

# Neuropathology of white matter disease in Leber's hereditary optic neuropathy

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## Summary

Leber's hereditary optic neuropathy (LHON) is associated with point mutations in the mitochondrial DNA (mtDNA), coding for a mitochondrial respiratory chain complex I subunit. It is characterized by bilateral, usually sequential, optic neuropathy and may co-occur with multiple sclerosis-like white matter lesions. Despite repeated clinical reports including MRI and histopathological examination of the visual system, neuropathological descriptions of LHON associated with multiple sclerosis-like syndrome are lacking. We present here the case of a female patient with a point mutation at nucleotide position T14484C, who suffered from relapsing episodes of visual loss of both eyes and consecutively developed Hashimoto thyroiditis as well as widespread demyelinating CNS lesions outside the visual system. She died of bronchopneumonia at the age of 44 years, after a disease duration of 19 years, with

progressive deterioration, epileptic seizures and immobility. Immunohistochemical analysis on formalin-fixed and paraffin-embedded tissue reveals a spectrum of neuropathological changes, including actively and inactively demyelinating plaques in the white matter and optic nerve, vacuolation and cystic necrosis with CD8-positive T cells in the frontal lobe, axonal damage, and vacuolation of white matter. Tissue destruction is associated with upregulation of mitochondrial manganese superoxide dismutase within the lesions and an increase in the expression of inducible nitric oxide synthase within macrophages and microglia. This variable phenotype of extraoptic LHON disease suggests that mtDNA mutations may affect the nervous system on a common metabolic basis and occasionally may aggravate or initiate autoimmune pathology.

**Keywords:** LHON; mitochondrial disease; multiple sclerosis; demyelination; CD8-positive T cells

**Abbreviations:** LHON = Leber's hereditary optic neuropathy; mtDNA = mitochondrial DNA; WM = white matter

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## Introduction

Leber's hereditary optic neuropathy (LHON) is a mitochondrially inherited disease with male predominance (Riordan-Eva *et al.*, 1995). It is characterized by bilateral optic atrophy with loss of central vision due to degeneration of the retinal ganglion cells and optic nerve axons. More than 30 mitochondrial DNA

(mtDNA) mutations have been associated with LHON (for a complete list, see MITOMAP, <http://www.mitomap.org>). Mutations in complex I of the oxidative phosphorylation (OXPHOS) system at nucleotide positions ND4/11778, ND1/3460 and ND6/14484 are considered as high risk or so-called

'primary' LHON mutations and are present in nearly 90% of LHON patients (Huopen, 2001; McFarland *et al.*, 2002).

In general, pathology in LHON is limited to the optic system; however, in rare cases, complication by a Leigh-like encephalopathy, another mitochondrial disease which primarily affects the grey matter, or more frequently a multiple sclerosis-like syndrome may occur (Harding *et al.*, 1992; Vanopdenbosch *et al.*, 2000; Funalot *et al.*, 2002). The latter is mainly associated with mutation 11778 and extremely rarely with mutation 14484 (Horvath *et al.*, 2000; Vanopdenbosch *et al.*, 2000). The neurological characteristics, including MRI features and/or oligoclonal bands in CSF, of the multiple sclerosis-like syndrome associated with LHON are indistinguishable from those of multiple sclerosis in general (Vanopdenbosch *et al.*, 2000).

There have been only a few neuropathological studies of LHON. These consistently show degeneration of the retinal ganglion layer and optic nerve with axonal loss, occasionally with mild inflammation (Saadati *et al.*, 1998; Howell, 1999). Others describe evidence of myelin splitting and reactive astrocytosis with vacuolar degeneration in the optic nerves (Carelli and Sadun, 2001). Additional findings include demyelination in the gracile columns of the spinal cord as well as some demyelination in peripheral nerves of the lower extremities (Kwittken and Barest, 1958).

