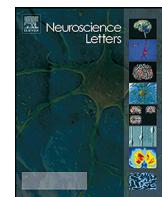




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### Review article

# Mitochondria in neuroinflammation – Multiple sclerosis (MS), leber hereditary optic neuropathy (LHON) and LHON-MS

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### HIGHLIGHTS

- Mitochondrial damage can be both inherited and acquired.
- The role of mitochondria as drivers of neuroinflammation remains unresolved.
- There is overlap in the clinical and molecular mechanisms of MS and LHON.
- Mitochondrial pathways may represent potential therapeutic targets in MS.

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### ABSTRACT

Mitochondrial dysfunction is associated with neuroinflammation and neurodegenerative disease, but its role as a driver in these processes is uncertain. Understanding the pathogenesis of inherited mitochondrial disorders may help us to uncover mechanisms involved during acquired mitochondrial dysfunction. We review the mechanisms of mitochondrial dysfunction in Leber's hereditary optic neuropathy and multiple sclerosis and discuss shared clinical and molecular features in both conditions. Targeting mitochondrial pathways involved in inflammation or apoptosis may be a possible therapeutic approach in multiple sclerosis.

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**Abbreviations:** ATP, adenosine triphosphate; LHON, Leber's Hereditary Optic Neuropathy; MS, multiple sclerosis; mtDNA, mitochondrial DNA; SNP, single nucleotide polymorphism.

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## 1. Introduction

Inflammation involving the nervous system, termed neuroinflammation, is emerging as a key driver of pathology in many neurological diseases, and is thought to both trigger and exacerbate neuronal injury. Genome-wide studies in patients with common neurodegenerative diseases, such as multiple sclerosis (MS) [1] or Alzheimer's disease (AD) [2,3], have identified risk loci in genes predicted to alter immune function and these have subsequently been experimentally validated [4,5]. Similarly, damage to mitochondria, the energy-producing organelle within the cell, is present in many neurodegenerative diseases. Deletions and point mutations in mitochondrial DNA (mtDNA), a 16.5 kb circular molecule separate from the nuclear genome, occur in neurons and localise to areas of pathological change in MS and AD [6]. However, it remains unclear whether mitochondria are drivers or innocent bystanders during neuroinflammation and whether mitochondrial damage is the cause or consequence of neurodegeneration.

In this review, we describe the interaction between mitochondria and neuroinflammation, focussing on multiple sclerosis, a canonical neuroimmune disease, and Leber's Hereditary Optic Neuropathy (LHON), a primary mitochondrial disorder. In doing so, we aim to explore the extent to which mitochondrial dysfunction is a primary or secondary phenomena in neuroinflammation and the downstream mechanisms by which this leads to neurodegeneration.

## 2. Multiple sclerosis and mitochondrial dysfunction

Multiple sclerosis (MS) is an autoimmune, neurodegenerative disease characterised by inflammatory demyelination and axonal loss within the central nervous system. It is one of the most common cause of neurological disability in young adults and affects over 2 million people worldwide [7]. Most patients suffer from a relapsing-remitting course (RRMS), characterised by inflammatory attacks that manifest as neurological deficits followed by partial recovery. After a period of time patients transition to a progressive disease course (secondary progressive, SPMS) with accumulating neurological disability that correlates with neuronal loss in the brain and spinal cord [8–10].

The diagnosis of MS requires evidence of central nervous system lesions disseminated in time and space with the exclusion of alternative diagnoses that may present in a similar manner. The McDonald 2010 criteria [11] allow a diagnosis to be established on the basis of clinical history and examination alone, but magnetic resonance imaging (MRI) is commonly used to confirm presence of new lesions (dissemination in time) at different sites within the neuraxis (dissemination in space). The brain MRI is almost always abnormal in established MS cases and the presence of contrast enhancement, suggesting loss of BBB integrity, alongside typical lesion localisation (periventricular, juxtacortical, infratentorial and within the spinal cord), are helpful to exclude alternative diagnoses. As these diagnostic criteria have evolved, so too has the list of differential diagnoses, but MS still remains sufficiently non-specific for it to remain a diagnosis of exclusion. Amongst this differential diagnosis list are primary mitochondrial disorders, which, given the spectrum of mitochondrial dysfunction seen in MS, is perhaps unsurprising. Due to the heterogeneous presentation of MS, there is considerable overlap between MS and primary mitochondrial disorders, in both clinical signs and symptoms and imaging features (see Table 1 for details). However, prominent muscle, peripheral nerve or cardiac involvement, seizures, cerebrovascular disease, retinal pigmentation, irreversible optic neuropathy and diabetes mellitus are useful signs to help differentiate mitochondrial disorders from MS [12]. With the advent of cheap molecular genetic

testing, nuclear and mitochondrial DNA sequencing represents a cost-effective investigation to exclude a proportion of these primary mitochondrial MS mimics, however, a molecular diagnosis is unable to be reached in approximately a third of cases of mitochondrial disease, leaving considerable uncertainty about the cause and recurrence risks within families.

