MITOCHONDRIAL MYOPATHIES: CURRENT DIAGNOSIS (I)

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ABSTRACT

Mitochondrial diseases can present at any age and include a combination of multisystemic symptoms. Major manifestations of muscle involvement include infantile hypotonia, weakness, and lactic acidosis; severe exercise intolerance and easy fatigability, variable fixed weakness, often involving the extraocular muscles. Additionally, infantile or childhood encephalomyopathies have been identified in which CNS (e.g., seizures, ataxia, stroke-like episodes) and muscle symptoms coexist.

Paraclinical investigations support the diagnosis and help in categorizing the mitochondrial myopathies (MM). Serum CK level may be mildly elevated in MM but are often normal; blood lactate concentration is usually elevated at rest. Electroneuromyographic features are not pathognomonic: myopathic EMG findings and reduced sensory response amplitudes. Muscle biopsy is a more specific test of mitochondrial myopathies, tipically showing the presence of ragged red fibers, COX negative and SDH positive, with ultrastructurally abnormal mitochondria (electron microscopy). Enzymatic and genetic tests are sometimes useful.

After overviewing the current literature, a brief clinical case report is presented.

Key words: mitochondrial myopathies, diagnosis

Mitochondrial defects involving the Krebs cycle (fumarase deficiency, succinate dehydrogenase deficiency) and abnormal coupling of adenosine diphosphate phosphorylation to oxygen uptake (Luft's diseases) have been described, but the most common mitochondrial myopathies are associated with respiratory chain abnormalities (1).

The clinical presentation in these disorders is heterogeneous. The presence of progressive external ophtalmoplegia is very suggestive, as is the presence of central nervous (CNS) dysfunction such as ataxia, seizures, or sensorineural deafness. In most cases myopathy is present along with a cluster of CNS symptoms suggesting the well-defined syndromes – Table 1 (2, 3). The retina, in particular the retinal pigment epithelium, is highly vulnerable to be involved by mtDNA defect, and the retinopathy is phenotypically variable and frequently subclinical, depending to some extent on the type or site of mt DNA defect (4).

Peripheral neuropathy is also a frequent association. (3) Mitochondrial myopathy with cardiomyopathy has been re ported with a variety of point mutations in DNA. Cardiac conduction blocks are more frequent in patients with KSS. Intraventricular conduction defects show an unusually rapid progression to potentially fatal complete atrioventricular block and are an indication for prophylactic cardiac pacing (5, 6, 7).

Muscle wasting is not restricted to the eyes. The face and neck can also be affected, leading to incomprehensible speech and swallowing difficulties. Overall musculature wasting pervades many affected individuals, requiring wheelchairs and, in

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severe cases, assisted living requirements. Exercise-induced pain can also result.

Sometimes, exercise intolerance is associated with painful muscle cramps and/or injury-induced pain. The cramps are actually sharp contractions that may seem to temporarily lock the muscles, while the injury-induced pain is caused by rhabdomyolysis, leading to myoglobinuria. Cramps or myoglobinuria usually occur when someone with exercise intolerance "overdoes it," and can happen during the overexertion or several hours afterward (8).

Symptoms of mitochondrial myopathies are largely variable, even within the same family, and are dependent on the amount and type of genetic mutations present. These disorders can occur in infancy, childhood, or adulthood.

Until recently it was generally thought that nuclear DNA abnormalities present in childhood and mtDNA abnormalities (primary or secondary to a nuclear DNA abnormality) present in late childhood or adult life; however, recent advances have shown that many mtDNA disorders present in childhood, and many nuclear genetic mitochondrial disorders present in adult life (1, 9).

