Ultrastructural Analysis of Extraocular Muscle in Chronic Progressive External Ophthalmoplegia

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Extracocular muscles are primarily involved in many mitochondrial diseases, but no reports exist regarding the morphological appearance of the muscles in cases of long-standing ocular myopathies. For this reason, muscle samples obtained from surgery in a sporadic case of chronic progressive external ophthalmoplegia (CPEO) were used for ultrastructural investigation and molecular analysis of mitochondrial DNA. Genetic testing revealed a heteroplasmic macrodeletion of about 5.0 kilobases in length, localized between the 9570– and 14619–base pair regions. Electron microscopy revealed focal areas of both disruption and abnormality of mitochondria in only some of the muscle fibers, producing “selective vacuolization.” This ultrastructural pattern was highly selective and limited to some extraocular muscle fibers, sparing all the others. The “selective damage” observed in this case of CPEO resembles that case occurring in another mitochondrial disease, Leber hereditary optic neuropathy, where damage occurs only in the papillomacular bundle of the retina, sparing peripheral axons. It is possible that some anatomical and physiological factors play a leading role in both Leber hereditary optic neuropathy and ocular myopathies. The ultrastructural aspect herein observed needs to be further investigated to better understand whether a particular muscle fiber type is the target of mitochondrial impairment in CPEO.

Chronic progressive external ophthalmoplegia (CPEO) is a mitochondrial disease that principally affects the extracocular muscles. Different types of macrodeletions in mitochondrial DNA (mtDNA) have been associated with CPEO and its variants, ranging from 3.0 to 8.5 kilobases (kb) in length. Besides mtDNA testing, diagnosis of CPEO is supported by histopathological evidence of “ragged red fibers” on modified Gomori trichrome stain in muscle biopsy specimens. Biochemical abnormalities of oxidative phosphorylation may be found as well. However, few studies have been performed with attention to the ultrastructural features of the extracocular muscles. Indeed, the relationship between mtDNA abnormalities and the functional and morphological changes of mitochondria that lead to the phenotypical manifestations of the disease is still poorly understood. To our knowledge, no studies correlating mtDNA deletion and muscle features in CPEO have been performed. To investigate the relationship existing between mtDNA abnormalities and the ultrastructural aspects of extracocular muscles in long-standing CPEO, we performed a molecular assessment of mtDNA and an electron microscopy study of the medial rectus in a patient with isolated CPEO who underwent ptosis and strabismus surgery.
ing technique, skeletal biopsy specimens of the left bicep muscle performed at age 28 years revealed ragged red fibers. The patient was not previously tested for mtDNA. Visual acuity was 20/20 OU and funduscopic ex-

Molecular assessment of muscle mtDNA revealed a heteroplasmic macrodeletion 5.0 kb in length (Figure 1 and Figure 2). In particular, Figure 1 shows the presence of 2 clearly separate bands in lane 1 (CPEO case) accounting for heteroplasmy. The up-
per band corresponds to a normal mtDNA amount, whereas the lower band reveals the presence of deleted mtDNA. Heteroplasmy was estimated to be as high as 55%. In a second blotting with several restriction enzymes, deletion was localized between the 9570 and the 14619 positions (Figure 2). This type of deletion is called “common deletion” because it is the type most frequently associated with CPEO. The deleted region (from position 9570 to 9990) encodes for several transfer RNAs (codons for glutamine, arginine, histidine, serine, and leucine), as well as for many subunits of complex III of cytochrome c oxidase. Furthermore, the deletion (from position 14149 to 14619) involves mitochondrial subunits for nicotinamide adenine dinucleotide activity (ND3, ND4, ND5, and pars of the ND6).

Low-magnification electron microscopy of the resected muscle revealed a heterogeneous ultrastructural pattern, with normal muscle fibers flanked by other fibers that have diffuse sarcoplasm vacuolization (Figure 3). At high magnification, ultrastructurally normal fibers displayed peripherally located nuclei, granular sarcoplasm containing regularly arranged myofibrils, tubules of smooth endoplasmic reticulum, glycogen deposits, and mitochondria characterized by numerous inner cristae (Figure 4). On gross estimation, the number of mitochondria was not increased. High-magnification analysis of the vacuolized fibers revealed alterations of the mitochondrial compartment, responsible for ultrastructural defects. In fact, the sarcoplasm of damaged muscle fibers was filled with ultrastructurally normal myofibrils, whereas mitochondria displayed different degrees of disruption of their cristae, leading from partial to complete mitochondrial matrix emptying, with preservation of the external membrane.

