Mitochondrial Myopathies

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Abstract
Mitochondrial diseases, defined restrictively as disorders due to defects of the mitochondrial respiratory chain, are notoriously heterogeneous, both clinically and genetically. Here, we review only disorders affecting exclusively or predominantly skeletal muscle (mitochondrial myopathies). As the respiratory chain is under dual genetic control, we consider first myopathies due to defects in mitochondrial DNA (mtDNA), distinguishing those due to defects of mitochondrial protein synthesis *in toto* from those due to mutations in protein-encoding genes. We then divide disorders due to nuclear gene defects into six groups and discuss mitochondrial myopathies due to genetic defects affecting: respiratory chain components; the protein importation machinery; the inner membrane lipid milieu; and intergenomic signaling. We conclude with some considerations on therapy.
Key words: mitochondrial myopathy, mtDNA, nuclear DNA, ophthalmoplegia.

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There are papers in the history of medicine that will never become obsolete because, by combining original thinking with the best technology available at the time, they open up new vistas. One such paper is the report in 1962 by Luft and coworkers of a young Swedish woman with severe hypermetabolism, mild weakness, and normal thyroid function [39]. The genial intuition of Rolf Luft, an endocrinologist, that the problem was to be sought in skeletal muscle rather than in the thyroid gland, combined with the expertise in mitochondrial bioenergetics of the late Lars Ernster and the morphological acumen of Björn Afzelius, led the Karolinska group to discover the first mitochondrial disease and the first example of "organellar medicine". The fact that Luft disease is one of the rarest human metabolic disorders is a historical curiosity that does not diminish the importance, indeed the beauty, of that observation.

What Luft may not have suspected at the time was that he was opening a seemingly bottomless Pandora's box of human diseases. By 1994, it was clear that mitochondrial dysfunction could affect every tissue in the body, thus amply justifying the term "mitochondrial medicine" that Luft introduced in the title of a review article [38].

Of course, one of the unique characteristics of mitochondrial diseases is that mitochondria are relics of independent bacteria-like intruders (welcome intruders, as it turned out) that took permanent residence in our cells over a billion years ago. As such, mitochondria possess their own DNA (mtDNA) and are under dual genetic control. However, the role of mtDNA is limited to the "business end" of mitochondrial energy metabolism, the respiratory chain, where 13 of its approximately 90 proteins are encoded by the mitochondrial genome and are synthesized within the organelle. All other respiratory chain subunits are encoded by nuclear DNA (nDNA): after being synthesized in the cytosol, they are imported into the organelle, where they are assembled, together with their mtDNA-encoded counterparts, into the respective holoenzymes in the mitochondrial inner membrane (Figure 1). By a generally accepted convention, terms like mitochondrial diseases, mitochondrial cytopathies, or mitochondrial encephalomyopathies refer restrictively to primary genetic defects in the respiratory chain.

Although mtDNA was discovered 39 years ago [47] and human mtDNA had been fully sequenced by 1981 [2], clinicians paid no attention to this genetic relic until 1988, when mutations in mtDNA were first associated with human disease [27, 80]. Three major features distinguish mitochondrial from mendelian genetics. First, mtDNA is inherited from the mother, and so are diseases due to pathogenic point mutations in this genome (maternal inheritance). Second, mtDNA molecules are present in multiple copies (polyplasmy), pathogenic mutations usually affect only a proportion of mtDNAs (heteroplasmy), and a minimum critical percentage of mtDNAs has to be mutated to impair oxidative phosphorylation (threshold effect). Third, during subsequent cell generations the degree of heteroplasmy can change (mitotic seg-

