# Clinical Diagnostic and Therapeutic Approach to the Patient with Suspected Mitochondrial Disease

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## ABSTRACT

1. This chapter focuses on the clinical approach to diseases with impaired respiratory chain function. Diseases of other mitochondrial pathways, such as disorders of fatty acid oxidation (FAO), the Krebs cycle, the urea cycle, and pyruvate oxidation are addressed in other chapters.

2. Respiratory chain diseases are clinically very heterogeneous. They include multisystem or monosystem disorders, and can have congenital, infantile, or late-onset presentations. They may be familial or sporadic. Familial cases may result from mutations of nuclear DNA (nDNA), which show Mendelian transmission, or of mitochondrial DNA (mtDNA), which show maternal transmission. Mitochondrial diseases are frequently but not invariably associated with characteristic laboratory abnormalities (e.g., elevated blood or CSF lactate, characteristic findings on muscle biopsy and brain MRI, and known pathogenic genetic changes).

3. Syndromology. Certain signs or combinations of signs should alert clinicians to a possible mitochondrial disorder. Many are due either to mtDNA mutations or to nuclear mutations affecting mtDNA maintenance. Progressive external ophthalmoplegia (PEO) is often a telltale sign of mitochondrial dysfunction, present in isolation or associated with multiple symptoms, manifesting as a “PEO-plus” syndrome often with involvement of extramuscular systems excluding the CNS, or as “mitochondrial encephalomyopathy” such as in Kearns–Sayre syndrome (KSS). Two mitochondrial syndromes frequently cause optic atrophy: autosomal dominant optic atrophy (DOA) and Leber hereditary optic neuropathy (LHON). The former is due to mutations in the gene (*OPA1*) encoding a crucial dynamin-like mitochondrial fusion protein; the latter is predominantly due to mutations in three mtDNA genes encoding complex I subunits (*ND1*, *ND4*, and *ND6*). Stroke-like episodes with mitochondrial encephalomyopathy and lactic acidosis suggest MELAS syndrome, which appears most commonly in older children or adults and can result from a variety of mtDNA mutations. A common infantile mitochondrial disorder is hepatocerebral syndrome (Alpers–Huttenlocher syndrome), a devastating condition with liver failure and intractable seizures, due to *POLG* mutations causing mtDNA depletion. The absence of a classical mitochondrial syndrome does not exclude a mitochondrial disease, and many mitochondrial disease patients present with a single complaint.

4. Molecular prenatal diagnosis is straightforward for nuclear encoded conditions if the causal mutations are known. For diseases caused by mtDNA mutations, some degree of prenatal prediction of clinical outcome is possible for certain mutations in protein-coding mtDNA genes. An example is m.8993T>G, which causes neuropathy, ataxia, and retinitis pigmentosa (NARP), and maternally inherited Leigh syndrome (MILS). For NARP/MILS, a mutation level below 30% in chorionic villus samples (CVS) or in amniocytes suggests a favorable outcome, but a mutant fraction exceeding 70% is an ominous sign, and intermediate mutation loads leave us uncertain about the outcome. Prenatal diagnosis is considerably less reliable for mutations in mtDNA genes of protein synthesis, such as tRNA genes (e.g., the MELAS m.3243A>G mutation), because the mutation load in prenatal samples may differ considerably from that of fetal tissues and the mutant fraction can shift during gestation and after birth.

5. There are three major therapeutic strategies for mitochondrial diseases. They focus on root causes, on downstream mechanisms, and on clinical endpoints. Therapies directed toward root causes include the following. (a) Mitochondrial replacement therapy (MRT): MRT is a promising technique, particularly for reproductive genetics. In MRT, a cell nucleus from a person with a pathogenic mtDNA mutation is transferred into an enucleated cell (e.g., an oocyte) from a donor who has normal mtDNA. That cell and its progeny will have normal mtDNA. (b) Heteroplasmic shifting has been attempted in cells that are heteroplasmic for mtDNA mutations, using custom-designed endonucleases specific for mutant DNA. (c) Stem cell therapy: One potential role is the replacement of a defective enzyme that acts on a diffusible substrate, for instance allogeneic hematopoietic stem cell transplantation (AHST) in patients with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) due to thymidine phosphorylase (TP) deficiency. Treatments focused on downstream mechanisms employ strategies such as removing noxious compounds (e.g., hyperlactatemia with systemic acidosis), boosting ATP synthesis by enhancing mitochondrial biogenesis (e.g., stimulating the PGC-1α pathway),

removing excessive reactive oxygen species (ROS) (e.g., coenzyme $Q_{10}$ or idebenone), and providing "cocktails" of vitamins and cofactors ($CoQ_{10}$; L-carnitine; vitamins B; vitamin E). Symptomatic treatments, directed toward clinical endpoints, are very important. They can prolong and improve the quality of the lives of mitochondrial patients. Examples include the pharmacological treatment of epilepsy and pacemaker placement for cardiac arrhythmias. Although currently, there is no efficient therapy that is generally applicable to all mitochondrial diseases, some promising treatments apply to specific conditions (e.g., MRT in mtDNA-related diseases; idebenone in LHON; $CoQ_{10}$ supplementation in $CoQ_{10}$ deficiencies; AHSCT in MNGIE syndrome; deoxynucleoside treatment in TK2-deficiency). The only such therapy with documented effectiveness is idebenone in LHON. MRT has advanced to the stage of assessment by national regulatory bodies in the United Kingdom and in the United States. There is hope and need for the development of more general approaches to mitochondrial encephalomyopathies in the future.

# 1. INTRODUCTION

It is essential to agree on a definition of mitochondrial diseases because, strictly speaking, they encompass defects in any one of the multiple metabolic pathways contained within the mitochondrion, this tiny but extraordinarily complex organelle. However, following conventional wisdom, we will only consider defects involving directly or indirectly the mitochondrial respiratory chain (RC), the "business end" of energy metabolism, where most cellular ATP is generated. We will not discuss genetic defects of other mitochondrial pathways also involved in energy production "upstream" of the RC, including deficiencies of pyruvate carboxylase and pyruvate dehydrogenase (PDH), and fatty acid oxidation (FAO) defects, which are described in separate chapters.

Despite this drastic censorship, we still have a huge job ahead of us because defects of the RC are arguably the most clinically and genetically heterogeneous disorders in medicine. The main reason for this polymorphism is that the RC is the only metabolic pathway under the control of two different genomes, the nuclear DNA (nDNA) and the mitochondrial DNA (mtDNA). Additional reasons include the peculiar rules of mitochondrial genetics, the structural and functional complexity of the RC, and the rich dialogue needed to keep the mitochondrial genome in sync with the nuclear genome, which has the overarching authority over the good functioning of the RC.

The morass of clinical symptoms and signs associated with mitochondrial diseases makes inexperienced clinicians either underdiagnose them ("what is this confusing disorder?") or probably more often, overdiagnose them ("this disorder is so confusing that it must be mitochondrial").

When confronted with a patient with a probable mitochondrial disease, one of the first useful diagnostic questions is whether the primary defect involves the mtDNA or the nDNA. After establishing differential clinical criteria between mtDNA-related and nDNA-related mitochondrial diseases, we will discuss common symptoms and signs tissue by tissue, starting from the most frequently affected tissues in a sort of hierarchical order.

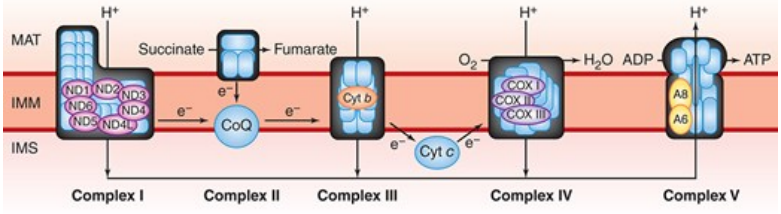
## 1.1 mtDNA-Related Mitochondrial Diseases

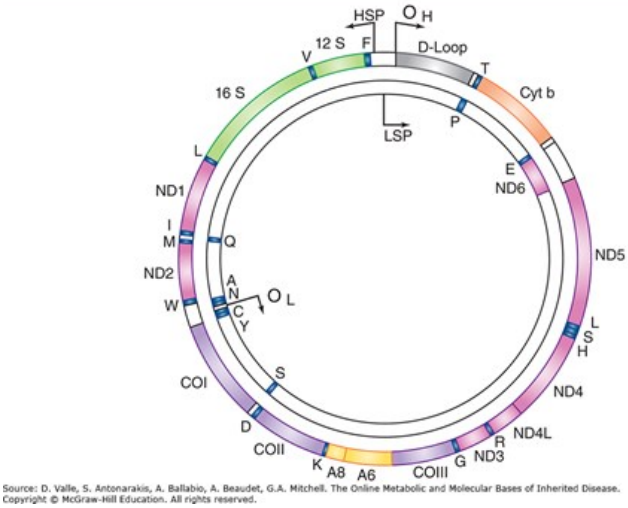
It is impossible to explain the protean clinical manifestations of mtDNA-related diseases without outlining the principles of mitochondrial genetics, which is distinct from Mendelian genetics but ought to be equally familiar to the practicing physician. This is just an outline. The details of mtDNA genetics are discussed in other chapters of this book. Mitochondrial DNA is located in mitochondria and observes different rules of transmission and segregation than nuclear DNA.

(i) Physically, human mtDNA (Fig. 129-1) is a 16,569 base circular, double-stranded molecule, which contains 37 genes: 2 rRNA genes, 22 tRNA genes, and 13 structural genes encoding subunits of the mitochondrial RC. Mitochondria and mtDNA are present in all cells of the body, none excluded (mature circulating red blood cells are devoid of mitochondria, but their progenitors in the blood marrow are not).

**Figure 129-1.**

The respiratory chain (top) and mitochondrial DNA (mtDNA, bottom). Genes and corresponding gene products are similarly color-coded. ND denotes the subunits of NADH-coenzyme Q oxidoreductase (complex I); cyt *b*, cytochrome *b*; subunits of cytochrome *c* oxidase are labeled CO in the mtDNA scheme and COX in the respiratory chain rendition. A6 and A8 indicate subunits 6 and 8 of ATP synthase. The 22 tRNA genes are denoted by one-letter amino acid nomenclature; 12S and 16S denote ribosomal RNAs (rRNAs). $O_H$ and $O_L$ are the origins of heavy- and light-strand replication; HSP and LSP are the promoters of heavy- and light-strand transcription. ADP, 5 adenosine diphosphate; ATP, 5 adenosine triphosphate; IMM, inner mitochondrial membrane; IMS, intermembrane space; MAT, mitochondrial matrix.




Source: D. Valle, S. Antonarakis, A. Ballabio, A. Beaudet, G.A. Mitchell. The Online Metabolic and Molecular Bases of Inherited Disease.


(ii) *Maternal inheritance.* At fertilization, all mtDNA derives from the ovum. Therefore, the mode of transmission of mtDNA and of mtDNA point mutations (single deletions of mtDNA are usually sporadic events) differs from Mendelian inheritance. A mother carrying an mtDNA point mutation will pass it on to all her children (males and females), but only her daughters will transmit it to their progeny. A hereditary disease expressed in both sexes but with no evidence of paternal transmission is strongly suggestive of an mtDNA point mutation.

(iii) *Heteroplasmy and threshold effect.* Each cell contains hundreds or thousands of mtDNA copies, which, at cell division, distribute randomly among daughter cells. In normal tissues, all mtDNA molecules are considered identical (homoplasmy), although supersensitive new generation sequencing techniques have revealed small numbers of mutations in normal individuals, a situation dubbed “universal heteroplasmy.” However, to all intents and purposes, most deleterious mutations of mtDNA affect only a percentage of genomes within a cell, a tissue, an individual (heteroplasmy). A minimum critical number of mutant mtDNAs is required to cause mitochondrial dysfunction in a particular organ or tissue and mitochondrial disease in an individual (*threshold effect*).

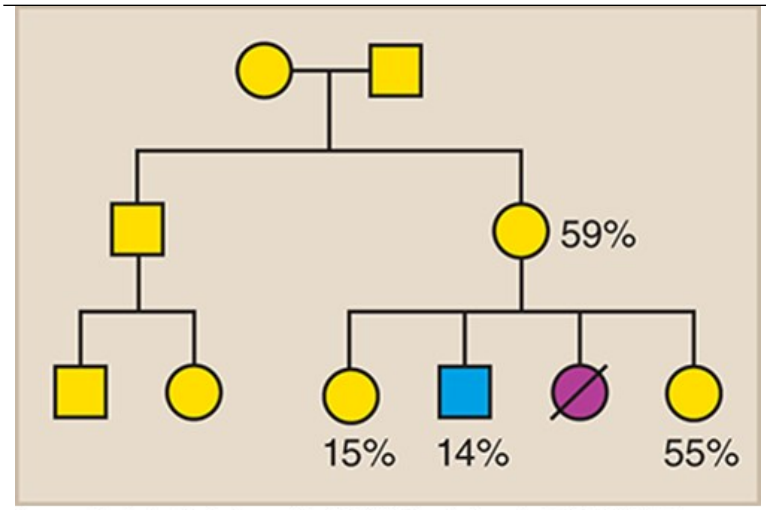
(iv) *Mitotic segregation.* At cell division, the proportion of mutant mtDNAs in daughter cells may shift and the phenotype may change accordingly.

Let us see how the rules of mitochondrial genetics can help us reach a diagnosis and explain some of the diagnostic conundrums of mitochondrial diseases.

Clear evidence of maternal inheritance points not only to a mitochondrial disorder but, more precisely, to an mtDNA-related disorder. Here, two words of caution are in order. First, maternal transmission is often not apparent because of heteroplasmy and the threshold effect (see below and Fig. 129-2).

**Figure 129-2.**

Pedigree of a girl (purple circle) who died at age 12 years of MELAS syndrome due to a typical m.3243A>G mutation (Hirano M, Ricci E, Koenigsberger MR, et al. MELAS: an original case and clinical criteria for diagnosis. *Neuromusc Disord.* 1992;2:125-135). The mutation levels in leukocyte DNA are indicated. Her older brother (blue square) suffered from slight mental retardation while her mother and two sisters were asymptomatic. The blood mutation load is not available for the proband but varied tremendously among family members.




Source: D. Valle, S. Antonarakis, A. Ballabio, A. Beaudet, G.A. Mitchell.
The Online Metabolic and Molecular Bases of Inherited Disease.


Second, maternal transmission may be apparent but not obviously so. Thus, family history has to be examined with a fine-tooth comb, without neglecting isolated “soft” signs in maternal relatives, such as short stature, migraine headache, hearing loss, exercise intolerance, psychiatric symptoms, or diabetes mellitus, including gestational diabetes. One case from our experience illustrates this point. Patient III-3 in Figure 129-2, the brother of a child with typical mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes (MELAS) syndrome, had mental retardation, which was ascribed to perinatal asphyxia but was much more likely due to the relatively low mutation load of the m.3243A>G mutation in tissues available for testing, with higher levels in brain

The bewildering variety of diseases attributed to the over 250 known pathogenic mutations of mtDNA is not surprising when one considers that mitochondria are ubiquitous organelles and that all human tissues, in isolation or in various combinations, can be affected by mtDNA mutations. This concept is illustrated in Table 129-1, a compilation of symptoms and signs described in mitochondrial encephalomyopathies due to single mtDNA rearrangements, to point mutations in genes affecting protein synthesis *in toto*, or a protein-coding gene.

Table 129-1.

