Practical Approaches to a Mito Diagnosis

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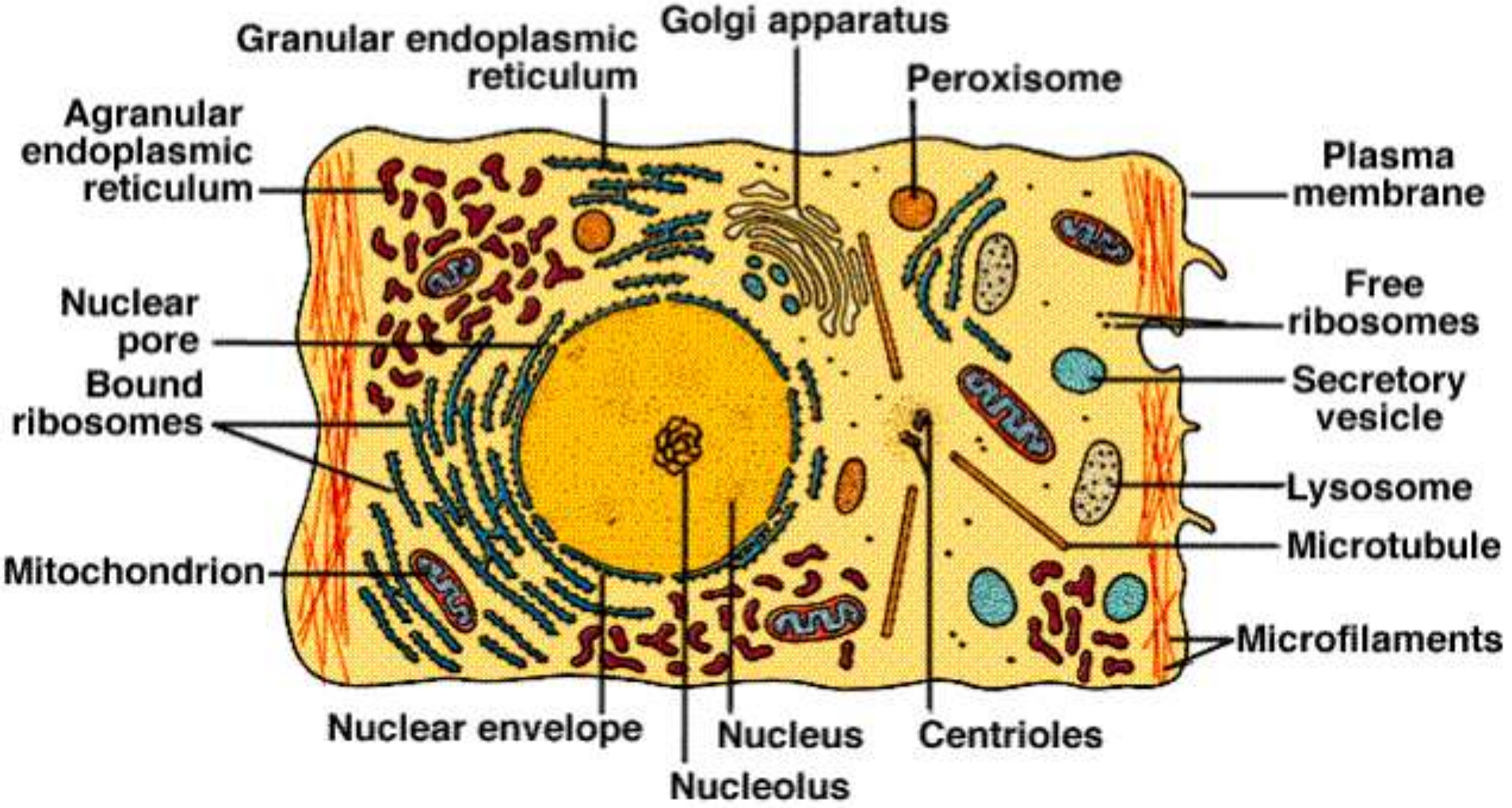
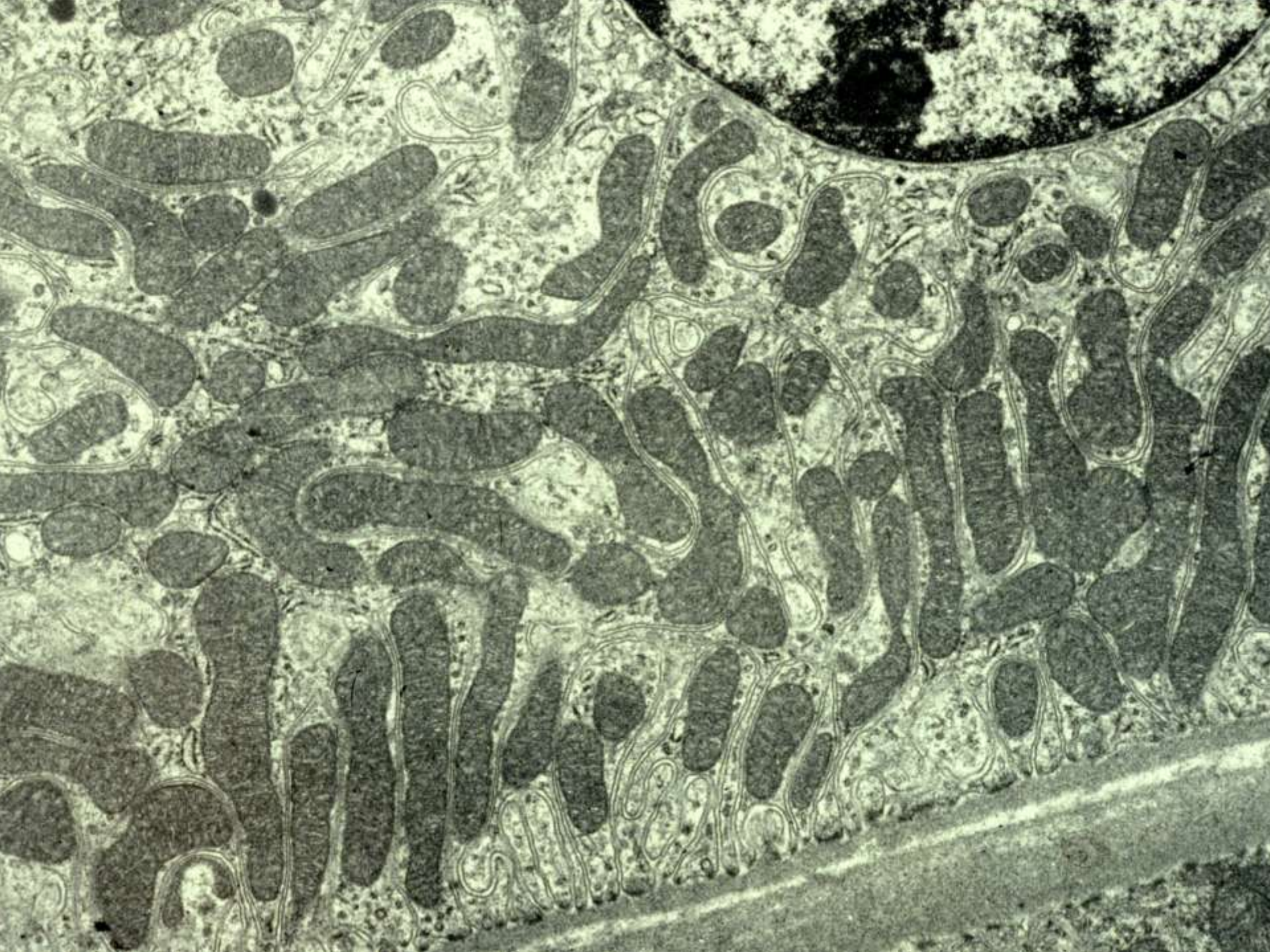
UNIVERSITY *of* CALIFORNIA, SAN DIEGO
MEDICAL CENTER

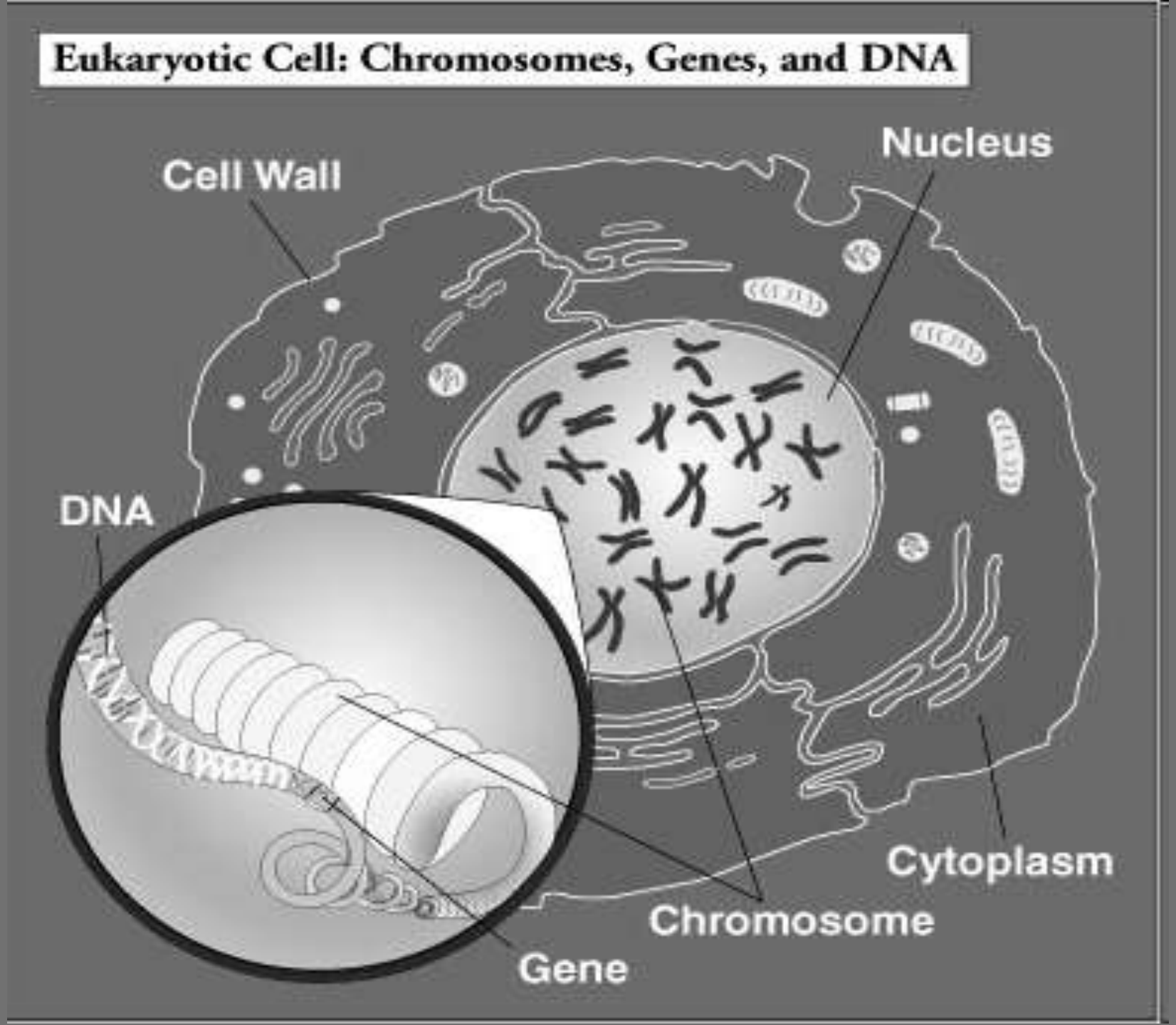
Overview of Mitochondrial Diagnosis

- 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?