Although the exact trigger of MS is unknown, clues regarding its aetiology have been derived from pathological and imaging studies. It is clear that following a central or peripheral activating event, infiltration of peripheral immune cells occurs either across a leaky blood-brain barrier (BBB), across the blood-CSF barrier at the choroid plexus or via the subarachnoid space [13]. Subsequent parenchymal damage manifests as gadolinium-enhancing lesions on MRI and correlate reasonably with episodic clinical disease activity, termed relapses [14,15]. Autoreactive T cells, directed against myelin epitopes, are thought to play a central role in driving immune-mediated damage in MS, leading to demyelination and underlying axonal damage. However, whether this process represents a primary initiating event, or exacerbates an earlier, more generalised inflammatory response, is unclear.

Clonally-expanded somatic mtDNA deletions have been found in respiratory-deficient neurons of secondary-progressive MS patients [16], but as with other neurodegenerative diseases that also harbour increased neuronal mtDNA mutations [6], the timing of these genetic changes and their effect on downstream processes is unclear. Studies to date interrogating the role of specific inherited mtDNA single nucleotide variants have been limited by small sample sizes and the majority have focussed on LHON mtDNA mutations (reviewed in [17]), though reports of an MS-like syndrome in patients with mutations in nuclear-encoded mitochondrial genes, such as *OPA1* [18–20] and *POLG* [21], have also been described. Following an earlier effort to understand the role of mtDNA variation in MS and other common human disease [22], a recent large study has evaluated the association between MS and mitochondrial SNPs using >7000 MS cases and >14000 controls from 7 countries [23]. No single mtDNA variant was associated with MS risk but an association between mitochondrial haplogroups J, T and JT with MS risk was reported in a discovery cohort, though this was not able to be replicated in an independent cohort. A similar association between haplogroup J and T carriers and MS risk has been described in smaller cohorts [24,25] and is of particularly interest given that some of the single nucleotide polymorphisms (SNPs) that define these haplogroups encode subunits of mitochondrial complex I. Mitochondrial haplogroups represent groupings of shared polymorphisms, differentiated through sequential addition of single nucleotide variants over the course of human history [26]. It is difficult to establish the functional consequence of each individual SNP within each haplogroup, due to the challenge of defining 'haplogroup-defining' SNPs and because some variants are in almost complete linkage disequilibrium with each other. However, evidence from cytoplasmic hybrid (cybrid) cell lines, in which mitochondrial variants are compared against the same nuclear background, suggest that different mitochondrial haplogroups can alter inflammatory responses [27–29]. Further work is required to clarify the pathways involved in modulating this response, and how this translates to clinical differences in MS risk and disease progression for patients with each mitochondria haplogroup.

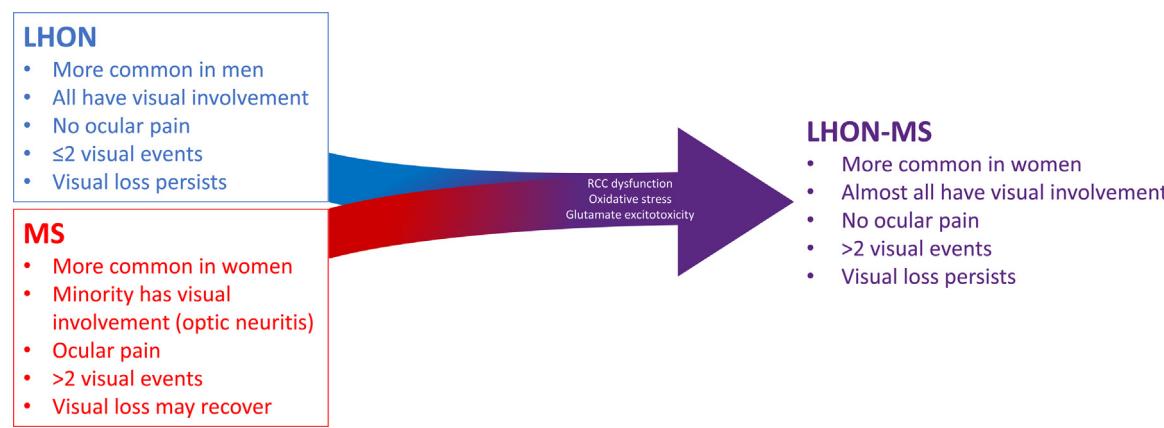
Accumulation of mtDNA damage, either as a primary or secondary event, can affect the transcription and assembly of respiratory chain subunits, resulting in defects in ATP production. Mitochondrial respiratory chain complex I activity is reduced in chronic active MS lesions [30] and both complex I and III gene transcripts are decreased in non-lesional motor cortex of MS patients when compared to healthy controls [31]. The numbers of gene transcripts for nuclear-encoded respiratory chain complex I, II, IV and V subunits were also found to be reduced in this group, though the

