Chapter 6

Altered mitochondrial fission and fusion in MS cortex

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In preparation
Abstract

Currently, evidence is emerging that mitochondrial dysfunction is a key feature of multiple sclerosis (MS) pathology and contributes to neurodegeneration and axonal injury. Mitochondria are highly dynamic organelles that continuously divide and merge, processes known as mitochondrial fission and fusion, respectively. Fission and fusion of mitochondria are essential for maintaining a healthy, functional mitochondrial population and are of utmost importance in neurons, since these cells heavily rely on aerobic respiration. Here, we describe for the first time changes in the expression of mitochondrial fission and fusion in MS cortex. In general, we observed reduced mRNA expression of fission 1 (Fis 1) and dynamin-like protein 1 (DLP1), which are involved in fission, and reduced mRNA of the fusion genes mitofusin 1 and 2 (Mfn1 & 2) and optic atrophy 1 (OPA1) in normally myelinated cortex of MS patients compared to controls. Fission and fusion protein levels, however, were less markedly reduced. DLP1, essential in mitochondrial fission, was significantly decreased in MS cortex, whereas fission1 and mitofusin1 protein levels were only slightly reduced. Notably, we identified reduced PPARγ co-activator-1α (PGC-1α), a transcriptional co-activator of many nuclear encoded mitochondrial genes, as a probable cause for the observed decrease in fission and fusion gene expression in MS cortex. Conclusively, our data point towards a PGC-1α-driven decrease of mitochondrial fission and fusion in MS cortex. Over time, disturbed mitochondrial dynamics can affect mitochondrial morphology and function and thereby contributes to neurodegeneration in MS.

Keywords
Mitochondria; multiple sclerosis; neurodegeneration; PGC-1α; fission and fusion
Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) affecting both white and gray matter. It is generally believed to be caused by an autoimmune reaction against a specific myelin component, resulting in loss of myelin and oligodendrocytes. Although not the primary targets, axons and neurons are also severely damaged in the CNS of MS patients, especially in the progressive phase of the disease. These days, neurodegeneration is widely accepted as the primary cause of permanent clinical disability. However, therapeutics which are currently used in the clinic fail to reduce neurodegeneration in MS patients.

Processes leading to neurodegeneration in MS are thus far not well understood, but several papers have pointed towards an important role for mitochondria. In the experimental autoimmune encephalomyelitis (EAE) model, it was shown that intra-axonal mitochondrial swelling is the first step towards focal axonal degeneration. Reactive oxygen and nitrogen species (ROS & RNS) produced by activated microglia and infiltrating macrophages appeared to cause mitochondrial pathology, which preceded axonal injury. Previously, several papers have described mitochondrial changes in white matter MS lesions, including alterations in oxidative phosphorylation, which likely contribute to axonal degeneration. Importantly, mitochondrial changes in MS are not restricted to the white matter as we and others showed the occurrence of mitochondrial alterations in neurons in normally myelinated cortex in MS brains. Particularly the expression of proteins involved in oxidative phosphorylation and maintenance of the mitochondrial redox balance are severely reduced in MS cortex. Moreover, neuronal mitochondria in MS cortex harbour multiple deletions in mitochondrial DNA (mtDNA), which further hampers mitochondrial functioning. Recently, we described decreased levels of PGC-1α, a transcriptional co-activator involved in transcription of many nuclear-encoded mitochondrial proteins, as a likely cause for many of the mitochondrial changes observed in MS. Taken together, evidence is emerging that mitochondrial dysfunction is extensive in MS brain tissue and contributes to neuronal injury and disease progression MS brains.

