**Genotype–Phenotype Correlation of Maternally Inherited Disorders Due to Mutations in Mitochondrial DNA**

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**SUMMARY**

Mitochondrial disorders are heterogeneous systemic ailments that are most often caused by maternal inheritance of a variety of mutations of the mitochondrial (mt) DNA. Paternal inheritance and somatic mutation are rare. The disorders are well recognized not only for the genotypic heterogeneity, but also the phenotypic variation among the affected members of a single family. The genotype–phenotype correlation of the diversity of the syndromic and non-syndromic features of mitochondrial disorders are discussed. Some aspects of the molecular mechanisms of this heterogeneity, and the histopathologic findings are highlighted. [Taiwanese J Obstet Gynecol 2006;45(3):201–207]

**Key Words:** encephalopathy, epilepsy, mitochondrial disease, mitochondrial DNA, mutation, myopathy, oxidative phosphorylation, OXPHOS disease

**Historical Overview**

The discovery of DNA in human mitochondria by Nass and Nass in 1963 [1], followed by the successful complete sequencing of human mitochondrial DNA (mtDNA) almost two decades later by Anderson et al [2], heralded a new era of mitochondrial medicine. mtDNA is a circular DNA of 16,569 base pairs, with one light strand and one heavy strand. Absence of histone structure and inefficient DNA repair render it susceptible to “genetic error” or mutation. Mutations of mtDNA in humans cause many disorders, collectively known as oxidative phosphorylation (OXPHOS) disorder [3], or mitochondrial disease (MtD) [4–9]. The concept of mitochondrial cytopathy emerged from a report by Luft et al in 1962 [10], which described a young woman with hypermetabolism of nonthyroid origin, morphologically abnormal muscle mitochondria and “loose coupling” of oxidation and phosphorylation of the isolated muscle mitochondria.

The histopathologic findings of *ragged red fibers* (RRF) on modified Gomori trichrome histochemical stain was then described by Engel and Cunningham in 1963 [11]. The ultrastructural alterations of mitochondria of the skeletal muscles of patients with MtD/OXPHOS were elucidated in the reports by Shy and Gonatas, and described as pleoconial myopathy and megacconial myopathy in 1964 and 1966, respectively [12,13]. In 1977, the nosology of mitochondrial encephalomyopathy was introduced [14]. This terminology remains widely used nowadays, although the brain and muscles are not the only two organ systems involved in most cases of mitochondrial disorders.

Affections of OXPHOS involve multiple organs, such as the brain, eyes, ears, heart, liver, kidneys, endocrine systems, integument, peripheral nerves, muscles and so on. A dozen clinical syndromes have been reported (Table), and they are called the syndromic MtD/syndromic OXPHOS disease. Genotypic and phenotypic
heterogeneity are notorious to MtD/OXPHOS disease [9,15]. However, non-syndromic neurologic manifestations or secondary forms of central nervous system (CNS) dysfunction may also occur. Regardless of whether it is syndromic or non-syndromic MtD, mtDNA mutations are frequently caused by mtDNA rearrangement [16], mtDNA deletion [17–20], duplication, or point mutation [21–24]. Dual point mutations and large-scale deletion plus a critical point mutation are rare [25,26].

Genotype–Phenotype Correlation of Human Syndromic MtD

Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS)

MELAS syndrome [27] is the most common syndromic manifestation of mitochondrial encephalomyopathies that occur in all age groups [24,28]. The clinical course of MELAS is slowly progressive with stepwise deterioration. The severity of lactic acidosis correlates with the severity of neurologic deficit [29]. Seizures are frequent in patients with MtD. However, intractable epilepsy, epilepsy partialis continua or Koshevnikov syndrome may occur in patients with MELAS syndrome [30].

Calcification of bilateral basal ganglia is a frequent neuroimaging finding in patients with MELAS [31]. The stroke-like episodes often involve the parieto-occipital lobes and posterior brain regions crossing two or more arterial territories [32]. Deep white matter changes in watershed areas and subcortical regions are common in patients with MELAS [33,34]. Reversible vasogenic edema, focal brain ischemia, neuronal metabolic derangement and cellular energy failure have been accruled to the characteristic lesions seen on brain magnetic resonance imaging (MRI) [35]. Findings from brain single photon emission computed tomography in patients with MELAS may be anecdotal. Focal hyperperfusion (hyperemia) [36] or regional hypoperfusion [37] may be seen at different stages of the disease. The latter has been proposed to be due to a “mitochondrial vasculopathy” and can be seen in MELAS/A3243G family members who manifest clinically as chronic progressive external ophthalmoplegia (CPEO) (CPEO/A3243G)

