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Mini Review

MITOCHONDRIAL DISORDERS

Salvatore DiMAURO* and Kurenai TANJI

Columbia University College of Physicians & Surgeons,
630 West 168th Street, New York, NY 10032, USA

Summary In this minireview, we attempt to survey the three main group of mitochondrial disorders, defects of nuclear DNA, defects of mitochondrial DNA, and defects of intergenomic signaling, with emphasis on recent contributions and pathogenetic mechanisms. In so doing, we have tried to point out some of the numerous unsolved problems in genotype/phenotype correlation and to indicate future directions of research.

Key Words mitochondria, mitochondrial DNA, intergenomic signaling, respiratory chain, mitochondrial encephalomyopathies

The fascinating and still unfolding story of mitochondrial disorders is not only a "tale of two genomes," but also a tale of their sometimes less than harmonious interaction. Thus, we have to review succinctly three types of human diseases: 1) Defects of nuclear genes, inherited as mendelian traits; 2) Defects of mitochondrial genes, which can be sporadic conditions or maternally-inherited disorders; and 3) Defects of intergenomic communication, in which the still unknown culprits are nuclear genes and inheritance is mendelian. Though in our review we will consider these three groups in sequence, a unified biomolecular classification of the mitochondrial disorders, including acquired conditions, is offered in Table 1. Because of the concise nature of these minireviews, we will limit references to the most recent contributions. Other references are provided in recent comprehensive reviews, including three chapters in a row in the 2nd edition of *The Molecular and Genetic Basis of Neurological Disease* (Schon, 1997; DiMauro and Bonilla, 1997; Wallace, 1997).

1. Defects of Nuclear Genes

All mitochondrial proteins except 13 subunits of the respiratory chain are encoded in the nuclear genome. Most of these proteins are synthesized as larger

* To whom correspondence should be addressed.

Table 1. Biomolecular classification of mitochondrial diseases.

Inherited conditions
Nuclear DNA defects:
Defects of substrate transport
Defects of substrate utilization
Defects of the Krebs cycle
Defects of the electron transport chain
Defects of oxidation-phosphorylation coupling
Defects of mitochondrial protein importation
Defects of intergenomic signaling
Mitochondrial DNA defects:
Sporadic large-scale rearrangements
Inherited large-scale rearrangements
Point mutations affecting protein-coding genes
Point mutations affecting protein synthesis gene
Acquired conditions
Infectious (*e.g.* Reye syndrome)
Toxic (*e.g.* MPTP)
Iatrogenic (*e.g.* zidovudine)
Aging

precursors containing "leader peptides" that direct them to mitochondrial receptors: the actual transfer of proteins across the outer and inner mitochondrial membranes and their eventual localization in the appropriate mitochondrial compartment depends on the concerted action of multiple factors, some cytoplasmic, others membrane-bound, and still others located in the mitochondrial matrix. It stands to reason, then, that most mitochondrial diseases are due to defects of nuclear genes: these have been commonly classified according to the area of metabolism that is affected (Table 1).

Defects of substrate transport impair the mechanisms of active transport that are needed to import even simple metabolites from the cytoplasm across the finicky filter represented by the inner mitochondrial membrane. Examples include defects in any of the several components of the "carnitine cycle," including carnitine itself, CPT-I or CPT-II, and the carnitine-acylcarnitine translocate proper. All of these disorders are transmitted as autosomal recessive traits and there have been great strides in our understanding of their molecular bases (DeVivo *et al.*, 1996). The adenine nucleotide translocator (*Ant*) deserves special mention because recently a "knockout" mouse was generated with markedly deficient expression of the muscle/heart isoform of *Ant* (*Ant1*) (Graham *et al.*, 1997). This is the first animal model with the morphological and biochemical features of mitochondrial myopathy and cardiomyopathy.