A detailed *family history* is important in making the diagnosis and in directing molecular genetic testing. Most adults with PEO or KSS represent single occurrences in a family. Many of the childhood-onset encephalomyopathies are single occurrences

TABLE 1. *Clinical Syndromes of Mitochondrial Diseases (3)*

Disorder	Primary Features	Additional Features
Alpers-Huttenlocher syndrome	• Hypotonia • Seizures • Liver failure	• Renal tubulopathy
Chronic progressive external ophthalmoplegia (CPEO)	• External ophthalmoplegia • Bilateral ptosis	• Mild proximal myopathy
Kearns-Sayre syndrome (KSS)	• PEO onset at age <20 years • Pigmentary retinopathy • One of the following: CSF protein >1g/L, cerebellar ataxia, heart block	• Bilateral deafness • Myopathy • Dysphagia • Diabetes mellitus • Hypoparathyroidism • Dementia
Pearson syndrome	• Sideroblastic anemia of childhood • Pancytopenia • Exocrine pancreatic failure	• Renal tubular defects
Infantile myopathy and lactic acidosis (fatal and non-fatal forms)	• Hypotonia in 1st year of life • Feeding and respiratory difficulties	• Fatal form may be associated with a cardiomyopathy and/or the Toni-Fanconi-Debre syndrome
Leigh syndrome (LS)	• Subacute relapsing encephalopathy • Cerebellar and brain stem signs • Infantile onset	• Basal ganglia lucencies • Maternal history of neurologic disease or Leigh syndrome
Neurogenic weakness with ataxia and retinitis pigmentosa (NARP)	• Late-childhood or adult-onset peripheral neuropathy • Ataxia • Pigmentary retinopathy	• Basal ganglia lucencies • Abnormal electroretinogram • Sensorimotor neuropathy
Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS)	• Stroke-like episodes at age <40 years • Seizures and/or dementia • Ragged-red fibers and/or lactic acidosis	• Diabetes mellitus • Cardiomyopathy (initially hypertrophic; later dilated) • Bilateral deafness • Pigmentary retinopathy • Cerebellar ataxia
Myoclonic epilepsy myopathy sensory ataxia (MEMSA)[1]	• Myopathy • Seizures • Cerebellar ataxia	• Dementia • Peripheral neuropathy • Spasticity
Myoclonic epilepsy with ragged-red fibers (MERRF)	• Myoclonus • Seizures • Cerebellar ataxia • Myopathy	• Dementia • Optic atrophy • Bilateral deafness • Peripheral neuropathy • Spasticity • Multiple lipomata
Leber hereditary optic neuropathy (LHON)	• Subacute painless bilateral visual failure • Males:females ~4:1 • Median age of onset 24 years	• Dystonia • Cardiac pre-excitation syndromes

[1]Also referred to as mitochondrial recessive ataxia syndrome (MIRAS) and spinocerebellar ataxia with epilepsy (SCAE)

in a family and may be caused by autosomal recessive nuclear gene defects or mtDNA defects. A clear maternal inheritance pattern (no male transmissions) may indicate an underlying mtDNA defect. The range of clinical features of mtDNA disease is broad, and there may be many oligosymptomatic family members (e.g., some with diabetes mellitus or mild sensorineural deafness as the only feature). A clear autosomal dominant pattern of inheritance may be seen in individuals with PEO.

Mitochondrial dysfunction should be considered in the differential diagnosis of any progressive multisystem disorder. The diagnosis is most challenging when only one symptom is present; the diagnosis is easier to consider when two or more seemingly unrelated symptoms are present, involving more than one organ system.

The investigation can be relatively straightforward if a person has a recognizable phenotype and if it is possible to identify a known pathogenic mtDNA mutation. The difficulty arises when no mtDNA defect can be found or when the clinical abnormalities are complex and not easily matched to those of more common mitochondrial disorders (1, 10).

Paraclinical tests are used to support a diagnosis of mitochondrial disease and to categorize the mitochondrial disorder into one of the specific syndromes (1, 3, 11).

Neuroimaging is indicated in individuals with suspected CNS disease.