Dysfunction of single mitochondria occurs in every mammalian cell, not in the least because mitochondria are the main site of ROS production and mtDNA lacks protective histones. Therefore, cells are equipped with an intricate system of mitochondrial quality control to ensure a healthy mitochondrial population. Essentially, mitochondrial quality control consists of three distinct processes termed mitochondrial fission, fusion and mitophagy. Mitochondrial fission is a process by which a mitochondrion divides into two or more mitochondria. The antonym of mitochondrial fission is mitochondrial fusion, being the fusion of two or more separate mitochondria into one large mitochondrion. Autophagy of mitochondria is generally referred to as mitophagy, and serves as a way for cells to remove (dysfunctional) mitochondria. Collectively, these processes are referred to as mitochondrial dynamics. Previously, it has been shown that fusion of damaged mitochondria can restore function by intermixing mitochondrial contents. Subsequent fission can then lead to one fully functional and one dysfunctional mitochondrion, and the latter is subsequently targeted for mitophagy. Thus far, several proteins have been identified that mediate fission and fusion. Fission is executed by fission 1 (Fis1) and dynamin-1 like protein (DLP1), whereas mitofusin 1 & 2 (MFN1&2) mediate fusion of the outer mitochondrial membrane and optic atrophy 1 (OPA1) is essential for inner
mitochondrial membrane fusion. Mitochondrial dynamics is especially important in neuronal cells as illustrated by autosomal dominant optic atrophy, which can be caused by mutations in OPA1 and Charcot-Marie-Tooth disease 2a, which is caused by MFN2 mutations. Moreover, abnormalities in the neuronal fusion and fission machinery have been described in Alzheimer's and Parkinson's disease, both neurodegenerative diseases characterized by impaired mitochondrial metabolism. Considering the central role of mitochondrial dysfunction in MS, we here set out to explore the expression of fission and fusion proteins in the cortex of MS patients.

In summary, we observed major changes in the expression of all fission and fusion genes in MS cortex, whereas at the protein level only DLP1 was significantly reduced. Moreover, we showed that silencing of neuronal PGC-1a results in decreased mRNA levels of fission and fusion proteins, indicating that PGC-1a regulates the production of proteins involved in mitochondrial dynamics. Since PGC-1a expression is significantly reduced in MS cortex we postulate that decreased PGC-1a levels might underlie the fission and fusion changes observed in MS cortex.

Material and Methods

Brain tissue

Brain tissue from frontal cortex and cingulate gyrus was obtained post-mortem in collaboration with the Netherlands Brain Bank, Amsterdam, The Netherlands. In this study we included 15 patients and 13 controls which were carefully matched for age, sex and post-mortem delay; patients & controls with Braak stage >1 were excluded from this study. Clinical data of MS patients and controls are summarized in table 1. Immediately after excision, frontal cortex (FC) and cingulate gyrus (CG) tissue was cut in half. One part was fixed in formaldehyde and embedded in paraffin for immunohistochemistry (IHC); the other part was snap-frozen in liquid nitrogen for IHC, RNA and protein isolation.

The study was approved by the institutional ethics review board (VU University Medical Center, Amsterdam) and all donors or their next of kin provided written informed consent for brain autopsy, use of material and clinical information for research purposes.

Immunohistochemistry

Paraffin or frozen sections (5 µm) were stained as described previously. In short, after deparaffinization and antigen retrieval (paraffin sections) or fixation in acetone (frozen sections), sections were incubated with the appropriate primary antibody (for details, see table 2) in phosphate buffered saline (PBS) supplemented with 1% bovine serum albumin (BSA; Roche Diagnostics GmbH, Mannheim, Germany) overnight at 4°C and stained with the EnVision horseradish peroxidase (DAKO, Glostrup, Denmark) kit followed by 3,3’-diaminobenzidine-tetrahydrochloridedihydrate (DAB; DAKO). All sections were counterstained with haematoxylin. Images were taken on a Leica DM4000B microscope (Leica Microsystems Heidelberg GmbH, Mannheim, Germany).

Immunofluorescence

To confirm neuronal localization of fusion and fission proteins, paraffin and frozen sections (5 µm) were stained using immunofluorescent techniques. After deparaffinization and antigen retrieval (paraffin sections) or fixation in acetone (frozen sections), sections were incubated with primary antibody mix containing one of the fission and fusion
antibodies together with either an antibody directed against NeuN or β3-tubulin (for details, see table 2) in phosphate buffered saline (PBS) supplemented with 1% bovine serum albumin (BSA; Roche Diagnostics GmbH, Mannheim, Germany) overnight at 4°C. Hereafter, primary antibodies were detected with appropriate secondary antibodies conjugated with Alexa Fluor (Invitrogen, Carlsbad, CA, USA). Nuclei were stained with DAPI. Images were taken on a Leica DM6000 microscope (Leica Microsystems Heidelberg GmbH).

