MITOCHONDRIAL DISORDERS IN NEUROLOGY

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In the opening lecture of this course, Dr. Eric Schon will describe the molecular genetic and pathogenic bases of mitochondrial diseases. In the second lecture, Dr. Hirano will link those scientific principals to the clinical presentations of mitochondrial diseases. I will address the following key questions.

1. What are mitochondrial diseases?
2. What are the typical clinical features of mitochondrial diseases?
3. What are the clinically recognizable mitochondrial syndromes?
4. When should I suspect a mitochondrial disease?
5. If I suspect that a patient has a mitochondrial disease, what tests should I order?
6. If standard diagnostic testing for mitochondrial disease is inconclusive, what should I do?

1. What are mitochondrial diseases?

Mitochondrial diseases are a challenge because they are among the most heterogeneous human disorders at every level: clinical, biochemical, and genetic.[1, 2] This is due to the multiple functions of mitochondria besides the classic textbook definition of being the "powerhouse of the cell," i.e. the main generators of ATP, and to their dual genetic control, dependent on both nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). A pragmatic definition of the mitochondrial diseases has emerged during the past 25 years, restricting them to defects of the final energetic pathway, the "business end," of mitochondrial metabolism, that is, oxidative phosphorylation (OXPHOS).[3, 4] Although this definition does reduce significantly the number of diseases under consideration, it still allows for extraordinary heterogeneity, in part because of the OXPHOS's intrinsic complexity and in part because OXPHOS is, in fact, the only mitochondrial pathway under the control of both nDNA and mtDNA. Of the approximately 90 proteins that compose the OXPHOS pathway, only 13 are encoded by mtDNA, and yet mutations in mtDNA are responsible for a disproportionate number of mitochondrial diseases. This clinical heterogeneity often leads to confusion and, in turn, to the frequent overdiagnosis and underdiagnosis of mitochondrial diseases. Overdiagnosis occurs because complicated multisystemic disorders are often presumed to be mitochondrial diseases while underdiagnosis occurs because physicians are not aware of these conditions.

2. What are the typical clinical features of a mitochondrial disease?

Fortunately, there are clinical red flags that often enable neurologists to identify mitochondrial diseases and to pinpoint the specific diagnoses and genetic mutations. Although typically multisystemic, mitochondrial disorders frequently affect brain and skeletal muscle and are therefore often described as "mitochondrial encephalomyopathies".

The neurological manifestations of mitochondrial disease are diverse. In infants or young children, psychomotor retardation or regression is characteristic of many mitochondrial diseases and particularly Leigh syndrome, which can be diagnosed by brain T2 or FLAIR MRI showing symmetrical brain lesions in the basal ganglia, brainstem, or both. Seizures, cerebellar ataxia, and dementia in children or young adults are also frequent manifestations of mitochondrial encephalopathies. Sensorineural hearing loss and migraine headaches are very common in mitochondrial disease patients.

The most common neuromuscular feature of mitochondrial disease is myopathy causing proximal muscle weakness often accompanied by exercise intolerance that is often out-of-proportion to the weakness. Extra-ocular weakness (ptosis and chronic progressive external ophthalmoparesis [CPEO]) is another common neuromuscular manifestations and is often accompanied by oropharyngeal weakness causing dysphagia or nasal dysarthria. Unlike myasthenia gravis, the extra-ocular weakness does not fluctuate dramatically and may not cause diplopia. The ptosis and CPEO can be asymmetric. Peripheral neuropathy is common and is typically an axonal sensorimotor neuropathy.

Ophthalmological involvement is also frequent in mitochondrial diseases. In addition to ptosis and CPEO, optic neuropathy is common and may be severe causing centrocecal or cecal scotomas. Pigmentary retinopathy is also frequent.

Cardiopathy is also common among mitochondrial disease patients and often manifests as cardiac conduction defects or cardiomyopathy. In Kearns-Sayre syndrome, heart block is a defining clinical feature and is typically progressive so placement of a cardiac pacemaker should be considered. Mitochondrial cardiomyopathy

in often hypertrophic at the onset before evolving into dilated cardiomyopathy. Wolff-Parkinson-White syndrome is another common finding on electrocardiograms, but is usually asymptomatic.

Gastrointestinal dysmotility is being recognized with increasing frequency among mitochondrial disease patients. Oropharyngeal weakness causes dyphagia and nasal speech and is frequently associated with ophthalmoparesis. Endocrinopathies, particularly diabetes mellitus, are common in mitochondrial diseases; therefore, it is important to screen annually for diabetes and thyroid disease. Nephropathies usually present as renal tubular acidosis (de Toni-Fanconi syndrome), but steroid-resistant nephrotic syndrome is another clinical manifestation that is particularly frequent in juvenile patients with coenzyme Q_{10} deficiency.

