Infantile mitochondrial encephalopathy

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Abstract
Individually rare, when taken as a whole, genetic inborn errors of metabolism (IEM) account for a significant proportion of early onset encephalopathy. Prompt diagnosis is crucial to assess appropriate investigation and can sometimes warrant successful therapy. Recent improvements in technology and expansion of knowledge on the biochemical and molecular basis of these disorders allow astute child neurologists and paediatricians to improve the early diagnosis of these genetically determined defects. However, because of rarity and heterogeneity of these disorders, IEM-encephalopathies are still a formidable challenge for most physicians. The most frequent cause of childhood IEM encephalopathy is mitochondrial disease, whose biochemical “signature” is faulty energy supply due to defects of the last component of the oxidative pathways residing within mitochondria, i.e. the mitochondrial respiratory chain (MRC).

Key words:
Mitochondrial disorders; Encephalomyopathies; Mitochondrial DNA; Respiratory Chain Deficiencies

Introduction
Mitochondria are double-membrane organelles of eukaryotic cells, crucial in a number of essential processes like heat generation, calcium, iron and sulfur homeostasis, and apoptosis. Mitochondria are also the major source of adenosine triphosphate (ATP), synthesized through the process of oxidative phosphorylation (OXPHOS) and required for all endoergonic cellular processes. ATP deficiency leads to cellular dysfunction and ultimately death. OXPHOS is carried out by MRC, which is composed of multi-heteromeric complexes located in the mitochondrial inner membrane (MIM). Briefly, the dehydrogenation of the electron acceptors nicotinamide adenine dinucleotide (NADH+H^+) and flavin adenine dinucleotide (FADH_2) generates a flow of electrons
that, through a series of redox reactions, liberates energy, which is exploited to extrude hydrogen ions (protons), from the mitochondrial matrix (MM), through MIM, to the mitochondrial intermembrane space (IMS). The proton translocation is carried out by pumps contained in MRC complexes I, III and IV. Electrons are eventually fixed to molecular oxygen by complex IV, to produce water, whereas the energy stored as the proton-gradient based membrane potential (ΔY), generated across the MIM, can then be utilized by Complex V, or ATP synthase, to condensate inorganic phosphate (Pi) and adenosine diphosphate (ADP) into ATP, the energy “currency” of the cell. In addition to the MRC complexes, two electron carriers (ubiquinone or coenzyme Q, CoQ, and cytochrome c, cyt c) and a set of other enzymatic activities that can supply electrons to CoQ, take part in this process, including complex II (succinate-ubiquinone reductase, SQR), the electron-transfer flavoprotein-ubiquinone reductase (ETF-QR) and dihydroorotate dehydrogenase (DHODH). Mitochondria contain their own DNA (mtDNA), a circular molecule of double-stranded DNA, which is present in multiple copies within each organelle and, in sexuate species, is inherited exclusively through the maternal lineage. Human mtDNA is 16.569 basepairs in size, and encodes 13 OXPHOS polypeptide subunits and 24 RNAs, (22 tRNAs and 2 rRNAs), essential for intra-mitochondrial protein synthesis. MtDNA-encoded subunits interact with over 70 nuclear-encoded subunits on the inner mitochondrial membrane to form four of the five MRC complexes. Seven mtDNA genes encodes for the (ND) subunits of complex I, three for subunits of complex IV (cytochrome c oxidase, COX), one, cytochrome b, is a subunit of complex III, and two, ATPase 6 and 8, are subunits of the F0 particle of complex V. Many additional nuclear-encoded factors are essential for the maintenance, replication and expression of mtDNA, for the structural formation, quality control, and turnover of the MRC itself, as well as for the biogenesis of the organelles and their organization in a dynamic network (1).
As a result, genetic defects affecting mtDNA or nuclear DNA (nDNA) can compromise ATP synthesis and cause human disease. Tissue and organ function critically depends on adequate ATP production, especially when energy demand is high, like in neurons and muscle fibers. This explains why primary disorders of mitochondrial function usually cause neurodegeneration and/or muscle weakness, leading to neuromuscular disease in children and adults. However, specific mitochondrial syndromes can involve any other organ, either individually or in combination with brain and muscle impairment. Mutations of mtDNA and of the vast repertoire of nuclear genes converging on the formation and function of MRC are responsible for mitochondrial disorders, a group of rare, heterogeneous, usually inherited but occasionally sporadic, conditions affecting humans at any age. Individually rare, when taken as whole mitochondrial disorders are among the most frequent genetic diseases in humans, with a minimum prevalence of ~1 in 5,000 individuals in the European population (2). In spite of substantial progress made in the last two decades, we still face major limitations for the development of new treatments and preventing mitochondrial disorders, which mainly concern (i) the complete molecular definition of mitochondrial disease, and (ii) the mechanistic understanding of mitochondrial disease gene products, (iii) the development of rational therapies.

There is no effective treatment for mitochondrial disorders, and current clinical management is focused on treating complications (3). Although this can have a major impact on quality of life, these disorders are usually progressive, leading to major disability and premature death. There is therefore a clear need to develop new management strategies.

In a large proportion of mitochondrial disease cases it is still not possible to reach a molecular genetic diagnosis. In fact, more than 50% of adult patients, and an even greater percentage of paediatric cases, remain undefined genetically, so that diagnosis is based solely on biochemical
and/or morphological findings, in skeletal muscle or, more rarely, in cultured fibroblasts. The lack of a genetic diagnosis prevents the patients from receiving reliable family counseling, impedes the parents to obtain reliable prenatal diagnosis, and obliges the physicians to refer to biochemical skills, expertise and methodologies that vary from lab to lab, and are often incomplete. Whilst the analysis of mtDNA is a relatively well-standardized procedure in most specialized Centers, the list of known disease genes associated with mitochondrial dysfunction is constantly increasing. This problem, which is directly linked to the complexity and heterogeneity of mitochondrial medicine, makes the diagnostic workout of these disorders a formidable burden for standard methodological approaches. In addition, the identification of new genes, which has largely relied so far on homozygosity mapping of multiconsanguineous families, has intrinsic limitations due to the availability of informative kindreds. New technology based on next-generation sequencing offer the concrete hope to rapidly fill the gap between clinical and biochemical outline and genetic diagnosis in these diseases.

