

Involvement of extraocular muscle in mitochondrial encephalomyopathy

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Summary. We carried out a histological examination of the extraocular muscles (EOMs) in a case of myoclonus epilepsy associated with ragged-red fibers (MERRF) and two cases of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), which did not manifest external ophthalmoplegia clinically. By light microscopy, many granular and vesicular fibers were seen associated with endomysial fibrosis. Electron microscopy revealed that the fibers showed prominent accumulation of abnormal mitochondria, extensive loss of myofibrils, proliferation of free sarcoplasmic reticulum and an increased amount of lipid vacuoles. These changes were more pronounced in MELAS than in MERRF. Hirano bodies were often seen in the subsarcolemmal area of muscle fibers and also in the intramuscular myelinated nerve fibers and axon terminals. These findings suggest the presence of mitochondrial myopathy of the EOMs in cases of MELAS and MERRF.

Key words: Mitochondrial encephalomyopathy – Extraocular muscle – Ultrastructure – Mitochondrial myopathy

Mitochondrial encephalomyopathy is a heterogeneous disorder encompassing three distinctive clinical syndromes, Kearns-Sayre syndrome (KSS), myoclonus epilepsy associated with ragged-red fibers (MERRF), and mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), and other disorders. KSS shows progressive external ophthalmoplegia, the extraocular muscle (EOMs) undergoing severe degeneration with endomysial fibrosis and containing many ragged-red fibers (RRFs) [3, 4, 12, 25].

On the other hand, MERRF and MELAS do not manifest external ophthalmoplegia, and this is one of the important clinical features which differentiate them from KSS [5, 21]. The EOM in MERRF and MELAS has not been examined in previously reported autopsy cases [6,

7, 10, 13, 15, 23, 24]. In this report, we describe the histological features of the EOM in MERRF and MELAS. The nervous system components concerned with ocular movement were also examined.

Materials and methods

All of the present three cases examined (one case of MERRF and two cases of MELAS) have been reported elsewhere with regard to their detailed clinical histories and histopathological findings [7, 17–20, 28, 29].

The MERRF patient was a 30-year-old woman who died after a clinical course of 16 years. She was found to have saccadic eye movement at the age of 25 years. However, the limitation of extraocular movement was not followed. The postmortem examination was performed 11 h after death.

MELAS patient 1 was a 14-year-old boy who died after a 13-year clinical course. He was found to have alternate external strabismus at the age of 2 years. MELAS patient 2 was a 16-year-old girl who died after a total clinical course of 10 years. She did not show limitation of extraocular movement. They were autopsied 2 h after death.

The superior and medial rectus muscles, superior colliculus, periaqueductal gray matter, medial longitudinal fasciculus, and nuclei and nerve roots of the IIIrd, IVth and VIth cranial nerves in these three cases were examined by light microscopy. Specimens of the EOM were taken at autopsy and fixed in 3% glutaraldehyde-1% paraformaldehyde. They were then processed for electron microscopy and examined using a Hitachi HS11-B electron microscope.

Four cases were selected for the control study. They died aged 41 to 46, and had no abnormal ocular movement. They included a case of liver cirrhosis, a case of multiple cerebral infarcts and two cases of subarachnoid hemorrhage. They were autopsied 2 to 8 h after death. The superior rectus muscles were also taken at autopsy and fixed in 3% glutaraldehyde-1% paraformaldehyde, embedded in epoxy resin and examined by the light microscope.

Results

Extraocular muscles (EOM)

The superior and medial rectus muscles in MERRF and MELAS showed essentially the same histological