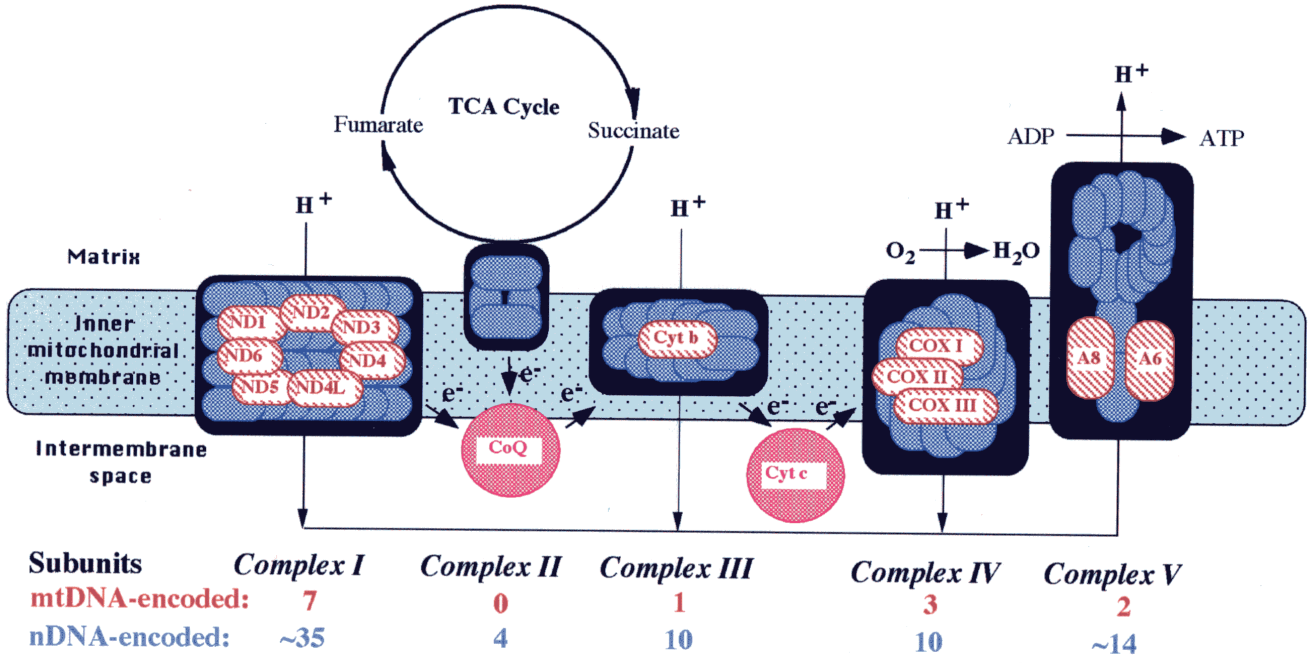

Figure 1. Schematic view of the mitochondrial respiratory chain, showing nuclear DNA-encoded (blue) and mtDNA-encoded red) subunits. Protons (H^+) are first pumped from the matrix to the intermembrane space through complexes I, III, and IV, then flow back into the matrix through complex V to produce ATP. Coenzyme Q (CoQ) and cytochrome c (Cyt c) are electron carriers.

regation) and, with it, also the clinical expression of the disease can vary both spatially (tissues affected) and temporally. These basic rules explain – at least in part – the extraordinary inter- and intra-familiar clinical and biochemical heterogeneity of mtDNA-related disorders.

As the title of this article implies, we will confine our discussion to the mitochondrial myopathies, namely, disorders of the respiratory chain affecting exclusively of predominantly skeletal muscle. Like all mitochondrial diseases, mitochondrial myopathies can also be classified genetically into two major groups, those due to mutations in mtDNA and those due to mutations in nDNA.

Before considering individual clinical entities in these two groups, we can ask ourselves a more general question: How can mitochondrial diseases be confined to skeletal muscle? At first sight, tissue specificity appears incompatible with the fact that mitochondria are ubiquitous organelles. However, selective muscle involvement can be explained by at least three different mechanisms:
Mutations in tissue-specific nuclear genes. This is a logical concept, which, however, still lacks clinical verification. Two infantile myopathies associated with severe cytochrome *c* oxidase (COX) deficiency, one invariably fatal, the other spontaneously reversible (discussed in more detail below) are transmitted as autosomal recessive traits, but the affected genes remain unknown.
Somatic mtDNA mutations. These are spontaneous, *de novo* mutations in the mitochondrial genome occurring in the oocyte or in the embryo but affecting myoblasts after germ-layer differentiation, thus sparing other tissues.
Skewed heteroplasmy. In this situation, garden variety pathogenic point mutations in mtDNA affecting some but not all mitochondrial genomes (that is, heteroplasmic) are ubiquitous, but have an unusually skewed predominance in skeletal muscle, such that they surpass the pathogenic threshold only in this tissue, resulting in myopathic phenotypes.