This review is focused on mitochondrial conditions presenting in first years of life with a prevalent involvement of the central nervous system. The classification of mitochondrial encephalopathy displayed in Table 1, which is organized according to clinical criteria, will be followed in the presentation of individual syndromes.
Alpers Huttenlocher and Hepatoencephalopathic mtDNA-depletion syndromes

Clinical definition

This disorder, which was described long before being recognized as a mitochondrial disease, is hallmarked by diffuse, progressive degeneration of the gray matter of the cerebrum, associated with variable degree of liver involvement, ranging from increased levels of hepatic enzymes in plasma, to severe liver failure. Neuropathology reveals widespread, patchy involvement of the cortex with astrocytosis, vacuolization, neuronal loss and capillary proliferation. The disease onset is typically in infancy or early childhood, although juvenile cases have been reported (4, 5), and the initial symptoms are usually severe hypotonia and refractory seizures (6). Status epilepticus is common but valproate therapy should be avoided as it may precipitate hepatic failure (7). In most patients with early onset the course of the disease is rapidly progressive, leading to death usually before three years of age. Brain magnetic resonance imaging (MRI) shows severe and progressive cortical and subcortical atrophy, but involvement of other gray structures, particularly the thalami, is frequent.

Molecular genetics

The disease is inherited as an autosomal recessive trait; liver and, sometimes, muscle, are characterized by depletion of mtDNA that in the former tissue can decrease by >90% of the norm. Reduced activity of mtDNA polymerase gamma and mutations in POLG1 are the most frequent cause of this severe encephalopathy (8, 9). POLG1 is a gene composed of 22 exons located on chromosome 15 that encodes pol-gamma A, the 145 kDa polypeptide that carries out the polymerase and proofreading activities of the enzyme. In mitochondria, one copy of pol-gammaA binds two copies of a second, 55 kDa subunit, termed pol-gamma B, which confers processivity to the holoenzyme. The physical interaction between pol-gamma A and the two pol-gamma B
subunits occurs in the intermediate “spacer region” that separates the N-terminal region, containing the mitochondrial targeting sequence and the exonucleolytic “proofreading” activity of pol-gamma A, from the C-terminal region, that performs the polymerase activity. The result is the formation of a functionally active heterotrimeric complex, that binds DNA and also interacts with other components of the mitochondrial replisome, including the mitochondrial single-stranded DNA binding protein, mtSSB, the mtDNA helicase, termed Twinkle, and other factors, such as topoisomerases, ligases, etc.

More than a hundred mutations have been identified in POLG1, associated with a number of different clinical presentations. Alpers-Huttenlocher’ syndrome is in fact at the end of a clinical spectrum that also includes juvenile-onset spino-cerebellar ataxia and epilepsy (SCAE) syndrome, its variant sensory-ataxic neuropathy, deafness and ophthalmoplegia (SANDO, OMIM 607459), and adult-onset, autosomal recessive (ar) or dominant (ad) progressive external ophthalmoplegia (PEO, OMIM 157640 and 258450), with or without additional features, such as generalized myopathy, peripheral sensory-motor neuropathy, parkinsonism, bipolar affective disorder, ovarian failure with precocious menopause, etc. Whilst liver mtDNA depletion is the molecular hallmark of Alpers-Huttenlocher’ syndrome, and has also been occasionally documented in SCAE, the POLG1-associated PEO syndromes are characterized by the accumulation of multiple mtDNA deleted species in skeletal muscle (and in brain as well). This is a common molecular signature of mendelian PEO, irrespective of the primary genetic cause.

Mutations in POLG1 are relatively specific to different clinical presentations. In particular, Alpers-Huttenlocher’ syndrome is frequently, but not exclusively, associated with the presence of two mutations, either mutation A467T or mutation W748S, affecting aminoacid residues residing in the intermediate “spacer” region of the pol-gamma A subunit. In general, one allele carries either mutation, whereas the other contains mutations in other aminoacid residues. In most cases, but
not always, the second mutation is in the polymerase-domain of the protein but many exceptions have been reported (see http://tools.niehs.nih.gov/polg for a complete and continuously updated list of POLG1 mutations associated with different syndromes).

Early-onset hepato-encephalopathy with mtDNA depletion (mitochondrial depletion syndrome, MDS) has also been associated with two additional nuclear genes, besides POLG1: deoxyguanosine kinase (DUOGK) (10) and MPV17 (11). In these patients the hepatic involvement prevails on the neurological impairment, determining severe, connatal or very early-onset metabolic acidosis, severe episodes of hypoglycemia, rapidly progressive liver failure with evolution into cirrhosis, usually leading to early death (10-11). DGUOK encodes dGK, the mitochondrial deoxy-guanosine kinase that recycles purine nucleosides by phosphorylation, being part of the salvage pathway of the intramitochondrial nucleotide pools. Impairment of dGK activity, and of its partner enzyme specific to pyrimidine nucleosides, thimidine kinase 2, TK2, determines shortage and imbalance of nucleotides, the “building blocks” of mtDNA synthesis, leading to mtDNA depletion. For reasons that are still unclear, whilst mutations in dGK are associated with hepatocerebral MDS (variant 3, OMIM 251880), mutations in TK2 cause early-onset myopathic, or encephalomyopathic MDS (variant 2, OMIM 609560). Mutations in MPV17, a protein of unknown function of the inner mitochondrial membrane, are responsible for a peculiar form of hepato-cerebral MDS (variant 6, OMIM 256810), which also includes Navajo Familial Neurohepatopathy, NNH, a condition restricted to the Navajo population, caused by a “founder” missense MPV17 mutation, the W50Q. (12)