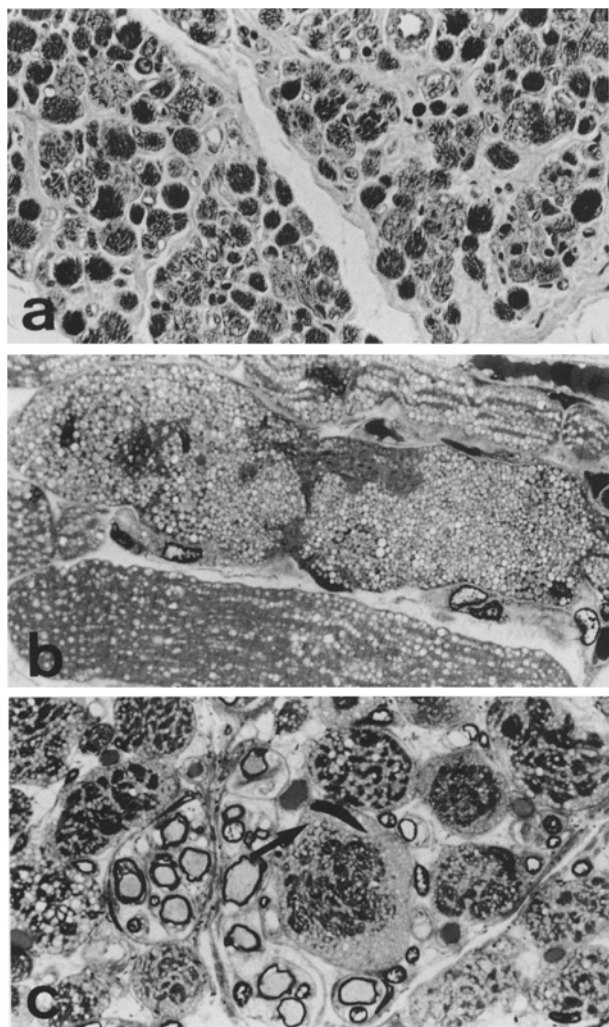


Fig. 1 a–c. Extraocular muscle (EOM) by light microscopy. **a** Many muscle fibers appear fine granular and vesicular. Moderate endomysial fibrosis is evident. Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), Masson trichrome stain; $\times 250$. **b** Abundant vesicular structures and extensive loss of myofibrils are evident. MELAS, Epon section, toluidine blue and safranin (TBS) staining; $\times 500$. **c** Homogeneous rod-like structures (*arrow*) are sometimes present in the subsarcolemmal area where vesicular structures have accumulated. Myoclonus epilepsy associated with ragged-red fibers (MERRF), Epon section. TBS staining; $\times 500$

findings. Many muscle fibers showed a granular and clear appearance in ordinary paraffin sections (Fig. 1 a). Moderate endomysial fibrosis was noted in many fasciculi. In epoxy resin-embedded sections, all of the granular and clear fibers were revealed to have abundant vesicular structures, which were markedly accumulated in the subsarcolemmal regions, and variable loss of myofibrils (Figs. 1 b, c). Almost all of the muscle fibers had many vesicular structures. The surface of these fibers was often irregular. These features were more pronounced in MELAS. However, no neurogenic changes were observed. Homogeneous rod-like structures were often seen in the subsarcolemmal regions of the vesicular fibers (Fig. 1 c). They were eosinophilic and appeared red with

Masson's trichrome stain, gray with Klüver-Barrera stain and purple with phosphotungstic acid-hematoxylin stain. They showed round, oval or spindle-like profiles. No significant abnormalities were noted in the nerve fibers within the muscle (Fig. 1 c).

The control superior rectus muscles preserved their sarcoplasm even in the two cases, who were autopsied 8 h after death. We observed only a small number of the vesicular structures, which were seen abundant in the muscle fibers in MERRF and MELAS. Rod-like structures were not seen in the fibers.

In MERRF and MELAS, electron microscopy revealed that the vesicular appearance of muscle fibers by light microscopy was due to prominent accumulation of abnormal mitochondria (Fig. 2 a). These mitochondria were variable in size and shape, having tubulo-vesicular cristae or a proliferated inner membrane. Giant mitochondria were often seen, especially in MELAS. The morphological changes in the mitochondria were more prominent in MELAS than in MERRF (Fig. 2 b). We could not see the crystalline or globular mitochondrial inclusions in EOMs. The granular and sharply demarcated mitochondrial inclusions which were seen sometimes (Fig. 2 b) were considered to be glycogen particles. Lipid droplets were often seen in the fibers filled by mitochondria (Fig. 2 c). There was variable loss of myofibrils in muscle fibers. Some fibers showed extensive myofibril loss and prominent accumulation of glycogen particles (Fig. 2 d). Even fragments of the remaining myofibrils in these fibers retained their cross-striations. Disorganization of myofibrils was sometimes observed, and some fibers showed almost complete myofibril loss. They were filled with abnormal mitochondria and sarcoplasmic reticulum (Fig. 2 e).

