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NEWS and VIEWS: mitochondrial encephalomyopathies

Elena Caldarazzo Ienco, Daniele Orsucci, Vincenzo Montano, Elena Ferrari, Lucia Petrozzi, Marta Cheli, Annalisa LoGerfo, Costanza Simoncini, Gabriele Siciliano and Michelangelo Mancuso

Department of Clinical and Experimental Medicine, Neurological Clinic, University of Pisa

In recent years, there has been a surge of interest in mitochondrial diseases, a group of metabolic conditions caused by impairment of the oxidative phosphorylation system. The ubiquitous presence of mitochondria in all the cells of the body, their role as cell powerhouse and their particular genetic characteristics explain the phenotypic complexity and the diagnostic difficulties, bridging from paediatrician to neurologist.

Key words: mitochondria, mitochondrial DNA, nuclear DNA, encephalopathy, myopathy

Introduction

The mitochondrion is a membrane-enclosed cytoplasmic organelle which has evolved from a primitive aerobic bacteria by means of a symbiotic relationship that started 1.5 billions years ago (1). Mitochondria are described as "cellular power plants" because they produce most of the cell's supply of adenosine triphosphate (ATP), by means of the oxidative phosphorylation (OXPHOS) machinery, which comprises electron transport chain (ETC) and ATP synthase (complex V). The ETC provides the cell with the most efficient energetic outcome in terms of ATP production. It consists of four multimeric protein complexes (complex I to IV) located in the inner mitochondrial membrane together with complex V (2) and also requires two electron carriers, coenzyme Q10 (CoQ10, or ubiquinone) and cytochrome c (cyt c). This metabolic pathway is under control of both nuclear (nDNA) and mitochondrial (mtDNA) genomes (2). Mitochondria, in fact, have their own DNA, the mitochondrial DNA (mtDNA), represented by a circular molecule of 16.5 kb without introns; one mitochondrion can contain two to ten copies of mtDNA (2). Mitochondrial diseases (MD) are the commonest inherited neuromuscular disorders with a prevalence of 1-2 in 10000 (3). They are a heterogeneous group of disorders caused by impairment of the mitochondrial ETC and, although initially considered neuromuscular disorders, MD were soon recognized to be more than just myopathies, when pediatric neurologists placed attention to the frequent occurrence of brain disease in children with mitochondrial alterations in their muscle biopsies and coined the term mitochondrial encephalomyopathies (4, 5), today widely accepted and reserved for defects of the respiratory chain (6).

Molecular and clinical features

The genetic classification of mitochondrial encephalomyopathies distinguishes disorders due to defects in mtDNA, which cause maternally inherited or sporadic disorders, from those due to defects in nDNA, which show a Mendelian inheritance pattern. A more practical way of categorizing mitochondrial encephalomyopathies includes defects of mtDNA maintenance (impairment of intergenomic communication) (7).

Mitochondrial genetics differs from Mendelian genetics in three major aspects: maternal inheritance, heteroplasmy and threshold effect, and mitotic segregation. This genetic complexity explains the great phenotypic variability of mitochondrial disorders and the lack of specific genotype-phenotype correlations. Deleterious mutations of mtDNA usually don't affect all mtDNAs (heteroplasmy) being required a minimum critical mutation load to cause mitochondrial dysfunction in a particular organ or tissue and mitochondrial disease in an individual (threshold effect); moreover, at cell division, the propor-

Address for correspondence: Michelangelo Mancuso, Department of Experimental and Clinical Medicine, Neurological Clinic, University of Pisa, Via Roma 67, Pisa, Italy. Tel. +39 050 992440. Fax +39 050554808. E-mail: mancusomichelangelo@gmail.com

tion of mutant mtDNAs in daughter cells may change, varying the phenotype (mitochondrial segregation) (6). The effects of mtDNA defects may have a wide phenotypic variability leading to heterogeneous multisystemic disorders and, although many overlapping syndromes have been described, mitochondrial medicine identified well-defined syndromes: Kearns-Sayre syndrome (KSS), chronic progressive external ophthalmoplegia (CPEO), Pearson syndrome (PS), these first three two syndromes due to large-scale deletions; mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), myoclonus epilepsy with ragged-red fibres (MERRF), caused by transfer RNA (tRNA) mutations that globally affect mitochondrial protein synthesis; and Leber hereditary optic neuropathy (LHON), neuropathy, ataxia and retinitis pigmentosa (NARP), maternally inherited Leigh syndrome (MILS) (8), attributable to mutations in a protein-coding gene.