Another, exceptionally rare, early-onset encephalopathic variant of hepato-encephalopathic mtDNA depletion is infantile onset spino-cerebellar ataxia (IOSCA or MTDPS7, OMIM 271245), a disease due to a single, recessive mutation (Y508C) in the C10ORF2 gene, encoding the Twinkle helicase, which is part of the Finnish disease heritage (13). These patients are characterized by a
severe neurodegenerative disorder with a combination of ataxia, athetosis, hypotonia, sensorineural deafness and severe epilepsy. They develop progressive atrophy of the cerebellum, brainstem, and spinal cord, and a sensory axonal neuropathy, associated with mtDNA depletion in brain and liver (14). Neurons of cerebellum and frontal cortex have decreased activity of respiratory chain complex I. A different homozygous mutation (T457I) in C10ORF2 has also been identified in 3 Algerian consanguineous patients with a similar condition, including severe hepatocerebral phenotype characterized by neonatal hypotonia, mild liver insufficiency, increased serum and CSF lactate, psychomotor regression, seizures, and peripheral neuropathy (15).

Fulminant hepato-cerebral failure has also been reported in several consanguineous patients characterized by neonatal ketoacidotic coma and profound COX deficiency. Two allelic mutations, a 2-bp frameshift deletion and a P174L, were identified in SCO1 (16), standing for synthesis of cytochrome oxidase 1, a COX assembly factor that promotes the incorporation of copper atoms in the catalytic sites of nascent complex IV.

Finally, a progressive hepatoencephalopathy in patients with severe defect of mitochondrial protein synthesis has been associated with mutations in the EFG1 gene, encoding mitochondrial elongation factor, EF-G1 (17). As expected, the biochemical profile of these patients consists of severe, multiple defects of mtDNA-dependent MRC complexes in muscle and cultured fibroblasts.

**Leigh Encephalopathy - Leigh Disease (LD)**

*Clinical definition*

This is by far the most frequent mitochondrial encephalopathy in infancy and childhood. Leigh disease (LD) is primarily a neuropathological-neuroradiological entity, characterized by focal bilateral lesions in one or more areas of the deep gray matter, including rostral spinal cord, the brainstem, thalamus, cerebellum, and the basal ganglia (Fig. 1A-B). The lesions consist of areas of
demyelination, gliosis, necrosis with spongiotic vacuolization, and capillary proliferation. Although the typical LD brain lesions, and corresponding symptoms, are associated with a huge number of different gene mutations, their presence should nevertheless alert the astute child neurologist and pediatrician to orient the diagnosis toward mitochondrial impairment of energy supply. In most cases LD has in fact been acknowledged as the final common neuropathological outcome of an early failure in the bioenergetic system that sustains the survival and activity of nerve cells and their connections in the central nervous system. Accordingly, lactic acidosis, due to a block in cellular respiration, is a virtually invariant finding, although it may fluctuate in severity. The clinical neurological symptoms are related to the areas involved in neurodegeneration and necrosis, initially consisting in hypotonia and psychomotor regression, followed by variable onset of dystonia, involuntary movements, ataxia, and eventually quadriparetic spasticity, accompanied by general symptoms that include failure to thrive and recurrent vomiting, the latter being possibly associated with worsening of the lactic acidosis. The peripheral nervous system is also frequently affected, mainly as an axonal demyelinating polyneuropathy. The nervous system, including skeletal muscle, is by far the apparatus predominantly, sometimes exclusively, affected, with the exception, in a few cases, of the proximal renal tubule, determining a De Toni Debre Fanconi syndrome.

**Molecular genetics**

Severely impaired activity in any of the MRC complexes can lead to LD (OMIM 256000), the most frequent falling into four groups (i) isolated defects of Complex I (Fig. 1A-B), or (ii) Complex IV, or (iii) multiple MRC defects, and (iv) mutations in ATPase 6, a mtDNA encoded subunit of complex V. To our knowledge, a single homozygous mutation in SDHA, the gene encoding the 70 kDa flavoprotein subunit of Complex II (18), and a single S45F homozygous substitution in the small
UQCRQ subunit of complex III (19), are the only LD cases associated with genetically-defined defects of the corresponding enzymes so far reported. Contrariwise, in our experience, defects of pyruvate dehydrogenase complex (PDHC) activity are also relatively frequent in LD, in particular those associated with mutations in the X-linked PDHA1 gene encoding the E-1α catalytic subunit of the complex (20). However, PDHC deficiency can also be found in a number of early-onset encephalopathy cases with neuroradiological features that differ from or only partially overlap to those of typical LD.

i- Defects of complex I

Mitochondrial complex I (NADH dehydrogenase ubiquinone–ubiquinol reductase) catalyzes the proton-motive oxidation of reduced nicotinamide–adenine dinucleotide (NADH) by ubiquinone. It consists of ≈45 subunits with a total molecular mass of 10^3 kDa (21). Seven subunits (MTND1-6, MTND4L) are encoded by the mitochondrial genome, the others being encoded by nuclear genes. Leigh syndrome has been associated with several mutations affecting either mtDNA encoded subunits (e.g. mutations in MTND2, MTND3, MTND5, MTND6), or nuclear encoded subunits (NDUFS1, NDUFS3, NDUFS4, NDUFS7, NDUFS8, NDUFA2, NDUFV1), and also with mutations in specific complex I assembly factors (NDUFAF2, C8ORF38, C20ORF7, FOXRED1).