The homogeneous rod-like structures observed by light microscopy (Fig. 1 c) were revealed to be filamentous inclusions, of which three types were evident. The first type showed association with sarcoplasmic reticulum-like structures (Fig. 3 a). The second consisted of a regular array of filaments which were sometimes arranged in perpendicular planes (Fig. 3 b). The third type showed a peculiar herring-bone or ladder-like pattern (Fig. 3 c). A few zebra bodies were seen in the subsarcolemmal regions without any apparent connection with myofibrils. In MELAS cases, smooth muscle cells of a few arterioles among the muscle fibers showed accumulation of mitochondria, some of which had abnormal cristae.

Nervous system

The superior colliculus from the MELAS patient 1 showed two foci of minute necrotic lesions associated with macrophages and capillary proliferations, and from the MELAS patient 2 and the patient with MERRF showed mild neuronal loss with gliosis. The periaqueductal gray matter showed mild gliosis in all three cases. In the axon terminals and myelinated axons of the EOM, Hirano bodies were often seen. The IIIrd, IVth and VIth cranial nerve trunks and nuclei showed no

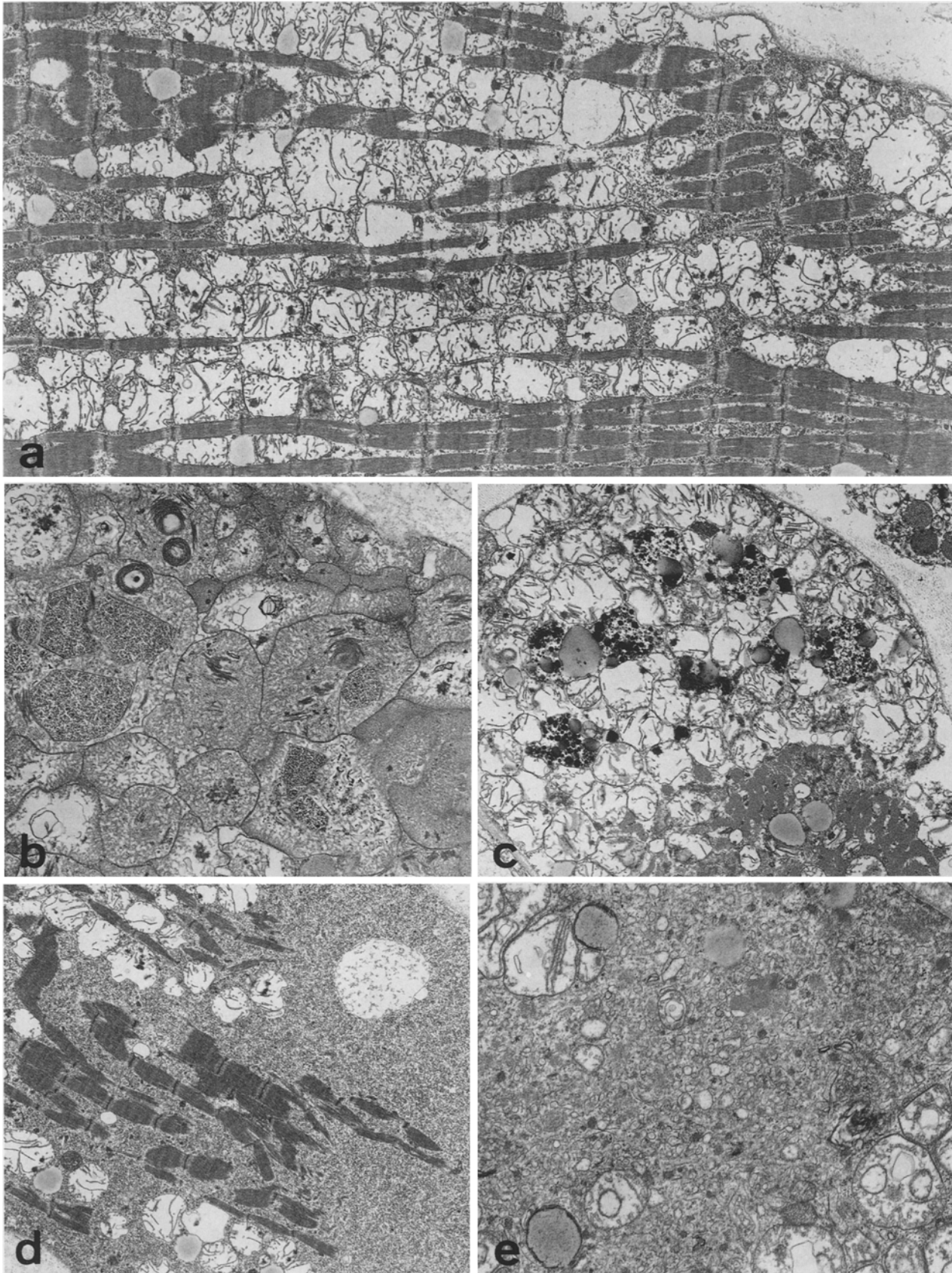


Fig. 2a–e. Ultrastructural changes in the EOM. **a** Prominent accumulation of mitochondria is evident in a muscle fiber. MERRF, $\times 7,200$. **b** Accumulated abnormal mitochondria show proliferation of the inner membrane, tubulo-vesicular cristae and a variable size and shape. The granular mitochondrial inclusions were seen sometimes. MELAS, $\times 6,800$. **c** An increased amount of lipid droplets is

often present among accumulated mitochondria in muscle fibers. MERRF, $\times 6,700$. **d** Extensive loss of myofibrils and prominent accumulation of glycogen are evident. The remaining myofibrils retain their cross-striations. MERRF, $\times 5,000$. **e** Myofibrils have almost disappeared and the sarcoplasmic reticulum is present freely in the muscle fiber. MELAS, $\times 13,300$