Disorders of nDNA defects refers to genes that encodes approximately 1,700 mitochondrial proteins; pathogenic mutations can directly affect nDNA-encoded respiratory chain subunits, especially in the gigantic complex I and complex II, or indirectly affecting pathways such as proteins expression, translation, import into mitochondria, assembling with their mtDNA encoded counterparts (9, 10). Complex I deficiency is the commonest biochemical defect found in mitochondrial disorders and it is associated with a broad range of clinical phenotypes ranging from lethal neonatal disease to adult onset neurodegenerative disorders (11). Mutations in complex I assembly proteins can manifest as Leigh syndrome (NDUFAF2 and NDUFAF5), encephalopathy (NDUFAF4) and cardioencephalomyopathy (NDUFAF1) with a high level of genetic heterogeneity and weak genotype–phenotype correlations (10). Leigh syndrome, also defined as subacute necrotizing encephalomyelopathy, is a devastating progressive neurodegenerative disorder of infancy or early childhood associated by specific neuropathological features: bilaterally symmetrical foci of cystic cavitation, vascular proliferation, neuronal loss, and demyelination in the basal ganglia, brainstem, and posterior columns of the spinal cord. It is characterized by decompensation during an intercurrent illness and typically associated with psychomotor retardation or regression, often followed by transient or prolonged stabilization or even improvement, but inevitably resulting in eventual stepwise progressive neurologic decline (12). Mutation in complex II proteins are rarely associated with Leigh's syndrome but are a common cause of inherited paragagliomas and phaeochromocytomas (13). Complex III deficiency typically causes a severe multisystem early onset disorder, which is recessively inherited and rare (14). Mutations in complex IV or in complex IV assembly factors result in severe, typically fatal, infantile disease. Complex IV assembly gene disorders include *SURF1* (Surfeit locus protein 1), associated with Leigh Syndrome. Mutations in nDNA-encoded complex V subunit genes also appear very rare (10).

The role of mtDNA maintenance, including replication and integrity, is under control of the nucleus; when the dialogue between the two genomes becomes impaired, the resulting diseases are characterized by mtDNA depletion, multiple mtDNA deletions, and site-specific mtDNA point mutations (15). Mutations in several genes have been associated with defects in mtDNA maintenance, these include *ANT1*, which encodes the adenosine nucleoside translocator; *PEO1*, which encodes a helicase called Twinkle; *TYMP*, which encodes the cytosolic enzymem thymidine phosphorylase (TP); *POLG*, which encodes the mitochondrial polymerase γ catalytic subunit and is by far the commonest cause of mtDNA stability disorders; and *POLG2*, which encodes the dimeric accessory subunit of POLG (16). *POLG* gene example shows how a single mutant gene can cause either mtDNA depletion or multiple mtDNA deletions and result in diverse syndromes from a severe hepatocerebral disorder of infancy or childhood (Alpers syndrome), through to adult-onset autosomal dominant or recessive progressive external ophthalmoplegia (PEO), to parkinsonism and other clinical phenotypes, including sensory ataxic neuropathy, dysarthria and ophthalmoparesis (SANDO), or mitochondrial recessive ataxia syndrome (17-18). MtDNA depletion may be linked to other syndromes: encephalomyopaty (linked to mutations in *SUCLA2*, *SUCLG1* or *RRM2B*), mitochondrial neurogastrointestinal encephalomyopathy (MNGIE; caused by mutations in *TYMP*), myopathy (caused by mutationsin *TK2*), hepatoencephalopathy (associated also to mutations in *DGUOK* and *POLG*).