ii- Defects of complex IV

Defects of genes encoding COX assembly factors are the main cause of complex IV deficiency, and are usually transmitted as autosomal recessive trait, presenting early in life as Leigh syndrome. Mutations have been reported in SURF1, a protein involved in the formation of COX holocomplex through a mechanism that is still poorly understood (22); COX10 (23) and COX15 (24), two enzymes involved in the biosynthesis of the COX-specific heme a moiety; and TACO1 (25), a
mitochondrial translational activator required for efficient translation of COX subunit I. In addition a single mutation (A354V) in LRPPRC, (26), a mitochondrial pentatricopeptide involved in mtDNA gene expression or in RNA stability and processing causes the French-Canadian type of Leigh syndrome (OMIM 220111).

iii- Combined MRC defects

By genetic investigation of patients with combined biochemical MRC deficiency and defective mitochondrial translation, mutations in the mitochondrial elongation factor EFG1 gene were identified in one subject with early-onset Leigh syndrome (27). Homozygous mutations in the c12orf65 gene, encoding a member of the peptide-release-factors protein family were found in patients with Leigh syndrome associated with optic atrophy and ophthalmoplegia (28).

iv- Defects of complex V (MTATP6)

ATP synthase (complex V) comprises an integral membrane component F0 and a peripheral moiety F1. Only two F0 proteins (ATP6 and 8) are encoded by mtDNA (29).

Different heteroplasmic mutations in MTATP6 (9176T-C, 9185T-C, 9176T-G) have been associated to Leigh syndrome (30). Depending on the percentage of heteroplasmy, the most frequent mutation, a T8993G transversion, can lead to a severe, early onset maternally inherited Leigh syndrome (MILS), or to milder, juvenile- or adult-onset NARP (Neurogenic muscle weakness, ataxia, retinitis pigmentosa. OMIM 551500) phenotype. MILS typically occurs when the T8993G mutation is >90%, whereas NARP is commonly associated with percentages around 50-60%. A spectrum of conditions of progressively increasing severity can occur with intermediate percentages of heteroplasmy. The second most frequent mutation is a transition T->C in the same position, associated with juvenile Leigh- or NARP-syndromes (31). The percentage of heteroplasmy
in both mutations is similar in different tissues, including chorionic villi; this observation has prompted several Centers, including ours, to perform genetic prenatal diagnosis in pregnant women carrying the NARP mutations, with high degree of predictive reliability.

**Leukoencephalopathy**

The presence of diffuse white matter involvement has been reported in an increasing number of patients with mitochondrial encephalopathy. In our series of 320 affected children leukoencephalopathy is the predominant or exclusive MRI feature in ≈20% of the cases, consisting of severe involvement of the brain white matter with hardly any significant alteration in basal nuclei or brainstem. Some patients develop early-onset, large cystic lesions within the affected white matter, in others progressive vacuolization can ensue later in the course of the disease. In still other cases, particularly those associated with PDHC deficiency, severe delay of central myelination is frequently combined with cortical malformations of variable severity, e.g. micropolygyria.

Mitochondrial leukoencephalopathy in infancy is often associated with defects of Complex I or Complex II (Fig. 1C-D), although a few patients with COX deficiency and SURF1 mutations have also been reported to be affected by predominantly leukoencephalic lesions. Irrespective of the biochemical defect, two major clinical presentations are known: (i) infants with psychomotor delay since the first months of life, failure to thrive, growth impairment, who undergo rapidly progressive downhill course resulting in severe spastic quadripareisis and mental impairment; (ii) children characterized by a disease-free period during the first years of life, followed by acute onset of focal motor disturbances, sometimes seizures, and undergoing slowly progressive
downhill course; impairment of motor abilities is marked, whereas the cognitive functions are relatively spared.

The differential diagnosis, which is primarily based on the MRI pattern, must consider the spectrum of early-onset leukodystrophy, including Alexander’s disease, Canavan’s disease, magalencephalic leukoencephalopathy with subcortical cysts, and the vanishing white matter disorder. An additional help for the differential diagnosis comes from brain proton spectroscopy (H\(^+\)-MRSI). In mitochondrial diseases this technique can well detect lactate accumulation also in brain regions that are not yet anatomically altered and therefore fail to be identified by MRI. However, the presence of a H\(^+\)-MRSI lactate peak is not specific for mitochondrial disorders, being also found in the active phase of other inherited leukoencephalopathies, or in ischemic and inflammatory lesions. Contrariwise, a H\(^+\)-MRSI peak corresponding to accumulated succinate can be considered as a hallmark in patients with complex II deficiency, particularly those carrying mutations of \textit{SDHAF1} (32).

A peculiar mitochondrial white matter disease of childhood or young adulthood is Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL) (33). LBSL is caused by mutations in the \textit{DARS2} gene, which encodes the mitochondrial aspartyl-tRNA synthetase (see below). The first symptom, typically consisting of gait disturbance, is followed by slowly progressive cerebellar ataxia, increasingly severe pyramidal signs, and sensory abnormalities referable to neurodegeneration of the long tracts of the dorsal columns of the spinal cord. The diagnosis relies on a virtually pathognomonic MRI pattern consisting of signal abnormalities that involve the cerebral white matter, the dorsal columns and the corticospinal tracts of the spinal cord, the pyramids, the cerebellar peduncles, the intraparenchimal tract of the V cranial nerve, the posterior arm of the internal capsule, and the splenium of the corpus callosum.
Molecular genetics

MRC defects are not uncommon in patients with leukodystrophy.

Leukoencephalopathy, associated with specific Complex I deficiency can be caused by mutations in either structural subunit or in assembly factor. For instance, mutations in NDUFS1, encoding the 51kDa subunit, or NDUFS1, encoding the FMN-associated 70 kDa subunit of complex I, can cause leukodystrophy and myoclonic epilepsy (34), in addition to Leigh syndrome; whereas a mutation in NUBPL, that participates in respiratory chain complex I assembly by incorporating the Fe/S clusters into complex I subunits, has been found in a single patient with leukodystrophy and elevated lactate in the CSF (35).

