

SCIENTIFIC REPORT

Human extraocular muscles in mitochondrial diseases: comparing chronic progressive external ophthalmoplegia with Leber's hereditary optic neuropathy

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Aims: To compare the ultrastructural aspects of human extraocular muscles in two types of mitochondrial disease: chronic progressive external ophthalmoplegia (CPEO) and Leber's hereditary optic neuropathy (LHON).

Methods: Muscle samples of the medial rectus obtained from surgery in a sporadic case of CPEO associated with deleted mitochondrial DNA, and post mortem in a case of 3460/ND1 LHON were processed for electron microscopy (EM). The medial rectus from an autoptic time to fixation matched control was used to exclude postmortem artefacts.

Results: The CPEO specimen revealed focal areas of disruption and abnormalities of mitochondria in some muscle fibres, creating a "mosaic-like" pattern. In the LHON specimen a diffuse increase in both number and size of mitochondria (mean diameter 0.85 μm v 0.65 μm of control, $p < 0.0001$) with swollen appearance and disorganised cristae filled all spaces of sarcoplasmic reticulum. In some areas the excessive number of mitochondria slightly distorted myofibrils.

Conclusion: EM investigation of extraocular muscles in CPEO and LHON reveals marked differences. A "mosaic-like" pattern caused by a selective damage of muscle fibres was evident in CPEO, whereas a diffuse increase in mitochondria with preservation of myofibrils characterised the LHON case. These ultrastructural changes may relate to the different expression of the two diseases, resulting in ophthalmoplegia in CPEO and normal eye movements in LHON.

The extraocular muscles (EOMs) are among the fastest and yet most fatigue resistant skeletal muscles in the body. The complexity of actions performed by the EOMs is reflected in their cytoarchitecture and fibre type composition, which differ from ordinary skeletal muscles in many respects, including motor unit properties.¹ Their activity (of both saccades and pursuit movements) is continuous and highly energy dependent. Fatigue resistance may be the result of their use of glycolytic or oxidative enzymes, their mitochondrial content, and the extensive capillary network associated with individual myofibres. However, these biochemical characteristics and the elevated energy requirements expose EOMs to be targeted by mitochondrial dysfunction.

Both chronic progressive external ophthalmoplegia (CPEO) and Leber's hereditary optic neuropathy (LHON) are the result of mitochondrial DNA (mtDNA) mutations that lead to mitochondrial dysfunction. CPEO is characterised by progressive loss of muscles activity and near complete ophthalmoplegia at the late stages.² On the other hand, LHON leads to optic neuropathy but is not associated with EOM

dysfunction.³ However, ultrastructural alterations in EOMs have been reported in a single case described at necropsy of LHON carrying the 11778/ND4 mtDNA mutation.⁴ In a previous report we studied the medial rectus muscle in a case of sporadic CPEO carrying a heteroplasmic mtDNA macrodeletion.⁵

Here we report the ultrastructural features in the medial rectus (MR) of a further LHON case carrying the 3460/ND1 mutation in comparison with the previously reported CPEO case and with an age matched control. The quantitative and qualitative differences in myofibrillary damage, in relation to the different pathophysiology of these mtDNA defects, may explain the phenotypical expression in LHON and CPEO.

MATERIALS AND METHODS

The medial recti (MR) from three patients were obtained at surgery or necropsy and prepared for ultrastructural analysis. Muscle was obtained from a 48 year old white male, affected since his 20s by a sporadic form of CPEO and carrying the heteroplasmic mtDNA "common deletion," who underwent strabismus surgery because of a high degree of exotropia, as already detailed.⁵ The second case was obtained at necropsy from a previously reported 75 year old white female with LHON carrying the mtDNA mutation at position 3460/ND1 in homoplasmic form.⁶ The third muscle (normal control) was obtained from a 72 year old white female cornea donor after her death, which had been secondary to a cardiac infarction. The postmortem times to fixation for the normal and LHON cases were 20 hours and 24 hours, respectively. All muscle specimens were immediately fixed in Karnovsky solution for 3 hours at room temperature, postfixed in 1% osmium tetroxide, dehydrated in graded acetone, and embedded in Araldite (Fluka Chemie, AG, Buchs, Switzerland). Ultrathin (80 nm thick) sections were stained with uranyl acetate-lead citrate, and examined with a Philips EM 208S electron microscope (Fei Electron Optics BV, Eindhoven, Netherlands). In LHON and in the normal control case the diameter of a randomly chosen sample of mitochondria was calculated using the software analySIS (Soft Imaging System GmbH, Munster, Germany). Differences in the median diameter values between the two groups have been evaluated with the non-parametric Mann-Whitney test for independent samples. Two sided p values less than 0.05 have been considered statistically significant.