Another early pathological analysis, carried out before genetic testing was introduced, reported diffuse fibrillary gliosis in the white matter of the cerebral hemispheres, and demyelinated zones lacking active degradation in the optic nerves, chiasma and optic tracts in addition to myelin loss with Marchi-positive lipid in the crossed and uncrossed pyramidal tracts, and central chromatolysis of neurons of the cervical and thoracic segments of the spinal cord (Adams *et al.*, 1966). Although they could not demonstrate intraparenchymal inflammation or multiple sclerosis-like changes, diffuse mononuclear infiltration of the leptomeninges was mentioned.

In sum, despite repeated clinical reports, histopathological descriptions of brain white matter (WM) and optic nerve changes in LHON associated with multiple sclerosis-like syndrome are lacking. Neuropathological studies of the optic nerve also lack information about the kinetics of degeneration in LHON as these investigations are carried out with patients who lost vision many years prior to death. Here we demonstrate a case with WM disease associated with the rare T14484C LHON mutation which consists histopathologically of a spectrum of changes reminiscent of both multiple sclerosis and mitochondrial WM disease, as in Kearns–Sayre syndrome (KSS) (Muller *et al.*, 2003). Lesions include demyelination, vacuolation, cystic necrosis, plaques, and inflammatory cell infiltrates involving the optic nerve as well.

## Material and methods

### Clinical findings

Some aspects of the case history were published previously (Horvath *et al.*, 2000). Briefly, the family history of the patient is

unremarkable. In 1984, at the age of 25 years, left-sided retrobulbar neuritis was diagnosed during her first pregnancy. This was followed by several similar episodes on both sides. Initially corticosteroid and vitamin B treatment resulted in good recovery. Later, progressive amblyopia developed in the left eye.

In 1993, cranial MRI examination revealed no pathological findings. Two years later, evaluation of the CSF showed neither oligoclonal bands nor intrathecal immunoglobulin G (IgG) production. In 1997, the patient developed paraesthesia of the left leg and was subjected to a cranial MRI. WM alterations were found in the corpus callosum and paraventricular region (Horvath *et al.*, 2000).

In 1998, the patient complained about left lower limb weakness, ataxic gait, painful paraesthesia on the trunk and urinary retention. In addition, she was diagnosed with Hashimoto thyroiditis and she received levothyroxine treatment (50 µg/day). In 1999, personality changes, emotional lability, paranoid thoughts, anxiety, mood alterations, paraparesis and bilateral Babinski sign were observed.

In March 2000, a high sedimentation rate (63 mm/h) was noted; antinuclear antibody and rheumatoid factor titres were negative. Cranial MRI (Fig. 1) revealed moderately space-occupying, partly cystic, bilateral alterations hyperintense on T2 and hypointense on T1 sequences in the frontal lobe. Spotted contrast enhancement and perifocal oedema were also present. Brain biopsy taken from the frontal region showed signs of demyelination.

In May 2000, she was treated for acute renal insufficiency due to urinary retention. From February 2001, occasional epileptic seizures were observed. The patient died of bronchopneumonia at the age of 44 years. According to genetic analysis, she was homoplasmic for the T14484C mutation (Horvath *et al.*, 2000).

