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Extraocular Mitochondrial Myopathies and their Differential Diagnoses

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ABSTRACT The diagnosis of mitochondrial myopathy depends upon a constellation of findings, family history, type of muscle involvement, specific laboratory abnormalities, and the results of histological, pathobiochemical and genetic analysis. In the present paper, the authors describe the diagnostic approach to mitochondrial myopathies manifesting as extraocular muscle disease. The most common ocular manifestation of mitochondrial myopathy is progressive external ophthalmoplegia (PEO). To exclude myasthenia gravis, ocular myositis, thyroid associated orbitopathy, oculopharyngeal muscular dystrophy, and congenital fibrosis of the extraocular muscles in patients with an early onset or long-lasting very slowly progressive ptosis and external ophthalmoplegia, almost without any diplopia, and normal to mildly elevated serum creatine kinase and lactate, electromyography, nerve conduction studies and MRI of the orbits should be performed. A PEO phenotype forces one to look comprehensively for other multisystemic mitochondrial features (e.g., exercise induced weakness, encephalopathy, polyneuropathy, diabetes, heart disease). Thereafter, and presently even in familiar PEO, a diagnostic muscle biopsy should be taken. Histological and ultrastructural hallmarks are mitochondrial proliferations and structural abnormalities, lipid storage, ragged-red fibers, or cytochrome-C negative myofibers. In addition, Southern blotting may reveal the common deletion, or molecular analysis may verify specific mutations of distinct mitochondrial or nuclear genes.

KEYWORDS Mitochondrial DNA mutation; nuclear DNA mutation; progressive external ophthalmoplegia; extraocular myopathy; differential diagnosis

INTRODUCTION

Extraocular and neurological symptoms are the most frequent clinical presentation of mitochondrial disorders. Mitochondria are cytoplasmic organelles synthesizing the energy donor (ATP) of the cell. The term mitochondrial disorders is restricted to abnormalities of oxidative phosphorylation (Smeitink et al., 2001; Zeviani & Di Donato, 2004). The respiratory chain encompasses five enzymatic complexes (I, II, III, IV, V), embedded in the inner membrane of mitochondria. Coenzyme Q and cytochrome c serve as additional electron shuttles between the complexes. The formation of the respiratory chain is under control

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of both genetic systems, the nuclear genome and the mitochondrial genome (mitochondrial DNA (mtDNA)) with the exception of complex II, which is strictly nuclear encoded (DiMauro & Hirano, 2005; Larsson & Olfords, 2001; Smeitink et al., 2001; Schmiedel et al., 2003; Zeviani & Di Donato, 2004). Complexes I, II and IV pump protons from the mitochondrial matrix into the intermembrane space, forming an electrochemical gradient across the inner mitochondrial membrane, and the energy which this creates is harnessed by ATP synthase for the production of ATP from ADP (Mitchell, 1979). This production of ATP from the reduction of oxygen, known as oxidative-phosphorylation coupling, is what generates the energy needed for cellular function (DiMauro & Hirano, 2005; Larsson & Olfords, 2001; Mitchell, 1979; Smeitink et al., 2001; Schmiedel et al., 2003; Zeviani & Di Donato, 2004). Extraocular muscle is significantly different from limb skeletal muscle, but it is not precisely known why extraocular muscles are preferentially affected by mitochondrial disorders. Extraocular muscles have smaller motor unit sizes, higher motor neuron discharge rates, higher blood flow, and higher mitochondrial volume fractions compared to skeletal muscle. This suggests that the energy demands and therefore the susceptibility to mitochondrial dysfunction are superior (reviewed by Richardson et al., 2005).

