

Mitochondrial Dysfunction in Multiple Sclerosis: A Systematic Review

Alia Saberi¹, Samaneh Kazemi²

¹ Neuroscience Research Center, Department of Neurology, Poursina Hospital, Guilan University of Medical Sciences, Rasht, Iran

² Deputy of Research and Technology, Guilan University of Medical Sciences, Rasht, Iran

Received: 20 Dec. 2017; Accepted: 05 Nov. 2018

Abstract- Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder of the central nervous system (CNS) and is characterized by a high degree of heterogeneity in progression and treatment response. Mitochondrial dysfunction is increasingly recognized as an important feature of MS pathology and may be relevant for clinical disease progression. This paper systematically reviews published evidence concerning the role of mitochondrial abnormalities with MS. Literature searched using the Web of Science, PMC/Medline via PubMed and Scopus databases up to May 2017 with no time and language limitation. After quality assessment, 9 articles were included in the study. All data extraction was conducted by two reviewers independently. Based on the results of the studies, it seems that mitochondrial DNA abnormality and mitochondrial dysfunction may be due to primary inflammation in MS or may be occurred itself before any inflammation, but definitely contributes to axonal degeneration and disease progression. Mitochondrial abnormality contributes to axonal degeneration in MS and disease progression.

© 2019 Tehran University of Medical Sciences. All rights reserved.

Acta Med Iran 2019;57(1):5-16.

Keywords: Multiple sclerosis; Disease progression; DNA; Mitochondria

Introduction

MS is traditionally considered an inflammatory-mediated demyelinating disease of the CNS. However, MS is increasingly viewed as a neurodegenerative disease in which axonal damage, neuronal death, and atrophy of the CNS are the principal causes of irreversible neurological disability in patients. Although the mechanisms of neurodegeneration in MS are poorly understood, several lines of evidence point to secondary mitochondrial dysfunction as a key player in the process (1).

A number of MS post-mortem studies have now reported mitochondrial abnormalities within neurons, detailed at the level of enzyme activity, protein, transcripts, and DNA. At a functional level, the important aspect of mitochondrial abnormalities in MS is their consistent impairment of the activity of mitochondrial respiratory chain enzymes, namely complex I, complex III and complex IV (three of the five complexes that make up the mitochondrial respiratory chain). In terms of causation of the mitochondrial respiratory chain enzyme deficiency in the neuronal cell body, molecular explanations include a decrease in a large number of nuclear DNA-encoded transcripts of mitochondrial

respiratory chain complexes and a high level of mitochondrial DNA (mtDNA) deletions (2).

In recent years, it has become increasingly clear that dysfunctional mitochondria are important contributors to the damage and loss of both axons and neurons, and observations in animals and histopathological studies have suggested that infiltrating leukocytes and activated microglia play a central role in neuronal mitochondrial dysfunction (3).

A systematic review was conducted to have a clear answer and deep understanding of the topic of concern.

Materials and Methods

Literature search

This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (4). Web of Science, PMC/Medline via PubMed and Scopus databases were searched with same search strategies for studies published up to May 2017. The search strategy using Boolean Operators and keywords was: "Mitochondrial Dysfunction" AND "Multiple Sclerosis." Combination of these terms without filters regarding time and language limits, study design and

Corresponding Author: S. Kazemi

Deputy of Research and Technology, Guilan University of Medical Sciences, Rasht, Iran
Tel: +98 13 33330939, Fax: +98 13 33330939, E-mail address: kazemi_s@gums.ac.ir

Multiple sclerosis and mitochondrial dysfunction

type were used to obtain the search results.

Criteria for inclusion

Studies with human model of MS and any study concern of mitochondrial dysfunction or genetic change of mitochondrial DNA were considered.

We appraised titles and abstracts of the retrieved articles to determine the initial eligibility; and if necessary, the full articles were studied in detail in order to be selected for the review. Two reviewers extracted data. After a detailed study, the remaining articles were included.

Quality assessment

Assessment of the quality of the articles was conducted using the modified quality assessment checklist for observational studies (STROBE). The checklist was applied to the appraisal of each study by two reviewers independently.

Methods for data extraction

After screening databases, the initial articles were selected, and their data were extracted uniformly. All data extraction conducted by two reviewers independently and any disagreements were resolved by discussion.

Results

A total of 874 reports were screened for the analysis of patients with MS and Mitochondrial Dysfunction. After eliminating the duplicate articles, 433 articles were obtained for this review. After removing 409 unrelated records (based on the contents of the abstracts for each article), 24 full texts were assessed for eligibility. Fifteen Full-text articles excluded, with reasons that those were Animal model study, Case report, Letter to editor, Review article as explained in figure 1. The study selection process is described in figure 1. Characteristics of the included articles are summarized in table 1.