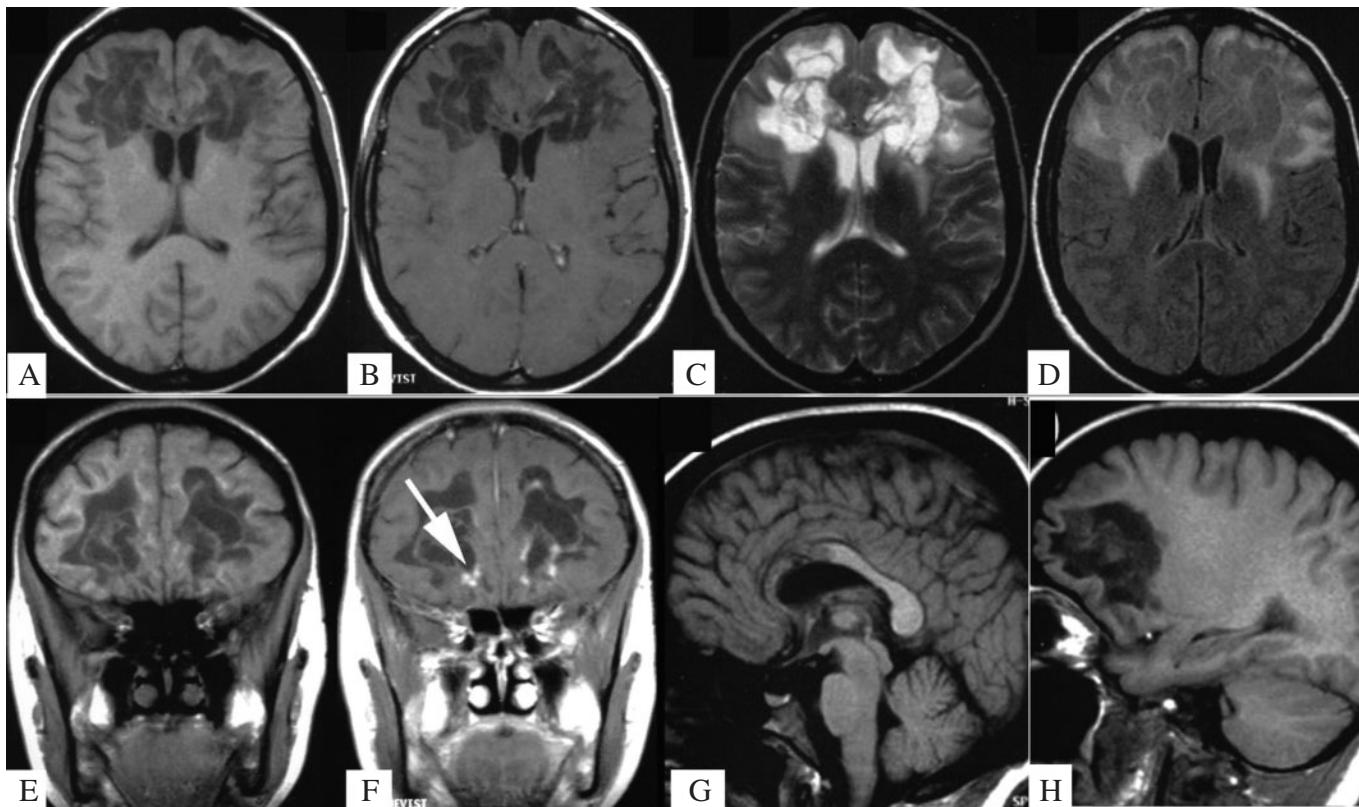
### Neuropathology

After widespread sampling of formalin-fixed tissue, including brain-stem, basal ganglia, cerebral cortical areas, cerebellum, gross hemispheric sections of five levels, spinal cord and dorsal roots, blocks were embedded in paraffin and routinely stained using haematoxylin and eosin (HE), Luxol fast blue–periodic acid Schiff (PAS), Woelcke myelin stain, Bielschowsky, van Gieson elastica and cresyl violet. Formalin-fixed dorsal roots of the cervical and lumbar level were embedded in epon and processed for semi-thin sections.

We used well characterized antibodies to evaluate inflammation and tissue damage (Table 1). To detect DNA fragmentation *in situ* (by terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling; TUNEL), a commercial kit (In Situ Cell Death Detection Kit, AP, Roche Diagnostics, Mannheim, Germany) was used. Immunohistochemistry for light microscopy and double immunolabelling for confocal laser microscopy were performed according to previously described protocols (Höftberger *et al.*, 2004). Double immunolabelled sections were evaluated with a Zeiss LSM laser scanning microscope with appropriate operating conditions (Höftberger *et al.*, 2004).

### Methods of quantification

Evaluation of CD8-, CD4- and CD20-positive cells was performed on serial sections in corresponding fields, with a size of 1 mm<sup>2</sup> in the plaque centre, plaque edge and adjacent WM, defined by an ocular morphometric grid.



