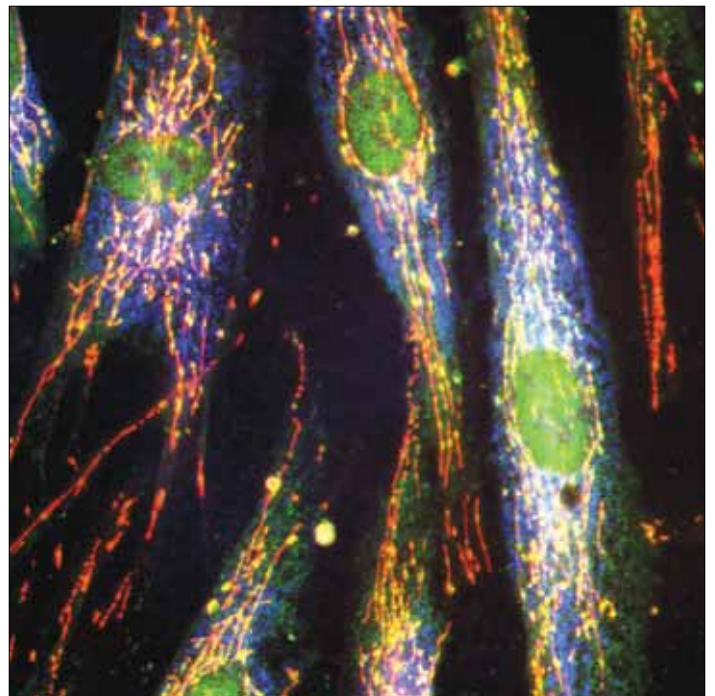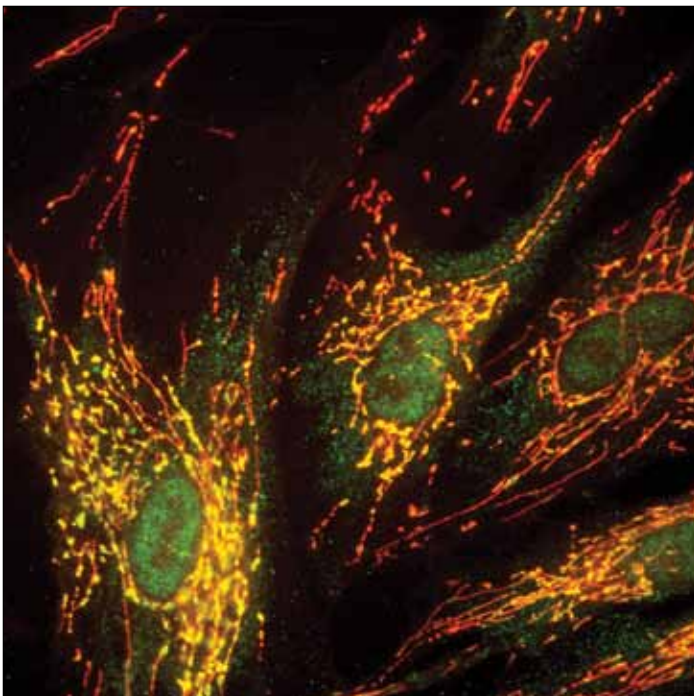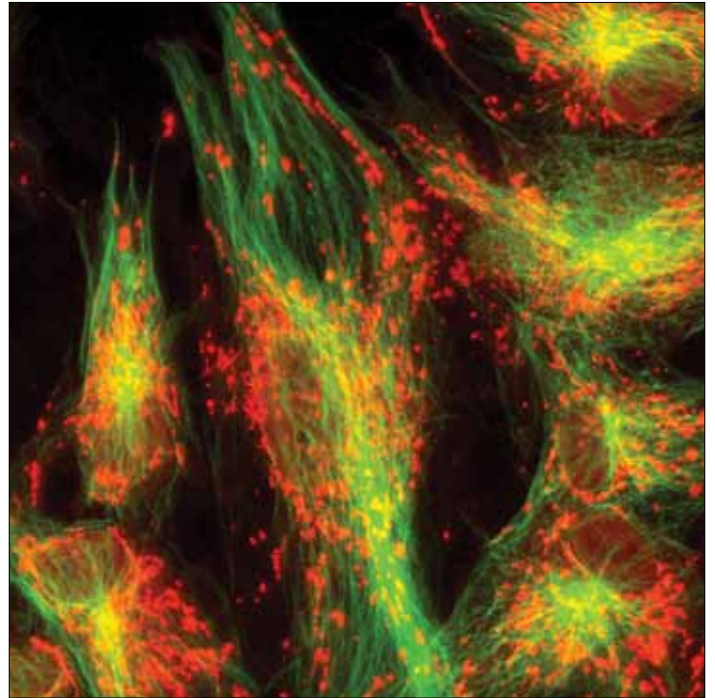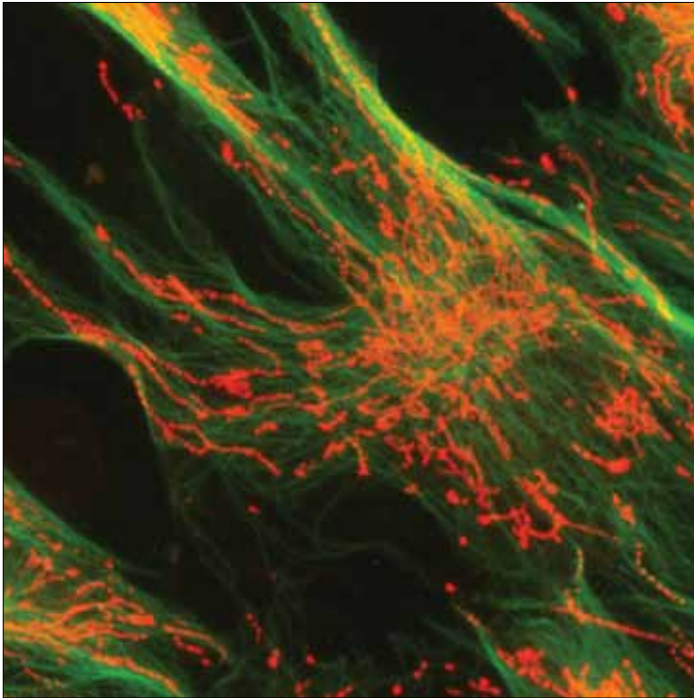COLUMBIA UNIVERSITY