The concept of multi-system disease is crucial in mitochondrial medicine and makes the molecular diagnosis challenging, as many different medical specialties are involved. Tissues and organs with high energy demands are most severely affected. Accordingly, besides clinical pictures that primarily affect a specific tissue such as primary mitochondrial myopathy, mitochondrial dysfunction typically affects also brain (e.g., seizures, stroke–like episodes, diffuse encephalopathy, ataxia, parkinsonism, dementia), sense organs (deafness, optic atrophy, retinal pigmentary degeneration), extraocular muscle (ptosis, external ophthalmoplegia), skeletal muscle (myopathy, exercise intolerance), heart, liver (hepatopathy), kidney (renal tubular acidosis) and endocrine system (diabetes).

Diagnosis

The diagnosis of MD diseases is complex and requires several investigations including routine and par-

ticular laboratory tests, electrophysiological studies, neuroimaging studies, muscle biopsy and genetic test.

The first step in the diagnosis is represented by the knowledge of patient and family history and physical and neurologic examination of the patients (19), in order to research the "mitochondrial red flags" often overlooked (19).

Generally, serum creatine kinase levels are normal or mild elevated; one exception is the myopathic variant of the mtDNA depletion syndrome (19). Elevated lactate and/or elevated lactate to pyruvate ratio can suggest the presence of mitochondrial dysfunction but they can also be caused by other conditions such as organic acidemias, other inborn errors of metabolism, toxins, tissue ischemia, and certain other diseases (20). Simple, non-invasive test for metabolic myopathies are the forearm exercise test and incremental exercise test on a cycle ergometer, which can reveal an exaggerated increased production of lactate with muscle activity in the first case and an anticipation of the anaerobic threshold in the second case (21-24). Several amino acids including alanine, glycine, proline, and threonine found to have high levels in mitochondrial disorders, conversely citrulline, was found to be significantly decreased in the plasma of subjects with mitochondrial disease (25-27). The exact sensitivity and specificity of amino acid elevations in patients with mitochondrial disease are not yet known. Lactic aciduria is often seen in mitochondrial diseases and the major biomarkers of mitochondrial dysfunction are 3-methylglutaconic acid, dicarboxylic aciduria, 2-oxoadipic aciduria, 2-aminoadipic aciduria, and methylmalonic aciduria, malate and fumarate (28-32).

Recently, Lehtonen and coworkers analyzed serum values of FGF21 and GDF15, two new promising biomarkers, from patients with mitochondrial diseases and non-mitochondrial disorders partially overlapping with mitochondrial disorder phenotypes providing Class III evidence that elevated FGF21 accurately distinguishes patients with mitochondrial myopathies from patients with other conditions (33-35), and FGF21 and GDF15 mitochondrial myopathy from other myopathies (33-36). However, the validation of this evidences and the definition of a reliable specific biomarker of mitochondrial disease, also for prognosis and, especially, form monitoring drug-response to treatment is still strongly needed.

Usually electromyography shows specific myopatich pattern but may be also normal (24-37). Electroneurography is abnormal in those forms of mitochondrial myopaties presenting with neuropathy (38).

Neuroimaging may play a significant role in the diagnosis of mitochondrial disorders, especially some patterns of magnetic resonance imaging (MRI) of the brain. MELAS syndrome is characterized by stroke-like lesions that do not respect vascular territories (39), while more florid white matter abnormalities are seen in MNGIE, Leigh syndrome, and mitochondrial disorders due to defects in the aminoacyl-tRNA synthetases. Also Proton magnetic resonance spectroscopy (MRS) is useful and may demonstrate high levels of lactate or succinate (39-41).