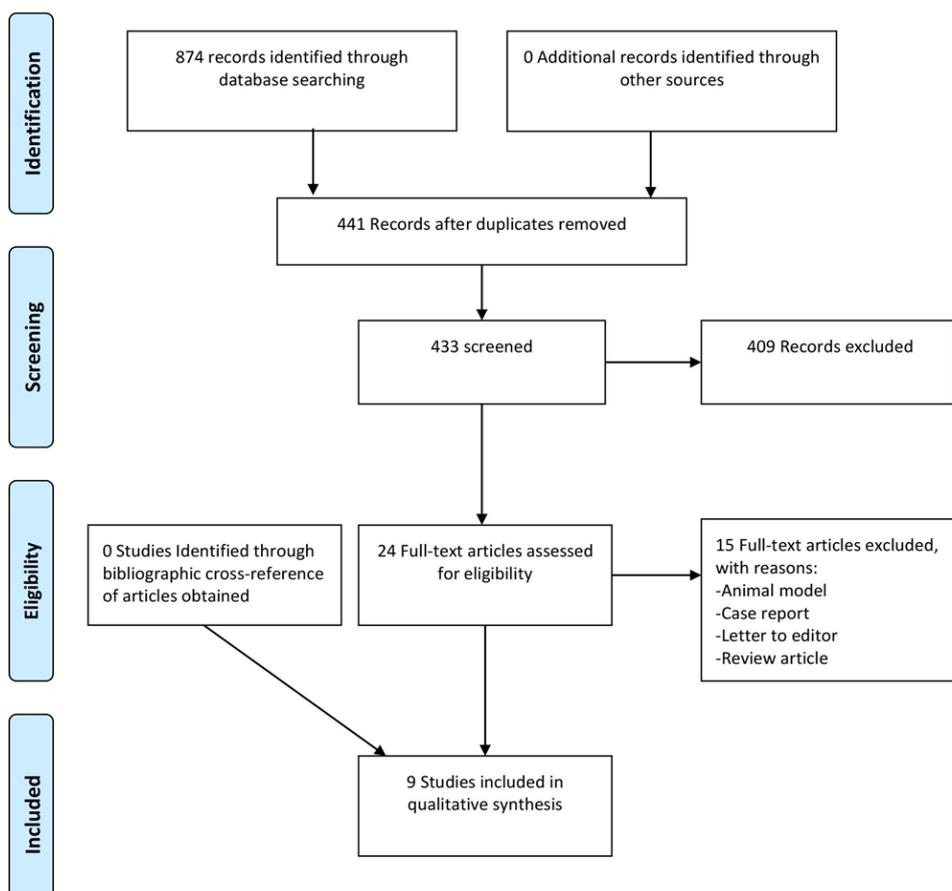


Figure 1. Flow chart of the selection process of eligible studies

Table 1. Summary of the included studies

Author, Year, Country	Study type	Number of participants	The course of multiple sclerosis	EDSS	Age (year)	Case group	Control group	Lab method	Main Results
Witte et al., Netherlands, 2009	Original	33	SPMS PPMS ND		Case group (34–80, mean 60) Control group: (24–84, mean 63)	26 MS patients	7 non-neurological controls	-Brain autopsy and frozen in liquid nitrogen -Classification of MS lesions by immunohistochemical analysis using antibodies against PLP -Detection of mitochondrial protein expression and enzyme activity using immunohistochemical and biochemical techniques -Quantification of colocalization of mitochondria and their co-localization with axons and astrocytes within MS lesions and NAWM using a standard confocal scanning microscope (TCS SP2, Leica Microsystems, Heidelberg GmbH, Mannheim, Germany) -Autopsy using Snap frozen blocks of CP epithelium -A histochemical assay of Complex IV/complex II. -Immunofluorescent histochemistry -Immunohistochemistry -DNA extraction by Laser microdissection and real-time PCR	The enhanced density of mitochondria in MS lesions might contribute to the formation of free radicals and subsequent tissue damage.
Campbell et al., UK, 2012	Original	19	-	-	-	10 patients with MS	9	-A two-step long-range PCR was carried out on the major arc of the mitochondrial genome using the Expand Long Template PCR System to detect D-mtDNA. -Electrophoresis with agarose gels using the Qiagen gel extraction kit -Sequencing the mtDNA -Clonally expanded D-mtDNA in single respiratory enzyme-deficient CP epithelial cells using single-molecule PCR -Determination the extent of respiratory enzyme deficiency and D-mtDNA at a single cell level	Clonal expansion of mitochondrial DNA deletions (Δ -mtDNA) is prominent within the choroid plexus epithelium in MS.
Andalib et al., Iran, 2017	Original	200 Female: 126 Male: 74	RRMS	-	Case group (32.47 \pm 8.13) Control group (30.24 \pm 15.49)	100 RRMS	100 unrelated healthy controls	-DNA extraction by salting-out method -Amplification of mtDNA fragment with PCR -RFLP by Mva I for PCR products -Electrophoresis with 3% agarose gel -Imaged restriction products in a UV transilluminator -Genotyping by sequencing -DNA extraction by salting-out method -Amplification of mtDNA fragment with PCR	The G13708A mtDNA variation is not associated with MS in the selected Iranian population.
Andalib et al., Iran, 2015	Original	200 Female: 126 Male: 74	RRMS	-	Case group (32.47 \pm 8.13) Control group (30.24 \pm 15.49)	100 RRMS	100 unrelated healthy controls	-RFLP by Nla III and Acc I restriction endonuclease enzymes for mtDNA T4216C and A4917G variations -Electrophoresis with 3% agarose gel -Imaged restriction products in a UV transilluminator -Genotyping by sequencing -DNA extraction by salting-out method -Amplification of mtDNA fragment with PCR	The mtDNA T4216C variation in mitochondrial ND1 gene and mtDNA A4917T in mitochondrial ND2 gene are not associated with MS in selected Iranian population.
Andalib et al., Iran, 2015	Original	200 Female: 126 Male: 74	RRMS	-	Case group (32.47 \pm 8.13) Control group (30.24 \pm 15.49)	100 RRMS	100 unrelated healthy controls	-RFLP by Acc I and Rsa I restriction endonuclease enzymes for mtDNA G15257A and G15812A variations -Electrophoresis with 3% agarose gel -Imaged restriction products in a UV transilluminator -Genotyping by sequencing	The G15257A and G15812A variations in the mtDNA tRNA(Thr) gene are associated with susceptibility to MS in the selected Iranian population.

Continuance of Table 1

Author	Study Type	Sample Size	MS Type	RRMS (n, range)	SPMS (n, range)	PPMS (n, range)	Other	Methods	Findings
Leurs et al., Netherlands, 2017	Original	187	RRMS, SPMS, PPMS	92 (3.0-4.0)	27 (6.0-7.0)	13 (4.0-6.0)	50 various neurologic disease controls, 5 healthy controls	Digital PCR	No significant difference between the concentration of mtDNA in CSF between RRMS and SPMS and significantly decreased in RRMS patients. A non-specific elevation of concentration of mtDNA in PMS patients.
Haile et al., Canada, 2017	Original	12 Female: 9, Male: 3	RRMS, SPMS, PPMS	-	????	-	Dead patient with MS	-Autopsy using Snap frozen blocks of postmortem cerebral sub-ventricular deep white matter samples -Preparing tissue lysates from the human frozen brain as well as from the spinal cords of EAE mice and producing cell lines -Immunohistochemistry -Extraction of total RNA by RT-PCR. -Assessing the Rab32 expression	Rab32 links endoplasmic reticulum (ER) stress and mitochondrial dysfunction. ER stress is strongly associated with Rab32 upregulation in the progression of MS, leading to mitochondrial dysfunction and neuronal death
Witte et al., Netherlands, 2013	Original	24 Female: 10, Male: 14	SPMS, PPMS, NA, ND	Case group (40-88)	The control group (51-78)	-	15 progressive MS patients (dead)	-Brain autopsy -Immunohistochemistry -Isolation of mRNA from frozen tissue blocks by Real-time quantitative PCR -Protein analysis by Western blot	Neuronal loss in NAGM of MS patients significantly correlated with the extent of PGC-1a decrease. Reduced neuronal PGC-1a expression in MS cortex partly underlies mitochondrial dysfunction and neurodegeneration in MS cortex.
Zambonin et al., UK, 2011	Original	15 Female: 7, Male: 8	RPM, SPMS, PPMS, NA	Case group (34-84)	Control group (57-95)	-	10 patient with MS (dead)	-Brain autopsy -Immunohistochemistry -Immunofluorescently labeled axons (SMI31-positive) and mitochondria identified using porin and myelin (myelin basic protein for remyelinated regions in MS tissue) were imaged on a Leica scanning fluorescent microscope with an oil immersion lens at x63 (Confocal microscopy) -Detection of mitochondrial respiratory chain complex IV active elements in unfixed cryostat sections from snap frozen tissue blocks, and Sequential complex IV histochemistry and immunofluorescent labeling of axons	-Mitochondrial content within remyelinated axons was significantly less than in acutely and chronically demyelinated axons but more numerous than in myelinated axons. -The number of mobile mitochondria in remyelinated axons was similar to myelinated axons, although significantly greater than in demyelinated axons. -Demyelination increases the energy demand of axons. But remyelination partially ameliorates the energy demand of axons.