**Fig. 1** MR examination with native and contrast-enhanced axial T1 (A and B, respectively), T2 (C), FLAIR (D), native and contrast-enhanced coronal T1 (E and F, respectively), axial and sagittal T1-weighted images (G and H). A widespread symmetric lesion in the frontal lobes involves the deep and the subcortical white matter, with concentric layers of low and intermediate (T1) or intermediate and high signal intensity (T2). Scattered contrast enhancement can be seen at the margins of these layers (arrow, F). The genu and the rostral third of the corpus callosum are severely involved. The destructive as well as the space-occupying nature of the lesion is presented by the isolated widening of the frontal horns and the compression of the fronto-polar sulci, respectively.

## Results

### Histopathological findings

In the WM of the brain, the most striking finding is a bilateral, destructive, cystic brain lesion within the frontal lobe partly affecting U fibres (Fig. 2D), which is characterized by demyelination accompanied by loss of oligodendrocytes, axons and astrocytes. This extensive tissue destruction results in cystic necrosis and prominent glial reactivity (Figs 2E–G and 3A). Demyelination is characterized by ongoing myelin destruction at the lesion edge, with myelin-associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP), and Luxol fast blue-positive degradation products within macrophages (Figs 2F, and 3B and C). Around the periphery of the lesion, vacuolation of WM with microglial proliferation and astrocytic hypertrophy is apparent (Fig. 3D). Inflammation is characterized by the presence of macrophages and lymphocytes within the lesion, which diffusely infiltrate the parenchyma and form an inflammatory rim at the actively demyelinating edge of the lesion (Table 2 and Fig. 3E). Some perivascular T cells can be found beyond the lesional border in the adjacent, vacuolated WM (Table 2). Additionally, large amounts of lipopigment are

apparent within perivascular macrophages (data not shown). The meninges, normal white and grey matter are not involved. The majority of T cells are CD8 positive, but do not express granzyme B. Furthermore, deposition of complement components, including C5b-9 and C9neo, is lacking. Major histocompatibility complex (MHC) class I ( $\beta_2$ -microglobulin) can be identified on endothelial cells, macrophages, lymphocytes and some microglia. Acute cell death, visualized by TUNEL staining, was absent; however, staining for  $\alpha$ -B-crystallin, a marker for cell stress, reveals pronounced immunolabelling of oligodendrocytes in the vacuolated periplaque WM as well as of astrocytes within the lesion (Fig. 3F). Immunoreactivity for inducible nitric oxide synthase (iNOS) within the cytoplasm of macrophages as well as microglia in the adjacent WM was found, most consistently in actively demyelinating lesions (Fig. 3G). Additionally, massive upregulation of the mitochondrial manganese superoxide dismutase (MnSOD) within the plaque region was present (Fig. 3H). Axonal damage is reflected by massive loss of axons in the plaque centre (Fig. 3I) and acute axonal damage, with accumulation of amyloid precursor protein in axonal spheroids, in the actively demyelinating border (Fig. 3J).

**Table 1** Characteristics of antibodies used in this study

Antibody	Source	Type	Specificity
α-B-crystallin	DAKO	pAb	Stress protein
APP	Chemicon	mAb	Amyloid precursor protein
β <sub>2</sub> -microglobulin	DAKO	pAb	MHC class I
C5b-9	Quidel	mAb	Terminal complement component
C9neo	DAKO	mAb	Terminal complement component
CD3	DAKO	mAb	T lymphocytes
CD8	DAKO	mAb	Cytotoxic/suppressor T cells
CD20	DAKO	mAb	Most B cells
CD45RO	DAKO	mAb	Helper T cells
CD68	DAKO	mAb	Macrophages
CNPase	SMI	mAb	Oligodendrocytes
GFAP	DAKO	pAb	Astrocytes
Granzyme B	NeoMarkers	mAb	Granzyme B
iNOS	Chemicon	pAb	Inducible nitric oxide synthase
MAG	Dobersen <i>et al.</i> (1985)	mAb	Myelin-associated glycoprotein
MBP	DAKO	pAb	Myelin basic protein
MnSOD	StressGen	pAb	Mitochondria
MOG	Piddlesden <i>et al.</i> (1993)	mAb	Myelin oligodendrocyte glycoprotein
PLP	Piddlesden <i>et al.</i> (1993)	pAb	Proteolipid protein
SMI31	SMI	mAb	Phosphorylated neurofilament protein