The gold standard to demonstrate mitochondrial dysfunction in vivo is muscle biopsy (42, 43). The main pathological features of MD are ragged red fibers (RRF) (obtained trought modified Gomori trichrome stain) or ragged blue fibers (RBF) (when using succinate dehydrogenase staining) and COX negative fibers (44). RRF or RBF consist of a subsarcolemmal accumulation of enlarged, abnormal mitochondria with ultrastructurally dense cristae and paracrystalline inclusions (45), probably an attempt to compensate the respiratory chain dysfunction. Mitochondrial proliferation with RRF is typically found in patients with deletions, depletion or point mutations in tRNA genes (MELAS, MERRF) (46). In contrast, RRF are almost never observed in patients with mtDNA point mutations of structural genes (LHON, NARP, Leigh) (47). On the contrary, RRF may be found in Leigh syndrome (48). Importantly, normal muscle histology does not rule out a MD. Biochemical spectrophotometric investigations can be performed in tissue homogenates to measure the activity of respiratory chain (RC) enzymes. A mutation in a nDNA or mtDNA gene encoding a structural subunit of the RC commonly results in deficiency of the solitary affected enzyme, whereas the impairment of mitochondrial protein synthesis (mutations in tRNA, single or multiple deletions, and mitochondrial depletion) reduces the activity of respiratory complexes I, III, and IV while sparing complex II (SDH) which is entirely encoded by nDNA (19).

Genetic studies can be performed on muscle biopsy, to detect single or multiple deletions of mtDNA and/or mtDNA depletion and to sequence the entire mtDNA for point mutations (49). Molecular studies on peripheral circulating cells or other easily accessible tissues (like urinary sediment, oral mucosa, hair follicles and cultured skin fibroblasts) can be performed. Genetic studies on blood cells are more useful in nDNA than in mtDNA-associated disorders because, as a result of the mitotic segregation, mtDNA mutations (especially mtDNA deletions) are more easily detected in muscle than other tissues (49-19). Interestingly, urine sediment often contains mtDNA mutations at higher levels than blood, buccal swabs, or even fibroblasts so screening urine for mtDNA mutations may be recommended before muscle biopsies (24-37).

Therapy

Currently, there is no available disease-modifying therapy for mitochondrial disorders. Therefore, treatment of mitochondrial disease involves predominantly supportive care, early treatment of organ-specific manifestations

with pharmacological therapy (for example, antiepileptic drugs for seizures, dietary or pharmacological therapy for diabetes mellitus) and surgical remedies (such as blepharoplasty, cochlear implants for hearing loss and placement of a cardiac pacemaker or ablation of secondary conduction pathways) which are useful in prolonging and improving the quality of life of patients (50).

The most obvious strategy to treat mitochondrial disorders is to enhance RC function mitigating both energy crisis and oxidative stress, and remove noxious metabolites (like lactate, thymidine). Several agents (mostly nutritional supplements) have been investigated with double-blind, placebo-controlled studies. These include antioxidants (such as CoQ, α-lipoic acid, vitamin C and vitamin E), agents that modulate mitochondrial electron transfer flux (such as riboflavin), ROS scavengers (Coenzyme Q10, MitoQ, glutathione) nitric acid precursors (such as l-arginine), energy buffers (such as creatine and l-carnitine) and drugs involved mitochondrial biogenesis (such as vitamin B3) (51).

None has demonstrated a striking efficacy in clinical trials, although numerous non-blinded studies, anecdotal case reports and small series have suggested modest efficacy (52-54). Coenzyme Q10 is specifically indicated in patients with defects of CoQ10 biosynthesis who show a dramatic improvement following high doses and long-term CoQ10 supplementation (55). Idebenone, a short-chain benzoquinone, is the only disease-specific drug recently approved to treat visual impairment in adolescents and adults with Leber's hereditary optic neuropathy. Early and prolonged idebenone treatment may improve significantly the frequency of visual recovery and possibly change the natural history of the disease, as demonstrated in two clinical trials and retrospective case series (56, 57). Patients with stroke like episodes seem to benefit from oral/intravenous arginine or citrulline (nitric oxide precursors) supplementation with improvements on clinical symptoms associated with stroke-like episodes and decreased severity and frequency of these episodes (58, 59).

Children with liver failure due to hepatocerebral syndromes associated with mtDNA depletion and mutations in *DGUOK* or *POLG* may also benefit from liver transplantion, especially if the brain and other organs are relatively spared (60).

There has been great interest in exercise regimen and their benefit on both biochemical and clinical end-points in mitochondrial disorders. Aerobic, endurance, and resistance training programs have been studied. It is likely the benefits of exercise are due to reversal of deconditioning, which is a common feature of many muscle diseases. Furthermore exercise seems to alter the underlying pathology by promoting mitochondrial biogenesis, increasing antioxidant enzyme activity, muscle mitochondrial enzyme activity, maximal oxygen uptake, and peripheral muscle strength (61, 62).

