Introduction

Living things undergo aging process over time. This is nature. During the process of natural senescence, alterations in the genetic signals dictate the direction, the rate, and the consequences of aging. Aging process also occurs in the mitochondrial genome. The genetic components of human mitochondria lie within the circular structure of the mitochondrial DNA (mtDNA) within the mitochondria itself1,2. However, interactions of nuclear DNA (nDNA) and mtDNA dictate the integrity of the structural and functional proteins of mitochondrial machinery and cells. Lack of histone protection and low efficiency of mtDNA repair render mtDNA more susceptible than nDNA to “genetic error” (mutation). As a consequence, mutated mtDNA or nDNA causes alterations in mitochondrial functions, one of which is derangement of oxidative phosphorylation (OXPHOS) and cellular energy failure. Disorders of OXPHOS3,4 and mitochondrial disease (MtD)5–7 are 2 common names used interchangeably for “mitochondrial cytopathy”8. Therefore, knowing the molecular basis of mitochondria, interaction between nDNA and mitochondria, and recognition of the variety of phenotypes of MtD are important steps to the understanding of mitochondrial medicine.

Awareness of the Molecular Basis and Clinical Manifestations of MtD

Aberration from the normal functions of mitochondrial respiratory chain or OXPHOS causes disorders that involve multiple organs, such as the brain, ears, endocrine systems, eyes, heart, kidneys, liver, muscles, peripheral nerves, and skin. Normal functions of the mitochondrial respiratory systems are dictated by the integrity of both nDNA and mtDNA. Direct hits of either the mutation of mtDNA itself or nDNA may cause defects in cellular or mitochondrial OXPHOS. Two forms of mtDNA mutations are recognized9. They are: (1) mtDNA mutations that interfere with mitochondrial protein synthesis; and (2) mtDNA mutations that impair specific protein-encoding genes, and thus mitochondrial protein transport10. In contrast, there are 3 patterns of nDNA mutations that affect the mitochondrial respiratory
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chain. They are: (1) nDNA gene mutations that encode subunits of respiratory chain complexes such as complexes I and II; (2) nDNA mutations that interfere with the genes encoding ancillary proteins needed for correct assembly and function of the respiratory chains such as complexes III, IV, and V; and (3) nDNA mutations that interrupt intergenomic signaling and thus the quality of mtDNA. The “double hits” theory may apply to the latter. Regardless of the mechanism, the final consequence would be derangement of cellular OXPHOS, which manifests clinically as MtD. More than a dozen clinical syndromes of MtD have been recognized\(^\text{11}\) and they are collectively called the syndromic MtD/OXPHOS disease. Recognition of the manifestations of syndromic MtD is of utmost importance to the diagnosis of the majority of MtD.

Characteristics of Syndromic MtD

1. Chronic progressive external ophthalmoplegia (CPEO)\(^\text{12,13}\) manifests by chronic insidious onset of bilateral ophthalmoparesis (Figure 1C) with or without familial occurrence. CPEO can be misdiagnosed as ocular myasthenia gravis or other forms of congenital ocular myopathy. The lack of diurnal change of weakness, and disproportionate paralysis of the extraocular muscles and levator palpebrae are the key features to distinguish CPEO from myasthenia gravis. Diabetes mellitus (DM) is a common association in Taiwanese patients with CPEO and A3243G genotype\(^\text{14,15}\). However, A3243G genotype may have diverse clinical phenotypes\(^\text{16}\). In essence, mtDNA rearrangement, deletion, and single point substitution are common molecular mechanisms of CPEO\(^\text{16–18}\).

2. Kearns-Sayre syndrome (KSS)\(^\text{19–21}\) has onset of symptoms at childhood or adolescence. The clinical manifestations are ocular symptoms and signs of CPEO with pigmentary retinopathy, such as retinitis pigmentosa (Figure 2), ataxia with cerebellar atrophy, dementia or mental retardation, short stature, deafness, cardiac arrhythmia, multiple endocrine disorders, and myopathy. Lactic acidosis and cerebral white matter lesions\(^\text{22}\) may occur. The diagnostic criteria are progressive external ophthalmoplegia, Lactic acidosis and cerebral white matter lesions may occur. The diagnostic criteria are progressive external ophthalmoplegia,