These “ghost” mitochondrial profiles were responsible for the selective vacuolization observed on low-magnification electron microscopy (Figure 5). Also, the cristae of many mitochondria were arranged in an onion ring-like shape (Figure 6). The histological architecture of muscle fibers was otherwise normal, without any evidence of inflammatory infiltration. Moreover, we did not see any fibrosis among the muscle fibers. No rectangular- or ring-shaped paracrystalline inclusions were found and “woolly bodies” were not observed in our case of CPEO.
To date, investigations of extraocular muscles in mitochondrial diseases reported in the literature describe abnormalities of the mitochondria in the muscle analyzed. Ultrastructural studies of these abnormalities revealed longer than normal and irregularly shaped inner cristae, increased surface-volume ratio, paracrystalline inclusions of uncertain nature, increased number of mitochondria filling up to two thirds of the muscle cell cytoplasm, and presence of vacuoles in the mitochondria. All those abnormalities are presumed to be related to cell energy needs in response to a genetically determined impairment of adenosine triphosphate production.

In our patient, we found several other features never reported in previous studies: mtDNA macrodeletion involving genes encoding for cytochrome c oxidase and for several transfer RNAs, is associated with a selective vacuolization of some muscle fibers as observed under electron microscopy (Figure 3, A). Also, high magnification of the same areas showed that the vacuolization is caused either by total disruption of the mitochondrial structure or of their inner cristae (Figure 3, C). Moreover, we did not detect any evidence of muscle fibrosis. Indeed, we were unable to find any paracrystalline inclusions or vacuoles within the mitochondria. Paracrystalline inclusions have been reported as early ultrastructural changes in CPEO whereas they have never been described in long-standing cases of mitochondrial diseases nor in our case. The significance of paracrystalline inclusions is still a matter of debate since they are assumed to be an early sign of mitochondrial matrix changes, or abnormal condensation of the matrix produced during laboratory procedures. In contrast, cristae disruption and autolytic digestion are almost always present during the entire clinical evolution of those diseases. The selective damage found in the medial rectus of our patient may be only partially explained by heteroplasmy, since this is estimated to be as high as 55%, whereas a lower percentage of fibers is involved. The selective damage found in our case resembles the selective damage occurring in LHON, where point mutations of mtDNA are associated with progressive atrophy of the papillomacular bundle, sparing all other axons in the retina.

Why the damage is so selective remains unclear. A possible explanation is that in LHON, as well as in CPEO, the thinnest structures (axons and muscle fibers) are the targets of the mitochondrial impairment. Also, some anatomical features may play a key role. In fact, the papillomacular bundle fibers are the thinnest axons in the human organism. In LHON, this may give rise to an impossibility of mitochondrial duplication in such a small neuronal bag in response to an energy request, thus creating a block of the axonal flow with subsequent optic atrophy (catastrophe theory). Perhaps some unknown physiological features such as energy request, heteroplasmy, and molecular dimension of the muscle fibers involved play a similar role in our case of CPEO, and may be responsible for the selective damage we observed.

To our knowledge, no studies correlating mtDNA testing and ultrastructural aspects of extraocular muscles in cases of long-standing CPEO have been previously published. In this regard however, 2 re-
Recent studies using Gomori stain compared mtDNA defects with light microscopy appearance of muscle biopsy specimens, but ultrastructural features have not been investigated. Kiyomoto et al found a decrease in cytochrome c oxidase subunit IV activity in some muscle fibers in skeletal biopsy specimens of patients with CPEO with heteroplasmic common deletion. Lindner et al found cytochrome c oxidase-negative fibers in 74% of their patients affected by mitochondrial encephalomyopathies; among those patients, 42% tested positive for mtDNA common deletion. Anyway, the architectural structure of the muscle itself has not been tested so we cannot conclude that only some fibers are selectively damaged in these studies. Although our case shares the same common deletion with these 2 cited studies, we did not perform any biochemical study on cytochrome c oxidase activity to characterize muscle fibers. In planning future studies, further morphological investigations in mitochondrial diseases are important as they may provide insight about the pathogenesis of those clinical entities. With regard to CPEO, to confirm the ultrastructural pattern observed in our case and to identify the muscle fiber type that may be selectively damaged, additional studies correlating mtDNA testing with ultrastructural and biochemical features of extraocular muscles are needed.

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