Departments of Neurology and
Pathology & Cell Biology

The Diagnosis of
Mitochondrial Diseases

The Diagnosis of Mitochondrial Diseases at Columbia University Medical Center

Department of Pathology & Cell Biology

Department of Neurology

Laboratory of Molecular Neurogenetics

Clinical consultation and diagnosis

Full range of enzyme diagnostics

Analysis of mitochondrial DNA

Research on mitochondria and their roles in diseaese

Physician education

Please see inside back cover
for contact information.

Front cover: Mitochondrial morphology in human fibroblasts (cover micrographs). The panel at top left is a wild-type cell. Mitochondria (red structures) are labeled with MitoTracker Red™; tubulin, labeled in green, helps indicate overall cell structure. The panel at top right, labeled in a similar manner, shows a fibroblast from a patient with a mitochondrial disease. Note the appearance of many mitochondria as punctate structures, compared to the more tubular mitochondria in the wild-type cell. The panel at lower left shows mitochondria (stained with MitoTracker Red) co-localizing with a mitochondrial-associated protein (stained green with a fluo-rescently-labeled antibody); the regions of red-green co-localization appear yellow. The panel at lower right is stained in the same way but, in addition, the endoplasmic reticulum is also stained, in blue. Photographs courtesy of Dr. Estela Area-Gomez.

Introduction

Columbia University scientists pioneered the study of diseases caused by mitochondrial malfunctions and have written the major treatises in the field. This area continues to be a focus of research at Columbia University Medical Center, which, in addition to research and clinical evaluation, provides superb molecular and biochemical diagnostic tests. Perhaps most important, we offer experience and clinical judgment. The diagnostic laboratory is now managed by the Department of Pathology and Cell Biology, which has a staff of 400 clinical and basic scientists and technicians. It is structured as a service organization. The purpose of this publication is to provide clinicians with a review of mitochondrial diseases and how our scientists diagnose them.

Mitochondrial Diseases

The mitochondrial encephalomyopathies comprise a diverse group of disorders characterized by defects in mitochondrial function. Because the mitochondrion is the only non-nuclear organelle in the animal cell that contains proteins encoded by its own DNA, inherited defects causing mitochondrial dysfunction can be due to mutations either in nuclear DNA (nDNA) or in mitochondrial DNA (mtDNA). Since there are several (or many) mitochondria per cell, depending on the percentage of mutated genomes and their distribution in the various organs, mitochondrial syndromes vary in their severity. Further complicating the diagnosis of mitochondrial disorders is the finding that environmental factors can also cause mitochondrial malfunction. This wide variation in the clinical expression of mitochondrial diseases is due not only to the unique role that mitochondria play in the cell, but also to the unusual genetic interplay between the mitochondrion and the nucleus.

Salvatore DiMauro, MD
Lucy G. Moses
Professor of Neurology
H. Houston Merritt Clinical
Research Center for
Muscular Dystrophy and
Related Diseases
Associate Chair for Laboratory
Research and Training

Michio Hirano, MD
Professor of Neurology
Director, H. Houston Merritt
Clinical Research Center
for Muscular Dystrophy and
Related Diseases
Associate Director, Adult
Muscular Dystrophy Association
Neuromuscular Clinic

Ali B. Naini, PhD
Director, Laboratories of
Metabolic and Mitochondrial
Diseases, Departments of
Pathology & Cell Biology and
Neurology

Kurenai Tanji, MD, PhD
Associate Professor
of Pathology & Cell Biology
*Director, Neuromuscular*
Pathology Laboratory

Eric A. Schon, PhD
Lewis P. Rowland
Professor of Neurology and
Genetics & Development
H. Houston Merritt Center