- CT may show basal ganglia calcification and/or diffuse atrophy.
- MRI may show focal atrophy of the cortex or cerebellum, or high signal change on T2-weighted images, particularly in the occipital cortex. There may also be evidence of a generalized leukoencephalopathy. Cerebellar atrophy is a prominent feature in the pediatric age group (12, 13).

Neurophysiologic studies *(12, 13, 14):*

- Peripheral neurophysiologic studies are indicated in individuals with limb weakness, sensory symptoms, or areflexia. Electromyography (EMG) is often normal but may show myopathic features. Nerve conduction velocity (NCV) may be normal or may show a predominantly axonal sensorimotor polyneuropathy.
- Magnetic resonance spectroscopy (MRS) and exercise testing (with measurement of blood concentration of lactate) may be used to detect evidence of abnormal mitochondrial function non-invasively (elevated lactate level in brain or muscle at rest, or a delay in the recovery of the ATP peak in muscle after exercise).
- Electroencephalography (EEG) is indicated in individuals with suspected encephalopathy or seizures. Encephalopathy may be associated with generalized slow wave activity on the EEG. Generalized or focal spike and wave discharges may be seen in individuals with seizures.

Electrocardiography and echocardiography *(12,14)* may indicate cardiac involvement (cardiomyopathy or atrioventricular conduction defects).

Laboratory evaluation *(1, 11, 12, 14):*

- Serum CK level may be mildly elevated in mitochondrial myopathies but are often normal.

– High: Chronic progressive external ophthalmoplegia (CPEO) & Ptosis; Limb weakness - Very high: Mitochondrial DNA depletion.

- Lactate and pyruvate level (better to asses it in arterial than venous blood).

– Measurement of blood lactate concentration is indicated in individuals with features of a myopathy or CNS disease. Fasting blood lactate concentrations above 3 mm/L support a diagnosis of mitochondrial disease.

– Measurement of CSF lactate concentration is indicated in individuals with suspected CNS disease. Fasting CSF lactate concentrations above 1.5 mm/L support a diagnosis of mitochondrial disease.

– Lactate/Pyruvate ratio

- High (> 50:1): Suggests metabolic block in respiratory chain
- Normal: Metabolic block is upstream, e.g. pyruvate dehydrogenase complex

– Normal values do not exclude mitochondrial disorders (examples: NARP; MILS; may become elevated with short exercise).

– Lactic acidosis. It is important to exclude other causes of lactic acidosis when interpreting these values. For example, the concentration of lactate may be elevated in the blood and CSF of affected individuals following a seizure. CSF lactate concentration may be elevated following an ischemic stroke.

Muscle biopsy (1, 2, 12, 14)***:*** a more specific test of mitochondrial myopathies, it must be analyzed for histologic or histochemical evidence of mitochondrial disease. Respiratory chain complex studies are then usually carried out on skeletal muscle or skin fibroblasts.

"Ragged red" fibers (RRFs) on muscle biopsy are observed in a wide variety of mitochondrial diseases including mtDNA depletion, mtDNA deletions, and mitochondrial transfer RNA mutations.

RRFs are not seen in disorders due to defects of substrate transport, substrate utilization, and the Kreb's cycle. RRFs are not pathognomonic of mitochondrial diseases, but can also be seen in other conditions like dystrophies, dermatomyositis and in older individuals and also in zidovudine-associated myopathy in HIV-infected patients. However, in these diseases, the intensity and the proportion of muscle biopsies showing RRFs will be sparse.

Presence of more than 2% RRFs in skeletal muscle biopsy is taken as one of the criteria for the diagnosis of mitochondrial disease (15, 16).

Distinctive features of muscle biopsy in mithochondrial myopathies (1, 2, 12, 14):

- Succinate dehydrogenase (SDH) stain
- Usual abnormality in mitochondrial disorders: Increased staining of muscle fibers
- Most sensitive & specific stain for mitochondrial proliferation in muscle fibers
- Specific confirmation of mitochondrial dysfunction & proliferation
- Muscle fibers with increased staining

– Mitochondrial disease: Mitochondrial proliferation.