RRMS: relapsing-remitting MS; SPMS: secondary progressive MS; RPMS: relapsing progressive MS; PPMS: primary progressive MS; CIS: clinically isolated syndrome; ND: not determined; NA: not applicable; LBSL: Leukoencephalopathy with brain stem and spinal cord involvement and high brain lactate; CP: choroid plexus EAE: experimental autoimmune encephalomyelitis; NAWM: normal-appearing white matter; PLP: proteolipid protein

Discussion

Mitochondria DNA can be possessed independently. Mitochondria provide energy for cells by synthesizing adenosine triphosphate (ATP). Moreover, they play a role in programmed cell death (apoptosis). The mitochondrial respiratory chain on its inner membrane consists of four complexes (complex I-IV), the fifth complex contributes directly to ATP synthesis (5-7). These complexes are built of multiple subunits, most parts are proteins coded by mitochondrial DNA (mtDNA), and only complex II is

encoded by nuclear DNA. The most amount of ATP is produced by oxidative phosphorylation; in which large amounts of reactive oxygen (ROS) and nitrogen species (RNS) are formed. Production of cellular antioxidants is defense against this process. Mitochondrial dysfunction resulted in a decrease in ATP synthesis impaired Ca²⁺ content and increased ROS and RNS at the same time. Due to increased lipid peroxidation as a result of elevated ROS levels, membrane injuries occur, secondary failures accumulate in mtDNA (as secondary de novo mutations). Mitochondrial damage in MS was found to play an

important role in the progression of the disease (8). Energy deficits and mitochondrial dysfunction due to inflammation involved in neuronal degeneration in MS. Mancini *et al.*, showed that during the acute relapsing phase of experimental autoimmune encephalomyelitis (EAE), neuronal vulnerability to mitochondrial complex IV inhibition is enhanced, and the activation of NO pathway and microglia lead to an increased neuronal susceptibility to this kind of inhibition (9). The neurotoxicity of NO is more likely mediated by peroxynitrite rather than NO itself, Lan *et al.*, hypothesize that NO takes part in MS through impairing the function of monocarboxylate transporter 1, to cause axonal degeneration (10).

Křištofiková *et al.*, for the first one reported the enhancement of the mitochondrial enzyme 17 β -hydroxysteroid dehydrogenase type 10 (17 β -HSD10) in the CSF of patients with MS up to 179%. The result is not especially surprising since recent research shows the involvement of mitochondria in the development of the disease, e.g. via mutations in mitochondrial deoxyribonucleic acid (11).

Witte *et al.*, claimed that mitochondrial alterations might occur as a response to demyelination and inflammation. Since demyelination leads to increased energy demand in axons and could thereby affect the number, distribution, and activity of mitochondria. The number of mitochondria and their co-localization with axons and astrocytes within MS lesions and adjacent normal-appearing white matter (NAWM) was quantitatively assessed. In both active and inactive lesions, an increase in mitochondrial protein expression, as well as a significant increase in the number of mitochondria, was found. Mitochondrial density in axons and astrocytes was significantly enhanced in active lesions compared to adjacent NAWM, whereas a trend was observed in inactive lesions. Complex IV activity was strikingly up-regulated in MS lesions compared to control white matter and, to a lesser extent, NAWM. Finally, they demonstrated increased immunoreactivity of the mitochondrial stress protein mtHSP70 in MS lesions, particularly in astrocytes and axons (12,13).

Current research revealed that the following mitochondrial abnormalities are involved in the development and progression of multiple sclerosis: 1) mitochondrial DNA defects, 2) abnormal mitochondrial gene expression, 3) defective mitochondrial enzyme activities, 4) deficient mitochondrial DNA repair activity, and 5) mitochondrial dysfunction.

Mao and Reddy (14) proposed that abnormal mitochondrial dynamics (increased fission and decreased

fusion in neurons) are affected by MS. Further, they also propose that mitochondrial abnormalities and mitochondrial energy failure may impact other cellular pathways, including increased demyelination and inflammation in neurons and tissues that are affected by MS

Mitochondrial DNA alternations in MS

The mtDNA mutations may increase the risk of MS (14). Campbell *et al.*, proposed the clonally expanded mitochondrial DNA deletions within the choroid plexus in MS patients by studying on snap frozen blocks of CP (Cerebral palsy) from 10 autopsied MS patients 5 PD (Parkinson's disease), 5 AD (Alzheimer's disease) and 9 control cases. Clonal expansion of D-mtDNA is the process by which a mutant mtDNA molecule increases to high levels within a single cell containing both wild-type and mutant mtDNA. Unlike in AD and PD, the diffuse inflammatory process in MS involves the choroid plexus, and mitochondria are exposed to reactive oxygen and nitrogen species over a prolonged period. They determined the extent of respiratory enzyme deficiency and D-mtDNA at a single cell level within choroid plexus epithelial cells in MS as well as in AD, PD, and controls. The respiratory enzyme-deficient (lacking complex IV and with intact complex II activity) cells were more prevalent within the choroid plexus in AD, MS and PD compared with controls. The main catalytic subunit of complex IV (subunit-I of cytochrome c oxidase) lacked in significantly more respiratory enzyme-deficient cells in MS compared with AD, PD, and controls. The single cell analysis showed a fourfold increase in the percentage of respiratory enzyme deficient choroid plexus epithelial cells harboring clonally expanded D-mtDNA in MS. Our findings establish clonal expansion of D-mtDNA as a feature relatively more prominent within the choroid plexus epithelium in MS than AD, PD or controls. We propose clonal expansion of D-mtDNA as a molecular link between inflammation and Campbell *et al.*, (2012) proposed the clonally expanded mitochondrial DNA deletions within the choroid plexus in multiple sclerosis patients by studying on snap frozen blocks of CP from 10 autopsied MS patients 5 PD, 5 AD and 9 control cases. Clonal expansion of D-mtDNA is the process by which a mutant mtDNA molecule increases to high levels within a single cell containing both wild-type and mutant mtDNA. Unlike in AD and PD, the diffuse inflammatory process in MS involves the choroid plexus, and mitochondria are exposed to reactive oxygen and nitrogen species over a prolonged period. They determined the extent of respiratory enzyme deficiency and D-mtDNA at a single cell level within choroid plexus epithelial cells in MS as well as in AD, PD, and controls. The respiratory

Multiple sclerosis and mitochondrial dysfunction

enzyme-deficient (lacking complex IV and with intact complex II activity) cells were more prevalent within the choroid plexus in AD, MS and PD compared with controls. The main catalytic subunit of complex IV (subunit-I of cytochrome c oxidase) lacked in significantly more respiratory enzyme-deficient cells in MS compared with AD, PD and controls. The single cell analysis showed a fourfold increase in the percentage of respiratory enzyme deficient choroid plexus epithelial cells harboring clonally expanded D-mtDNA in MS. Our findings establish clonal expansion of D-mtDNA as a feature relatively more prominent within the choroid plexus epithelium in MS than AD, PD or controls. We propose clonal expansion of D-mtDNA as a molecular link between inflammation and part of a delayed cellular energy failure in MS (15).

