Mitochondrial diseases: a review
Doenças mitocondriais: uma revisão

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ABSTRACT
Mitochondria are organelles responsible for production of most energy through oxidative phosphorylation process (OXPHOS). It contains a double strand DNA (mitDNA) of about 16,500 bp encoding two ribosomal RNAs and 37 mitochondrial proteins. Mutation in mitDNA may result in multisystem syndromes known as mitochondrial diseases, affecting predominantly tissues that require high levels of ATP such as skeletal muscle (mitochondrial myopathies), brain (e.g. MELAS, MERRF, LHON e NARP), liver, kidney (Fanconi syndrome), heart and endocrine glands (Pearson syndrome). A case of mitochondrial disease was first reported in 1962 and correlation of such disease with mutations in mitDNA gained scientific importance in late 1980’s. There are 150 alterations reported in mitDNA capable of producing metabolic dysfunctions of clinical relevance. To date, no standard protocol for diagnosis of mitochondrial diseases has been established, partially due to the wide amplitude of clinical manifestation generally observed. A combined analysis of clinical data, biochemical, morphological and laboratory tests must be performed to evaluate mitochondrial respiratory chain activity and integrity of nuclear and mitochondrial genomes. Currently, there are no effective treatments available for mitochondrial diseases, but only palliative therapeutics using conventional strategies to relieve symptoms. Thus, gene therapy emerges as potential therapeutic strategy for more efficient treatment of mitochondrial diseases.

Keywords: Mitochondrial diseases; Metabolic diseases; Review

INTRODUCTION
Mitochondria are organelles responsible for most energy production in the body, through oxidative phosphorylation process (OXPHOS). Since this process and ATP biosynthesis are under nuclear genetic and
mitochondrial control (mitDNA) and the information found in the mitDNA is directly related to organelle characteristics and behavior, genome mitochondrial mutations may produce primary genetic defects in the respiratory chain, whose phenotypical expression is generically denominated as mitochondrial disease. Mitochondrial diseases are clinical and genetically heterogeneous and mainly affect tissues that require great energy consumption. Development and improvement of biomolecular techniques have increased the number of diagnostic tools for identification of mitDNA changes that result in human diseases, greatly elucidating these disorders, although, so far, there is no scientifically effective treatment for them. The purpose of this study is to describe mitochondrial morphology, physiology and genetics, as well as mitDNA changes and relate them to the main clinical syndromes resulting from deficiencies of mitochondrial respiratory chain.

MITOCHONDRIA

The mitochondria is a cytoplasmic organelle that oxidates pyruvate (originated from metabolism of sugar, proteins and fatty acids) and releases chemical energy (ATP), water and carbon gas (CO₂). As an organelle found only in eukaryotic organisms, it was primarily identified in the 19th century, although its functions were only understood after 1948, when isolation of intact mitochondria was possible.

Origin

Life organization on Earth was structured after development of primitive unicellular organisms. It is believed that anaerobic heterotrophic cells (protoeukaryotic) involved aerobic bacteria, determining a symbiotic relation: bacteria were fed with nutrients by their host and released energy, ensuring energetic supply for activities in both structures. Certain evidences corroborate with the theory of the bacterial origin of mitochondria, as its average size, ribosomal RNA sequence, proportion of mitDNA nitrogen bases and presence of mitochondrial double membrane are similar to those found in the bacteria(1).

Mitochondrial morphology

The mitochondria is formed by two membranes presenting different functions and delimiting two mitochondrial compartments: one between the outer and inner membranes - the intermembranous space, and the other internally to internal membrane, where the mitochondrial matrix is found. The outer membrane, due to presence of porin (a protein that forms water channels), is permeable to molecules smaller than 5,000 Daltons, allowing them into the intermembranous space. Therefore, the intermembranous space content is similar to that found in cytosol, presenting several enzymes involved in nucleotide phosphorylation originated from ATP found in the mitochondrial matrix.