– Other: Regenerating muscle fibers.

- Strongly SDH reactive blood vessels: MELAS
- SDH positive (Ragged red) muscle fibers with prominentlipid accumulation: Coenzyme Q_{10} deficiency

– Gomori trichrome stain

- Stains "Ragged red" muscle fibers
- Much less specific & sensitive for mitochondrial proliferation than SDH

– Cytochrome oxidase (COX) stain changes

– Absent or reduced staining of muscle fibers: Reduced COX activity.

– May be diffuse or in scattered fibers.

– Increased staining of fibers: mitochondrial proliferation; variably present depending on syndrome.

- Scattered COX- fibers ± Ragged red fibers: Suggests

– mtDNA mutation affecting mitochondrial protein synthesis.

– Mutations in COX I, COX II, or COX III genes.

– Heteroplasmic mutation.

- Muscle fibers with both COX-positive and increased SDH staining suggests specific mutations: MELAS: many SDH+ fibers are COX+; mutations in mtDNA protein encoding genes, except COX genes; cytochrome b: all SDH+ fibers are COX+;
- COX reduction: diffuse

– Severe: sparing spindles and smooth muscles of vessels (fatal or benign infantile myopathy, homoplasmic mutation).

– Moderate: Including spindles and smooth muscles of vessels (Leigh syndrome with COX deficiency).

- Ragged red COX- fibers with reduced immunocytochemical detection of cytochrome oxidase II: Suggests mutations affecting mitochondrial protein synthesis generally
- Reduced mtDNA-encoded COX subunits I and II in COX-deficient muscle fibres: mtDNA mutations
- Reduction of all COX subunits in all muscle fibers: ? Nuclear mutations

– Sudan black: Increased lipid in muscle fibers.

- Lipid pattern: Droplets enlarged
- Lipid increase: Muscle fiber patterns

Scattered fibers with mitochondrial proliferation (SDH+)

– Selectively increased: Type I muscle fibers.

– Increased in all muscle fibers.

– Inflammation.

- Uncommon in most mitochondrial disorders
- Present with: Inclusion body myositis; polimyositis COX-

– Muscle fiber necrosis & regeneration: Uncommon.

– Immunohistochemistry.

- Abnormal protein accumulation in ragged red fibers: mainly desmin and αβcrystallin, but also heat shock proteins, dysferlin, emerin, utrophin, caveolin;
- Apparently related to impaired extra-lysosomal protein degradation;

– Electron microscopy.

- Usually not specific or sensitive in adults with non-diagnostic histochemistry results
- Children

– Ultrastructure may be only evidence of mitochondrial pathology in 6%

– No data regarding specificity of these morphologic changes for mitochondrial disease.

- May be helpful in confirming mild pathologic changes on histochemistry
- Not helpful in mitochondrial differential diagnosis

Unfortunately, some patients with mitochondrial myopathy do not show any of the above characteristic

muscle changes. Enzymology and molecular genetic studies are often required to establish a definite diagnosis of mitochondrial diseases (17).

Biochemistry analysis (1, 2)

- Muscle preferable to cultured fibroblasts
- Fresh or Frozen muscle may be used
- Combined partial defects of respiratory enzymes containing mtDNA-encoded subunits: Suggests mtDNA mutations
- Can be normal in mitochondrial disorders: Especially with multiple mtDNA deletion syndromes

Molecular genetics: (1, 2)

Molecular genetic testing may be carried out on genomic DNA extracted from blood (suspected nuclear DNA mutations and some mtDNA mutations) or on genomic DNA extracted from muscle (suspected mtDNA mutations). Studies for mtDNA mutations are usually carried out on skeletal muscle DNA because a pathogenic mtDNA mutation may not be detected in DNA extracted from blood.

- Southern blot analysis may reveal a pathogenic mtDNArearrangement. The deletion or duplication breakpoint may then be mapped by mtDNA sequencing.
- Targeted mutation analysis of a panel of genes may be performed.
- If a recognized point mutation is not identified, the entire mitochondrial genome may be sequenced.