Peter L. Nagy, M.D., PhD
Assistant Professor of
Pathology and Cell Biology

Mitochondrial Medicine

Edited by
Salvatore DiMauro
Michio Hirano
Eric A Schon

Mitochondrial Genetics

The human mitochondrial genome is a circular double stranded DNA molecule 16,569 base pairs (bp) in length:
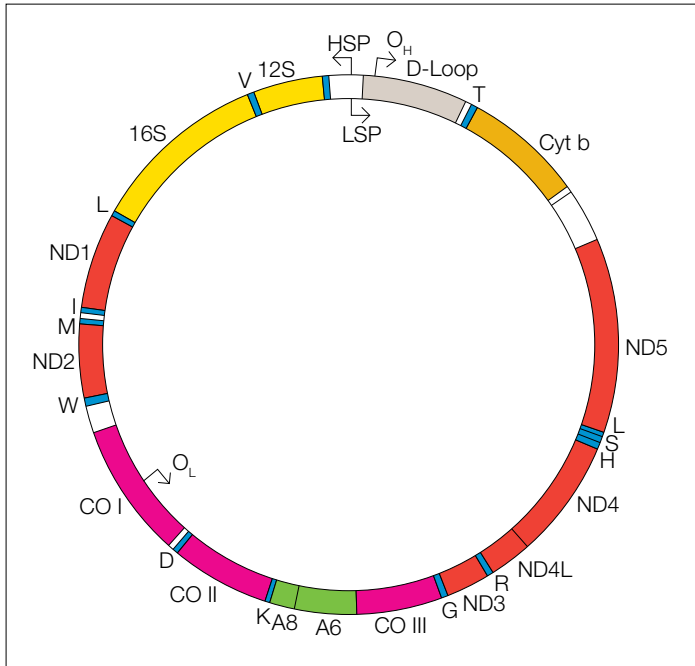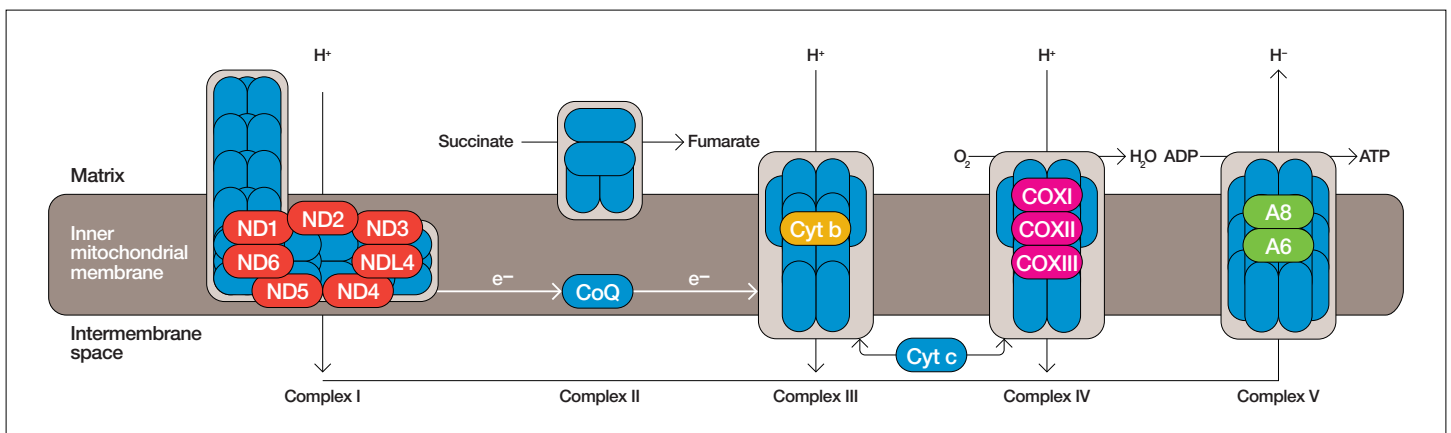

Figure 1

The human mitochondrial genome encodes for 13 polypeptides, 22 tRNAs, and 2 rRNAs. The subunits of NADH-ubiquinone oxidoreductase (ND), cytochrome c oxidase (COX), cytochrome b (Cyt b), ATP synthase (A), 22 tRNAs, and two rRNAs (12S and 16S) are shown. The origins of heavy (O_H) and light (O_L) strand replication, and the heavy (HSP) and light (LSP) strand transcriptional promoters are also shown.

It contains 37 genes, which specify 2 ribosomal RNAs (rRNA), 22 transfer RNAs (tRNAs), and 13 polypeptides, all of which are components of complexes of the respiratory oxidative phosphorylation system (*Figure 2*).

These components include 7 subunits - ND1, ND2, ND3, ND4, ND4L, ND5, and ND6 - of complex I (NADH coenzyme Q oxido-reductase); 1 subunit - cytochrome b - of complex III (CoQ-cyto-chrome c oxidoreductase); 3 subunits - COX I, COX II, and COX III - of complex IV (cytochrome c oxidase, or COX) and 2 subunits - ATPase 6 and ATPase 8 - of complex V (ATP synthase). These four complexes also contain subunits encoded by nDNA, which are imported from the cytoplasm into the mitochondria. Complex II (succinate dehydrogenase-CoQ oxidoreductase) contains only nuclear DNA-encoded subunits. Reducing equivalents produced in the Krebs cycle and in the ß-oxidation spirals are passed along a series of multimeric protein complexes (the electron transport chain) and the energy produced by the reactions of the electron transport chain is utilized to condense inorganic phosphate and adenosine diphosphate to produce adenosine triphosphate (ATP).[2]

Mitochondrial genetics is unusual in many ways. First, mitochondria and mtDNAs are unique in that they are inherited only from the mother. Thus, most pathogenic errors in mtDNA are *maternally-inherited:* women will transmit the defect to all of their children (males and females), but only the daughters will transmit the disease to their children. A disease expressed in both sexes but with no evidence of paternal transmission is strongly suggestive of a mtDNA point mutation.[2] Second, cells contain hundreds or thousands of mitochondria; each mitochondrion contains approximately 5 mtD-NAs. Thus, both normal and mutated mtDNAs may coexist within the patient's tissues, a condition known as *heteroplasmy*. If the patient is heteroplasmic, the clinical phenotype can vary among tissues and can even change within a tissue during the course of time, because of the random distribution of mutated mtDNAs to daughter cells during each cell division; this phenomenon is known as *mitotic segregation*.[2] Virtually every human organ system can be affected, but tissues with high requirements for oxidative energy metabolism, such as muscle, heart, eye, and brain are particularly vulnerable; this is referred to as the *threshold effect*. Relatively low levels of mutated mtDNAs can affect the respiratory capacity of these tissues, and high levels can be devastating. It is therefore no surprise that mito-chondrial disorders are predominantly encephalomyopathies.

Figure 2

Schematic representation of the respiratory chain system. Subunits encoded by mtDNA are shown in red and subunits encoded by nuclear DNA are shown in blue. Electrons (e-) flow along the electron transport chain, and protons (H+) are pumped from the matrix to the intermembrane space through complexes I, III, and IV, then back into the matrix through complex V, producing ATP. Coenzyme Q (CoQ) and cytochrome c are electron carriers.