Figure 1. (A, B) Magnetic resonance imaging (T2WI) of a young man with mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes phenotype and A3243G genotype, (C) whose mother shows a chronic slowly progressive external ophthalmoplegia (CPEO) phenotype with bilateral blepharoptosis and A3243G genotype (CPEO/A3243G).
pigmentary retinopathy, and one of the 3 other features such as complete heart block, cerebrospinal fluid protein > 100 mg/dL, and ataxia. Phenotypic mimicry has been reported in KSS and Lowe syndrome. The prognosis is poor. MtDNA deletion occurs in more than 70% of patients with KSS. Approximately 99% of mitochondrial deletions occur within the common area between nucleotide positions (np) 5,000 and 16,000. Deletions are of different sizes in different people. Rapidly dividing cells lose the deleted mitochondria over time. A sporadic somatic cell mutation (germ line is normal), mitochondrial duplication, and single point mutation at tRNA have also been reported.

3. Leber’s hereditary optic neuropathy (LHON) is the most frequent cause of maternally inherited, painless, bilateral blindness that occurs in young adults. Pattern reversal visual evoked potentials (VEP) confirm the severity of the optic neuropathy (Figure 3). Movement disorders, dementia, and cardiomyopathy may occur. Cerebral white matter changes resemble multiple sclerosis. It is estimated that 75–95% of LHON are caused by mutations of G11778A (40–60%) and G3460A (20%) that affect complex I, and G15257A (15%) that affect complex III. At least 8 major mutations have been recognized.

4. Mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes (MELAS) syndrome is the most common syndromic manifestation of MtD that occurs in all age groups. The clinical course of MELAS is slowly progressive with stepwise deterioration. The severity of lactic acidosis correlates with the severity of neurologic deficit. Epilepsy is frequent in patients with MELAS. Epilepsia partialis continua or Koshevnikov syndrome may occur. Calcification of bilateral basal ganglia, ischemic stroke-like lesions (Figure 1A and B) involving the posterior temporal and parieto-occipital lobes crossing 2 or more arterial territories, and deep white matter changes in watershed areas and subcortical regions are common in patients with MELAS. Brain single-photon emission computed tomography (SPECT) shows focal hyperperfusion (hyperemia) or regional hypoperfusion at different stages of the disease. Reversible vasogenic edema, focal brain...
ischemia or “mitochondrial vasculopathy”\textsuperscript{35,36}, neuronal metabolic derangement, and cellular energy failure have been accrued to the characteristic lesions seen on brain magnetic resonance imaging (MRI). Cortical atrophy with dementia is a late consequence of MELAS. A-to-G transition of tRNA\textsubscript{Leu} (UUR) gene at np 3243 is the most frequent mutation (> 90% of patients) found in both Caucasians and Orientals with MELAS (MELAS/A3243G)\textsuperscript{37–43}.

5. The syndrome of myoclonic epilepsy and ragged red fibers (MERRF)\textsuperscript{41,44} occur more often in children and young adults. Photosensitive myoclonus, myopathy, and epilepsy are cardinal features. Myopathy occurs in 70% of patients carrying the A8344G mutation\textsuperscript{41}. Ataxia may occur in 50% of patients. Short stature, optic neuropathy, and lipomata are uncommon associations. A8344G mutation in the tRNA\textsubscript{Lys} gene occurs in 80–90% of patients with MERRF\textsuperscript{41,45,46}. T-to-C transition at np 8356 (T8356C) in the tRNA\textsubscript{Lys} gene\textsuperscript{47} and point mutation in the tRNA\textsubscript{Ser} gene\textsuperscript{46} have also been reported.

6. Maternally inherited myopathy and cardiomyopathy syndrome occurs in A3260G in tRNA\textsubscript{Leu} and T3250C in tRNA\textsubscript{Leu}\textsuperscript{48}. Under very rare circumstances, dual mutations with 3,399 large-scale deletion and T5814C substitution\textsuperscript{49} may present with a clinical syndrome of oculopharyngeal somatic myopathy. Further, patients with MtD may develop neuropathy with ataxia and retinitis pigmentosa (NARP). T8993G (leu to arg) and mutation at ATPase subunit 6 (complex V) have been accrued to cause NARP\textsuperscript{50}.