CLINICAL MANIFESTATIONS OF EXTRAOCULAR MITOCHONDRIAL MYOPATHY

Progressive External Ophthalmoplegia

The most common form is late-onset bilateral progressive external ophthalmoplegia (PEO) (Bau & Zierz, 2005; Chinnery et al., 2004; Petty et al., 1986; Richardson et al., 2005; Von Graefe, 1867). PEO was first described in 1867 by Albert von Graefe as a clinical syndrome and disease entity. PEO onset ranges between the ages of 11 and 82 years (Bau & Zierz, 2005; Chinnery et al., 2004). PEO is characterized by ptosis and weakness of extraocular muscles leading to limitation of extraocular movements with relative sparing of downgaze, and occasionally dysconjugate ocular movements. Although transient diplopia may occur, the majority of patients seldom complain of diplopia and are mostly unaware of their restrictions (Bau & Zierz, 2005; Petty et al., 1986; Richardson et al., 2005; Von Graefe, 1867). Ptosis and ophthalmoplegia may occur together, but each can occur alone. The ptosis is often asymmetric. Up to 90% of PEO patients have additional weakness of the facial, bulbar or limb muscles. Thus, many patients may be classified as "PEO plus" because they present additional multisystemic symptoms such as other neurological symptoms, hearing disturbances, or diabetes (Bau & Zierz, 2005; DiMauro & Hirano, 2005; Larsson & Olfords, 2001; Mitchell, 1979; Richardson et al., 2005; Smeitink et al., 2001; Schmiedel et al., 2003; Zeviani & Di Donato, 2004). In about 15% of patients with PEO, autosomal dominant or recessive inheritance is noted. Autosomal dominant PEO (adPEO) is characterized by accumulation of multiple deletions of mtDNA in the patient's tissues. Rarely, autosomal recessive PEO is found. Clinically, no differences between hereditary and sporadic PEO can be demonstrated; thus, only the family history may provide further information (Bau & Zierz, 2005; Zeviani & Di Donato, 2004).

Kearns-Sayre Syndrome/PEO Plus

Kearns and Sayre first described PEO as a key feature of the Kearns-Sayre syndrome (KSS) in 1958 (Kearns & Sayre, 1958). This syndrome is characterized by onset before age 20 years and encompasses PEO, atypical pigmentary retinopathy (salt- and pepper-like appearance), and frequently myopathic weakness, heart block, cerebellar ataxia and high cerebrospinal fluid (CSF) protein levels. KSS may include incomitant strabismus, mental deterioration, pyramidal signs, short stature, diabetes or delayed sexual maturation (Bau & Zierz, 2005; DiMauro & Hirano, 2005; Kearns & Sayre, 1958; Larsson & Olfords, 2001; Smeitink et al., 2001; Schmiedel et al., 2003; Zeviani & Di Donato, 2004). An analogous syndrome-like entity of KSS is ptosis and ophthalmoplegia with additional organ involvement (PEO Plus). This syndrome was first described by Drachman in 1968, indicating the multisystemic nature of the mitochondrial disease (Drachman, 1968).

Mitochondrial Myopathy and Encephalopathy, Lactate Acidosis, Stroke-like Episodes (MELAS)

The acronym MELAS was introduced by Pavlakis in 1984 to denote probably the most common maternally inherited mitochondrial syndrome, encompassing

mitochondrial myopathy, encephalopathy, lactate acidosis and stroke-like episodes. At an age of onset between 3 to 40 years, early symptoms include muscle weakness (87%), easy fatigability (15–18%), recurrent headaches and seizures (28%). The typical clinical manifestations of MELAS include stroke-like episodes (99–100%), seizures (85–96%), short stature (55–100%), muscle weakness (87–89%), headache/vomiting (77–92%), hearing loss (27–75%), encephalopathy (20–95%), optic atrophy (20%), pigmentary retinopathy (16%) and PEO (13%) (Hirano & Pavlakis, 1994; Thambisetty & Newman, 2004). Biochemical and histopathological features encompass the presence of elevated lactate in serum and cerebrospinal fluid (50–100%), ragged-red fibers in muscle biopsies (80–100%) and strongly succinate dehydrogenase (SDH)-positive blood vessels. Several mtDNA point mutations have been associated with MELAS. An A→G mutation in the transfer RNA (tRNA) Leu (UUR) gene at position 3243 of the mtDNA accounts for approximately 80% of MELAS cases. Some other mtDNA mutations have been reported. In addition, southern blot analysis is recommended for identifying rearrangements of mtDNA, such as large-scale partial deletions and duplications (reviewed by Thambisetty & Newman, 2004).