CNPase = 2'3'-cyclic nucleotide 3' phosphodiesterase; GFAP = glial fibrillary acidic protein; MnSOD = mitochondrial manganese superoxide dismutase; mAb = monoclonal antibody; pAb = polyclonal antibody; DAKO, Glostrup, Denmark; Chemicon, Temecula, USA; Quidel, San Diego, CA; SMI: Sternberger Monoclonals Incorporated, Lutherville, MD; NeoMarkers, Westinghouse Drive, Fremont, CA; StressGen, Glanford Ave., Victoria, BC Canada.

Similar but less destructive lesions can be found in the optic chiasm, corpus callosum and periventricular WM; additionally, diffuse myelin pallor and moderate vacuolation in the cerebral hemispheres, cerebellar WM, pontine basis and pyramids as well as degeneration of the cervical gracile fascicle can be observed (Table 2). In contrast, grey matter structures are well preserved. Small vessel vasculitis, thrombosis, capillary proliferation or infarction is lacking.

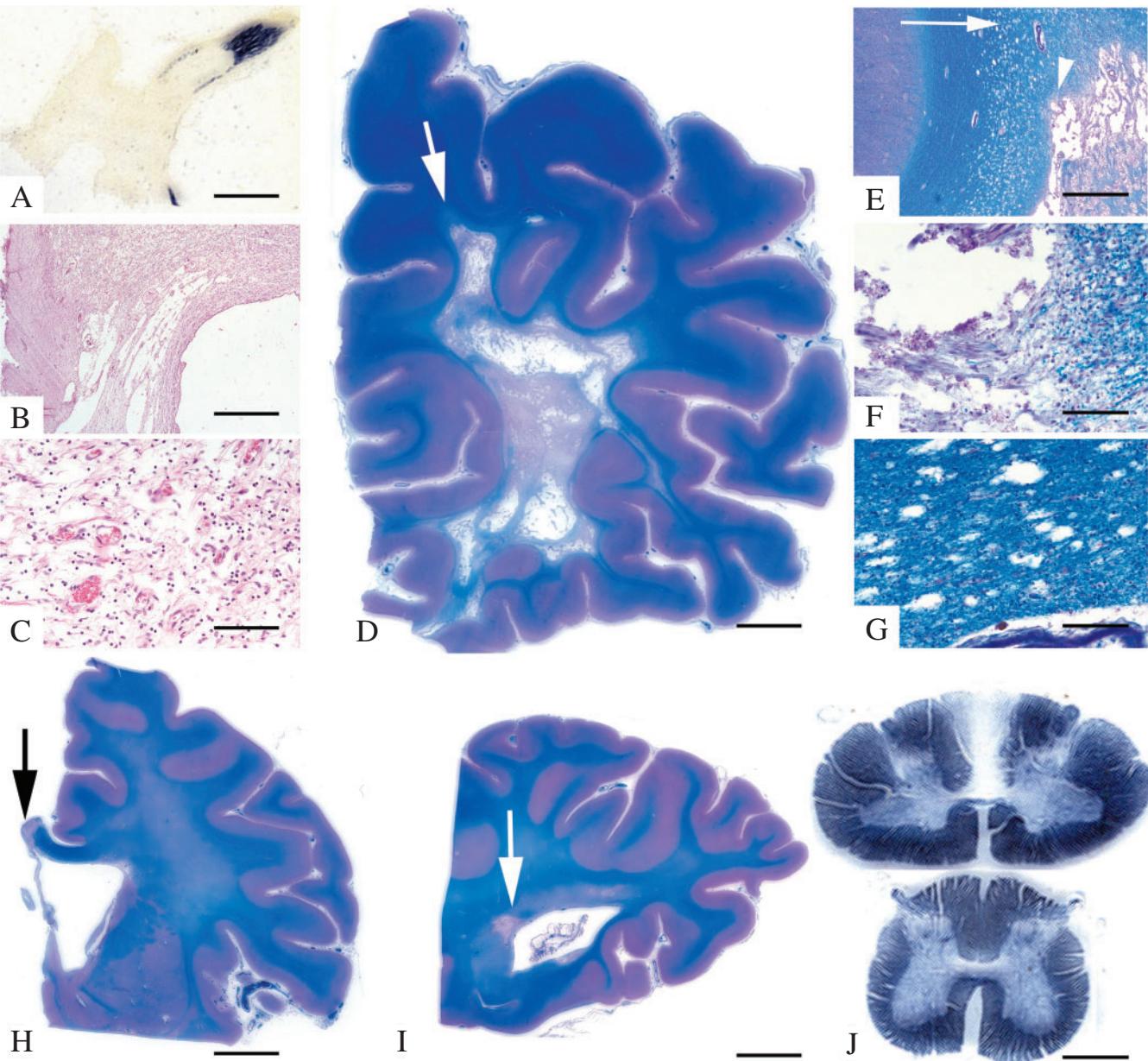
## Discussion

The spectrum of neuropathological changes of the present case with T14484C LHON mutation includes axonal damage, active demyelination, partly extensive tissue destruction (cystic necrosis), inactive demyelinated areas and vacuolation of WM. Thus the features are reminiscent of both classical and atypical lesions of multiple sclerosis (Lassmann, 1998). Our observations suggest that the extensive and unselective tissue damage is mediated predominantly by T cells and activated macrophages/microglia. However, the low number of perivascular inflammatory infiltrates may be due to immunosuppressant treatment. The reason for the predominantly frontal localization with relative sparing of other brain areas remains obscure.

Nonetheless, WM changes are not exclusively due to a multiple sclerosis-like demyelinative process. Vacuolation and diffuse myelin pallor, reminiscent of that seen in KSS (Oldfors and Tulinius, 2003), might also contribute to abnormalities picked up by MRI. Interestingly, vacuolation