7. Overlap syndromes of MtD have been reported. They are LHON/MELAS overlap syndrome with G13513A mutation\textsuperscript{51}, MERRF/MELAS with A3243G overlap syndrome\textsuperscript{52}, CPEO/MELAS overlap syndrome\textsuperscript{53}, and CPPDM3243/MELASDM3243\textsuperscript{15}.

8. Polyneuropathy, ophthalmoplegia, leukoencephalopathy, and intestinal pseudo-obstruction (POLIP), also known as mitochondrial neurogastrointestinal encephalomyopathy, is characterized by dysmotility and paresis of the gastrointestinal tract, episodic vomiting, and progressive malnutrition that may lead to death in early or middle age life\textsuperscript{54}. Postmortem pathologic studies showed neuronal loss of the celiac ganglion, fibrosis of the mesenteric and Auerbach plexuses, and leukoencephalopathy\textsuperscript{54,55}. Multiple mtDNA deletion is one of the causes of POLIP\textsuperscript{56}.

**Nonsyndromic Manifestations of Mitochondrial Disease (NSMMtD)**

OXPHOS diseases may present with a number of NSMMtD ranging from an isolated neurologic symptom or sign to various combinations with non-neurologic features. Movement disorders manifested by chorea, tremor, hemiballism, ataxia, or dystonia, migraine-like headache, neuropsychiatric manifestations, and endocrine disorders such as hypopituitarism, deficiency of secretion of antidiuretic hormone, hypogonadism, and DM are common combinations. Some other features of central nervous system manifestations may be secondary to organ failure such as hepatic encephalopathy and nephropathy.

**Work-up of Suspected MtD**

The work-up of suspected MtD, in the order of increasing strength of evidence and difficulty of the techniques and their invasiveness, are listed below.

1. As MtD is notoriously caused by maternal inheritance of a variety of mtDNA mutations, obtaining a detailed family history and pedigree of the index case is of utmost importance. Specific attention must be paid to the family history of a wide range of symptomatology, such as visual disturbance, hearing deficit, migraine-like headache, early onset of stroke-like episodes, familial occurrence of epilepsy, involuntary movements, symptoms suggestive of myopathy or recurrent myoglobinuria, exercise intolerance, unexplained endocrine disorders and renal dysfunction, familial occurrence of early-onset DM, thyroid disorders, etc.

2. Blood tests must include plasma levels of glucose, lactate and pyruvate ratio, creatinine phosphokinase, liver and renal function tests, etc. Following informed consent, collection of mtDNA from among the family members, especially from siblings and maternal relatives, is a key step to the molecular diagnosis. Searching for common mtDNA mutations, such as mtDNA rearrangement, mtDNA deletion, mitochondrial duplication, or point mutation, can be laborious. Determination of heteroplasmy, mosaicism, and mutant load is critical for explaining the heterogeneity of phenotype-genotype correlation\textsuperscript{57–60}. Moreover, the mtDNA mutation loads are selectively amplified through generations\textsuperscript{60} and aging\textsuperscript{37}. 

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3. Work-up of the specific organ systems depend on the degree of suspicion of the syndromes which the index case may have. Examinations of the eye ground for retinitis pigmentosa, optic atrophy, and cataract. Evaluations of the auditory systems, gastrointestinal systems, kidneys, endocrine systems, central and peripheral nervous systems, neuromuscular systems, and cerebrospinal fluid examinations, etc. must be individualized. The extent of examinations depends on the specific syndromes suspected.

4. Neuroimaging and electrophysiologic studies include brain MRI, and brain SPECT for those with epilepsy, dementia, and stroke-like episodes. Magnetic resonance spectroscopy and positron emission tomography are used for the evaluation of regional brain metabolism, electroencephalography for seizure evaluation, VEP for objective assessment of the optic pathways, and nerve conduction studies for quantification of peripheral nerve involvement.