Sensory Ataxic Neuropathy, Dysarthia and Ophthalmoparesis (SANDO)

External ophthalmoparesis, ataxia, sensory neuropathy and dysarthria are not normally associated in a single mitochondrial syndrome. In 1997, Fadic et al. first reported this rare entity, which is now termed SANDO (Fadic et al., 1997). Up to now, at least 18 cases in different families have been reported worldwide (OMIM 607459). In patients with SANDO, compound heterozygosity for two mutations and homozygous mutations in the POLG gene have been found (Rantamaki et al., 2001; Van Goethem et al., 2003, 2004). Recently, in a patient with SANDO, a heterozygous mutation in the C10ORF2 gene was found (Hudson et al., 2005). This finding indicated that SANDO is a variant of autosomal recessive PEO.

Moreover, external ophthalmoplegia may also occur as a symptom in mitochondrial syndromes, such as myoclonic epilepsy, myopathy with ragged-red fibers (MERRF), mitochondrial neurogastrointestinal encephalopathy (MNGIE), and neuropathy, ataxia and retinopathia pigmentosa (NARP) (Bau & Zierz, 2005; DiMauro & Hirano, 2005; Larsson & Olfords, 2001; Smeitink et al., 2001; Schmiedel et al., 2003; Zeviani & Di Donato, 2004).

In summary, there is a wide phenotypic and genotypic overlap between PEO, KSS, PEO plus, MELAS, and SANDO; it is therefore uncertain whether these syndromes really represent different entities or are to a certain extent clinical variants of one condition only (Bau & Zierz, 2005).

DIFFERENTIAL DIAGNOSES OF EXTRAOCULAR MITOCHONDRIAL MYOPATHIES

Ocular Myasthenia Gravis

The term 'ocular myasthenia contrasting generalized myasthenia' is used to define the clinical subtype of myasthenia gravis with isolated eye muscle weakness and blepharoptosis or ophthalmoparesis, resulting in diplopia. Ocular dysfunction accounts for 50–80% of the initial manifestations of myasthenia gravis (Elrod & Weinberg, 2004; Romi et al., 2005). Asymmetric ptosis is most often associated with acquired and autoimmune myasthenia gravis. Furthermore, in young patients, inherited congenital myasthenic syndromes are associated as an early feature with ptosis and ophthalmoplegia (Beeson et al., 2005). The eyelid ice test, edrophonium test and nerve conduction studies with repetitive nerve stimulation of the facial nerves are helpful in making the diagnosis. Additionally, in 40–60% of the patients, acetylcholine receptor antibodies are present (Beeson et al., 2005; Elrod & Weinberg, 2004; Romi et al., 2005).

Ocular Myositis

In primary idiopathic orbital myositis, one or more extraocular muscles are affected. Acute onset, severe pain in and around the eye, pain on movement, occasional diplopia, chemosis and globe displacement are typical features. Beyond the acute form, a more chronic relapsing-remitting form is seen. Transorbital ultrasound and coronal MRI scans are helpful to further define the disease. Usually, there is a rapid response to systemic steroid therapy (Berkhoff et al., 1997; Lacey et al., 1999).

Thyroid Associated Orbitopathy

Thyroid orbitopathy is thought to be an organ-specific autoimmune process and is associated with thyroid disease in 80–90%of cases (Lacey et al., 1999; Scott & Siatkowski, 1999). Bilateral muscle involvement including inflammation, edema, and secondary fibrosis is very common. Imaging reveals fusiform posterior enlargement of extraocular muscles (Lacey et al., 1999; Scott & Siatkowski, 1999).

Oculopharyngeal Muscular Dystrophy OPMD

The combination of ptosis and pharyngeal weakness combined with ophthalmoparesis, dysphagia and weakness and wasting of face, neck, and distal limb muscles is quite characteristic for autosomal dominant, late-onset OPMD (Brais, 2003; Ruegg et al., 2005). OPMD is caused by expansions in a 6-GCG trinucleotide repeat tract located in the first exon of the polyadenylate binding protein nuclear 1 gene on chromosome 14q. Genetic testing is available (Brais, 2003; Ruegg et al., 2005).

Myotonic Dystrophy Type 1 (DM1)

Among the numerous well-known multisystemic features of the classic form of myotonic dystrophy (DM1), e.g., muscle weakness and myotonia, the eye is in any case affected. Not only myotonic cataracts but also retinal abnormalities, ptosis and blepharospasm are common features of the disease. DM1 is caused by expansions in the CTG trinucleotide repeat tract located in the myotonic dystrophy protein kinase gene on chromosome 19q. Genetic testing is available (Harper, 2001; Machuca-Tzili et al., 2005).