Myopathies Due to Defects in mtDNA

Mutations in mtDNA fall into two categories, those affecting mitochondrial protein synthesis *in toto* and those affecting specific protein-coding genes.

Defects of mitochondrial protein synthesis

Mutations in tRNA or rRNA genes, large-scale deletions (which always encompass one or more tRNA genes), and mtDNA depletion impair protein synthesis and result, biochemically, in defects of all respiratory chain complexes containing mtDNA-encoded subunits (I, III, IV, and V). Muscle histochemistry is characterized by scattered fibers with massive mitochondrial proliferation (ragged-red fibers [RRF] in the Gomori trichrome stain), which are hyper-reactive with the succinate dehydrogenase (SDH) stain ("ragged-blue fibers") but hypo- or un-reactive with the cytochrome *c* oxidase (COX) stain (Figure 2A, B). The only exception

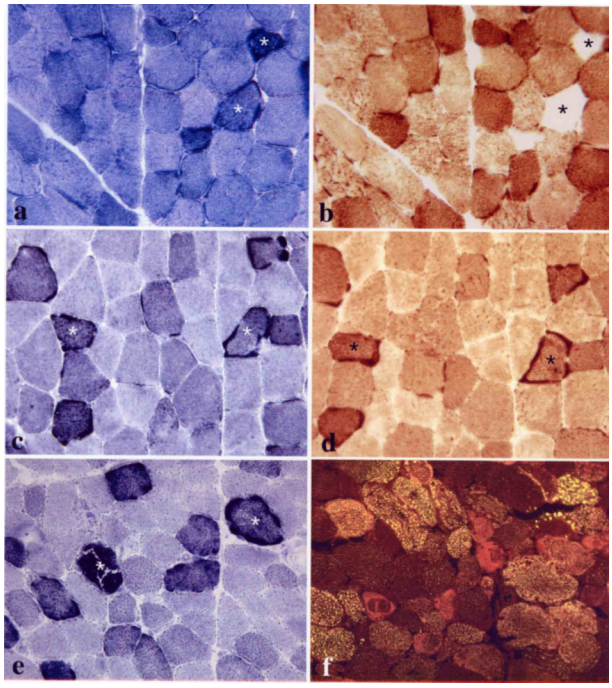

Figure 2. Serial frozen cross sections of muscle biopsies from a patient with PEO and single mtDNA deletion (a, b); a patient with a cytochrome b mutation and complex III deficiency (c, d), and a patient with the myopathic presentation of CoQ10 deficiency (e, f). Sections were stained for the following reactions: SDH (a, c, and e), COX (b and d), and with Nile blue, a stain for neutral lipids (f). Asterisks indicate SDH-hyperintense ("ragged blue") fibers in a,c, and e, COX-negative fibers in b, and intensely COX-positive fibers in d.

to this "rule" is that RRF in patients with typical MELAS syndrome are often COX-positive.

Disorders due to mitochondrial protein synthesis are usually multisystemic, although brain and skeletal muscle are almost invariably affected (hence the term "mitochondrial encephalomyopathies"). However, myopathy dominates the clinical picture in a few situations, either due to somatic mutations or to skewed heteroplasmy (see above).

Single, large-scale deletions of mtDNA are associated with three clinical phenotypes: (i) Kearns-Sayre syndrome, a devastating multisystemic disease of early onset (before age 20), characterized by progressive external ophthalmoplegia (PEO), pigmentary retinopathy, and heart block, plus a variety of less consistent additional symptoms, such as ataxia, dementia, nephropathy, or endocrinopathy; (ii) Pearson syndrome (PS), a predominantly hematological and almost invariably fatal disease of infancy, with sideroblastic anemia and exocrine pancreas dysfunction; (iii) a pure myopathy with PEO, ptosis, and proximal limb weakness, usually slowly progressive and compatible with a normal lifespan. Single deletions are spontaneous event occurring in oogenesis and are very rarely transmitted because only a small minority of maternal mtDNA repopulates the fetus. When the few deleted mtDNAs present in the blastocyst segregate to muscle, myopathy is the clinical outcome. Thus, in sporadic patients with PEO and weakness, Southern blot of muscle mtDNA is the prime diagnostic test, showing two bands, one corresponding to normal mtDNA and the other to partially deleted mtDNA. It is important to keep in mind that muscle is needed for the diagnosis because the mutation is not detectable in other tissues.