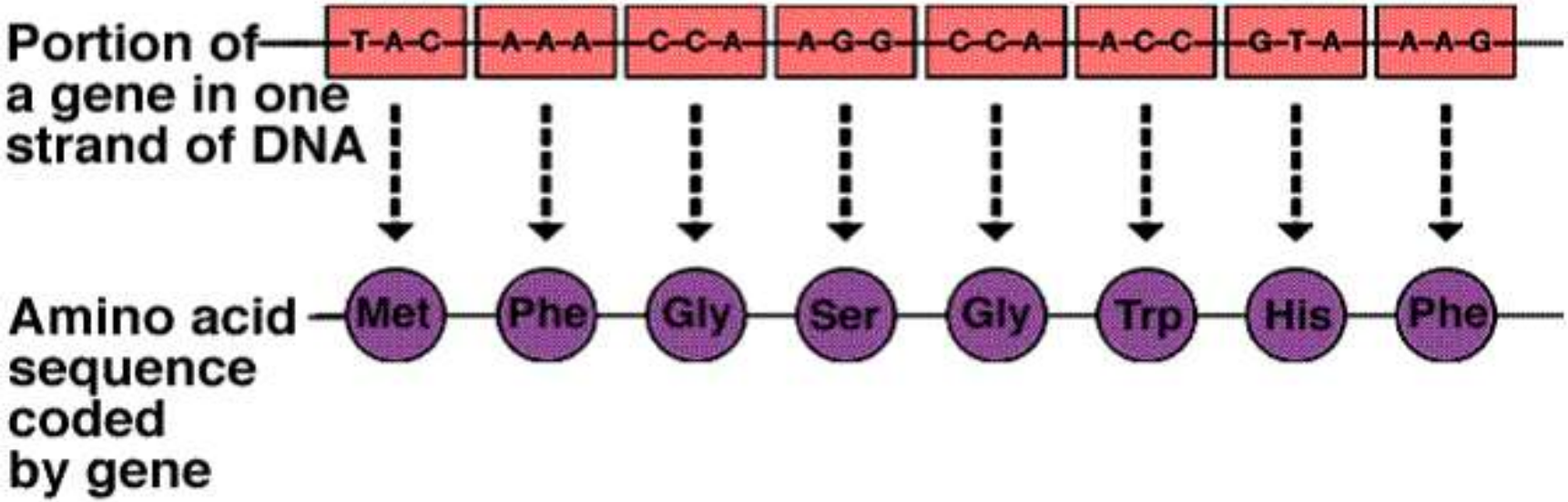
Human Cell






Eukaryotic Cell: Chromosomes, Genes, and DNA
Cell Wall
Nucleus
DNA
Cytoplasm
Chromosome
Gene

Triplet Codes



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

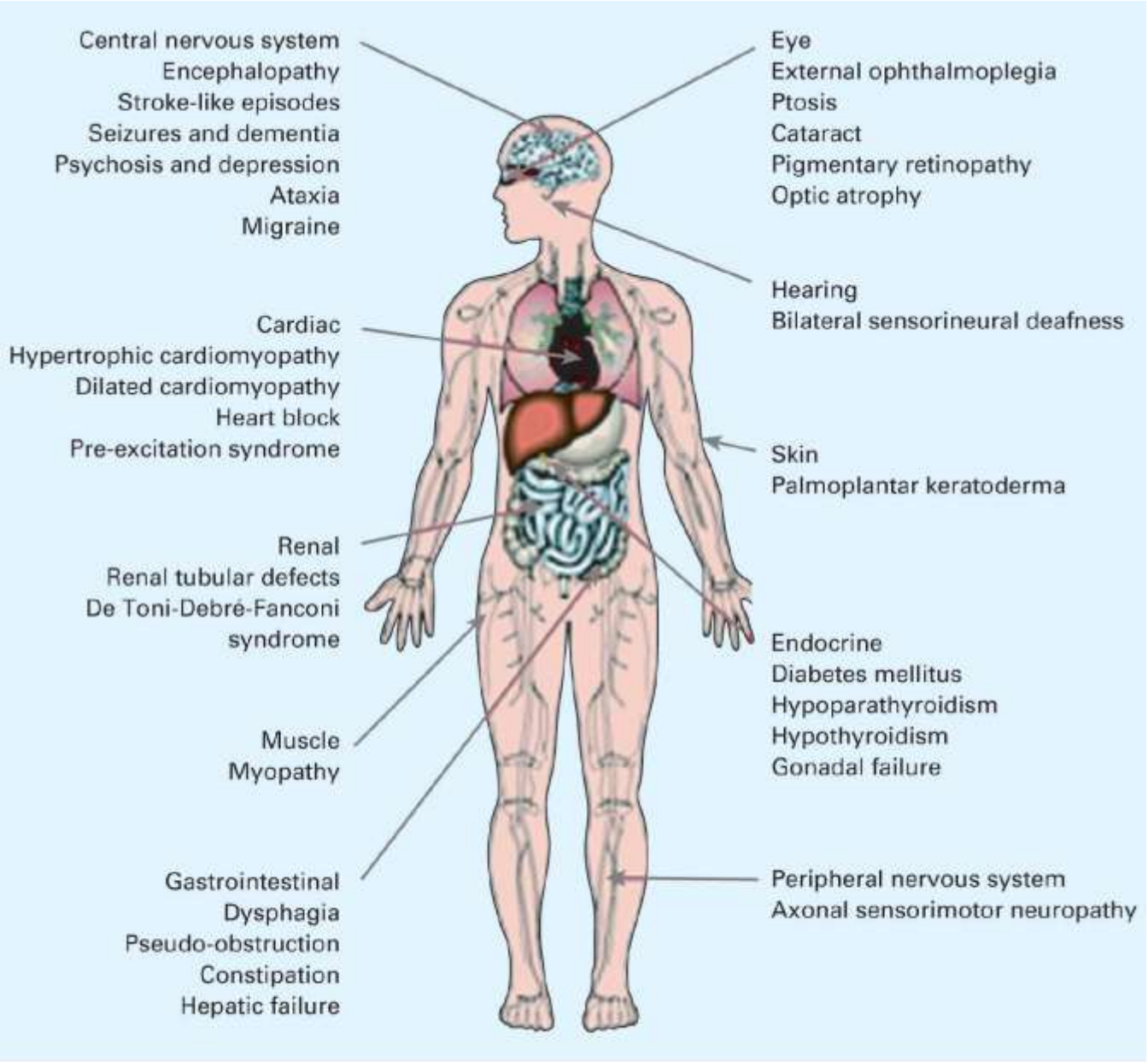


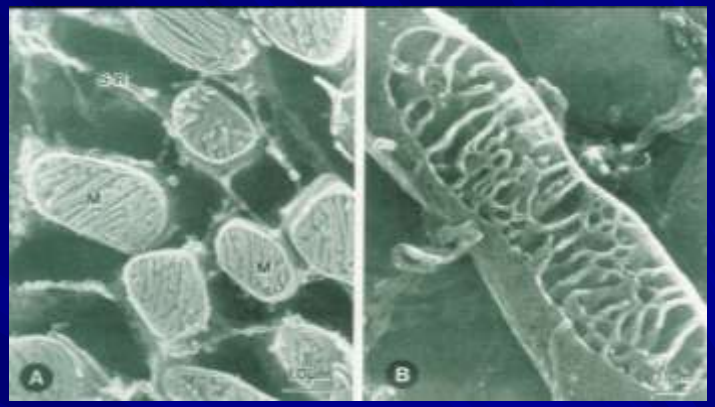
Fig 2. The clinical features of mitochondrial disorders. Reproduced with permission from BMJ Publishing Group.[7]

Kirkman MA, Yu-Wai P, Chinnery PF Clin Med. 2008 Dec;8(6):601-6.

Naviaux, 2001

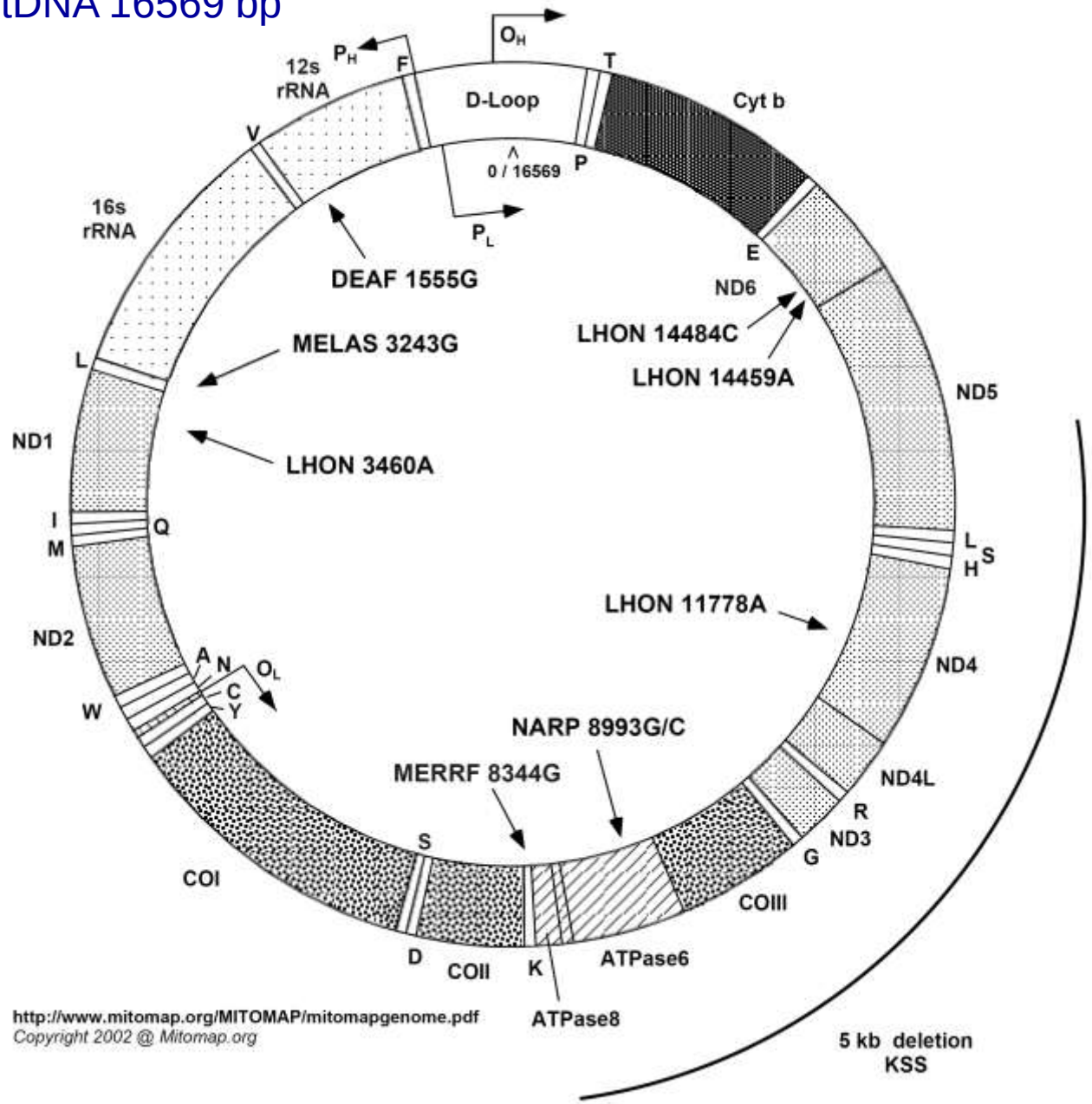
Major Mitochondrial Functions

- Make ATP for cellular energy – oxidative phosphorylation
- Metabolize
  - fats
  - carbohydrates
  - amino acids



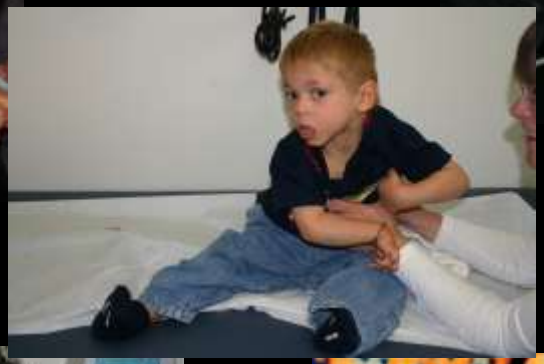
- Interconvert carbohydrates, fats and amino acids
- Synthesize some proteins
- Reproduce themselves (replicate), fusion/fission
- Participate in apoptosis
- Make free radicals
- Innate Immunity