Congenital Cranial Dysinnervation Disorders (CCDDs) and Congenital Fibrosis of Extraocular Muscles CFEOM

The congenital cranial dysinnervation disorders (CCDDs) encompass congenital, non-progressive, sporadic, or familial abnormalities of cranial musculature that result from developmental abnormalities or complete absence of one or more cranial nerves with primary or secondary muscle dysinnervation. Among the CCDDs, congenital fibrosis of the extraocular muscles (CFEOM) leads to rare inherited strabismic syndromes presenting with congenital, non-progressive bilateral ophthalmoplegia with active and passive restriction of globe movement and fibrosis of the extraocular muscles innervated by the oculomotor and/or trochlear nerves. At least three distinct syndromes are recognized: CEFOM1 is an autosomal dominant form and caused by mutations in the KIF21a gene on chromosome 12p. CFEOM2 is an autosomal recessive form caused by homozygous mutations in the ARIX gene on chromosome 11q. CFEOM3 has been mapped in separate families to different loci on chromosomes 12q, 13q and 16q (Engle, 2002; Hanisch et al., 2005).

LABORATORY ANALYSIS

Analyzing serum creatine kinase and lactate levels may reveal slight elevations, but in sporadic PEO, CK and lactate levels may be within the normal range. Elevated CSF lactate levels are found almost only in generalized mitochondrial cytopathies. The clinical symptom of exercise intolerance leads to the performance of a non-ischemic forearm test (Hogrel et al., 2001) or, possibly better, a maximal cycle exercise test (Taivassalo et al., 2003). The degree of exercise intolerance in mitochondrial myopathies correlates directly with the severity of impaired muscle oxidative phosphorylation as indicated by the peak capacity for muscle oxygen extraction. Exaggerated circulatory and ventilatory responses to exercise are direct consequences of the level of impaired muscle oxidative phosphorylation and increase exponentially in relation to an increasing severity of oxidative impairment (Taivassalo et al., 2003). The diagnostic value of a constant workload protocol is superior to an incremental cycle test, but this test is less sensitive for MM than simple testing of resting lactate and muscle morphology. Therefore, cycle testing of MM patients remains an important research tool, but should not be a standard diagnostic procedure for MM (Jeppesen et al., 2003).

Electrophysiology

For the detection of subclinical neuropathy or myasthenia gravis, nerve conduction studies with repetitive nerve stimulation are performed. Electromyography may help to exclude myotonia and myositis, and sometimes in choosing the muscle for biopsy, e.g. by detection of a subclinical myopathic EMG pattern in up to 30% of the patients (Girlanda et al., 1999). The

ERG is usually normal or demonstrates attenuated a- and b-wave amplitudes.

Muscle Biopsy and Biochemical Analysis

The histological and histochemical analysis of a muscle biopsy remains the most important diagnostic screen for detecting mitochondrial abnormalities (Barron et al., 2005; Bau & Zierz, 2005; Taylor et al., 2004; Yu Wai Man et al., 2005). We recommend taking a biopsy of a proximal limb muscle instead of an extraocular muscle (Barron et al., 2005; Girlanda et al., 1999; Karppa et al., 2004; Taylor et al., 2004; Yu Wai Man et al., 2005). One common method of detecting mitochondrial accumulation is the Gomori trichrome stain, which may show the subsarcolemmal accumulation of mitochondria, the so called ragged-red fibers. A more specific way to evaluate mitochondria is the histochemical enzyme reactions for the mitochondrial enzyme succinate dehydrogenase (SDH) and cytochrome-C oxidase (COX). The COX reaction is especially useful in the evaluation of mitochondrial myopathies because it contains subunits encoded for by both the mitochondrial and nuclear genome. A mosaic pattern of COX activity is highly indicative of heteroplasmic mtDNA disorders. Single COX-negative fibers, frequently corresponding to ragged-red fibers, with additional lipid storage in some cases can be demonstrated (Fig. 1). In cases with a low percentage of COX-negative fibers, sequential staining for COX/SDH may be helpful. SDH is encoded exclusively by the nuclear genome and therefore its activity is independent of mtDNA. A global decrease in COX activity is usually suggestive of a nuclear mutation in one of the ancillary proteins required for COX assembly and function. Electron microscopy is helpful in selected cases by revealing enlarged mitochondria, absent or abnormal cristae and the presence of paracrystalline inclusions (Fig. 1). The respiratory chain complex deficiencies are detectable by measurement of the enzyme activity in muscle homogenates. PEO and KSS have been reported with deficiencies of complexes I, II, III, IV, and I +IV (Barron et al., 2005; Bau & Zierz, 2005; Chinnery et al., 2004; DiMauro & Hirano, 2005; Karppa et al., 2004; Larsson & Olfords, 2001; Mitchell, 1979; Petty et al., 1986; Richardson et al., 2005; Smeitink et al., 2001; Schmiedel et al., 2003; Taylor et al., 2004; Yu Wai Man et al., 2005; Zeviani & Di Donato, 2004). Nevertheless, some patients will have completely normal histology and histochemistry. The presence of low levels of COX-negative fibers or ragged-red fibers must also be interpreted with caution, since clonal expansion of mtDNA in single fibers is well-known in aging muscle.

