

Genetics of Mitochondrial Myopathies

Jin-Hong Shin and Dae-Seong Kim*

Department of Neurology, Pusan National University Yangsan Hospital, Yangsan, Korea

Mitochondrion is an intracellular organelle with its own genome. Its function in cellular metabolism is indispensable that mitochondrial dysfunction gives rise to multisystemic failure. The manifestation is most prominent with tissues of high energy demand such as muscle and nerve. Mitochondrial myopathies occur not only by mutations in mitochondrial genome, but also by defects in nuclear genes or secondarily by toxic insult on mitochondrial replication. Currently curative treatment modality does not exist and symptomatic treatment remains mainstay. Administration of L-arginine holds great promise according to the recent reports. Advances in mitochondrial RNA import might enable a new therapeutic strategy.

Key words: Mitochondrial myopathies, MELAS syndrome, MERRF syndrome, Progressive external ophthalmoplegia, Heteroplasmy, Histochemistry

Introduction

The mitochondrion is an intracellular organelle with double membrane residing in most of the eukaryotic cells. Citric acid cycle and beta oxidation take place in mitochondrial matrix, and most of the adenosine triphosphate (ATP) pool is produced on the mitochondrial inner membrane by way of oxidative phosphorylation.¹⁾ Its main function is *cellular power plants*, that the influence of mitochondrial dysfunction is most prominent in tissues of high energy demand, such as muscle and nerve. However, the phenotype may not be limited to the tissue of major concern as mitochondria exist in every type of tissue in human body.

The recognition of family with mitochondrial DNA mutation is straightforward when the inheritance is clearly through maternal lineage. However, the pattern can be obscured in families with wide variation of mutational load,²⁾ and sporadic cases with *de novo* mutation do occur.³⁾ Manifestations between family members may vary significantly and even one phenotype can change into another as the child survives critical period, for example, in

Pearson syndrome. Clinical spectrum of mitochondrial disorders is broad, so consensus diagnostic criteria for mitochondrial disorders have been proposed for children⁴⁾ and adults.⁵⁾ Sequence map of human mitochondrial genome with its normal and pathogenic variants is publicly available.⁶⁾

In this review, we will discuss genetic features of mitochondrial myopathies as well as their key findings. Representative syndromes will be described in detail and the less common entities will be summarized in Table 1.

Mitochondrial myopathies by mitochondrial genomic mutation

Mitochondrion is a unique organelle in that it contains its own circular genome of 16.5 kb.⁷⁾ Mitochondrial genome encodes only 13 of 93 subunits for oxidative phosphorylation system. All 22 tRNAs and 2 rRNAs are on mitochondrial genome, while the other proteins including ribosomal proteins are imported

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*Corresponding author: Dae-Seong Kim, M.D., Ph.D.

Department of Neurology, Pusan National University Yangsan Hospital, 20 Geumo-ro Mulgeum-eup Yangsan-si, Gyeongsangnam-do 626-770, Korea
Tel: 055-360-2122, Fax: 055-360-2152, E-mail: dskim@pusan.ac.kr

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Table 1. Mitochondrial myopathies by primary genetic defects

Phenotype	Related genes	
	Mitochondrial	Nuclear
MELAS	<i>MTTL1</i> , other mitochondrial tRNAs, NADH dehydrogenase subunits	
MERRF	MTTK, <i>MTTL1</i> , other mitochondrial tRNAs	
PEO	Single large deletion, duplication, <i>MTTL1</i>	<i>POLG1</i> , <i>POLG2</i> , <i>SLC25A4 (ANT1)</i> , <i>C10ORF2 (TWINKLE)</i> , <i>RRM2B</i> , <i>DNA2</i>
KSS	Single large deletion, large tandem duplication	
MNGIE		<i>ECGF1</i>
myopathy	Single large deletion, <i>MTTL1</i> , <i>cytochrome b</i>	<i>TK2</i>

MELAS, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibers; PEO, progressive external ophthalmoplegia; KSS, Kearns-Sayre syndrome; MNGIE, mitochondrial neurogastrointestinal encephalo myopathy

from cytoplasmic translation. Some mutations in mitochondrial genome can significantly affect mitochondrial function. Each mitochondrion contains several copies of DNA molecule, and each cell is powered by many mitochondria, over 100,000 in case of an oocyte. Mitochondrion replicates its own DNA and fuses or divides in response to cellular metabolic needs.