<table>
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<tr>
<td>CPEO</td>
<td>mtDNA rearrangement</td>
<td>16</td>
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<tr>
<td></td>
<td>mtDNA del, tRNA_{Leu} (A3243G, spm), MTMTN*CPEO5692G</td>
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<td>mtDNA rearrangement, del</td>
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<tr>
<td></td>
<td>tRNA_{Leu} (spm)</td>
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<tr>
<td>LS</td>
<td>NARP, SURF1 (spm)</td>
<td>56–61</td>
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<td></td>
<td>ND3, NDS, ATPase 6 (spm)</td>
<td></td>
</tr>
<tr>
<td>LHON</td>
<td>G11778A (spm), G3460A, G15257A, ND1, 4, 6 (spm); dual mutations</td>
<td>63–70</td>
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<tr>
<td>MELAS</td>
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</tr>
<tr>
<td></td>
<td>3302, 3303, 3243, 3250, 3260 (spm), tRNA_{Leu} and tRNA_{Glu}</td>
<td>38–43</td>
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<tr>
<td></td>
<td>dual mutations</td>
<td></td>
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<tr>
<td>MERRF</td>
<td>tRNA_{A8344G} (A8344G, spm)</td>
<td>26, 46, 47</td>
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<td>T8356C, POLG (spm)</td>
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<td>OPSM</td>
<td>3,399 bp del plus T5814C mutation</td>
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<td>MCM</td>
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Overlap syndrome

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<tr>
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<td>LS/MELAS</td>
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<td>MELAS/MERRF</td>
<td>tRNA_{Leu}, tRNA_{Glu}</td>
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*Refer also to references 3, 6, 8, 9, 17–29, 35, 44, 49, 65, 66 and 76. CPEO = chronic progressive external ophthalmoplegia; mtDNA = mitochondrial DNA; del = deletion; spm = single point mutation; KSS = Kearns–Sayre syndrome; LS = Leigh’s syndrome; LHON = Leber’s hereditary optic neuropathy; MELAS = mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episode; MERRF = myoclonic epilepsy, ragged red fibers; MNGIE = mitochondrial neurogastrointestinal encephalomyopathy; OPSM = oculopharyngeal somatic myopathy; MCM = myopathy/cardiomyopathy.
Dementia with cortical atrophy is a late consequence of MELAS.

A-to-G transition in the tRNA^{Leu(UUR)} gene at nucleotide position (np) 3243 is the most frequent mutation (>90% of patients) found in both Caucasians and Orientals with MELAS (MELAS/A3243G) [23, 24, 38-43]. However, single point mutation at np 3271 (MTTL1* MELAS 3271C), at np 3302, 3303, 3250 and 3260 [8, 38, 43, 44], and double mutations at tRNA^{Leu} and tRNA^{Glu} [25] have also been reported. The mutant genes of MELAS were amplified through generations [45] and anticipation may occur.

**Myoclonic epilepsy and ragged red fibers (MERRF)**

MERRF [9, 24, 46-49] occurs 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 [26]. RRF on histopathologic study of muscle biopsy is an important clue to the diagnosis. However, it could be absent in some proven cases. Ataxia may occur in 50% of patients. Short stature, optic neuropathy and lipomata are uncommon associations. The A8344G mutation in the tRNA^{Lys} gene occurs in 80-90% of patients with MERRF [24, 46]. T-to-C transition at np 8356 (T8356C) in the tRNA^{Lys} gene [47] and point mutation in the tRNA^{Ser} gene [48] have also been reported.

**Chronic progressive external ophthalmoplegia (CPEO)**

CPEO [7, 8, 16-20, 37, 49, 50] can sometimes be misdiagnosed as ocular myasthenia gravis or other forms of congenital ocular myopathy. Disproportionate paralysis of the extraocular muscles and levator palpebrae, and lack of diurnal change of the ophthalmopleasis are the key features to distinguish CPEO from myasthenia gravis. CPEO with carnitine deficiency may overlap with MELAS [50]. mtDNA rearrangements and large-scale deletion of mtDNA are common in CPEO [16, 19, 20]. A combination of 3,399 bp deletion and T5814C mutation has been reported in a Taiwanese patient with slowly progressive oculopharyngeal somatic myopathy resembling CPEO plus syndrome [26]. Heteroplasmic A3243G may also manifest clinically as CPEO with diabetes mellitus (CPPDM3243) in one affected family member and MELAS with diabetes mellitus (MELASDM 3243) in another (CPPDM3243/MELASDM3243 syndrome) [15].