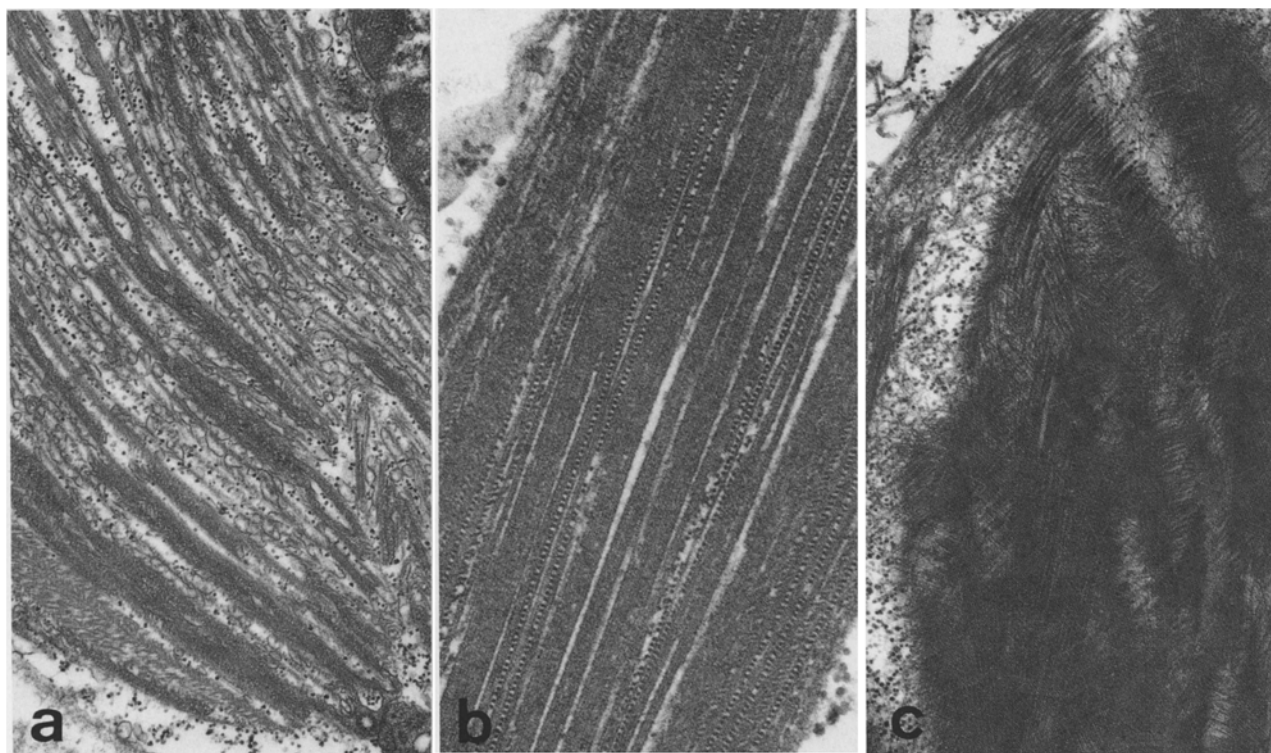


Fig. 3a–c. Filamentous inclusions within the muscle fiber. **a** Inclusion associated with a sarcoplasmic reticulum-like structure. MERRF, $\times 21,600$. **b** Inclusion consisting of a regular array of

filaments. MERRF, $\times 64,000$. **c** Inclusion showing a herring-bone or ladder-like pattern. MERRF, $\times 21,600$

degeneration. The medial longitudinal fasciculus was well preserved.

Discussion

In the autopsy cases of MERRF and MELAS reported previously, mitochondrial myopathy was noted in the limb muscles [6, 7, 10, 13, 16, 23, 26, 28], or in the heart muscle [15, 18, 19].

In the present study, we carried out a histological examination of the EOMs in MERRF and MELAS. Although frozen tissues of the muscles for modified Gomori trichrome staining were not obtained, the features of the EOMs by light and electron microscopy corresponded to RRFs. We also observed the vesicular structures in the control muscle fibers. However, the number of these structures was smaller than that in MERRF or MELAS. A few fibers showed RRFs or contained increased amount of mitochondria in the normal EOMs [2, 9, 22]. Ultrastructurally, however, the EOMs in the normal cases showed normal mitochondria which were increased in number [9]. We considered that the high frequency of RRFs and the abnormal profiles of increased mitochondria in MERRF and MELAS were significant. The frequency of RRFs, myofibrillar degeneration and endomysial fibrosis of the EOMs were more pronounced in MELAS than in MERRF. In the present cases, defects of the complex I in the mitochondrial respiratory chain have been revealed in the MELAS patient 2

[17, 27] and defects of the complex IV in the MERRF patient (Yoneda et al., personal communication). It is still unclear whether the difference in the deficient enzyme in MELAS and MERRF is correlated with the severity of the histopathological changes in the EOMs. We have already performed histological examinations of the limb muscles from our present patients [17, 27, 28], and RRFs were less frequent than in the EOMs. This situation is the same as in KSS cases, in which degeneration of muscle fibers associated with RRFs is more marked in the EOMs than in limb muscles [3, 4, 8, 11, 12, 22].