A peculiar leukoencephalopathy with accumulation of lactate and succinate in the white matter, is the neuroradiological hallmark of in patients with severe reduction of complex II activity and amount, caused by mutations in SDHAF1, a specific complex II assembly factor (Fig. 1C-D) (32).

Leukodystrophic features have occasionally been described also in patients with isolated complex IV deficiency. A loss of function SURF1 mutation, a gene usually associated to Leigh syndrome (see above) has been reported in isolated leukodystrophy with involvement of the corticospinal tracts (36). Moreover, the only case due to a mutation in a nuclear encoded COX subunit (37), affecting COX6B1, was characterized by a combination of early-onset leukodystrophic encephalopathy, myopathy and growth retardation associated with COX deficiency.

Finally, in one patient with severe infantile macrocystic leukodystrophy with micropolygyria and multiple MRC defects, a homozygous mutation in the mitochondrial elongation factor Tu (EF-Tu) (27) was demonstrated to compromise the EF-Tu binding to its tRNA substrate.

LBLS. As reported above, DARS2 mutations are the cause of LBLS (38); this has been the first disorder due to mutations in a gene encoding a mitochondrial aminoacyl-tRNA synthetase.
Almost all patients with LBSL are compound heterozygotes, sharing a complex rearrangement in one allele that involves a T-C stretch upstream from exon 3 (228-20/-21delTTinsC) and a second variable mutation. The 228-20/-21delTTinsC is a hypomorphic mutation that partially interferes with the splicing of exon 3, leading to frameshift and premature truncation (R76SfsX5) of a fraction of DARS transcripts. The normal aliquot of DARS transcript explains why LBLS is a slowly progressive condition, compared to early-onset, rapid, sometimes fulminant syndromes associated with other mutations in nuclear genes involved in mtDNA translation (e.g. EFG1, EF-Tu, etc.).

**Encephalocardiomyopathy**

Combined encephalo-cardiomyopathy is a severe, usually fatal, mitochondrial condition of early infancy. Children are frequently critically ill at birth, with severe heart failure and lactic acidosis. Clinical features include hypertrophic cardiomyopathy, severe myopathic and/or central hypotonia, failure to thrive, and early respiratory distress. Other signs are occasionally reported, including microcephaly, hepatomegaly, facial dysmorphism, such as low-set ears, retrognathia and prominent nasal bridge with hypertelorism. MRI is rather unspecific, but can include abnormal signal intensity in the periventricular white matter, and, inconsistently, lesions of the deep gray nuclei. The clinical course may be fulminant with fatal outcome in the neonatal period. The patients who survive the first months of life present psychomotor delay, with a variable collection of other signs including oculomotor disturbances, such as nystagmus, cognitive impairment, ataxia and myopathy. High levels of lactate are frequently detected in plasma, CSF and urine. In a specific condition, X-linked Barth syndrome, severe dilating cardiomyopathy with myocardial non-compaction is accompanied by fluctuating neutropenia and hallmarked by increased excretion of 3-methylglutaconic acid.
Like most of the early onset mitochondrial disorders, the muscle biopsy is usually unrevealing and only biochemical investigation of the respiratory chain in muscle or fibroblasts can lead to the diagnosis.

**Molecular genetics**

The most frequent biochemical abnormalities are isolated defect of Complex I, Complex IV or Complex V. However, a homozygous mutation (G555E) in SDHA, part of complex II, that was previously associated with Leigh syndrome, has recently been found in two large consanguineous families with neonatal isolated cardiomyopathy (39).

Complex I deficiency was reported in patients with encephalocardiomyopathy, carrying mutations in genes encoding structural subunits (NDUFS2, NDUFV2, NDUFA11) or assembly factors (NDUFAF4, ACAD9) specific for complex I. Whereas single cases/families are described for NDUFV2, NDUFA11, NDUFAF4, different mutations have been found in NDUFS2 (40) and ACAD9. ACAD9 encodes a poorly understood member of the mitochondrial acyl-CoA dehydrogenase protein family, and has been demonstrated to interact with other complex I assembly factors (41); mutations in ACAD9 gene are consistently associated with severe, connatal lactic acidosis, which is sometimes fatal, followed by hypertrophic cardiomyopathy. Although the latter is the predominant symptom in the surviving patients, encephalopathy with mental retardation and poor growth have also been reported (42).

SCO2, in concert with SCO1, enables the first two subunits of complex IV to be incorporated into the holoprotein. Eight mutations in SCO2 have been described in patients with fatal infantile cardioencephalomyopathy and COX deficiency. The cardiac hypertrophy reported in SCO2-defective patients seems to be rather specific. Interestingly, all patients reported were compound heterozygotes; even more remarkably, one particular mutation, E140K, was present in all affected
individuals (43). Albeit without the same genotype-phenotype specificity, also mutations in another assembly factor for complex IV, COX15, can cause fatal infantile hypertrophic cardiomyopathy.

Mutations in TMEM70, a putative assembly factor of complex V, were found in patients, mostly of Gipsy origin, with cardiomyopathy and isolated deficiency of ATP synthase (44); the prevalent homozygous mutation, a A-to-G transition in intron 2 of the TMEM70 gene, resulting in aberrant splicing and loss of the mRNA transcript, can give an high degree of intra-familial variability in the severity of symptoms.

A deficiency of ATP synthetase was reported also in two siblings with lactic acidosis, hypertrophic cardiomyopathy, and muscular hypotonia (45); a homozygous mutation in the SLC25A3 gene, encoding a mitochondrial phosphate carrier, was identified. The mutation affects the alternatively spliced exon 3A, expressed in muscle.

Other mitochondrial disorders with cardiac involvement, but without a specific biochemical deficiency, include a mutation of DNAJC19, which encodes a putative mitochondrial import protein. The mutation causes autosomal recessive dilated cardiomyopathy with ataxia (46). In addition to mutations of carrier or import proteins, alteration of lipid milieu of the inner mitochondrial membrane, which is composed predominantly of cardiolipin, could cause OXPHOS dysfunction.