Mao and Reddy reviewed some studies which consider mitochondrial structural and functional changes in the pathogenesis of MS as follow. SNP analysis has shown that genetic variants of complex I genes may influence the response of tissues to inflammation in the CNS (16). Further, genetic alterations in uncoupling proteins are reported to be implicated in patients with MS. Uncoupling protein 2 (UCP2) is a member of the mitochondrial proton transport family that uncouples proton entry to the mitochondria from ATP synthesis. Vogler *et al.*, reported that the UCP2 common-866G/A promoter polymorphism is associated with susceptibility to MS in a German population. In a study of 1097 MS patients and 462 control subjects, they found the common G allele associated with disease susceptibility ($P=0.0015$). The UCP2 -866G allele was correlated with lower levels of UCP2 expression in vitro and in vivo. Thus, UCP2 may contribute to MS susceptibility by regulating the level of UCP2 protein in the CNS and/or in the immune system (17). Defects in mtDNA have been associated with late-onset MS. Ban *et al.*, (18) sequenced the mtDNA from 159 patients with MS and completed a haplogroup analysis of 835 MS patients and 1506 controls. They found a trend towards over-representation of super-haplogroup U as the only evidence for association with MS. In a parallel analysis of nuclear-encoded mitochondrial protein genes in the same subjects, they also found a trend towards association with the complex I gene, NDUFS2 (18).

A study of MS patients in Europe showed that a potentially functional mtDNA SNP, nt13708 G/A, was significantly associated with an increased risk of MS ($P=0.0002$). The study identified the nt13708A variant as an allele susceptible to MS, which may suggest a role in MS pathogenesis (19). An increasing number of case reports on Leber's hereditary optic neuropathy (LHON) associated mtDNA point mutations, and some patients

with MS and LHON share the same mtDNA mutation, suggesting that mitochondrial determinants may contribute to genetic susceptibility in MS and LHON (20). In a recent study, 58 unrelated Bulgarian patients with RRMS and 104 randomly selected healthy individuals were analyzed for the presence of 14 mtDNA polymorphisms determining major European haplogroups as well as three (4216, 14798, 13708) secondary LHON mutations. However, 21 of the 58 patients (36.2%) were positive for the T4216C mutation, while only 11.3% of the controls carried this mutation ($P=0.01$; $OR=4.38$), suggesting that the 4216C base substitution may be a predisposing marker for MS. These findings also supported the hypothesis that particular mtDNA variants may contribute to the genetic susceptibility of some people with MS (14).

Andalib *et al.*, (21) investigated mtDNA G13708A variation by PCR-RFLP and restriction enzyme of *Mva I* and it was identified that there is no significant association between MS and G13708A variation ($P=0.7$, $OR=0.8$). Mayr-Wohlfart *et al.*, (22) and Hanefeld *et al.*, (23) with the same restriction enzyme in two different German populations and a study by Mihailova on a Bulgarian population (24) with restriction enzyme BstNI also found the same results. The results of 4 above mentioned studies are based on the small sample size populations, but Yu *et al.*, (19) based on six European case-control cohorts and a total of approximately 5000 participants resulted differently. In three well-matched cohorts; G13708A was found to be significantly associated with susceptibility to MS ($OR:1.71$, $P=0.0002$). But the interesting finding by subsequent mtDNA sequencing of 50 subjects was that the association depended on a nucleotide at position 13708 rather than the variations (21).

Andalib in another study (25) assessed T4216C variation in the ND1 mtDNA gene in an Iranian population and found no association between MS and these variations [$P=0.61$, $OR=1.1$ (95% $CI:0.5-2.4$) and $P=0.637$, $OR=1.2$ (95% $CI:0.4-3.5$) respectively].

Yu *et al.*, (19) by pooled analysis of previously mention cohort studies found no association between MS and the mtDNA T4216C variation ($P=0.078$, $OR=1.06$, 95% $CI=0.70-1.01$). Such that of Penissen-Besnier *et al.*, in a Caucasian population (26) and Chalmers *et al.*, (27) Vyshkina *et al.*, (28) in Caucasian population by using Sequenom Mass ARRAY System also reported no association between mtDNA T4216C variation and MS. But T4216C mtDNA variation was seen to be a predisposing marker for MS in a study by Mihailova *et al.*, in a Bulgarian population ($P=0.01$; $OR=4.38$) (24). In a German study, Mayr-Wohlfart *et al.*, (22) found that mtDNA T4216C variation frequency in MS subjects (18%)

was higher than in the controls (11%).

Andalib in the same study (25) assessed A4917G variation in the mtDNA ND2 gene and found no association between MS and this variation [$P=0.637$, $OR=1.2$ (95% $CI:0.4-3.5$)]. Such as the results of Penissen-Besnier *et al.*, in a Caucasian population (24) and Chalmers *et al.*, (27). However, Vyshkina *et al.*, (28) in the Caucasian population found that mtDNA A4917G variation was associated with MS ($P=0.006$). In a German study, Mayr-Wohlfart *et al.*, found a high frequency of the A4917T variation in MS cases (11%), compared to controls (4%) (22). Therefore, the relation with no mutation is conclusive. No association was seen between T14798C variations, and MS. T14798C variations were found to be less in MS subjects (10%) than in the controls (6.9%) by Mihailova *et al.*, (24).

Andalib in his third study assessed mtDNA G15257A and mtDNA G15812A variation in MS in an Iranian population and did not find any association. Interestingly only one patient in each case and control group demonstrated mtDNA G15257A variation ($P=0.637$, $OR=1$, 95% $CI:0.0-79.2$) and no patient in each studied group had mtDNA G15812A variation. ($P=1$, $OR=1$, 95% $CI:0.0-79.2$) (25). Such that of Hwang *et al.*, (29) who found no association between MS and the LHON mutations (including mtDNA mutations at nucleotide (nt) 11778, and nt 14484, 3460, and 15257) in a Korean population by analyzing 12 MS subjects. Such that of Japanese MS patients (30).

Moreover, the mtDNA G15257A variation was shown to have no pathogenetic significance in Italian MS subjects (31). 5.4% of the MS subjects presenting with early and prominent optic nerve involvement and 5 out of 99 (5.1%) healthy controls had the mtDNA G15257A variation in a homoplasmic state. According to these studies, it can be concluded that these variations are not associated with MS.