Human mtDNA 16569 bp



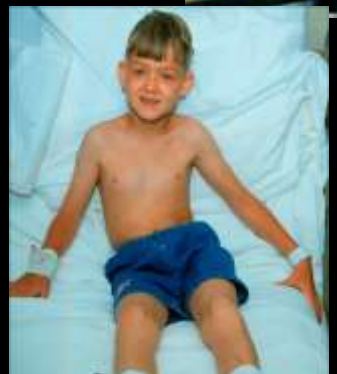












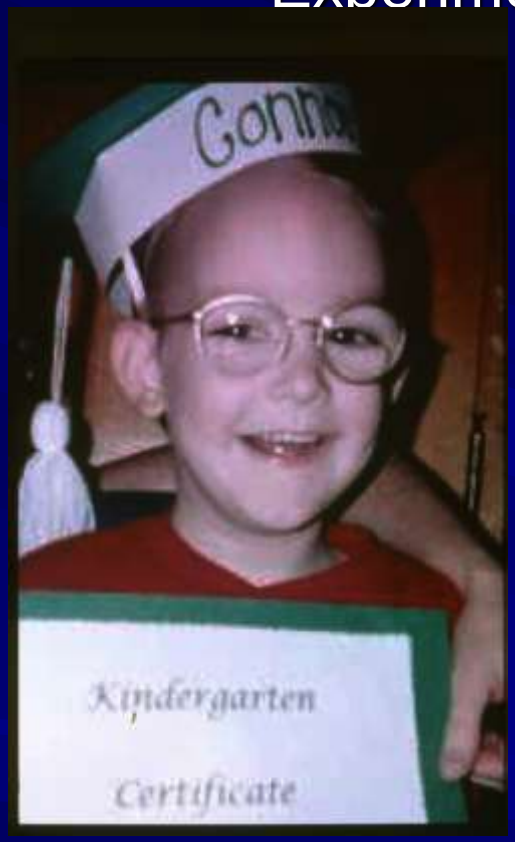

Oxphos Disease

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

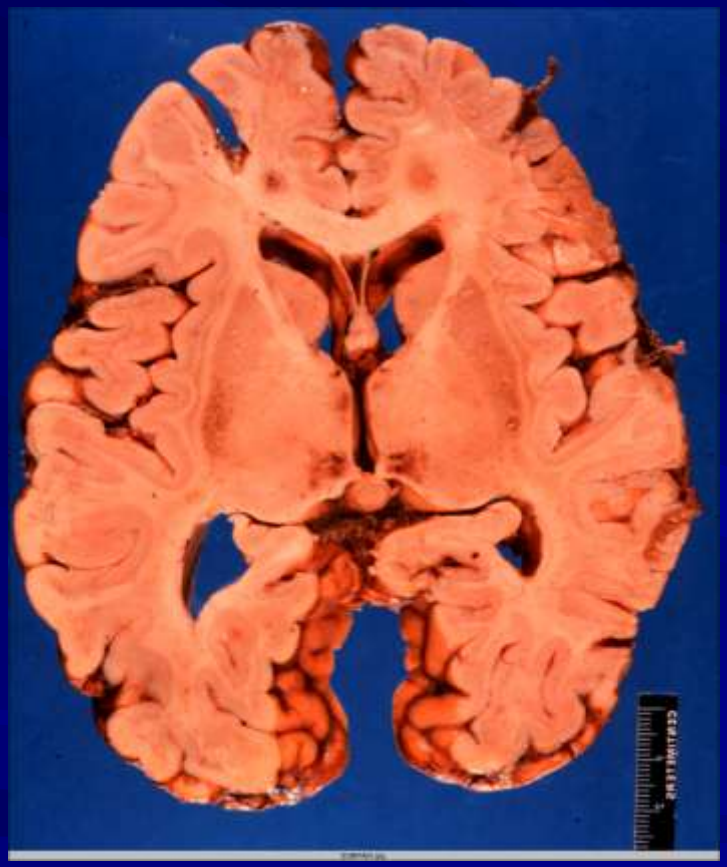


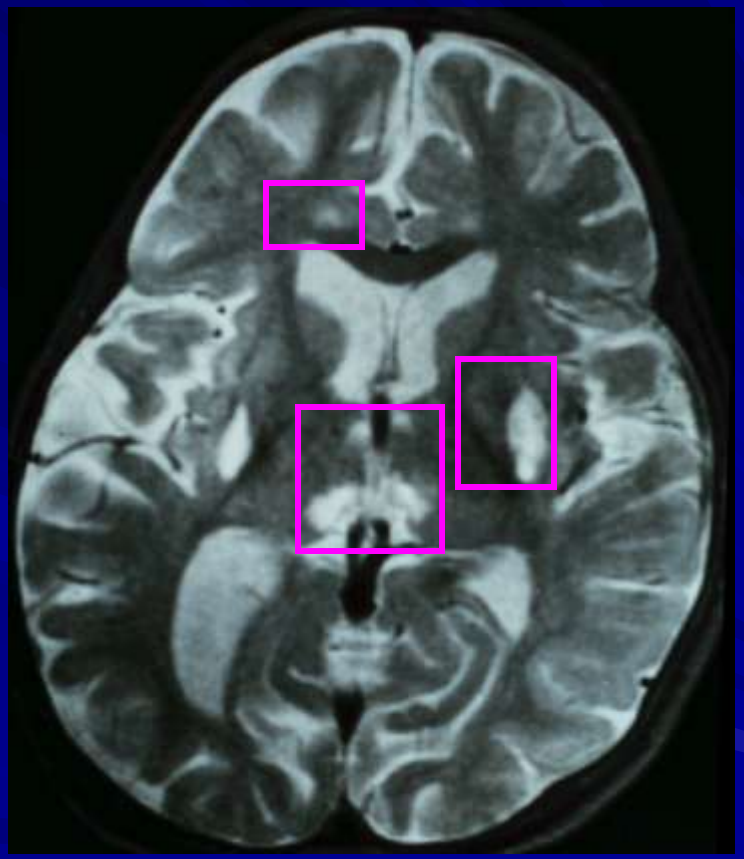
Age 5

Age 8

Age 16
Graduating from HS
In June 2011

Leigh Syndrome: Subacute Necrotizing Encephalomyelopathy





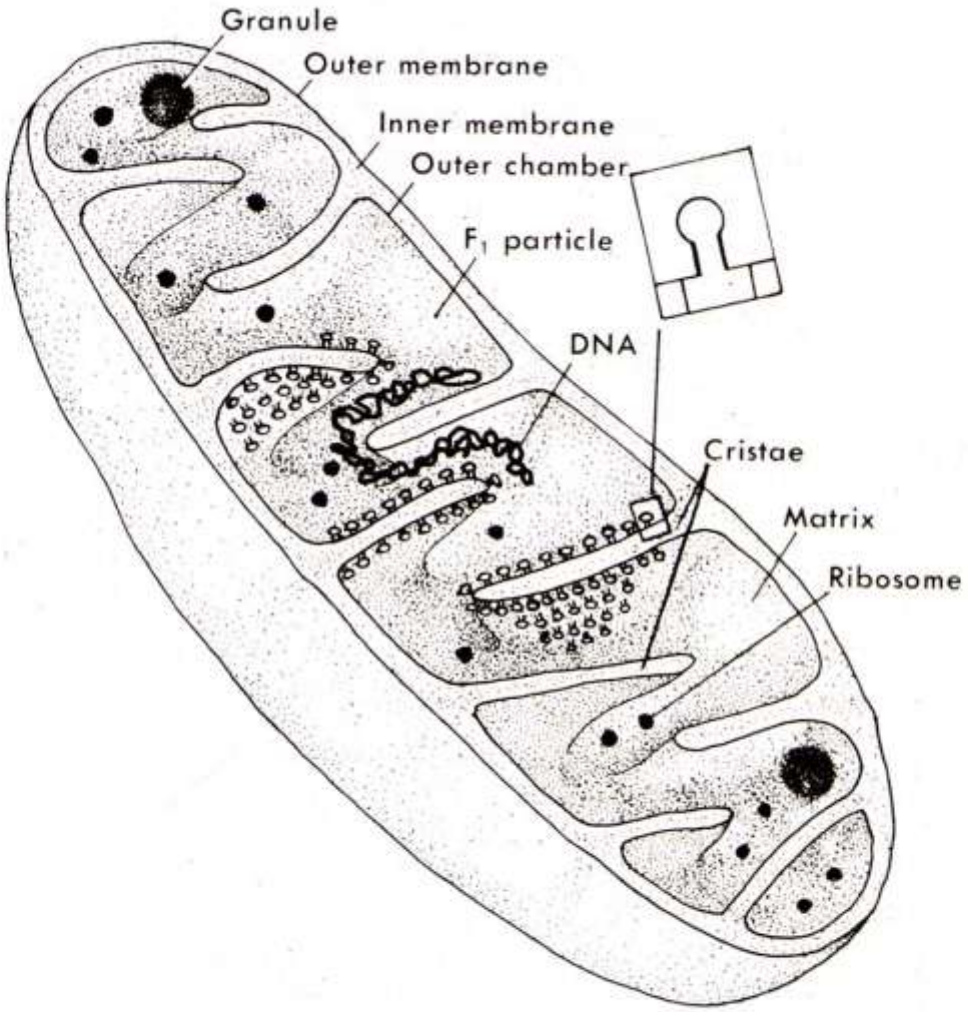
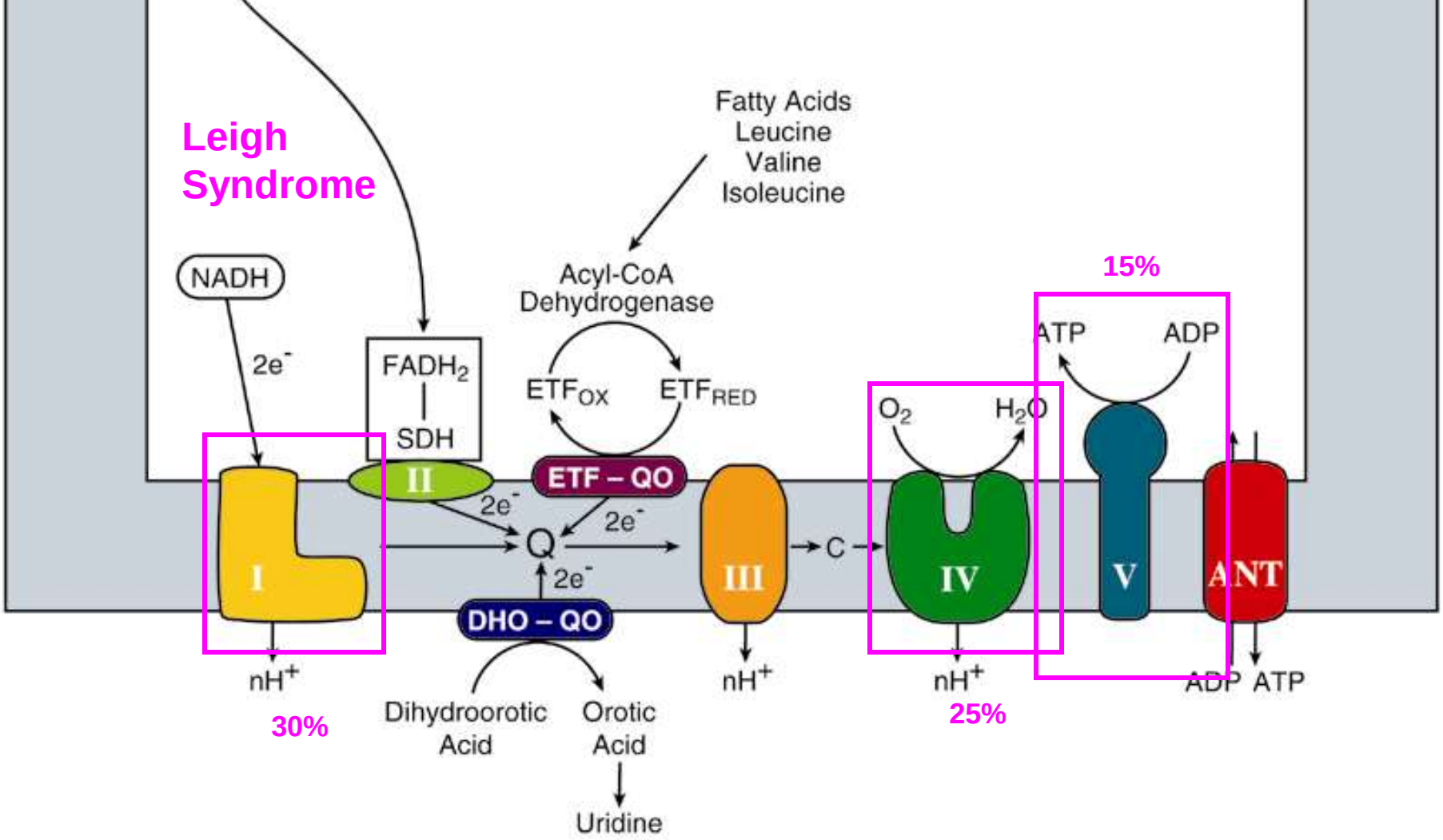


Figure 12–3 Three-dimensional diagram of a mitochondrion cut longitudinally. The main features are shown. Observe that the cristae are folds of the inner membrane and that on their matrix side they have the F_1 particles. The inset shows an F_1 particle with the head piece and stalk.



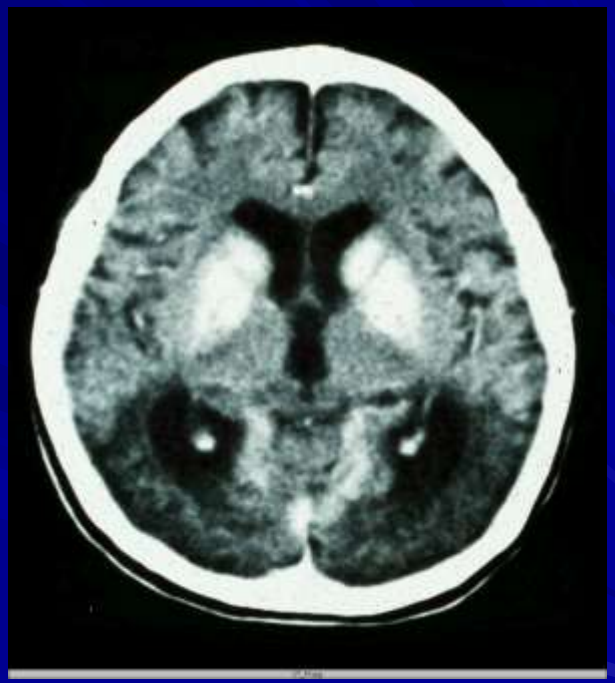
ETC Genes					
mt DNA	7	0	1	3	2
nuclear DNA	36	4	10	10	13

MELAS

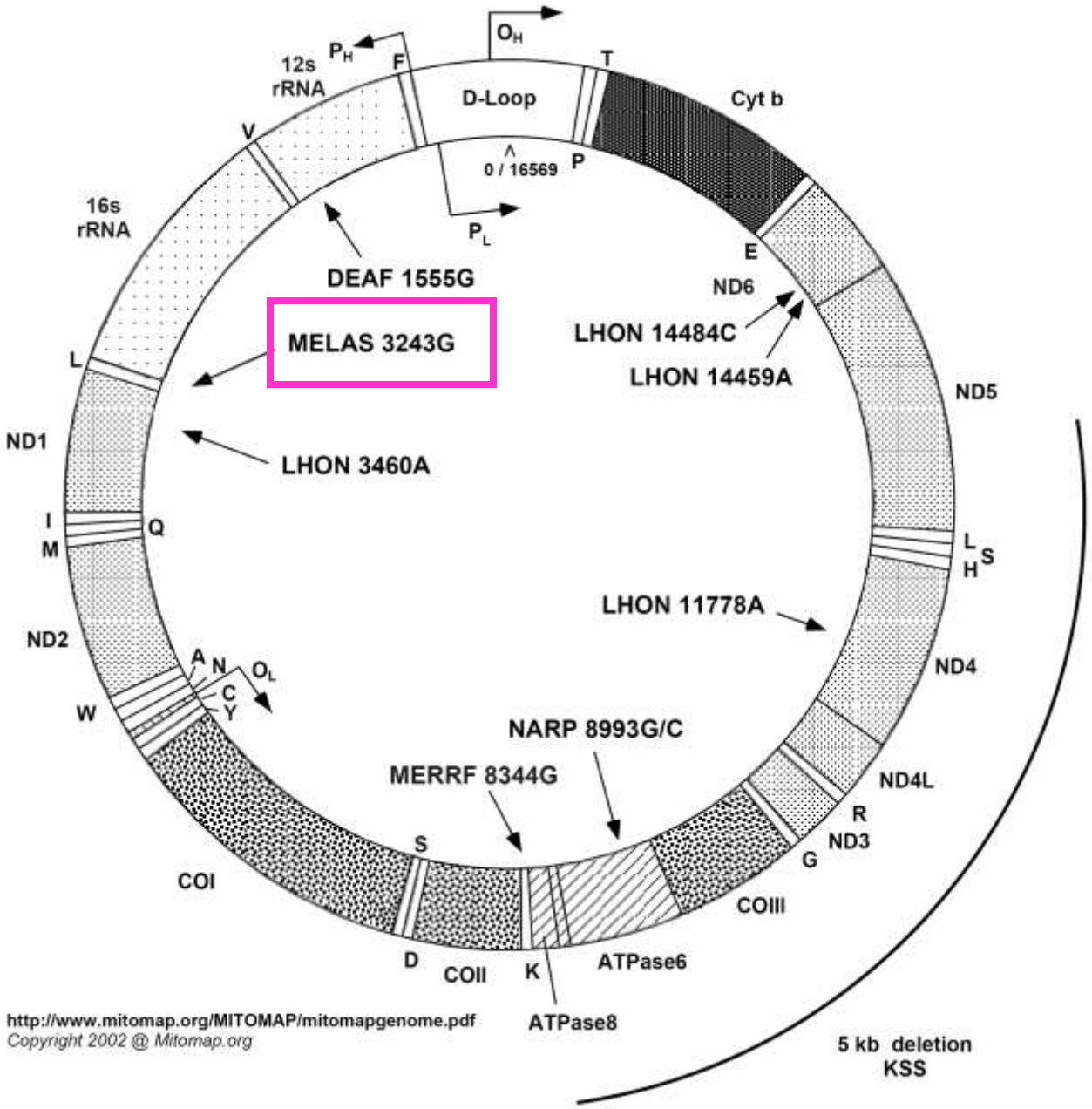
- Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like Episodes