Real-time quantitative PCR

To assess the expression of the various genes of interest, mRNA from frozen tissue blocks was isolated as follows. First, two frozen sections were stained for proteolipid protein (PLP) and MHC-II, to determine non-demyelinated cortical areas and assess infiltration of immune cells and microglial activation. Selected areas were subsequently outlined with a scalpel on the tissue block. After cutting 20µm sections, outlined areas were collected until >10 mg, snap-frozen and kept at -80°C. To ascertain that no white matter, meninges or demyelinated cortex was collected, 2 frozen sections cut at the end of tissue collection were stained for PLP and MHC-II. Subsequently, mRNA was isolated from these samples using the RNeasy® Lipid Tissue Mini Kit (Qiagen Sciences, Maryland, USA). Total RNA from cell cultures was isolated using Trizol (Invitrogen) as previously described. mRNA concentrations were measured using Nanodrop (Nanodrop Technologies, Wilmington, DE, USA).

cDNA was synthesized with the Reverse Transcription System kit (Promega, Madison, WI, USA) following manufacturer’s guidelines. Quantitative PCR (qPCR) reactions were performed in an ABI7900HT sequence detection system using the SYBR Green method (Applied Biosystems, Foster City, CA, USA) as described previously. Obtained mRNA expression levels were normalized to GAPDH expression levels. Alls primers were synthesized by Ocimum Biosolutions (Ocimum Biosolutions, IJsselstein, the Netherlands) and can be found in supplementary data.

Western blot

For protein analysis of frontal cortex and cingulate gyrus, 30-50 mg of non-demyelinated cortical tissue was collected as described above. Tissue was homogenized by incubating the samples with M-PER buffer (Thermo Scientific, Rockford, IL, USA) with protease & phosphatase inhibitors (Roche diagnostics GmbH) on ice for 30 min and passing the samples 10 times through an 0,8 mm² needle (21G x 1 1/2”; Terumo, Leuven, Belgium). Protein isolation from cultured cells was performed using M-PER buffer supplemented with protease & phosphatase inhibitors according to manufacturer’s protocol. Protein concentrations were measured using the BCA protein assay (Thermo Scientific).

Western blot was executed as previously described. In short, equal amounts of protein (25-100µg) were separated on 10% SDS-PAGE gels and transferred to PVDF membranes (Bio-Rad Laboratories, CA, USA). After blocking in Odyssey blocking buffer (LI-COR Biosciences, Lincoln, AK USA), membranes were incubated with appropriate primary antibody (for details, see table 2) overnight in Odyssey blocking buffer at 4°C. Primary antibodies were detected by incubation with appropriate IRDye secondary antibodies (LI-COR Biosciences) for 1 hour at RT in Odyssey blocking buffer and quantified using the Odyssey infrared imaging system (LI-COR Biosciences). Actin quantification was used to
correct for total protein loading variation.

**Cell culture and lentivirus-mediated delivery of shRNA**

The human neuroblastoma cell line, SH-SY5Y, was cultured in Opti-MEMα/HAM F-12 (1:1, Life Technologies, Vienna, Austria) containing 10% foetal calf serum (FCS, Life Technologies), 2 mM L-glutamin (Life Technologies), and penicillin/streptomycin (50mg/ml; Life Technologies) in 24-well plates (Greiner Bio-One, Frickenhausen, Germany) at 5% CO₂ and 37°C. To establish knockdown of selected proteins we used a vector-based shRNA technique as described previously. Expression plasmids encoding specific shRNAs targeting human PGC-1α and plasmids encoding non-targeting shRNA were obtained from SigmaAldrich (MISSION® shRNA library; St. Louis, MO, USA). Recombinant lentiviruses were produced by co-transfecting subconfluent human embryonic kidney (HEK) 293T cells with the shRNA lentivirus expression plasmid and packaging plasmids (pMDLg/pRRE and pRSV-Rev) using calcium phosphate as a transfection reagent. HEK 293T cells were cultured in Dulbecco’s modified Eagle’s medium (Life Technologies) supplemented with 10% FCS and 1% penicillin/streptomycin, in a 37 °C incubator with 5% CO₂. Infectious lentiviruses were collected 48 h after transfection. The supernatant was centrifuged to remove cell debris and stored at -80°C. SH-SY5Y cells were transduced with the lentivirus-containing shRNA. Forty-eight hours after infection of SH-SY5Y cells with the shRNA-expressing lentiviruses, stable cell lines were selected by puromycin treatment (2 µg/mL; SigmaAldrich). The expression knockdown efficiency was determined by qPCR and western blotting. As control, SH-SY5Y cells stably transduced with non-targeting shRNA were used.