**Kearns–Sayre syndrome (KSS)**

KSS [51] has cardinal features of pigmentary retinopathy, CPEO, cerebellar atrophy and cerebral white matter lesions [32-34]. Additional features are cardiac arrhythmia, mental retardation and multiple endocrine disorders. Onset of symptoms usually occurs in the teens and the prognosis is poor [49]. mtDNA deletion [16, 19, 20] occurs in >70% of patients with KSS. Single point mutation at tRNA^{Leu} has also been reported [36, 39, 52]. Approximately 99% of mitochondrial deletions occur within the common area between base pair at the position 5000 to 16000. Deletions are of different sizes in different people [17]. Rapidly dividing cells lose the deleted mitochondria over time. A sporadic somatic cell mutation (germ line is normal) and mitochondrial duplication occur rarely. The diagnostic criteria for KSS includes: CPEO, pigmentary retinopathy, and one of the three other features such as complete heart block, cerebrospinal fluid (CSF) protein ≥ 100 mg/dL and ataxia. A variety of some other associated features are short stature, deafness, dementia, myopathy, lactacidosis and endocrinopathy. KSS with Lowe syndrome [53] and other overlap syndrome (see below) may occur.

**Leigh’s syndrome (LS)**

LS [54] is a genetic disorder with mendelian recessive inheritance. It affects males more often than females [54-60]. Based on the age at onset of disease, it can be divided into four types. The “infantile form” [54, 55] occurs before the age of 1 year, the “early childhood form” develops by the age of 2 years, the “adolescent form” in the first two decades of life, and the “chronic form” [56, 57]. Clinical features include: (1) psychomotor developmental arrest; (2) ataxia, hypotonia, other cerebellar signs; (3) loss of vision; (4) dysphagia and other pyramidal signs; (5) central neurogenic hyperventilation; (6) neuropathic signs. Computed tomography (CT) of the brain show symmetrical low density in the lenticular nucleus, called “holes in the brain”. CSF may be abnormal with elevated CSF protein. LS has been considered an inborn error of gluconeogenesis [58]. A variety of metabolic abnormalities can be found, such as deficiency of mitochondrial enzyme, thiamine triphosphate phosphoryltransferase (TTP) in the brain, deficiency of pyruvate carboxylase in the liver, decreased Krebs cycle activity, deficiency of pyruvate dehydrogenase and endophile system, and accumulation of pyruvate and lactic acids [58]. Co-enzyme Q [59] and antioxidants [60] may be useful for some patients with LS and encephalopathy. LS has a diverse genetic basis. Single point mutations in the NARP, SURF1, ND3, NDS, and ATPase 6 genes have been reported (Table). NARP mutation accounts for about 20% of LS, especially when the percentage mutant load is high. Certain cases have a point mutation, T-to-C transition, at np 9176 [61].
Autopsy of the brain and spinal cord of an infant with LS [54] shows: (1) vascular (capillary) or endothelial proliferation with less prominent neuronal changes (necrotizing encephalopathy); (2) degenerative changes in neuronal processes and myelin sheath resulting in loosening of neuropil; (3) astrocytosis with microglial proliferation; (4) mitochondrial changes. LS predominantly affects gray matter in a symmetrical pattern, especially involving the basal ganglia, diencephalon, substantia nigra, cerebellum, brainstem nuclei and spinal cord [54, 62]. It may rarely affect the centrum semiovale and the peripheral nervous systems. It differs from Wernicke’s encephalopathy in that it involves the substantia nigra more extensively and involves the mammillary bodies much less. Histopathology of the skeletal muscles shows RRF and abnormal mitochondria. Cardiomyopathy, renal tubular dysfunction and growth hormone deficiency may also be present.

Leber’s hereditary optic neuropathy (LHON)
LHON is the most common cause of maternally inherited blindness that occurs in adolescence. Painless bilateral vision loss of subacute onset is the main feature. Brain MRI shows white matter changes resembling multiple sclerosis [33, 34, 63]. Involuntary movements such as chorea, dementia [64] and cardiomyopathy may occur [65, 66]. At least eight major mutations have been recognized [63–70]. It is estimated that 75–95% of LHON are caused by mutations in the following three genes: G11778A (40–60%) and G3460A (20%) that affect complex I, and G15257A (15%) that affect complex III. Dual mutations have been reported [68]. Phosphorous magnetic resonance spectroscopy may be abnormal in patients with LHON [70].