Mitochondrial Morphology

The two main biochemical features in most mtDNA disorders are *respiratory chain deficiency* and *lactic acidosis*. Skeletal muscle is the tissue of choice in which to study the pathogenic consequence of mtDNA mutations because in many pathogenic situations it signals "mitochondrial distress" through marked mitochondrial proliferation, with formation of massive accumulation of mitochondria – resulting in the so-called *ragged-red fibers (RRF)*. RRF can be detected in two ways. Staining with modified Gomori trichrome shows mitochondrial proliferation as red or purple areas in a subset of fibers, often concentrated in the subsarcolemmal region; the normal or less-affected fibers stain blue or turquoise. Staining for the activity of *succinate dehydrogenase (SDH)* shows accumulations of mitochondria as deep purple regions in the case of mitochondrial mutation while normal or less-affected fibers stain light purple (Figure 3A).
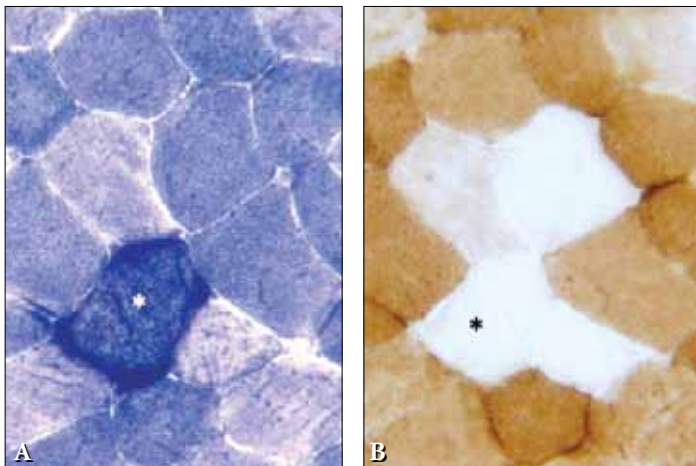
Figure 3
Histochemistry in muscle serial sections to detect: A. SDH activity (RRF) and B. COX activity. Note that the RRF is COX-negative. Simultaneous staining for SDH and COX provides an even more sensitive way of detecting COX-deficient fibers.

In the electron microscope, mitochondria with abundant mutations can have abnormal structures[3] and in many cases contain paracrystalline inclusions (Figure 4).
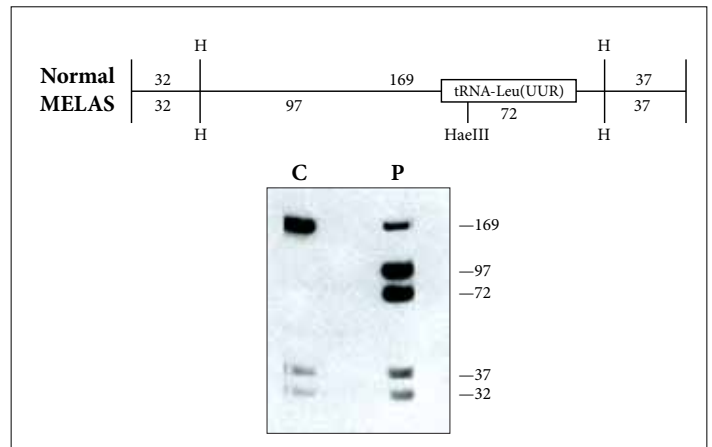

Figure 4
Electron micrograph of abnormal mitochondria. Note mitochondria with packed cristae and the paracrystaline inclusions.

Muscle is often a mosaic for respiratory chain deficiency, which is most easily observed by histochemistry for COX activity (Figure 3B). Because COX subunits are encoded by both the mitochondrial or by the nuclear genome, the COX reaction is particularly useful in the evaluation of mitochondrial myopathies[4]. COX activity staining of normal muscle shows dark brown or brownish-orange type I (highly oxidative) fibers and light brown type II (glycolytic) fibers. In most mitochondrial disorders, many fibers - usually type I fibers - are almost completely devoid of COX activity (i.e. little or no stain present). A similar histochemical stain can be performed to detect mitochondrial ATPase. Besides histochemistry, immunohistochemistry can be used to detect specific polypeptides For example, antibodies are available to detect mtDNA-encoded polypeptides (e.g. ND1 and COXII) as well as nDNA-encoded polypeptides that are imported into mitochondria (e.g. COX IV).

Genetic Analysis of mtDNA Mutations

There are three types of mtDNA mutations: point mutations, large-scale rearrangements (deletions and duplications), and depletion.

Point mutations are detected by *restriction fragment length polymorphism (RFLP)* analysis of regions of mtDNA which have been amplified by the *polymerase chain reaction (PCR)*. In brief, a region flanking the suspected point mutation is amplified by PCR, and the resulting PCR product is digested with a *restriction endonuclease* that cleaves the DNA diagnostically at the site of mutation (Figure 5).

Figure 5
PCR/RFLP analysis to detect a MELAS mutation in the tRNA Leu(UUR) gene. An A→G mutation at m.3243 creates a HaeIII (H) polymorphism. P = patient; C = control.
Numbers denote DNA fragment sizes in base pairs.

The resulting fragments are visualized by gel electrophoresis. This test yields three types of information: (1) presence or absence of the mutation; (2) if present, whether the mutation is heteroplasmic or homoplasmic; (3) if heteroplasmic, the proportion of mutant mtDNAs.

Large-scale rearrangements of mtDNA (deletions and duplications), as well as quantitative errors in the amount of mtDNA present in a tissue, are analyzed by Southern blot hybridization. In brief, total DNA is digested with a restriction enzyme, electrophoresed through an agarose

gel, transferred to nitrocellulose or nylon filter paper, and hybridized to an appropriately labeled probe (usually cloned mtDNA). The localization of the mtDNA probe bound to the membrane is visualized by autoradiography. This test yields four types of information: (1) the relative amount of mtDNA present; (2) presence or absence of rearrangement; (3) if present, whether the rearrangement is heteroplasmic or homoplasmic; (4) if heteroplasmic, the proportion of rearranged mtDNAs and if homoplasmic, the nature of the rearrangement.