**Statistical analysis**

If D’Agostino and Pearson omnibus normality test was passed Student’s t-test was performed to compare expression levels between patients and controls, otherwise Mann-Whitney U-test was used. mRNA and protein expression levels of shRNA treated cells were compared using Student’s t-test.

**Results**

*Decreased expression of mitochondrial fission genes and proteins*

Mitochondrial fission and fusion are essential cellular processes to maintain a healthy mitochondrial population. Previously, we and others have described severe mitochondrial alteration in the cortex of MS patients. Here we set out to investigate if genes and proteins involved in mitochondrial dynamics are differentially expressed in MS cortex. Therefore, we first performed quantitative RT PCR on cortical samples from cingulate gyrus (CG) and frontal cortex (FC) of MS patients and control for genes involved in mitochondrial fission (Fig 1A). We observed significantly reduced mRNA expression of Fis1 in FC samples. Fis1 mRNA expression in GC was also reduced in MS cortex compared to control albeit not significantly. DLP1 mRNA levels were decreased in both GC and FC of MS patients compared with grey matter from non-neurological controls. Next, we analyzed protein expression of Fis1 and DLP1 in protein samples from the same patients and controls using western blot. DLP1 protein expression was significantly reduced in MS FC by 27%, whereas DLP1 expression in CG and Fis1 expression in CG and FC were only slightly reduced (by 10%, 12% and 9%, respectively) (Fig 1B & C). Western blot analysis of porin, a mitochondrial
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marker, and β3-tubulin, a neuronal marker, revealed no differences between patient and control samples, indicating similar mitochondrial and neuronal content in MS and control cortices. This implies that reduced expression of fusion proteins was not due to a reduced number of mitochondria or neurons in MS cortex. Immunohistochemistry revealed expression of Fis1 in neurons and in a subset of glia, whereas DLP1 was predominantly expressed in neuronal cell bodies (Fig 1D & E). No differences were observed between MS and control cortex with regard to distribution pattern or staining intensity. In general, we found a consistent reduction in mitochondrial fission genes and to a lesser extent proteins in MS cortex.

Altered expression of mitochondrial fusion genes and proteins
Quantitative RT PCR on the same set of cortical samples as described above revealed an overall reduction of genes involved in mitochondrial fusion in MS cortex. However, statistical significance was only observed in Mfn1 and OPA1 gene expression in FC samples (Fig 2A). Protein levels of Mfn1 were consistently but non-significantly reduced in MS cortex with 12% and 28% in GC and FC, respectively. While, no marked changes in Mfn2 and OPA1 protein levels were observed comparing MS cortex with non-neurological control samples (Fig 2B & C). Immunohistochemistry revealed a prominent neuronal expression pattern of Mfn1, Mfn2 and OPA1 in MS cortex, similar to control. Neuronal localization of fusion proteins was confirmed by fluorescent double labelling with NeuN or β3-tubulin (Fig 2D, E & F).

Mitochondrial fission and fusion transcription is regulated by PGC-1α
Previously, we described reduced levels of PGC-1α in neurons in non-demyelinated MS cortex. Here, we investigated if decreased PGC-1α expression in MS cortex could explain the changes we observed in mitochondrial fission and fusion genes and proteins. Therefore, we silenced PGC-1α expression in neuronal cells by transducing SH-SY5Y cells with short-hairpin RNA directed at PGC-1α. PGC-1α-silenced neuronal cells exhibited a 70% reduction in PGC-1α protein expression when compared with SH-SY5Y cells transduced with non-targeting short-hairpin RNA (Fig 3A & B). In general, PGC-1α silencing in neuronal cells resulted in a significantly reduced expression of genes involved in mitochondrial fission and fusion. Fis1 mRNA levels were also decreased upon PGC-1α silencing albeit not significantly.

Discussion
We studied for the first time the expression of mitochondrial fission and fusion genes and proteins in the cortex of MS patients. In general, we observed a downregulation of genes involved in fission and fusion. Remarkably, the marked decrease in mitochondrial fission and fusion genes only led to profound changes in protein expression in MS cortex for dynamin-like protein 1 (DLP1). Expression of the other fission and fusion proteins in MS cortex was only slightly decreased or even similar to control. Silencing of PGC-1α revealed that the reduced PGC-1α levels in MS cortex could account for the decrease in mitochondrial fission and fusion gene expression.