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


O_H
P_H
12s rRNA
F
D-Loop
T
Cyt b
V
0 / 16569
P
16s rRNA
P_L
DEAF 1555G
E
ND6
MELAS 3243G
LHON 14484C
LHON 14459A
L
ND5
ND1
LHON 3460A
I
Q
M
L
H
S
LHON 11778A
ND2
ND4
A
N
O_L
C
Y
W
NARP 8993G/C
MERRF 8344G
ND4L
R
ND3
S
G
COI
COIII
D
COII
K
ATPase6
ATPase8
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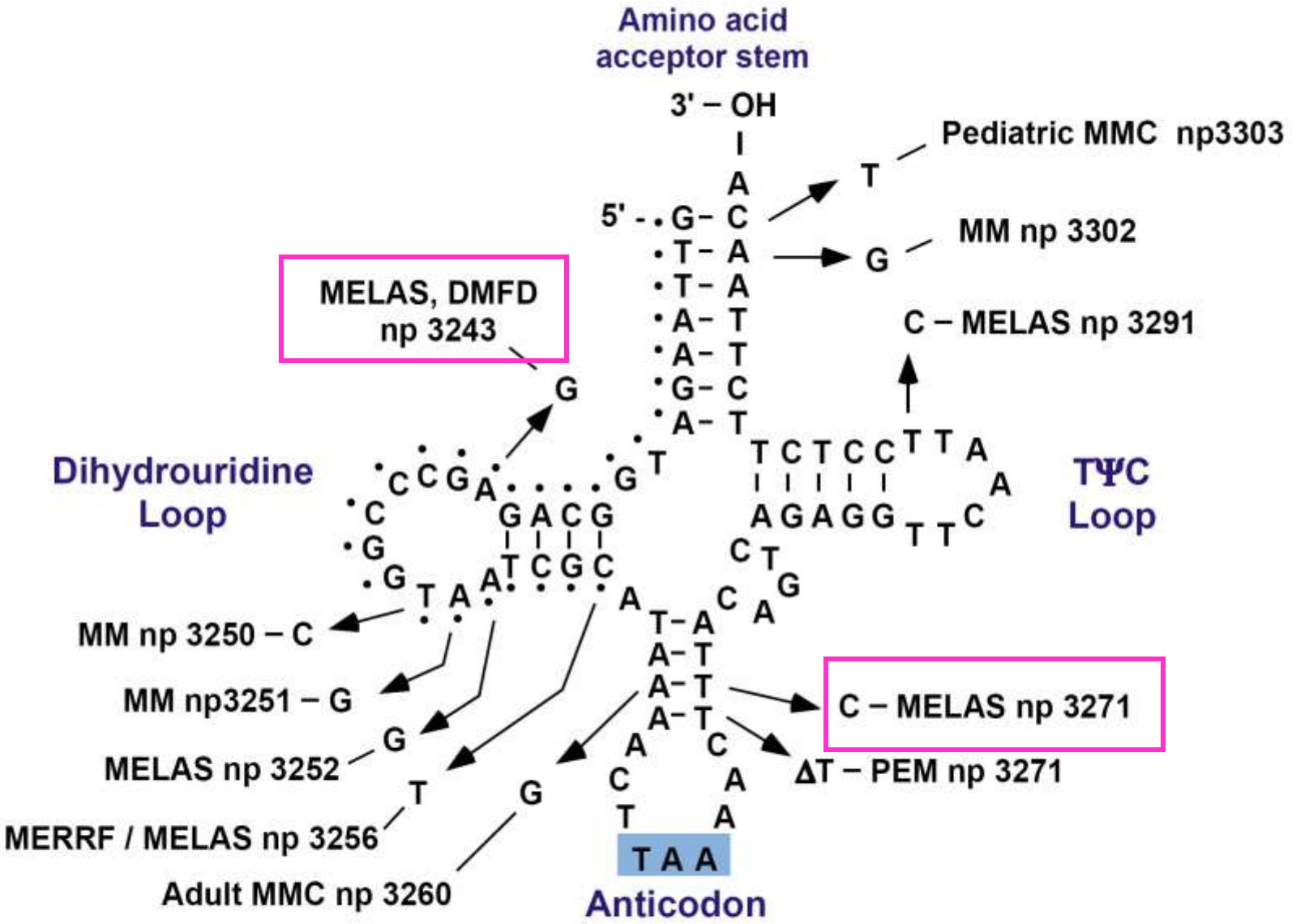
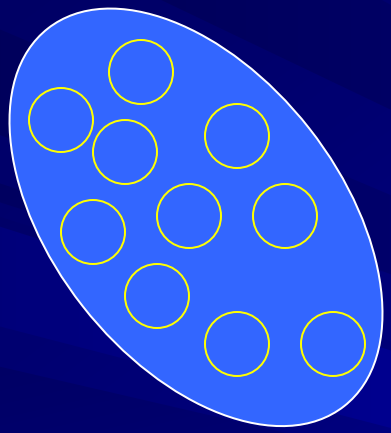
KSS



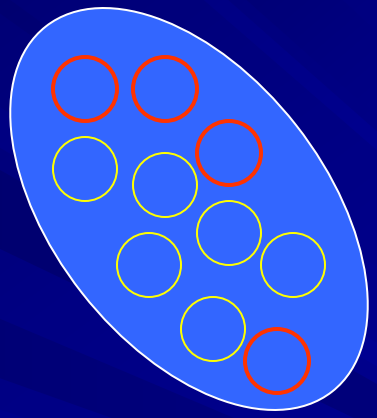
Pathological Mutations in tRNA$^{Leu(UUR)}$

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

Heteroplasmy

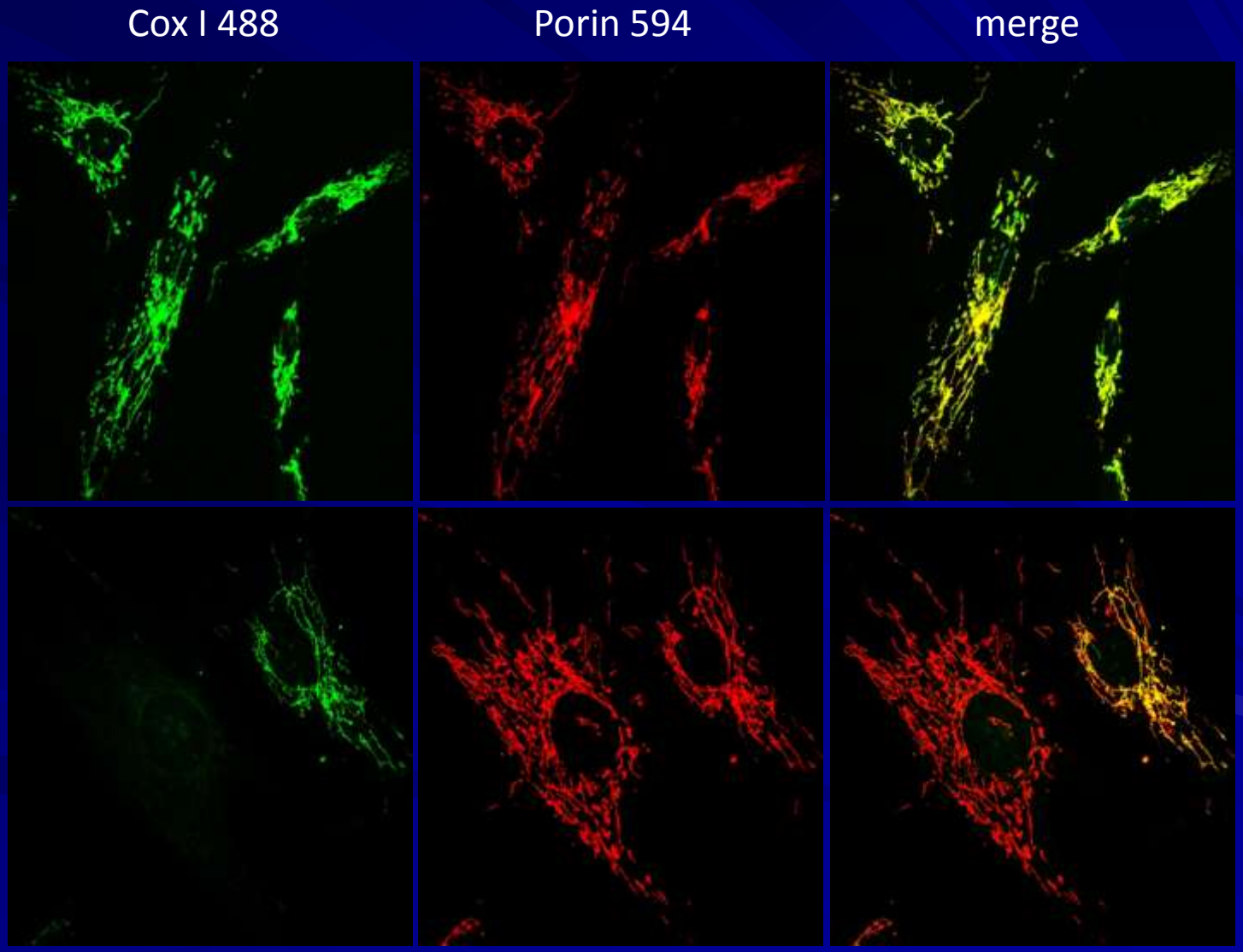


Wild Type



Mutant

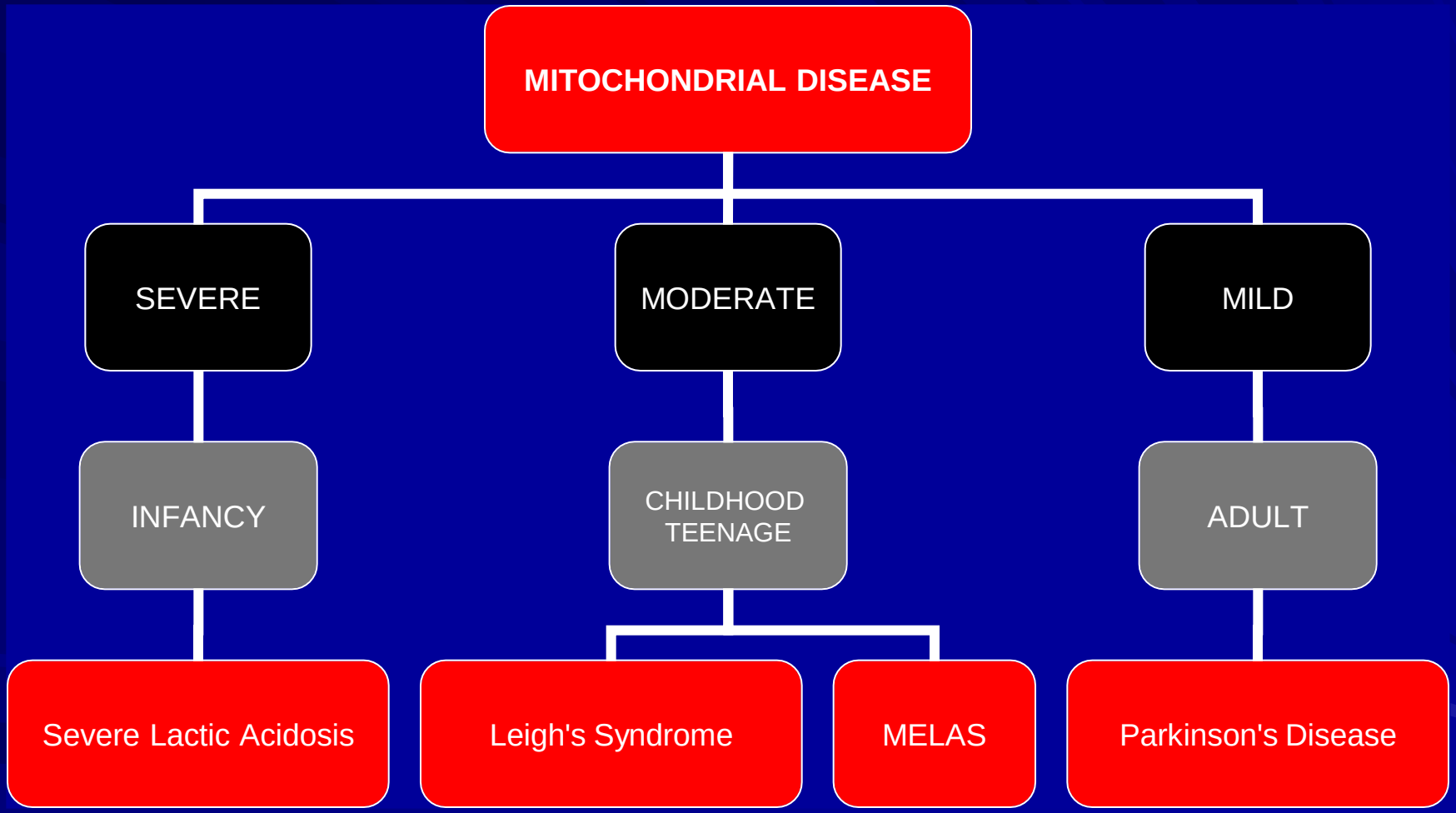
Heteroplasmy in Fibroblasts



How Does Genetic Mitochondrial Disease Present ?