Barth syndrome (OMIM 302060) is caused by mutations in TAZ (or G4.5), an X-linked gene encoding a cardiolipin-specific acyl–coenzyme A synthetase (tafazzin) involved in the biosynthesis and structural diversification of this crucial phospholipid of the inner mitochondrial membrane. Accordingly, cardiolipin is markedly decreased in skeletal and cardiac muscle and in platelets from affected patients (47).
Miscellaneous Disorders

There is a huge number of early-onset mitochondrial encephalopathies that do not meet the definition of specific phenotypes, and must therefore be included in a miscellaneous group of conditions (Table 2). Some of them are very rare, having been described only in single families or isolated populations. The most important information about this group of disorders is summarized in table 2. In spite of their rarity, these disorders well illustrate the complex relationship between mitochondria dysfunction and human diseases. Clinicians should be aware of their existence in order to appropriately address the molecular investigation.

In addition to the clinical/radiological features, the presence of specific biochemical defects or molecular alterations (MRC complex activities, mitochondrial protein synthesis, reduced mtDNA amount, mtDNA deletions) may help in the identification of the genetic cause of the disease.

Complex III deficiency

- BCS1L: GRACILE and Bjornstad syndromes

Various nuclear genes have been implicated in the assembly of yeast CIII. However, only one such gene, BCS1-like (yeast) (BCS1L), has been identified in the human genome. Several BCS1L gene mutations have been reported in complex III deficiency, associated with different clinical presentations: neonatal proximal tubulopathy, hepatopathy and encephalopathy and isolated progressive infantile encephalopathy, sometimes with brainstem and basal ganglia lesions consistent with a diagnosis of Leigh syndrome (48). The acronym GRACILE stands for growth retardation, aminoaciduria, cholestasis, iron overload, lactic acidosis and early death and designates an infantile condition caused by a specific BCS1L mutation, S78G, which is part of the Finnish disease heritage (49). A second autosomal recessive condition associated with BCS1L
A missense mutation is Björnstad syndrome, characterized by sensory-neural deafness and pili torti (50).

- TTC19

A second, putative assembly factor for complex III, TTC19 (tetratricopeptide repeats 19) responsible for a mitochondrial disorder, has been recently discovered. TTC19 mutations have been identified in patients with slowly progressive mitochondrial encephalopathy with onset in late infancy characterized by ataxia, dysarthria, dystonia, degeneration of peripheral motor axons, psychomotor regression, and eventually global neurological failure, due to the accumulation of multiple necrotic lesions of the brain. Severe reduction of cIII activity was present in skeletal muscle. One patient was reported with an adult onset of a subacute, rapidly progressive neurological failure with fatal outcome three years later (51).

- UQCRB

A single case with mutation in UQCRB (or QP-C), the human ubiquinone-binding protein of complex III has been reported. The mutation consisted of a complex rearrangement at the C terminus, and resulted in severe complex III deficiency in the liver. The patient had no permanent liver dysfunction but only metabolic crises after fasting, with hepatomegaly, hypoglycaemia, lactic acidaemia and mild elevation of liver enzymes (52).

Complex IV deficiency

-FASTKD2 encephalomyopathy

FASTKD2 nonsense mutation was found in an infantile mitochondrial encephalomyopathy, characterized by developmental delay, hemiplegia, convulsions, with peculiar asymmetrical brain atrophy. The activity of Cytochrome c oxidase was reduced in muscle. FASTKD2 is mitochondrial
protein, member of the FAST kinase domain-containing protein (FASTKD) family, and it may play a role in apoptotic processes (53).

- **ETHE1: Ethylmalonic Encephalopathy**

Ethylmalonic Encephalopathy (EE) is an autosomal recessive disorder characterized by early-onset encephalopathy, microangiopathy, chronic diarrhea, defective cytochrome c oxidase (COX) in muscle and brain, and high excretion of ethylmalonic acid in urine. EE is caused by mutations in the **ETHE1** gene. The ETHE1 protein is a mitochondrial sulfur dioxygenase that takes part in the aerobic energetic exploitation of, and detoxification from, sulfide (H$_2$S). ETHE1 mutations lead to accumulation of H$_2$S, a biologically active compound that inhibits the activity of enzymes such as COX and short-chain acylCoA dehydrogenase (SCAD), acts as a potent vasodilator and damages the colonic mucosa and the endothelia of small vessels. EE is the first example of a mitochondrial disorder caused by genetically-determined poisoning of the respiratory chain (54).

*Multiple MRC complex deficiency and mtDNA depletion/abnormalities*

- **SUCLA2 and SUCLG1:** mutations in these genes cause encephalomyopathic mitochondrial DNA depletion syndrome with methylmalonic aciduria.

Succinyl-CoA synthetase (SCS) is composed of two subunits, one of which (the beta subunit) determines whether the enzyme is GTP-specific (SCS-G) or ATP-specific (SCS-A). The **SUCLA2** gene encodes SCS-A, a TCA cycle enzyme that catalyzes the formation of succinate and ATP from succinyl-CoA and ADP. Mutations in **SUCLA2** cause encephalomyopathic mitochondrial DNA depletion (55). A further biomarker of this condition is mild methylmalonic aciduria, a derivative of propionate, due to the block of the TCA-dependent catabolism of propionyl-CoA derived from the $\beta$-oxidation of odd-number fatty acids. The **SUCLG1** (also reported as **SUCLA1**) gene encodes the alpha subunit of SCS. Mutations in this gene have been associated with infantile
encephalomyopathic syndromes with high excretion of methylmalonic acid. The patients with SUCLG1 mutations had a more severe, albeit variable (56), phenotype compared to those with SUCLA2 mutations.