**Clinically Recognizable Mitochondrial Syndromes***

| Mitochondrial Syndromes | | | |
|---|---|---|---|
| *Condition* | *Classic Features* | *Other Common Manifestations* | *Molecular Findings* |
| ***mtDNA Syndromes*** | | | |
| Kearns–Sayre syndrome (KSS) | 1. Onset before age 20 yrs<br>2. Ptosis<br>3. Progressive external ophthalmoplegia (PEO)<br>4. Pigmentary retinopathy<br>5. Heart block<br>6. Myopathy | 1. Cerebellar ataxia<br>2. Short stature<br>3. Sensorineural hearing loss<br>4. Mental retardation/dementia<br>5. CSF protein >100 mg/dl<br>6. Hyperparathyroidism<br>7. Cardiomyopathy<br>8. Dysphagia<br>9. Cricopharyngeal achalasia | 1. Often, large-scale mtDNA single deletion<br>2. Rarely, maternally inherited mtDNA point mutation |
| Chronic progressive external ophthalmoplegia (CPEO) | 1. Ptosis<br>2. Progressive external | 1. Dysphagia<br>2. Myopathy | 1. Often, large-scale mtDNA single deletion |

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| | ophthalmoplegia (PEO) | 3. Lactic acidosis<br>4. Exercise intolerance | 2. Less commonly, maternally inherited mtDNA point mutation |
| Leber hereditary optic neuropathy (LHON) | 1. Optic atrophy<br>2. Central or centrocecal scotoma (papillomacular bundle fiber loss)<br>3. Red color vision defect<br>4. Loss of visual acuity<br>5. Predominantly affects males | 1. Acute/subacute visual recovery<br>2. LHON/dystonia<br>3. No RRF in muscle biopsy | 1. Pathogenic mtDNA point mutation |
| Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) | 1. Stroke-like episodes (typically <age 40)<br>2. Encephalopathy (seizures, dementia, or both)<br>3. Mitochondrial myopathy (lactic acidosis and RRF) | 1. Exercise intolerance<br>2. Weakness<br>3. Short stature<br>4. Hearing loss<br>5. Increased CSF protein<br>6. Cardiomyopathy<br>7. Ataxia<br>8. Optic atrophy<br>9. Intestinal dysmotility<br>10. Diabetes mellitus<br>11. PEO<br>12. Nephropathy<br>13. Recurrent headache and vomiting | 1. One common mutation, m.3243A>G, in $tRNA^{Leu(UUR)}$ gene (*MTTL*); mutation load evaluated in urinary sediment<br>2. Over 22 different point mutations in six genes |
| Maternally inherited diabetes mellitus and deafness (MIDD) | 1. Early onset diabetes<br>2. Hearing loss<br>3. Lactic acidosis | 1. Perimacular retinal atrophy<br>2. Loss of vision<br>3. Loss of vestibular function<br>4. Late-onset neuropathy | 1. Typical MELAS mutation m.3243A>G in *MTTL* |
| Myoclonic epilepsy with ragged-red fibers (MERRF) | 1. Myoclonus<br>2. Seizures<br>3. Myopathy<br>4. Ataxia | 1. Hearing loss<br>2. Dementia<br>3. Lipomatosis<br>4. Neuropathy<br>5. Exercise intolerance<br>6. Ptosis/ophthalmoparesis<br>7. Optic atrophy<br>8. Cardiomyopathy<br>9. Diabetes<br>10. Tremor<br>11. Migraine | 1. 3 mutations in $tRNA^{Lys}$ (*MTTK*)<br>2. One common mutation m.8344A>G |
| Neuropathy, ataxia, retinitis pigmentosa/maternally inherited Leigh syndrome (NARP/MILS) | 1. Neurogenic weakness<br>2. Ataxia<br>3. Retinitis pigmentosa<br>4. Maternally inherited LS | 1. Seizures<br>2. Peripheral neuropathy<br>3. Cerebellar atrophy<br>4. No RRF in muscle biopsy | 1. 2 mutations, m.8993T>G, m.8993T>C in ATPase 6 gene (*MTATP6*) account for 75%–90% of NARP and >90% of MILS |

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| Pearson syndrome (PS) | 1. Infantile onset<br>2. Sideroblastic anemia, neutropenia, thrombocytopenia<br>3. Vacuolization of marrow precursors<br>4. Pancreatic dysfunction | 1. Hepatic failure<br>2. Proximal tubulopathy<br>3. Watery diarrhea<br>4. Early death after blood transfusions.<br>5. Surviving children develop KSS | 1. Large-scale single mtDNA deletions<br>2. Rarely maternally inherited |
| PEO-plus | 1. Ptosis<br>2. Ophthalmoplegia<br>3. Myopathy<br>4. Plus at least one of other common manifestations | 1. Dysphagia<br>2. Hearing loss<br>3. Cardiopathy<br>4. Ataxia<br>5. Encephalopathy<br>6. Does not fulfill criteria for KSS or SANDO | 1. mtDNA point mutation or single deletion |
| ***nDNA Syndromes*** | | | |
| Alpers–Huttenlocher syndrome (AHS) | 1. Childhood refractory seizures<br>2. Psychomotor regression<br>3. Cortical blindness<br>4. Hepatopathy | 1. Valproic acid exposure can cause fulminant liver failure<br>2. Liver cirrhosis | 1. *POLG* mutations causing mtDNA depletion syndrome (MDS) |
| PEO-plus syndromes (dominant) | 1. Ptosis<br>2. Ophthalmoplegia<br>3. Myopathy<br>4. Plus at least one of other common manifestations | 1. Optic atrophy<br>2. Spastic paraparesis<br>3. Sensorineural deafness<br>4. Cerebellar ataxia<br>5. Endocrinopathies<br>6. Sensory ataxia<br>7. Sensory-motor polyneuropathy<br>8. Psychiatric illnesses | 1. Multiple mtDNA deletions in skeletal muscle<br>2. Mutations in *OPA1*, *ANT1*, or in *PEO1 (*Twinkle)* |
| Barth syndrome | 1. X-linked<br>2. Mitochondrial cardiomyopathy<br>3. Mitochondrial myopathy<br>4. Cyclic neutropenia<br>5. Growth retardation | | 1. Mutations in *TAZ*, encoding monolysocardiolipin transacylase<br>2. High monolysocardiolipin/cardiolipin ratio in blood and other tissues |
| Axonal Charcot–Marie–Tooth (CMT) | 1. CMT 2A<br>2. Axonal autosomal dominant<br>3. Early onset<br>4. Severe course | | 1. Mutations in *MFN2*, required for mitochondrial outer membrane fusion |
| | 1. CMT2A1<br>2. Axonal autosomal dominant<br>3. Motor greater than sensory involvement | | 1. Mutations in KIF1B required for anterograde transport of mitochondria |

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| | 1. CMT2K<br>2. Childhood-onset<br>3. Severe axonal autosomal recessive | | 1. Mutations in *GDAP1*,controlling fusion, fission, and transport of mitochondria |
| | 1. CMT2K<br>2. Childhood- or adult-onset<br>3. Moderate to severe autosomal dominant | | 1. Mutations in *GDAP1* |
| Demyelinating Charcot–Marie–Tooth | 1. CMT 4A<br>2. Autosomal recessive demyelinating<br>3. Childhood-onset<br>4. Severe-course<br>5. Motor>sensory involvement | | 1. Mutations in *GDAP1* |
| Intermediate (axonal and demyelinating) Charcot–Marie–Tooth | 1. CMTRIA<br>2. Autosomal recessive<br>3. Childhood-onset | | 1. Mutations in *GDAP1* |
| | 1. CMTRID<br>2. Autosomal recessive | | 1. Mutation in *COX6A1* |
| Hereditary sensory neuropathy | 1. Hereditary sensory neuropathy 2C (HSN2C)<br>2. Autosomal recessive<br>3. Childhood-onset<br>4. Severe distal sensory loss and milder distal motor weakness | | 1. Mutations in *KIF1A* responsible for anterograde transport of mitochondria |
| Dominant optic atrophy (DOA) (Kjer syndrome) | 1. Autosomal dominant optic atrophy | 1. Similar to mtDNA-related LHON | 1. Mutation in *OPA1* |
| Infantile onset spinocerebellar ataxia (IOSCA) | 1. Ataxia<br>2. Hypotonia<br>3. Athetosis<br>4. Areflexia | 1. PEO<br>2. Optic atrophy<br>3. Hearing loss<br>4. Dementia<br>5. Neuropathy | 1. Recessive mutations in *PEO1 (Twinkle)*<br>2. mtDNA depletion |
| Mitochondrial gastrointestinal encephalomyopathy (MNGIE) | 1. Ptosis<br>2. Ophthalmoplegia (PEO)<br>3. Peripheral neuropathy<br>4. Gastrointestinal dysmotility | 1. Hearing loss<br>2. Hepatopathy<br>3. Endocrinopathies<br>4. Early death | 1. Elevated plasma deoxyuridine thymidine, or both<br>2. Mutations in *TYMP*<br>3. Muscle mtDNA depletion, multiple |

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| | 5. Cachexia<br>6. Leukoencephalopathy | | deletions, or both |
| Sengers syndrome | 1. Congenital cataracts<br>2. Hypertrophic cardiomyopathy<br>3. Mitochondrial myopathy<br>4. Lactic acidosis | | 1. Mutations in *AGK* |
| Sensory ataxic neuropathy dysarthria and ophthalmoparesis (SANDO) | 1. Progressive sensory ataxia<br>2. Dysarthria<br>3. PEO<br>4. Ptosis | 1. Decreased or absent sensory nerve action potentials<br>2. Seizures<br>3. Cognitive impairment<br>4. Hearing loss | 1. Recessive *POLG* mutations<br>2. Multiple mtDNA deletions |
| ***Syndromes Resulting from mtDNA or nDNA Mutations*** | | | |
| Leigh syndrome (LS) | 1. Infantile-onset<br>2. Typically, repeated neurological episodes, often during or following an infection, with partial recuperation<br>3. Brain stem or basal ganglion signs particularly respiratory (hypo- or hyperpnea; irregular pauses, apnea) | 1. Magnetic resonance imaging: bilaterally symmetrical T2-hyperintense lesions of basal ganglia and/or brainstem<br>2. Elevated lactate in blood, CSF, and/or on magnetic resonance spectroscopy of brain<br>3. Other neurological (hypotonia, movement disorders, convulsions, motor/developmental delay, spasticity, ataxia)<br>4. Ocular (optic atrophy, retinopathy, abnormal eye movements)<br>5. Visceral (cardiomyopathy, hepatic steatosis, episodes of lactic acidosis) | 1. Mutations in numerous mtDNA or nDNA genes |

*The table provides a list of the principal signs or combinations of signs that should arouse suspicion of mitochondrial disease. The list, compiled from the authors' clinical experience, is a baseline guide for clinical identification of mitochondrial diseases. It is not exhaustive: each condition can have other signs not listed and other distinct mitochondrial syndromes have been reported. Commonly used abbreviations are indicated.

The table also presents clinical features in mitochondrial diseases associated with mtDNA mutations. Boxes highlight typical features of specific syndromes (except Leigh syndrome, which is defined by neuroradiological and neuropathological alterations). CNS, central nervous system; ENT, ear-nose-throat; GI, gastrointestinal system; mtDNA denotes deleted mtDNA; PNS, peripheral nervous system.

Table 129-1 highlights the clinical features of the most common syndromes, which are difficult to miss in typical patients. It is also a reminder that any combination of these symptoms and signs ought to alert the astute clinician to the possibility of an mtDNA-related disorder. A third function of the table is to stress the fact that most mtDNA-related disorders are multisystemic, a useful diagnostic clue indicating that multiple brain areas or multiple organ systems are affected.

As mentioned above, some mtDNA syndromes are so characteristic that they do not pose a diagnostic challenge. Others, however, are rare and unusual syndromic combinations, sometimes mimicking Mendelian disorders, such as Lowe syndrome,[1] Usher syndrome,[2] spinal muscular atrophy,[3,4]

congenital disorders of glycosylation (CDG),[5] the EAST syndrome,[6] or dominant acute necrotizing encephalopathy (ANE).[7]

The striking variation of clinical severity in members of the same family harboring one and the same mtDNA mutation is largely determined by the relative proportion of normal and mutant genomes in different tissues (degree of heteroplasmy; mutation load). The best example is offered by the m.8993T>G mutation in the ATPase 6 gene associated with two conditions, a syndrome of juvenile-onset neuropathy, ataxia, and retinitis pigmentosa (NARP)[8] and an infantile Leigh syndrome (maternally inherited Leigh syndrome, MILS).[9] Whereas NARP patients have a mutation load of about 70%, infants with Leigh syndrome have mutation loads around 90%.[10]

Another example from our experience is that of two sisters who died in infancy of Leigh syndrome whereas their mother, who was normal until 26 years of age, developed a typical myoclonus epilepsy ragged-red fiber (MERRF) syndrome as a young adult: they all harbored the m.8363G>A mutation in the $tRNA^{Lys}$ gene, but the mutation load in skeletal muscle was 98% in the children and 84% in their mother.[11]

The phenomenon of mitotic segregation can explain how certain patients with mtDNA-related disorders may actually shift from one clinical phenotype to another as they grow older. This is exemplified by a few infants who survive Pearson syndrome (PS), a form of sideroblastic anemia that is usually fatal in the first year of life. Those who survive go through a healthy period only to develop the devastating encephalomyopathy Kearns–Sayre syndrome (KSS) as they approach adolescence. How can this shift be explained? As both PS and KSS are due to single large-scale deletions in mtDNA, there is initially a negative selection of deleterious deletions in the actively dividing bone marrow cells, which is followed by a selective advantage (and a gradual increase) of the same mutations in postmitotic tissues, including muscle and brain.[12,13]

We stated that most mtDNA-related diseases are multisystemic and this is a useful diagnostic clue. However, it would be a serious mistake for a clinician to exclude an mtDNA mutation solely on the basis of mono-organ involvement. Examples abound and the most commonly tissue affected in isolation seems to be skeletal muscle.[14] There are two possible explanations for this apparently paradoxical phenomenon. First, it may be due to somatic mutations, spontaneous, *de novo* mutations in the mitochondrial genome occurring in the embryo myoblasts after germ-layer differentiation, thus sparing other tissues. Second, it can be an example of skewed heteroplasmy, a situation in which heteroplasmic mutations are, in fact, ubiquitous but greatly prevail in skeletal muscle, such that they surpass the pathogenic threshold only in this tissue, resulting in myopathies. We hope that this overview and the clinical examples we employed will help the clinician make sense of the all too often confusing and apparently contradictory clinical features of patients harboring mutations in mtDNA.

## 1.2 nDNA-Related Disorders

Mutations in nDNA can affect subunits of RC complexes (“direct hits”), ancillary factors needed for the proper assembly and function of RC complexes (“indirect hits”), or the phospholipid composition of the inner mitochondrial membrane (IMM) in which the RC resides. By and large, there is a more stereotypical association between these genetic defects and clinical phenotypes than in mtDNA-related disorders, presumably due to the lack of heteroplasmy and to the “all-or-none” nature of the underlying RC defect. For the same reasons, these disorders tend to appear early in life and to run a rapidly downhill course, with death in infancy or early childhood. A good example of this situation is the psychomotor retardation or early regression that is typical of Leigh syndrome, the most common clinical (and neuropathological) expression of both direct and indirect hits to the RC.

Defects in nDNA can also affect mtDNA maintenance, with mtDNA depletion and/or multiple mtDNA deletions (defects of intergenomic signaling). Although disorders of mtDNA maintenance are unequivocally Mendelian, they share many features of mitochondrial genetics, including heteroplasmy and the threshold effect. Accordingly, onset may be early or later in life and clinical severity may vary. For example, mutations in genes involved in mtDNA replication, including *ANT1* encoding adenine nucleotide translocator 1, *PEO1* encoding a helicase called Twinkle, or *POLG* encoding the catalytic subunit of polymerase γ can cause autosomal dominant adult-onset progressive external ophthalmoplegia (PEO) associated with multiple mtDNA deletions but can also cause infantile or childhood encephalomyopathies or hepatocerebral syndromes with mtDNA depletion. Conversely, mutations in genes controlling the homeostasis of the mitochondrial nucleoside pool (*DGUOK*, *TK2*, *RRM2B*, *SUCLA2*, *SUCLG1*) typically cause mtDNA depletion resulting in infantile or childhood-onset myopathy or hepatocerebral syndrome, but they can also manifest later in life in association with multiple mtDNA deletions.[15,16]

These disorders also exemplify how the distinction between pediatric and adult syndromes is often blurred, at least from an etiologic standpoint. In pediatrics, most mitochondrial diseases (MDs) caused by mutations in nuclear genes have an autosomal recessive inheritance. Accordingly, family history is usually not contributive unless there are similarly affected siblings or parental consanguinity which would strongly suggest an nDNA-related

disorder.