Skewed heteroplasmy explains eight cases of isolated myopathy due to mutations in tRNA genes [11, 22, 23, 34, 43, 68, 77, 81]. None of these patients had PEO, but the three with mutations in the $tRNA^{Leu(UUR)}$ gene had predominant involvement of respiratory muscle, which may be a useful diagnostic clue. Family history was negative in three of these patients, suggesting that the mutations occurred *ex novo*. Skewed heteroplasmy of mutations in different tRNA genes caused maternally inherited PEO with or without limb weakness in numerous patients [26]. In a few patients with PEO, mutations in tRNA genes were probably both skewed and *de novo* because there was no evidence of maternal inheritance [26].

Defects in protein-coding genes

During the past 15 years, experience with a rapidly increasing population of patients harboring mtDNA mutations has led clinical investigators to formulate a number of generalizations that are too often interpreted as "dogmas". Following is a short list of such dogmas.

a. Point mutations in mtDNA are transmitted by maternal inheritance and negative family history is evidence against such etiology.
b. Point mutations in mtDNA are associated with multisystem disorders and are rare causes of pure myopathies.
c. RRF are typically seen in patients with mtDNA mutations that impair overall mitochondrial protein synthesis while they are absent in muscle biopsies from patients with mutations in mtDNA protein-coding genes, such as the NARP/MILS mutations in the ATPase 6 gene, or the various mutations in genes encoding complex I subunits (ND genes) associated with Leber's hereditary optic neuropathy (LHON).
d. In patients with mtDNA point mutations, RRF do not show cytochrome *c* oxidase (COX) activity (i.e. are histochemically COX-negative), except in typical MELAS syndrome.

All of these dogmas have been shattered, or at least cracked, by recent experience with myopathic patients harboring mutations in protein-coding genes. Specifically, all of these patients have been sporadic, negating the first dogma; no patient has had clinical involvement of tissues other than muscle, negating the second dogma; all patients have had RRF and "ragged blue" fibers (that is, fibers hyperreactive with the SDH stain – Figure 2,C,), negating the third dogma; patients with point mutations in genes encoding subunits of complex I or complex III showed COX-positive RRF (Figure 2D,

and only patients with mutations in COX genes showed – predictably – COX-negative RRF as well as non-RRF, thus contradicting the fourth dogma.

Among *complex I* gene defects, three have been found thus far in sporadic patients with myopathy: a nonsense mutation in the ND4 gene [7], an intragenic inversion in the ND1 gene altering three highly conserved amino acids [45], and a 2-bp deletion in the ND2 gene [64]. All three patients suffered from exercise intolerance. The third patient became a "cause celébre" when it was found that this man's muscle mtDNA was mostly of *paternal* origin. Although this situation, which broke yet another central dogma of mitochondrial genetics, does not negate the general rule that mtDNA is transmitted maternally, it raises the intriguing possibility that tissue-specific paternal mtDNA inheritance might somehow favor mtDNA mutagenesis [64].

Mutations in cytochrome *b*, the only mtDNA-encoded subunit of *complex III*, offer the best example of this group of myopathies. Eleven sporadic patients, all with exercise intolerance and two with recurrent myoglobinuria, had pathogenic mutations in the cytochrome *b* gene [5-6, 20, 33, 35, 40]. However, not all pathogenic mutations in the cytochrome *b* gene associated with myopathy result from *de novo* mutations: small amounts of mutant mtDNA were detected in blood from one asymptomatic mother [31] and in urinary sediment cells from another [40].

Three patients have been described with isolated myopathy, *complex IV* deficiency, and mutations in mtDNA COX genes. A 16-year-old girl with recurrent myoglobinuria triggered by prolonged exercise or viral illness had a 15-bp microdeletion in the COX III gene [32]; a 34-year-old man with life-long exercise intolerance and recurrent myoglobinuria induced by intense or repetitive exercise had a nonsense mutation (G5920A) in the COX I gene[29]; and a 14-year-old boy with proximal limb weakness and premature fatigue of five-year duration had a missense mutation (T7671A) in the COX II gene[54]. All three patients were sporadic.