In many individuals in which molecular genetic testing does not yield or confirm a diagnosis, further investigation of suspected mitochondrial disease can involve a range of different clinical tests, including muscle biopsy for respiratory chain function.

Mutation screening

Positive result: Confirms diagnosis

- Screen for most common mutations associated with syndrome

– e.g. MELAS A3243G then T3271C.

- Blood DNA: Adequate for

– Point mutations in tRNA genes: MELAS; MERRF.

– Some mutations in structural genes: NARP; Lebers.

– Single large mtDNA deletions with systemic disorders: Kearns-Sayre; Pearson

- Muscle DNA: Required for

– Multiple deletions.

– Single mtDNA deletions in PEO & other localized disorders.

– MELAS point mutation in oligo- or asymptomatic relatives.

– Some point mutations in structural genes.

Several diagnostic schemes were proposed to improve the diagnostic sensitivity, as the clinical and paraclinical features are suggestive rather than pathognomonic of mitochondrial diseases.

Clinical case exemplification

Case No 4593: C.M., female, 56 years.

Clinical onset: about 20 years ago.

Signs and symptoms: bilateral palpebral ptosis, muscular weakness (predominant proximal) in inferior limbs, bilateral hipoaccusia, distal limb global decrease of sensibility, generalized decrease of deep tendon reflexes.

Paraclinical tests: EMG – myopathic pattern; decreased sensory-motor nerve conduction velocity. EEG – III degree permanent atrio-ventricular block (permanent cardiostimulation).

Cranian CT normal.

Muscle biopsy:

- Gomori trichrome stain: "ragged red" muscle fibers (fig. 1)
- Succinate dehydrogenase (SDH) stain: increased staining of muscle fibers (scattered) (fig. 2)
- Cytochrome oxidase (COX) stain: Absent or reduced staining of muscle fibers (scattered); 33% COX negative fibers (fig. 3)
- Immunohistochemistry: abnormal protein accumulation in ragged red fibers (mainly desmin) (fig. 4)
- Electron microscopy: abnormal ultrastructure of mithocondria (spiral cristae and lipid inclusions (fig. 5).

