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

A Carta, V Carelli, T D’Adda, F N Ross-Cisneros, A A Sadun

**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 autopic 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 $0.85 \pm 0.65 \mu m$, 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.

**Results**

Low magnification electron microscopy (EM) of the CPEO specimen showed normal myofibrils adjacent to abnormal myofibrils that had diffuse sarcomeric ultrastructural dysfunction. However, ultrastructural alterations in EOMs have been reported in a single case described at necropsy of LHON carrying the 11778/ND4 mtDNA mutation. 

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.

In LHON, 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.

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

---

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. Their activity (of both saccades and pursuit movements) is continuous and respects, including motor unit properties. 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. Their activity (of both saccades and pursuit movements) is continuous and respects, including motor unit properties. 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. Their activity (of both saccades and pursuit movements) is continuous and respects, including motor unit properties. 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. Their activity (of both saccades and pursuit movements) is continuous and respects, including motor unit properties.
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 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 μm. This increase was very significant if compared to the normal autoptic control (p<0.0001). We were unable to identify any 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. In fact, this mutation is lethal in homoplasmy and is always found heteroplasmy—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. 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
The general notion that an increase in mitochondria is, in disorganised or impoverished myofibrils, nor inflammation of the muscle was essentially preserved without evidence of currently studied 3460/ND1 LHON case the cytoarchitecture.

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 heteroplasmia 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.

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, heteroplasmia 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. 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.

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. 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 constraints may limit the size 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.

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.

ACKNOWLEDGEMENTS
The authors thank Ms Antonella Piazza from the University of Parma for technical assistance in reviewing the English version of this paper.

REFERENCES