Practical Approaches to a Mito Diagnosis

Richard H. Haas M.B., B.Chir., M.R.C.P.
Director UCSD Mitochondrial Disease Laboratory
Co-Director UCSD Mitochondrial and Metabolic Disease Center

UNIVERSITY of CALIFORNIA, SAN DIEGO
MEDICAL CENTER
Overview of Mitochondrial Diagnosis

- Basic Mito Facts and Background, including what mitochondria do
- Types of problems that can be caused by Mito dysfunction
- Genetics, in brief
- Mito Diseases - what are they and how are they classified (OXPHOS, Leigh's, MELAS, etc.)
- Inherent problems in diagnosis/diagnostic approaches of both OXPHOS and mtDNA disease - Heteroplasmy
- Testing, in brief, including advantages and limitations of new nDNA gene sequencing
- Clues to the diagnosis of mitochondrial disease for clinicians (and families)
- How does one arrive at a diagnosis of Mito disease? Combination of clinical testing, biochemical testing, personal and family history, and symptoms/clinical presentation
- Why are more invasive tests (i.e. muscle biopsy) sometimes necessary?
- How is the field of mitochondrial medicine changing? Are there new types of mitochondrial disease? What may the future look like for this field and for patients/families?
Eukaryotic Cell: Chromosomes, Genes, and DNA

- Cell Wall
- Nucleus
- Cytoplasm
- Chromosome
- Gene
- DNA
Triplet Codes

Portion of a gene in one strand of DNA

Amino acid sequence coded by gene


Met  Phe  Gly  Ser  Gly  Trp  His  Phe
Basic Mito Facts

- Prokaryote (Bacterial) origin of mitochondria & mtDNA – symbiotic relationship
- 1500 nuclear mitochondrial genes
- 2-10 mtDNA molecules per mitochondrion
- 100 – 10,000 mitochondria per nucleated cell
- mtDNA is maternally inherited
Fig 2. The clinical features of mitochondrial disorders. Reproduced with permission from BMJ Publishing Group.7
Where is Mitochondrial Disease?

- Diabetes Clinics
- Muscular Dystrophy Clinics
- Epilepsy Clinics
- Atypical Leukodystrophy
- Psychiatric Clinics, Bipolar Depression, Schizophrenia
- Sudden Infant Death Syndrome (SIDS)
- GI Dysmotility Clinics
- Rheumatology/Multiple Sclerosis, Autoimmune Disease
- Unexplained Liver Failure
- Cancer/Hematology Clinics
- AIDS and Chronic Virus Infections
- Macular Degeneration/Unexplained Blindness
- Unexplained Kidney Disease
- Heart Disease, Heart Failure
- Autism, Atypical Autism, and ASD
- Hearing Loss/Language Delay Clinics
- Alzheimer and Parkinson Clinics
- Atypical Learning Disabilities, ADD

Naviaux, 2001
Major Mitochondrial Functions

- Make ATP for cellular energy – oxidative phosphorylation
- Metabolize
  - fats
  - carbohydrates
  - amino acids
- Interconvert carbohydrates, fats and amino acids
- Synthesize some proteins
- Reproduce themselves (replicate), fusion/fission
- Participate in apoptosis
- Make free radicals
- Innate Immunity
Human mtDNA 16569 bp
Oxphos Disease
A disease of energy metabolism resulting in impairment of oxidative phosphorylation

Nuclear Gene Defects (80% of Child disease)

mtDNA Defects (60% of Adult disease)
Leigh Syndrome—Cytochrome Oxidase Deficiency
Experimental Treatment with TAU and DCA

Age 5

Age 8

Age 16
Graduating from HS
In June 2011
Leigh Syndrome: Subacute Necrotizing Encephalomyelopathy
Figure 12-3 Three-dimensional diagram of a mitochondrion cut longitudinally. The main features are shown. Observe that the cristae are folds of the inner membrane and that on their matrix side they have the $F_1$ particles. The inset shows an $F_1$ particle with the head piece and stalk.
Leigh Syndrome