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**Table 1**  
Clinical overlap between multiple sclerosis and primary mitochondrial disorders.

Mitochondrial disorder	Clinical features of multiple sclerosis													Neuroimaging			Visual evoked potentials	Oligoclonal Excluded by		
	Ataxia	Bowel/bladder dysfunction	Cognitive disturbance	Dizziness/vertigo	Dysarthria	Dysphagia	Fatigue	Headache	Mood disturbance	Numbness/Seizures tingling	Sexual dysfunction	Spatial neglect	Tremor	Visual disturbance	Weakness	T1 lesions	T2 lesions	Atrophy		
Leber hereditary optic neuropathy (LHON)	–	–	–	–	–	–	–	–	–	+	–	–	–	+	+	+	±	Absent/delayed	mtDNA sequencing, fundal examination, retinal photography	
Leigh syndrome	+	–	–	–	–	+	–	–	–	+	+	–	+	–	+	+	–	–	–	increased CSF/plasma lactate or pyruvate, mtDNA/nuclear gene sequencing
MELAS	+	–	+	–	–	–	–	–	–	+	+	–	–	–	+	+	–	+	–	mtDNA sequencing, muscle biopsy (ragged red fibres and respiratory chain complex defects – usually partial complex I/IV)
MERRF	+	–	+	–	–	–	+	–	–	+	+	–	+	–	+	+	–	–	–	mtDNA sequencing, muscle biopsy (ragged red fibres and respiratory chain complex defects – all complexes)
Optic atrophy, type 1	+	–	–	–	–	–	–	–	–	+	–	–	–	–	+	+	–	+	Absent/delayed	molecular testing of <i>OPA1</i>
<i>POLG</i> -related disorders	+	–	–	–	+	–	–	+	–	+	–	–	–	–	+	+	–	+	–	molecular testing of <i>POLG</i> , nerve conduction studies
Pyruvate dehydrogenase (PDH) deficiency	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	increased CSF/plasma lactate or pyruvate, molecular testing of <i>PDHA1</i>	

CSF, cerebrospinal fluid; LHON, Leber's hereditary optic neuropathy; MELAS, mitochondrial encephalopathy, lactic acidosis and stroke-like episodes; MERRF, myoclonic epilepsy with ragged-red fibres; mtDNA, mitochondrial DNA; MS, multiple sclerosis; *OPA1*, optic atrophy 1 (mitochondrial dynamin-like GTPase); *POLG*, polymerase gamma (mitochondrial DNA polymerase); RCC, mitochondrial respiratory chain complex.



**Fig. 1.** Overlap in clinical and molecular features in LHON-MS.

activity of complex IV was unaffected [31]. In normal-appearing grey matter of MS patients, mtDNA abundance (copy number) is increased, which may be a mechanism to compensate for reduced respiratory complex transcripts, by providing sufficient mtDNA-encoded subunits to allow for complex assembly [32]. Alteration in respiratory chain function may be context-dependent, requiring accompanying insults to manifest as a biochemical defect [33]. A detectable loss of the mtDNA-encoded complex IV catalytic subunit (COX-1) is seen in pattern III MS lesions but not in acute pattern II lesions [34,35]. Pattern III lesions are seen in fulminant disease and aggressive MS subtypes and are associated with hypoxia-like tissue injury [35], which may provide the additional insult required to drive damage above a biochemical threshold [33]. With inhibition of oxidative phosphorylation, electron flux is diverted to generate reactive oxygen (ROS) and reactive nitrogen species (RNS), causing further damage to respiratory chain subunits, resulting in a self-perpetuating cycle that is especially damaging to vulnerable, demyelinated axons [36,37]. Indeed, oxidative stress, resulting from an imbalance between the cell's production of ROS and ability to compensate via antioxidant activity, may be a key driver for cell loss in acute lesions. *In vitro* oligodendrocytes are more sensitive to reactive oxidative and nitritative species and this may account for selective loss of oligodendrocyte in demyelinating lesions [38].