On the other hand, Mayer-Wohlfart *et al.*, (22) found a possible association of mtDNA variations in MS with optic nerve involvement. Also, Kalman *et al.*, (32) investigated LHON associated mtDNA mutations in MS patients in the USA and concluded that certain sets of the mtDNA variations are associated with, and predispose to, MS, in which that 53 MS subjects (20.8%) were shown to be positive for at least two (4216 and 4917 or 13,708) or three (4216,13,708, 15,257) simultaneous secondary LHON mutations, along with 7(9.5%) controls ($P=0.036$).

Additional evidence was provided by Schoenfeld *et al.*, who demonstrated through microarray analysis that both mtDNA content and mitochondrial gene expression were significantly increased during oligodendrocyte differentiation in rats and humans (33).

Some human studies proposed abnormal mtDNA as a marker of MS and disease progression or response to medication:

Leurs *et al.*, in a study assessed 2 cohort studies in Deutschland and Sweden. Patients with PMS showed a significant increase in CSF mtDNA compared to non-inflammatory neurologic disease controls. In the Dutch cohort, mtDNA concentration was found to have significant increased level in progressive MS cases. Patients with higher T2 lesion volumes (ratio=2.13, $P=0.03$) and lower normalized brain volumes (ratio=0.42, $P=0.02$) showed increased concentration of mtDNA. But T1-hypointense lesion volumes (black hole lesion volume (BHLV)) and gadolinium-enhanced lesions on MRI did not relate to mtDNA concentration in Dutch cohort. Also, EDSS was not related to mtDNA copies/ μL . In Swedish cohort, Patients treated with fingolimod had significantly lower mtDNA copy levels at follow-up compared to baseline. For fingolimod users, mtDNA copy number concentrations were almost 50% lower on follow-up (median=9.6 copies/ μL) compared to baseline (median=17.9 copies/ μL), $z=-2.52$, $P=0.012$, $r=-0.37$). There was no significant difference in mtDNA copy levels in the fingolimod-RRMS patients ($n=23$) compared to the RRMS group using other DMT (dimethyl fumarate: $n=7$, interferon-beta: $n=12$; ratio=1.65, $P=0.089$). But in the Dutch cohort, there was no significant difference in the concentration of mtDNA copies between the groups with and without DMT (ratio=1.12, $P=0.624$). The median CSF mtDNA concentration was 16 copies/ μL (IQR, 7.75-65.25) in the group without DMT and 18 copies/ μL (IQR, 12-77) in the interferon using groups. It can be concluded that mitochondrial dysfunction has a role in MS and its clinical progression (34).

Mitochondrial dysfunction in MS

Zambonin *et al.*, the study determined the mitochondrial content within demyelinated, remyelinated and myelinated axons in post-mortem tissue from patients with multiple sclerosis. The following demyelination both in acute and chronic phase, the number of mitochondria which was labeled by Immunofluorescence increases in the axons in the CNS, maybe due to the increase in energy needs. In the remyelination phase, its number decreases but is yet more than normal myelinated axons. It is due to an increase in mitochondrial respiratory chain complex IV activity. Although according to this study the total mitochondrial number increases in demyelinated fibers, the number of mobile mitochondria in remyelinated and myelinated axons is similar and, significantly greater than in demyelinated axons (35).

Multiple sclerosis and mitochondrial dysfunction

In the other studies, ultrastructural analysis of demyelinated spinal cord lesions showed dramatically reduced numbers of mitochondria and microtubules and demonstrated Ca²⁺-mediated destruction of chronically demyelinated axons and axonal swelling (36,37). As reduced energy production is a major contributor to Ca²⁺-mediated axonal degeneration, authors focused on changes in oxidative phosphorylation and inhibitory neurotransmission. Compared with controls, 488 transcripts were decreased, and 67 were increased in the MS cortex. Twenty-six nuclear-encoded mitochondrial genes and the functional activities of mitochondrial respiratory chain complexes I and III were decreased in the MS motor cortex. Reduced mitochondrial gene expression was specific for neurons. In addition, synaptic components of GABAergic neurotransmission and the density of inhibitory interneuron processes also were decreased in the MS cortex. In addition, recently a number of mitochondrial respiratory chain proteins in active lesions from acute MS were analyzed using immunohistochemistry (38). Functionally important defects of mitochondrial respiratory chain complex IV [cytochrome c oxidase (COX)] including its catalytic component (COX-I) are present in some active MS lesions (Pattern III) (39). The lack of immunohistochemically detected COX-I is apparent in oligodendrocytes, hypertrophied astrocytes, and axons, but not in microglia. These findings suggest that hypoxia-like tissue injury in Pattern III MS lesions may be initiated from mitochondrial impairment. On the other hand, in inactive areas of chronic MS lesions the complex IV activity and mitochondrial mass, judged by porin immune reactivity, are increased within approximately half of large chronically demyelinated axons compared with large myelinated axons in the brain and spinal cord. Mitochondria are generated in the cell body and transported to the axon (anterograde movement) to replace those that are worn out. In turn, the damaged axonal mitochondria are transported back to the cell body (retrograde movement) for degradation. Furthermore, nuclear DNA encodes both the majority of mitochondrial proteins and the superfamily of motor proteins required for transport of mitochondria along the axon: kinesins for anterograde movement and dyneins for retrograde transport back to the cell body. Both kinesins and dyneins bind to microtubules, which form the tracks for mitochondrial transport among other functions. The targeting of motile mitochondria to high energy-demanding sites and the maintenance of stationary mitochondrial sites require docking proteins. In progressive MS, Kinesins are decreased at the protein (KIF5A) and transcript levels (KIF1B, KIF5A, and

KIF21B) in the deep layers (layer V–VI) of the non-lesional cortex. This effect is most prominent in cases with short disease duration. A recently identified protein is syntaphilin, a member of the syntaxin family of proteins encoded by nuclear DNA, as an axon-specific mitochondrial docking protein. Overexpression of syntaphilin in neurons leads to the cessation of mitochondrial movement and their docking in the axon (2). Campbell *et al.*, have detected increased syntaphilin in surviving chronically demyelinated axons from MS cases, suggesting increased mitochondrial docking and stationary mitochondria (2). Mahad *et al.*, also found syntaphilin and phosphorylated neurofilament -H were increased in chronic lesions (40).

Recently, Regenold *et al.*, investigated the relationship between disturbed CNS mitochondrial energy metabolism and MS disease progression by measuring cerebrospinal fluid (CSF) concentrations of sorbitol, fructose, and lactate, all metabolites of extra-mitochondrial glucose metabolism (41). They found that concentrations of all three metabolites, but not concentrations of glucose or myoinositol, were significantly increased in CSF from secondary progressive and, to a lesser degree, relapsing-remitting patients, compared to healthy controls. Furthermore, CSF concentrations of sorbitol and fructose (polyol pathway metabolites), but not lactate (anaerobic glycolysis metabolite), correlated positively and significantly with Expanded Disability Status Scale (EDSS) score, an index of neurologic disability in MS patients. These findings suggest that abnormal mitochondrial glucose metabolism is increased in MS patients and is associated with disease progression (41).