All the mitochondrial DNAs in a cell are normally identical, a state called *homoplasmy*. It may not be the rule, especially with pathologic mutations, and it is called *heteroplasmy*.⁸⁾ Mutational load is defined as a ratio of mutant to normal sequences. It can strikingly vary between generations or between siblings, as early in the germline development the cells undergo the mitochondrial bottleneck, where a very small pool of mitochondria split into daughter cells.⁹⁾

The mutational load can be different in tissues of one individual either due to selective survival or random distribution. Cellular dysfunction emerges if the mutational load exceeds a threshold. This quantitative nature of mitochondrial disorders, opposed to qualitative affection in autosomal inherited disorders, makes the inheritance pattern hard to be recognized.

Inheritance of mitochondrial mutation is almost strictly matrilineal.¹⁰⁾ It is not only because the mitochondria in a sperm are a thousand times outnumbered by those of an oocyte at the moment of fertilization, but also selectively degraded during development. Anecdotal reports leave open the rare possibility of paternal transmission of mitochondrial mutation.^{10,11)}

Mitochondrial myopathy by nuclear genomic mutation

Over 85% of the mitochondrial proteins are actually encoded in nuclear genome. They are synthesized in cytoplasmic machinery and imported through mitochondrial membranes. In case of mitochondrial dysfunction by defects of nuclear genome, the

inheritance pattern should be like any of autosomal disorders while the clinico-pathologic features overtly indicate mitochondrial disorders.

Mitochondrial myopathy not by primary genetic defect

Toxic insult is another possibility to be considered in evaluation of mitochondrial myopathy. The well-documented example is zidovudine-induced necrotizing myopathy.¹²⁾ Zidovudine, a nucleoside analogue reverse transcriptase inhibitor, is an antiretroviral drug to treat HIV infection. In relation to its cumulative dose, it may cause proximal myopathy accompanying fatigue and myalgia. Its histologic features include ragged red fiber (RRF) and cytochrome c oxidase (COX)-negative fibers like in other mitochondrial myopathies.¹³⁾ Molecular analysis shows mitochondrial depletion supposedly by polymerase γ inhibition.^{14,15)} The pathology is reproduced in murine model.¹⁶⁾

Another example is the myopathy by clevudine, a pyrimidine nucleoside analogue for treatment of chronic hepatitis B.¹⁷⁾ We found patients on long-term usage of clevudine complained slowly progressive proximal muscular weakness over several months. The laboratory findings revealed elevated serum creatine kinase and lactic acid. Muscle biopsy revealed numerous COX-deficient RRFs and selective type 2 fiber necrosis with marked myonecrosis. Depletion of mitochondrial DNA is also shown by quantitative PCR analysis. The symptom was reversible within 16 weeks after discontinuation of clevudine.¹⁸⁾

Skeletal muscle biopsy in mitochondrial myopathy

Basic morphological findings of mitochondrial disorder on hematoxylin-eosin stain may show non-specific myopathy such

as excessive variability of muscle fiber size and scattered necrotic/regenerating fibers. The "ragged red fiber (RRF)" is the histological hallmark of mitochondrial disorders, shown on modified Gomori-trichrome stain (Fig. 1A).^{19,20} It is a muscle fiber in which compensatory proliferation of dysfunctional mitochondria accumulates in subsarcolemmal and intermyofibrillar space. The bright red masses around the sides of the muscle fiber contrasts with green sarcoplasm just as the name describes. Infrequent RRF in patients with advanced age should be considered normal.

Succinate dehydrogenase (SDH) is the only enzyme encoded exclusively by nuclear genome among the mitochondrial respiratory complexes. Normally type I (oxidative) fibers contain more mitochondria than type II (glycolytic) fiber; thus type I fibers are darker than type II fiber on SDH stain. Mitochondrial dysfunction does not compromise SDH activity, that SDH activity on histochemical stain reflects mitochondrial proliferation. RRF on modified Gomori-trichrome stain is depicted as ragged "blue" fiber on SDH stain (Fig. 1B). Vascular endothelium may also get highlighted blue with mitochondrial dysfunction.²¹

COX is encoded by both mitochondrial and nuclear genome. It reliably reflects mitochondrial respiratory activity in many types of mitochondrial mutation. Like on SDH stain, it is normal that type I fibers are stained darker than type II fibers on COX stain.