was also mentioned in the optic nerves in another study (Carelli and Sadun, 2001). However, previous pathological studies usually described end-stage changes, while we could demonstrate an active phase of the process. WM involvement without neurological manifestation, oligoclonal bands in CSF and response to steroid therapy may be associated with LHON (Lev *et al.*, 2002). The presence of inflammatory cells within lesions of LHON is unusual since only rarely may mild inflammation be detected (Saadati *et al.*, 1998). In contrast, in our patient, introduction of corticosteroid intermittently improved visual and neurological function, suggesting an early immunological mechanism in addition to the primary degeneration of the optic nerve (Kwittken and Barest, 1958).

The mechanisms leading to inflammatory demyelination and tissue damage in LHON so far have not been determined. It may be due to a coincidental association of multiple sclerosis in a patient with LHON. In particular, reactive oxygen and nitrogen species, produced by activated macrophages in multiple sclerosis lesions, may impair mitochondrial function (Beltran *et al.*, 2000; Lu *et al.*, 2000). This may be potentiated in the presence of a genetic defect of mitochondrial function. In this case, the mitochondrial dysfunction may aggravate or modify the pathogenesis of the lesions (Harding *et al.*, 1992; Mojon *et al.*, 1999). However, similar mitochondrial DNA mutations were not found in an unselected multiple sclerosis population (Kellar-Wood *et al.*, 1994). Alternatively, tissue injury in LHON patients may by itself provoke an autoimmune response in genetically susceptible individuals. Molecular mimicry was implicated in the precipitation of an autoimmune process in mtDNA mutations