The inner membrane presents selective permeability to molecules and ions that are metabolized or required by matrix enzymes, and is related to oxidative phosphorylation. Its impermeability to certain ions is due to presence of cardiolipin, a phospholipid rarely found in other plasmatic membranes. Its structure is composed by folds projecting to the matrix, known as mitochondrial crests, increasing the total area of inner membrane. The amount of crests is directly proportional to cell energy demand. Three types of protein complexes are found in the crests and are related to the electron transporting chain, ATP synthesis and metabolite passing to matrix.

The mitochondrial matrix is a concentrated watery solution which contains hundreds of enzymes and substrates involved in energy production, among which are found those related to pyruvate and fatty acid oxidation of the citric acid cycle. Mitochondrial enzymes related to energetic processes catalyze oxidation of organic nutrients by molecular oxygen. Several copies of mitochondrial genome, ribosomes and RNAs are also found in the matrix, as well as enzymes involved in mitochondrial gene expression.

Energetic processes

The energetic need of eukaryotic organisms is not compatible with glycolytic processes, which convert monosaccharide in pyruvate, releasing two ATP molecules. In order to fulfill their needs, there are processes that may potentialize the energetic performance originating from glycolysis products. Processes such as citric acid cycle and oxidative phosphorylation occur inside the mitochondria. The first consists of a sequence of cyclic relations using pyruvate, acetyl-CoA and water to produce GTP (chemical energy) and high-energy electrons, carried by NAD+ and FADH complexes for the respiratory chain.

A set of membrane proteins and electron carriers of the inner mitochondrial membrane is involved in ordered electron transfer. Energy produced in this transport is used to generate an electrochemical gradient of protons by means of hydrogen pumps found in the membrane. Potential energy stored in this gradient is converted into chemical energy through
ATPase, using kinetic energy of the proton flow inside it to yield energetically unfavorable reaction between ADP and inorganic phosphate (figure 1).

Mitochondrial DNA

Mitochondrial DNA was discovered in 1966 by Van Bruggen, Sinclair, Steven and Nass, but it was only sequenced in 1981, by Anderson et al. It is a double-strand DNA with nearly 16,500 bp and 37 genes encoding two ribosomal mitRNA, 22 mitRNA carriers and 13 polypeptide chains, which are essential components of the complexes I, III, IV and V in oxidative phosphorylation process (complex II components are fully encoded by the nuclear genome) (figure 1). It corresponds to approximately 0.3% of cellular DNA amount and is considered semi-autonomous, since mitochondrial maintenance and functioning depend on proteins encoded by genes of nuclear DNA, although it has self-control mechanisms for duplication, transcription and translation.

The mit DNA is grouped in protein complexes denominated nucleoids. Each mitochondria presents nearly 10 nucleoids, each one containing up to 8 mitDNA. Therefore, there are up to 100 molecules of mitDNA in mitochondrial matrix, presenting a total of over 2000 copies for each cell. The mitDNA replication does not occur exclusively in the S-period of cell cycle. Some mitDNA molecules replicate more times than others during a single cell cycle, but in the end of the process, the number of total mitDNA is doubled.

DNA mitochondrial point mutations may occur, such as substitution, deletion or duplication, at a 10-fold higher rate than in nuclear DNA. Several factors seem to contribute for mutation rate increase: most mitDNA is composed by codifying sequences, intermediated by a few regulatory sequences; mitochondria presents DNA repair mechanisms of low effectiveness; mitDNA is found near the electron carrier chain, and is exposed to great amounts of free radicals and presence of small amounts of histone proteins, which are responsible for DNA condensation and protection.

The mitDNA mutations are frequently heteroplasmic mutations, that is, mutant and normal mitDNA molecules coexist in one single cell. Mutant mitDNA proportion varies among different tissue cells and according to the individual age. These mutations are not of mendelian inheritance, once cell division, after a randomized process of mitDNA replication, each one of the four daughter cells resulting from a meiosis process receive integral and mutated molecules, apparently in an randomized manner.