The filamentous structures observed in the muscle fibers were identical with those described in the EOMs from a patient with exotropia [14] and from aged men [30] as intramuscular Hirano bodies. Our present examination revealed that these structures were associated with a sarcoplasmic reticulum-like membrane. The development of these structures might be related in some way to myofibrillar degeneration. Zebra bodies have been reported in skeletal muscles and the EOMs in various conditions. The significance of zebra bodies present in MERRF and MELAS is not known. Hirano bodies within myelinated nerve fibers and axon terminals was described previously in a case of progressive external ophthalmoplegia [1]. Although myelinated nerve fibers in the EOMs and the IIIrd, IVth and VIth cranial nerve trunks of our three patients were well preserved, Hirano bodies within the nerve fibers and axon terminals in the EOMs might indicate some degeneration of the intramuscular nerve fibers.

Daroff et al. [4] considered that the external ophthalmoplegia in KSS was neurogenic. However, in some patients with KSS who showed external ophthalmoplegia and marked degeneration of the EOMs associated with RRFs, the anatomical structures in the brain concerned with ocular movement including the IIIrd, IVth and VIth cranial nerves and nuclei were well preserved [3, 12, 26]. The presence of RRFs, loss of myofibrils and other alterations of the EOMs described here may cause external ophthalmoplegia in cases of MERRF and MELAS.

Accumulation and structural changes of the mitochondria were seen in the smooth muscle cells of the arterioles within the EOMs in our MELAS patients, although much milder than those in the brain [18]. This indicates that the blood vessels in the EOMs are also involved by mitochondrial angiopathy in MELAS [18].

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