Interestingly, analysis of mitochondrial enzymes on human muscle showed that in people with MS, there were fewer type I fibers, and that fibers of all types were smaller and had lower succinate dehydrogenase (SDH, component of the respiratory chain complex II) and SDH/alpha-glycerol-phosphate dehydrogenase (GPDH) but not GPDH activities, suggesting that muscle in this disease is smaller and relies more on anaerobic than aerobic-oxidative energy supply than does muscle of healthy individuals (42). Similar to the brain, muscles are also highly dependent on mitochondrial oxidative energy metabolism, so it is reasonable that there is a weaker muscle in MS patients, indicating muscle is also one of the targets of MS. In some rare cases, MS could have a mitochondrial myopathy combination, in which MRI showed widespread white matter lesions, muscle biopsy showed ragged red fibers and COX (complex IV) deficiency, Southern blot analysis revealed a large deletion of mtDNA (43). Probably the severe mitochondrial genomic deletion is the key cause or

initiation factor for this special case.

Another interesting key issue of mitochondria must be discussed below. The mitochondrial permeability transition leads to mitochondrial swelling, outer membrane rupture and, enhances ROS generation, induces mitochondrial calcium perturbation and facilitates mitochondrial pro-apoptotic molecule release. The mitochondrial permeability transition pore (PTP) is thought to consist of the adenine nucleotide translocator, a voltage-dependent anion channel, and cyclophilin D (CyPD, the Ppif gene product), a prolyl isomerase located within the mitochondrial matrix. CyPD is a key regulator of the PTP, and they are required for mediating Ca²⁺- and oxidative damage-induced cell death (44,45). In experimental animal MS disease model, EAE mice lacking CyPD showed that neurons missing CyPD, are resistant to oxidative agents thought to be the mediators of axonal degeneration observed in both EAE and MS and have mitochondria that are able to more effectively handle elevated Ca²⁺. Consistent with this neuronal resistance, animals missing CyPD are able to recover, clinically, following the induction of EAE (46). These results directly implicate pathological activation of the mitochondrial PTP in the axonal damage occurring during MS, in another word, PTP and mitochondria are the critical target of EAE, perhaps multiple sclerosis (47,14,8).

Patergnani *et al.*, showed that oligodendrocyte differentiation is particularly sensitive to mitochondrial toxins, and this observation corroborated the importance of mitochondria for proper oligodendrocyte maturation and consequent myelination. They additionally assessed the other aspects of mitochondrial abnormalities in MS (48). mtDNA mutations, the expression of key nuclear-and mitochondrial-encoded subunits of the ETC has been linked to MS susceptibility. Peroxisome proliferator-activated receptor (PPAR) gamma coactivator-1 α (PGC-1 α) is a transcription factor that regulates the expression of OxPhos subunits and mitochondrial defense. The latter function is achieved by controlling the expression of mitochondrial antioxidant proteins. Interestingly, the mRNA levels of PGC-1 α were found to be significantly reduced in cortical samples from MS patients, and this reduction was associated with a significant loss of pyramidal neurons in deep cortical layers and with an increase in ROS production (49). Thus, the reduction of the neuronal PGC-1 α levels may contribute to neuronal loss via impairment of OxPhos and mitochondrial redox imbalance. Furthermore, recent findings indicate that altered expression of nuclear respiratory factor1/2 (NRF-1/2), estrogen-related receptor α (ESR1), and PPARs affect the expression of OxPhos genes and cause oxidative as

well as nitrosative damage. In particular, changes in NRF-2 associated with down-regulation of mETC genes and increased production of ROS have been found in postmortem MS brains (50). As the binding of NRF-2 to DNA is redox-regulated (51), the overall increment in oxidative damage could affect NRF-2 outcomes. As a consequence, NRF-2 fails to bind to the promoter region of mETC genes, and the transcription and production of protein subunits of the mETC complexes are consequently affected (48).

In EAE, it has been found that nitric oxide, superoxide, and peroxynitrite can impair mitochondrial function, thereby inhibiting mitochondrial complexes I to V, aconitase, manganese superoxide dismutase, and creatine kinase, which can lead to damage of mtDNA, lipid peroxidation and increased mitochondrial proton permeability (52-54). It has been reported in EAE, that mitochondrial dysfunction can be found in the early stage of MS disease (55). In a recent study (based on histological evidence), the excessive production of nitric oxide by activated microglia and macrophages can be a cause of reversible conduction block, which is observed in demyelinated axons (56,57). A new study found that mitochondrial damage can precede inflammation in EAE, suggesting that mitochondrial dysfunction is primary in the disease (58).

Endoplasmic reticulum (ER) stress is a hallmark of neurodegenerative diseases such as multiple sclerosis (MS). However, this physiological mechanism has multiple manifestations that range from impaired clearance of unfolded proteins to altered mitochondrial dynamics and apoptosis. The membranous contacts between the ER and mitochondria, called the mitochondria-associated membrane (MAM), could provide a functional link between these two mechanisms. Haile *et al.*, found that the induction of guanosine triphosphatase (GTPase) Rab 32 a known regulator of the MAM correlates with ER stress proteins in MS brain, as well as in EAE, and occurs in multiple CNS cell types. They identified Rab 32, known to increase in response to acute brain inflammation, as a novel unfolded protein response (UPR) target. High Rab32 expression shortens neurite length, alters mitochondria morphology, and accelerates apoptosis/necroptosis of human primary neurons and cell lines and lead to the progression of MS (59).

Mitochondrial medicine for neurodegenerative diseases

A study has been done by utilizing electrophysiological techniques in brain striatal slices, to evaluate the potential protective effects of interferon β -1a against acute neuronal

Multiple sclerosis and mitochondrial dysfunction

dysfunction induced by mitochondrial toxins. Interferon β -1a was found to exert a dose-dependent protective effect against the progressive loss of striatal field potential amplitude induced by the mitochondrial complex I inhibitor rotenone. Interferon β 1a also reduced the generation of the rotenone-induced inward current in striatal spiny neurons. Conversely, interferon β 1a did not influence the electrophysiological effects of the mitochondrial complex II inhibitor 3-nitropropionic acid. The protective effect of interferon β 1a against mitochondrial complex I inhibition was found to be dependent on the activation of STAT1 signaling. Conversely, endogenous dopamine depletion and the modulation of the p38 MAPK and mTOR pathways did not influence the effects of interferon β 1a. During EAE striatal rotenone toxicity was enhanced but the protective effect of interferon β 1a was still evident (60).