Defects of substrate utilization include enzyme defects in pathways needed for the processing of substrates to acetyl-CoA, such as the pyruvate dehydrogenase complex (PDHC) or the β-oxidation pathway. Numerous molecular defects have been identified in both pathways (DeVivo *et al.*, 1996), and inheritance is autosomal recessive except for defects of the E1α subunit of PDHC, which is

encoded by a gene on the short arm of the X-chromosome. A multitude of different mutations have been described in the E1α gene, causing a wide spectrum of clinical presentations and affecting males and females in almost equal numbers, more like an X-linked dominant than a recessive trait (DeVivo *et al.,* 1996).

Defects of the Krebs cycle are rare and often partial, a reflection of the vital role of this pathway in intermediary metabolism. The best studied is probably fumarase deficiency, which causes a devastating encephalopathy of early infancy associated with a characteristic organic acid profile in the urine. There was remarkable genetic heterogeneity among the handful of patients studied at the molecular level (DeVivo *et al.,* 1996).

Defects of the electron transport chain can be due to mutations in either the nuclear or the mitochondrial genome because seven of the over 40 subunits of complex I, one of the 11 subunits of complex III, and three of the 13 subunits of complex IV are encoded by mtDNA. Complex II is entirely encoded by the nuclear genome. Several enzyme defects of individual complexes, both generalized and confined to muscle, have been attributed to nuclear gene mutations because family history suggested autosomal rather than maternal transmission. However, molecular evidence is thus far limited to two children of a consanguineous union, who had Leigh syndrome and a homozygous C toT transition at nucleotide 1684 of the flavoprotein subunit gene of SDH (Bourgeron *et al.*, 1995).

We would like to single out Coenzyme Q10 (CoQ10) deficiency as a probably overlooked disorder of the electron transport chain. Primary CoQ10 deficiency was first reported by the late Ogasahara from the Mayo Clinic in two sisters with exercise intolerance and slowly progressive weakness (Ogasahara *et al.*, 1989). Around age 5 years, brain involvement was manifested by learning disability in both sisters, seizures in one, and cerebellar syndrome in the other. In addition, both sisters had episodes of myoglobinuria and muscle biopsy showed excessive accumulation of mitochondria (ragged-red fibers [RRF]) and lipid storage. Laboratory abnormalities included lactic acidosis, increased serum CK levels, and myopathic EMG. Polarographic analyses showed decreased respiration of muscle mitochondria with both NADH-dependent substrates and succinate. CoQ10 concentration in muscle was about 5% of normal and both sisters responded to CoQ10 administration. We have recently seen a very similar case, a 35-year-old woman with delayed motor milestones, proximal weakness, exercise intolerance, episodes of exercise-induced myoglobinuria, and complex partial seizures (Sobreira *et al.*, 1997). Muscle biopsy showed RRF and excess lipid. Biochemical analyses showed defects of complexes I+II and I+III, and CoQ10 concentration was less than 25% of normal. She also improved markedly with CoQ10 replacement. In collaboration with Dr. Servidei (Catholic University, Rome, Italy) we have studied two more cases in young brothers with mitochondrial myopathy, recurrent myoglobinuria, and seizures, who responded dramatically to CoQ10 administration (Servidei *et al.*, 1996). Thus, after a lull of six years from Ogasahara's paper, several new cases

of CoQ10 deficiency are cropping up, which suggests that this diagnosis may have been neglected. In addition, considering the complexity of the CoQ10 synthetic pathway, it is likely that multiple distinct defects may occur, possibly with different clinical syndromes, which remain to be defined.

Defects of oxidative phosphorylation and oxidation/phosphorylation coupling. Defects of mitochondrial ATP synthesis (complex V of the respiratory chain) have been proven in patients with mutations in one of the two mtDNA-encoded subunits of ATP synthase (see below), but there is no disease convincingly linked to defects in any of the 12 nuclear genes.