Other management considerations in mitochondrial disease include the avoidance of agents, which may worsen the patient's condition (51). Statins often cause toxic effects on skeletal muscle, although the precise mechanisms remain unclear. Statin should therefore be used cautiously in mitochondrial disease, with careful monitoring of symptoms and the serum creatine kinase. Antiretroviral agents are known to cause reversible and dose-dependent mitochondrial toxicity. Valproic acid is known to interfere with mitochondrial function and in clinical practice may aggravate symptoms in patients with mitochondrial disease, and valproate-induced hepatotoxicity may be more common. Antibiotics, (specifically minocycline, chloramphenical, and aminoglycosides), can be harmful to the mitochondria because they inhibit mtDNA translation and protein synthesis, causing hearing loss as well as cardiac and renal toxicity. Mitochondrial patients may be at a higher risk for propofol infusion syndrome and propofol use should be avoided or limited to short procedures; narcotics and muscle relaxants can create respiratory depression, and caution must be used in mitochondrial patients who may already have hypotonia, myopathy, or an altered respiratory drive.

Although therapies for specific mitochondrial diseases, such as MNGIE, are emerging, treatment for the vast majority of mitochondrial disorders is limited and relies on symptomatic management, so new treatment approaches are strongly needed. One of the most promising strategy is the use of molecules able to enhance mitochondriogenesis. Biotechnology companies in the US and the Netherlands have already launched early phase I and II studies for drugs targeted at MD patients. In 2014, Cerutti and Coworkers showed here that supplementation with nicotinamide riboside, a natural NAD(+) precursor, or reduction of NAD(+) consumption by inhibiting the poly(ADP-ribose) polymerases, leads to marked improvement of the respiratory chain defect and exercise intolerance of the Sco2 knockout/knockin mouse, a mitochondrial disease model characterized by impaired cytochrome c oxidase biogenesis, highlithing this strategy as potentially translatable into therapy of mitochondrial disorders in humans (63). Future strategies are also expected for MNGIE, such as liver transplantation (64) which has been demonstrated capable of rapidly normalize serum levels of toxic nucleosides, or gene therapy using a liver-targeted AAV vector transferring of the human *TYMP* coding sequence (65).

Future perspective

While enormous progress has been made in diagnostics and pathomechanisms in MD, major advances in treatment

have unfortunately not paralleled this so far and the clinical management is mainly focused on symptom control. The heterogeneity of MD, the contribution of two genomes, the little knowledge of their natural history, lack of awareness among general practitioners and sometime specialists and the complexity of the diagnostic approach all contribute to the unsuccessful management of these diseases and limit the correct interpretation, reproducibility and comparability (including the possibility of meta-analyses) of clinical trials. Thus, it is crucial to better define all of the clinical, biochemical, histological and molecular factors involved in MD as well as genotype-phenotype correlations and the natural history of the different syndromes.

In the last several years mitochondrial medicine has been extremely successful in the characterization of the molecular and genetic basis of disease. However, "deep phenotyping" in mitochondrial disorders is a challenging but necessary task as well. Clinical variability is broad even in individuals with the same genotype and the statistical power is low in single-center studies, owing to the rarity of these conditions. Large and comprehensive patient registers may represent the right instruments to fill this lack and put fundamental basis to launch collection of longitudinal clinical data and to build controlled clinical trials. Granted by Telethon-UILMD in 2009, the nation-wide Italian collaborative network has been established a web-based registry of patients with MD harmonized with other European Databases and Networks, collecting and characterizing clinically, histologically and genetically more than 1400 patients so far, with both adulthood and childhood onset of the disease. This Network has been instrumental to redefine the clinical features of common mtDNA mutations (e.g., m.3243A > G (66), m.8344A > G (67), single deletions (68)) and to better elucidate some signs and symptoms of mitochondrial diseases including myoclonus (69) and peripheral neuropathy (38). The register has also allowed the dissemination of new knowledge and highlight MD to public opinion makers.

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