## 2. CLINICAL MANIFESTATIONS OF RESPIRATORY CHAIN DISORDERS IN PEDIATRICS

### 2.1 Clinical History

#### 2.1.1 Antenatal signs and symptoms

In infants and children, it is unusual to elicit a history of fetal distress, and fetal loss due to RC dysfunction is infrequent, possibly because anaerobic glycolysis is the major source of cellular ATP in fetal tissues.[17] A glaring exception is represented by mutations in a cytochrome *c* oxidase (COX) assembly gene, *SCO2*, associated with a rapidly fatal infantile cardioencephalopathy and with frequent miscarriages.[18] A similar association with fetal loss and stillbirth has been described in Barth syndrome.[19] At the most severe end of the spectrum, major impairment of the RC has also been associated with fetal hydrops and antenatal cardiomyopathy[20] or with cerebral dysgenesis (including polymicrogyria and agenesis of the corpus callosum) and mild dysmorphism.[21,22] These cases exhibited multiple RC deficiencies caused by defects of the mitochondrial translation machinery, and were associated with severe neonatal presentation including fulminant lactic acidosis, profound encephalopathy, and early death. With these exceptions and a few others,[23-25] in our experience, dysmorphic features are infrequent in babies with RC defects. However, low birth weight (≤10th percentile for gestational age) is often noted, especially in infants with complex I deficiency.[26] Other antenatal problems have been reported (including polyhydramnios, oligoamnios, gestational diabetes, maternal hypertension or preeclampsia, and HELLP syndrome).[27,28] However, a causal relationship with RC dysfunction remains to be established and, given the extreme molecular diversity, no known correlation exists with one or another type of RC disease. Pregnancies at risk for an affected fetus (such as couples with a previous affected child) should be carefully monitored. In most cases, pregnancy and birth are uneventful, and the newborn appears normal.

#### 2.1.2 Neonatal presentations

Some patients with MDs present immediately at (or a few hours after) birth with fulminant lactic acidosis, frequently associated with neurological distress. They exhibit profound metabolic acidosis, major lactic acidemia (sometimes with ketosis), tachypnea and respiratory distress, hypotonia, lethargy or coma, poor skin perfusion and frequent hemodynamic instabilities.[29] Intractable seizures can occur and patients can rapidly develop multi-organ failure. This overwhelming presentation usually requires full resuscitation and intensive care. Hyperammonemia has also been observed in fulminant neonatal cases, probably resulting from generalized disturbances of mitochondrial functions (including ureagenesis), due to severe energy deprivation.[30,31] Severe neonatal cardiomyopathies can also be observed, associated with poor prognosis.[32] In other cases, less dramatic clinical features occur in the first weeks, associating feeding difficulties, recurrent vomiting, and failure to thrive. Neonatal hypoglycemia can be observed in cases of mitochondrial hepatopathy[33] and as a nonspecific symptom of impaired energy metabolism.[29,30,34] Usually, these patients have multisystemic disease. Several subgroups have been recognized: an encephalomyopathic form (sometimes rapidly evolving to Leigh disease), a cardiac form (mainly early lethal cardiomyopathy), and a hepatodigestive form with progressive cholestatic liver disease.[26] Such specific patterns of organ involvement help to identify RC dysfunction as the cause of the pathology. However, when a clinician is faced with a newborn showing early profound lactic acidosis and collapse, perinatal asphyxia is usually the first consideration. Lack of a birth history suggestive of asphyxia (normal fetal cardiac monitoring and absence of obstetrical complications), severe acidosis in the face of a rather short period of asphyxia, and persistence of lactic acidemia despite normalization of hemodynamics and oxygenation, are clues pointing to a primary lactic acidemia.

Proximal tubulopathy is frequent in patients with hepatopathy. Massive neonatal proteinuria in the context of fulminant lactic acidosis and multisystemic disease suggests a possible coenzyme $Q_{10}$ synthesis defect.[35] Severe neonatal presentations including congenital lactic acidosis have been reported in nearly all types of MD, including individual RC complex deficiencies (both nuclear and mtDNA-related), as well as in multiple RC deficiencies (including mtDNA depletion syndromes and defects of mitochondrial translation). It can also occur in defects of earlier steps of energy metabolism, including PDH complex and pyruvate carboxylase deficiencies.

#### 2.1.3 Neurological presentations

Due to its high metabolic rate and energy requirement,[36] the central nervous system is one of the commonest tissues involved in RC defects both in

children and in adults, and brain dysfunction is often the cause of presenting symptoms. In neonates, it manifests as nonspecific neonatal distress with hypotonia, lethargy, poor sucking, and sometimes loss of respiratory control. Abnormal movements and seizures can also occur. In infancy and childhood, neurological presentation can include nonspecific developmental delay or progressive psychomotor regression with various combinations of neurological symptoms including muscle weakness, seizures, ataxia, pyramidal syndrome, or extrapyramidal movement disorders.[37,38]

#### 2.1.3.1 Developmental delay and psychomotor regression

Most commonly, evidence of psychomotor regression occurs after an initial well period of weeks or months: dramatically for the family, the child loses acquired skills, including sitting or standing alone, babbling, smiling, and tracking objects. Such a regression reflects the ravages of energy failure on the developing nervous system. At the same time, the child may refuse feeding, have recurrent vomiting (leading to failure-to-thrive), and develop additional neurological symptoms.

Depending on the age-at-onset and the constellation of clinical features, several patterns of neurodegeneration have been recognized. An infantile or early childhood presentation with clinical and neuroradiological evidence of brainstem and basal ganglia involvement is characteristic of Leigh syndrome (see Section 2.1.3.2). Later onset with predominant intractable seizures associated with cerebral atrophy and liver disease was identified as Alpers–Huttenlocher syndrome. Neuropathological examination in these cases reveals degeneration of the gray matter in the cerebral cortex, with loss of neurons and reactive astrocytosis. Severe multifocal and myoclonic epilepsy can be complicated by status epilepticus and epilepsia partialis continua. Electroencephalographic findings typically include high-voltage slow-wave activity and superimposed polyspikes with occipital predilection.[39,40] Most cases of Alpers syndrome are associated with mtDNA depletion caused by autosomal recessive *POLG* mutations. A similar presentation can also occur with mutations in other genes associated with mtDNA depletion and hepatocerebral syndromes.[41]

In addition, many pediatric patients with MD exhibit obvious psychomotor regression, rapidly developing a nonspecific encephalopathy with various combinations of hypotonia, ataxia, spasticity, extrapyramidal syndrome, seizures, and mental retardation. They do not fit the classical definitions of Leigh syndrome or of other typical mitochondrial syndromes.[37] However, neuroimaging can reveal basal ganglia lesions and cerebral or cerebellar atrophy. In some cases, peripheral neuropathy (mainly sensory-motor axonal neuropathy) also occurs, although it is frequently subclinical. Electrophysiological studies suggest that the involvement of the peripheral nervous system is often underestimated in MD.[42] Some mitochondrial encephalopathies with psychomotor regression can present as leukodystrophy, with widespread white matter lesions.[43] Leukodystrophy has been reported with nuclear-related RC deficiencies[44-49] as well as in mtDNA-related disorders.[50-52] In rare cases, progressive cavitating leukoencephalopathy occurs,[53] with encephalomalacia mimicking vanishing white matter disease.[54,55]

By contrast, other patients present with chronic developmental delay without rapid regression and sometimes evolving to progressive spasticity. Such a nonspecific course can mimic cerebral palsy[56,57] and patients remain undiagnosed until long-term follow-up reveals slow progression and the presence of additional symptoms (e.g., ptosis, ophthalmoplegia, retinitis pigmentosa, or myoclonus). In these cases, cerebral imaging can be an important clue to MD, showing for example unsuspected basal ganglia lesions. Lactate in the cerebrospinal fluid (CSF) can be increased, even when normal in blood. Finally, MR spectroscopy may reveal a lactate doublet both in the CSF and in the cerebral parenchyma.[58]

Importantly, valproate therapy should be strictly avoided in patients with suspected Alpers syndrome (and other types of mtDNA depletion) because it can trigger fulminant hepatic failure (even in patients without previous apparent liver involvement).

#### 2.1.3.2 *Brainstem and basal ganglia disease*

One of the commonest and most specific presentations of MD in infancy and childhood is acute or subacute neurodegeneration with severe axial hypotonia, dystonia, drowsiness, and signs of brainstem involvement, including abnormal eye movement, ophthalmoplegia, and abnormal breathing pattern (hyperventilation alternating with apnea and ataxic breath). This clinical constellation, most commonly associated with neuroradiological evidence of brainstem (and/or basal ganglia) lesions and lactic acid accumulation in the central nervous system, is called Leigh syndrome (LS).[59] These patients often present with recurrent decompensations and bouts of drowsiness with loss of developmental skills. Of note, these deteriorations usually occur after stresses, such as minor infections during which the metabolic demand is presumably increased, compromising energy homeostasis in tissues with impaired energy production. This downhill evolution is usually rapidly fatal over weeks, months, or years. In many cases, LS is associated with lactic acidosis, sometimes with acute metabolic decompensation eventually leading to death.[24] In other cases, it occurs in the absence

of systemic lactic acidemia, with death resulting from complete loss of respiratory control.[60] When a notable but rare clinical sign, retinitis pigmentosa, is present, it should orient the diagnosis to the NARP/MILS syndrome due to mutations in the ATPase 6 gene (*MTATP6*) of complex V. Neuropathologically, LS consists of symmetrical bilateral lesions of the deep gray matter from the rostral spinal cord to the brainstem, thalamus, cerebellum, and sometimes the basal ganglia. It should be noted that a diagnosis of LS is only the beginning of a potentially long search for the molecular cause, as LS has been associated with mutations in mtDNA (maternally inherited LS or MILS) or mutations in nDNA affecting any RC complex (but predominantly complex I and complex IV) as well as the PDH complex.

Regarding neuroimaging, a word of caution is mandatory: basal ganglia lesions are relatively common in MD. Their presence has been used excessively in the medical literature as synonymous with LS. However, the definition of LS implies clinical criteria, including acute or subacute (and recurrent) attacks of psychomotor regression, signs and symptoms of brainstem (and/or basal ganglia) dysfunction, and raised lactate in blood and/or CSF.[61] Although these criteria should not be taken too strictly (Leigh-like vs. typical Leigh), the clinical component of the definition is important in order to properly orient etiological investigations. Concerning cerebral imaging, brainstem involvement is much more specific for LS than are basal ganglia lesions. It has been suggested that lesions of the subthalamic nuclei (in association with brainstem) are specifically associated with *SURF1*-related LS,[62] but there are many exceptions. Predominant or exclusive involvement of the thalami in patients with Leigh-like syndrome is indicative of acute necrotizing encephalopathy,[63] an autosomal dominant condition with incomplete penetrance whose pathophysiology and possible relationship with oxidative phosphorylation remains unresolved. A specific combination of T2-weighted hyperintensities in the substantia nigra, medial lemniscus, spinothalamic tract, medial longitudinal fasciculus, and mammillothalamic tracts has been specifically associated with mutations in the *NDUFAF2* gene and complex I deficiency.[64] In practice, it is rare for the pattern of cerebral lesions in LS to strongly suggest a specific gene defect.

Recurrent acute encephalopathy with bilateral striatal necrosis can be also observed in the mitochondrial thiamine transporter deficiency syndromes, with or without lactate increase in the CSF.[65,66] Importantly, in such cases, these diagnoses should be considered along with RC disorders because patients can improve with thiamine and biotin therapy.[67,68]

#### 2.1.3.3 *Stroke-like episodes*

Stroke-like episodes (SLEs) are acute neurological events associated with hemispheric cerebral lesions mimicking vascular infarct but not conforming to the regional distribution of major vascular territories. In addition, diffusion-weighted MRI have suggested pathophysiological differences between SLE and vascular lesions, showing normal or increased apparent diffusion coefficient in SLE whereas this is decreased in ischemic stroke. Clinically, SLE usually presents as a combination of focal neurological dysfunctions (transient hemiparesis, cortical blindness, aphasia), seizures, confusion, and alteration of consciousness. Headache also frequently occurs early in the course of the episodes. SLEs are rare in infancy and uncommon in early childhood. Mostly, they occur in adolescence or adulthood. In the context of MD, occurrence of an SLE usually indicates the presence of an mtDNA-related disorder. However, SLEs have also been reported exceptionally in autosomal recessive MD, including isolated complex IV deficiency,[69] mtDNA depletion syndrome,[70,71], mtDNA multiple deletions due to *POLG* mutations,[72] and coenzyme $Q_{10}$ deficiency.[73,74] In mtDNA depletion, what has been reported is the association between intractable seizures and large hemispheric edematous lesions. It should be noted that SLEs are suggestive but not specific of MD, as they can be observed in other inherited metabolic and non-metabolic conditions including, for example, CDG,[5] hyperammonemia,[75] and Charcot–Marie–Tooth (CMT) disease.[76]

#### 2.1.3.4 *Progressive ataxia*

Although ataxia is a frequent nonspecific component of the neurological presentation of MD, in a few disorders it is the predominant clinical sign. In familial cerebellar ataxia with muscle coenzyme $Q_{10}$ deficiency, patients present with progressive walking difficulties due to gait ataxia starting in late infancy or childhood. The course is slowly progressive, leading to severe disabilities over one or two decades. Cerebellar ataxia can be isolated but is more frequently associated with exercise intolerance, mild peripheral neuropathy or pyramidal signs, with or without mild mental retardation. Blood lactic acid concentration is normal or minimally increased. Neuroimaging studies show cerebellar atrophy. This autosomal recessive cerebellar ataxia (SCAR9) was related to mutations in *ADCK3* (also called *CABC1*), which encodes a kinase that plays a regulatory role in the ubiquinone synthesis pathway.[77] Other patients with mutations in the same gene have a more complex neurological disease, including seizures, dysarthria, progressive intellectual regression, and SLE.[74,78] Unfortunately, current data suggest that oral $CoQ_{10}$ treatment fails to improve the clinical condition of these patients.

A similar presentation of slowly progressive cerebellar ataxia starting in childhood (or adolescence), but complicated by spasticity, dorsal column dysfunction, and dysarthria (with normal intellectual function or minimal mental impairment) has been observed in association with a characteristic pattern of white matter abnormalities, including diffuse T2-hyperintensities in the supratentorial white matter (sparing the subcortical U-fibers), in the brainstem (pyramids in the medulla oblongata), and in the spinal cord (dorsal columns).[79] This specific clinical syndrome with autosomal recessive inheritance is associated with a mitochondrial translation defect caused by mutations of the aspartyl-tRNA synthetase gene (*DARS2*).[48] Of note, muscle RC enzyme activities are normal and blood and CSF lactate concentrations are usually also in the normal range. However, MR-spectroscopy in most patients reveals increased lactate in the abnormal white matter.

Ataxia also dominates some clinical presentations of *POLG* mutations, associated or not with multiple mtDNA deletions or mtDNA depletion.[70,80] The phenotypes are described by the acronyms MIRAS (mitochondrial recessive ataxia syndrome) and SANDO (sensory ataxic neuropathy, dysarthria, and ophthalmoparesis).[81] In these cases, peripheral neuropathy with hypo/a-reflexia and loss of proprioceptive and vibratory sensations, contributes markedly to the gait ataxia ("sensory ataxic neuropathy"). Especially in children, *POLG*-related autosomal recessive ataxia is usually associated with seizures, myoclonus, dysarthria, abnormal eye movements, and progressive cognitive impairment. Such clinical features are similar but less severe than those seen in the IOSCA (infantile-onset spinocerebellar ataxia) syndrome, a recessive mitochondrial encephalopathy prevalent in the Finnish population and caused by a founder mutation in *PEO1*, which encodes a DNA helicase required for mtDNA maintenance.[82]

#### 2.1.3.5 *Muscle*

Isolated myopathy is an extremely common presentation of mitochondrial disease in infancy, manifesting with congenital or severe early infantile hypotonia and generalized weakness, weak cry and suck, respiratory stridor, and intercostal recession while breathing. Here, the differential diagnosis is staggering, as it includes spinal muscular atrophy, congenital muscular dystrophy, congenital metabolic (non-mitochondrial) myopathies, and congenital myasthenia. In fact, mitochondrial myopathies are certainly not the most common causes of the "floppy baby syndrome," although they should be given careful consideration. This is also a clear illustration of how the diagnosis of mitochondrial disease cannot rely on clinical features alone but requires laboratory data: in the case of a floppy infant, lactic acidosis as a rule accompanies mitochondrial dysfunction and distinguishes mitochondrial myopathy from other causes of congenital weakness.