Fatty Acids Leucine Valine Isoleucine

Acyl-CoA Dehydrogenase

ETF<sub>OX</sub> ETF<sub>RED</sub>

ETF-QO

SDH

FADH<sub>2</sub>

NADH

2e<sup>-</sup>

Dihydroorotic Acid

Orotic Acid

Uridine

O<sub>2</sub>

H<sub>2</sub>O

ATP

ADP

ADP ATP

ETC Genes

<table>
<thead>
<tr>
<th>mt DNA</th>
<th>7</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>nuclear DNA</td>
<td>36</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>13</td>
</tr>
</tbody>
</table>
MELAS

Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like Episodes
Severe Pediatric MELAS—90% Heteroplasmy

- Age 19: prepubertal, short stature, ataxia, dementia, seizures
- Multiple occipital infarcts cortical blindness
- Deafness, myopathy, cardiomyopathy
- Plasma lactate 3.5 mM, CSF lactate 5.5 mM
- Calcified Basal Ganglia

Skoglund RR. Neurology. 1979 May;29(5):717-20
Pathological Mutations in tRNA^{Leu(UUR)}

http://www.mitomap.org/MITOMAP/tRNAleu.pdf

Copyright 2002 @ Mitomap.org
Heteroplasmy

Wild Type

Mutant
Heteroplasmic in Fibroblasts

Control

MELAS 3243

Cox I 488 Porin 594 merge
How Does Genetic Mitochondrial Disease Present?

**Acute/ Subacute**
- Severe Metabolic Crisis
- Encephalopathy
- Arrhythmia, Heart block
- Ophthalmoplegia, Blindness
- Stroke

**Chronic**
- Growth Retardation
- Developmental Delay
- ‘Strabismus’
- Diabetes
- Irritable Bowel Syndrome
- Cardiomyopathy
- Neuropathy, Ataxia
- Hypotonia and Weakness
- Exercise Intolerance
- Dementia
Severity of Disease Affects Onset

MITOCHONDRIAL DISEASE

SEVERE
INFANCY
Severe Lactic Acidosis

MILD
ADULT
Parkinson's Disease

MODERATE
CHILDHOOD TEENAGE
Leigh's Syndrome

MELAS
Mitochondrial Disease: A Practical Approach for Primary Care Physicians

Richard H. Haas, MB, BChir, MRCPa,b, Sumit Parikh, MDc, Marni J. Falk, MDd, Russell P. Saneto, DO, PhDs, Nicole I. Wolf, MDf,g, Niklas Darin, MDh, Bruce H. Cohen, MDc

aDepartments of Neurosciences and Pediatrics, University of California San Diego, La Jolla, California; bDepartments of Neurosciences and Pediatrics, Rady Children’s Hospital and Health Center, San Diego, California; Division of Neuroscience, Cleveland Clinic, Cleveland, Ohio; dDivision of Human Genetics, Children’s Hospital of Philadelphia and University of Pennsylvania, Philadelphia, Pennsylvania; eDivision of Pediatric Neurology, Children’s Hospital and Regional Medical Center, University of Washington, Seattle, Washington; fDivision of Child Neurology, University Children’s Hospital, Heidelberg, Germany; gDivision of Child Neurology, University Children’s Hospital, Zürich, Switzerland; hDivision of Child Neurology, Queen Silvia Children’s Hospital, Göteborg, Sweden

PEDIATRICS Volume 120, Number 6, December 2007
DIAGNOSTIC EVALUATION OF MITOCHONDRIAL DISEASE