The laboratory is working on the development and introduction of various methodologies that will allow identification of mutations throughout the mitochondrial genome. The *MitoChip* from Affimetrix will provide such information (http://genome.cshlp.org/content/14/5/812.full). New high throughput sequencing methods will potentially replace this methodology and will also allow for sequencing of the approximately 1500 nuclear encoded genes that have significant effects on mitochondrial biogenesis and function.

Mitochondrial Diseases

Mitochondrial genetics is population genetics, not Mendelian genetics. This fact, coupled with interplay between the nucleus and the mitochondria, causes a surprisingly wide variation in the clinical expression of mitochondrial disease.

Diseases associated with maternally-inherited mutations in mtDNA

Leber hereditary optic neuropathy (LHON)
LHON is a maternally-inherited form of blindness due to an optic neuropathy, with onset typically in the second or third decade of life. The loss of vision begins in the central visual field, usually in one eye, and affects the other eye soon after. Both patients and asymptomatic individuals frequently have peripapillary telangiectasia.

Genetics:
Three point mutations in Complex I are observed more frequently and are strongly associated with the LHON phenotype. A second group of "secondary mutations" are also associated with LHON, but these may interact with other mtDNA mutations to cause LHON.

Morphology:
Muscle biopsies appear normal. RRF are absent.

Molecular Diagnosis:
The PCR-RFLP-based genetic screening in blood DNA for the three primary LHON mutations (m.3460G>A *MTND1*, m.11778G>A *MTND4*, and m.14484T>C *MTND6*) are indicated for all LHON referrals. The m.11778G>A mutation is the most prevalent, accounting for 45-91% of white and Japanese LHON patients.[5,6] Because PCR-RFLP screening for LHON mutation has false positive rates between 2 to 7 per cent[7], all positive tests should be confirmed by direct sequencing to rule out false results. Screening for secondary LHON mutations is not warranted, as their pathogenic significance is currently unknown.

Myoclonus epilepsy with ragged-red fibers (MERRF)
MERRF is characterized by myoclonus, ataxia, generalized seizures, and myopathy with ragged-red fibers. Onset is usually in childhood. Other clinical features include hearing loss, neuropathy, short stature, dementia, and optic atrophy. The family history is typically consistent with maternal inheritance, but family members may be asymptomatic or oligosymptomatic. Pathological studies have revealed neuronal loss in the dentate and inferior olivary nuclei, diffuse cerebellar and brain stem white matter gliosis, and spinal cord posterior column degeneration.

Genetics:
Most patients with MERRF harbor heteroplasmic mutations in the *tRNA^Lys (MTTK) gene*.

Morphology:
RRF are present. Many fibers, including the RRF, are COX-negative.

Molecular Diagnosis:
The m.8344A>G transition is detected in ~80% of MERRF patients. Analysis of the m.8356T>C mutation is warranted only in cases where m.8344A>G mutation is absent despite strong indication of a MERRF diagnosis.

Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS)
MELAS is defined clinically by stroke usually before age 40, encephalopathy characterized by dementia, seizures, or both, and evidence of mitochondrial dysfunction, with RRF, lactic acidosis, or both. The strokes commonly cause hemianopia or cortical blindness. Other clinical features include recurrent vomiting, limb weakness, exercise intolerance, migraine-like headaches, and short stature. MELAS families also often have oligosymptomatic and asymptomatic maternal relatives. Stroke is the clinically distinctive feature of MELAS, but the etiology of the strokes is unknown. Computerized tomography and magnetic resonance imaging studies have shown brain lesions, but they do not conform to the distribution of major cerebral arteries. Excessive accumulations of mitochondria detected by histochemistry for SDH activity have been seen in the walls of small arteries and capillaries in muscle and pia mater.

Genetics:
MELAS is associated most often with heteroplasmic point mutations in the *tRNA^Leu(UUR)* MTTL1 gene. A few mutations have been found in complex I and complex IV polypeptides, most notably in ND5.

Morphology:
RRF are present. However, in distinction to most other disorders in which RRF are present, most fibers, including RRF, are positive for COX activity. In fact, the presence of COX-positive RRF can be considered strong evidence for the MELAS phenotype. A second morphological hallmark of MELAS is the presence of strongly SDH-positive vessels (SSV).

Molecular Diagnosis:
Of the 29 mutations that have been found associated with MELAS to date, 7 are located in ND5 and 5 are located in tRNA^Leu(UUR), one

of the two tRNAs that are required for the incorporation of leucine into mtDNA-encoded polypeptides[8]. The m.3243A>G mutation is the most common MELAS mutation and is present in approximately 80% of patients with typical MELAS presentation.

Neuropathy, ataxia, and retinitis pigmentosa (NARP); maternally inherited Leigh syndrome (MILS)

NARP is characterized by developmental delay, seizures, proximal neurogenic muscle weakness, ataxia, dementia, sensory neuropathy, and retinitis pigmentosa. MILS is a multisystem degenerative encephalopathy with onset in infancy, characterized by hypotonia, myoclonus, brainstem dysfunction, peripheral neuropathy, developmental delay, psychomotor regression, ataxia, seizures, and optic atrophy. The diagnosis can be confirmed by the characteristic pathological findings of symmetric necrotic foci in the thalamus, basal ganglia, brainstem, and dentate nuclei. In addition, retinitis pigmentosa, present in about half of MILS patients, is a distinguishing clinical feature.

Genetics:
NARP is associated with mutations in the ATPase 6 gene (*MTATP6*; subunit 6 of complex V). When the proportion of mitochondria with the NARP mutation is very high (>90%), the clinical phenotype is not NARP but MILS.