In fact, hypotonia and weakness are rarely the sole manifestation of a mitochondrial disease and are more often associated with, or are the precursors of, other symptoms (psychomotor delay, seizures, dystonia, vomiting, nystagmus, as in Leigh syndrome). However, there are important exceptions. Thus, profound weakness present at birth or developing early in life can be the only manifestation of one mtDNA depletion syndrome, due to mutations in the gene (*TK2*) encoding thymidine kinase 2. To make the differential diagnosis from congenital muscular dystrophy more difficult, this is one of the few mitochondrial diseases in which serum CK levels can be markedly elevated.[3] Survival has varied from months to several years and even to young adulthood, and death is due to pulmonary failure.

Another isolated mitochondrial myopathy is extremely rare but ought to be kept in mind for its potentially positive outcome. It presents at or soon after birth with profound hypotonia and generalized weakness (requiring tube feeding and often assisted ventilation) but – if these fragile infants are vigorously supported – it resolves spontaneously over months, such that most patients are normal by 2 or 3 years of age.[83] This "infantile reversible COX deficiency myopathy" is associated with a maternally inherited homoplasmic mtDNA mutation in the $tRNA^{Glu}$ gene (m.14674T>C), which, however, in and by itself, does not explain the reversible weakness.[84,85] In fact, we now know that this is a disorder of mtDNA translation in which mutations in the nuclear gene *TRMU* impair the 2-thiouridylation of the mutant $tRNA^{Glu}$, thus exacerbating its pathogenic effect.[86] However, from a practical point of view, in floppy infants suspected to have this reversible mitochondrial myopathy, temporary supportive measures are lifesaving. Knowledge of the mtDNA defect, which is pathognomonic – if not pathogenic – facilitates diagnosis at early stages.

Another uncommon isolated myopathy presents in childhood or adolescence with exercise intolerance, muscle cramps and fatigue, sometimes associated with chronic muscle weakness and occasionally with recurrent myoglobinuria. There is chronic lactic acidosis, and lactate increases excessively after aerobic exercise. In most cases, such clinical presentation results from sporadic mutations in mtDNA genes, including those coding for the three COX subunits (*MTCO1*,[87] *MTCO2*,[88] *MTCO3*[89]), the cytochrome *b* of complex III (*MTCYB*[14]), or in tRNA genes.[90] Isolated myopathy with exercise intolerance starting in early childhood, exercise-induced myoglobinuria, and lactic acidosis has also been reported in patients originating from northern Sweden. These patients have succinate dehydrogenase (SDH) and aconitase deficiencies, and multiple abnormalities in iron-sulfur (FeS)-

containing RC complexes I, II, and III, which are caused by a homozygous founder mutation in the *ISCU* gene.[91] Clinically, exercise intolerance is also associated with tachycardia, palpitation, and shortness of breath caused by greatly increased cardiac output and reduced muscle oxygen uptake. A similar presentation of exercise intolerance and muscle weakness in association with sideroblastic anemia and lactic acidosis is also observed in the MLASA (mitochondrial myopathy and sideroblastic anemia) syndrome.[92,93]

### 2.1.4 Liver disease in MD

A relatively frequent clinical history that ought to alert the pediatrician to the possibility of an underlying mitochondrial dysfunction is the association of brain and liver involvement (hepatocerebral syndrome). Neurological symptoms are similar to those described above for Leigh syndrome, with developmental delay or regression but with more prominent and difficult-to-control seizures. Liver involvement leads rapidly to liver failure. An important diagnostic clue is provided by valproate toxicity, which can be very severe and orients the diagnosis toward Alpers–Huttenlocher syndrome. In general, fulminant liver involvement is rare in mitochondrial diseases, and any child with hepatocerebral dysfunction should be suspected of having one of several mtDNA depletion syndromes, including those due to mutations in *DGUOK*, *MPV17*, *POLG*, and *PEO1* (*Twinkle*). Liver involvement can also occur as a part of the classic mitochondrial PS (caused by heteroplasmic single mtDNA deletions). Because the liver has a central role in lactic acid recycling, it is usually assumed that mitochondrial liver diseases must be associated with systemic lactic acidosis. While this is true most of the time, there are exceptions and the clinician cannot exclude mitochondrial hepatopathy on the basis of the absence of lactic acidemia.[94]

Are there clinical clues that may help us sort out the most likely diagnosis?[33,95] Virtually isolated hepatopathy in infants is well documented in deoxyguanosine kinase deficiency (*DGUOK* mutations). However, most cases of deoxyguanosine kinase deficiency present hepatocerebral syndromes with early developmental regression. Of note, patients with the multisystemic form of DGUOK deficiency can have a typical and recognizable pattern of abnormal eye movements, with rotary nystagmus progressively evolving to opsoclonus.

When liver dysfunction in an infant or child is associated with seizures, ataxia, or peripheral neuropathy, mutations in *POLG* should be suspected, especially if the seizures are difficult to control and if valproate administration has worsened liver insufficiency (Alpers–Huttenlocher syndrome). Interestingly, heterozygous *POLG* variants have been associated with valproate liver toxicity in otherwise healthy people.[96]

Onset with hypoglycemia and later development of brain dysfunction has been associated with mutations in *MPV17*.[97] A clinical variant due to a homozygous mutation in *MPV17* affects the peripheral more than the central nervous system (with sensory and motor neuropathy). However, the diagnosis in this case is dictated by ethnicity and geography: the so-called Navajo neurohepatopathy (NNH) is reported to date only in the Navajo population of Southwestern United States.[98]

Predominant brain involvement (cerebrohepatic syndrome) with ataxia, sensory neuropathy, epilepsy, and especially ophthalmoplegia suggests the possibility of autosomal recessive *PEO1* mutations.[99,100] Early-onset liver disease also occurs in isolated RC deficiencies, including complex III deficiency caused by *BCS1L* mutations (in association with proximal tubulopathy and severe encephalopathy),[101] and complex IV deficiency due to *SCO1* mutations.[102]

In addition, early mitochondrial hepatopathy has also been described together with multiple RC complexes deficiencies and attributed to mitochondrial translation defects caused by mutations of the *EFG1, TSFM,* and *TRMU* genes.[103-105] Patients with *EFG1* and *TSFM* mutations typically have neonatal hepatocerebral disease with encephalopathy and early death. In contrast, patients with mutations in *TRMU* start between the age of 2 and 6 months with isolated liver failure without neurological or other extrahepatic symptoms. With intensive medical support, liver disease often resolves spontaneously and long-term follow-up in surviving patients showed absence of neurological involvement. Whatever the mechanism for this unexpected clinical course, from a practical point of view, reversible infantile liver failure and favorable outcome should be considered as a possible evolution in mitochondrial hepatopathies, especially in the absence of extrahepatic involvement.

Finally, secondary neonatal hemochromatosis is seen in patients with severe hepatic failure and massive iron overload in the GRACILE (growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death) syndrome, caused by mutations in the complex III assembly gene *BCS1L*, but also in at least one mtDNA depletion syndrome.[106] Currently, most cases of neonatal hemochromatosis are attributed to gestational alloimmune liver disease in which iron overload is considered secondary to antenatal liver failure that impairs the regulation of iron metabolism in the feto-placental unit.[107] Severe antenatal liver involvement in MD can produce a similar phenotype.

In infants with severe liver failure, the biochemical diagnosis of MD can be challenging. Poor clinical and neurological condition as well as lactic acidemia may be caused by liver failure but the reduction in RC enzyme activities can be secondary to the liver pathology itself, making the interpretation of RC spectrophotometric assays difficult in damaged tissues.[108] Measurement of mtDNA content in liver and muscle tissues can yield clearer results and help establish the diagnosis.[109] Normal concentration of mtDNA in the presence of multiple RC enzyme deficiencies suggests a mitochondrial translation defect.

### 2.1.5 Cardiac pathology

Heart disease is a frequent manifestation of MD in pediatrics.[110] In some cases, for example those caused by mutations in *SCO2*, it can be the major presenting symptom.[111] This is also the case in patients with Barth syndrome, in some forms of complex IV deficiency (*COX15*,[112,113] *C2ORF64*[114] mutations) and complex I deficiency (*NDUFS2*,[115] *NDUFV2*,[116] *NDUFAF1*[117]), as well as in multiple RC deficiencies caused by mitochondrial translation defects.[118-122] Hypertrophic cardiomyopathy is also a leading feature of autosomal recessive complex V deficiency caused by *TMEM70* mutations, in association with 3-methylglutaconic aciduria.[123] The association of hypertrophic cardiomyopathy, lactic acidosis, and congenital cataract defines Sengers syndrome, caused by recessive mutations in the *AGK* gene.

In the neonatal period, cardiomyopathy is a poor prognostic factor and congestive heart failure can be rapidly fatal.[32] However, in rare cases, if patients benefit from intensive support, they can survive this critical neonatal phase, and long-term prognosis may be surprisingly favorable. This was observed for example in patients with mitochondrial phosphate carrier deficiency (*SLC25A3* gene), a disorder in which newborns present with severe hypertrophic cardiomyopathy and lactic acidosis, but who may survive without mental impairment at least until 17 years of age.[124,125]

In early infancy, cardiac disease frequently occurs in association with neurological disease (including Leigh or Leigh-like syndrome) and is called cardioencephalopathy. In other cases, cardiac disease is part of a multi-organ pathology or it appears as a secondary complication later in the course of the disease. This is observed in many classical mtDNA-related syndromes, such as MELAS and MERRF, more commonly in adults but also in children.[126-128]

In most cases, cardiac involvement manifests as a concentric, non-obstructive, hypertrophic and hypokinetic cardiomyopathy. Dilated cardiomyopathy is less frequently observed and may result from dilatation secondary to progressive heart failure.[129] Nevertheless, some primary dilated cardiomyopathies are also observed, for example in patients with Barth syndrome and in some cases of complex V deficiency, as well as in a Barth-like syndrome caused by disturbances of the mitochondrial protein import machinery.[130] A non-compacted aspect of the myocardium with prominent trabeculations and deep intertrabecular recesses at the internal face of the left ventricle has been associated with Barth syndrome and mutations in the *TAZ* gene (with or without 3-methylglutaconic aciduria). However, ventricular non-compaction is not specific to Barth syndrome and can be observed in other mitochondrial diseases,[131] in mutations of genes coding for cytoskeletal proteins,[132] or as a secondary event in congenital heart malformations.

Histiocytoid cardiomyopathy is a rare form of infantile dilated cardiomyopathy characterized by subendocardial nodules of histiocytoid myocytes originating from the conduction system. It is strongly suggestive of MD and has been attributed to mutations in nuclear and in mtDNA genes.[133] It manifests as severe cardiac arrhythmia. Cardiac conduction abnormalities, including Wolff–Parkinson–White (WPW) syndrome and heart block, are not rare in mitochondrial cardiomyopathies. Although cardiac conduction blocks may be asymptomatic, they can also be life-threatening complications requiring placement of a pacemaker, even in childhood and particularly in KSS.[134]

### 2.1.6 Renal diseases

Kidney dysfunction in pediatric MD is not rare and it usually manifests as proximal tubulopathy (De Toni–Debré–Fanconi syndrome), a relatively minor clinical feature in infants with widespread multisystemic involvement. De Toni–Debré–Fanconi syndrome is characterized by impairment of proximal tubular reabsorption of variable severity, which leads to urinary losses of amino acids, glucose, proteins, phosphate, bicarbonate, and other electrolytes. These tubular losses are often moderate and have little clinical impact (e.g., aminoaciduria) in the short term. However, in surviving patients, progressive nephropathy with interstitial fibrosis may lead to chronic renal failure.[135] End-stage renal disease can also occur with tubulointerstitial nephritis in the absence of tubulopathy.[50] Exceptionally, severe and acute metabolic acidosis is due to major bicarbonate loss and

has no relationship with lactic acidemia.[136] Given its high metabolic rate, it is not surprising that the proximal tubule is a hotspot of mitochondrial renal pathology. Proximal tubules generate more than 95% of their ATP through oxidative metabolism.[137] *In vitro* experiments have shown that the nephrotoxicity of some drugs is related to their inhibition of mitochondrial respiration, resulting in ATP depletion and secondary tubular damage.[138] Functional coupling between ion transport and aerobic metabolism was demonstrated *in vitro*.[139] Moreover, even in normal proximal tubule cells, the energy demand is close to their capacity for ATP generation, as shown by the rapid decrease in ATP level after stimulation of epithelial sodium transport.[140] Tubular dysfunction has been reported both in mtDNA-related MD (including large deletions in PS and KSS) and in autosomal recessive diseases of nuclear origin (including complex III[101,141] and complex IV deficiencies[46]). A second form of tubulopathy associated with MD is "Bartter-like" renal tubular acidosis with hypercalciuria, which has been associated with KSS.[142,143]

Another renal manifestation of MD is glomerulopathy. Proteinuria rapidly progressing to steroid-resistant nephrotic syndrome in infancy is a typical presentation of coenzyme $Q_{10}$ ($CoQ_{10}$) biosynthetic defects. The pathology ranges from focal segmental glomerulosclerosis to severe crescentic glomerulonephritis and diffuse mesangial sclerosis. Nephrotic proteinuria can occur as early as the neonatal period.[35] Mitochondrial nephrotic syndrome is typically steroid-resistant and is frequently associated with progressive neurological disease, including intractable seizures, optic atrophy, basal ganglia disease, Leigh syndrome, diffuse cerebral atrophy, and focal neurological symptoms (associated with stroke-like lesions).[35,73,144] However, an apparently isolated glomerular nephropathy has also been described.[145] The current lack of long-term data about MD patients with isolated renal disease does not allow for accurate prediction of outcome, including whether patients will develop regression and neurological symptoms. Congenital (or early-onset) sensorineural deafness seems specifically associated with nephrotic syndrome in patients with *COQ6* mutations.[146] Importantly, and unlike other genetic forms of steroid-resistant nephrotic syndrome, in $CoQ_{10}$ deficiency, kidney disease can be improved by $CoQ_{10}$ supplementation, with some reduction of proteinuria and stabilization of renal function. However, extrarenal manifestations seem less responsive to this treatment.