- Acute/ Subacute
  - Severe Metabolic Crisis
  - Encephalopathy
  - Arrhythmia, Heart block
  - Opthalmoplegia, 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: A Practical Approach for Primary Care Physicians

Richard H. Haas, MB, BChir, MRCP[a,b], Sumit Parikh, MD[c], Marni J. Falk, MD[d], Russell P. Saneto, DO, PhD[e], Nicole I. Wolf, MD[f,g], Niklas Darin, MD[h], Bruce H. Cohen, MD[c]

[a]Departments of Neurosciences and Pediatrics, University of California San Diego, La Jolla, California; [b]Departments of Neurosciences and Pediatrics, Rady Children's Hospital and Health Center, San Diego, California; [c]Division of Neuroscience, Cleveland Clinic, Cleveland, Ohio; [d]Division of Human Genetics, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia, Pennsylvania; [e]Division of Pediatric Neurology, Children's Hospital and Regional Medical Center, University of Washington, Seattle, Washington; [f]Division of Child Neurology, University Children's Hospital, Heidelberg, Germany; [g]Division of Child Neurology, University Children's Hospital, Zürich, Switzerland; [h]Division of Child Neurology, Queen Silvia Children's Hospital, Göteborg, Sweden

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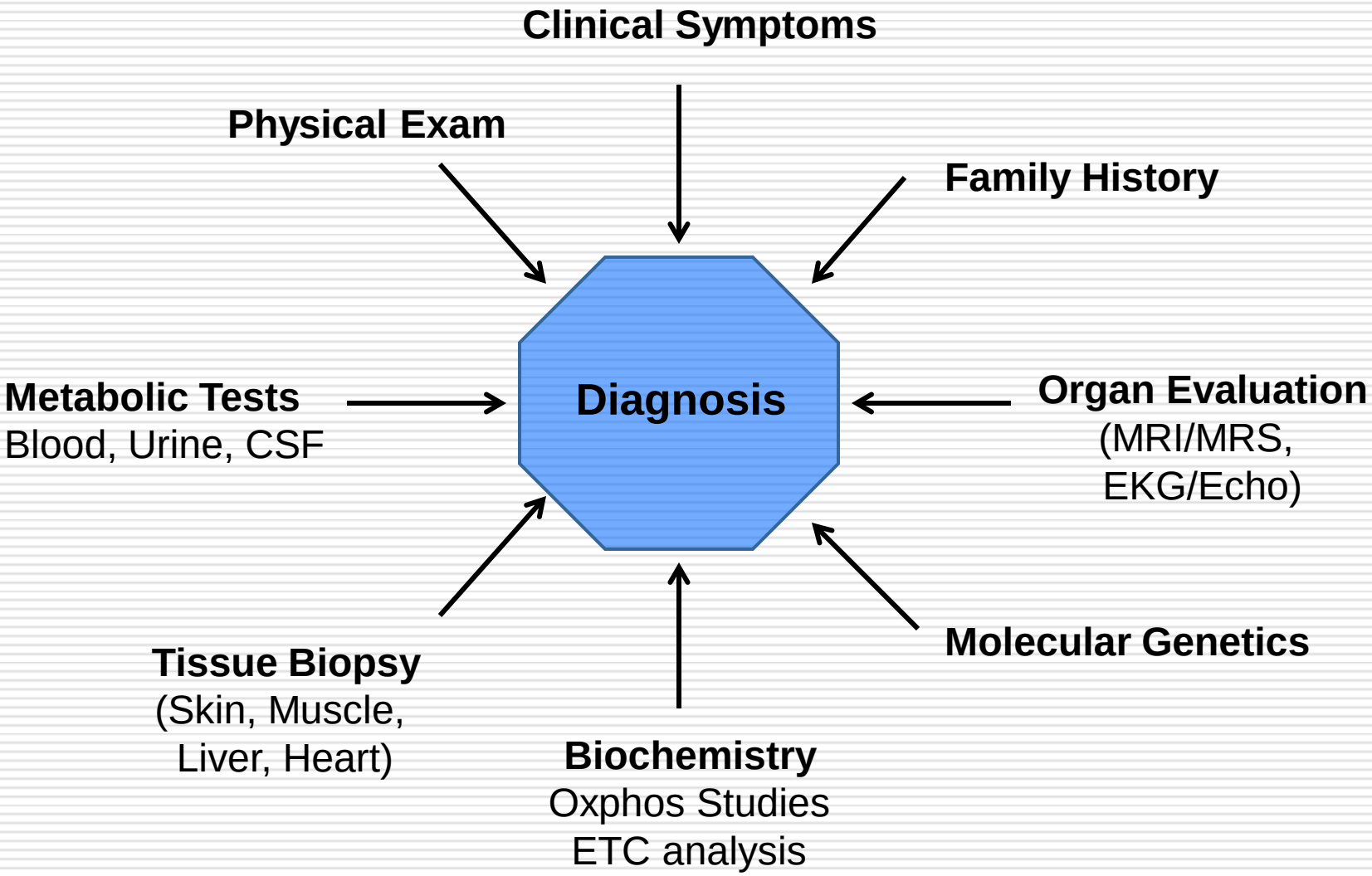
DIAGNOSTIC EVALUATION OF MITOCHONDRIAL DISEASE

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



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

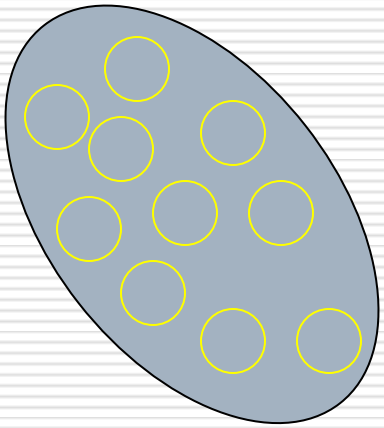
Available Mito Tests

Test	Tissue
Histology/EM	Muscle, Liver, Heart
Carnitine, CoQ	
Nuclear DNA	Blood, All Tissues
mtDNA	All Tissues, Muscle Best
Electron Transport Assays	Muscle Fresh/Frozen, Fibroblasts, Liver, Heart
Polarographic Assay	Fresh muscle, Liver, Heart or Mitochondria
Enzyme Assay	All Tissues, Mitochondria, Fibroblasts
Protein Immunoassay	Mitochondria (Blue Native)
Immunocytochemistry	Tissue (Clear Native)
Immunohistochemistry	Fibroblasts/ Muscle Tissue

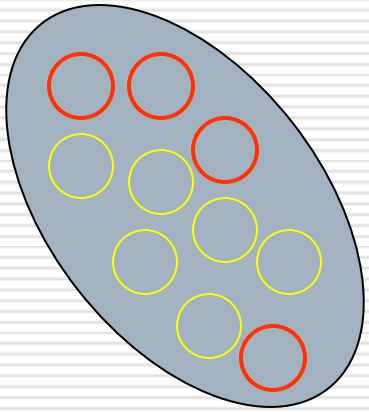
Tissue Diagnosis

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

Heteroplasmy



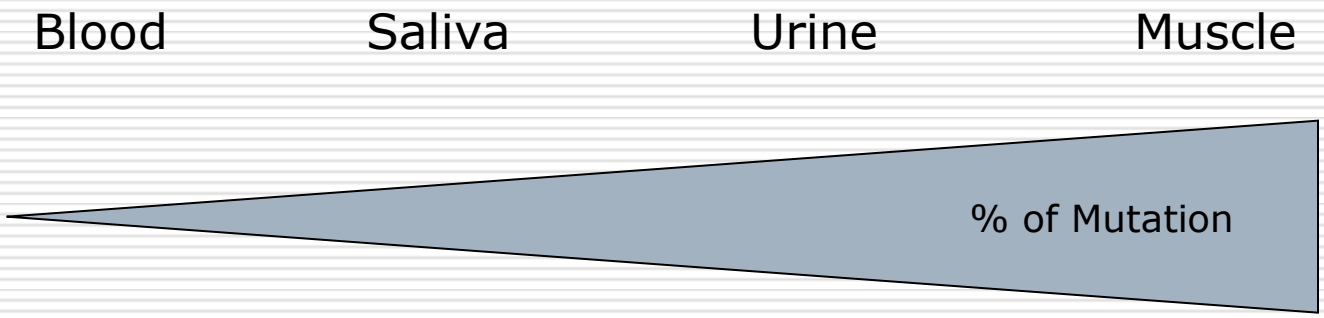
Wild Type



Mutant

The Basics of Tissue Testing for Mitochondrial Disease

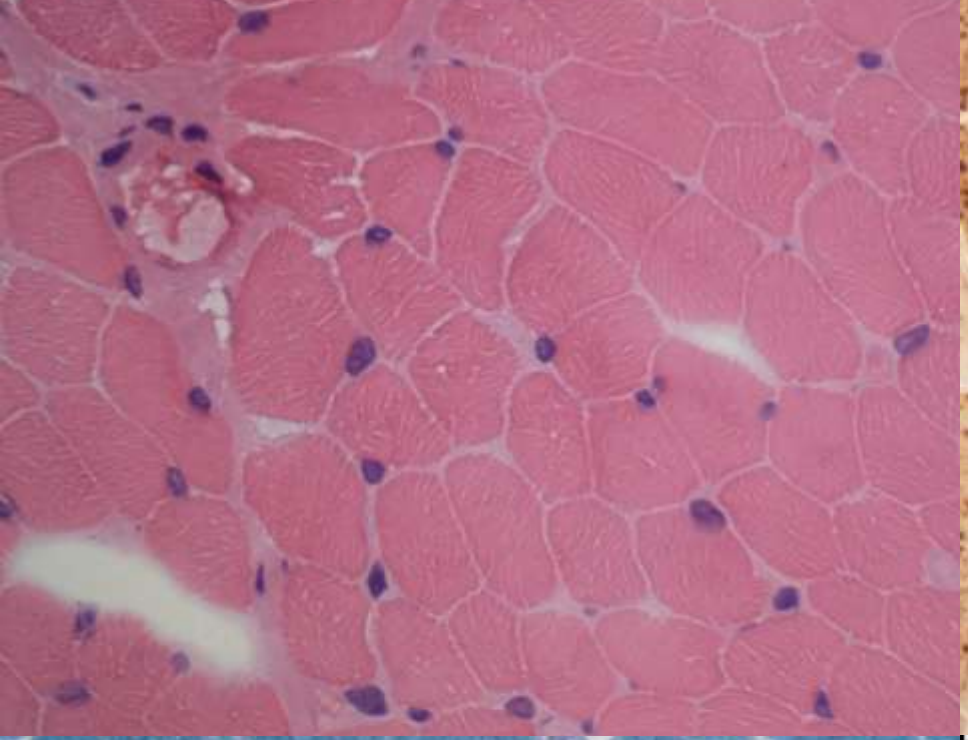
- Tissues for mtDNA Testing –
  The Heteroplasmy Problem