3. What are the clinically recognizable mitochondrial syndromes?

Mitochondrial Diseases due to mtDNA mutations

Clinical classification of the mitochondrial disorders is of pragmatic significance in guiding the diagnostic evaluation and in directing the therapy. Among the many syndromes caused by mtDNA mutations, six multisystem disorders that occur frequently are: Kearns-Sayre syndrome (KSS)/chronic progressive external ophthalmoplegia (CPEO); mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS); myoclonus epilepsy ragged-red fibers (MERRF); neuropathy, ataxia, retinitis pigmentosa (NARP)/maternally inherited Leigh syndrome (MILS); and Leber hereditary optic neuropathy (LHON).

In 1988, our understanding of mitochondrial encephalomyopathies was radically reformed by the identification of the first mtDNA mutations[5, 6]; single large-scale deletions of mtDNA were identified in KSS/CPEO patients[5, 7] while a mtDNA point mutation (adenine-to-guanine at nucleotide 11778, m.11778A>G, in the ND4 gene encoding subunit 4 of complex I) was observed in many LHON patients[6]. Thus, these initial mtDNA mutations demonstrated that clinical phenotypes can be associated with particular genotypes. Like most diseases due to mtDNA point mutations, LHON is maternally inherited; however, in contrast, KSS/CPEO is usually sporadic because the mtDNA rearrangements seems to originate in oogenesis or early zygote formation.

Like LHON, MERRF and MELAS are maternally inherited disorders, however, the mutations differ. As noted, LHON is due to a mutation in a gene encoding a structural polypeptide while MERRF and MELAS are usually caused by point mutations in transfer RNA genes. MERRF is typically caused by A-to-G mutation at nucleotide 8344 (m.8344A>G) of the mtDNA $tRNA^{Lys}$ gene[8] while about 80% of MELAS patients have a m.3243A>G mutation in the $tRNA^{Leu(UUR)}$ gene[9], thus, both syndromes are due to defects of mitochondrial protein synthesis. NARP is a multisystem disorder usually due to mtDNA point mutations (m.8993T>G or m.8993T>C) in the ATP6 gene encoding subunit 6 of ATPase[10]. Within families with NARP patients, relatives may be affected by the more devastating maternally inherited Leigh syndrome (MILS)[11-13]. The NARP-MILS spectrum illustrates the importance of mtDNA heteroplasmy as NARP patients usually have 70-90% mutation load while individuals afflicted more severely with MILS generally harbor >90% mutation.

Nuclear DNA Mutations

The first nuclear DNA (nDNA) causing a mitochondrial respiratory chain defect was described 20 years ago in a pair of siblings with Leigh syndrome who were found to have compound heterozygous mutations in the flavoprotein subunit of complex II[14]. Since then, additional mutations encoding structural subunits as well as assembly factors for complexes I, III, IV, and V Leigh syndrome[15-18].

A growing number of autosomal diseases have been associated with mtDNA depletion, multiple deletions, or both[19, 20]. These disorders are due to defects in mtDNA maintenance, which requires replication machinery and substrates to synthesize this small genome. Of particular clinical importance, the POLG gene (*POLG*), which encodes the mitochondrial DNA polymerase gamma (POLG) required for mtDNA replication, is the most common cause of mendelian mitochondrial diseases, which have multiple clinical presentations including: autosomal dominant progressive or recessive external ophthalmoplegia (AD/AR-PEO), sensory ataxic neuropathy, dysarthria, and ophthalmoplegia (SANDO), and Alpers syndrome an early childhood onset encephalopathy with intractable seizures, and hepatopathy. AD/AR-PEO and SANDO are associated with multiple deletions in muscle while Alpers is associated with mtDNA depletion.

MtDNA depletion syndrome was originally reported in infants with severe hepatopathy or myopathy. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) has been associated with depletion, multiple deletions, and site-specific point mutations of mtDNA[21]. This autosomal recessive disorder is due to mutations in the *TYMP* gene encoding thymidine phosphorylase, demonstrating the importance of maintaining the nucleotide precursor pool for mtDNA replication. Allogenic stem cell transplantation (e.g. bone marrow transplantation) can correct biochemical derangements in MNGIE, but clinical efficacy and safety remain unproven[22].

Coenzyme Q_{10} (CoQ_{10} or ubiquinone) is another vital component of the inner mitochondrial membrane where it serves to shuttle electrons to complex III from complexes I and II. Deficiency of CoQ_{10} in skeletal muscle was originally described in two sisters with the triad of myoglobinuria, encephalopathy (seizures, ataxia, mental

retardation), and ragged-red fibers[23]. In addition to this encephalomyopathy, CoQ_{10} deficiency has been been associated with cerebellar ataxia, infantile-onset multisystemic disorders (typically encephalopathy and nephrotic syndrome), isolated nephrotic syndrome, and isolated myopathies[24]. Primary CoQ_{10} deficiency is caused by defects in CoQ_{10} biosynthesis while secondary forms are due to defects in genes not directly related to ubiquinone synthesis[25].