RESULTS

Low magnification electron microscopy (EM) of the CPEO specimen showed normal myofibrils adjacent to abnormal myofibrils that had diffuse sarcomeric ultrastructural

Abbreviations: CPEO, chronic progressive external ophthalmoplegia; EM, electron microscopy; EOMs, extraocular muscles; LHON, Leber hereditary optic neuropathy; MR, medial recti

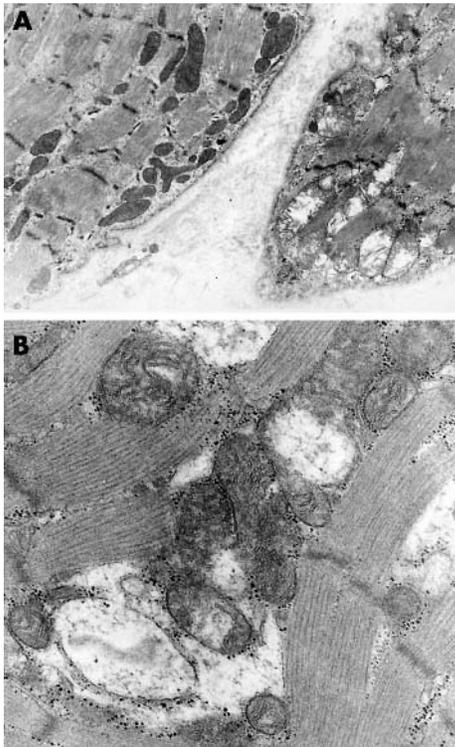


Figure 1 CPEO case. (A) Electron micrograph showing a normal fibre (left side) in close proximity to an altered, swollen one. This pattern of distribution is observed within all the muscle examined, creating a “mosaic-like profile” (original magnification $\times 24\,000$). (B) High magnification electron micrograph shows an altered muscle fibre. Many mitochondria have lost their inner cristae, thus creating a “ghost profile,” with a swollen appearance producing muscle displacement (original magnification $\times 40\,000$).

alterations which resulted in a “mosaic-like” pattern (fig 1A). Areas of mitochondrial swelling were often close to the normal mitochondria in an adjacent myofibril. At high magnification EM, abnormal fibres showed sarcomeric swelling and the presence of “ghost” mitochondrial profiles with partial to complete matrix emptying, whereas the external membrane was generally preserved (fig 1B). In other instances a profound rearrangement of the cristae with an “onion ring-like” appearance was also observed. The damage within muscle fibres appeared selectively distributed. The number of mitochondria appeared not to be increased without any evidence of inflammation or fibrosis.

In the LHON specimen there were an increased number of mitochondria which took up to two thirds of the sarcomeric space producing in some areas local distortion of myofibril organisation (fig 2). Otherwise the architecture of the muscle fibres was normal with a regular alternation of actin and myosin filaments and empty mitochondria forming dense clusters organised in columns in the sarcoplasmic reticulum (fig 3). Frequent abnormal disruption and re-arrangement of the cristae characterised the LHON mitochondria, without any evidence of inflammation or fibrosis. The abnormalities of mitochondrial populations in LHON were seen homogeneously distributed throughout the specimen. Many mitochondria were characterised by homogeneous electron dense inclusions and flocculent material (fig 4). Moreover, most mitochondria had an increased size and a “swollen appearance” with a median diameter of $0.85\ \mu\text{m}$. This increase was very significant if compared to the normal autoptic control ($p < 0.0001$). We were unable to identify any

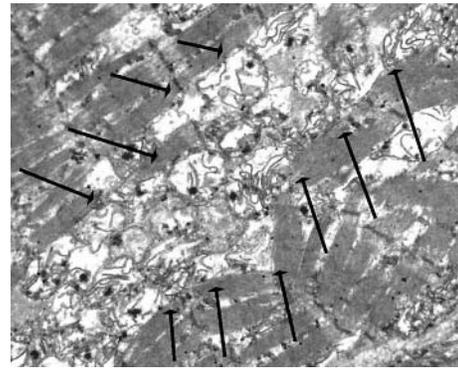


Figure 2 LHON case. All sarcoplasmic spaces are filled by mitochondria which occupy up to two thirds of the total muscle cells cytoplasm. The accumulation of mitochondria creates a “mitochondrial stream” (see between arrows) with distortion of myofibrils in some areas. Within many mitochondria can be seen elaboration and stacking of cristae (original magnification $\times 8900$).

paracrystalline structure in either disease after computer retrieval of all images was performed.