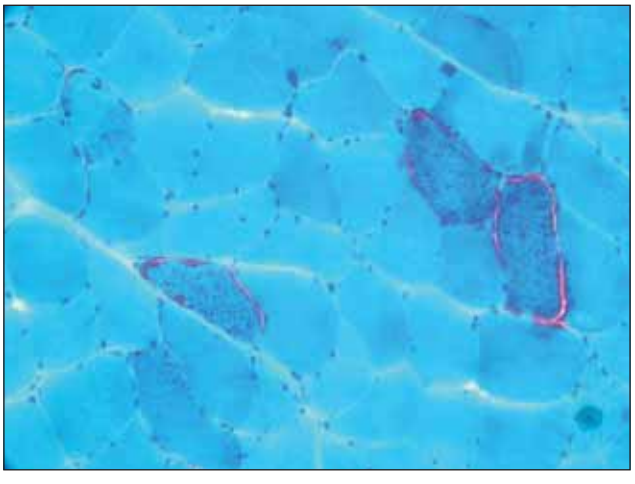

FIGURE 1. *Gomori trichrome X 40*

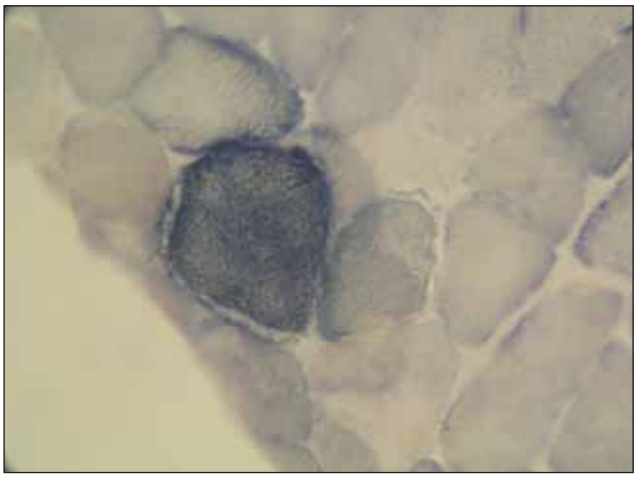

FIGURE 2. *SDH X 100*

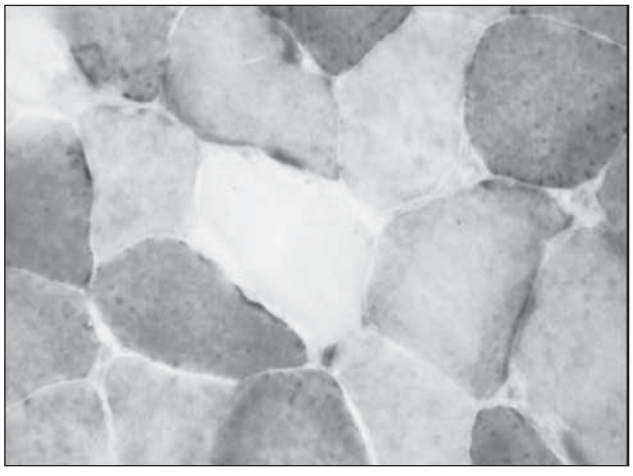

FIGURE 3. *COX X 200*

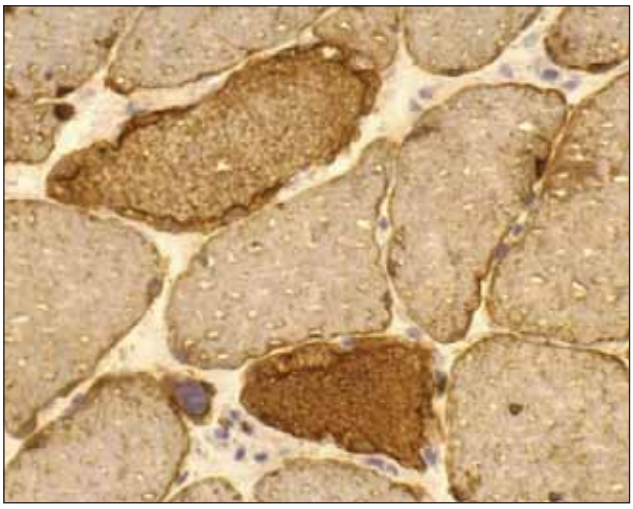

FIGURE 4. *Immunohistochemistry X 200*

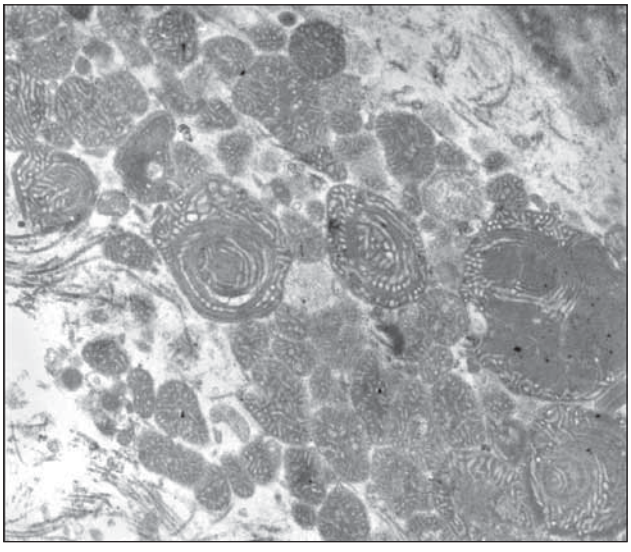

FIGURE 5. *Electron microscopy*

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