The only convincing defect of oxidation/phosphorylation coupling remains "nonthyroidal hypermetabolism," or Luft disease, whose historical importance as the first disease documentedly due to mitochondrial dysfunction (Luft *et al.*, 1962) contrasts with its extreme rarity (two bona fide patients described). Both patients with Luft disease were sporadic cases and it is generally assumed that the defect involves a nuclear gene, although neither the gene nor the protein responsible for the "loose coupling" have been identified.

New mitochondrial disorders. Before leaving the topic of mitochondrial disorders due to defects in nuclear genes, we should mention that this area is getting increasingly exciting as the products of newly discovered disease-related genes turn out to be mitochondrial proteins. In the latest issue of *Nature Genetics* two studies document the mitochondrial nature of frataxin, the protein encoded by the gene that is mutated in Friedreich's ataxia (Koutnikova *et al.*, 1997; Wilson and Roof, 1997).

2. Defects of Mitochondrial Genes

Considering that the first mutations in mtDNA were described in 1988 (Wallace *et al.*, 1988; Holt *et al.*, 1988), progress in "mitochondrial genetics" has been astoundingly rapid. In less than a decade, more than 50 point mutations and a multitude of large-scale rearrangements have been associated with a bewildering array of clinical manifestations. Figure 1 is an updated version of the mtDNA "morbidity map," but new pathogenic mutations are still been described at a brisk pace rendering this map quickly obsolete. In addition, while mtDNA-related disorders were initially considered almost exclusively of neurological interest (hence the popular label "mitochondrial encephalomyopathies"), it is now clear that every tissue of the body (and every subspecialty of medicine) can be involved. To illustrate this point, Table 2 lists symptoms and signs reported in patients with four common mtDNA mutations, including deletions, two representative tRNA mutations (A3243G, typically associated with MELAS, and A8344G, typically associated with MERRF), and one representative mutation in a protein-coding gene (T8993G, typically associated with NARP or MILS). The data in this table amply vindicate Prof. Luft's contention that we are dealing with "mitochondrial medicine" (Luft, 1994).

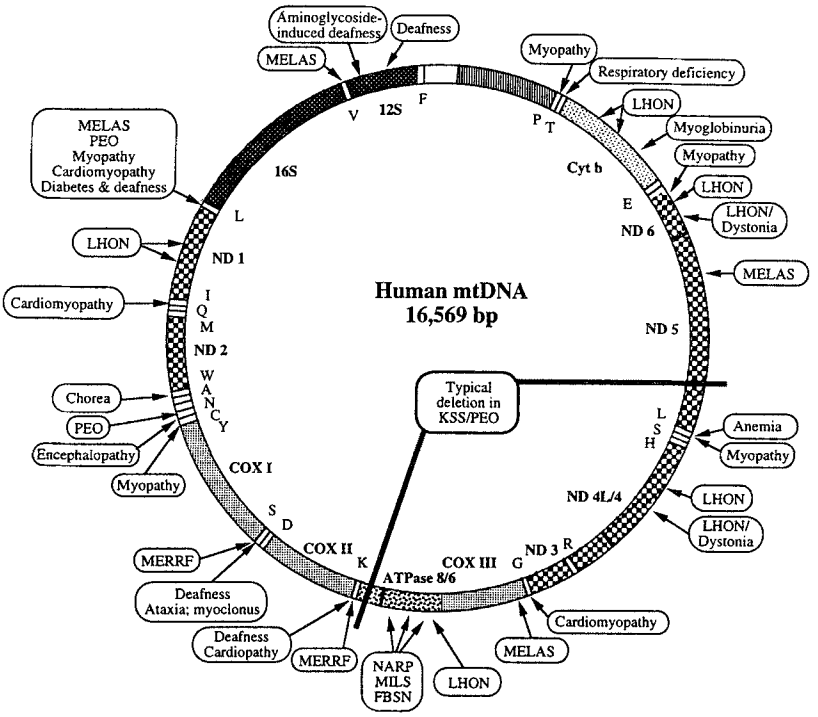

Fig. 1. "Morbidity map" of human mitochondrial DNA. The map of the 16.5 kb mtDNA shows differently shaded areas representing the structural genes for the seven subunits of complex I (ND), the three subunits of complex IV (COX), cytochrome *b* (cyt b), and the two subunits of ATP synthetase (ATPase 6 and 8), the 12S and 16S ribosomal RNAs (rRNA), and the 22 transfer RNAs (tRNA) identified by one-letter codes for the corresponding amino acids. For acronyms, see List of abbreviations. Modified from DiMauro and Bonilla (1997) with permission.