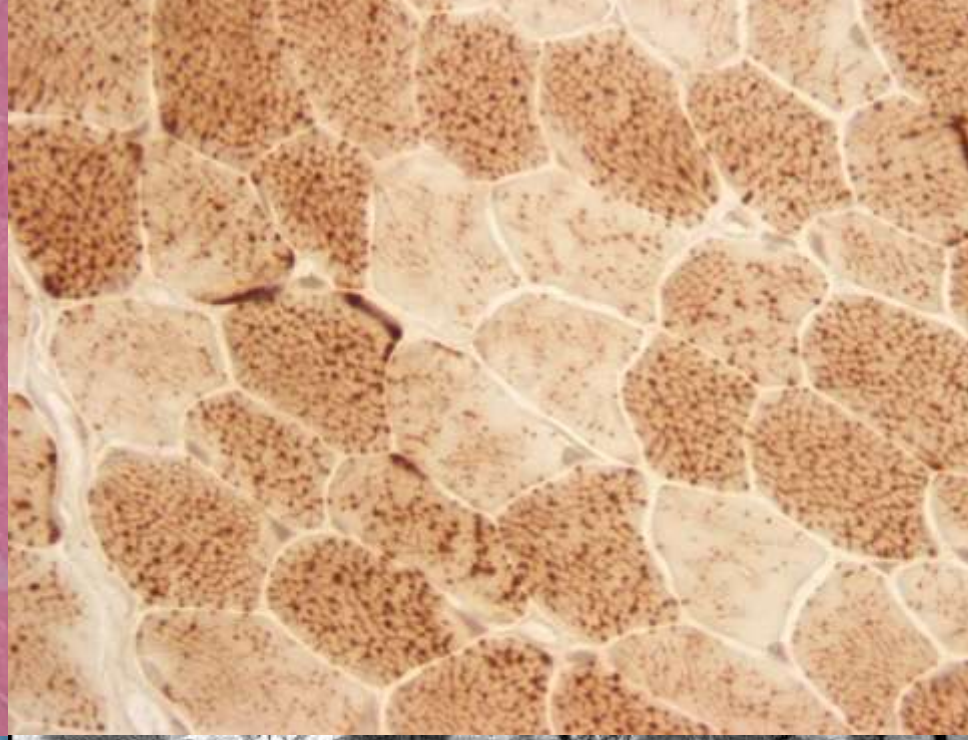


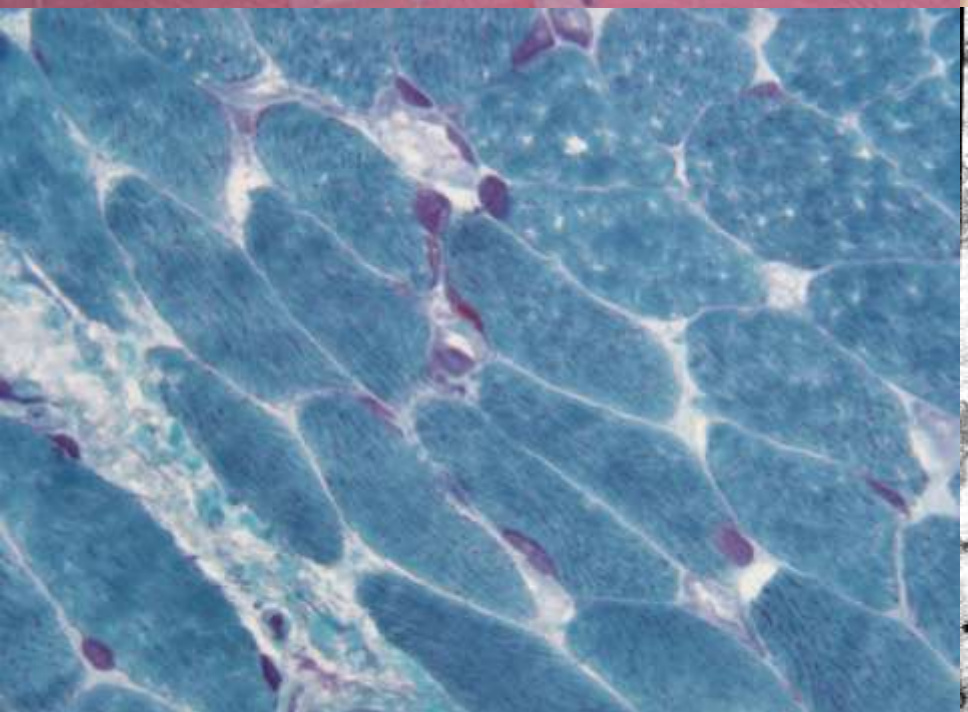
Saliva Collection (Oragene)

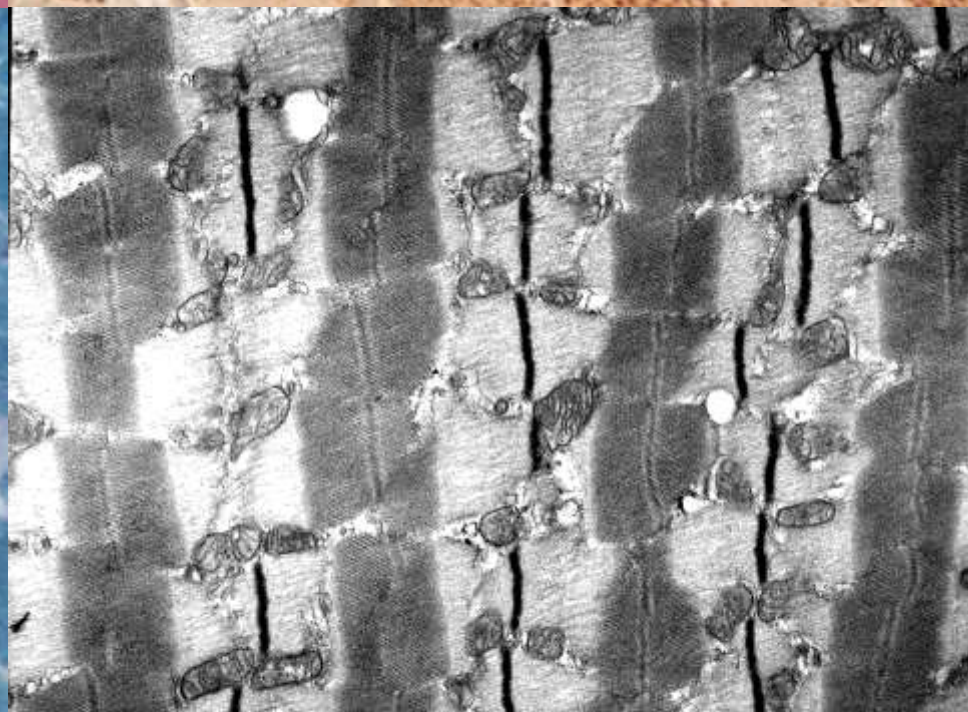
Muscle Biopsy Problems

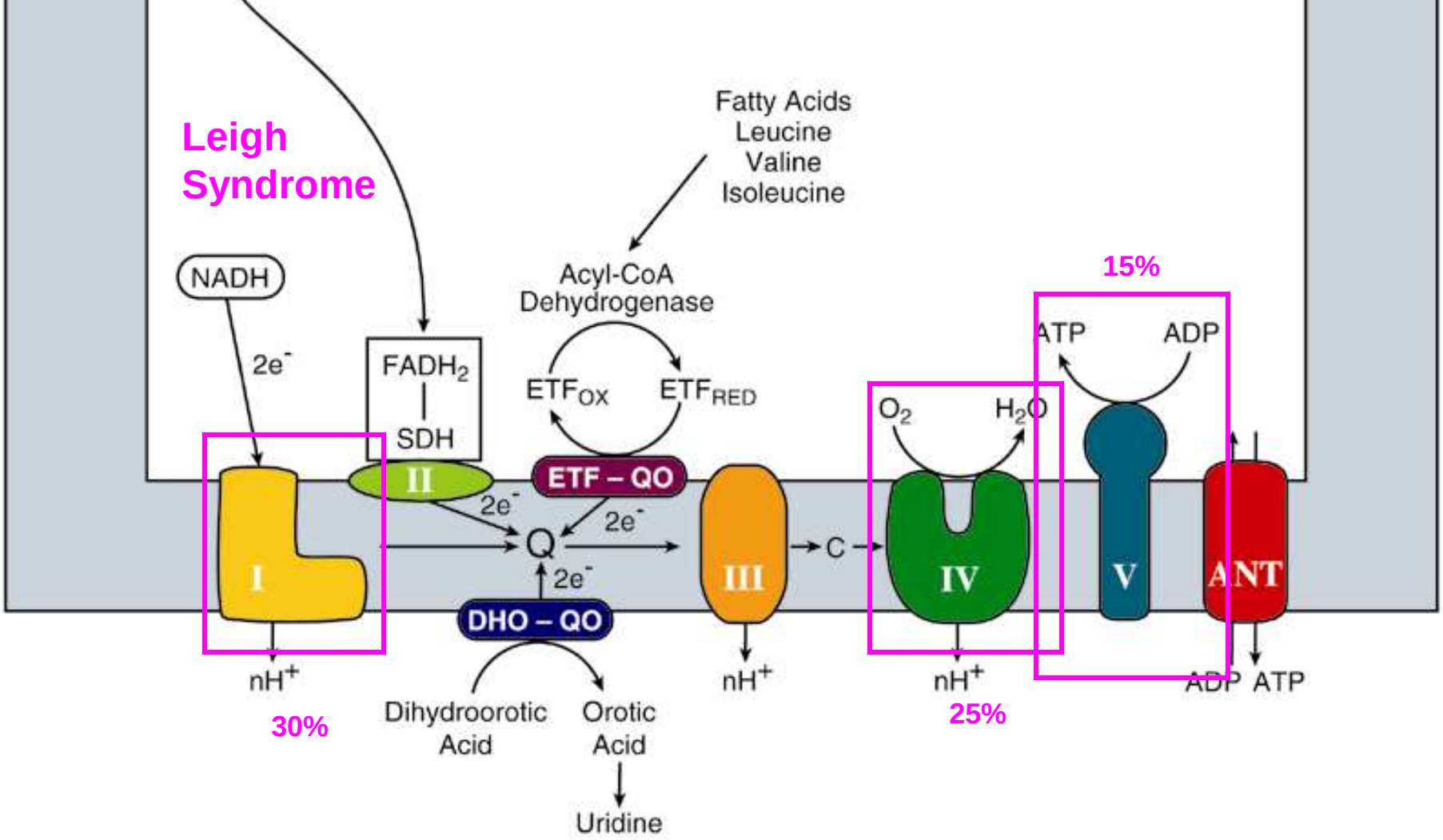
- Histochemistry
  - Often normal in Pediatric cases
- Electron Microscopy (EM)
  - May help but often difficult to get
- ETC Assays
  - Lab to lab variation
  - Very susceptible to sample handling