The major challenge to properly establishing mitochondrial dysfunction as the cause of a patient’s presentation is the absence of a definitive biomarker that characterizes mitochondrial disease in all patients. Thus, the diagnostic evaluation is necessarily multitiered and broad-based, with a focus on integrating information from many avenues: the complete medical and family history, clinical findings that may be suggestive of mitochondrial disease (see Tables 1 and 2), biochemical laboratory abnormalities such as lactic acidosis (which, as discussed above, is neither sensitive nor specific as a single biomarker for many mitochondrial disorders), tissue-biopsy evidence of abnormal electron-transport chain enzyme activity or impaired respiratory capacity, and, if possible, the identification of a pathogenic mtDNA or nDNA mutation.
Diagnosis of Mitochondrial Disease

Clinical Symptoms

Physical Exam

Family History

Metabolic Tests
  Blood, Urine, CSF

Organ Evaluation
  (MRI/MRS, EKG/Echo)

Tissue Biopsy
  (Skin, Muscle, Liver, Heart)

Molecular Genetics

Biochemistry
  Oxphos Studies
  ETC analysis
Metabolic & Other Tests
Blood, Urine & CSF

- CPK
- Lactate and Pyruvate
- Ammonia
- Plasma Amino Acids
- Plasma Acyl-carnitine profile
- Plasma Carnitine
- Urine Organic Acids
- DNA Studies
Available Mito Tests

- **Test**
  - Histology/EM
    - Carnitine, CoQ
  - Nuclear DNA
  - mtDNA
  - Electron Transport Assays
  - Polarographic Assay
  - Enzyme Assay
  - Protein Immunoassay
    - Immunocytochemistry
    - Immunohistochemistry

- **Tissue**
  - Muscle, Liver, Heart
  - Blood, All Tissues
  - All Tissues, Muscle Best
  - Muscle Fresh/Frozen, Fibroblasts, Liver, Heart
  - Fresh muscle, Liver, Heart or Mitochondria
  - All Tissues, Mitochondria, Fibroblasts
  - Mitochondria (Blue Native)
    - Tissue (Clear Native)
    - Fibroblasts/ Muscle Tissue
Tissue Diagnosis

- Available Tissues
  - Blood
  - Saliva (Buccal Epithelial Cells)
  - Urine Sediment
  - Muscle
  - Skin Fibroblasts
  - Other Tissues
    - Liver
    - Heart
Heteroplasmy

Wild Type

Mutant
The Basics of Tissue Testing for Mitochondrial Disease

- **Tissues for mtDNA Testing – The Heteroplasmy Problem**

<table>
<thead>
<tr>
<th>Blood</th>
<th>Saliva</th>
<th>Urine</th>
<th>Muscle</th>
</tr>
</thead>
</table>

% of Mutation
Saliva Collection (Oragene)
Muscle Biopsy Problems

- Histochemistry
  - Often normal in Pediatric cases

- Electron Microscopy (EM)
  - May help but often difficult to get

- ETC Assays
  - Lab to lab variation
  - Very susceptible to sample handling
Figure 3. Residual activity of complex I CS ratios in the 66 skeletal muscle biopsies analyzed in this patient series.
Ragged Red Fiber Myopathy
Neurometabolic Evaluation

- Referred age 16 months with
  - Global delay & hypotonia
  - Plasma lactate $\uparrow$ 4.1 mM
  - CPK $\uparrow$ 155 U/L
  - Urine organic acids – mild increase in 3-OH isovalerate and glutamate.
  - Plasma acylcarnitines $\uparrow$ C5OH, C3 and C2.
  - Biotinidase normal
  - Leukocyte carboxylases normal

SS Age 23 months
Muscle PCR Msp-I Digest

NARP 8993 T>C or G
60-70%
Summary

- Tissue sampling for mitochondrial disease is dictated by the tests required. Nuclear DNA testing requires only blood.
- Blood, saliva and urine for mtDNA testing are all feasible but heteroplasmy presents a problem.
- Muscle biopsy remains the ‘Gold Standard’ for electron transport chain assay and for mtDNA testing.
- Fresh muscle offers the opportunity to perform functional polarography and to isolate mitochondria for electron transport and protein study.
Probability of Mitochondrial Disease