Mitochondria are highly dynamic organelles that can be found in the cytosol and cellular processes where they continuously divide and merge, also known as fission and fusion, respectively. Mitochondrial fission and fusion are processes that shape the mitochondrial
Figure 1 (previous page). Expression of genes and proteins involved in mitochondrial fission in MS cortex. (A) qRT-PCR analysis revealed reduced mRNA levels of Fis1 in cortex of cingulate gyrus (CG; 89.6% ± 5.1) and frontal cortex (FC; 85.1% ± 6.2) of MS patients. DLP1 was significantly decreased (67.3% ± 12.3 for CG; 58.1% ± 6.4 for FC) in MS cortex. mRNA levels were normalized against GAPDH mRNA levels. (B) Representative images of western blots revealed a marginal reduction in Fis1 expression and a marked reduction in DLP1 expression in MS cortex. Porin and β3-tubulin expression remained unchanged in patient samples. (C) Quantification of western blot data revealed a non-significant reduction in Fis1 expression in CG (89.6% ± 4.3) and FC (87.8% ± 14.6) and DLP1 in CG (91.1% ± 5.3). DLP1 expression was significantly reduced in frontal cortex of MS patients (72.8% ± 8.9). No changes were observed between MS and control regarding cortical porin and β3-tubulin expression, indicating equal number of neurons and mitochondria. Protein levels were normalized against actin. (D) Immunohistochemical analysis revealed that a large proportion of Fis1 was present in neuronal cytoplasm,
whereas only a subset of glial cells was positive for Fis1 (arrow). Neuronal expression of Fis1 was confirmed by immunofluorescent doublestaining with NeuN (inset; Fis1 in green, NeuN in red). (E) DLP1 immunohistochemistry revealed neuronal localization of DLP1. Neuronal expression of DLP1 was confirmed by co-staining with β3-tubulin (inset; DLP1 in red, β3-tubulin in green). Values denote mean ± SEM. * P<0.05 as determined by student’s t-test or Mann-Whitney U-test. For cingulate gyrus n=9; for frontal cortex n=6.

Figure 2. Expression of genes and proteins involved in mitochondrial fusion in MS cortex. (A) qRT-PCR data showed significantly reduced mRNA levels of Mfn1 (77.0% ± 9.1) and OPA1 (62.8% ± 3.7) in frontal cortex of MS patients. Mfn1 and OPA1 levels were non-significantly decreased in cingulate gyrus (CG; 88.9% ± 7.5 for Mfn1; 68.9% ± 15.3 for OPA1), as was Mfn2 in both CG and FC (85.9% ± 6.0 and 82.9% ± 5.6, respectively). (B) Representative images of western blots showing reduced Mfn1 expression and unchanged Mfn2 and OPA1 levels in MS cortex. (C) Quantification of western blot data revealed a non-significant reduction in Mfn1 protein expression (79.9% ± 6.2 for CG; 64.2% ± 3.2 for FC) in MS cortex. Mfn2 levels were similar to control in MS CG (104.2% ± 3.1) and FC (100.6% ± 4.9), whereas OPA1 protein expression was slightly increased in CG (107.8% ± 6.0) and slightly decreased in FC (89.0% ± 9.6) in MS patients. (D, E & F) Immunohistochemical analysis of Mfn1,
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Mfn2 and OPA1 in control and MS cortex (data not shown) demonstrated a specific neuronal staining (x 200). This was confirmed with double labelling with NeuN for Mfn1 (inset in D; Mfn1 in green, NeuN in red), and with β3-tubulin for Mfn2 and OPA1 (insets in E & F; Mfn2/OPA1 in red, β3-tubulin in green). Values denote mean ± SEM. * P<0.05, **P<0.01 as determined by student’s t-test or Mann-Whitney U-test. For cingulate gyrus n=9; for frontal cortex n=6.

Figure 3. Silencing of neuronal PGC-1α expression leads to decreased transcription of mitochondrial fission and fusion genes. (A) Western blot images revealed lower PGC-1α protein levels in SH-SY5Y cells transduced with PGC-1α shRNA compared to those transduced with non-targeting shRNA. (B) Quantification of images shown in (A) demonstrated significantly reduced PGC-1α expression in SH-SY5Y cells transduced with PGC-1α shRNA (29.7% ± 1.8; n=2). (C) This reduction in PGC-1α led to significantly decreased gene expression of DLP1 (63.6% ± 4.5), Mfn1 (69.6% ± 4.5), Mfn2 (91.5% ± 2.0) and OPA1 (52.4% ± 4.0), Fis1 was non-significantly reduced in SH-SY5Y cells transduced with PGC-1α shRNA (85.3% ± 6.1) (n=6). mRNA levels were normalized against GAPDH mRNA levels Values denote mean ± SEM. * P<0.05, **P<0.01 as determined by student’s t-test or Mann-Whitney U-test. For cingulate gyrus n=9; for frontal cortex n=6.