Morphology:
RRF are absent, but electron microscopy of muscle biopsies has revealed small subsarcolemmal aggregates of mitochondria and occasional mitochondria with abnormal cristae. Muscle fibers are COX-positive, but a subset of fibers may be either negative or deficient for mitochondrial ATPase.

Molecular Diagnosis:
Routine referrals for MILS or NARP, investigation of the m.8993T>G and m.8993T>C mutations.

Maternally-inherited progressive external ophthalmoplegia (PEO)

PEO is characterized by extraocular muscle weakness with ptosis in childhood and adolescence, and is often accompanied by limb weakness. PEO is also seen in the multisystem disorder called Kearns-Sayre syndrome (KSS), which is typically sporadic. However, about 20% of PEO patients have a positive family history consistent with maternal inheritance.

Genetics:
Point mutations in a number of tRNA genes have been associated with PEO.

Morphology:
RRF are present. RRF may be COX-positive (as in MELAS), but a significant number may be COX-negative.

Molecular Diagnosis:
Routine referrals for PEO, should be screened for the m.3243A>G mutation in addition to testing for mtDNA rearrangements.

Type II diabetes; diabetes and deafness

A surprisingly large number of patients harboring mitochondrial mutations have symptoms of diabetes. Usually, these patients have type II diabetes mellitus which can present relatively early in life. In addition, familial cases of maternally-inherited diabetes, often in conjunction with deafness or severe hearing loss (MIDD), have been described. These patients often harbor the same mutation usually found in MELAS, but other pedigrees have shown large-scale mtDNA rearrangements.

Genetics:
Point mutations in a number of tRNA genes.
Morphology: RRF are present. RRF may be a mixture of COX-positive and COX-negative fibers.

Molecular Diagnosis:
For routine referrals for MIDD, only testing for the m.3243A>G mutation is recommended.

Aminoglycoside-induced deafness (AID)

The syndrome of maternally-inherited aminoglycoside induced deafness causes hypersensitivity to aminoglycoside drugs, such as kanamycin, gentamicin, and streptomycin. A related disorder is familial non-syndromic deafness, which is also maternally-inherited.

Genetics:
AID and non-syndromic deafness are associated with a point mutation in the 12S rRNA gene.

Morphology:
No information available at present.

Molecular Diagnosis:
For routine referrals for AID, only testing for the m.1555A>G mutation is recommended.

Maternally-inherited diabetes mellitus

A number of pedigrees of maternally-inherited diabetes have been identified. Clinical, the unusual feature of affected members is the early onset of the endocrine dysfunction. Most cases of maternally inherited diabetes are due to point mutations of mtDNA. While most DNA rearrangements arise spontaneously, in rare cases a mother with sporadic PEO or KSS and deleted mtDNA will transmit the mtDNA rearrangement to her child. In addition, maternally-inherited mtDNA duplications have been observed in a syndrome of brain, kidney, and endocrine dysfunction.

Genetics:
Point mutations in the tRNA^Leu(UUR) gene; large-scale mtDNA duplications are detectable by Southern blot analysis of blood DNA.

Morphology:
The muscle may or may not be abnormal (e.g. RRF, COX-negative RRF), depending on the amount of mutation present.

Molecular Diagnosis:
For routine referrals we recommend Southern blot or long range PCR for the detection of mtDNA rearrangement and analysis to detect the m.3243A>G mutation.

Infantile myopathy with COX deficiency

In the fatal form, the disorder appears soon after birth, with severe diffuse weakness, respiratory distress, and lactic acidosis. Respiratory insufficiency and death ensues before one year of age. Inheritance is apparently autosomal recessive. In the reversible or "benign" form of infantile myopathy with COX deficiency, patients at birth are indistinguishable from patients with the fatal myopathy. However, if supported aggressively, they improve spontaneously and are usually normal by 2 or 3 years of age. The recovery is accompanied by a gradual return of COX activity in muscle, which can be demonstrated both biochemically and histochemically. The reversible form of infantile myopathy with COX deficiency is due to a maternally inherited homoplasmic m.14674T>C mutation in tRNA^{Glu}.

Morphology:
In both the fatal form and in the early stages of the reversible form, muscle is completely devoid of COX activity and RRF are present. Of practical importance, early differential diagnosis can be provided by immunocytochemistry of muscle biopsy specimens. The immunoreactivity to COX II (mtDNA-encoded) is positive in the fatal form but negative in the reversible form. Immunoreactivity to COXI-Iab (nDNA-encoded) is negative in both forms. Screening for the M.14674T>C mutation identifies patients with the reversible form.

Molecular Diagnosis:
Screening for m.14674T>C in tRNA^{Glu} is recommended.

Other disorders

A number of other mitochondrial mutations have been associated with limb-girdle myopathy, myopathy plus cardiomyopathy, cardiopathy plus a multisystem disorder, sensorineuronal deafness, and fatal infantile respiratory chain deficiency.

Genetics:
These disorders have been associated with point mutations in a number of tRNA genes.

Morphology:
RRF are usually present, and are often COX-negative.

Diseases associated with spontaneous errors in mtDNA

Some mtDNA mutations can arise spontaneously, with no apparent genetic component (i.e. neither mothers nor siblings harbor any observable mtDNA mutations). These sporadic mutations are associated with large-scale partial deletions and duplications of mtDNA. There is only one specific type of mtDNA rearrangement in each patient. The particular type of deletion varies among patients, but about one third of all patients harbor the same deletion, called the "common deletion". More than two hundred species of rearranged mtDNAs have been described. Three disorders have been associated with these mtDNA rearrangements.

Sporadic Kearns-Syre syndrome (KSS)

KSS is defined by the invariant triad of progressive external ophthalmoplegia (PEO), pigmentary retinopathy, and onset before age 20, with at least one of the following features: cerebellar ataxia, cardiac conduction block, or cerebrospinal fluid protein greater than 100 mg/dL. Other associated clinical features include: proximal limb weakness, renal tubular dysfunction, hearing loss, and endocrinopathies (particularly diabetes mellitus and hypoparathyroidism).