Clinical + Biochemical Criteria

- Definite
- Probable
- Possible
- Unlikely

Mitochondrial disorders
A proposal for consensus diagnostic criteria in infants and children

Nicole I. Wolf, MD; and Jan A.M. Smeitink, MD, PhD

NEUROLOGY 2002;59:1402–1405
Table 2 The MDC: Combination of biochemical and general criteria and final patient assignment

<table>
<thead>
<tr>
<th>Biochemical criteria</th>
<th>General criteria:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unlikely</td>
</tr>
<tr>
<td>Unlikely</td>
<td>Unlikely</td>
</tr>
<tr>
<td>Possible</td>
<td>Possible</td>
</tr>
<tr>
<td>Probable</td>
<td>Possible</td>
</tr>
<tr>
<td>Definite</td>
<td>Probable</td>
</tr>
</tbody>
</table>
The in-depth evaluation of suspected mitochondrial disease

The Mitochondrial Medicine Society’s Committee on Diagnosis
Richard H. Haas a,*, Sumit Parikh b, Marni J. Falk c, Russell P. Saneto d, Nicole I. Wolf e, Niklas Darin f, Lee-Jun Wong g, Bruce H. Cohen b, Robert K. Naviaux h

a Departments of Neurosciences & Pediatrics, University of California San Diego, La Jolla, CA and Rady Children’s Hospital San Diego, San Diego, CA, United States
b Division of Neurosciences, The Cleveland Clinic, Cleveland, OH, United States
c Division of Human Genetics, The Children’s Hospital of Philadelphia and University of Pennsylvania, Philadelphia, PA, United States
d Division of Pediatric Neurology, Children’s Hospital and Regional Medical Center, University of Washington, Seattle, WA, United States
e Department of Child Neurology, University Children’s Hospital, Heidelberg, Germany
f Division of Child Neurology, The Queen Silvia Children’s Hospital, Göteborg, Sweden
g Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, United States
h Departments of Medicine and Pediatrics, Division of Medical and Biochemical Genetics, University of California San Diego, La Jolla, CA and Rady Children’s Hospital San Diego, San Diego, CA, United States

Received 8 October 2007; received in revised form 21 November 2007; accepted 21 November 2007
Available online 1 February 2008

Abstract

Mitochondrial disease confirmation and establishment of a specific molecular diagnosis requires extensive clinical and laboratory evaluation. Dual genome origins of mitochondrial disease, multi-organ system manifestations, and an ever increasing spectrum of recognized phenotypes represent the main diagnostic challenges. To overcome these obstacles, compiling information from a variety of diagnostic laboratory modalities can often provide sufficient evidence to establish an etiology. These include blood and tissue histochemical and analyte measurements, neuroimaging, provocative testing, enzymatic assays of tissue samples and cultured cells, as well as DNA analysis. As interpretation of results from these multifaceted investigations can become quite complex, the Diagnostic Committee of the Mitochondrial Medicine Society developed this review to provide an overview of currently available and emerging methodologies for the diagnosis of primary mitochondrial disease, with a focus on disorders characterized by impairment of oxidative phosphorylation. The aim of this work is to facilitate the diagnosis of mitochondrial disease by geneticists, neurologists, and other metabolic specialists who face the challenge of evaluating patients of all ages with suspected mitochondrial disease.

© 2008 Elsevier Inc. All rights reserved.