Du *et al.*, focused on mitochondrial permeability transition pore (mPTP) for the treatment of MS. The protective effects of mPTP blockade have been studied in animal models of a variety of neurodegenerative diseases, and current studies are focusing on inhibition on cyclophilin D (CypD) or voltage-dependent anion channel (VDAC). In experimental MS mouse model, it has been shown that CypD depletion ameliorates the severity of symptoms and preserves axonal functions. Several CypD inhibitors such as cyclosporine A (CsA), Sanglifehrin A (SfA), and FK506 have been developed and are reported to be capable of inhibiting mPTP formation and its consequent damages. Cyclosporine A (CsA) has been experienced in Amyotrophic lateral sclerosis (ALS), FK506, in an MS and HD animal models, and VDAC in ALS mice (47).

Based on the results of the studies, it seems that mitochondrial DNA abnormality and mitochondrial dysfunction may be due to primary inflammation in MS or may occur itself before any inflammation, but definitely contribute to axonal degeneration and disease progression.

References

1. Morató L, Bertini E, Verrigni D, Ardisson A, Ruiz M, Ferrer I, et al. Mitochondrial dysfunction in central nervous system white matter disorders. *Glia* 2014;62:1878-94.
2. Campbell GR, Worrall JT, Mahad DJ. The central role of mitochondria in axonal degeneration in multiple sclerosis. *Mult Scler* 2014;20:1806-13.
3. Witte ME, Mahad DJ, Lassmann H, van Horssen J. Mitochondrial dysfunction contributes to neurodegeneration in multiple sclerosis. *Trends Mol Med* 2014;20:179-87.
4. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol* 2009;62:1006-12.
5. DiMauro S; Schon EA. Mitochondrial respiratory-chain diseases. *N Engl J Med* 2003;348:2656-68.
6. Reddy PH. Mitochondrial medicine for aging and neurodegenerative diseases. *Neuromolecular Med* 2008;10:291-315.
7. Reddy PH. Mitochondrial oxidative damage in aging and Alzheimer's disease: Implications for mitochondrially targeted antioxidant therapeutics. *J Biomed Biotechnol* 2006;3:31372.
8. Rajda C, Pukoli D, Bende Z, Majláth Z, Vécsei L. Excitotoxins, mitochondrial and redox disturbances in multiple sclerosis. *Int J Mol Sci* 2017;pii:E353.
9. Mancini A, Tantucci M, Mazzocchetti P, de Iure A, Durante V, Macchioni L. Microglial activation and the nitric oxide/cGMP/PKG pathway underlie enhanced neuronal vulnerability to mitochondrial dysfunction in experimental multiple sclerosis. *Neurobiol Dis* 2018;113:97-108.
10. Lan M, Tang X, Zhang J, Yao Z. Insights in pathogenesis of multiple sclerosis: nitric oxide may induce mitochondrial dysfunction of oligodendrocytes. *Rev Neurosci* 2018;29:39-53.
11. Kristofiková Z, Bocková M, Hegnerová K, Bartos A, Klaschka J, Rícný J, et al. Enhanced levels of mitochondrial enzyme 17 β -hydroxysteroid dehydrogenase type 10 in patients with Alzheimer disease and multiple sclerosis. *Mol Biosyst* 2009;50:1174-9.
12. Witte ME, Bø L, Rodenburg RJ, Belien JA, Musters R, Hazes T, et al. Enhanced number and activity of mitochondria in multiple sclerosis lesions. *J Pathol* 2009;219:193-204.
13. Witte ME, Geurts JJ, de Vries HE, van der Valk P, van Horssen J. Mitochondrial dysfunction: a potential link between neuroinflammation and neurodegeneration? *Mitochondrion* 2010;10: 411-8.
14. Mao PZ, Reddy PH. Is multiple sclerosis a mitochondrial disease? *Biochim Biophys Acta* 2010;1802:66-79.
15. Campbell GR, Kraysberg Y, Krishnan KJ, Ohno N, Ziabreva I, Reeve A, Trapp BD, et al. Clonally expanded mitochondrial DNA deletions within the choroid plexus in multiple sclerosis. *Acta Neuropathol* 2012;124:209-20.
16. Vyshkina T, Banisori I, Shugart YY, Leist TP, Kalman B, et al. Genetic variants of complex I in multiple sclerosis. *J Neurol Sci* 2005;228:55-64.
17. Vogler S, Goedde R, Mitterski B, Gold R, Kroner A, Koczan D, et al. Association of a common polymorphism in the promoter of UCP2 with susceptibility to multiple sclerosis. *J Mol Med* 2005;83:806-11.