During fecundation, cytoplasmic organelles for formation of the zygote originate from the feminine gamete (ovule), because the paternal mitDNA presents a small ubiquitin molecule that induces its proteolysis.
during oocyte penetration. This has to do with a maternal uniparental inheritance; therefore, a woman presenting a mitDNA mutation may transmit a variable amount of mutant mitDNA to her descendents, resulting in either healthy individuals or carriers of functional disorders(7).

Maternal mitDNA inheritance, its presence in multiple copies in the organelle (polyplasmia), the possibility of mutation in only a few copies of mitDNA (heteroplasma) and grade variation of these mutations in cell generations (mitotic segregation) turn the clinical expression of mitochondrial diseases both spatially and temporally variable (affected tissues). These peculiarities partially explain the great clinical and biochemical inter and intra-familial heterogeneity found in expression of mitochondrial diseases(8).

MITOCHONDRIAL DISEASES

Mitochondrial diseases consist of a group multisystem disorders resulting from deficiency in ATP mitochondrial synthesis(9), which is a consequence of mitDNA or nuclear DNA mutations (chart 1). Since 1962, when a case of mitochondrial disease was first described and, particularly since the late 1980’s, when specific mitDNA was associated to human disease mutations(9), at least 150 alterations capable to produce metabolic dysfunctions have been identified in the mitochondrial genome, thus comprising a group of genetic diseases of statistical significance(10).

As these syndromes present involvement of mitochondrial energy production, tissues with high ATP demand and higher oxidative turnover are more affected. Thus, some organs are primarily affected - such as skeletal striated muscles, brain, liver, heart and endocrine glands, with eventual secondary involvement in other organs. Despite this diversity of clinical expression of mitochondrial diseases (chart 2; figure 2), the investigation of some syndromes has been particularly important for the improvement and understanding of mitochondrial medicine. Descriptions of prevalent syndromes are as follows:

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes syndrome (MELAS)

It is characterized by strokes with hemipareses and hemianopsia, and affects usually individuals aged under 40 years and frequently during childhood. It may present migraine, recurrent vomiting, dementia and lactic acidosis. In approximately 80% of cases the molecular defect is a punctual mutation(10). Some studies reported a prevalence of 16/100,000(11).

Syndrome of myoclonic epilepsy with ragged-red fibers (MERRF)

The clinical characteristics include myoclonias, generalized seizures, cerebellar ataxia and myopathy. In young individuals, the disease is triggered by a rate of mutated mitDNA of 95% and in patients between 60-70 years of age, at a rate of 60%.(9). Most MERRF patients present a mutation (A8344G) in a gene for t mitRNA of mitDNA. Some studies demonstrate a prevalence of approximately 0.25/100,000 in the United Kingdom(9).