Glomerulopathy has also been reported in patients harboring mtDNA mutations or deletions and without $CoQ_{10}$ deficiency. Although this occurs especially in adolescents or adults,[147] it has been rarely observed in infancy.[148]

### 2.1.7 Gastroenterological manifestations, nutritional issues, growth, and endocrinopathies

Infants with MD frequently present with failure to thrive and feeding problems, including recurrent vomiting and anorexia. In unselected pediatric series, up to 50% of patients present growth delay. Gastro-esophageal reflux (GER) is a common nonspecific symptom. Many children with failure to thrive, anorexia, and encephalopathy require nasogastric tube feeding.[28] Later in childhood or adolescence, failure to thrive and short stature are frequent manifestations of MD, without relationship to digestive problems. In most of these cases, short stature is likely due to the general energy shortage of the MD itself rather than to an endocrinopathy. However, growth hormone deficiency has been occasionally reported, especially in patients with mtDNA deletions.[149]

Chronic diarrhea in infancy due to villous atrophy is a rare clinical presentation of MD, associated for example with PS and heteroplasmic large-scale single mtDNA deletions.[150] Pancreatic exocrine insufficiency is a typical manifestation of PS,[151] but it has also been reported in an autosomal recessive form of COX deficiency characterized by dyserythropoietic anemia.[152] Acute, recurrent, and chronic pancreatitis has been reported in several MD, including MELAS m.3243A>G,[153] MERRF m.8344A>G,[154] and KSS.[155]

Severe gastrointestinal dysmotility typically is a key feature of mitochondrial neuro-gastrointestinal encephalomyopathy (MNGIE) syndrome, caused by thymidine phosphorylase (gene *TYMP*) deficiency.[156,157] It causes progressive alimentary tract dysfunction with vomiting, early satiety, borborygmi, constipation and/or diarrhea, intestinal pseudo-obstruction, intestinal diverticuli (frequently in the small bowel), and abdominal distention contributing to severe cachexia, sometimes requiring long-term parenteral nutrition. In MNGIE, symptoms are usually mild before the age of 10 years. Although chronic intestinal pseudo-obstruction can also occur in adults with other MD,[158] it has also been identified in children, especially in patients with Alpers syndrome caused by *POLG* mutations or with encephalomyopathy due to mtDNA point mutations.[159,160]

Various endocrinopathies have been occasionally reported in MD, including hypoparathyroidism, hypothyroidism, hypogonadism, and Addison disease. Overall, these are uncommon complications, with the possible exception of KSS, in which periodic hormonal testing (calcium; TH, TSH; T4; sex

hormones; cortisol/ACTH) is required.[161] Diabetes mellitus is a frequent manifestation of MD in adults but is not common in children. An unusual combination of recurrent ketoacidotic episodes with hyperglycemia (requiring insulin therapy) and hyperlactacidemia in infancy has been reported in patients with complex III deficiency and recessive mutations in *CYC1*, coding for cytochrome *c*1.[162]

### 2.1.8 Ophthalmological signs and pathology

The eye is also a vulnerable target of mitochondrial pathology. In heterogeneous cohorts of pediatric patients with MD, the frequency of ophthalmological manifestations varies from 30% to 80%.[28,110,163] In infancy and childhood, the commonest manifestations are strabismus, nystagmus, and other abnormal eye movements frequently associated with Leigh or Leigh-like syndrome and other progressive mitochondrial encephalopathies. They can be the presenting symptom.[164] A specific pattern of rotary nystagmus and opsoclonus is observed in patients with hepatocerebral disease and mtDNA depletion caused by *DGUOK* mutations.[165] In early encephalopathy, optic atrophy is a frequent nonspecific finding but it does not point to any specific MD. In families with Leigh and Leigh-like syndrome, some members have optic atrophy while others present later with typical LHON signs. Such intrafamilial variability is explained by variable levels of heteroplasmy for mutations in *MTND1*, *MTND4*, or *MTND6* genes. Later in childhood and adolescence (and more frequently in adulthood), ptosis and ophthalmoplegia can occur, especially in patients with mtDNA deletions (or point mutations). The spectrum of ophthalmological manifestations of MD also includes pigmentary retinopathy, cortical blindness, macular dystrophy, cataract, and corneal dystrophy.

### 2.1.9 Hematology

Hematological manifestations of MD in pediatrics include aplastic, megaloblastic and sideroblastic anemia, neutropenia, thrombocytopenia, and pancytopenia. Hematological abnormalities are rarely the predominant symptom of MD. Infants with PS usually present sideroblastic anemia and bone marrow failure, in addition to exocrine pancreatic insufficiency and encephalopathy. Transfusion-dependent macrocytic anemia in infancy has also been reported as an accompanying symptom in some patients with systemic COX deficiency, including *COX10*[166] and *COX4I2* deficiency (in association with dyserythropoiesis),[152] and in patients with multiple RC defects.[167] In childhood and adolescence, sideroblastic anemia (requiring repeated transfusion) is a cardinal manifestation of the MLASA syndrome, with mitochondrial myopathy, exercise intolerance, and lactic acidosis.[92,93] Finally, cyclic, intermittent, or permanent neutropenia typically occurs in boys with Barth syndrome, predisposing them to severe bacterial infections. Antibiotic prophylaxis and granulocyte colony-stimulating factor therapy may be required in these cases.[168]

### 2.1.10 Biological phenotype and metabolic profile

Lactate (lactic acid), the end product of anaerobic glycolysis, accumulates when aerobic metabolism is impaired. Lactic acidemia has been considered the hallmark of oxidative phosphorylation disorders, especially in pediatrics. However, lactate has a central position in the metabolic network at the crossroad of multiple pathways of intermediary metabolism. Consequently, lactic acid is also increased in other metabolic conditions, including disorders of pyruvate metabolism, organic acidemias, FAO defects, and disorders of gluconeogenesis. Of course, lactic acidosis is also observed in many non-metabolic and nongenetic conditions associated with inadequate tissue oxygen supply. On the other hand, several authors outlined that patients with MD may present with normal lactic acid in blood and many MD patients do not have hyperlactacidemia, even some patients with severe infantile presentations.[60,73,94,169,170] Lactate concentration should be considered as a dynamic biological marker, with large intraindividual variability over time. In long-term follow-up studies, whereas most patients have increased mean blood lactate concentration, up to 50% have at least one lactate concentration in the normal range.[28] In children with MD, postprandial lactate is often elevated. In addition, in many MDs patients experience recurrent bouts of metabolic crises with huge increases of lactate and severe metabolic acidosis. Such metabolic decompensations are life-threatening crises associated with high mortality.[24,32] Although the triggers of these crises are frequently not identified, it has been hypothesized that patients have just sufficient residual OXPHOS function to sustain energy metabolism in basal conditions, but they rapidly develop energy failure under conditions requiring increased energy flux such as fever, infection, or exercise. Sensitivity and specificity of lactate measurement for the diagnosis of MD is better in CSF than in blood. It is well known that MD patients can have normal blood lactate levels despite having a clear increase of CSF lactate.

A spurious increase of lactate is frequently observed in infants and young children due to poor blood sampling conditions. It is a common artifact if the child struggles during sampling or if a tourniquet is used for phlebotomy, causing stasis and lactate accumulation from erythrocyte glycolysis. CSF lactate measurement is far less sensitive to such artifacts. Blood sampling through an indwelling intravenous catheter allows repeated measurements at rest with simultaneous determination of other energy substrates (amino acids, ketone bodies, and pyruvate).

The lactate to pyruvate (L/P) molar ratio reflects the equilibrium between product and substrate of the reaction catalyzed by lactate dehydrogenase. The L/P ratio correlates with the cytoplasmic NADH/$NAD^+$ ratio and is used as a surrogate measure of the cytosolic oxidoreduction state. When cellular respiration is impaired, as in hypoxia, pyruvate oxidation is reduced, resulting in lactic acidemia. In this case, the reduced forms of the oxidoreduction coenzymes (NADH, $FADH_2$) predominate and the L/P ratio is increased – typically above 20. A similar impairment of oxidative metabolism occurs in inborn errors of the mitochondrial RC. In contrast, in PDH deficiency, the metabolic block is upstream of the RC, the cytosolic redox potential is predicted to be unaltered and the L/P ratio is typically normal or low. A shift in cytosolic redox conditions toward a more reduced state is accompanied by elevation of circulating levels of lactate, and the predictive value of the L/P ratio as a marker of MD increases at higher lactate concentrations.[171] In our opinion, the importance and usefulness of the L/P ratio in evaluating patients suspected to have MD has been overestimated. PDH deficiency is associated with a low L/P ratio, but in many cases, the risk of PDH deficiency can be evaluated on clinical grounds. In addition, pyruvate determination is technically challenging due to the instability of pyruvate under routine phlebotomy conditions, which requires collection on ice, immediate deproteination of the blood sample, and rapid analysis. Inadequate handling and processing can result in pyruvate loss and spurious L/P ratio elevation.

Plasma amino acid chromatography can show an increase of alanine, the product of pyruvate transamination. As in the case of lactate, the sensitivity and specificity of hyperalaninemia for diagnosis of MD are low. However, in contrast to the determination of lactate and pyruvate levels, alanine is relatively resistant to artifact from improper specimen collection.

Elevations of other amino acids such as proline, glycine, and sarcosine are also nonspecific and inconstant markers of MD. The inhibition of proline oxidase by lactate may explain the hyperprolinemia. Sustained hyperglycinemia may occur in disturbances of FeS cluster metabolism and primary or secondary defects of lipoic acid synthesis, because lipoate is a cofactor of the glycine cleavage system. Accordingly, multiple RC deficiencies are also associated with hyperglycinemia and lactic acidosis (the PDH enzyme complex also requires lipoate) in several MD with multiple mitochondrial dysfunctions.[172-174] Of note, glycine concentration cannot be interpreted if the patient is treated by valproic acid which strongly inhibits glycine cleavage.

Urinary organic acids may reveal excretion of lactate, but also of several Krebs cycle intermediates. Usually, these nonspecific findings do not help in identifying a specific disease or candidate gene. A notable exception is mild methylmalonic aciduria, which points to succinyl CoA ligase complex deficiency, especially in the clinical context of early encephalomyopathy, Leigh-like syndrome, and deafness.[175]

Urinary organic acid chromatography may show elevated excretion of ethylmalonic or 3-methylglutaconic acids. Mild elevations of each can be seen nonspecifically in mitochondrial disease. Greater elevation of ethylmalonic acid occurs in ethylmalonic encephalopathy (due to *ETHE1* gene mutations).[176] In this context, clinical findings like orthostatic acrocyanosis, petechiae, and diarrhea strongly suggest this diagnosis. In addition to RC diseases, ethylmalonic aciduria also occurs in short chain acyl-CoA dehydrogenase deficiency, which is usually caused by a frequent polymorphism of the *ACADS* gene, and is without clear clinical significance.

The combined presence of ethylmalonic acid, glutaric acid, and isovalerylglycine suggests multiple acyl-CoA dehydrogenase deficiency due to deficiency of electron transfer flavoprotein or of electron transfer flavoprotein dehydrogenase.[177] Ethylmalonic acid can also be found in hereditary defects of riboflavin transport or synthesis, which are potentially treatable disorders with phenotypic overlap with the signs of mitochondrial disease.[178]

Although the mechanism of its production remains unknown, persistent excretion of 3-methylglutaconic acid (3-MGA) is a key biochemical marker for several MD, and is helpful for focusing diagnostic investigation on a small number of genes.[179] From a clinical point of view, several recognizable phenotypes are associated with elevated urinary levels of 3-MGA. In the neonatal period, patients may be acutely ill, with muscle hypotonia, metabolic acidosis, and hypertrophic cardiomyopathy caused by TMEM70 deficiency.[180] Later in infancy, males affected by Barth syndrome (mutation in the X-linked gene *TAZ*) develop cardiomyopathy with left ventricular non-compaction, growth failure, muscle weakness, and neutropenia.[181] Some patients with Barth syndrome do not excrete elevated amounts of 3-MGA. In a Canadian Hutterite population, an autosomal recessive Barth-like phenotype plus ataxia has been reported, due to *DNAJC19* mutation.[182] In Sengers syndrome, characterized by cataract and cardiomyopathy, 3-MGA excretion is mild and inconstant.[183] In the absence of cardiomyopathy, an elevated 3-MGA excretion with a neurodegenerative phenotype including psychomotor regression, Leigh-like lesions, dystonia, and deafness, suggests MEGDEL syndrome (*ME*thyl*G*lutaconic aciduria-*D*eafness-*E*ncephalopathy and *L*eigh-

like), caused by *SERAC1* mutations.[184] A similar neurodegenerative phenotype (without deafness but with congenital cataract) is also observed in patients with *CLPB* mutations, in whom 3-MGA is also observed.[185] Slowly progressive ataxia and extrapyramidal signs with optic atrophy and 3-MGA suggest Costeff syndrome (*OPA3* mutations).

### 2.2 Variability of Clinical Presentation According to Age

Mitochondrial disorders span the spectrum from congenital to adult-onset conditions that are often divided into pediatric versus adult diseases, but this distinction is often blurred by the fact that patients with MDs often experience onset in childhood, but live with the disease as adults. Nevertheless, some mitochondrial diseases, both mtDNA-related and Mendelian, do not manifest until adolescence or adulthood. We will discuss their clinical features below. Here, we will consider why alterations in the same metabolic pathway, and even within the same gene, may have delayed expression.

In general, adult-onset mitochondrial diseases tend to be associated with mtDNA mutations or with defects of mtDNA maintenance. An important determinant of the age of clinical onset is provided by the mutant fraction of mtDNA, which is presumably related to the level of residual OXPHOS activity and ATP production. The best example is provided by the NARP/MILS syndrome: in the same family, individuals harboring ~70% to 90% mutant mtDNAs usually present as young adults with the acronymic features of neuropathy, ataxia, and retinitis pigmentosa, whereas infants with ≥90% mutant genomes show LS.

However, there are also examples to the contrary, showing that the mutation load in and by itself does not explain the penetrance of mtDNA-related diseases. The best illustration of this concept comes from LHON, which is always due to homoplasmic mutations in one of three *MTND* genes and yet almost always manifests in young adults. Clearly, other causes, including nuclear modifier genes and epigenetic factors influence the expression of the disease. In fact, there is good evidence that upregulation of mitochondrial biogenesis and increased mitochondrial mass is associated with milder signs of ocular pathology.[186] The protective effect of estrogens may explain the high male-to-female ratio of LHON patients and exemplifies the importance of epigenetics.[187]

The tissue distribution of a pathogenic mtDNA mutation may also affect age-at-onset or, at least, age-at-presentation. For instance, mutations confined to skeletal muscle, either because of skewed heteroplasmy or because of their somatic and *de novo* origin, can be better tolerated in childhood than in later years, so that patients are adults when they seek medical attention.[188] Similarly, patients harboring single large-scale mtDNA deletions are more likely to present in childhood if all tissues are affected, as in KSS, whereas patients in whom single mtDNA deletions are limited to skeletal muscle present with adult-onset PEO.[189]

Compared to the “loose” rules of mitochondrial genetics, the “tighter” rules of Mendelian genetics explain why mitochondrial diseases due to mutations in nDNA tend to be more stereotypical and to appear earlier in life. However, we have to make an important distinction between those mutations that affect the RC either directly or indirectly and those that affect mtDNA maintenance, resulting in mtDNA depletion or multiple mtDNA deletions. Although disorders of mtDNA maintenance are unequivocally inherited as Mendelian traits, they share much of the clinical heterogeneity of primary mtDNA-related diseases because the polyploid nature of mtDNA is involved in both conditions. One consequence of this situation is that disorders of mtDNA maintenance may present in adult life, often as adult variants of more severe infantile diseases.[190] Thus, for example, while mutations in *TK2*, *DGUOK*, or *MPV17* typically cause mtDNA depletion syndromes characterized by severe and rapidly progressive myopathy or hepatocerebral disease in infancy or childhood, they can also cause adult-onset multisystemic syndromes often associated with PEO.[191-198] In these adult cases, the severity of the mtDNA depletion is less than in infantile cases and multiple mtDNA deletions are usually an associated or even predominant feature.

Autosomal dominant mitochondrial diseases often present in adulthood as is the case of autosomal dominant PEO due to *POLG* mutations. Another example is the unusual cause of late-onset Mendelian mitochondrial disorders attributed to a heterozygous mutation in a subunit of SDH, in two elderly sisters with progressive optic atrophy, ataxia, myopathy, and partial complex II deficiency.[199,200]

## 3. CLINICAL MANIFESTATIONS OF RESPIRATORY CHAIN DISORDERS IN ADULTS

### 3.1 Clinical History

#### 3.1.1 Adult presentations

While pediatric and adult MD patients share clinical features, in childhood-onset cases, the manifestations are often more severe and more frequently comprise multisystemic disorders than adult-onset individuals.