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) syndrome
MNGIE has also been recognized as POLIP (polynuropathy, ophthalmoplegia, leukoencephalopathy and intestinal pseudo-obstruction) [71–74]. Dysmotility and paresis of the gastrointestinal tract with episodic vomiting and progressive malnutrition may lead to death in early or middle age life [71]. Postmortem pathologic studies showed neuronal loss of the celiac ganglion, fibrosis of the mesenteric and Auerbach plexuses, leukoencephalopathy [71–74] and neuromyopathy. Multiple mtDNA deletion, point mutation in the thymidine phosphorylase gene and missense mutation in the NDS gene have been reported [71–75].

Neuropathy, ataxia, retinitis pigmentosa (NARP)
NARP is characterized by neuropathic weakness of the limbs, neuropathic sensory deficit, ataxia and retinitis pigmentosa [60, 76]: T8993G transition with leucine to arginine substitution and ATPase subunit 6 (complex V) defect [60].

Myopathy/cardiomyopathy syndrome
Myopathy/cardiomyopathy syndrome is rare, and it is caused by a point mutation at A3260G in tRNA^Leu, or T3250C in tRNA^Leu [77]. Mitochondrial myopathy may also be caused by chronic use of zidovudine [78].

Overlap syndromes
Overlap syndromes with diverse combinations, such as KSS/CPEO [19], MELAS/MERRF [79], LHON/MELAS [80], CPEO/MELAS [50], CPPDM/MELASDM [15], Leigh-MELAS [81] and KSS/Lowe [53] overlap syndromes and many others have been described.

Non-syndromic Manifestations of MtD
MtD/OXPHOS disease may present with a number of non-syndromic manifestations ranging from an isolated neurologic symptom or sign to various combinations with non-neurologic features, e.g. involuntary movements such as chorea, tremor, hemiballism, ataxia and dystonia, migraine-like headache, neuropsychiatric manifestations and endocrine disorders such as hypopituitarism, deficiency of secretion of anti-diuretic hormone, hypogonadism and diabetes mellitus. Some other features of CNS manifestations may be secondary to organ failure, such as hepatic encephalopathy and nephropathy.

Regardless of the mechanisms of the underlying genetic defects, a common molecular pathway is either a loss-of-function or a gain-of-a-new-function of the mitochondrial machinery that results in uncoupling of cellular oxidative phosphorylation. Apart from mtDNA point mutation, mtDNA deletion or duplication, nuclear DNA and mtDNA (nDNA/mtDNA) communication syndrome, and mtDNA depletion syndrome (MDS) may occur. nDNA/mtDNA communication syndrome is an autosomal dominant disorder with multiple nDNA/mtDNA deletions, however, MDS is an autosomal recessive disorder.

Mitochondrial depletion syndrome (MDS)
MDS most likely involves the brain, liver, kidney and muscles. The hepatocerebral form has been reported to be due to DGK gene mutations [82] and mtDNA copy number depletion. Mutation of the SUCLA2 gene encoding succinyl coenzyme A synthase of Krebs cycle has been reported [49].
Molecular Mechanisms Explaining the Heterogeneity of the Phenotypes of Mitochondrial Disorders

Heterogeneity of the clinical expressions of mtDNA defects can be explained by the following mechanisms. First, widespread distribution of the mutant mtDNA in different tissues varies among individuals [83]. The proportion of mutant mtDNA (percentage mutant load) in different tissues of the affected individual varies as well [5,8,16,24,40,84]. Phenotypes depend on the mutant load of different tissues [24,40,84]. It has been shown that the mutant load of muscles, but not of blood cells, of patients with A3243G mutation correlates with the occurrence of several clinical features [24]. Positive correlation between the frequencies of occurrence of the clinical features of recurrent stroke, dementia, epilepsy, and ataxia and percentage mutant load has been observed in the muscles. The higher the percentage of mutant load in muscles, the higher the frequency of occurrence of these specific features [24]. Conversely, an inverse correlation is valid for tissues with the myoclonic epilepsy and deafness (the higher percentage of A3243G mutant load has the lower frequency or percentage of occurrence of these features) [24]. Second, an intercellular heteroplasmy (cells carry both mutant and wildtype mtDNA) [85,86] and mosaicism exists. Different cells have different thresholds (safety margin) to the effects of mutant load. The amplification of mutant mtDNA through successive generations has been documented [45]. Finally, interactions among environmental factors and genes, communication and signal transduction between nDNA and mtDNA, and many other unknown modifying factors modulate the expression of the primary mtDNA defect.

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References