5. Muscle biopsy with a rapid modified Gomori-trichrome stain looking for the presence of “ragged red fibers” (RRF) is the classical standard procedure. An immunohistochemical stain can be very helpful for assessment of the severity of respiratory enzyme deficiency and molecular diagnosis. A deficiency of coenzyme Q in the muscle, but not in blood, has been reported. Oral supplementation of coenzyme Q may be helpful either in familial mitochondrial encephalomyopathy with coenzyme Q deficiency or in patients with Leigh’s disease. Furthermore, RRF and ultrastructural changes of mitochondria are also seen in non-MtDs. The ultrastructural alterations of mitochondria in the skeletal muscles of patients with MtD/OXPHOS disease were elucidated in the papers of Shy and Gonatas and Shy et al., and were described as “giant mitochondria”, “pleoconial myopathy”, and “macroconial myopathy” in 1964 and 1966, respectively.

6. Molecular diagnosis must include the whole mitochondrial genomic screening of the different tissues. The mutant load and mosaicism of mitochondrial mutations will determine the phenotypes of the patients. Mitochondrial genomic DNA from muscle tissue, peripheral white blood cells, and hair follicles are samples most often examined in patients suspected to have MtD and in screening for subclinical cases or unaffected family members. The percentage of mutant load of different tissues can be determined that correlate with the clinical manifestation.

Pathologic Changes of MtD

The histopathologic characteristics of MtD have been well studied in skeletal muscle. Three aspects of assessment include light microscopic findings, electron microscopic findings, and molecular diagnosis.

Light microscopic findings

1. The histopathologic finding of RRF of skeletal muscles on modified Gomori-trichrome histochemical stain is a hallmark of mitochondrial disorders. The mitochondrial dysfunctions could lie within the respiratory chain or outside it. Examples of some conditions of primary MtD due to defects outside the respiratory chain are carnitine palmityl transferase, pyruvate dehydrogenase complex, beta-oxidation, or fumarase deficiencies. Conversely, in some types of defects of the respiratory chain, RRF are not seen. Examples are cytochrome c oxidase (COX) deficiency in Leigh’s syndrome, and Kojewnikoff syndrome with MELAS. Supplementation of coenzyme Q may be helpful either in familial mitochondrial encephalomyopathy with coenzyme Q deficiency or in patients with Leigh’s disease. Furthermore, RRF and ultrastructural changes of mitochondria are also seen in non-MtDs. The ultrastructural alterations of mitochondria in the skeletal muscles of patients with MtD/OXPHOS disease were elucidated in the papers of Shy and Gonatas and Shy et al., and were described as “giant mitochondria”, “pleoconial myopathy”, and “macroconial myopathy” in 1964 and 1966, respectively.

2. Abnormal histochemical stains for mitochondrial enzymes such as succinate dehydrogenase, NADH-tetrazolium reductase, COX, etc. can be semiquantified.

Electron microscopic findings

1. Megaconial myopathy (enlarged mitochondria with disoriented cristae).
2. Pleoconial myopathy (abnormal proliferation of normal-looking mitochondria).
3. Intramitochondrial “paracrystalline” inclusions or osmiophilic inclusions. There are 2 types of crystalline inclusions in MtDs: (a) typical type 1 paracrystalline inclusions with “parking lot” appearance (Figure 4), usually found in the intracristal spaces; and (b) type 2 large rectangular crystallloid inclusions.
which are usually found between the inner and outer mitochondrial membranes. Paracrystalline inclusions may also be seen in normal human muscle, in patients with neuromuscular disorders other than primary MtD, such as in zidovudin myopathy, muscle ischemia, and in certain conditions caused by mitochondrial toxins such as the uncoupling agent 2,4-dinitrophenol. The nature and origin of these inclusions remain unclear. They are composed of proteins that are enzymatically inert, but may be immunoreactive to mitochondrial creatine kinase.

4. Abnormally branched and distorted cristae, concentrically arranged cristae, flattened cristae peripherally, or straight transverse cristae.

5. Mitochondrial matrix may appear empty or vacuolated or contain osmiophilic or other electron-dense bodies. Rarely, the electron-dense bodies consist of amorphous or granular inclusion, which are rich in iron.

6. “Needle-like” crystal inclusions (Figure 5) in the mitochondria with deformed mitochondrial cristae. In essence, understanding of the current concepts of mitochondrial medicine is a milestone in the diagnosis and management of patients with MtD.

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References