<table>
<thead>
<tr>
<th>mitDNA alterations</th>
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<tbody>
<tr>
<td>Isolated (sporadic) deletions</td>
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<td>Duplications or duplications/deletions (maternal inheritance)</td>
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<td>Punctual mutations (maternal inheritance)</td>
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<td>Nuclear DNA alterations (mendelian inheritance)</td>
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<td>Mutations in genes encoding enzymes or translocases related to:</td>
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<td>-Substrate transport</td>
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<td>-Substrate use</td>
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<td>-Krebs cycle</td>
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<td>-Electron carrier chain</td>
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<td>-Oxidation/phosphorylation coupling</td>
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<td>Deficient import of mitochondrial proteins</td>
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<td>Deficiency of intergenic signaling:</td>
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<tr>
<td>-Multiple mitDNA deletions</td>
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<td>-mitDNA deletion</td>
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</tbody>
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Chart 2. Examples of disorders resulting from mitDNA mutations and their inheritance (Adapted from: Nussbaum RL, McInnes RR, Willard HF. Genética médica. 6th ed. Trad. de Motta PA. Rio de Janeiro: Guanabara Koogan; 2002.)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Phenotype</th>
<th>Mutation more frequent in mitDNA molecule</th>
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<tbody>
<tr>
<td>Leber’s hereditary optical neuropathy (LHON)</td>
<td>Fast death of the optic nerve, leading to blindness in young adults</td>
<td>Substitution of complex I gene of the electron carrier chain (A11778G, A3460G e T14484C).</td>
</tr>
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<td>Neuroptathy, ataxia and retinitis pigmentosa (NARP), Leigh’s syndrome</td>
<td>Neurupothy, ataxia, retinitis pigmentosa, delayed development, mental retardation, lactic acideemia</td>
<td>Punctual mutations in the ATPase subunit of gene 6 (T8993G/C).</td>
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<tr>
<td>MELAS</td>
<td>Mitochondrial encephalomyopathy, stroke episodes before 40 years of age, lactic acidosis, ragged-red fibers in skeletal muscles (negative Cox), diabetes melilitus. Myoclonic epilepsy, ragged-red fibers in muscles, ataxia, neurosensory deafness</td>
<td>Punctual mutation in tRNA^Leu(UUR).</td>
</tr>
<tr>
<td>MERRF</td>
<td>Progressive or not-syndromic neurosensory deafness</td>
<td>Punctual mutation in tRNA^Leu(UUR). (A3243G).</td>
</tr>
<tr>
<td>Deafness</td>
<td>Progressive weakness of extraocular muscles</td>
<td>Mutation in rRNA(12S).</td>
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<td>Chronic progressive external ophthalmoplegia (CPEO)</td>
<td>Pancreatic insufficiency, macrocyt anemia, trombocytopenia and neutropenia</td>
<td>Punctual mutation in rRNA(12S) and major deletions.</td>
</tr>
<tr>
<td>Pearson syndrome</td>
<td>CPEO of late onset with cardiac block, pigmented retina.</td>
<td>Major deletions</td>
</tr>
<tr>
<td>Kearns Sayre syndrome (KSS)</td>
<td></td>
<td>Isolated deletion of mitDNA</td>
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Neuropathy, ataxia and retinitis pigmentosa (NARP)

Simultaneous occurrence of neuropathy, ataxia and retinitis pigmentosa characterizes a multisystem disorder of maternal inheritance, frequently followed by dementia, seizures and axonal sensory neuropathy. It concerns a punctual mutation (T8993G) in the gene that encodes ATPase 6, subunit of complex V of electron carrier chain (figure 1). The literature suggests a prevalence of 1/12000\(^{(12)}\).

Lebers Hereditary Optic Neuropathy (LHON)

It is a maternal-transmission disorder characterized by loss of bilateral central vision due to degeneration of the optic nerve, predominantly in men. The molecular basis for predominance in men has not been established. In 95% of cases, there is a punctual mutation (G3460A, G11778A or T14484C), related to problems in the gene sequence encoding polypeptides for complex I of the respiratory chain\(^{(9)}\). Approximately 90% of cases present homoplasmatic pattern (all mtDNA molecules are mutated), while 10% present heteroplasmatic pattern\(^{(13)}\). One study presents a world prevalence of 6.5/100,000\(^{(14)}\).
Leigh syndrome

This is a progressive neurodegenerative condition typically occurring in childhood, which mainly include dysfunctions of the brainstem, basal ganglia, demyelination, psychomotor regression, delayed development, ataxia, seizures and peripheral neuropathy. Heteroplasmy is observed in 95% of affected children. Although several mutations are associated with this syndrome, punctual mutations in ATPase 6 gene are the most common\(^{(7)}\). One study indicates a world prevalence of 2.25/100,000\(^{(3)}\).