These ultrastructural aspects were not likely related to postmortem artefacts, as suggested by the comparison with the postmortem time to fixation matched control.

DISCUSSION

The results of the present study point to the differences in the underlying pathophysiology between CPEO and LHON. The mtDNA “common deletion” in CPEO has the potential to produce much more severe disruption of mitochondrial oxidative phosphorylation (OXPHOS) than the point mutations in LHON.² In fact, this mutation is lethal in homoplasmic form and is always found heteroplasmic—that is, a mixture of wild type and mutant mtDNA genomes which may distribute with different loads in mitochondria within cells and tissues.

In contradistinction, the present case of LHON was homoplasmic mutant in relation to the milder OXPHOS impairment in LHON.³ Moreover, not only homoplasmic mutant mtDNA is compatible with life, but in most cases does not even induce the optic nerve pathology for which the co-occurrence of a further nuclear genetic modifying factor or of environmental exposure to trigger factors is currently

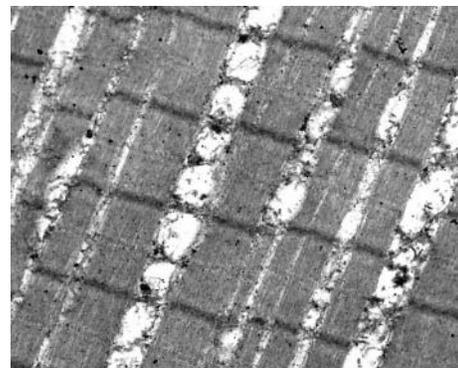


Figure 3 LHON case. The regular cytoarchitecture of the muscle fibre is appreciable. The anomalous ultrastructural features are the presence of swollen mitochondria which have increased their size two to three times (average median diameter is $0.85\ \mu\text{m}$ as measured on random sample). They are organised in columns between myofilaments forming dense clusters in the sarcoplasmic reticulum (original magnification $\times 11\,000$).

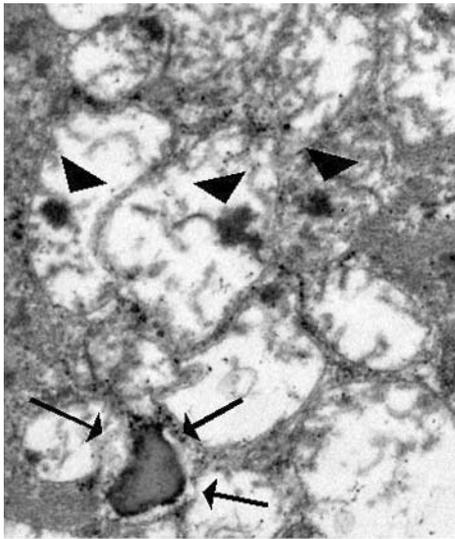


Figure 4 LHON case. Electron microscopic appearance of woolly bodies (arrowheads) and electron dense inclusion (arrows) of uncertain interpretation, probably related to mitochondrial matrix degeneration (original magnification $\times 41\,000$).

postulated.⁷ Thus, each cell in the LHON case carries all mtDNA molecules harbouring the 3460/ND1 mutation.

The hallmark of the CPEO case was the “selective damage” of specific myofibrils: while some of the fibres were clearly abnormal, others showed a regular sarcoplasmic architecture without any evidence of damage. This selectivity is best explained by mosaic heteroplasmy with clonally expanded high mutant load and under-threshold low mutant load in different myofibrils. The same “mosaic-like” pattern is usually found in skeletal muscles biopsies of CPEO patients, which reveal the presence of classic “ragged red fibres” with cytochrome c oxidase negative histoenzymatic staining intercalated with normal fibres.²

However, in CPEO there may be a complete loss of function in EOMs (strabismus fixus is a clinical landmark for CPEO) yet most other skeletal muscle is clinically not affected. Thus, heteroplasmy can only partially explain the prevalent involvement of EOMs. A possible explanation may be that 80% of EOMs fibres are type IIA singly innervated or global red singly innervated (myosin isoform expression and high mitochondrial content). These muscle fibres are strictly aerobic and may be more vulnerable in CPEO.