Myoglobinuria, and especially recurrent myoglobinuria, is commonly associated with blocks in the utilization of the two major sources of energy for muscle contraction, glycogen or fatty acids [17]. Strangely, blocks in oxidative phosphorylation, the final common pathway for energy production, were not considered until recently in the differential diagnosis of recurrent myoglobinuria. However, two patients with *complex III deficiency* did have each one episode of myoglobinuria [4, 6] whereas two of the three patients with myopathy and *COX deficiency* had multiple episodes of myoglobinuria related to unusually intense or repeated exercise [29, 32]. The difference in the frequency and intensity of myoglobinuria attacks between patients with defects in complex III and IV suggests that COX deficiency causes a more severe "energy crisis".

Myopathies Due to Defects in nDNA

Not surprisingly, given the "supremacy" of nDNA in the control of mitochondrial structure and function, respiratory chain disorders due to nDNA mutations fall into several categories, including (i) mutations in genes encoding components of the respiratory chain; (ii) mutations in genes encoding ancillary proteins needed for the correct assembly of respiratory chain complexes; (iii) mutations in genes encoding components of the protein importation machinery; (iv) mutations in genes encoding enzymes responsible for the lipid milieu of the inner mitochondrial membrane; (v) mutations in genes encoding factors needed for mitochondrial motility or mitochondrial fission; (vi) mutations in genes encoding factors needed for mtDNA integrity and replication (intergenomic communication). There are examples of myopathies in categories (i), (iii), (iv), (v), and (vi), and these we will consider in more detail.

Mutations in genes encoding components of the respiratory chain

These include coenzyme Q10 deficiency and the fatal and the benign variants of COX deficiency myopathy.

The myopathic form of *Coenzyme Q10 (CoQ10) deficiency* was first described in 1989 in two sisters, who, after normal early development, manifested exercise intolerance, slowly progressive axial and limb muscle weakness, and recurrent myoglobinuria [52]. However, in addition to myopathic symptoms, both patients also had evidence of central nervous system involvement, with seizures, ataxia, and mental retardation. Muscle biopsies showed RRF and excessive accumulation of lipid droplets (Figure 2E, F). The concentration of CoQ10 was markedly decreased (about 5% of normal) in muscle, but was normal in serum and cultured skin fibroblasts. This characteristic triad, myopathy with recurrent myoglobinuria, RRF with lipid storage in muscle, and brain dysfunction, was seen in three other patients with CoQ10 deficiency in muscle [16, 69], and all five patients responded rather dramatically to oral CoQ10 supplementation. Not only did strength and endurance return to normal in the two brothers described by DiGiovanni et al, but a repeat muscle biopsy after 8 months of therapy showed that CoQ10 levels had normalized, lipid storage had disappeared, respiratory chain enzyme activities had increased, and, interestingly, the proportion of fibers undergoing apoptosis had markedly decreased [16]. Although rare, this condition has to be considered in patients with recurrent myoglobinuria because it is amenable to replacement therapy.

There are at least three other clinical presentations of primary CoQ10 deficiency, in which myopathy is overshadowed by other symptoms. An *ataxic* form, dominated by cerebellar ataxia and cerebellar atrophy, appears to be rather frequent, as 19 patients have already been described [37, 46]. Probably because residual levels of CoQ10 are higher than in the myopathic form, muscle

biopsy from patients with ataxia usually does not show RRF or biochemical abnormalities. Two rare presentations include infantile-onset encephalomyopathy (with ataxia) and renal disease in three siblings [55], and adult Leigh syndrome in two sisters with encephalopathy, growth retardation, ataxia, deafness, and lactic acidosis [76]. Again, an important common characteristics of all patients with CoQ10 deficiency is that they respond to CoQ10 supplementation, at least to a certain extent.