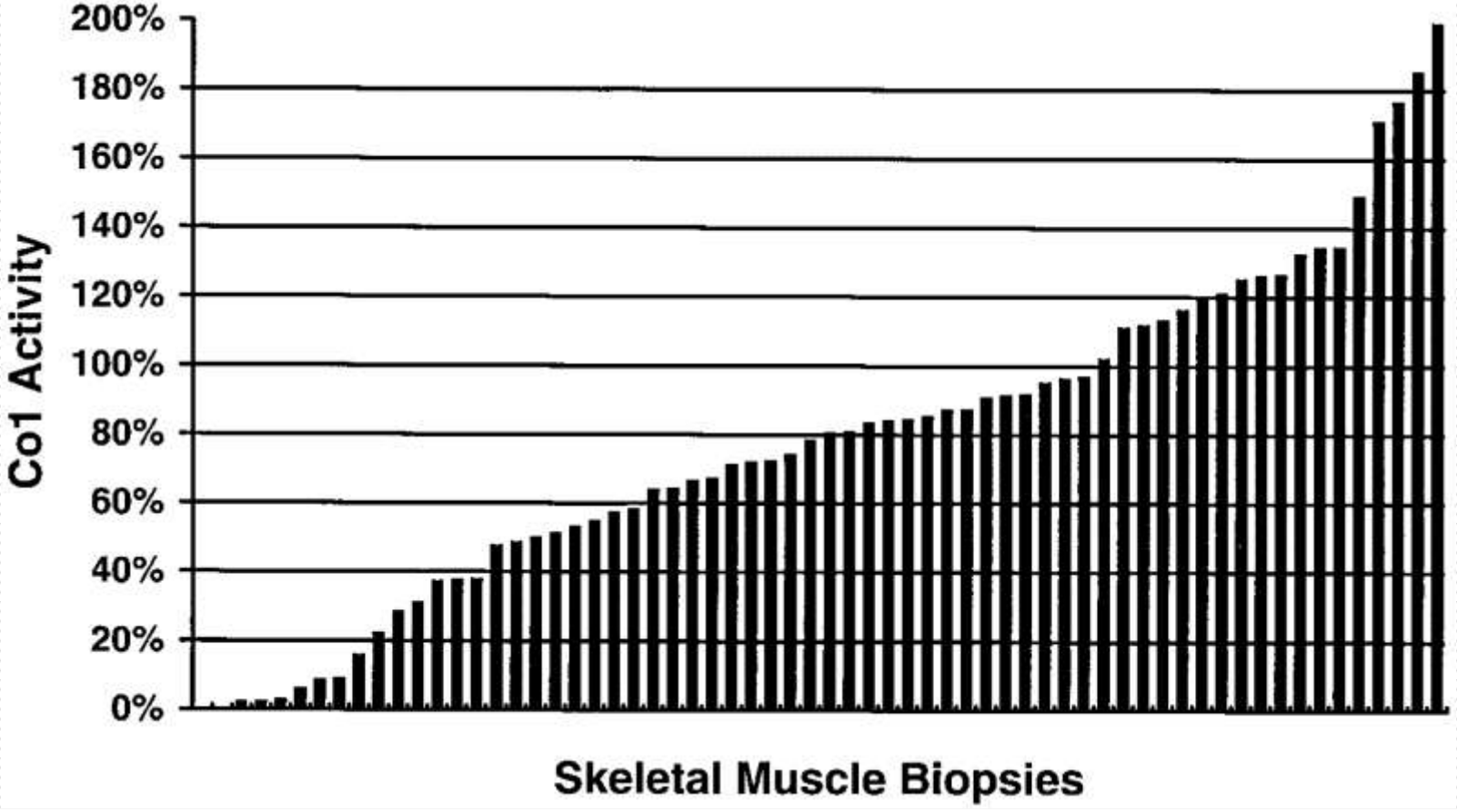






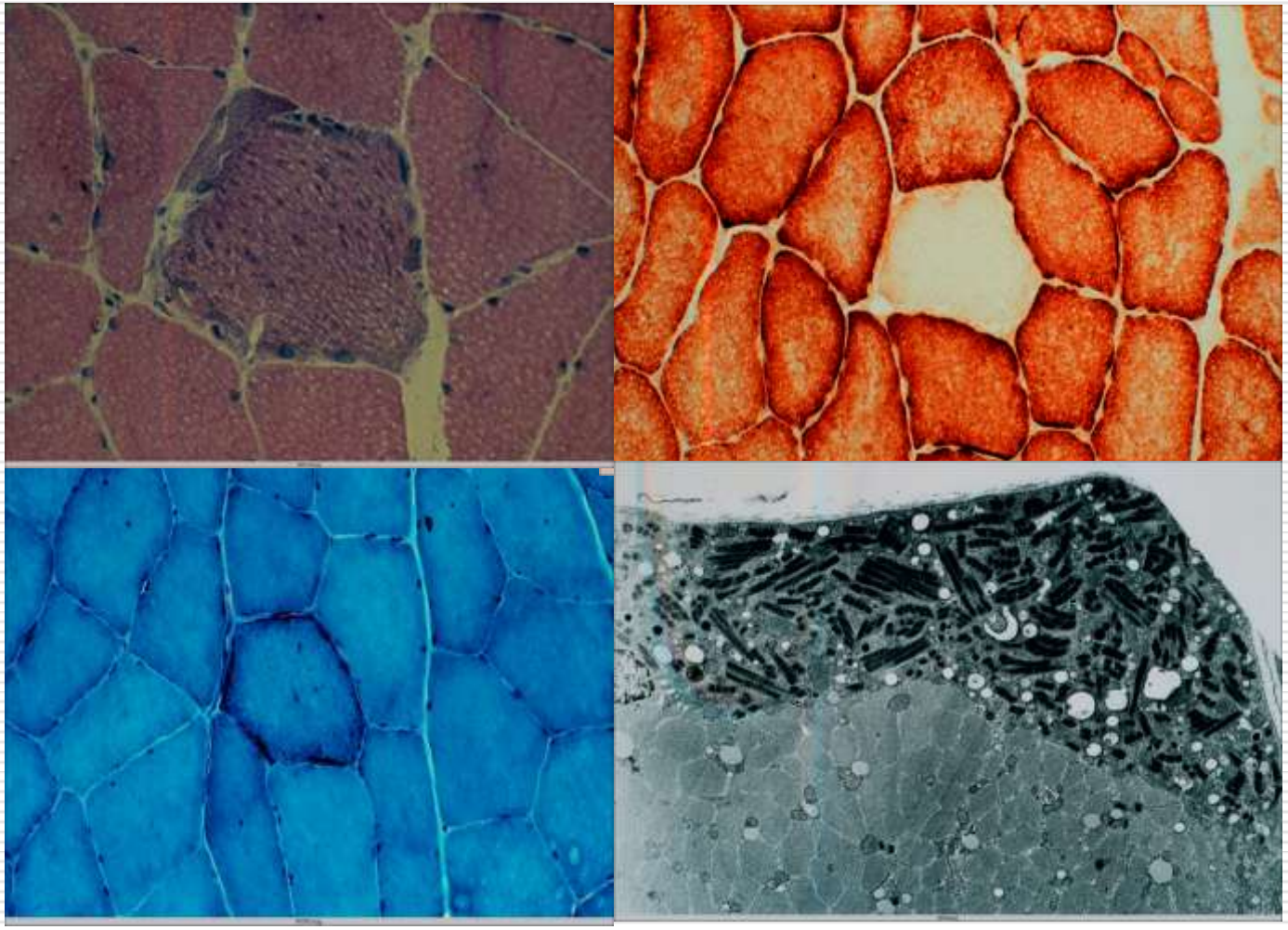
ETC Genes					
mt DNA	7	0	1	3	2
nuclear DNA	36	4	10	10	13

Figure 3. Residual activity of complex I CS ratios in the 66 skeletal muscle biopsies analyzed in this patient series



Bernier, F.P. et al. Neurology 2002;59:1406-1411

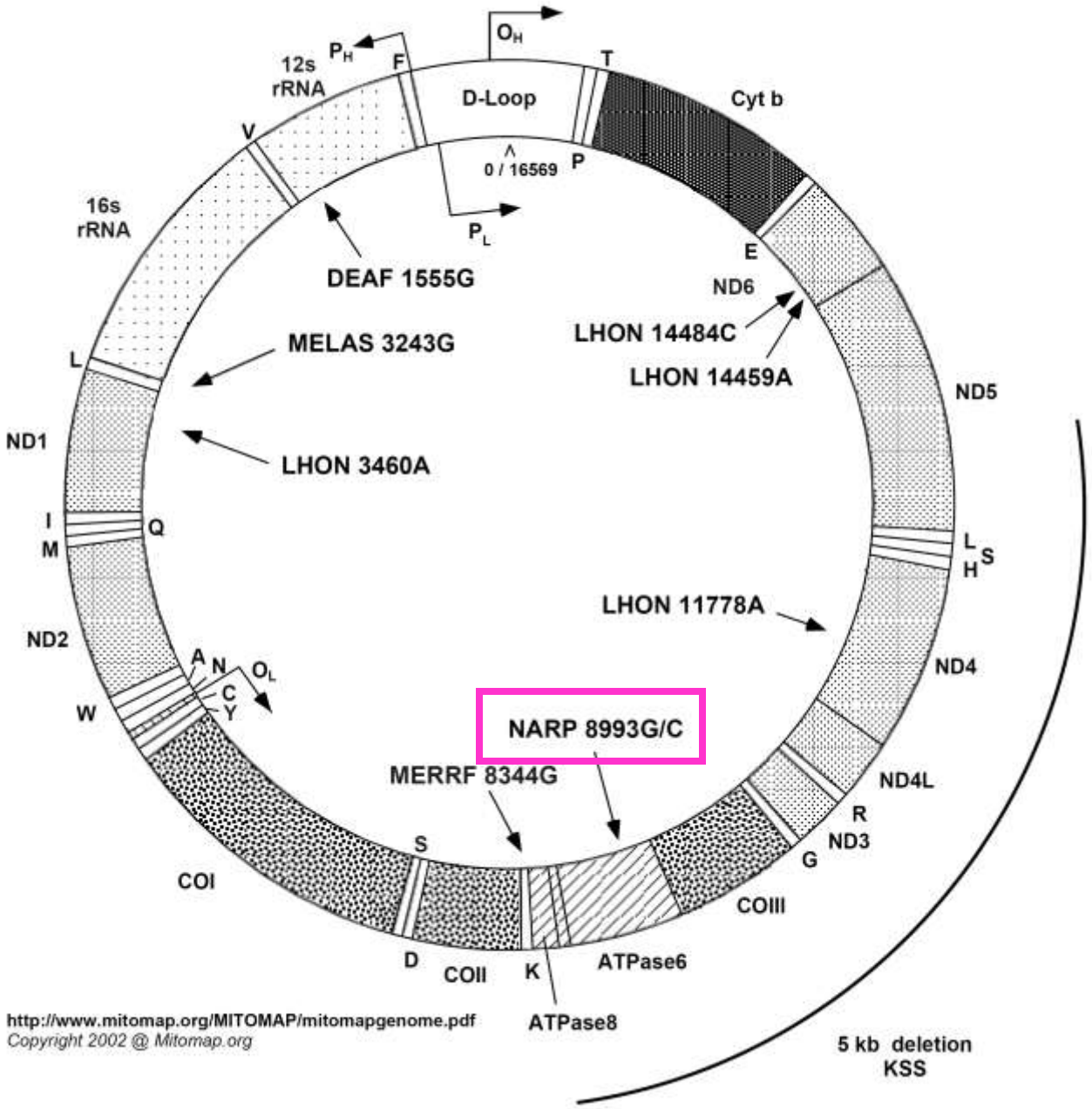
Ragged Red Fiber Myopathy



Neurometabolic Evaluation

SS Age 23 months

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


O_H
P_H
12s rRNA
F
D-Loop
T
Cyt b
V
0 / 16569
P
16s rRNA
P_L
DEAF 1555G
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ND6
LHON 14484C
MELAS 3243G
LHON 14459A
L
ND5
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ND2
ND4
A
N
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C
Y
W
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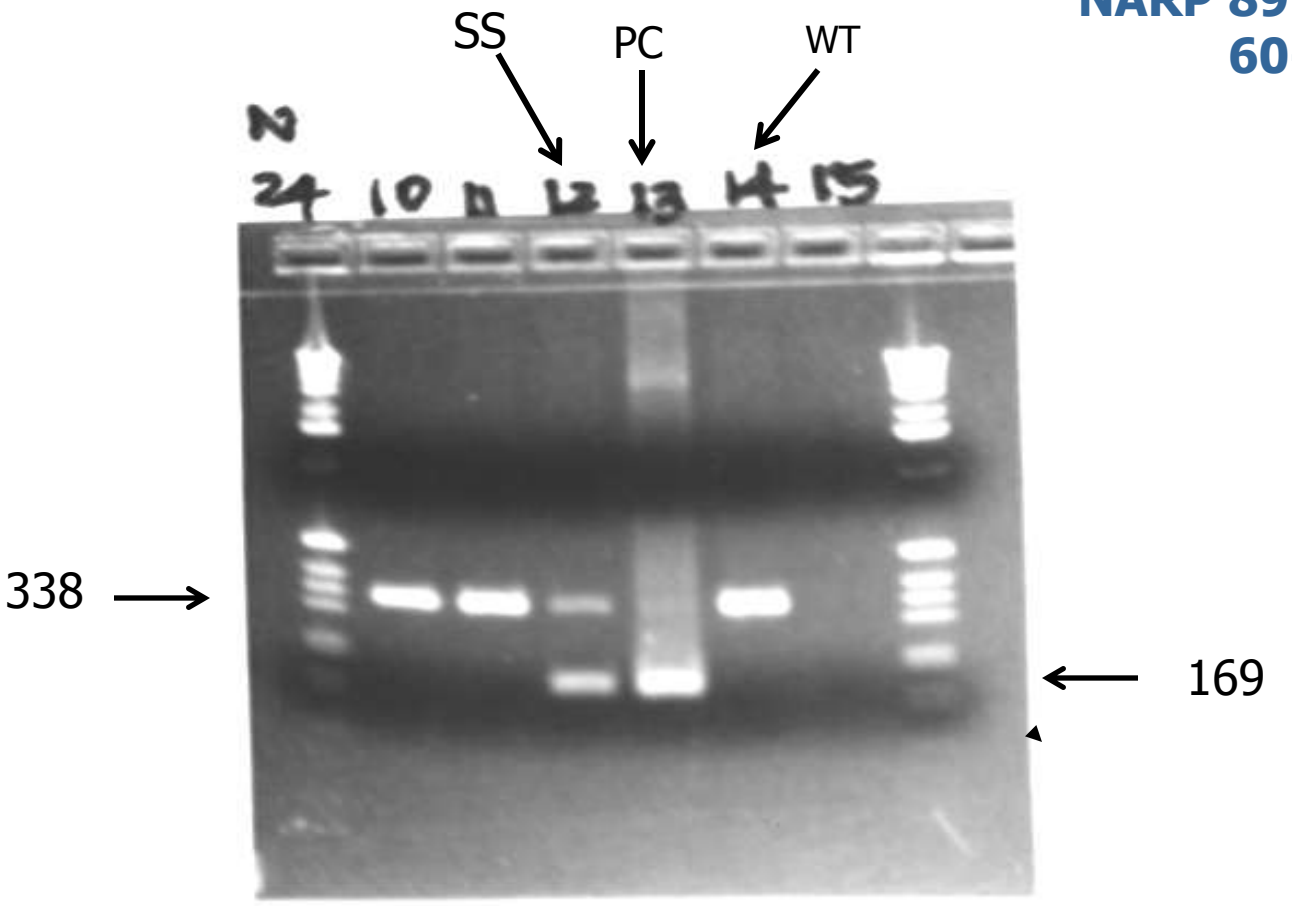
MERRF 8344G
ND4L
R
ND3
S
G
COI
COIII
D
COII
K
ATPase6
ATPase8
http://www.mitomap.org/MITOMAP/mitomapgenome.pdf
Copyright 2002 @ Mitomap.org
5 kb deletion
KSS

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*

Biochemical criteria	General criteria:			
	Unlikely	Possible	Probable	Definite
Unlikely	Unlikely	Possible	Possible	Probable
Possible	Possible	Possible	Probable	Probable
Probable	Possible	Probable	Probable	Definite
Definite	Probable	Probable	Definite	Definite

Wolf N., Smeitink J.A.
Neurology. 2002 Nov 12;59(9):1402-5

Available online at www.sciencedirect.com
ScienceDirect
Molecular Genetics and Metabolism 94 (2008) 16–37
Molecular Genetics and Metabolism
www.elsevier.com/locate/ymgme

The in-depth evaluation of suspected mitochondrial disease

The Mitochondrial Medicine Society's Committee on Diagnosis
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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.

Keywords: Mitochondrial disease; Laboratory diagnosis; Review

Epidemiology

>1:200 Children are Born with Potentially Pathogenic mtDNA Mutations

ARTICLE

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. Chinnery[1,3,*]

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.

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Screened for just 10 (5%) of >200 known pathogenic mtDNA mutations.