18. Ban M, Elson J, Walton A, Turnbull D, Compston A, Chinnery P, et al. Investigation of the role of mitochondrial DNA in multiple sclerosis susceptibility. *PLoS One* 2008;3:e2891.
19. Yu X, Koczan D, Sulonen AM, Akkad DA, Kroner A, Comabella M, et al. mtDNA nt13708A variant increases the risk of multiple sclerosis. *PLoS One* 2008;3:e1530.
20. Harding AE, Sweeney MG, Miller DH, Mumford CJ, Kellar-Wood H, Menard D, et al. Compston, Occurrence of a multiple sclerosis-like illness in women who have a Leber's hereditary optic neuropathy mitochondrial DNA mutation. *Brain* 1992;115:979-89.
21. Andalib S, Talebi M, Sakhinia E, Farhoudi M, Sadeghi-Bazargani H, Emamhadi MR, et al. Mitochondrial DNA G13708A variation and multiple sclerosis: Is there an association? *Rev Neurol* 2017;173:164-8.
22. Mayr-Wohlfart U, Paulus C, Henneberg A, Rödel G. Mitochondrial DNA mutations in multiple sclerosis patients with severe optic involvement. *Acta Neurol Scand* 1996;94:167-71.
23. Hanefeld FA, Ernst BP, Wilichowski E, Christen HJ. Leber's hereditary optic neuropathy mitochondrial DNA mutations in childhood multiple sclerosis. *Neuropediatrics* 1994;25:331.
24. Mihailova SM, Ivanova MI, Quin LM, Naumova EJ. Mitochondrial DNA variants in Bulgarian patients affected by multiple sclerosis. *Eur J Neurol* 2007;14:44-7.
25. Andalib S, Talebi M, Sakhinia E, Farhoudi M, Sadeghi-Bazargani H. Mitochondrial DNA T4216C and A4917G variations in multiple sclerosis. *J Neurol Sci* 2015;356:55-60.
26. Penisson-Besnier I, Moreau C, Jacques C, Roger J, Dubas F, Reynier P. Multiple sclerosis and Leber's hereditary optic neuropathy mitochondrial DNA mutations. *Rev Neurol* 2001;157:537-41.
27. Chalmers RM, Robertson N, Compston DAS, Harding AE. Sequence of mitochondrial DNA in patients with multiple sclerosis. *Ann Neurol* 1996;40:239-43.
28. Vyshkina T, Sylvester A, Sadiq S, Bonilla E, Canter JA, Perl A, et al. Association of common mitochondrial DNA variants with multiple sclerosis and systemic lupus erythematosus. *Clin Immunol* 2008;129:31-5.
29. Hwang JM, Chang BL, Park SS. Leber's hereditary optic neuropathy mutations in Korean patients with multiple sclerosis. *Ophthalmologica* 2001;215:398-400.
30. Nishimura M, Obayashi H, Ohta M, Uchiyama T, Hao Q, Saida T. No association of the 11778 mitochondrial DNA mutation and multiple sclerosis in Japan. *Neurology* 1995;45:1333-4.
31. Leuzzi V, Carducci C, Lanza M, Salvetti M, Ristori G, Giovanni S, et al. LHON mutations in Italian patients affected by multiple sclerosis. *Acta Neurol Scand* 1997;96:145-8.
32. Kalman B, Lublin F, Alder H. Mitochondrial DNA mutations in multiple sclerosis. *Mult Scler* 1995;1:32-6.
33. Schoenfeld R, Wong A, Silva J, Li M, Itoh A, Horiuchi M, et al. Oligodendroglial differentiation induces mitochondrial genes and inhibition of mitochondrial function represses oligodendroglial differentiation. *Mitochondrion* 2010;10:143-50.
34. Leurs CE, Podlesniy P, Trullas R, Balk L, Steenwijk MD, Malekzadeh A, et al. Cerebrospinal fluid mtDNA concentration is elevated in multiple sclerosis disease and responds to treatment. *Mult Scler* 2018;24:472-80.
35. Zamboni JL, Zhao C, Ohno N, Campbell GR, Engeham S, Ziabreva I, et al. Increased mitochondrial content in remyelinated axons: implications for multiple sclerosis. *Brain* 2011;134:1901-13.
36. Dutta R, McDonough J, Yin X, Peterson J, Chang A, Torres T, et al. Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. *Ann Neurol* 2006;59:478-89.
37. Trapp BD, Nave KA. Multiple sclerosis: an immune or neurodegenerative disorder? *Annu Rev Neurosci* 2008;31:247-69.
38. Mahad D, Lassmann H, Turnbull D. Review: mitochondria and disease progression in multiple sclerosis. *Neuropathol Appl Neurobiol* 2008;34:577-89.
39. Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann Neurol* 2000;47:707-17.
40. Mahad DJ, Ziabreva I, Campbell G, Lax N, White K, Hanson PS, et al. Mitochondrial changes within axons in multiple sclerosis. *Brain* 2009;132:1161-74.
41. Regenold WT, Phatak P, Makley MJ, Stone RD, Kling MA. Cerebrospinal fluid evidence of increased extra-mitochondrial glucose metabolism implicates mitochondrial dysfunction in multiple sclerosis disease progression. *J Neurol Sci* 2008;275:106-12.
42. Kent-Braun JA, Ng AV, Castro M, Weiner MW, Gelinas D, Dudley GA, R. et al. Strength, skeletal muscle composition, and enzyme activity in multiple sclerosis. *J Appl Physiol* 1997;83:1998-2004.
43. Bet L, Moggio M, Comi GP, Mariani C, Prella A, Checcarelli N, et al. Multiple sclerosis and mitochondrial myopathy: an unusual combination of diseases. *J Neurol* 1994;241:511-6.
44. Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, et al. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* 2005;434:658-62.

Multiple sclerosis and mitochondrial dysfunction

45. Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Yamagata H, et al. Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature* 2005;434:652-8.
46. Forte M, Gold BG, Marracci G, Chaudhary P, Basso E, Johnsen D, et al. Fowlkes, M. Rahder, K. Stem, P. Bernardi, D. Bourdette, Cyclophilin D inactivation protects axons in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis. *Proc Natl Acad Sci U. S. A.* 2007;104:7558-63.
47. Du H, Yan SS. Mitochondrial medicine for neurodegenerative diseases. *Int J Biochem Cell Biol* 2016;42:560-72.
48. Patergnani S, Fossati V, Bonora M, Giorgi C, Marchi S, Missiroli S, et al. Mitochondria in Multiple Sclerosis: Molecular Mechanisms of Pathogenesis. *Int Rev Cell Mol Biol* 2017;328:49-103.
49. Witte ME, Nijland PG, Drexhage JA, Gerritsen W, Geerts D, Van Het Hof B, et al. Reduced expression of PGC-1alpha partly underlies mitochondrial changes and correlates with neuronal loss in multiple sclerosis cortex. *Acta Neuropathol* 2013;125:231-43.
50. Pandit A, Vadnal J, Houston S, Freeman E, McDonough J. Impaired regulation of electron transport chain subunit genes by nuclear respiratory factor 2 in multiple sclerosis. *J Neurol Sci* 2009;279:14-20.
51. Kelly DP, Scarpulla RC. Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. *Genes Dev* 2004;18:357-68.
52. Cassina AM; Hodara R; Souza JM; Thomson L; Castro L, Ischiropoulos H, et al. Cytochrome C nitration by peroxynitrite. *J Biol Chem* 2000;275:21409-15.
53. Brown GC; Borutaite V. Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols. *Biochim Biophys Acta* 2004;1658:44-9.
54. Yamamoto T, Maruyama W, Kato Y, Yi H, Shamoto-Nagai M, Tanaka M, et al. Selective nitration of mitochondrial complex I by peroxynitrite: Involvement in mitochondria dysfunction and cell death of dopaminergic SH-SY5Y cells. *J Neural Transm* 2002;109:1-13.
55. Qi X, Lewin AS, Sun L, Hauswirth WW, Guy J. Mitochondrial protein nitration primes neurodegeneration in experimental autoimmune encephalomyelitis. *J Biol Chem* 2006;281:31950-62.
56. Redford EJ, Kapoor R, Smith KJ. Nitric oxide donors reversibly block axonal conduction: Demyelinated axons are especially susceptible. *Brain* 1997;120:2149-57.
57. Shrager P, Custer AW, Kazarinova K, Rasband MN, Mattson D. Nerve conduction block by nitric oxide that is mediated by the axonal environment. *J Neurophysiol* 1998;79:529-36.
58. Sadeghian M, Mastrolia V, Rezaei Haddad A, Mosley A, Mullali G, Schiza D, et al. Mitochondrial dysfunction is an important cause of neurological deficits in an inflammatory model of multiple sclerosis. *Sci Rep* 2016;6:33249.
59. Haile Y, Deng X, Ortiz-Sandoval C, Tahbaz N, Janowicz A, Lu JQ, et al. Rab32 connects ER stress to mitochondrial defects in multiple sclerosis. *J Neuroinflammation* 2017;14:19.
60. Di Filippo M, Tozzi A, Tantucci M, Arcangeli S, Chiasserini D, Ghiglieri V, et al. Interferon-β1a protects neurons against mitochondrial toxicity via modulation of STAT1 signaling: Electrophysiological evidence. *Neurobiol Dis* 2014;62:387-93.