population to the metabolic demands of the cell and are of utmost importance in neurons, since these cells heavily rely on aerobic respiration. The observed reduction in Mfn1, Fis1 and DLP1 expression and the predominant neuronal localization of all fission and fusion proteins suggests that rates of mitochondrial fission and fusion are lower in MS cortical neurons. Although it remains unclear whether the observed changes involved in mitochondrial dynamics affect mitochondrial morphology, it is not hard to envision that over time reduced rates of both fission and fusion lead to a less functional mitochondrial population in MS brain tissue and thereby contribute to neurodegeneration in MS.

Over the last few years, the involvement of impaired mitochondria function has received
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growing attention in MS research. As a result, mitochondrial dysfunction is now widely accepted to contribute to both axonal degeneration in the white matter and neuronal injury in the grey matter. Recently, we have uncovered an important causative role for reduced neuronal PGC-1α, a transcriptional co-regulator of many nuclear encoded mitochondrial proteins, in mitochondrial dysfunction. Previously, PGC-1α was identified as a transcriptional regulator of Mfn2, and it has been suggested that PGC-1α also regulates expression of the other fission and fusion proteins. We here provide strong evidence that in neuronal cells PGC-1α regulates the expression of all fission and fusion proteins, suggesting that decreased levels of PGC-1α might be a likely explanation for reduced fission and fusion in MS cortex. However, based on our in vitro experiments one would expect that besides Mfn1 and DLP1, OPA1 expression in MS cortex would also be decreased. And although this is the case for OPA1 mRNA, protein expression of OPA1 is unaltered in MS cortex. Reasons for this apparent discrepancy could be changes in posttranscriptional handling of OPA1 mRNA or reduced OPA1 protein degradation. In general, the decrease in fission and fusion mRNA levels is more pronounced compared to changes in protein expression. Again, this could be caused by posttranscriptional changes in mRNA or reduced protein degradation.

Future research is needed to unravel the functional consequences of the collective changes in fission and fusion expression in MS cortex. It is known, however, that reduced levels of neuronal DLP1, an important molecule in mitochondrial fission, leads to longer but fewer mitochondria. Similarly, Fis1 silencing increases average mitochondrial size, but does not affect total mitochondrial content. In contrast, reduced expression of Mfn1 results in smaller mitochondria in neuronal cells. Notably, reduced expression of fission or fusion proteins causes an abnormal mitochondrial distribution pattern with mitochondria concentrated around the nucleus and less mitochondria in the more remote cytoplasmic regions and dendrites. This could have severe implications for MS, where demyelination changes local metabolic needs of axons, and mitochondrial fission, fusion and transport are thus needed to fulfil these changing metabolic demands. Moreover, mitochondrial fission and fusion are required for maintenance of mitochondrial DNA, which means that the reduced rates of mitochondrial fission and fusion may contribute to the increased number of mitochondrial DNA deletions found in neurons in MS cortex. Thus, changes in mitochondrial fission and fusion in the cortex can contribute to both distant axonal degeneration and local neuronal damage in MS.

Changes in expression levels of mitochondrial fission and fusion genes and proteins are not specific for MS. In fact, altered mitochondrial dynamics have been implicated in the pathogenesis of Alzheimer’s disease (AD) and Parkinson’s disease (PD). In AD hippocampal neurons, DLP1, Mfn1, Mfn2 and OPA1 expression are decreased, whereas Fis1 levels are significantly higher, leading to severe metabolic dysfunction. In PD models, overexpression of Mfn2 or dominant-negative DLP1 mutant rescued neurons from cell death, indicating an important role for increased mitochondrial fission in PD. Although the aetiology of AD, PD and MS differs it is likely that alterations in mitochondrial dynamics are a common mechanism amplifying the already present mitochondrial dysfunction and concomitant neurodegeneration rather than initiating the neurodegenerative process. However, several studies in AD and PD models have shown that targeting mitochondrial fission and/or fusion can stop the neurodegenerative process. Therefore, further elucidation of altered mitochondrial dynamics in MS and subsequent studies aimed at
counteracting these changes in in vivo MS models might provide us with tools to bring neurodegeneration in MS to a halt.