The extreme clinical heterogeneity of mtDNA-related diseases, even among family members sharing the same mutation, can be largely explained by the peculiar features of mitochondrial genetics, especially heteroplasmy/threshold effect and mitotic segregation.

Heteroplasmy/threshold effect relate to the notion that mitochondrial genetics is population genetics. A pathogenic mutation of mtDNA usually affects only a certain proportion of the thousands of mitochondrial genomes that are present in each cell, resulting in the coexistance of mutant and wild-type mtDNAs (heteroplasmy). In each tissue, a minimum number of mutant mtDNAs will have to be present to impair oxidative phosphorylation and to cause clinical symptoms (threshold effect), and the threshold will be lower in tissues with higher oxidative demands. As the numbers of mutations can vary widely in different members of the same family and in different tissues of the same individual, this readily explains the

Table 2. Clinical features in mitochondrial diseases associated with mtDNA mutations.

Tissue	Symptom/sign	Δ-mtDNA		tRNA		ATPase	
		KSS	Pearson	MERRF	MELAS	NARP	MILS
CNS	Seizures	−	−	[+]	+	−	+
	Ataxia	+	−	[+]	+	[+]	±
	Myoclonus	−	−	[+]	±	−	−
	Psychomotor retardation	−	−	−	−	−	+
	Psychomotor regression	+	−	±	+	−	−
	Hemiparesis/hemianopia	−	−	−	[+]	−	−
	Cortical blindness	−	−	−	[+]	−	−
	Migraine-like headaches	−	−	−	[+]	−	−
	Dystonia	−	−	−	+	−	+
PNS	Peripheral neuropathy	±	−	±	±	[+]	−
Muscle	Weakness	+	−	+	+	+	+
	Ophthalmoplegia	[+]	±	−	−	−	−
	Ptosis	+	−	−	−	−	−
Eye	Pigmentary retinopathy	[+]	−	−	−	[+]	±
	Optic atrophy	−	−	−	−	±	±
	Cataracts	−	−	−	−	−	−
Blood	Sideroblastic anemia	±	[+]	−	−	−	−
Endocrine	Diabetes mellitus	±	−	−	±	−	−
	Short stature	+	−	+	+	−	−
	Hypoparathyroidism	±	−	−	−	−	−
Heart	Conduction block	[+]	−	−	±	−	−
	Cardiomyopathy	±	−	−	±	−	±
GI	Exocrine pancreatic dysfunction	±	[+]	−	−	−	−
	Intestinal pseudo-obstruction	−	−	−	−	−	−
ENT	Sensorineural hearing loss	−	−	+	+	±	−
Kidney	Fanconi syndrome	±	±	−	±	−	−
Laboratory	Lactic acidosis	+	+	+	+	−	±
	Muscle biopsy: RRF	+	±	+	+	−	−
Inheritance	Maternal	−	−	+	+	+	+
	Sporadic	+	+	−	−	−	−

Boxes highlight the typical clinical features of different syndromes, except for maternally-inherited Leigh syndrome, which is defined on the basis of neuroradiological or neuropathological criteria. Δ-mtDNA, deleted mtDNA. For other acronyms, see List of abbreviations. Reproduced from DiMauro and Bonilla (1997) with permission.

clinical heterogeneity observed in families with mtDNA mutations.