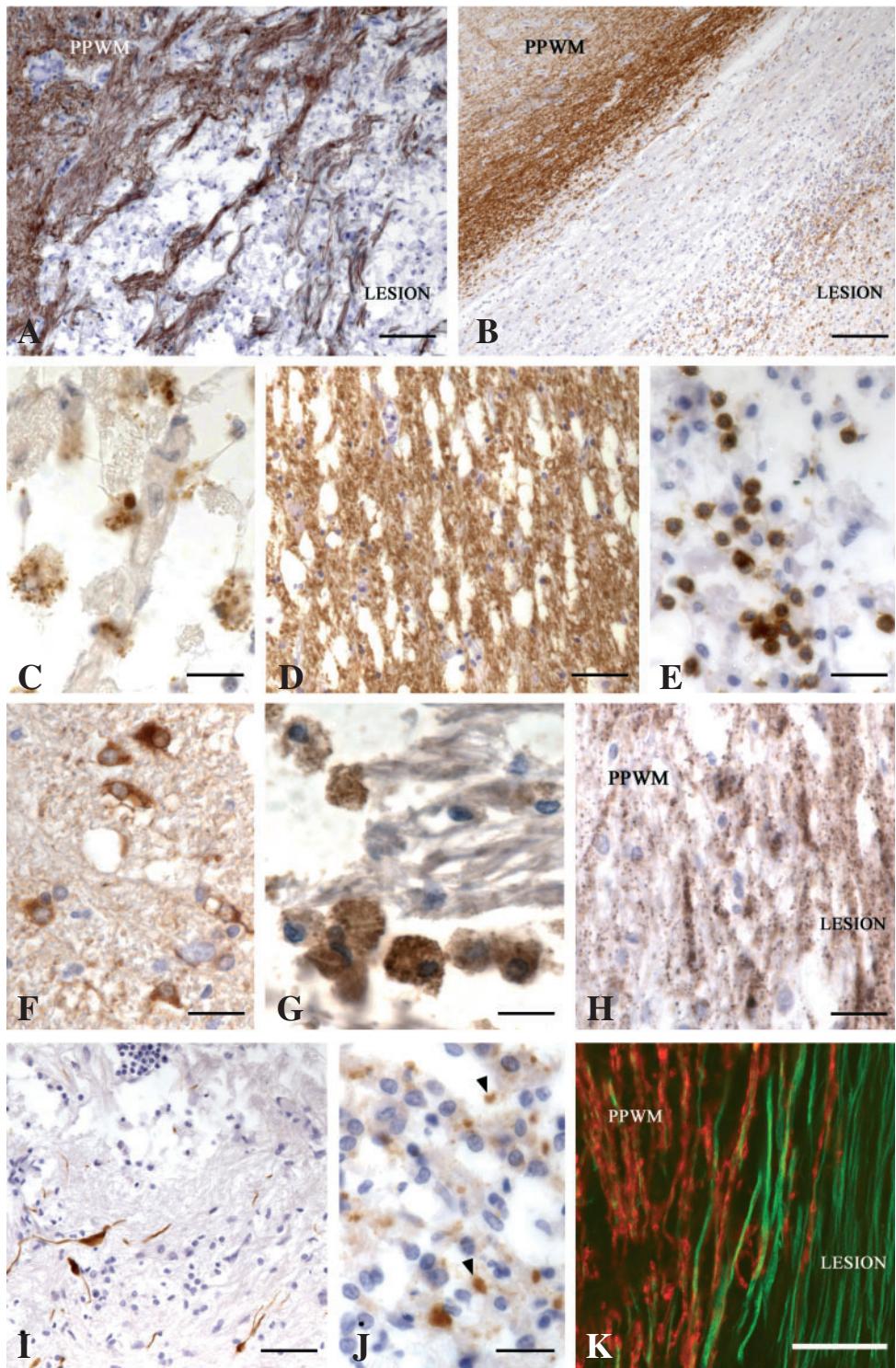


**Fig. 2** Histopathological findings in LHON associated with white matter disease. Demyelination (A, Woelcke stain), cystic lesion (B, H&E) and mononuclear infiltration (C, H&E) in the optic chiasma. There is a widespread cystic-necrotic white matter lesion in the frontal lobe (D). The arrow indicates the region enlarged in (E) demonstrating the edge of this with cystic destruction (F, indicated by an arrowhead in E) and vacuolation (G, indicated by an arrow in E). Plaques (indicated by arrows) in the corpus callosum (H) and around the occipital horn of the lateral ventricle (I), (D–I; Luxol-PAS staining). Degeneration of the gracile column in the cervical spinal cord (J, upper part), with sparing of the lumbar segment (J, lower part). Bars: A = 5 mm; B = 50  $\mu$ m; C, F and G = 25  $\mu$ m; D = 15 mm; E = 100  $\mu$ m; H and I = 6 mm; J = 1.5 mm.