Two myopathic variants of COX-deficient myopathy, apparently transmitted as autosomal recessive traits, are presumably due to mutations in muscle-specific and developmentally regulated COX subunits or COX-assembly proteins, although the molecular defects remain elusive in both conditions. The *fatal infantile myopathy* causes respiratory insufficiency and death before 1 year of age. Heart, liver, and brain are spared, but kidney is often affected, and patients suffer from DeToni-Fanconi syndrome [18]. Muscle biopsy shows RRF and diffuse lack of COX stain in muscle fibers, but normal reaction in intramuscular blood vessels and in muscle spindles. Electrophoresis of the mutant enzyme after immunoprecipitation failed to show any alteration in subunit pattern [13], but immunocytochemistry with antibodies against individual subunits showed a selective defect of COX VIIa in four patients [74]. The *benign infantile myopathy* also causes severe weakness early in life, often requiring assisted ventilation, but symptoms improve spontaneously and these children are usually normal by 2 or 3 years of age [19, 51, 57, 65, 82]. Muscle biopsies taken in the neonatal period show no RRF but diffuse absence of COX stain, except for blood vessels and spindles. However, biopsies taken at later times show increasing numbers of COX-positive fibers until the histochemistry returns to normal. Somewhat paradoxically, immunohistochemistry shows lack of both COX II and COX VIIa [74], although the genes of both subunits harbored no mutations (unpublished observations).

Mutations in genes encoding component of the protein importation machinery

A defect in protein importation was suggested, but not unequivocally documented, in two patients with myopathy. A 14-year-old girl with congenital myopathy had no RRF in the muscle biopsy, but excessive lipid droplets, scattered COX-negative fibers, and complete lack of the histochemical SDH reaction [61]. Biochemistry showed a specific defect of both the 27.7 kD iron-sulfur (FeS) protein of SDH and the Rieske protein of complex III. A defect of mitochondrial protein transport was suggested by the observation that the Rieske protein was present in the cytosol but not in isolated mitochondria. The second patient was a 22-year-old man with life-long exercise intolerance and recurrent myoglobinuria, whose muscle biopsy also showed complete lack of the histochemical stain for SDH [25]. Biochemically, there was a combined defect of SDH and aconitase (another enzyme containing FeS protein), and immunoblot showed that the 30-kD FeS protein of complex II was decreased. A second study of this same patient showed combined defects of complex I, complex III, and rhodanase, all of which contain FeS proteins [24]. Again, a defect of mitochondrial protein importation was suggested by immunoblot evidence that the Rieske protein was markedly decreased in mitochondria whereas its precursor was present in the cytosol.

Mutations in genes encoding enzymes responsible for t he lipid milieu of the inner mitochondrial membrane

Barth syndrome (BTHS) is an X-linked proximal myopathy and dilated cardiopathy associated with growth retardation, cyclic neutropenia, and 3-methylglutaconic aciduria [9, 10]. Muscle biopsy typically shows RRF and biochemical studies show variable combined defects of respiratory chain complexes. The gene responsible for BTHS, *G4.5*, encodes a family of proteins called tafazzins, which share conserved regions with phospholipid acyltransferases. This observation suggested that BTHS may be caused by defect (or defects) in lipid acid transfer, a concept supported by findings of decreased levels of cardiolipin and decreased incorporation of linoleic acid into polyglycerophospholipids in cultured skin fibroblasts from BTHS patients [78]. Cardiolipin is the only phospholipid exclusively localized to the inner mitochondrial membrane, and a marked deficiency of one cardiolipin species, tetralinoyl-cardiolipin, was documented in skeletal muscle, heart, and platelets from children with BTHS [63]. As cardiolipin is specifically enriched in muscle and heart and is essential for the correct assembly and functioning of the respiratory chain [62], these findings correlate nicely with the mitochondrial cardiopathy/myopathy that characterizes BTHS.

Mutations in genes encoding factors needed for mitochondrial motility or mitochondrial fission

Remembering their bacterial past, mitochondria replicate through fission and are not immobile within cells, but move even long distances (think, for example, about mitochondrial migration along the distance of an axon). Proof of principle of this pathogenetic concept was provided by the discovery that mutations in *OPA1*, a gene encoding a mitochondrial dynamin-related ATPase, were associated with an autosomal dominant form of optic atrophy and evidence of impaired mitochondrial motility in monocytes [1, 15].

Although mitochondrial motility in skeletal muscle may be somewhat constrained by the contractile apparatus, defective motility might cause myopathy. A predominantly myopathic syndrome that may be related to defective mitochondrial motility was described in 1998 by Nishino et al in four children from three unrelated families [48]. The patients had congenital myopathy, delayed psychomotor development, and, by late childhood, they had proximal limb weakness and severe mental retardation. Two of them also had dilated cardiomyopathy. Serum CK was moderately elevated and

Table 1. Mitochondrial disorders with PEO.