An important caveat is that while patients may come to medical attention with “adult-onset” conditions, careful review of the medical history may reveal manifestations had been present in childhood, but not recognized. For example, patients with PEO frequently present to ophthalmologists in adolescence or adulthood when ptosis and ophthalmoparesis are cosmetically bothersome; however, careful examination of photographs taken in childhood may reveal that the ptosis was, in fact, present many years earlier than originally thought. Alternatively, early signs and symptoms of MD may be present in childhood, but not recognized to be part of the MD until adulthood. This scenario occurs frequently with mtDNA point mutations, which may present in childhood with medical problems such as diabetes mellitus, hearing loss, or both, which are not recognized to be mitochondrial in origin until the patient develops manifestations that are more distinctive for mitochondrial disease (e.g., SLEs).

Some manifestations are age-specific. For example, developmental delay, Leigh syndrome, and growth retardation are frequent manifestations in children, whereas dementia or exercise-induced myoglobinuria are more frequent in adults. As many of the general clinical features of MD have already been described in detail in the preceding discussion of pediatric mitochondrial diseases, this section will highlight adult-specific manifestations.

### 3.1.2 Neurological presentations

As in children, in adults with MD, the central nervous system (CNS) is among the organs most frequently affected by RC dysfunction, and encephalopathy is often a presenting clinical feature[201]. While in children, psychomotor delay and regression are common CNS manifestations, the corresponding phenotypes in adults are cognitive impairment and dementia. Seizures, ataxia, and abnormal movements are common in both adult and childhood MD. Neuromuscular dysfunction with pyramidal disease, peripheral neuropathy, and myopathy is frequently present in adults with MD encephalomyopathies.

#### 3.1.2.1 *Cognitive impairment and dementia*

Adults with MD are frequently cognitively impaired; however, in contrast to infants and young children with MD, brainstem and basal ganglia lesions, characteristic of Leigh syndrome, are less frequent. Instead, adults with MD tend to show more cortical involvement with loss of cognitive functions (dementia) or relatively static cognitive involvement. MRI of the brain often shows nonspecific atrophy; however, detection of lesions of the basal ganglia, brainstem or both, basal ganglia calcification, leukoencephalopathy, cerebellar atrophy, or stroke-like lesions are suggestive of mitochondrial disease.[202,203] While leukodystrophy has been increasingly recognized as a manifestation of RC deficiencies in both children and adults[44-52], adult-onset leukodystrophy is uncommon.[204]

#### 3.1.2.2 *Brainstem and basal ganglia disease*

Although Leigh syndrome is rare in adult patients,[61,205] basal ganglia and brainstem lesions are common and manifest as movement disorders. In particular, patients with autosomal dominant or recessive CPEO due to *POLG* mutations often manifest parkinsonism with evidence of dopaminergic neuron loss on positron emission tomography (PET) scans and postmortem demonstration of loss of pigmented neurons with alpha-synuclein pathology and without Lewy bodies in the substantia nigra.[206-210] Autosomal dominant PEO with parkinsonism or with progressive supranuclear palsy-like parkinsonism have also been linked to mutations in *PEO1*, which encodes twinkle.[211,212]

#### 3.1.2.3 *Stroke-like episodes*

Although the SLEs that are the clinical hallmarks of MELAS typically occur in children and young adults, they may also initially present in older adults.[213] Because onset of SLEs is not common in adults over age 40, MELAS is often overlooked in older adults; however, alert clinicians may suspect the correct diagnosis when (1) the stroke does not conform to a large arterial distribution, (2) there are concomitant features of MD (such as sensorineural hearing loss, diabetes mellitus, and seizures), and (3) the family history is suggestive of mitochondrial disease.

#### 3.1.2.4 *Progressive ataxia*

As in pediatric MD patients, adults with MD often manifest ataxia as a prominent neurological feature of a multisystemic disease such as NARP, KSS, MERRF, MELAS, or SANDO.[214] The ataxia most often begins in childhood or adolescence, but occasionally may present in adults particularly with pathogenic mtDNA point mutations. The ataxias are typically due to cerebellar dysfunction, with the notable exception of patients with sensory ataxias due to *POLG* mutations.[70] Adult-onset cerebellar ataxia has been observed in patients with $CoQ_{10}$ deficiency due to autosomal recessive *ADCK3* mutations.[215]

#### 3.1.2.5 *Muscle*

In contrast to infants, who frequently present with isolated myopathy, adults with MD typically manifest myopathy in combination with brain involvement (encephalomyopathy) or as part of a multisystemic disease.[216] The myopathy usually presents as weakness of proximal limb and extraocular muscles.

The most common pure myopathic phenotype in adults is CPEO often in association with oropharyngeal and limb myopathy due to single large-scale deletions of mtDNA.[217] In addition, adult-onset CPEO or CPEO-plus may be inherited in autosomal dominant or recessive patterns due to nDNA gene mutations such as *POLG*, *TK2*, or *MPV17* that cause multiple deletions of mtDNA.[193,196,215] Less commonly, sporadic adults have presented with isolated skeletal myopathy with prominent exercise intolerance but without CPEO due to mtDNA mutations, particularly in the cytochrome *b* gene (*MTCYB*).[188,218] In such cases, muscle biopsies show RRFs that are COX-positive – a morphological clue to the molecular diagnosis. Another peculiar association is sporadic adult-onset mitochondrial myopathy with prominent dystrophic changes and RRF-COX-negative myofibers on histology due to apparently spontaneous mutations in *MTTM*, which encodes mitochondrial $tRNA^{Met}$.[219,220]

#### 3.1.2.6 *Peripheral nerve*

Like ataxia and myopathy, peripheral neuropathy is a common MD manifestation, which is most frequently observed in the setting of multisystemic disorders in adults as well as children.[216,221] For example, peripheral neuropathy is a defining clinical feature of NARP, SANDO, Navajo neurohepatopathy (and other *MPV17*-related disorders), and MNGIE and is often present in patients with MELAS, MERRF, and Leigh syndrome. The peripheral neuropathy is frequently mild and overshadowed by more severe involvement of other organs such as the brain in MELAS, MERRF, or KSS.

The neuropathy is typically chronic sensorimotor axonal in nature; however, in MNGIE, the neuropathy is characteristically demyelinating and can mimic chronic inflammatory demyelinating polyneuropathy.[222] With *POLG* mutations, large sensory fibers are preferentially affected causing loss of proprioception and sensory ataxia.[70]

Curiously, peripheral neuropathy is prominent in disorders of mtDNA maintenance such as MNGIE as well as *POLG*-related and *MPV17*-related disorders.

Inherited non-syndromic peripheral neuropathy (CMT disease) has been linked to mutations in over 40 genes including 5 that encode mitochondrial proteins: *MFN2* (causing CMT 2A), *GDAP1* (CMT 4A, CMT 2K, CMT RIA), *DHTKD1* (CMT 2Q), *AIFM1* (CMT X4), and *PDK3* (CMT X6).[223] Interestingly, two of these mitochondrial proteins are involved in mitochondrial dynamics; mitofusin-2 (encoded by *MFN1*) is required for fusion of mitochondrial outer membranes while ganglioside-induced differentiation-associated protein 1 (encoded by *GDAP1*) is involved in mitochondrial fission.[224,225]

### 3.1.3 Liver disease

In contrast to pediatric MD disorders, which often manifest severe hepatopathy and even frank hepatic failure as in Alpers syndrome, adult MD diseases less frequently show liver disease and when present, the hepatopathy is typically mild with fatty liver (hepatic steatosis) without liver dysfunction.[226] Curiously, hepatopathy has been observed occasionally in adults with mtDNA point mutations, but more commonly among adults with defects of mtDNA maintenance due to *POLG*, *MPV17*, and *TYMP* mutations.[70,156,196,227]

*POLG* genotypes may also predispose to sodium valproate susceptibility as indicated by a prospective study of 17 children and young adults who were initially healthy but developed valproic acid hepatotoxicity; heterozygous *POLG* variants were identified in 8 (47%).[96]

### 3.1.4 Cardiac pathology

Like children with MD, adults with MD frequently manifest cardiopathy; however, the specific phenotypes and etiologies differ in the two age groups.[228] In contrast to the severe and rapidly progressive cardiomyopathy that is characteristic of several infantile-onset multisystemic MDs (described in Section 2.1.5), adults with MDs manifest a wider range of cardiopathy that is of variable severity and progresses slowly. The major cardiac phenotypes in adults with MD include ventricular pre-excitation, WPW syndrome, cardiac conduction block, and cardiomyopathies (hypertrophic, dilated, and restrictive).

Cardiopathy is a common feature of several well-defined MD syndromes. Ventricular pre-excitation and WPW are particularly frequent in LHON (10%), MELAS (13%), and MERRF (17%) and rare mtDNA point mutations.**[127,228-230] Some patients with mtDNA point mutations, who are symptomatic from cardiac arrhythmias and have ventricular pre-excitation have had successful ablation of accessory pathways. Nevertheless, in most MD patients, WPW and ventricular pre-excitation are asymptomatic and presumed to be benign although their natural histories are unknown.

In contrast to pre-excitation syndromes, cardiac conduction block, a cardinal feature of KSS, is progressive and can be fatal if not treated with a pacemaker.[231] Heart block has also been reported in adults with mtDNA point mutations and rarely with MNGIE; however, the progression of this cardiac defect in those settings is unknown.[127,156,232]

Cardiomyopathy, which is often hypertrophic in the early stages, has been observed frequently in adult patients with mtDNA mutations, particularly the "MELAS" m.3243A>G (38% to 56% of patients) and "MERRF" m.8344A>G (33% of patients) mutations.[127,232,233] Overall, hypertrophic cardiomyopathy may be present in up to 40% of all MD patients.[110,234] The hypertrophic cardiomyopathy is typically predominant in the left ventricle and can progress to dilated cardiomyopathy with heart failure that may be fatal. Mutations in other mtDNA-encoded tRNA genes, and less commonly, ribosomal RNA genes have been associated with cardiomyopathy and the mt-tRNA$^{Ile}$ gene (*MTTI*) appears to be frequently associated with maternally inherited cardiomyopathy.[235] Particularly intriguing is the m.4300A>G *MTTI* mutation which has been identified in a homoplasmic state in multiple families with maternally inherited isolated hypertrophic cardiomyopathy of variable degrees of penetrance.[236] Less common than hypertrophic cardiomyopathy, dilated and restrictive cardiomyopathies have been reported in adult patients with mtDNA mutations.[227-239] Left ventricular non-compaction and histiocytoid cardiomyopathy have been mainly described in pediatric MD patients.[228]

### 3.1.5 Renal diseases

In contrast to nephropathy in pediatric MDs, renal involvement in adult MDs has not been characterized with the exception of patients harboring the m.3243A>G mutation.[240-242] Published case reports and small case series of patients with this mutation have documented renal disease, typically focal segmental glomerular sclerosis.[243,244] In addition, tubulointerstitial nephropathy and enlarged cystic kidneys have been observed. The renal disease often manifests in early adulthood and may be the initial manifestation of the disease.[242] Proteinuria is an early sign, but nephrotic syndrome is present in the minority of patients with renal disease. The nephropathy can be severe enough that at least four patients have received renal transplants.[245] It is the mtDNA mutation, rather than diabetes mellitus (which is also common with m.3243A>G), that seems to be the primary cause of the renal disease.[243,246] Some m.3243A>G patients with glomerulopathy and deafness have been misdiagnosed as having Alport syndrome.[245]

Urinary assessment of 117 adult patients with heterogeneous mitochondrial diseases revealed abnormalities of markers of tubular dysfunction (retinol binding protein) and glomerulopathy (albumin) in ~30% to 40% of patients with the m.3243A>G mutation and with other mitochondrial diseases.[241] Serum creatinine levels were normal in all patients, but hyponatremia (14%), hypophosphatemia (10%), and hypomagnesemia (14%) were not uncommon. The authors of this report concluded that kidney dysfunction is common in adult MD in the absence of elevated serum creatinine.

### 3.1.6 Gastrointestinal manifestations and nutritional issues

The gastrointestinal system is frequently affected in adults with mitochondrial diseases and most prominently in MNGIE patients.[156,247] All segments of the gastrointestinal system can be affected from the oropharynx through the esophagus, stomach, and small and large bowels. Nausea with or without vomiting is a common feature of many mitochondrial diseases, but the etiology may be difficult to pinpoint. Gastroesophageal dysfunction is a causal factor in many cases; however, vestibulopathy due to hyperlactatemia also contributes to nausea and vomiting.

Weakness of the oropharyngoesophageal muscles manifests as dysphagia, which is often accompanied by dysarthria with prominent nasal quality due to impaired soft palate mobility.[248] In a cross-sectional survey of 98 adult patients with genetically confirmed MD, difficulty swallowing was reported by nearly half (47 [48%]), with the highest frequency in patients with single mtDNA deletions (15/23 [65%]). In addition, dysphagia was common with other mtDNA defects: m.3243A>G (10/27 [37%]), m.8344A>G (3/9 [33%]), and multiple mtDNA deletions (14/28 [50%]). Among patients with *POLG* mutations, particularly those with SANDO, dysphagia is frequently present. In patients with MNGIE, dysphagia has been noted in 21%.[156] Dysphagia is often mild and may be overlooked, but can also be severe and manifest with nasal regurgitation.[249] Cricopharyngeal achalasia with incomplete opening of the upper esophageal sphincter has been noted to cause dysphagia in the pharyngeal phase of swallowing in patients with CPEO or KSS.[249]

Gastroparesis, presenting as early satiety, is a common feature of MNGIE, MNGIE-like cases, and other MDs.[156,250,251] Gastroparesis, independent of diabetes mellitus, has also been observed in patients with the m.3243A>G mutation.[252]

Intestinal dysmotility in adult MDs ranges from mild constipation or diarrhea to severe pseudo-obstruction. Abdominal pain can be a symptom of gastrointestinal dysmotility and varies in quality and severity from mild discomfort to excruciating crampy pain. Intestinal dysmotility is typically severe in MNGIE and has been associated with diverticulosis and diverticulitis in the small bowel.[156] Although both neurogenic and myogenic dysfunction can contribute to intestinal dysmotility in MDs, enteric myopathy, particularly severe in the outer muscle layer, appears to be the major cause of intestinal dysmotility in MNGIE.[253] Bacterial overgrowth is a common complication of gastrointestinal dysmotility.

Gastrointestinal dysfunction may cause weight loss and is a contributing factor to the thin body habitus of MD patients. Intake of food may be limited by recurrent nausea, vomiting, and early satiety caused by gastroesophageal dysmotility. Impaired transit of foods due to intestinal dysmotility can also limit nutritional intake in MD patients.

### 3.1.7 Endocrinopathies

While pediatric MD patients uncommonly manifest a variety of endocrinopathies, adults with MD frequently develop diabetes mellitus but rarely have other endocrine disorders.[254,255] The diabetes is often associated with hearing loss due to an mtDNA mutation, a syndrome commonly described as maternally inherited diabetes and deafness (MIDD) or diabetes and deafness (DAD). Typically, MIDD is caused by the m.3243A>G mutation; however, other mtDNA point mutations have been associated with this phenotype.[254,256] In a large longitudinal study of 85 individuals with the m.3243A>G mutation, 39% of individuals fully symptomatic with MELAS had diabetes mellitus while 30% of carrier relatives had diabetes.[257,258] With this mutation, diabetes may present at any age, but typically in adulthood with a mean age-at-onset of 37 to 38 years.[259,260] The onset is usually insidious, but can be acute with ketoacidosis. The diabetes is mainly due to insulin deficiency, but insulin resistance also contributes in some cases.

Besides diabetes mellitus, other endocrinopathies have been rarely described in adult MD patients. Hypoparathyroidism was observed in a 54-year-old woman with diabetes, deafness, muscle weakness, and the m.3243A>G mutation while hypergonadotrophic and hypogonadotrophic hypogonadism have been observed in patients with MNGIE.[261-263] Although hypothyroidism has not been documented to be a manifestation of MD, in a large cohort of predominantly adult patients harboring the m.3243A>G mutation, levels of total triiodothyroxine ($T_3$) and thyroxine ($T_4$) were lower both in patients with MELAS and in carrier relatives, compared to controls.