Contrary to SDH stain, it is characteristic to find COX-negative fibers in mitochondrial myopathy (Fig. 1C). RRFs, ragged blue fibers, and COX-negative fibers may or may not coincide.¹⁹

Other frequent histologic findings of mitochondrial myopathies include type-specific muscle fiber atrophy and fibers with lipid or glycogen accumulation. Electron microscopy reveals mitochondrial proliferation in subsarcolemmal or intermyofibrillar space. Mitochondria with abnormal shapes or with hypoplastic/dystrophic cristae can be seen. Paracrystalline inclusions show distinctive parking-lot appearance (Fig. 1D).²²

MELAS: mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes

It is a classic example of mitochondrial disease, first described in 1984.²³ The most prominent clinical feature is stroke-like episodes, which typically manifest as hemiparesis or hemianopsia. The lesion is usually on parieto-occipital cortex sparing underlying subcortex, and not confined to a specific vascular territory (Fig. 2A). Average age of onset is around 9 years old in juvenile form, and around 30 years old with adult form, far younger than usual stroke victims.²⁴ Seizure, headache, and muscle weakness are also

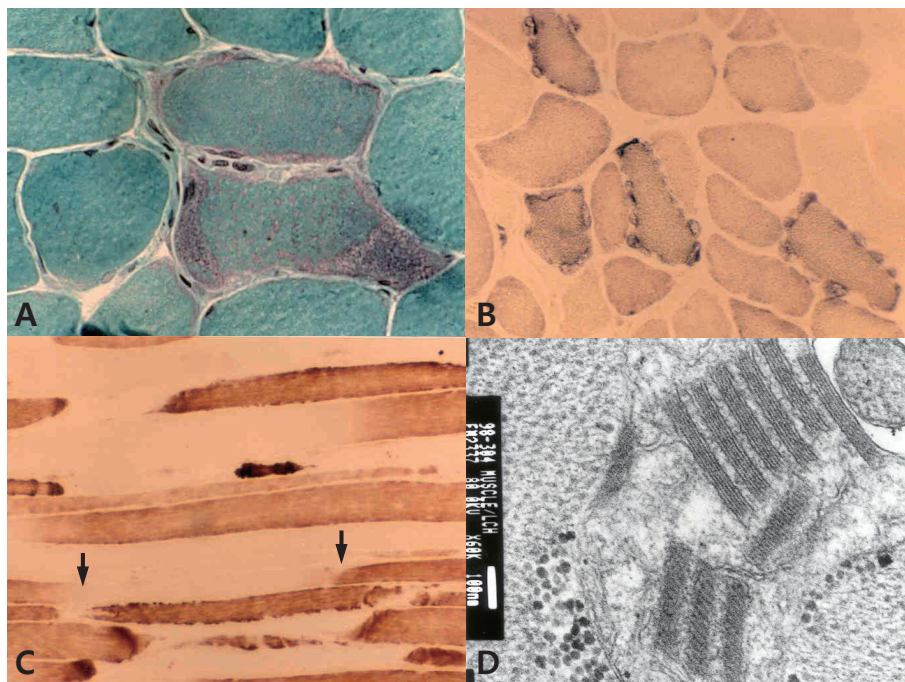


Fig. 1. Histochemical and electron microscopic findings of mitochondrial myopathy (DK, unpublished data). (A) Typical RRFs in a patient with mitochondrial myopathy (modified Gomori trichrome, x400). (B) Ragged blue fibers are highlighted (SDH, x200). (C) COX activity is partially lost in a muscle fiber (between arrows) from a patient with mitochondrial myopathy (COX, x100). (D) Paracrystalline inclusions in a swollen mitochondria in a patient with MELAS (electron microscopy, x60,000).

common at presentation. Half of the affected children have short stature. As the disease progresses, other symptoms common to mitochondrial disorders may appear such as deafness, diabetes, cardiac dysfunction, and cognitive decline.

Approximately 80% of the cases are with m.3243A>G mutation,²⁵⁾ and m.3271T>C accounts for another 10%.²⁶⁾ Both spots are on mitochondrial leucinet RNA (MTTL1, OMIM 590050). Allegedly due to mitotic segregation, the detection of m.3243A>G mutation is notoriously elusive with peripheral blood DNA preparation. DNA from muscle and urine sediment may retain the mutation until the advanced stage (Fig. 2B). Tens of other mitochondrial mutations are reported to cause the same syndrome. Majority of them are genes for other mitochondrial tRNAs or for subunits for respiratory complex I. A rare case with nuclear genomic defect has also been reported.²⁷⁾

Muscle biopsy may provide invaluable clues to the diagnosis of mitochondrial myopathy. The excessive number of RRF and COX-negative fibers is indicative of mitochondrial dysfunction in muscle. Strongly SDH-reactive vessel is a unique histological finding in MELAS implicating vasculopathy coexists.²¹⁾ In electron

microscopy, abnormal proliferation of mitochondria with paracrystalline inclusions is observed (Fig. 1D).¹⁹⁾