Genetics:
KSS is diagnosed by the presence of a single large-scale rearrangement of mtDNA (either a deletion or a duplication), as observed in Southern blot hybridization analysis of muscle DNA (see Figure 6). The analysis of DNA isolated from blood may also be informative.

Morphology:
RRF are present. Most RRF are COX-negative.

Molecular Diagnosis:
For routine referrals, Southern blot or long range PCR for the detection of mtDNA rearrangement is recommended.
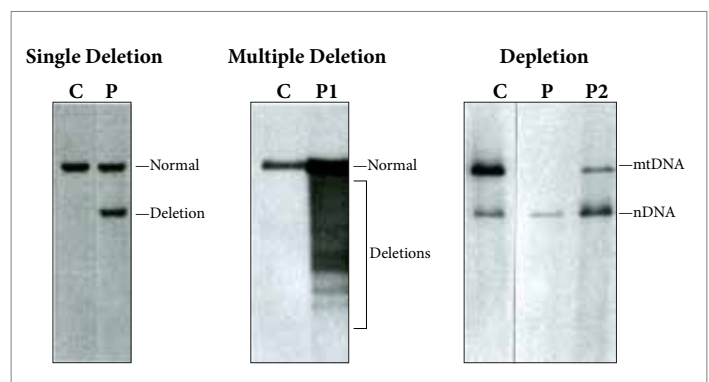

Figure 6
Southern blot hybridization to detect mtDNA rearrangements, including "single" deletions (left panel), "multiple" deletions (center panel), and mtDNA depletion (right panel). P,P1,P2 = patients, C = controls.

Sporadic progressive external ophthalmoplegia (PEO)

Giant deletions and duplications of mtDNA are observed not only in KSS, but also in sporadic PEO. This disorder, which is a milder version of KSS, is invariably associated with paralysis of eye movement and ptosis. There is often with proximal limb weakness. However, other organ systems are unaffected.

Genetics:
PEO is diagnosed by the presence of a single large-scale rearrangement of mtDNA, as observed in Southern blot hybridization analysis of muscle DNA.

Morphology:
RRF are often present. RRF are usually COX-negative.

Molecular Diagnosis:
For routine referrals, Southern blot or long range PCR for the detection of mtDNA rearrangement is recommended.

Pearson marrow/pancreas syndrome

Deletions and duplications of mtDNA are also present in a rare hematopoietic disorder called Pearson's marrow/pancreas syndrome.

The disease typically begins in infancy or early childhood with sideroblastic anemia and exocrine pancreatic dysfunction, and can be fatal despite blood transfusion therapy. Patients who present with Pearson's syndrome can later develop KSS.

Genetics:
Pearson's syndrome is diagnosed by the presence of a single large-scale rearrangement of mtDNA, as observed in Southern blot hybridization analysis of blood DNA.

Morphology:
Muscle may appear normal, especially in younger patients.

Molecular Diagnosis:
For routine referrals, Southern blot or long-range PCR for the detection of mtDNA rearrangement is recommended.

Diseases associated with Mendelian-inherited errors in mtDNA

Autosomal inherited PEO

Autosomal dominant-inherited myopathy is characterized by PEO (AD-PEO). Aside from the pattern of inheritance, the clinical presentation is fundamentally identical to maternally-inherited PEO and sporadic PEO. In some patients inheritance is autosomal recessive (AR-PEO).

Genetics:
Affected family members harbor large quantities of multiple species of large-scale deletion of mtDNA in their muscle, which are apparently generated during the life span of the patient. These are observable as multiple discreet bands or as a "smear" of hybridizing fragments migrating more rapidly than normal (full-length) mtDNA in Southern blot hybridization analysis.

Morphology:
RRF are present and are COX-negative.

Molecular Diagnosis:
For routine referrals, Southern blot or long range PCR for the detection of mtDNA rearrangement is recommended. Five nuclear genes, *POLG1, C10orf2/PEO1* (encoding Twinkle), *ANT1, POLG2,* and *OPA1* have been associated with AD-PEO and AR-PEO.

Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE)

MNGIE is a multisystem disorder affecting muscle (PEO is especially prominent), brain (leukodystrophy by MRI), peripheral nerves (demyelinating or axonal peripheral neuropathy) and the gastrointestinal system, with chronic diarrhea and intestinal pseudo-obstruction. Patients are thin and emaciated, and have lactic acidosis.

Genetics:
The disorder is transmitted as an autosomal recessive trait. In about half of patients, Southern blot shows multiple deletions of mtDNA in muscle. In addition, many tissues show depletion and site-specific somatic point mutations of mtDNA.

Morphology:
RRF are present and are COX-negative.

Molecular Diagnosis:
MNGIE is diagnosed by direct measurement of thymidine phosphorylase *(TP)* enzyme activity in buffy coat. Direct sequencing of the TP gene *(TYMP)* is recommended for confirmation of positive results.

Mitochondrial depletion syndrome (MDS)

Another type of mendelian-inherited disorder is a quantitative error in the amount of mtDNA present in specific tissues. In these mtDNA depletion syndromes, affected tissues show respiratory chain defects, massive mitochondrial proliferation (RRF in affected muscle), and severe depletion in the amount of mtDNA present. There may be no observable biochemical or genetic defect in unaffected tissues. In the early-onset form, symptoms begin at birth and death usually ensues before a year. The degree of depletion is severe (up to 98% reduction in mtDNA), but is confined to only one or two tissue types (e.g., muscle alone, liver alone, muscle and kidney). If the depletion is in muscle, it is uniform (i.e. all muscle fibers are devoid of observable COX enzyme activity, and have little or no immunoreactive mtDNA). In the later-onset form, symptoms begin around one year of age, and are usually confined to muscle.