### 3.1.8 Ophthalmological signs and pathology

Ocular pathology is common in adult MD diseases.[264-266] Ophthalmopathy can be sole clinical manifestation as exemplified by LHON and dominant optic atrophy (DOA or Kjer syndrome) due to *OPA1* mutations, or part of a syndrome such as KSS or MELAS. Optic neuropathy due to degeneration of retinal ganglion cells (RGCs) is the hallmark of maternally inherited LHON as well as autosomal DOA. LHON characteristically presents in young males in their teens or early adulthood with rapid and painless loss of vision in one eye, followed in a few weeks or months by loss of vision in the second eye. The visual defect typically begins with loss of color and fine vision as well as a central scotoma on visual field testing. In the early phase of the disease, peripapillary telangiectatic microangiopathy, swelling of the nerve fiber layer surrounding the optic disc, and absence of leakage on fluorescein angiography are characteristic of LHON. The vision loss usually stabilizes by one year after onset and most patients are legally blind. Spontaneous partial recovery of visual acuity can occur with variable frequency depending on the specific mtDNA mutation. Three mtDNA point mutations in complex I subunit genes account for over 90% of LHON cases: ND4 (m.11778G>A), ND1 (m.3460G>A), and ND6 (m.11484T>C). In contrast to most pathogenic mtDNA mutations, which are heteroplasmic, LHON mutations are typically homoplasmic. Haplogroups of mtDNA are genetic modifiers of

LHON; haplogroup J increases penetrance of the *MTND4* and *MTND6* LHON mutations.[267] *In vitro* studies demonstrating cytoprotective effects of estrogens in cytoplasmic hybrid (cybrid) cells harboring LHON mutations suggest that the male predominance of LHON is due to ameliorative effects of estrogens in women.

The Mendelian counterpart of LHON is DOA, which presents with childhood-onset slowly progressive bilateral loss of central vision with centrocecal scotomas, loss of color vision, and temporal optic nerve pallor on examination.[268] Both genders are equally affected and spontaneous recovery of vision is rare. The onset and progression are variable even within families. In addition to causing purely ophthalmological DOA, *OPA1* mutations also cause a DOA-plus syndromic condition characterized by optic neuropathy, sensorineural hearing loss, ataxia, myopathy, axonal sensory-motor peripheral neuropathy, ptosis, and ophthalmoplegia.[269,270] Muscle biopsies of DOA-plus patients show scattered ragged-red, COX-negative fibers, and multiple deletions of mtDNA. It remains to be determined why defects of OPA1, a mechanoenzyme needed for fusion of mitochondrial inner membranes, preferentially cause optic neuropathy as well as instability of mtDNA.

Curiously, subsets of patients with both LHON and DOA have been associated with white matter lesions that are indistinguishable from multiple sclerosis.[271,272]

Other mitochondrial optic neuropathy plus syndromes include Friedreich ataxia (due to autosomal recessive mutations in frataxin, which is required for mitochondrial FeS cluster biogenesis); optic atrophy 3 with cataracts (due to autosomal dominant *OPA3* mutations); optic atrophy 9 with or without deafness (due to recessive mutations in TMEM126A, a mitochondrial inner membrane protein); X-linked deafness-dystonia-optic atrophy (Mohr–Tranebjaerg syndrome caused by mutations in TIMM8A, a component of the mitochondrial intermembrane transport complex); and a late-onset recessive optic atrophy-ataxia syndrome (associated with a heterozygous mutation in the SDHA subunit of complex II).[200,273-276] Asymptomatic optic atrophy has been observed in patients with MERRF and MELAS.[213,277]

The retinal pigment epithelium is also frequently affected in adult MD patients and may cause difficulty with night vision. Pigmentary retinopathy is a defining feature of KSS and NARP, which typically begin in childhood, but the retinopathy may not be evident until adulthood.[8,278] The pigmentary retinopathy of KSS differs from retinitis pigmentosa and has been frequently described as “salt and pepper” pigment clumping with mottled hypopigmented and hyperpigmented areas producing a “moth-eaten appearance.”[278-280] The retinal degeneration often occurs in the peripapillary region. In NARP, the retinal dystrophy may appear as a “salt and pepper retinopathy” or as a “diffuse pigmentary retinopathy” which can cause severe night blindness.[281] In later stages of KSS and NARP, optic atrophy has been noted.

In contrast to KSS and NARP, which cause symptomatic pigmentary retinopathy, the retinopathy observed in MELAS and MIDD patients with the m.3243A>G mutation is typically asymptomatic and predominantly affects the posterior pole.[282-284] Perifoveal atrophy/hypopigmentation as well as pattern macular dystrophy with pale deposits and pigment clumping have been described.

### 3.1.9 Hematology

Hematological abnormalities are uncommon manifestations of childhood MDs, and are even rarer in adult MD patients. A small number of adults with MLASA due to *RARS2* or *PUS1* mutations have been reported mostly with childhood-onset and rarely with young adult-onset.[285-287] Infants with MD and anemia rarely survive to adulthood with the exception of patients with PS, who survive sideroblastic anemia and manifest CPEO or KSS in adulthood.[13,151,152]

### 3.1.10 Biological phenotype and metabolic profile

As well-documented in Section 2.1.10, lactic acidemia has been considered a hallmark of mitochondrial RC-oxidative phosphorylation disorders in pediatrics as well as in adult medicine. However, as in children with MD, elevated lactate is an insensitive biomarker of MD in adults. In two retrospective studies of adults with well-documented MD, sensitivity of lactic acidemia was 15% to 40%, while specificity was 92.7% to 98.5%.[288,289] In AD patients with central nervous system involvement, sensitivity was 9.1% with specificity of 92.8%.[289] In these studies, elevation of blood L/P ratio was even less sensitive, 9% to 11.5%, than lactic acidemia but sensitivity was 98.5% to 100%.

Nevertheless, L/P can be useful for distinguishing RC-oxidative phosphorylation defects from PDH complex deficiency.

Assessments of CSF lactic acid obtained by lumbar puncture or intracranial lactate by $^{1}$H-MRS can be helpful in diagnosing MD in adults with central nervous system pathology. In MELAS and oligosymptomatic patients with the m.3243A>G mutation, $^{1}$H-MRS has shown elevated ventricular lactate levels relative to healthy controls.[290]

Preliminary studies of serum fibroblast growth factor 21 (FGF-21) have indicated high sensitivity and specificity in adult MD patients particularly when muscle is clinically affected. In the original retrospective study, elevation of serum FGF1 in adult MD patients with muscle involvement showed a sensitivity of 89.7% with a specificity of 91.7% and a follow-up prospective study revealed a sensitivity of 68.5% and specificity of 95.5%.[288,289] While these findings are promising, additional validation of the diagnostic value of serum FGF-21 for MD is required. In contrast, a study of patients with the m.3243A>G mutation failed to detect correlations between serum FGF-21 levels with disease severity or with heteroplasmy levels in urinary epithelial cells or leukocytes suggesting that this biomarker is unlikely to be useful for monitoring and predicting disease progression with this mtDNA mutation.[291]

While children who are suspected of having mitochondrial diseases routinely undergo measurements of plasma amino acids, urine organic acids, and acyl-carnitine profiles, these tests are less frequently used in adults. Plasma amino acid assessments often reveal elevations of alanine, which is generated by transfer of an amino group from L-glutamate to pyruvate by alanine transaminase. In addition, glycine, proline, sarcosine, and tyrosine may be elevated.[292] Urine organic acids may show elevated lactic acid, tricarboxylic acid cycle intermediates, ethylmalonate, 3-methylglutaconic acid, and dicarboxylic acid but the abnormalities are not very striking or specific for mitochondrial disease.[293] Similarly, acylcarnitine profiles may reveal nonspecific abnormalities including low free carnitine, elevated acyl to free carnitine ratio, and increased acyl-carnitines suggestive of FAO defects.[292] Although the sensitivity and specificity of these abnormal amino acid, organic acid, and acylcarnitine profiles have not been formally assessed in MD patients, when present, they should be considered supportive of mitochondrial dysfunction.

A metabolic profiling study revealed elevated plasma creatine as a potential biomarker of mitochondrial dysfunction in MD patients; however, further validation of this metabolite is needed.[294]

### 3.1.11 Exercise physiology

In adult patients, cardiopulmonary exercise testing by cycle ergometry or treadmill assessments has been used to diagnose MD and to assess treatment efficacy.[295,296] Although these physiological tests have limited diagnostic utility, as studies have shown disappointingly low sensitivity and specificity, they are useful in clinical trials.[295,297-299] In contrast, measurement of venous oxygen levels in forearm muscles with handgrip exercise appears to have high sensitivity and may be of diagnostic value in patients with mitochondrial myopathies.[300,301]

## 4. DIAGNOSTIC ALGORITHM

The following rule-of-thumb concepts can be helpful in the differential diagnosis of mitochondrial diseases.

1. *Family history*. If maternal inheritance is evident, we must be dealing with an mtDNA-related disease, a diagnostic giveaway. However, because of heteroplasmy, maternal inheritance is not always obvious and family history has to be taken meticulously, trying not to ignore soft signs in the maternal lineage, such as short stature, migraines, hearing loss, diabetes, exercise intolerance, and psychiatric disorders (especially depression). On the other hand, we should not forget that disorders due to single mtDNA deletions (KSS, PS, CPEO) are almost always sporadic[302] as are diseases due to *de novo* mutations, such as most changes affecting *MTCYB*.[303] Conversely, parental consanguinity suggests autosomal recessive inheritance and homozygosity mapping has enabled identification of several mutated nuclear genes.

2. *Multisystem disorders*. This feature is common to both mtDNA- and nDNA-related diseases. However, some syndromes are so typically associated with mtDNA mutations (KSS, PS, MELAS, MERRF, NARP, MILS, LHON) or with mutations in specific nuclear genes (MNGIE, SANDO, Alpers, DOA) that they provide useful shortcuts to the molecular diagnosis. On the other hand, a diagnosis of LS tells us that we are dealing with a mitochondrial disease, but gives us little information about which genome is involved or to which subgroup of mitochondrial disorders the child belongs.

   Three organs are often involved, usually together with the brain but occasionally in the absence of encephalopathy. Hypertrophic cardiomyopathy can be the presenting and predominant problem. It is often rapidly fatal but it can also be chronic, as in Barth syndrome. Liver involvement is

typically associated with mtDNA depletion or with defects of mtDNA translation ("hepatocerebral syndromes") but rarely can be seen as part of direct or indirect hits to RC complexes. In children, nephropathy typically presents as proximal tubulopathy with Fanconi syndrome, but when it presents as nephrosis with proteinuria, it should raise the diagnostic suspicion of $CoQ_{10}$ deficiency.

3. *PEO*. After excluding myasthenia gravis and oculopharyngeal muscular dystrophy, PEO is a strong clue to mitochondrial dysfunction. Specifically, PEO is commonly associated with primary mtDNA large-scale rearrangements (KSS, CPEO) and with nDNA-related defects of mtDNA maintenance, also resulting in mtDNA deletions or depletion. Sporadic cases suggest primary mtDNA mutations whereas autosomal dominant or recessive inheritance suggests disorders of mtDNA maintenance. Maternal inheritance is rare but there are reports of PEO due to mutations in mtDNA tRNA genes, including the common MELAS mutation, m.3243A>G.[304]

   The extreme vulnerability of extraocular muscles to mitochondrial dysfunction correlates with their dependence on oxidative metabolism and this, in turn, correlates with their unique function, as both saccadic and smooth pursuit eye movements deal with the same mechanical load throughout life but occur uninterruptedly through the day.[305] In agreement with their sustained and energetically demanding contraction pattern, extraocular muscles are resistant to fatigue,[305] rich in mitochondria, and second only to the heart in vascularization.[306]

   A molecular basis for the preferential involvement of extraocular muscles has been offered by histochemical and single fiber studies from 13 patients with PEO, mostly due to single or multiple mtDNA deletions.[307] There were more COX-negative fibers and the mutation threshold for COX deficiency in single fibers was lower in extraocular than in limb muscles but this did not apply to one patient with the m.3243A>G MELAS mutation.[307]

4. *Onset* varies widely for mtDNA-related diseases, even within members of the same family, most likely due to varying degrees of heteroplasmy.[10] In most Mendelian disorders except for defects of mtDNA maintenance, onset is in infancy or early childhood, often following several months of normal development. Prenatal manifestations are uncommon, as shown by the rarity of signs like arthrogryposis and dysmorphisms. In a few instances, fetal cardiopathy is revealed by fetal bradycardia or by echocardiography. Adult onset is the rule in disorders of intergenomic communication, most notably multiple mtDNA deletions with PEO, probably because of the overlap of mitochondrial and Mendelian genetics (see Section 1.2).

5. *Laboratory exams.* Lactic acidosis is a hallmark of mitochondrial diseases. Although extremely common, it is neither invariably present nor necessarily severe. Increased lactate in the CSF is frequent and detectable by $^{1}$H-MRS.

   Serum CK is only mildly elevated in most mitochondrial disorders except in the mtDNA depletion myopathy due to TK2 deficiency, where increased serum CK serves as a diagnostic red flag.

   In 2011, FGF-21 was introduced as a valuable new serum or plasma biomarker for the detection of mitochondrial disorders, especially those involving skeletal muscle.[289] The diagnostic accuracy of FGF-21 was better than that of all conventional biomarkers, including lactate; this has been confirmed by a second study.[288] FGF-21 is not yet measured routinely, possibly because it requires an enzyme-linked immunosorbent assay (ELISA) that is not widely available.

6. *Muscle biopsy*. Although muscle biopsy is an invasive procedure, it remains a gold standard for the diagnosis of mitochondrial diseases, especially those due to primary mtDNA mutations.[308] Systematic analysis of frozen, cross-sectioned muscle with the histochemical reactions for COX, SDH, or both stains superimposed, provides valuable diagnostic clues (Fig. 129-3).

   It is important to remind the reader that the SDH stain reflects the activity of complex II of the RC (succinate-ubiquinone oxidoreductase), the only complex that is entirely encoded by nDNA. This means that the SDH stain is unaffected by deleterious mutations of mtDNA while at the same time is an excellent marker of mitochondrial abundance. In contrast, the three catalytic subunits of COX are encoded by mtDNA and COX stain is a very good index of mtDNA function. Not only mutations in *MTCO1*, *MTCO2*, or *MTCO3*, but also any mtDNA mutation impairing mitochondrial protein synthesis (i.e., large-scale deletions or point mutations in tRNA or rRNA genes) will result in a decrease or lack of COX stain.

   Another important reminder is that heteroplasmic mtDNA mutations are not uniformly distributed along syncytial muscle fibers, such that adjacent segmental sections may have widely different amounts of mutant mtDNAs. It follows that cross sections of the muscle biopsy from patients harboring pathogenic mtDNA mutations will show a mosaic pattern of COX-negative and COX-positive fibers (Fig. 129-3).

As mitochondria proliferate in response to energy failure (although the *primum movens* of this phenomenon remains obscure), COX deficiency is often accompanied by excess mitochondria, showing as hyperintense SDH stain (these fibers are dubbed "ragged-blue," by analogy with the more famous "ragged-red" fibers revealed with the modified Gomori trichrome stain) (Fig. 129-3). Thus, finding a mosaic pattern of normally stained fibers admixed with "ragged-blue" COX-negative fibers in a muscle biopsy is a robust clue to the diagnosis of an mtDNA-related disease affecting mitochondrial protein synthesis (more rarely, affecting one of the three mtDNA-encoded COX subunits). A mosaic pattern of ragged-blue but COX-positive fibers suggests a mutation in an mtDNA gene that encodes a RC subunit other than COX I, II, or III (e.g., a complex I subunit or cytochrome *b*).[188] This is discussed in greater detail in Chapter 99B.