The studies on MELAS pathogenesis focus on the most frequent m.3243A>G mutation. It is the 14th nucleotide in tRNA^{Leu(UUR)} gene and in highly conserved area. Experimental evidences suggest that this mutation disrupts tertiary foldings of the tRNA.^{28,29)} The mutant tRNA undergoes incorrect processing and maturation resulting in downstream biochemical defects. Disturbed processing of mitochondrial primary transcript is another possibility. In patients' skeletal muscle with m.3243A>G mutation, the amount of transcript 16S rRNA+tRNA^{Leu(UUR)}+ND1 genes, also known as RNA 19, is increased. It is proposed that RNA 19 transcripts produce stalling of translation by pseudoribosome formation.³⁰⁾

MERRF: myoclonic epilepsy with RRF

It is first described by Fukuhara et al in 1980.³¹⁾ Predominant feature of MERRF syndrome is myoclonic epilepsy and myopathy as the acronym stands for. The onset is in childhood to early adulthood. The myoclonus is either spontaneous or stimulus-sensitive. Progressive ataxia, muscle wasting, mental retardation, and optic atrophy may follow. Other symptoms common to mitochondrial disorders such as deafness, neuropathy, and external ophthalmoplegia can develop.

Muscle biopsy provides indispensable clue of mitochondrial dysfunction when the syndrome is not clear enough, although the findings are not specific for the diagnosis of MERRF.¹⁹⁾ Over 80% of the cases are with m.8344A>G mutation on mitochondrial lysine tRNA (MTTK, OMIM 590060).³²⁻³⁴⁾ Mutational load in peripheral blood DNA is lower than those from solid organ,³⁵⁾ but still remains high enough to be in detectable range. The m.8344A>G mutation is on MTTK. The same mutation has also been reported in cases of Leigh syndrome³⁶⁾ and mitochondrial Parkinson disease.³⁷⁾

Mutations of MTTK gene have been reported in a variety of phenotypes, some overlapping with and others distinctive from MERRF. On the other hands, several mitochondrial tRNA mutations have been implicated in MERRF. Interestingly m.3243A>G, the most frequent cause of MELAS syndrome, has also been reported to result in MERRF phenotype as well as other form of mitochondrial myopathy.³⁸⁾ This exemplifies the difficulty in genotype-phenotype correlation, possibly due to variation of mutational loads in different tissues, although influence from modifier genotype cannot be excluded.

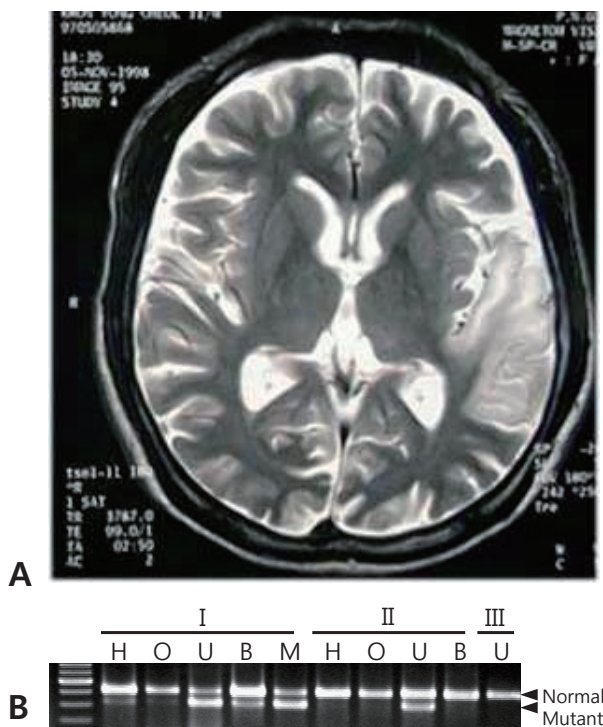


Fig. 2. (A) A stroke-like high signaled lesion in left temporal lobe on T2-weighted image of brain MRI in a patient with MELAS (DK, unpublished data). (B) Detection of heteroplasmy by PCR-RFLP in various tissue sample (I, 32 year-old patient; II, asymptomatic mother of the patient; III, normal control; H, hair follicle; O, oral mucosa; U, urine sediment; B, peripheral blood; M, muscle; Normal, uncut band; Mutant, cut by *ApaI* digestion, m.3243A>G) (JS, unpublished data).