(Baum, 1995). Alternatively, antigen or determinant spreading mediated through the liberation of autoantigen may induce the autoimmune response (Vanderlugt and Miller, 2002). The presence of another autoimmune disorder, Hashimoto thyroiditis, in our patient also supports this notion. It must be mentioned that we did not observe any signs of Hashimoto encephalitis. Vascular damage, angiopathy and Leigh disease-like neuropathology were also lacking (Funalot *et al.*, 2002).

In contrast to 11778, the 14484 LHON mutation is only exceptionally associated with multiple sclerosis-like disease (Horvath *et al.*, 2000; Vanopdenbosch *et al.*, 2000), thus additional genetic or epigenetic factors might be suspected for the unusual clinical course in our case.

In conclusion, the various phenotypes of extraoptic LHON disease suggest that mtDNA mutations may affect the nervous system on a common metabolic basis and occasionally may aggravate or initiate autoimmune processes.



**Fig. 3** Immunohistochemistry (A–J) and confocal laser scanning microscopy (K) in LHON associated with white matter disease. (A–C) Massive tissue destruction in the frontal lobe, with cystic necrosis [A, glial fibrillary acid protein (GFAP)] and demyelination [B, myelin basic protein (MBP)], with ongoing demyelinative activity, represented by myelin oligodendrocyte glycoprotein (MOG)-positive degradation products within macrophages (C). (D) Vacuolation and spongy myelin change in the peri-plaque white matter (MBP). (E) CD8-positive T cells at the lesion edge. (F–H) Evidence for oxidative stress with  $\alpha$ -B-crystallin-positive oligodendrocytes in the peri-plaque white matter (PPWM) (F), iNOS-positive macrophages (G) and MnSOD upregulation within the demyelinated lesion (H). (I and J) Massive axonal damage in the frontal lobe, with axonal loss and spheroids in the lesion centre (I, neurofilament protein) and acute axonal damage at the lesion edge (J, amyloid precursor protein). (K) Relative preservation of axons in the less destructive lesion of the corpus callosum (compact myelin, MBP, stained red; phosphorylated neurofilament protein, SMI-31, green). Bars: A = 80  $\mu$ m; B = 160  $\mu$ m; (C, E–H and J = 30  $\mu$ m; D and I = 50  $\mu$ m; K = 100  $\mu$ m.

**Table 2** Neuropathological findings in LHON associated with white matter disease

Location	Principal observation	Figure
Frontal lobe	Extensive demyelination, cystic-necrotic tissue destruction, inflammatory infiltration (89% CD8-positive T cells, 11% CD4-positive T cells and occasional B cells), axonal loss within the lesion centre and acute axonal damage at the lesion edge.	2D–G and 3A–C, E, I and J
Optic chiasm	Extensive demyelination, cystic loosening of tissue and inflammatory infiltration (71% CD8-positive T cells, 21% CD4-positive T cells and 8% CD20-positive B cells).	2A–C
Corpus callosum, periventricular WM	Demyelination with ongoing demyelination activity at the plaque border, inflammatory infiltration (98% CD8-positive T cells, 2% CD4-positive T cells and no B cells) and relatively preserved axons within the lesions.	2H and I, and 3K
Cerebral hemispheres, cerebellar WM, pontine basis, pyramids	Diffuse myelin pallor and moderate vacuolation, discrete, mostly perivascular inflammation (94% CD8-positive T cells, 6% CD4-positive T cells and no B cells).	2H and 3D
Spinal cord	Axonal degeneration of the cervical gracile fascicle and the dorsal roots. Active demyelination is lacking. Neither inflammation nor damage of spinal ganglia can be found.	2J

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