Disorder	Gene Product	Chromosome	**Mutation**
Autosomal Dominant PEO with multiple mtDNA deletions			
adPEO	Adenine nucleotide translocator 1	4q34-q35	Point mutations
adPEO	Twinkle	10q23.3-24.3	Various
adPEO	Polymerase gamma	15q22-q26	Point mutations
Autosomal Recessive PEO with multiple mtDNA deletions			
MNGIE	Thymidine phosphorylase	22q13.32-qter	Various mutations
PEO	Polymerase gamma	15q22-q26	Point mutations
ARCO	Unknown	Unknown	Unknown
Autosomal Recessive PEO with mtDNA depletion			
Severe myopathy	Thymidine kinase 2	16q22-q23.1	Point mutations
Sporadic PEO			
KSS, PEO, PEO-plus	tRNAs and mitochondrial proteins	mtDNA	Single large-scale deletion of mtDNA

there was no lactic acidosis. A defect of mitochondrial motility was suggested by the muscle biopsy, which showed, in addition to dystrophic features, abnormal distribution of mitochondria. These were absent in the center but abundant at the periphery of muscle fibers, and peripheral organelles were markedly enlarged. The activities of respiratory chain enzymes were normal and there was no evidence of mtDNA depletion.

Mutations in genes encoding factors needed for mtDNA integrity and replication (defects of intergenomic communication)

Defects of intergenomic signaling can be qualitative (multiple mtDNA deletions) or quantitative (mtDNA depletion) and the two may coexist in some conditions, such as the mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) syndrome [50].

Syndromes associated with multiple mtDNA deletions affect predominantly muscle and usually present as PEO (Table 1). Autosomal dominant PEO (adPEO), not unlike the sporadic form of PEO due to single mtDNA deletions described above, is a relatively benign condition with onset in adolescence or young adult life and slow progression. Bilateral ptosis, PEO, and proximal limb weakness dominate the clinical picture, but symptoms of multisystemic involvement can also occur, including dysphagia, dysphonia, neuropathy, hearing loss, cataracts and, in some families, severe depression [66, 71, 83]. Muscle biopsy usually shows RRF and COX-negative fibers, and biochemical analysis shows combined respiratory chain defects. Southern blot of total DNA isolated from muscle (blood DNA is not useful for diagnosis) reveals multiple mtDNA bands. Within the past thee years, mutations in three genes have been associated with adPEO: adenine nucleotide translocator 1 (*ANT1*) [30], *Twinkle* [70], and polymerase gamma (*POLG*) [75]. However, it is already clear that mutations in these three genes do not explain all cases of adPEO and other genes remain to be identified [36].

Autosomal recessive forms of PEO (arPEO) are more often multisystemic than adPEO, as illustrated by two syndromes: autosomal recessive cardiomyopathy and ophthalmoplegia (ARCO), and MNGIE. ARCO was reported in six patients from two unrelated Arab families, who presented with childhood-onset PEO, facial and proximal limb weakness, and severe cardiomyopathy requiring cardiac transplantation [12]. MNGIE is dominated by gastrointestinal symptoms (intestinal pseudo-obstruction, chronic diarrhea) leading to cachexia and early death [50]. Additional signs include ptosis, PEO, peripheral neuropathy, and leukoencephalopathy. MNGIE is caused by loss of function mutations in the gene encoding thymidine phosphorylase (*TP*), which may cause mtDNA abnormalities (both multiple deletions and depletion) by disrupting the mitochondrial nucleotide pool [49]. Interestingly, some families with arPEO without the characteristics of ARCO or MNGIE had mutations in *POLG* [36, 75]. Thus *POLG* mutations have to be considered in both adPEO and arPEO, though adPEO seems to predominate [36].