Mitotic segregation refers to the fact that the proportion of mutant mitochondrial genomes can vary in successive cell generations and provides an explanation for another peculiarity of mtDNA-related diseases, namely that symptoms and signs can change with time. The best example of this phenomenon is provided by the transition observed in some patients from Pearson marrow/pancreas syndrome (PS) to KSS: a few infants with PS survive their severe hematological disorder and may enjoy a brief period of well-being before developing symptoms and signs of KSS. This phenomenon is probably due to the simultaneous occurrence of two contrasting mitotic segregation processes in bone marrow and in postmitotic tissues such as muscle, heart, and brain: in rapidly-dividing hematopoietic cells, there is a selection favoring wild-type genomes, whereas in postmitotic tissues partially deleted mtDNAs seem to have a selective advantage over wild-type genomes and soon end up reaching pathological thresholds.

The clinical and biochemical heterogeneity of mtDNA-related disorders is such that straight pathogenetic relationships between a given mtDNA mutation and its often diverse phenotypes are difficult to establish. So difficult, in fact, that most papers discussing genotype/phenotype correlation conclude with a cautionary warning about possible synergistic influences of the nuclear genome (or of mtDNA polymorphisms) on the expression of the mtDNA mutation under study. However reasonable this may be, there is as yet precious little evidence of such factors. An X-linked factor that would explain the marked prevalence of affected males in Leber's hereditary optic neuropathy (LHON) remains elusive. Equally elusive is the nuclear factor that has been postulated to explain the more marked biochemical changes observed in lymphoblastoid cell lines containing homoplasmic levels of the A1555G mutation when the cells were obtained from symptomatic as compared to asymptomatic individuals (Guan *et al.*, 1996).

We believe that much of the clinical and biochemical heterogeneity can be explained by the rules of mitochondrial genetics and attributed to various factors (DiMauro, 1996).

(i) Type of gene affected. The once perplexing finding of multiple but partial respiratory chain enzyme defects in muscle from patients with mitochondrial encephalomyopathies is now explained by the overall impairment of mitochondrial protein synthesis that accompanies mutations in tRNA or rRNA genes, or large-scale deletions of mtDNA. In contrast, and following expectations, point mutations or microdeletions in protein-encoding genes usually cause specific enzyme defects, such as complex I deficiency in most cases of LHON, more or less severe defects in ATP synthesis in tissues from patients with NARP/T8993G or T8993C (Santorelli *et al.*, 1996a), and isolated COX deficiency in muscle of a patient with a microdeletion in the COX-III gene (Keightley *et al.*, 1996).

(ii) Abundance of the mutation. The degree of heteroplasmy clearly affects the severity of the phenotype. This is best illustrated by the T8993G mutation,

which causes NARP when it is present in moderate amount, but MILS when it is very abundant. Similarly, the abundance of the mutation in accessible tissues (blood, skin fibroblasts, hair roots, urinary tract cells) generally correlates reasonably well with clinical severity in oligosymptomatic maternal relatives of patients with MELAS or MERRF. An apparent exception to this rule is KSS, in that "partial cases" with some but not all the canonical diagnostic features seem to be very rare: we had found only 4 such "probable" cases in our series of 54 patients with PEO and single mtDNA deletions (Moraes *et al.*, 1989) and we have seen very few since then.

(iii) Distribution of the mutation in different tissues. Heteroplasmy/threshold effect can also explain how the same mutation can result in two different clinical syndromes, such as the devastating multisystemic KSS and the relatively benign sporadic progressive external ophthalmoplegia (PEO), both associated with single large-scale deletions of mtDNA. In KSS, the deletions are widespread whereas in PEO they appear to be confined to the musculature.