Mitochondrial myopathies

The mitDNA mutations resulting in respiratory chain alterations and limited exclusively to muscular tissues are denominated mitochondrial myopathies. Although it is predominant in skeletal striated musculature, it may also affect cardiac striated muscle. Structural alterations in genes encoding t mitRNA or mitDNA, large-scale mitDNA deletions and depletion of mitDNA prevent mitochondrial protein synthesis, consequently affecting structures of the respiratory chain complexes that contain subunits originated in this synthesis\(^{(8)}\). Diseases with this type of involvement usually affect multiple systems, even though the brain and skeletal muscles remain affected, which gave rise to the term “mitochondrial encephalomyopathy”. Large-scale mitDNA deletions are associated to three important clinical phenotypes: (I) Kearns-Sayre syndrome, an early multisystem disease (before 20 years of age), characterized by chronic progressive external ophthalmoplegia (CPEO), pigmented retinopathy, cardiac block and other minor symptoms, such as ataxia, dementia, neuropathy and endocrinopathy; (II) Pearson syndrome, a fatal disease in childhood that affects bone marrow functioning as well as the pancreas exocrine function (due to fibrosis and acinar atrophy), frequently followed by macrocytic anemia, thrombocytopenia and neutropenia; (III) pure myopathy associated with CPEO, palpebral ptosis and proximal weakness of the extremities. Mutations in specific encoding genes may lead to alterations in cytochrome b, which is the only complex III subunit of the respiratory chain decoded by mitDNA, as well as changes in the COX system (cytochrome c oxidase) of complex IV.

Other syndromes

Other syndromes, despite lower prevalence, represent significant physiological disorders resulting from mitDNA mutations, such as chronic progressive external ophthalmoplegia (CPEO), caused by multiple (Kearns-Sayre Syndrome) or single deletions, and characterized by a slow and progressive limitation of eye movements. It may progress to ophthalmoplegia (total ocular immobility) and is commonly followed by weakening of extrinsic ocular muscles.

The kidneys may be a point of mitochondrial disorders, as observed in Fanconi syndrome, caused by a mitDNA depletion (quantitative) leading to a proximal tubular dysfunction. The main clinical manifestations include polyuria, polydipsia, and elevated levels of glucose, amino acids, uric acid and electrolytes in urine. Its world prevalence is around 1/100,000\(^{(14)}\).

Hearing deficiency is another clinical manifestation frequently present in several mitochondrial syndromes, and may be caused by mutations either in mitDNA or nuclear DNA. Mitochondrial hearing deficiency may be of the syndromic type, with a multiple clinical picture, such as in the case of deafness (maternal inheritance), or non-syndromic, when deafness is presented as the single symptom, such as the occurrence of presbyacusia. Studies presenting involvement of the internal ear due to mitochondrial dysfunction demonstrate that the predominant type of hearing deficiency is neurosensory with energetic involvement of cochlea\(^{(15)}\). Other researches reported impairment of external ciliated cells or alterations in brainstem cochlear nuclei. This mitochondrial disorder presents a low prevalence, which corresponds approximately to 0.5% to 1% of all genetic hearing deficiencies\(^{(15)}\).

Two types of diabetes are related to mitochondrial disorders - maternal inheritance and diabetes mellitus. The first is caused by the same mutation present in MELAS (A3243G), with is a similar clinical picture of type I or type II diabetes, followed by maternal inherited bilateral hearing deficiency, with prevalence of roughly 1.5% of the total diabetic population. The second one is characterized by a global metabolic disorder of carbohydrates, lipids and proteins, with hyperglycemia resulting from deficient insulin secretion, deficient insulin action, or both. Some studies\(^{(16)}\) with patients presenting diabetes mellitus reported hyperexpression of mitochondrial genes for cytochrome oxidase (COX) I and III, NADH dehydrogenase 4 and r mitRNA, leading to alterations in the oxidative phosphorylation process, which is commonly verified in cases of uncontrolled diabetes.