The “mosaic-like profile” observed may also reflect the sparing of the fibre types with anaerobic metabolic capacity and fast type ATPase profile (myosin heavy chain profile type IIB and IIC of skeletal muscle fibres).

In contradistinction, our study of the 3460/ND1 LHON muscle demonstrated a different hallmark feature represented by the increased number of mitochondria filling up to two thirds of myofibrils sarcoplasm. Many of these mitochondria showed a swollen appearance with elongated and stretched inner cristae, similar to a previously described LHON case carrying the 11778/ND4 mutation.⁴ In the currently studied 3460/ND1 LHON case the cytoarchitecture of the muscle was essentially preserved without evidence of disorganised or impoverished myofibrils, nor inflammation or fibrosis. These ultrastructural findings in LHON support the general notion that an increase in mitochondria is, in

muscles, a compensatory response to a decreased OXPHOS efficiency, particularly in EOMs.^{2, 8}

The ultrastructural features we have described in this study may be related to the phenotypic differences in these two mitochondrial disorders. In CPEO the mitochondrial changes in EOMs seem more severe and lead to ophthalmoplegia, whereas in LHON the mitochondria are mostly increased in number and clinically the EOMs are never affected. Thus, it is plausible to hypothesise that in LHON the increase in mitochondrial number is a successful compensatory strategy for EOMs and skeletal muscle, despite evidence of subclinical impairment.^{3, 8} However, in LHON this compensatory strategy does not seem to work in the small calibre axons composing the papillomacular bundle of the optic nerve. In the latter physical constrains may limit the size and transport of mitochondria eventually leading to energy depletion and retinal ganglion cells degeneration.^{3, 9, 10} At the present time, there is no proved form of therapy for LHON, CPEO, or any other genetic mitochondrial disease. Further studies should dissect in deep the intracellular events induced by mitochondrial impairment and the strategies operated by different tissues leading to presence or absence of clinical symptoms as an essential step towards devising a strategy for treatment.

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REFERENCES

- Porter JD, Baker RS, Ragusa RJ, *et al*. Extraocular muscles: basic and clinical aspects of structure and function. *Surv Ophthalmol* 1995;**39**:451–84.
- Smeitink J, van den Heuvel L, DiMauro S. The genetics and pathology of oxidative phosphorylation. *Nat Rev Genet* 2001;**2**:342–52.
- Carelli V, Ross-Cisneros FN, Sadun AA. Mitochondrial dysfunction as a cause of optic neuropathies. *Prog Retin Eye Res* 2004;**23**:53–89.
- Sadun AA, Kashima Y, Wurdeman AE, *et al*. Morphological findings in the visual system in a case of Leber's hereditary optic neuropathy. *Clin Neurosci* 1994;**2**:165–72.
- Carta A, D'Adda T, Carrara F, *et al*. Ultrastructural analysis of extraocular muscle in chronic progressive external ophthalmoplegia. *Arch Ophthalmol* 2000;**118**:1441–5.
- Sadun AA, Win PH, Ross-Cisneros FN, *et al*. Leber's hereditary optic neuropathy differentially affects smaller axons in the optic nerve. *Trans Am Ophthalmol Soc* 2000;**98**:223–35.
- Carelli V, Giordano C, d'Amati G. Pathogenic expression of homoplasmic mtDNA mutations needs a complex nuclear-mitochondrial interaction. *Trends Genet* 2003;**19**:257–62.
- Wredenberg A, Wibom R, Wilhelmsson H, *et al*. Increased mitochondrial mass in mitochondrial myopathy mice. *Proc Natl Acad Sci USA* 2002;**99**:15066–71.
- Sadun AA. Mitochondrial optic neuropathies. *J Neurol Neurosurg Psychiatry* 2002;**72**:423–5.
- Sadun AA. Acquired mitochondrial impairment as a cause of optic nerve disease. *Trans Am Ophthalmol Soc* 1998:881–923.