However, there may be more subtle differences in the distribution of mtDNA mutations within an individual tissue, especially the brain. Classical neuropathology has already shown different types and sites of lesions in KSS, MELAS, and MERRF (Sparaco *et al.*, 1993), and the predominant involvement of the white matter in KSS may explain why seizures are so rarely seen in KSS patients while they are an obligatory feature in patients with MELAS and MERRF. Immunohistochemistry using antibodies against mtDNA-encoded and nuclear DNA-encoded subunits of the respiratory chain has documented selective involvement of the dentate nucleus in MERRF (Sparaco *et al.*, 1995). A combination of immunohistochemistry and *in situ* hybridization holds the promise of allowing us to draw "mutational maps" of the brain, possibly showing selective spatial enrichment of individual mutations in certain areas. However, even if this ideal scenario will be realized, this will still beg the question: why do specific mtDNA mutations tend to "prefer" some brain areas over others?

(iv) Pathogenesis of stroke in MELAS. Another conundrum regards the pathogenesis of strokes in MELAS: in fact there are at least two interrelated conundrums: 1) Why should the particular A to G base change at nt 3243 of the $tRNA^{Leu(UUR)}$ gene be so frequently associated with strokelike episodes? A certain "specificity" of the $tRNA^{Leu(UUR)}$ in causing strokes is suggested by the fact that at least three more mutations in the same gene (A3252G; T3271C; T3291C) have been associated with MELAS. On the other hand, MELAS mutations do not invariably cause stroke nor do several other mutations in the same gene. Conversely, typical MELAS has been associated to genes other than the $tRNA^{Leu(UUR)}$, some of them protein-coding genes (see below). 2) Are the strokes in MELAS vascular or metabolic in nature? Excessive accumulations of mitochondria have been described in the walls of arterioles and capillaries both in muscle and in brain in MELAS patients (Hasegawa *et al.*, 1991; Ohama *et al.*, 1987), but it is still unclear

whether the strokes are the consequence of "small vessel angiopathy" or are, in fact, independent of any vascular pathology.

As if the pathogenesis of MELAS were not complex enough, this disorder has been recently associated with three mutations in protein-coding genes, COX-III (T9957C) (Manfredi *et al.*, 1995), ND1 (T3308C) (Campos *et al.*, 1997), and ND5 (G13513A) (Santorelli *et al.*, 1997). Surprisingly, all three patients had not only typical clinical features but also ragged-red fibers (RRF) in their muscle biopsies, thus breaking a presumed "dogma" of mitochondrial pathology, namely, that RRF are characteristically associated with genetic errors affecting mitochondrial protein synthesis rather than individual subunits of the respiratory chain.

Although the rules of mitochondrial genetics provide satisfactory answers to many questions regarding clinical and biochemical heterogeneity, we are still far from a clear understanding of pathogenesis. The central question remains that of specificity: why are specific mutations associated (by and large) with specific syndromes? If all mutations in mtDNA affect respiratory chain/oxidative phosphorylation, why doesn't every mutation cause more or less the same syndrome? In a recent review (Schon *et al.*, 1997b), we suggest additional ways of looking at the problem: (i) the position of a point mutation in the tRNA "cloverleaf" may have pathophysiological implications; (ii) the pathological effects of some mtDNA mutations (the LHON mutations, perhaps?) may be "indirect," possibly mediated through a dysfunction of the immune system; (iii) there may be a selective vulnerability of "transporter tissues" (*i.e.* tissues involved in extracellular transport of small molecules and ions) to chronic energy defects, especially in the vicinity of the plasma membrane; (iv) related to this, there may be a crucial "compartmentalization" of mitochondrial ATP, especially in neurons, which might explain why mtDNA mutations cause specific cell dysfunction rather than cell death. Although these are largely hypothetical explanations, they offer ample food for thought and experimentation.

3. Defects of Intergenomic Signaling

According to the endosymbiotic hypothesis, mitochondria in animal cells are the descendants of protobacteria and retain only remnants of the original bacterial genome as mtDNA. The mitochondrial genome, therefore, depends heavily on the nuclear genome for the production of factors needed for mtDNA replication, transcription, and translation (Schon, 1997). Defects of the intergenomic "dialogue" represent a fascinating group of disorders in which the primary genetic errors are in nuclear DNA (and transmission is through mendelian inheritance), but the direct consequences of the nuclear mutations are qualitative or quantitative alterations of mtDNA.