PEO: progressive external ophthalmoplegia

Progressive ptosis and external ophthalmoplegia is a common feature in many mitochondrial disorders, while isolated PEO is not uncommon. It slowly evolves over several years, that the patients do not complain diplopia. Rather progressive ptosis can be troublesome necessitating corrective surgery sometimes. It is called Kearns-Sayre syndrome if the triad of PEO, onset before age 20, and one of the following features are met, such as pigmentary retinopathy, cerebellar ataxia, heart block, and elevated CSF protein. Sporadic cases are most commonly by a single clonal deletion in mitochondrial genome. A third of patients have 4.9 kb-common deletion flanked by 13 bp-repeat sequences called Bp1 and Bp2. In cases with point mutation showing maternal inheritance, m.3243A>G mutation is the most frequent.³⁹⁾

Noteworthy is the fact that indistinguishable phenotype can be due to autosomal genetic defect.⁴⁰⁾ The list of involved nuclear genes include γ -polymerases (POLG, OMIM 174763, catalytic subunit; POLG2, OMIM/604983, accessory subunit)^{41,42)} for mitochondrial DNA replication; a solute carrier family protein (SLC25A4, OMIM 103220),⁴³⁾ working as mitochondrial adenine nucleotide translocator (ANT1); chromosome 10 open reading frame 2 (C100 RF2, OMIM 606075),⁴⁴⁾ also known as T7 gene 4-like protein with intramitochondrial nucleoid localization (TWINKLE); M2 B ribonucleotide reductase (RRM2B, OMIM 604712),⁴⁵⁾ and a homolog of yeast DNA replication helicase 2 (DNA2, OMIM 601810),⁴⁶⁾ for the maintenance of mitochondrial and nuclear DNA stability.

The defects of these genes manifest as autosomal dominant disorder, while those of POLG can also assume autosomal recessive inheritance.⁴¹⁾ These genes are related to maintenance of mitochondrial genome, and their defects result in multiple deletions in mitochondrial genome. Multitude of symptoms may follow: neuropathy, ataxia, tremor, parkinsonism, depression, cataracts, pigmentary retinopathy, deafness, rhabdomyolysis, and hypogonadism.⁴⁷⁾ Muscle biopsy is not so different from other mitochondrial myopathies. Excessive numbers of RRFs, COX-negative fibers, and other non-specific myopathic features.⁴⁸⁾

Therapeutic approaches

So far no therapeutic approaches are considered established for mitochondrial myopathies.⁴⁹⁾ Symptomatic management resort to principles of common diseases, which includes antiepileptic medication for seizures, pacemaker for cardiac arrhythmia,

hypoglycemic agents for diabetes and hearing aids for deafness. Heart failure and respiratory failure in advanced cases are serious burden to be managed by specialists.

Pharmacological therapies which might help mitochondrial metabolism have been tried for many years, among which L-arginine gives a great promise in the treatment of MELAS. It is supposed to provide dual pharmacologic effect against 2 fundamental pathomechanisms: vasculopathy and cytopathy.^{30,50)} First, arginine as precursor of nitric oxide, induces vasodilation. It shows rapid clinical improvement on acute stroke-like episode⁵¹⁾ and prevents recurrence in chronic MELAS patients.⁵²⁾ In addition, arginine accelerates TCA cycle, which may reverse anaerobic metabolic shift in MELAS cells.⁵³⁾ Result from non-blinded study is encouraging. Controlled study on L-arginine therapy both in acute and chronic condition is expected.

Recent advances in artificial reproductive technique enabled researchers to explore the way to remove pathogenic mitochondria from fertilized egg. One approach is ooplasmic transfer, to add small amount of healthy cytoplasm to the fertilized egg with mitochondrial genetic defect.⁵⁴⁾ This will dilute the pathogenic mutational load, hopefully down below the threshold level. On the contrary, transfer of nuclear genome is also under investigation at various stages of oocyte maturation: pronucleus, metaphase chromosome, or germinal vesicle.⁵⁵⁾

Gene therapy is another strategy under active investigation.⁵⁶⁾ The mainstream gene therapy for muscle disease will be applicable for mitochondrial myopathy with nuclear genetic defects, while those with mitochondrial mutation will need more innovative approach. A recent advance in targeted RNA import is noteworthy. Transfer of exogenous corrective tRNA appended by ribonucleotide components of mitochondrial localization and import signal could successfully improve cell models of MELAS and MERRF.⁵⁷⁾

In summary, mitochondrial myopathies comes with broad spectrum of clinical manifestation. Once the clinical features indicate mitochondrial dysfunction, it is imperative to understand the genetics of mitochondrial myopathy for the proper diagnosis. While the management of mitochondrial myopathies has mainly been symptomatic therapy, it is promising that disease-modifying therapies are under trial and breakthroughs in innovative strategies are vigorously investigated.

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