- AIF encephalomyopathy

The apoptosis inducing factor (AIF) is a mitochondrial protein with a double function: it mediates programmed cell death, independently from the activation of caspases, but it is also a NADH-dependent oxidoreductase with an essential role in bioenergetic metabolism. A specific mutation (R201del) in the AIFM1 gene has been associated to an X-linked progressive mitochondrial encephalomyopathy in two first cousins. Both patients developed severe psychomotor regression, hypotonia and seizures associated with bilateral lesions in the striatum and brainstem. MRC multi-complex deficiency and mtDNA depletion (57) were found in muscle biopsies.

_Multiple MRC complex deficiency and reduced mitochondrial protein synthesis_

- MRPS16: Combined Oxidative Phosphorylation Deficiency 2

A genetic disease due to defective mitochondrial protein synthesis has been associated with mutations in _MRPS16_ gene, encoding the mitochondrial ribosomal protein subunit 16, a component of the small subunit (28S) of the mitochondrial ribosome. The only patient reported so far was an infant, born of consanguineous parents, with a homozygous mutation in MRPS16; she presented with agenesis of the corpus callosum, dysmorphisms, and died of intractable metabolic acidosis at 3 days of age (58).

- RARS2: Pontocerebellar Hypoplasia Type 6 (PCH6)

Another protein necessary for proper mitochondrial synthesis is arginyl-tRNA synthetase (RARS2). Homozygosity for an A-to-G change in intron 2 of the RARS2 gene (IVS2+5A-G) was detected in subjects affected by PCH6 from a consanguineous family (59). Serial brain MRI revealed
progressive atrophy of the cerebellum, pons, cerebral cortex, and white matter. The mutation causes exon 2 skipping and frameshift, albeit a faint normal-sized fragment was also seen on PCR amplification. It has been suggested that mis-splicing mutations preferentially affect the brain because of a tissue-specific vulnerability of the splicing machinery.

- GFER

A homozygous mutation in GFER (growth factor, ERV1 homolog) was found in children with progressive myopathy and partial combined respiratory-chain deficiency, congenital cataract, sensorineural hearing loss, and developmental delay (60). GFER is a sulfhydryl oxidase, part of a peculiar system, the disulfide relay system that allows the import of cysteine-rich proteins into the IMS.

**CoQ deficiencies**

Coenzyme Q10 (CoQ10), or ubiquinone, is a lipophilic component of the electron transport chain. Different syndromes associated with CoQ10 deficiency in muscle, have been described: an encephalomyopathic form with seizures, ataxia, or mental retardation (61); a multisystem infantile form with encephalopathy, cardiomyopathy and renal failure (62); a predominantly cerebellar form with ataxia and cerebellar atrophy (63); Leigh syndrome with growth retardation (64); and an isolated myopathic form (65). Several cases have been reported with recurrent myoglobinuria and with ragged-red fibers/lipid storage in muscle.

Mutations in the CoQ10 biosynthetic genes, COQ2, COQ9, PDSS1 and PDSS2 were reported in patients with severe infantile mitochondrial syndromes and tissue CoQ10 deficiency (66). Mutations in CABC1 (also know as ADCK3) (67), usually causing spinocerebellar ataxia type 9, and in APTX (aprataxin) (68), causing ataxia-oculomotor apraxia syndrome, were found in patients with
ataxia and low levels of CoQ in muscle biopsies, suggesting that the ataxic form is a genetically heterogeneous disease in which CoQ10 deficiency is secondary.

**Diagnosis**

Clinical signs and symptoms as recurrent vomiting, abnormal ocular motility or abnormal eye movements, hypotonia, focal seizures, failure to thrive, developmental delay or regression, are suggestive of, but unspecific to, mitochondrial disease; measurement of lactate and MRI analysis are important clues that are often abnormal in infantile mitochondrial encephalopathy. Unlike adult patients, lactate levels are usually elevated, in infants affected by mitochondrial disease, in either plasma, CSF, or both, the values being correlated to clinical severity. However, elevated lactate levels can also be detected in a broader series of early onset metabolic encephalopathies. MRI is as important as measurement of lactate to orient further diagnostic investigation. MRI may be initially normal but usually shows suggestive patterns later in the disease course. Leigh-like MRI pattern is rather specific and should prompt the child neurologist or pediatrician to perform investigation on mitochondrial bioenergetic pathways, particularly OXPHOS (and PDHC); however, in other MRI findings, for instance leukoencephalopathy, OXPHOS defects must be considered in the differential diagnosis with Alexander’s disease, CACH, organic acidurias and Canavan’s disease; likewise, FASTDK2 mutant patients develop a hemispheric atrophy resembling Rasmussen encephalopathy. Therefore, MRI evaluation must be integrated with the critical evaluation of clinical features. Signatures from analysis of urinary organic acids are important in some cases (i.e. ethylmalonic aciduria in ETHE1 patients; increased succinate in complex II deficiency; methylglutaconic aciduria in Barth’s syndrome; methylmalonic aciduria in SUCLA1 and SUCLG1 mutant patients), but in most case is a negative screening for organic acids that should suggest a defect of the respiratory chain rather than other metabolic pathways of intermediate metabolism,
e.g. beta oxidation defects, which are characterized by abnormal patterns of urinary organic acids. Unlike adult-onset mitochondrial encephalomyopathies, histological examination of muscle biopsy is seldom informative in early-onset mitochondrial encephalopathies. Ragged red fibers or abnormal distribution of succinate dehydrogenase are rarely seen in pediatric patients, whereas unspecific signs as fiber type disproportion or weak COX staining are more frequently observed. Thus, normal histological examination should not prevent the child neurologist or the pediatrician from carrying out biochemical analysis of the respiratory chain complexes.