Qualitative defects of mtDNA (multiple mtDNA deletions syndromes). The molecular hallmark of these disorders is the presence, in Southern blots of muscle mtDNA, of multiple bands representing species of mtDNA molecules harboring

Table 3. Disorders associated with mtDNA multiple deletions.

Disorder	Inheritance	Chrom. localiz.
Primary:		
PEO, myopathy	AD	?
PEO, myopathy	AD	3p
PEO, myopathy, depression	AD	10q23.3-24.3
PEO, myopathy, hypogonadism	AD	?
PEO, cardiomyopathy	AR	?
MNGIE	AR	?
Secondary:		
Late-onset myopathy	S	
IBM	S	

PEO, progressive external ophthalmoplegia; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; IBM, inclusion body myositis; AD, autosomal dominant; AR, autosomal recessive; S, sporadic.

deletions of different sizes (in contrast to the single abnormal band seen in Southern blots of mtDNA from patients with KSS, sporadic PEO, or PS). The clinical presentations vary considerably, but what we consider primary disorders are characterized by PEO, which is often part of multisystem syndromes inherited as autosomal dominant (AS-PEO) or autosomal recessive (AR-PEO) traits (Table 3).

AD-PEO syndromes tend to be dominated by myopathy, but hearing loss, tremor, cataracts, have been described in Italian families (Servidei *et al.*, 1991). Depression was an important feature in a large Finnish family (Suomalainen, 1997) and in an American family (Iannaccone *et al.*, 1974), in which we later documented multiple mtDNA deletions in muscle. Neuropathy and hypogonadism were the dominant additional features in a Swedish family with AD-PEO (Melberg *et al.*, 1996). Linkage analysis in the Finnish family localized the affected gene to chromosome 10 (Suomalainen *et al.*, 1995), but genetic heterogeneity is to be expected because the disorder is linked to a gene on chromosome 3 in some, but not all Italian families with AD-PEO (Kaukonen *et al.*, 1996).

Multisystemic involvement is more evident in the AR-PEO syndromes. Proximal weakness and severe cardiomyopathy (requiring cardiac transplantation) were associated with PEO in two families from the eastern seabord of the Arabian peninsula (Bohlega *et al.*, 1996). As the cacophonic acronym suggests, MNGIE involves multiple systems and includes myopathy, neuropathy, leukodystrophy, and severe gastrointestinal dysmotility with chronic diarrhea and intestinal pseudo-obstruction (Hirano *et al.*, 1994). Comparison of morphologic and molecular features in muscle biopsies from patients with AD-PEO and AR-PEO has shown that RRF and COX-negative fibers are more abundant in AD-PEO muscle specimens, which also contain greater proportions of deleted genomes (Carrozzo *et al.*, in press).

Neither the mutant genes nor the factors involved in the pathogenesis of

Table 4. Tentative classification of the clinical presentations of mtDNA depletion.

Primary:
1. Congenital myopathy (with or without nephropathy)
2. Congenital hepatopathy
3. Infantile myopathy (presenting as nonspecific myopathy with high serum CK)
4. Infantile or childhood myopathy with multisystem involvement
Secondary:
1. Other mitochondrial diseases (multiple mtDNA deletions)
2. Aging
3. Iatrogenic (zidovudine; other antiviral nucleoside analogues?)

multiple mtDNA deletions have been identified as yet, and linkage analysis is underway to localize additional genes in both AD-PEO and AR-PEO (Hirano *et al.*, 1997) families.

The pathophysiological role of multiple deletions is uncertain in sporadic conditions such as inclusion body myositis (IBM) (Griggs *et al.*, 1995; Santorelli *et al.*, 1996b) and late-onset myopathy (Johnston *et al.*, 1995). The latter seems to represent an exaggeration of the accumulation of mtDNA deletions that occurs in muscle during normal aging (Mendell, 1995).