Aging has also been related to mitochondrial diseases. Common neurodegenerative disorders, such as Alzheimer, Parkinson and Huntington diseases, atherosclerosis and diseases affecting motor neurons present mitochondrial anomalies, although a direct relation with mitDNA has not been fully elucidated\(^{(17)}\).
DIFFERENTIAL DIAGNOSIS

Currently, there is no standard protocol for diagnosis of mitochondrial diseases, partially due to the wide range and overlapping of clinical manifestations. However, myopathy, lactic acidosis, involvement of the central nervous system, ataxia, neurosensory bilateral deafness, low height, endocrinological and muscular changes, pigmented retinopathy and recurrent strokes are highly suggestive of mitochondrial disease and support differential diagnosis, particularly when they are concomitant.

Mitochondrial diseases may be divided into three groups: (i) those clearly recognized, associated to specific mtDNA anomalies, (ii) those whose symptoms indicate changes in mitochondrial genome and (iii) those presenting uncommon symptoms of mitochondrial diseases, but with potential mtDNA mutation. For family history, it is relevant to search for cases of deafness, diabetes, visual disorders, delayed development, neonatal or infant death in family, among others. In patients with symptoms compatible with changes in mitochondrial functions, a combined analysis of clinical data, biochemical, morphological and laboratory tests must be performed to evaluate respiratory chain activity and integrity of nuclear and mitochondrial genomes (figure 3). Useful data include levels of glucose, lactate and creatinine kinase, evaluation of renal filtration through excretion of amino acids and organic acids, CSF test, electrocardiogram and electromyography to evaluate electrical activity of cardiac and skeletal striated muscles, and magnetic nuclear resonance or computed tomography to assess integrity of the central nervous system.

The advances in molecular medicine enable a more specific investigation, including histochemical analyses and electronic microscopy (to study general muscle morphology, COX system activity and existence of ragged-red fibers in the subsarcolemma), biochemistry (to study the complexes I, III, IV and V of the respiratory chain) and molecular genetics (for mtDNA mutations and rearrangements).

TREATMENT

To date, there is no effective treatment for mitochondrial disorders, while there is few evidence of changing the course of disease. Therefore, current therapeutics is essentially palliative, to ameliorate symptoms of each patient through conventional techniques.

However, some studies show improvements when multivitamin complexes, cofactor supplements and medications are given to provide supplementary energy sources and reduce energy dependence produced by oxidative phosphorylation; to increase antioxidative cell capacity and reduce mitochondrial membrane damages caused by free radicals that are elevated in mitochondrial disorders; to eliminate intracellular cytotoxic products accumulated by malfunctioning of OXPHOS and to supply enzymes or their precursors, in order to compensate deficiency of respiratory chain elements. Among them, there are ubiquinone (coenzyme Q10), idebenone (ubiquinone synthetic analogue), vitamins C, K, (menadione) and E, thiamine (vitamin B1), lipoic acid, riboflavin (vitamin B2) and niacinamide (vitamin B3), nicotinamide, triacylglycerol, L-carnitine, succinate, folate, dichloroacetate (DCA), selenium and antioxidants or a combination of several supplements.

While there is no consensus regarding the benefit of therapy based on nutritional supplementation, other promising alternatives to treat mitochondrial diseases are under consideration. In theory, aerobic exercises would increase cardiac output, mitochondrial density, muscle capillarization and oxidative enzyme and antioxidant capacity. On the other hand, resistance exercises would reduce the proportion of mutant DNA in skeletal muscle, since they induce muscular damage and activate satellite cells, which transfer mitochondria and their wild mitDNA to damaged myofibrils. Molecular medicine and gene therapy have also presented expressive improvements in the field of mitochondrial diseases. Development of a method to insert normal self-replicative mitDNA in mitochondrial matrix, the attempt to recover the clinical phenotype through manipulation of mtDNA heteroplasmy, or even importing tRNA to mitochondrial matrix, replacing non-operative molecules resulting from alterations in its mitochondrial synthesis, are research lines already established. Therefore, scientific research is a key tool in the fight against this group of diseases, meanwhile medicine is still impotent.

REFERENCES