Molecular Genetics

The mitochondrial genome is small (16.6 kb) and encodes 13 proteins of the respiratory chain and 22 RNAs required for intramitochondrial protein synthesis. It is present in multiple copies within the muscle fibers and thus any defect may involve all copies of the mitochondrial genome (homoplasmy) or only a proportion (heteroplasmy) (Bau & Zierz, 2005; DiMauro & Hirano, 2005; Larsson & Olfords, 2001; Smeitink et al., 2001; Schmiedel et al., 2003; Taylor et al., 2004; Zeviani & Di Donato, 2004). Most common in approximately 80% of KSS and 40–70% of sporadic PEO patients are single, large-scale rearrangements of mtDNA. These rearrangements may be partial deletions or partial duplications. The most frequently identified large-scale deletion, the so called "common deletion," involves 4977 nucleotides. The majority of single large-scale rearrangements of mtDNA are sporadic and are therefore believed to be the result of the clonal amplification of a single mutational event, occurring in the oocyte of the mother or grandmother (Chinnery et al., 2004). The existence of multiple mtDNA deletions together with autosomal inheritance is characteristic of an underlying nuclear mutation, which is typical for adPEO (Agostino et al., 2003; Schmiedel et al., 2003; Tyynismaa et al., 2004).

Other patients with PEO show maternal inheritance, and in these patients mtDNA point mutations are detectable, particularly but not exclusively the 3243A→G MELAS mutation. In adPEO, most of the families carry heterozygous mutations in one of three genes: *ANT1*, encoding the heart-muscle-specific mitochondrial adenine nucleotide translocator, *Twinkle*, encoding a putative mtDNA helicase, and *POLG1,* encoding the catalytic subunit of the mtDNA-specific polymerase gamma (Agostino et al., 2003; Tyynismaa et al., 2004). Mutations in both *POLG1* alleles have been found in autosomal recessive PEO sibships (Agostino et al., 2003; Taylor et al., 2004; Tyynismaa et al., 2004; Van Goethem et al., 2003). Mutations in one of the three genes are also found in sporadic PEO patients. Thus, irrespectively of the inheritance, screening of these three