Neuronal cells are particularly sensitive to disruption to oxidative phosphorylation due to their reliance on large amounts of ATP to maintain their membrane potential, via the Na<sup>+</sup>/K<sup>+</sup> ATPase pump. Membrane ATPase dysfunction is thought to occur following demyelination where ATP production is inadequate, and results in increased intracellular sodium concentrations [39,40]. Axons compensate by upregulating production and redistribution of sodium channels along the axonal membrane and by reversing the Na<sup>+</sup>-Ca<sup>2+</sup> exchanger which expels sodium at the expense of increasing calcium influx [41]. In acute demyelinating lesions, accumulation of axonal calcium activates calcium-dependent cysteine proteases which disrupt cytoskeletal processes and lead to axonal injury [40,41]. These ionic imbalances promote a self-perpetuating cycle in which calcium directly modulates expression of sodium channels [42], further aggravating Na<sup>+</sup>/K<sup>+</sup> ATPase dysfunction. Increased sodium loading within the axon also drives axonal release of glutamate by reversing the action of sodium-dependent transporters [43]. The resulting excess glutamate concentration in the white matter cause myelin and oligodendrocyte toxicity via both direct and indirect pathways, involving AMPA and NMDA receptors signalling. AMPA activation leads to axonal damage by increasing mitochondrial calcium concentration via the mitochondrial permeability transition pore [44–46], as well as by triggering ERK-mediated oligodendrocyte apoptosis [47]. Similarly, NMDA stimulation induces mitochondrial toxicity by increasing calcium

levels and decreasing levels of superoxide dismutase 2 (SOD-2), mitochondrial membrane potential and ATP production [48]. These processes may be amplified in neurons with underlying sub-clinical mitochondrial defects, as noted in carriers of primary mitochondrial disorders.

### 3. Mitochondrial disease and MS: LHON and LHON-MS

Leber's hereditary optic neuropathy (LHON) is a maternally inherited mitochondrial disease characterised by bilateral, subacute visual loss in early adult life [49]. Three common pathogenic mitochondrial DNA variants account for 90–95% of LHON – m.3460G>A, m.11778G>A, m.14484T>C – and these are found in genes encoding subunits of respiratory chain complex I. Although retinal ganglion cells are preferentially affected, leading to optic nerve degeneration, additional extraocular abnormalities have been described in LHON pedigrees. These include non-neurological (cardiac arrhythmias, myopathy) as well as neurological abnormalities (tremor, dementia, movement disorders, peripheral neuropathy, and a multiple sclerosis-like presentation) [49].

The association between CNS inflammatory demyelination and LHON (hereby termed LHON-MS) was first described in 1926 by Mauksch and colleagues [50], but has since been eponymously named "Harding's disease", following a highly-cited case series by Anita Harding and colleagues. In their report they describe 8 unrelated women with a LHON family history presenting with bilateral optic neuropathy, six of whom developed neurological features compatible with a diagnosis of MS, while the remaining two had an isolated optic neuropathy and white matter lesions on imaging [51]. This series was followed by a number of further reports describing patients with both MS and LHON mutations [51–57] suggesting an association between the two diseases, however, LHON mutations were found very rarely when large MS populations were screened [53,58–60]. These screens rely on accurate estimates of the population carrier frequency of LHON mutations to determine whether the co-association of LHON mutations and MS occurs more often than would be expected by chance, thus supporting an argument of a causative interaction. As molecular genetic testing has become more readily available and affordable, larger cohorts of healthy individuals have been sequenced, resulting in refinement of LHON mtDNA mutation carrier frequency estimates [61–64]. Using these updated population-based prevalence estimates, Pfeffer and colleagues compared the numbers of affected LHON patients, unaffected carriers of LHON mutations and MS patients in the UK to the prevalence of LHON-MS, established via a national survey and a meta-analysis of published cases [65]. Although clinically-manifesting LHON is a rare disorder, affecting approximately 1

in 30,000–50,000 in Northern European countries, heteroplasmic LHON mtDNA mutations are found in approximately 1 in 300 of the population and homoplasmic mutations in approximately 1 in 1000 [63,64,66]. The prevalence of MS is approximately 2.6 in 1000 in the UK population [67]. Using these estimates the authors determine that approximately 150 people within the UK would be expected to have both homoplasmic LHON mutations and MS by chance, which far outnumbers the 12 patients found in their national survey. Similarly, there was no significant difference in prevalence of LHON mutations in MS patients compared to healthy controls as determined by analysis of published population screens [65].