Prevalence of Mitochondrial DNA Disease in Adults

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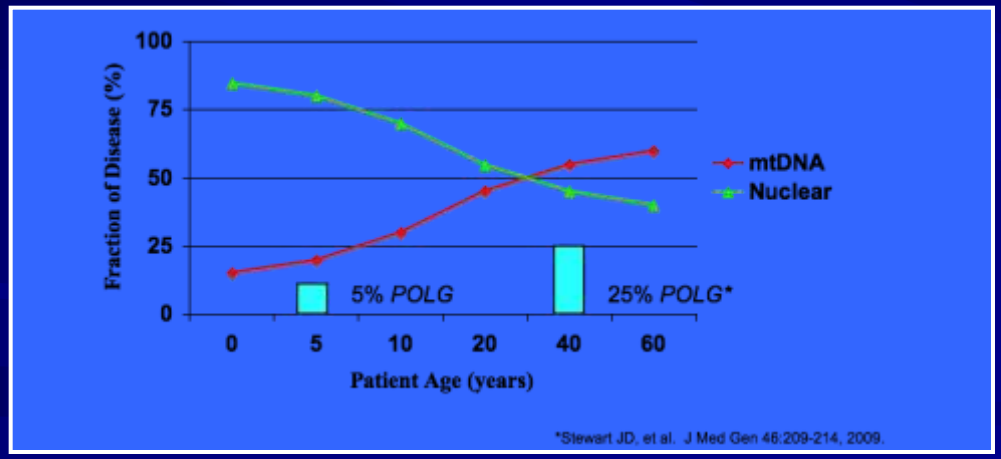
Objective: Diverse and variable clinical features, a loose genotype–phenotype relationship, and presentation to different medical specialties have all hindered attempts to gauge the epidemiological impact of mitochondrial DNA (mtDNA) disease. Nevertheless, a clear understanding of its prevalence remains an important goal, particularly about planning appropriate clinical services. Consequently, the aim of this study was to accurately define the prevalence of mtDNA disease (primary mutation occurs in mtDNA) in the working-age population of the North East of England.

Methods: Adults with suspected mitochondrial disease in the North East of England were referred to a single neurology center for investigation from 1990 to 2004. Those with pathogenic mtDNA mutations were identified and pedigree analysis performed. For the midyear period of 2001, we calculated the minimum point prevalence of mtDNA disease for adults of working age (>16 and <60/65 years for female/male patients, respectively).

Results: In this population, we found that 9.2 in 100,000 people have clinically manifest mtDNA disease, making this one of the commonest inherited neuromuscular disorders. In addition, a further 16.5 in 100,000 children and adults younger than retirement age are at risk for development of mtDNA disease.

Interpretation: Through detailed pedigree analysis and active family tracing, we have been able to provide revised minimum prevalence figures for mtDNA disease. These estimates confirm that mtDNA disease is a common cause of chronic morbidity and is more prevalent than has been previously appreciated.

Ann Neurol 2008;63:35–39



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

Epidemiology of Mitochondrial DNA Disease

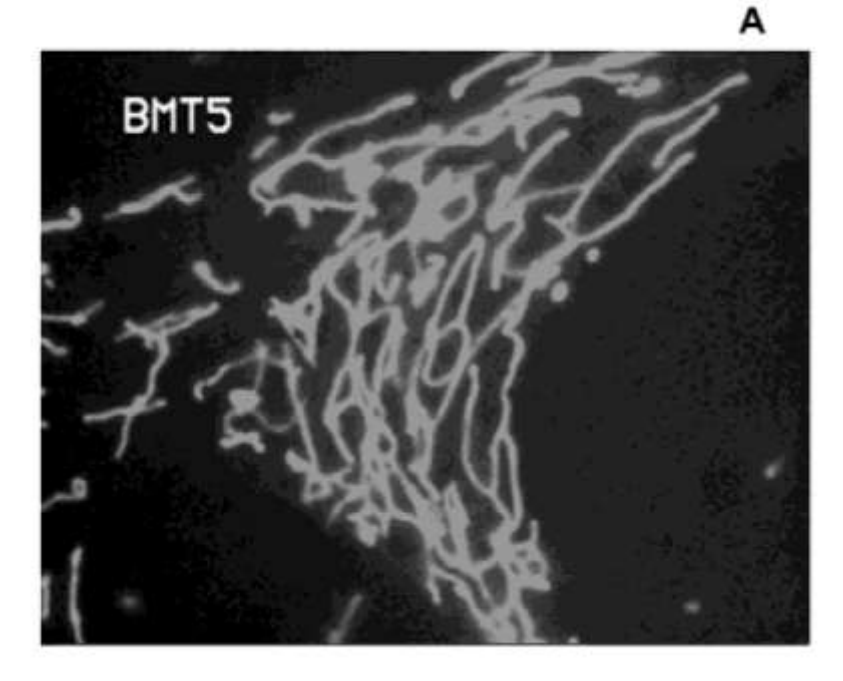
Expanding the Phenotype

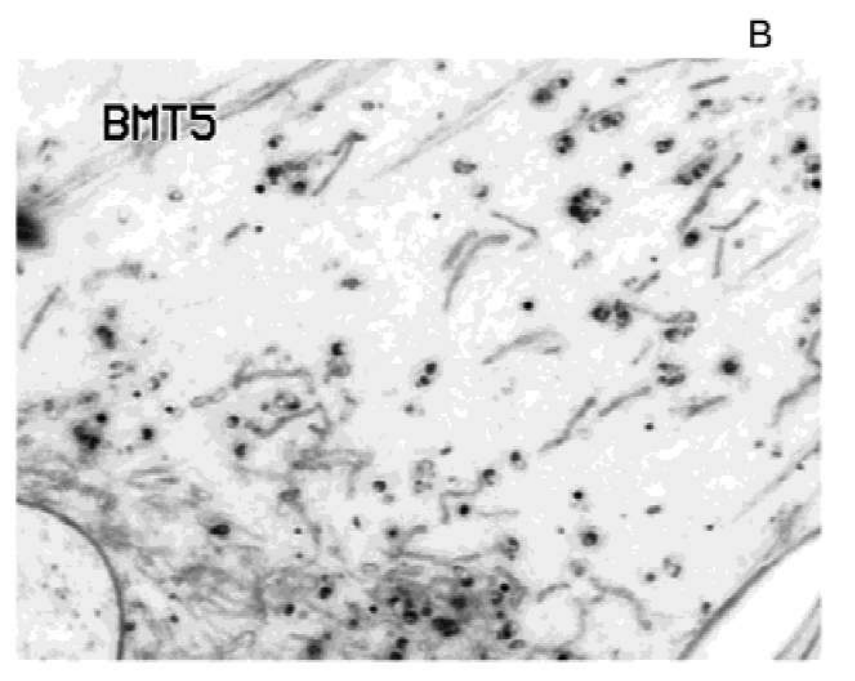
A never-ending process

The Dynamic Nature of Mitochondrial Networks

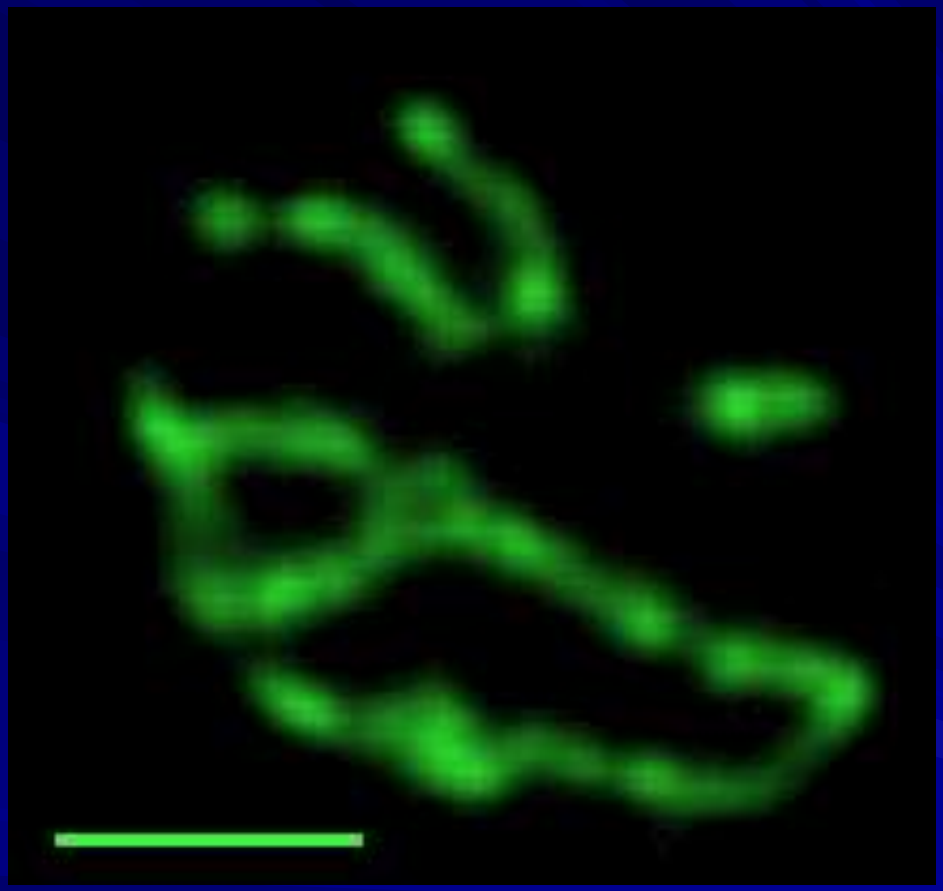
Control Fibroblast

Severe Complex I Deficiency





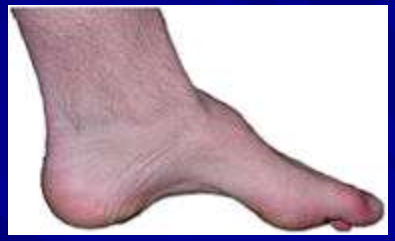
From Nhu-an Pham et al. Microsc.Microanal. 10, 247-260, 2004



David Chan
Caltech

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



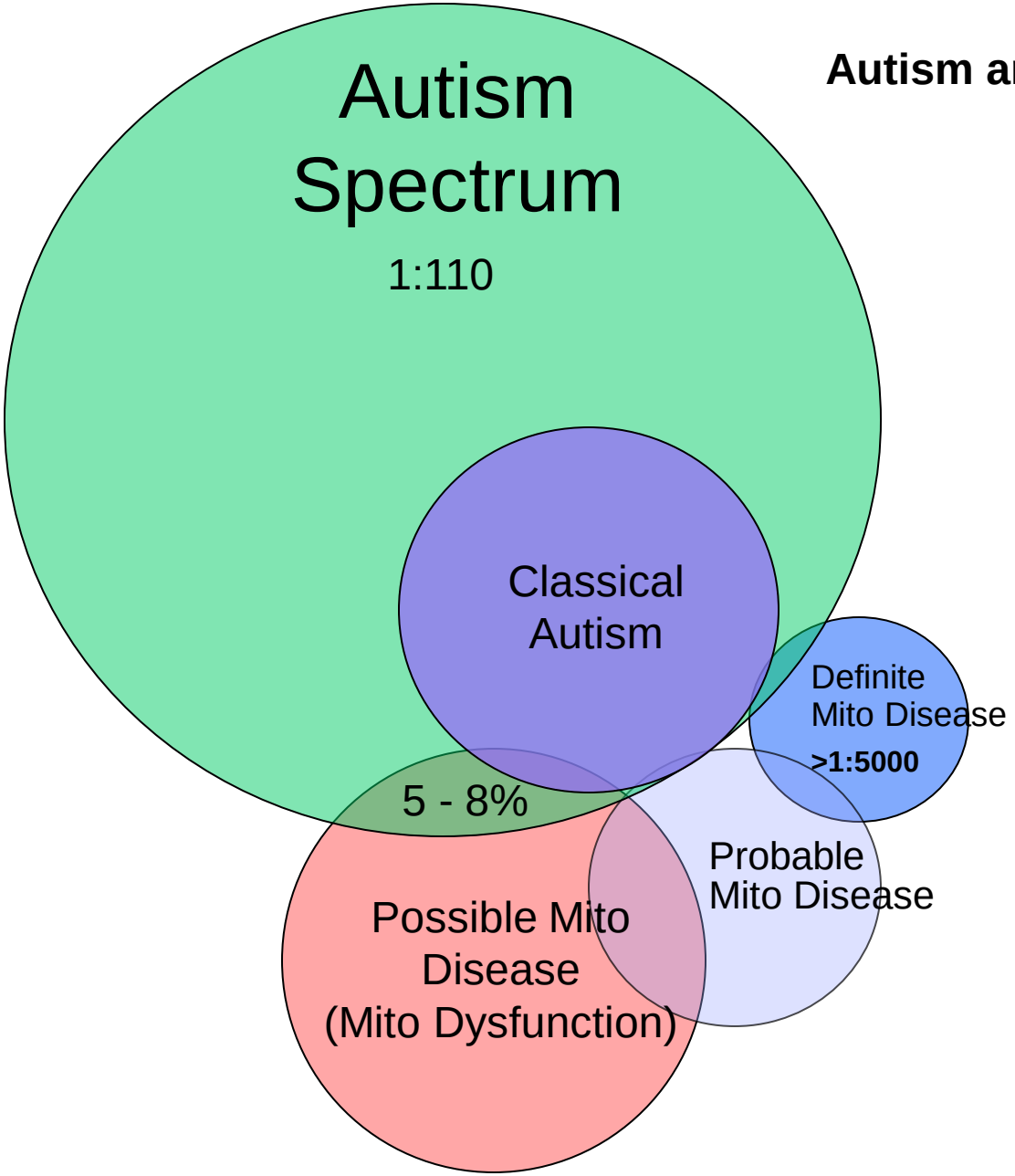
tRNA Lys G8363A Mitochondrial DNA Point Mutation

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

Graf W.D. et al. J Child Neurol. 2000 Jun;15(6):357-61

Autism and Mitochondria



DEVELOPMENTAL DISABILITIES
RESEARCH REVIEWS *16: 144–153 (2010)*

AUTISM AND MITOCHONDRIAL DISEASE

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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.
Dev Disabil Res Rev 2010;16:144–153.

dysfunction in the etiology of ASD, however, may be much more important than this Venn diagram would suggest.

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].