Taken together, we observed decreased expression of genes involved in mitochondrial fission and fusion in MS cortex, while changes in the protein levels were marginal. Moreover, we identified PGC-1α as a potential regulator of mitochondrial dynamics, suggesting that the observed decrease in fission and fusion genes might be the result of reduced neuronal PGC-1α levels in MS cortex. We speculate that the lower rates of mitochondrial fission and fusion are rather a consequence than a primary cause of mitochondrial dysfunction in MS cortex. Nevertheless, impaired mitochondrial dynamics will ultimately induce morphological and functional changes in the mitochondrial population and therefore contribute to neurodegeneration in MS.

Reference List

Mitochondrial dynamics in MS cortex


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### Tables

#### Table 1 Clinical data of MS patients and non-neurological controls

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<tr>
<td>Ctrl 11</td>
<td>70</td>
<td>NA</td>
<td>m</td>
<td>6:15</td>
<td>NA</td>
<td>FC</td>
</tr>
<tr>
<td>Ctrl 12</td>
<td>73</td>
<td>NA</td>
<td>f</td>
<td>6:45</td>
<td>NA</td>
<td>FC</td>
</tr>
<tr>
<td>Ctrl 13</td>
<td>60</td>
<td>NA</td>
<td>NA</td>
<td>6:50</td>
<td>NA</td>
<td>FC</td>
</tr>
</tbody>
</table>

SP = secondary progressive MS; PP = primary progressive MS; ND = not determined; m = male; f = female.

#### Table 2 Antibody details

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Dilution</th>
<th>IHC</th>
<th>WB</th>
<th>Antibody type</th>
<th>Source*</th>
</tr>
</thead>
<tbody>
<tr>
<td>proteolipid protein (PLP)</td>
<td>1:500</td>
<td>NA</td>
<td>NA</td>
<td>IgG2a</td>
<td>Serotec</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>1:50</td>
<td>NA</td>
<td>NA</td>
<td>IgG2b</td>
<td>eBioscience</td>
</tr>
<tr>
<td>mitofusin 1 (Mfn1)</td>
<td>1:500</td>
<td>1:200</td>
<td>NA</td>
<td>IgG2a</td>
<td>Abnova</td>
</tr>
<tr>
<td>mitofusin 2 (Mfn2)</td>
<td>1:350</td>
<td>1:100</td>
<td>NA</td>
<td>rabbit polyclonal</td>
<td>Sigma</td>
</tr>
<tr>
<td>optic atrophy 1 (OPA1)</td>
<td>1:1000</td>
<td>1:100</td>
<td>NA</td>
<td>IgG1</td>
<td>BD transduction</td>
</tr>
<tr>
<td>fission 1 (Fis1)</td>
<td>1:500</td>
<td>1:100</td>
<td>NA</td>
<td>rabbit polyclonal</td>
<td>BD transduction</td>
</tr>
<tr>
<td>dynamin-like protein (DLP1)</td>
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<td>1:100</td>
<td>NA</td>
<td>IgG1</td>
<td>BD transduction</td>
</tr>
<tr>
<td>PGC-1a</td>
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<td>1:100</td>
<td>NA</td>
<td>rabbit polyclonal</td>
<td>Santa Cruz</td>
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<tr>
<td>neuronal class III β3-tubulin</td>
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<td>1:100</td>
<td>NA</td>
<td>IgG2a</td>
<td>Covance</td>
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<tr>
<td>actin</td>
<td>NA</td>
<td>1:100</td>
<td>goat polyclonal</td>
<td>Millipore</td>
<td></td>
</tr>
</tbody>
</table>

IHC, immunohistochemistry; WB, western blot; HLA-DR, human leukocyte antigen-DR; PGC-1, peroxisome proliferator-activated receptor-γ coactivator-1; NA, not applicable

* Sources: Serotec, Oxford, UK; eBioscience, San Diego, CA; Abnova GmbH, Heidelberg, Germany; Sigma-Aldrich, St Louis, MO; BD Transduction Laboratories, Franklin Lake, NJ; Imgenex, San Diego, CA; Santa Cruz Biotechnology, Santa Cruz, CA; Abcam, Cambridge, UK; Millipore, Billerica, MA; Covance, Princeton, NJ;