Genetics:
The best and quickest test for mtDNA depletion in muscle is real-time PCR. In this assay, fragments of mtDNA and a nDNA gene are simultaneously amplified and quantitated to determine the relative amount of mtDNA compared to the reference nDNA gene. Depletion is also observed by performing a Southern blot hybridization analysis of DNA isolated from the affected tissue, using two probes simultaneously: a probe for mtDNA and a probe for nDNA. The depletion is detected as a severely reduced ratio of mtDNA:nDNA hybridization signal as compared to that found in DNA from the same tissue isolated from a normal individual (see Figure 6).

Morphology:
Histochemistry to detect COX, and immunohistochemistry to detect mtDNA encoded polypeptides (e.g., COX II, ND1) show an absence of signal in many or all fibers. Remarkably, numerous RRF (all COX-negative) are present. Proliferation of mitochondria in other tissues (e.g., liver, kidney) may also be observed by electron microscopy.

Molecular Diagnosis:
For routine referrals, quantification of mtDNA copy number by real-time PCR is recommended. Eight nuclear genes *(POLG1, TK2, DGUOK, RRM2B, MPV17, SUCLA2, SUCLG1,* and *PEO1)* have been associated with MDS: POLG1 and PEO1 mutations also cause AD- and AR-PEO (see above).

Mitochondrial diseases due to Mendelian-inherited mutations in nuclear DNA

There are almost 1600 nuclear genes required for proper mitochondrial function. Recent progress in rapid sequencing of DNA will soon allow us to offer diagnostic tests for defects in nuclear genes. Until such a test becomes available, the secondary effects of the nu-

clear mutations are evaluated either by studying the characteristics of the mitochondrial DNA or the biochemical and immunohisto-chemical characteristics of the mitochondria themselves. Recent advances in rapid sequencing of DNA will soon allow us to offer diagnostic tests for defects in nuclear genes.

Cardiac manifestations of oxidative phosphorylation system defects

Cardiomyopathy

Maternally inherited cardiomyopathy syndromes have been associated with a number of mtDNA point mutations, the majority of which lie within the tRNA-leucine and tRNA-isoleucine genes. Patients with cardiomyopathy and a complex I or IV defect but no identifiable mtDNA mutation are believed to harbor mutations within nuclear genes.[9]

Genetics:
A number of tRNA mutations have a propensity to cause cardiomyopathies.[8] Four of these mutations are located in tRNA[Leu(UUR)] and five in tRNA[Ile].

Molecular Diagnostics:
For routine referrals, screening of m.3243A>G, m.3260A>G, and m.3303C>T are recommended. Screening for the other four mutations (m.4269A>G, m.4300A>G, m.8363G>A, and T9997C is warranted when the first three mutations test negative.

References

1. DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. *N Engl J Med.* Jun 26 2003;348 (26):2656-2668.

2. DiMauro S, Davidzon G. Mitochondrial DNA and disease. *Ann Med.* 2005;37(3):222-232.

3. Tassin S, Walter GF, Brucher JM, Rousseau JJ. Histochemical and ultrastructural analysis of the mitochondrial changes in a familial mitochondrial myopathy. *Neuropathol Appl Neurobiol.* Sep-Oct 1980;6(5):337-347.

4. Taylor RW, Schaefer AM, Barron MJ, McFarland R, Turnbull DM. The diagnosis of mitochondrial muscle disease. *Neuromuscul Disord.* Apr 2004;14(4):237-245.

5. Mackey DA. Three subgroups of patients from the United Kingdom with Leber hereditary optic neuropathy. *Eye (Lond).* 1994;8 (Pt 4):431-436.

6. Yamada K, Oguchi Y, Hotta Y, al. e. Multicenter study on the frequency of three primary mutations of mitochondrial DNA in a Japanese pedigree with Leber's hereditary optic neuropathy:comparison with American and British counterparts. *Neuroophthalmology.* 1999;22:187-193.

7. Johns DR, Neufeld MJ. Pitfalls in the molecular genetic diagnosis of Leber hereditary optic neuropathy (LHON). *Am J Hum Genet.* Oct 1993;53(4):916-920.

8. Schon EA, Hirano M, DiMauro S. Molecular genetic basis of the mitochondrial encephalomyopathies. In: Schapira AHV, DiMauro S, eds. *Mitochondrial Disorders in Neurology* 2. Vol 26. Boston: Butterworth-Heinemann; 2002:69-113.

9. Hart PE, De Vivo DC, Schapira AHV. Clinical Features of the Mitochondrial Encephalomyopathies. In: Schapira AHV, DiMauro S, eds. *Mitochondrial Disorders in Neurology.* Boston: Butterworth Heinemann; 2002:35-68.

10. Wallace DC. Pathophysiology of mitochondrial disease as illuminated by animal models. In: Schapira AHV, DiMauro S, eds. *Mitochondrial Disorders in Neurology.* Boston: Butterworth Heinemann; 2002:175-212.

11. Schon, EA, DiMauro, S, Hirano, M and Gilkerson, RW. Therapeutic *Prospects for Mitochondrial Disease.* Trends in Molecular Medicine, Vol 16. 2010:268-276

Contact us:
http://www.columbiamitodiagnostics.org/contact.html
http://pathology.columbia.edu

For patient referral:
Michio Hirano, MD, *Medical Director*
212-305-3947
e-mail: mh29@columbia.edu

For biopsies and other samples:
Ali B. Naini, PhD, *Laboratory Director*
212-305-3947
abn2@columbia.edu

For biopsy preparation, visit our website

Please note our new address:
Laboratory of Molecular Neurogenetics
Department of Pathology and Cell Biology
Columbia University Medical Center
630 West 168th Street, VC-15th Floor, Room 208
New York, NY 10032

Phone: 212-305-3947
FAX: 212-305-3986
Administrator email: pa2120@columbia.edu *(Mr. Pablo Abreu)*