmol* 2021; 32: 225–232.

125. Natoli R, Valter K, Barbosa M, *et al.* 670nm Photobiomodulation as a novel protection against retinopathy of prematurity: evidence from oxygen induced retinopathy models. *PLoS One* 8: e72135.

126. Cheng Y, Du Y, Liu H, *et al.* Photobiomodulation inhibits long-term structural and functional lesions of diabetic retinopathy. *Diabetes* 2018; 67: 291–298.

127. Albarracin R, Eells J and Valter K. Photobiomodulation protects the retina from light-induced photoreceptor degeneration. *Investig Ophthalmol Vis Sci* 2011; 52: 3582–3592.

128. Lu YZ, Fernando N, Natoli R, *et al.* 670nm light treatment following retinal injury modulates Müller cell gliosis: evidence from in vivo and in vitro stress models. *Exp Eye Res* 2018; 169: 1–12.

129. Eells JT, Henry MM, Summerfelt P, *et al.* Therapeutic photobiomodulation for methanol-induced retinal toxicity. *Proc Natl Acad Sci U S A* 2003; 100: 3439–3444.

130. Beirne K, Rozanowska M and Votruba M. Red light treatment in an axotomy model of neurodegeneration. *Photochem Photobiol* 2016; 92: 624–631.

131. Rojas JC, Lee J, John JM, *et al.* Neuroprotective effects of near-infrared light in an in vivo model of mitochondrial optic neuropathy. *J Neurosci* 2008; 28: 13511–13521.

132. Hamblin MR. Mechanisms and mitochondrial redox signalling in photobiomodulation. *Photochem Photobiol* 2018; 94: 199–212.