4. When should I suspect a mitochondrial disease?

The wide clinical diversity of mitochondrial myopathies provides diagnostic challenges for even the most knowledgable and experienced neurologists. It is important to recognize the common mitochondrial syndromes described above; however, patients often present with disorders that do not fit with one of these classical phenotypes. Therefore, it is important to consider the diagnosis of mitochondrial disease when patients have red flag manifestations that are characteristic of mitochondrial disease.

Thus, in obtaining the medical history of patients suspected to have a mitochondrial encephalomyopathy, clinicians should inquire about the following clinical features: exercise intolerance, migraine headaches, diabetes mellitus, short stature, and hearing loss. Family history is important, but evidence of maternal-inheritance may be subtle when dealing with a mtDNA point mutation. For example, in families with MELAS syndrome, relatives in the maternal lineage may have migraine-like headaches or diabetes mellitus as the only manifestation of the genetic defect.

Careful physical examination may reveal subtle clues to the correct diagnosis. Patients are often short and thin. Cognitive dysfunction is common, variable in severity, and usually progressively worsens. Cranial nerve dysfunction includes ptosis, ophthalmoparesis, and hearing loss. Fundoscopy may reveal pigmentary retinopathy. Myopathy typically causes bulbar dysfunction, proximal limb muscle weakness, or both. Peripheral neuropathy is usually axonal sensorimotor, but may be demyelinating as in MNGIE.

5. If I suspect that a patient has a mitochondrial disease, what tests should I order?

Clinical Laboratory Tests

Laboratory evaluation should begin with routine blood tests including complete blood count, serum electrolytes (including calcium and phosphate), liver function tests, blood urea nitrogen, creatinine, lactate, and pyruvate. These tests may reveal parathyroid, kidney, or liver dysfunction. Lactate and pyruvate at rest are commonly elevated in patients with mitochondrial encephalomyopathies and these values may increase dramatically after moderate exercise. Formal exercise testing may reveal reduced workload capacity and early anaerobic threshold.

Electrocardiograms may reveal pre-excitation in MELAS or MERRF, and heart block in KSS or MELAS. Lumbar puncture may show elevated CSF protein, especially in KSS patients where it is often greater than 100mg/dl. CSF may also reveal elevated lactate and pyruvate levels, sometimes in patients with normal serum levels. Electromyography and nerve conduction studies are typically consistent with a myogenic process although neurogenic changes may be detected in MERRF or MELAS. Brain imaging with CT or MRI scans may reveal basal ganglia calcifications and atrophy in any of the three major syndromes.

Specialized Laboratory Tests

Translational research has greatly expanded our understanding of mitochondrial disorders and our diagnostic capabilities. Specialized evaluation for oxidative-phosphorylation defects has evolved from laboratory research to clinical muscle histochemistry and immunohistochemistry, measurement of oxidative-phosphorylation enzyme activities, and molecular genetic tests.

Genetic Testing

The identification of numerous mtDNA mutations including: duplications, depletions multiple deletions, and over 270 pathogenic point mutation as well as over 100 nuclear genes for mitochondrial diseases have revolutionized our understanding and classification of these disorders. For busy clinicians, it is impossible to keep up to date with the rapid progress of mitochondrial genetics; therefore, it may be necessary to consult specialists with expertise in these complex and diverse disorders. Nevertheless, it is important for clinicians to guide the molecular geneticists in the selection of the appropriate DNA tests from the increasingly complex and costly “menus” now available. Whole mtDNA sequencing of blood samples is now commercially available, and is a useful screening test for patients with evidence of a maternal inheritance. In patients with Alpers syndrome, SANDO, or AD/AR PEO, *POLG* sequencing is recommended. Panels of nuclear DNA genes are also commercially available, but must be applied judiciously in patients who show clinical hallmarks of mitochondrial disease with supportive laboratory evidence such as lactic acidosis, respiratory chain defects, mtDNA rearrangements/depletion, or mitochondrial histopathological changes in muscle.

Muscle Biopsies

Histological studies of patients with mitochondrial disorders have focused on skeletal muscle, but many characteristic microscopic changes have been noted in other tissues. The typical ultrastructural alterations seen in mitochondrial myopathies include: 1) an overabundance of ultrastructually normal mitochondria, “pleoconial myopathy”; 2) enlarged mitochondria with disoriented cristae, “megaconial myopathy”; and 3) inclusions within mitochondria, “paracrystalline” and “osmiophilic” inclusions. W.K. Engel and Cunningham developed a modified Gomori trichrome stain which has been commonly used to identify fibers with subsarcolemmal accumulations of mitochondria, which are referred to as "ragged-red fibers" (RRF)[26]. Histochemical stains for mitochondrial enzymes are also used to identify excessive mitochondrial proliferation and to demonstrate specific enzyme defects. These stains include: succinate dehydrogenase (SDH); nicotinamide dehydrogenase-tetrazolium reductase (NADH-TR); and cytochrome *c* oxidase (COX). Immunohistochemical techniques are used to identify defects in specific mitochondrial polypeptides.