Heteroplasmy explains not only the mosaic distribution of COX-negative fibers but also the different degrees of "COX-negativity," as some fibers have a deficiency rather than a lack of stain. To help reveal the COX-deficient fibers, the combined COX/SDH stain is extremely effective: when the COX and SDH stain are superimposed in normal fibers, the brown COX stain prevails and overshadows the blue SDH stain. However, even a small decrease in COX activity allows the SDH stain to shine through, making the COX-deficient fibers appear blue. Nor is this technique limited to skeletal muscle, as it has been successfully applied to reveal COX-deficient motor neurons in patients with ALS,[309] MELAS,[310] and other mtDNA-related diseases.[311]

As expected, in Mendelian disorders, for example COX-deficient LS, muscle histochemistry shows more or less severe but *diffuse* COX deficiency,[312] which is an important diagnostic clue (Fig. 129-3). In fact, as routine muscle histochemistry is usually non-informative in LS, COX histochemistry is a useful way to rule in or out one important cause of the disease (Fig. 129-3). There is one important exception to this rule: due to the overlapping features of Mendelian and mitochondrial genetics, muscle biopsies from patients with defects of mtDNA maintenance, and especially from cases of PEO with multiple mtDNA deletions, show the same mosaic pattern of COX-negative ragged-blue fibers as do primary mtDNA mutations. The presence of this morphological pattern, together with a family history suggestive of Mendelian inheritance, is an incentive to look for mutations in mtDNA maintenance genes even in clinically atypical cases.[313,314]

Ultrastructural studies of muscle are usually only confirmatory in that they show mitochondrial proliferation. However, the presence of paracrystalline inclusions, consisting of crystals of CK, reinforces the diagnosis of mitochondrial diseases (although it is still unclear why CK precipitates in pathological conditions)[315]; the presence of giant displaced mitochondria suggests problems with mitochondrial dynamics[316]; and abnormal cristae are typical of Barth syndrome.[317]

7. *Biochemistry*. Biochemical analysis is usually conducted in frozen muscle tissue or in cultured fibroblasts. A detailed, step-by-step procedure for the measurement of the activities of complexes I to IV has been published[318] and should facilitate comparison of data among laboratories, thus far a thorny issue.

   Individual respiratory complex deficiencies are expected to occur in patients with mutations in mtDNA protein-coding genes and with mutations in genes encoding single RC subunits (direct hits) or assembly genes for specific complexes (indirect hits). While this is largely the case, because the RC is organized in supercomplexes, it is more likely that a severe defect in one complex is associated with less severe defects in one or more additional complexes. Thus, for example, mutations in the *MTCYB* gene cause defects of complex III but also of complex I.[303]

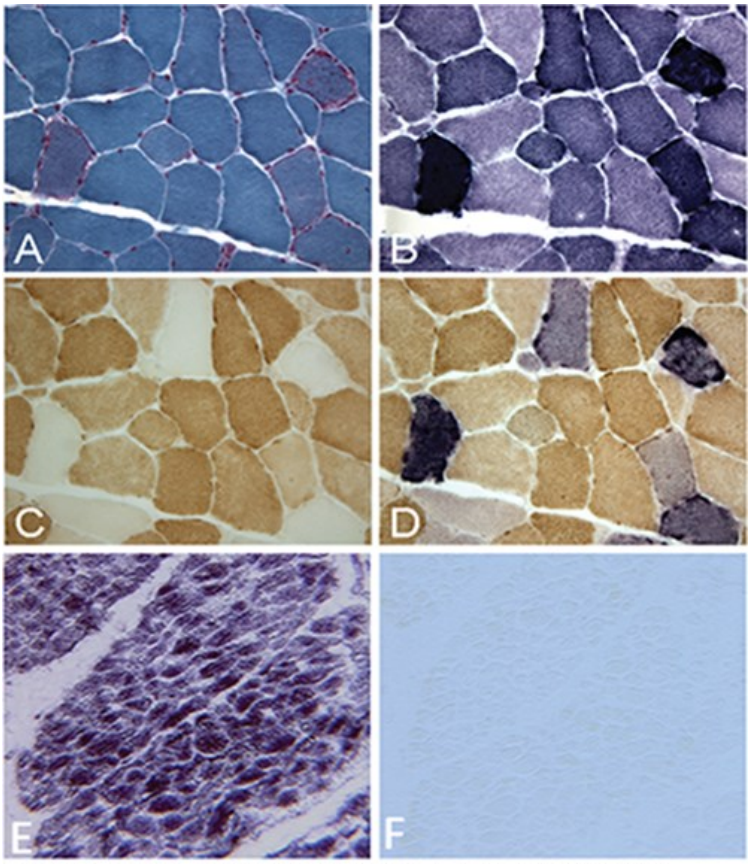
   Impaired biochemical activities of all complexes containing subunits encoded by mtDNA suggests either mutations in mtDNA genes controlling protein synthesis globally (tRNA or rRNA genes, single deletions) or mutations in nDNA genes controlling mtDNA maintenance (multiple deletions, depletion, defects of mtDNA translation, defects of the IMM lipid milieu).

   Some special patterns may be suggestive of specific problems. Combined defects of complex II + III are often associated with primary $CoQ_{10}$ deficiency whereas combined defects of complexes I, II, and III may be due to defective assembly of the FeS cluster proteins, especially when they are accompanied by aconitase deficiency.[319]

**Figure 129-3.**

Serial sections from muscle biopsies of patients with an mtDNA disease (MERRF syndrome, **A** to **D**) and an nDNA mitochondrial disease (deficiency of *SCO2*, an nDNA assembly gene for COX, **E** to **F**). **A** to **D**. With the modified Gomori trichrome stain (**A**), two ragged-red fibers are evident. The same

fibers appear “ragged-blue” with the succinate dehydrogenase (SDH) stain (**B**); are pale with the cytochrome *c* oxidase (COX) stain (**C**); and appear more or less intensely blue with the combined COX/SDH stain (**D**). **E** and **F**. A patient with mutations in *SCO2*, an nDNA assembly gene of COX. In contrast to the checkerboard pattern of the muscle biopsy in an mtDNA-related disease (above), this biopsy shows normal fiber type-related variable intensity with the SDH stain (**E**) but uniformly absent reaction with the COX stain (**F**).






## 5. OUTCOME AND PROGNOSIS

Given the genetic and clinical heterogeneity of mitochondrial diseases, it is extremely difficult to foresee the outcome of each disorder and even more difficult to offer a prognosis, which is frustrating to families. To complicate matters further, some genetically defined diseases have been reported only in a few patients or in a few families, thus making generalizations impossible.

In general terms, the outcome is less ominous in mtDNA-related than in Mendelian disorders because heteroplasmy allows for residual RC function, milder symptoms, and longer survival. This is best exemplified by the NARP/MILS syndrome, which, however, also tempers against generalizations, as family members with the relatively benign NARP syndrome may have relatives devastated by Leigh syndrome in infancy or early childhood.

In contrast, nDNA mutations tend to be more stereotypical and autosomal recessive disorders are usually more severe in their presentation, as shown by the Leigh syndrome presentation, which can be due to both direct and indirect hits on the RC and oxidative phosphorylation. Again, an exception has to be made for defects of mtDNA maintenance because of the modifying effects of mtDNA heteroplasmy on clinical severity, age at presentation, and disease course.

For the most common mtDNA-related diseases, such as KSS, MELAS, MERRF, and LHON, epidemiological studies, defining the natural history and identifying biomarkers may help in establishing prognosis. Thus, for example, an international multicenter study of 226 families in which a single mtDNA deletion had been associated in the proband with KSS, PEO, or PS showed that affected women have a small but measurable risk (1 in 24 births)

of having an affected child.[302]

For patients with MELAS (or, more accurately, for individuals carrying the m.3243A>G mutation), a natural history is emerging from studies of large family cohorts.[257,320,321] Although the natural history is useful and a prerequisite for the evaluation of therapeutic trials, it cannot by itself provide reliable prognostic information, which has to be based on predictive biomarkers. Simply measuring the mutation load in different easily accessible tissues (blood, saliva, urine) is not sufficient. Perhaps statistical modeling using multiple parameters including urinary mutation load[322] and age at onset will improve the accuracy of prediction of disease severity and progression, as described for large mitochondrial deletions.[323] A prospective cohort study of cerebral metabolic abnormalities assessed by proton magnetic spectroscopic imaging ($^1$H-MRSI) in 135 clinically heterogeneous m.3243A>G mutation carriers and 30 healthy volunteers allowed "converters" (i.e., carriers that would develop the MELAS syndrome during follow-up) to be distinguished from non-converters.[324] Clinically, converters and non-converters did not differ at baseline but brain lactate and total choline levels were reliable predictors of the risk of individual mutation carriers to develop the MELAS syndrome.

A recent retrospective analysis of 42 patients harboring the m.8344 A>G mutation has better defined the MERRF syndrome and established the frequency of symptoms and signs in the following descending order: myoclonus, weakness, ataxia, seizures, deafness, dementia, lipomatosis, neuropathy, exercise intolerance with hyperCKemia, PEO/ptosis, optic atrophy, cardiomyopathy, respiratory distress, diabetes, myalgia, tremor, migraine.[325] However, it was noted that none of these features had predictive value for the development of encephalopathy or myopathy, and neither did age at onset, disease duration, age at evaluation, blood lactate levels, or mutation load in blood. The need of predictive biomarkers was also noted.[325]

For LHON, an epidemiological study conducted in Northeastern England revealed a minimum point prevalence of visual deficit of 3.22 per 100,000 people and, predictably for this condition which has incomplete genetic penetrance, a higher prevalence of the three canonical LHON mutations (11.82 per 100,000).[326] This same study also disclosed an unexpectedly high frequency of heteroplasmy and recommended that each maternal family member seeking advice about the risk of visual loss should receive mtDNA molecular testing.

## 6. THERAPEUTIC APPROACH AND GENETIC COUNSELING

Although we have defined mitochondrial diseases as defects of the RC, there is no panacea for RC dysfunction and no universal therapy for mitochondrial disorders. First, it is important to state the obvious, that incurable diseases are not untreatable diseases and both pharmacological (e.g., antiepileptic drugs) and surgical (e.g., blepharoplasty) remedies are useful in prolonging and improving the life of mitochondrial patients. Throughout this chapter, we have emphasized the minority of known MDs for which a treatment response is proven or plausible.

However, we will briefly review here a few strategies aimed at curing (or even preventing) mitochondrial diseases, some of which have shown promise either *in vitro* or in animals and others have shown encouraging preliminary results in humans.

The most obvious therapeutic approach is to enhance RC function, thus mitigating both energy crisis (ATP deficit) and oxidative stress (toxic buildup of ROS). The central compound used almost universally in mitochondrial patients is $CoQ_{10}$, which makes sense considering its pivotal role in electron transport, its antioxidant properties, and its safety even at high doses. With the important exception of some – but not all – patients with primary $CoQ_{10}$ deficiency who respond dramatically to $CoQ_{10}$ supplementation,[327] the effect of $CoQ_{10}$ has been modest and subjective.[328] Two synthetic derivatives of $CoQ_{10}$ appear more promising. Idebenone, a short-chain benzoquinone, has shown positive results in two studies of LHON patients, in a retrospective analysis[264] and in a randomized placebo-controlled trial.[329] The second compound, a parabenzoquinone labeled EPI-743, has been tested thus far only in open studies: it reversed vision loss in 4 of 5 LHON patients,[330] showed clinical improvement in 12 children with a variety of mitochondrial disorders,[331] and arrested or reversed disease progression in 13 children with various genetically proven LS.[332]

The second logical therapeutic approach is to eliminate accumulating noxious compounds. To decrease brain lactate in patients with MELAS, we used dichloroacetate (DCA), which keeps PDH in the active form and favors lactate oxidation: unfortunately, DCA had unacceptable neurotoxicity and had to be discontinued.[333] A more promising "detoxifying" therapy – despite its inherent risks – appears to be allogeneic hematopoietic stem cell transplantation (AHSCT) aimed at restoring sufficient thymidine phosphorylase (TPase) activity in patients with MNGIE to normalize the circulating toxic levels of thymidine and deoxyuridine.[327] As of 2010, 5 of 11 patients with MNGIE who had undergone AHSCT were alive, had normal blood TPase

activity, and virtually undetectable levels of thymidine and deoxyuridine. An international controlled trial showed that 9 of 24 patients were alive at last follow-up with a median follow-up of those surviving patients was 1,430 days.[334] For mtDNA-related disorders, an obvious – but by no means simple to implement – goal is to shift heteroplasmy, that is, to lower the mutation load to subthreshold levels. Several strategies have been tried, often with good results in cybrid cell lines. When deprived of glucose and exposed to ketogenic media, cybrids harboring single mtDNA deletions shifted their heteroplasmy level and recovered mitochondrial function, probably through selective mitochondrial autophagy (mitophagy).[335] A genetic approach to heteroplasmic shifting involves using restriction endonucleases as silver bullets that target to the mitochondrial matrix and recognize sites of specific pathogenic mutations.[336] As the dormant stem cells in skeletal muscle (satellite cell) have fewer mtDNA mutations than adult muscle fibers, muscle regeneration after induced myonecrosis has been used to shift heteroplasmy, and with good results, at least in one patient with strabismus.[337]

Mitochondrial dynamics could be exploited therapeutically in two opposite ways. On the one hand, we could enhance mitochondrial fission and favor mitophagy, a natural "quality control" function that sequesters and eliminates dysfunctional mitochondria, perhaps sensing their abnormally low membrane potential.[335,338] On the other hand, enhancing mitochondrial fusion and "networking" would allow complementation of "bad" and "good" mitochondria and normalization of overall mitochondrial function, as shown by the use of mdivi-1 (mitochondrial division inhibitor 1[339,340]).

If it is true that mitochondrial proliferation (e.g., the ragged-red fibers in skeletal muscle) is a futile compensatory attempt, we could try to improve on nature's strategy by enhancing mitochondrial biogenesis through the activation of the transcriptional coactivator PGC-1α (peroxisome proliferator-activated receptor [*P*PAR] *G*amma *C*oactivator)-1 alpha. The advantage over disease-induced mitochondrial proliferation, which favors mutated mtDNAs, is that pharmacological upregulation of mitochondrial biosynthesis increases the number of *all* mtDNAs, thus allowing wild-type genomes to compensate for mutated ones. Encouraging results have been obtained in four mouse models of COX deficiency: one COX10-deficient animal benefited from treatment with bezafibrate, a PPAR panagonist,[341] as did three transgenic mice (with mutations in *Surf1*, *Sco2*, and *Cox15*) treated with the AMP-dependent kinase (AMPK) agonist AICAR, which phosphorylates PGC-1α.[342]

For nDNA-related mitochondrial disorders, "classical" gene therapy is – of course – an option, although it carries the well-known "baggage" of this approach, including the choice of appropriate viral or nonviral vectors, delivery to the affected tissues, and potential immunological reactions. Nonetheless, adeno-associated virus-mediated gene transfer has proven successful in two animal models, the *Ant1* mutant mouse that we mentioned above,[343] and in a mouse model of ethylmalonic encephalomyopathy.[344]

For mtDNA-related diseases, many of which are devastating and undiagnosable prenatally (at least as far as reliably predicting their clinical expression), the Holy Grail would be to prevent their occurrence altogether via "cytoplasmic transfer." We have long known that this was technically feasible, but recent advances raise the hope that this reproductive option will soon be available to women carrying mtDNA mutations. In this technique, the nucleus of an *in vitro*-fertilized oocyte from a carrier is transferred to an enucleated oocyte from a normal donor: the embryo will have the nDNA of the biological parents but the mtDNA of a normal mitochondrial donor. Experiments in primates using spindle-chromosomal complex transfer (ST) have documented that infants were healthy and devoid of maternal mtDNA.[345] The same technique has been applied to fertilized[346] and unfertilized but parthenogenically activated[347] human oocytes and has shown that cells develop into normal blastocysts and contain exclusively donor mtDNA. Even more excitingly, only donor mtDNA was detected after stem cell lines from blastocysts were differentiated into neurons, cardiomyocytes, and β-cells.[347] Similar results were obtained in the United Kingdom after pronuclear transfer in abnormally fertilized human oocytes developed to the blastocyst stage.[348] Despite some minor concerns,[349] the stage has been set for therapeutic application.[350] In fact, an asymptomatic woman who carries the m.8993T>G mutation has given birth to apparently healthy infant boy after oocyte MRT.[351]

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