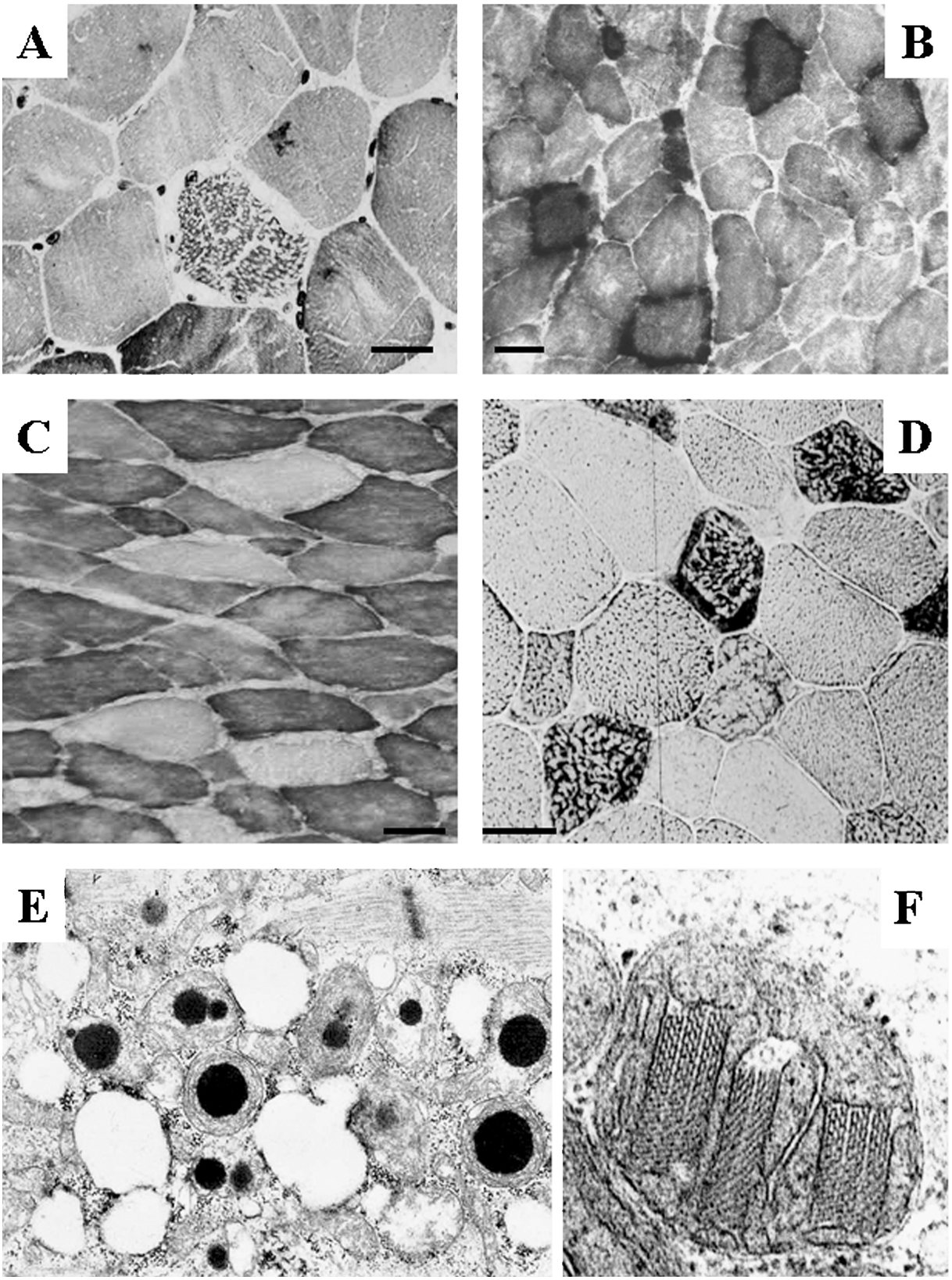

FIGURE 1 **Biopsy of the biceps brachii muscle from a sporadic PEO patient with the common large-scale deletion. (A) Classical ragged-red fiber (RRF) using the modified Gomori trichrome stain. The red product is selectively sequestered by mitochondria that accumulate at the sarcolemma. (B) SDH histochemistry detecting subsarcolemmal mitochondrial accumulation (arrow). (C) COX histochemistry reveals COX-negative RRFs. (D) Sudan black lipid histochemistry shows pronounced lipid storage in typical RRFs. (E) Electron microscopy of a RRF (X 7600). (F) Electron microscopy of paracrystalline inclusions. Typical "parking lot" type crystalloids. Bars in A–D 50 μm.**

genes may be performed in selected patients presenting with PEO (Agostino et al., 2003; Tyynismaa et al., 2004).

CONCLUSION

Although knowledge about mitochondrial disorders is now greatly increased, in patients with PEO and KSS/PEO Plus a muscle biopsy is still needed for the diagnosis. We recommend taking a biopsy of a proximal limb muscle instead of an extraocular muscle. Southern blot analysis of muscle DNA as a first step is useful to test for single or multiple deletions. In some cases with multiple mtDNA deletions and autosomal inheritance, molecular analysis of *POLG1*, *Twinkle* and *ANT1* are suggested.

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