In KSS, MELAS, and MERRF, RRF with ultrastructurally abnormal mitochondria are almost always identified in skeletal muscle by the Gomori trichrome stain. SDH histochemistry reveals “ragged-blue fibers” (RBF) with darker-than-normal staining in subsarcolemmal regions of muscle fibers. In MELAS patients, there is often excessive SDH staining within blood vessel walls, so-called strongly SDH-reactive vessels or SSVs. Another peculiar characteristic of skeletal muscle from MELAS patients is the relative preservation of COX staining in RRF, in contrast to muscle from KSS and MERRF patients, in which there are abundant COX-negative RRF on serial or double-stained (SDH and COX) sections.

Unfortunately for clinicians, the histological abnormalities described above may be absent as in cases of NARP, Leber’s hereditary optic neuropathy (LHON), and Leigh syndrome (LS). To further confuse the picture, in muscle of people over age 50, RRF/RBF and COX-deficient fibers are often detected at low levels (<4% RBF, <2% RRF, and <5% COX-negative fibers).

Biochemistry (Respiratory chain enzyme assays)

Activities of mitochondrial respiratory chain enzymes can be measured in crude muscle extracts or in isolated mitochondria. In KSS, MELAS, MERRF, and in syndromes associated with multiple deletions or depletion of mtDNA, there are multiple partial defects of respiratory chain enzymes; however, the patterns are not consistent and normal enzyme activities have been reported. Some mitochondrial disorders, such as LHON and NARP, may not have any detectable abnormalities of respiratory chain enzymes with routine assays.

Isolated monoenzymopathies can be diagnostic for some of the clinical syndromes described above, such as the COX-deficiencies. In addition, low activities for respiratory chain enzyme complexes I+III and II+III indicate a possible defect in complex III or a deficiency of coenzyme Q_{10}. CoQ can be measured most reliably in muscle to confirm this diagnosis.

MtDNA Rearrangements

Southern blot analysis is the most appropriate technique to identify single mtDNA duplications, and single or multiple mtDNA deletions. Patients with sporadic PEO or KSS are likely to have single mtDNA deletions while individuals with autosomal dominant or recessive PEO syndromes (AD-PEO, AR-PEO, MNGIE) often show multiple mtDNA deletions. It is important to screen muscle DNA for the presence of the deletions, because replicating cells, such as fibroblast and lymphocytes, generally do not have deletions detectable by Southern blot. Because multiple mtDNA deletions can be identified by polymerase chain reaction (PCR) in muscle from normal aged individuals, clinicians must be careful in their interpretation of multiple mtDNA deletions detected by PCR.

If there is a history of maternally inherited neurological disorders, then one should consider mtDNA point mutations. If a patient fulfills the clinical criteria for one of the more common mitochondrial encephalomyopathies, such as MELAS or MERRF, then the appropriate mtDNA point mutations can be screened. Leukocyte DNA is generally suitable for identifying mtDNA point mutations. If a patient does not have the common m.3243A>G “MELAS” mutation, then one can screen (by whole mtDNA sequencing) for over 20 other mutations which have been associated with the MELAS phenotype.

MtDNA Depletion

Depletion of mtDNA can be detected by quantitative PCR or Southern blot analysis of affected tissues, particularly muscle or liver. It is important to avoid screening blood or fibroblasts for mtDNA depletion because these cells almost always show normal mtDNA levels. Once mtDNA depletion is detected, nuclear genes can be selected for sequencing based on the clinical phenotype (e.g. *TK2* in cases of pure myopathy, *DGUOK* and *POLG* in patients with hepatocerebral disease).

6. If standard diagnostic testing for mitochondrial disease is inconclusive, what should I do?

Recent research studies suggest that elevated serum levels of FGF-21 (fibroblast growth factor 21) is a sensitive and specific biomarker in patients with mitochondrial myopathies; however, this test is not commercially available[27, 28].

Although still a research tool, whole exome sequencing can be performed to screen for nuclear gene mutations in patients with clear mitochondrial abnormalities such as Leigh syndrome with a respiratory chain enzyme defect.

Finally, patients who may have a mitochondrial disease may be referred to one of the sites in the North American Mitochondrial Disease Consortium (NAMDC), a NIH-sponsored network of centers of mitochondrial disease excellence in the United States and Canada. Further information about NAMDC is available at the website http://rarediseasesnetwork.epi.usf.edu/NAMDC/

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