Keywords: Mitochondrial disease; Laboratory diagnosis; Review
Epidemiology
Pathogenic Mitochondrial DNA Mutations Are Common in the General Population

Hannah R. Elliott,1 David C. Samuels,2 James A. Eden,3 Caroline L. Relton,3 and Patrick F. Chinnery1,3,*

Mitochondrial DNA (mtDNA) mutations are a major cause of genetic disease, but their prevalence in the general population is not known. We determined the frequency of ten mitochondrial point mutations in 3168 neonatal-cord-blood samples from sequential live births, analyzing matched maternal-blood samples to estimate the de novo mutation rate. mtDNA mutations were detected in 15 offspring (0.54%, 95% CI = 0.30–0.89%). Of these live births, 0.00107% (95% CI = 0.00087–0.0127) harbored a mutation not detected in the mother's blood, providing an estimate of the de novo mutation rate. The most common mutation was m.3243A→G. m.14484T→C was only found on sub-branches of mtDNA haplogroup J. In conclusion, at least one in 200 healthy humans harbors a pathogenic mtDNA mutation that potentially causes disease in the offspring of female carriers. The exclusive detection of m.14484T→C on haplogroup J implicates the background mtDNA haplotype in mutagenesis. These findings emphasize the importance of developing new approaches to prevent transmission.
Epidemiology of Mitochondrial DNA Disease

mtDNA Disease (<50% of Total)

9.2 per 100,000 Retired Adults
16.5 per 100,000 Working Adults and Children

Total Prevalence = 25.7 per 100,000
= 1 in 4,000 (3,891)

mtDNA + nDNA Disease
Birth Incidence

1 in 2,000 will Develop Disease
1 in 4,000 Before Age 10
1 in 4,000 After Age 10
Expanding the Phenotype

A never-ending process
The Dynamic Nature of Mitochondrial Networks

Control Fibroblast

Severe Complex I Deficiency

From Nhu-an Pham et al. Microsc.Microanal. 10, 247-260, 2004
Mitochondrial fusion and fission

Mitochondrial fusion GTPases
- Mitofusin 2
  - (MF2) Charcot-Marie-Tooth disease CMT2A
  - HMSN VI
- Optic atrophy 1 (OPA1)
  - Autosomal Dominant Optic Atrophy

Fission proteins
- Dynamin Related Protein 1 (DRP1)
  - Infantile mitochondrial cytopathy with lactic acidemia VLCFA, optic atrophy and hypotonia
Autism

- Four year-old boy with history of normal pre-, peri- and postnatal courses
- Normal development until 18 months of age
- Progressive loss of expressive language and language comprehension
- Gradual increase in disruptive behavior, hyperkinesis, and self injurious behavior
- Mild motor clumsiness but no ataxia
- Normal plasma lactate
- Sister with Leigh Disease

Autism Spectrum

1:110

Classical Autism

5 - 8%

Possible Mito Disease (Mito Dysfunction)

Definite Mito Disease

Probable Mito Disease

>1:5000

Autism and Mitochondria
Autism and Mitochondrial Disease

Richard H. Haas

1Department of Neurosciences, UCSD Mitochondrial and Metabolic Disease Center,
University of California San Diego, La Jolla, California

2Department of Pediatrics, UCSD Mitochondrial and Metabolic Disease Center,
University of California San Diego, La Jolla, California

Autism spectrum disorder (ASD) as defined by the revised Diagnostic and Statistical Manual of Mental Disorders: DSM IVTR criteria (American Psychiatric Association [2000] Washington, DC: American Psychiatric Publishing) as impairment before the age of 3 in language development and socialization with the development of repetitive behaviors, appears to be increased in incidence and prevalence. Similarly, mitochondrial disorders are increasingly recognized. Although overlap between these disorders is to be expected, accumulating clinical, genetic, and biochemical evidence suggests that mitochondrial dysfunction in ASD is more commonly seen than expected. Some patients with ASD phenotypes clearly have genetic-based primary mitochondrial disease. This review will examine the data linking autism and mitochondria.

Neurodegeneration in primary mitochondrial disease patients is frequently precipitated by infection, postulated to be mediated by metabolic decompensation and cytokine toxicity. More recently, autistic regression with resulting ASD in children who were thought to be previously normal has been reported following fever associated with infection or immunizations. Some of these children are subsequently recognized to have primary mitochondrial disease—"Mitochondrial Autism," a term suggested by Weissman et al. [2008].