Differences in the clinical presentation of LHON-MS patients and those with 'classical' LHON or MS alone, suggest that although LHON mtDNA mutations may not alter the risk of developing MS, instead, they may have a disease-modifying effect through common molecular pathways. This interplay is supported by an ocular phenotype in LHON-MS patients that is distinct to that of typical LHON patients. When compared to classical LHON patients, a larger proportion of LHON-MS patients have more than 2 visual events, persisting unilateral visual loss and an unusually long time interval before both eyes become affected – an average of 1.66 years in LHON-MS compared to LHON patients who have bilateral symptoms within 0.5 years [65,68]. An interaction between the two diseases would account for these differences, and may be additive or synergistic (Fig. 1). The presence of mtDNA LHON mutations as a background to MS may result in an atypical optic neuritis (which is severe, painless and irreversible) or alternatively, the presence of MS or its risk factors may precipitate LHON in patients who would otherwise have been asymptomatic or compound damage in tissues affected by both diseases.

Interaction between mtDNA mutations and MS at a molecular level may converge on shared pathways of oxidative damage and cell death (Fig. 1). Complex 1 dysfunction caused by LHON mtDNA mutations results in decreased ATP synthesis, increased ROS production and impairments in glutamate transport leading to retinal ganglion cell dysfunction and apoptosis [69,70]. Similar impairments are seen in demyelination and neurodegeneration in MS, whereby mitochondrial damage leads to respiratory chain complex dysfunction and glutamate excitotoxicity. Therefore, in patients with inherited (e.g. mtDNA) or acquired (neuroinflammation-induced mitochondrial dysfunction) defects in these overlapping pathways, there may be an increased likelihood of apoptosis, resulting in a more severe disease phenotype. This overlap gives hope for the treatment of multiple sclerosis with mitochondrial-targeted therapeutics that specifically address these shared pathways. Examples include mitoQ, a mitochondria-targeted antioxidant, shown to reduce inflammation and neuronal loss in spinal cord of EAE mouse models [71,72], and the enhancement of mtDNA repair processes in oligodendrocytes to protect against ROS and cytokine-induced apoptosis [73,74]. Future work is required to establish the timing of drug delivery relative to the initial insult; it is likely that therapeutic benefit can only be achieved if drugs are administered prior to irreversible cell processes, such as apoptosis. Furthermore, specific delivery of these compounds to their desired site of action within the cell remains a challenge and will ultimately require trials with human subjects to clarify issues of dosing and safety.

#### 4. Conclusion

Characterising the clinical and molecular features of primary mitochondrial disorders provides valuable insight into the role of mitochondrial dysfunction in neuroinflammation and neurodegenerative diseases. By interrogating pathways driving pathology common to both MS and LHON, we are able to identify down-

stream mediators of mitochondrial dysfunction and to distinguish between primary or secondary processes. Although the association of LHON mtDNA mutations in patients with MS is likely due to chance, the resulting superimposed clinical phenotype is instructive. The modulation of the ocular phenotype in LHON-MS patients suggests that mitochondrial damage from each risk factor interact to lower a threshold, triggering cell-specific damage within the optic nerve. Further work to clarify the molecular determinants of this threshold effect in patients with sub-clinical mtDNA defects is warranted. Untangling the cause from effect of mitochondrial dysfunction in neuroinflammation will require carefully designed *in vitro* and *in vivo* models that allow measurement of the downstream consequences of specific defects in mitochondrial function or mtDNA mutations. Much of the work to date assessing mitochondria in neurodegenerative disease has focussed on neuronal changes, however, modulation of immune cell function by mitochondria defects may represent an earlier, and perhaps more reversible, target for therapeutic intervention.

#### Author contributions

David Bargiela: design, drafting and revision of manuscript. Patrick F Chinnery: revision of manuscript and study supervision.

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#### Disclosures

The authors report no disclosures relevant to the manuscript.

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