MtDNA depletion syndromes (MDS) are inherited as autosomal recessive traits and fall into two major groups, one dominated by myopathy, the other by hepatopathy, although both presentations often involve other tissues, including brain, kidney, and heart [79]. The myopathic form can be apparent at birth (congenital myopathy) and cause fatal respiratory failure within months, or develop at about one year of age and progress more slowly, often simulating muscular dystrophy. The differential diagnosis from muscular dystrophy is made more difficult by the fact that mtDNA depletion syndromes are the only mitochondrial myopathies with markedly elevated serum CK. In the congenital form, muscle biopsy shows RRF and diffuse COX deficiency. In the later onset form, initial biopsies may show only non-specific changes (further complicating the diagnosis), while samples taken later show RRF and COX-negative fibers. Biochemical studies show combined

defects of respiratory chain complexes containing mtDNA-encoded subunits. The diagnosis is established by densitometry of Southern blots comparing mtDNA and nDNA (hybridized with a probe for nuclear 18S ribosomal DNA) [44], and is confirmed by immunocytochemistry with anti-DNA antibodies [3, 44].

In late 2001, mutations in two nuclear genes were identified in patients with MDS: changes in the deoxyguanosine kinase (*dGK*) gene were found in patients with the hepatic form [42], and changes in the thymidine kinase 2 (*TK2*) gene were associated with the myopathic form [56]. When we screened two large series of patients with predominantly hepatic or predominantly muscular symptoms, we found that 3 of 21 hepatic patients had *dGK* mutations [59] and 4 of 20 myopathic patients had *TK2* mutations [41]. These data confirmed the molecular etiology of the two clinical forms of MDS, but also showed that other genes must be involved and remain to be identified. It is noteworthy that one of two siblings with a homozygous I22M mutation in TK2 was a phenocopy of spinal muscular atrophy (SMA) type I (Werdnig-Hoffmann disease). We had described another child with the SMA phenotype and mtDNA depletion [53] and have recently seen an infant with Werdnig-Hoffmann, cardiomyopathy and mutations in the COX assembly gene *SCO2* [60]. It is, therefore, important to consider mitochondrial dysfunction in all children with clinical features of SMA but without mutations in the survival motor neuron gene (*SMN*).

Conclusions

Mitochondrial myopathies have come a long way from the seminal description of Luft and coworkers 41 years ago, but the field is still open and challenging. New pathogenetic concepts, such as defects in the mitochondrial membrane lipid milieu and defects of mitochondrial motility or fission remain to be explored in search of novel muscle disorders. “Old diseases”, such as the fatal and benign forms of COX-deficient myopathies, remain to be defined at the molecular level. In fact, the first mitochondrial myopathy of them all, Luft syndrome, is still a genetic puzzle.

More importantly, therapy is woefully inadequate. We give patients with mitochondrial myopathies various cocktails of vitamins, cofactors, and oxygen radical scavengers more out of frustration than in the real hope of helping. However, there are glimmers of hope. First, patients with primary CoQ10 deficiency, and especially the myopathic form, do benefit from high-dose CoQ10 supplementation. Second, somewhat counterintuitively, exercise itself may prove an effective therapy, in at least two different ways. Aerobic training not only protects patients against deconditioning but also improves their exercise capacity, though the molecular basis of this phenomenon remains to be clarified [73]. On the other hand, resistance training through isometric exercise might damage muscle fibers and promote regeneration. As satellite cells and myoblasts contain lesser amounts of pathogenic mutations than do mature muscle fibers, this “gene shifting” might lower the mutation load of regenerating fibers below the pathologic theshold [21, 67, 72]. Direct injection of myotoxic agents such as bupivacaine has the same effect [14], but more limited application in humans. Unfortunately, injection of bupivacaine in one levator palpebrae muscle in five patients with PEO or KSS did not cause any improvement [8].

The rapid progress in our understanding of the molecular bases of mitochondrial myopathies has important therapeutic implications. One immediate advantage is the availability of prenatal diagnosis for devastating disorders, such as the fatal infantile myopathic forms of mtDNA depletion. A more distant advantage is that this knowledge is the prerequisite for gene therapy. A third advantage is the development of pharmacological approaches directed at circumventing the biochemical defect. For example, addition of copper to the medium of myoblast cell cultures harboring mutations in SCO2, a copper transport protein needed for COX assembly, corrected COX deficiency *in vitro* [28, 58]. It is not farfetched to consider copper supplementation for infants with *SCO2* mutations, who would otherwise rapidly succumb to cardiopathy or SMA syndrome. Although a lot of work remains to be done, it seems appropriate to end this review on this hopeful note.

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