Management

Physicians dealing with mitochondrial disorders have to face a myriad of different problems that vary according to the age of the patients and to their clinical presentation. As a rule there are not definitive means to halt the progression of the disease and frequently symptom-based management is the only approach that can offer some relief to patients and their families. Supportive treatment includes correction of lactic acidosis with bicarbonate during acute phase of metabolic imbalance, prompt treatment of infections that, however trivial, may dramatically deteriorate neurological conditions; prevention of dehydration in cases characterized by difficulty of swallowing or recurrent vomiting, and aggressive treatment of seizures. Hypotonia, psychomotor delay and arthro-skeletal complications of motor disorders should be treated with physical therapy, postural management with orthotic aids and/or surgical correction. One of the major problems in infantile mitochondrial disorders is failure to thrive and poor somatic growth. As a collateral effect, these symptoms increase the anxiety of the parents, notably of that in charge of nourishing the child, usually the mother, that frequently develops a feeling of personal inadequacy and guilt. This dynamics has negative consequences in the relationship among family members and can interfere with the quality of life of the patient. Therefore, the feeding problems
of the child should be carefully assessed, distinguishing dysphagia from gastro-esophageal reflux, gastrointestinal dysmotility, anorexia or weakness of the feeding muscle system. A feeding program should be discussed with the parents, to devise the best feeding strategies, including, if necessary, gavage or enteral tube feeding.

In conclusion, because of their complexity and multisystemic involvement, mitochondrial disorders need a multidisciplinary approach, with the participation, beside the child neurologist, of physicians with competence in cardiology, ophthalmology, nephrology, gastroenterology and metabolic pediatrics. Therapists working on speech, respiration, nutrition, physical and cognitive problems need to cooperate in a coordinated action (69).

**Therapy**

Treatment of mitochondrial disorders is in general disappointing. Nevertheless in the last years the increasing knowledge on the pathogenic mechanisms of cell damage due to impaired mitochondrial have opened new therapeutic options. Defective energy is usually accompanied by other effects of impaired mitochondria, including free radical production, apoptosis, and in some cases accumulation of toxic substances. These aspects of mitochondrial disease can also be tackled by rational treatment. Available strategies may be summarized as follows: i) avoid toxic effect of some drugs (valproate, aspirin, amynoglycoside antibiotics, antiretroviral drugs) ii) supplementation with pharmacological doses of coenzymes or vitamins that take part in the RC complexes or buffer toxic metabolites (CoQ10, riboflavin, carnitine, thiamine) iii) provide antioxidant molecules to combat ROS production by abnormal mitochondria (Vitamin C, E, alpha-lipoic acid and N-acetylcysteine).

There is consistent evidence that dichloroacetate (DCA) has an effect in lowering lactate levels in different mitochondrial disorders. DCA maintains the pyruvate dehydrogenase complex in an
active form thus increasing the formation of acetyl-CoA from pyruvate and reducing the accumulation of lactate. However, these beneficial effects seem limited to the acute phase of lactic acidosis, whereas chronic exposure to DCA has been associated with the onset of progressive peripheral neuropathy (70). L-arginine, as a donor of nitric oxide, acts as a vasodilator of brain microcirculation, and has been used with some beneficial effect in MELAS, where it can limit the consequence of acute stroke-like episodes and decrease their recurrence (71).

Among the several cofactors and vitamins that have been used in mitochondrial disorders, CoQ10 and riboflavin have shown the highest effectiveness. Disorders due to CoQ10 biosynthetic defects partially respond to high doses of exogenous ubiquinone. Riboflavin can dramatically change the outcome of a limited number of patients with leukoencephalopathy due to complex I or II deficiency. The mechanism of action is likely the stabilization of a crippled, but however partially functioning, complex, by increasing the supply of the vitamin precursor of FMN and FAD, the two prosthetic moieties of complex I and II respectively.

Very recently the combined use of metronidazole and acetylcysteine has been effective to treat ethylmalonic encephalopathy due to Ethe1 mutations. The bactericidal effect of metronidazole on the intestinal anareobes and the increase availability of glutathione derived from acetylcysteine lead to a decrease of the toxic compound sulfide, which is predominantly produced by the anaerobes and fails to be detoxified if the sulfur dioxygenase activity of Ethe1 is missing.

The multiorgan involvement of mitochondrial disorders is a major hurdle for organ transplantation (72). However, myo-neuro-gastro-intestinal encephalopathy, MNGIE, a disorder leading to the accumulation of thymidine to toxic levels, has shown substantial improvement by increasing the ability of the organism to clear off the toxic substance by hemopoietic stem-cell transplantation. In principle, other disorders characterized by the accumulation of toxic compounds, including Ethylmalonic Encephalopathy, could also be treated by this approach.
In conclusion, with a few exceptions treatment of early onset mitochondrial encephalopathy is still inadequate and prevention through a correct genetic counselling and prenatal diagnosis is of the utmost importance. However, prenatal diagnosis requires the identification of the causative mutation, and can safely be applied to disorders due to nuclear gene mutations. Nevertheless, prenatal diagnosis has successfully been applied to several cases of NARP/MILS carrier mothers (73), since the heteroplasmy of the T8993G mutation is evenly distributed among tissues, including chorionic villi, so as to confer predictive value to the quantitative analysis in CVS. The application of the same approach to other heteroplasmic mtDNA mutations that instead vary in percentage from tissue to tissue, e.g. MELAS (74), is still a highly controversial, ethically sensitive issue.

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Bibliography


A, B: MRI of a patient presenting with a Leigh phenotype with Complex I deficiency due to T10158C mutation in MTND3 gene: coronal (A) and axial (B) T2 weighted images show bilateral putaminal hyperintense lesions and minimal posterior periventricular white matter hyperintensity (B).

C, D: MRI of a patient presenting with mitochondrial leukoencephalopathy with Complex II deficiency due to mutation G169 C of SDHAF1 gene: coronal (C) and axial (D) T2 weighted images show hyperintensity of the lobar white matter also involving corpus callosum and posterior limbs of the internal capsule (D), white matter is abnormal also in the cerebellar hemispheres (C).