Quantitative defects of mtDNA (mtDNA depletion). This autosomal recessive condition is characterized by the virtual absence or markedly decreased concentration of mtDNA in one or more tissues: a tentative classification of the clinical variants is offered in Table 4.

Three main syndromes can be distinguished, based on tissue involvement and amount of residual mtDNA (DiMauro and Bonilla, 1997; DeVivo *et al.*, 1996): (i) a congenital and rapidly fatal myopathy (sometimes associated with Fanconi-Debré syndrome), in which the amount of mtDNA in muscle is negligible; (ii) a congenital and rapidly fatal hepatopathy with extremely low levels of mtDNA in liver; and (iii) an infantile myopathy, usually presenting by 1 year of age, and causing death within a few years. The two myopathies are often diagnostic challenges. The congenital form is clinically indistinguishable from the fatal infantile myopathy due to COX deficiency: only immunohistochemical and biochemical studies of muscle provide differential features (virtual absence of mtDNA immunohistochemically; defects of multiple respiratory chain complexes biochemically in mtDNA depletion). The infantile form often presents in a nonspecific fashion, without lactic acidosis or RRF in the muscle biopsy; later in the course, the association of RRF and extremely high levels of serum CK provides an important diagnostic clue.

However, our experience suggests that the clinical spectrum of mtDNA depletion can be wider because we have seen several patients with later onset, longer survival, and evidence of multisystem involvement (Vu *et al.*, 1995). Again, these patients may be represent diagnostic riddles, such as a child that we followed for several years with a diagnosis of spinal muscular atrophy (SMA), until the

negative molecular data for the SMA mutations, the appearance of RRF in a second biopsy, and the unusually high serum CK values put us on the right diagnostic track (Pons *et al.*, 1996).

All forms of mtDNA depletion are transmitted as autosomal recessive traits and are probably due to mutations in nuclear genes encoding factors that are involved in mtDNA replication. The defective factor (or factors) remain unknown, although low levels of mitochondrial transcription factor A (mtTFA) have been found in tissues from patients with mtDNA depletion (Larsson *et al.*, 1994). However, it remains to be established whether the decreased amount of mtTFA is the cause or the effect of the mtDNA depletion. Knowledge of the genetic defect (or defects) in these conditions would be of crucial importance because prenatal diagnosis is not feasible and we can only offer these families standard genetic counseling for an autosomal recessive disease.

We will not discuss here the secondary forms of mtDNA depletion, except for warning that antiviral nucleoside analogues, including zidovudine (AZT) carry the risk of depleting mtDNA in muscle or liver (Arnaudo *et al.*, 1991; McKenzie *et al.*, 1995).

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List of abbreviations ATP, adenosine triphosphate; CPT, carnitine palmitoyl transferase; CK, creatine kinase; CoA, coenzyme A; CoQ, coenzyme Q; COX, cytochrome *c* oxidase; EMG, electromyography; FBSN, familial bilateral striatal necrosis; IBM, inclusion body myositis; KSS, Kearns-Sayre syndrome; LHON, Leber's hereditary optic neuropathy; MELAS, mitochondrial encephalomyopathy with lactic acidosis and strokelike episodes; MERRF, myoclonic epilepsy with ragged-red fibers; MILS, maternally inherited Leigh syndrome; MNGIE, myo-neuro-gastrointestinal-encephalopathy; mtDNA, mitochondrial DNA; mtTFA, mitochondrial transcription factor; NADH, nicotinamide adenine dinucleotide, reduced; NARP, neuropathy, ataxia, retinitis pigmentosa syndrome; PDHC, pyruvate dehydrogenase complex; PS, Pearson marrow/pancreas syndrome; PEO, progressive external ophthalmoplegia; SDH, succineate dehydrogenase.

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