Primary Mitochondrial Disease Evaluations:

The evolving role of muscle biopsy

Children's Hospital of Philadelphia
Mitochondrial Medicine Frontier Program
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COI Disclosures

- No financial disclosures

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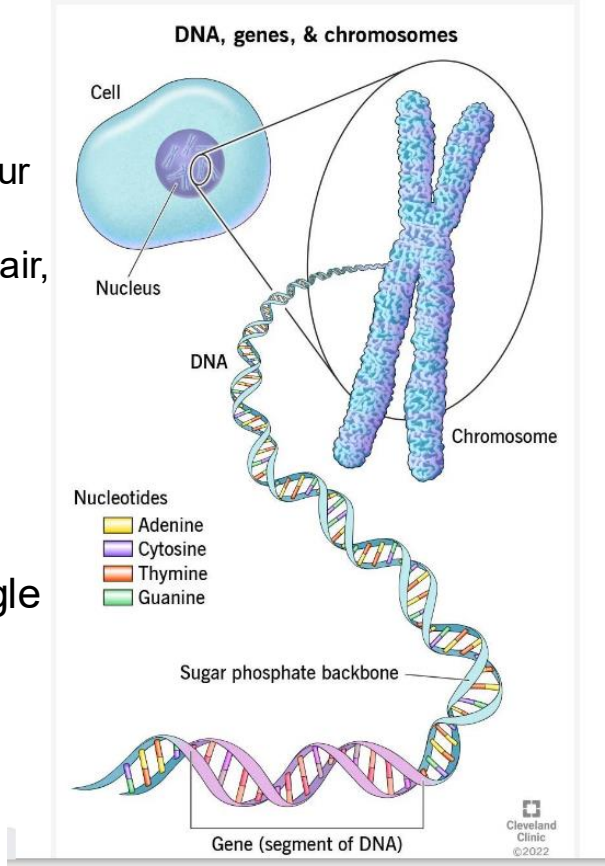
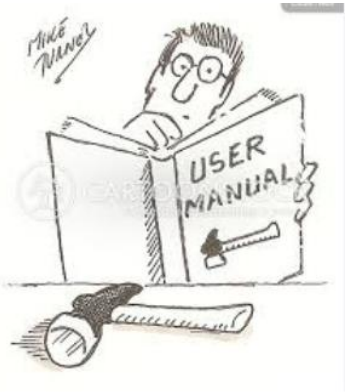
Genetics Background

- **DNA = D**eoxyribo**N**ucleic **A**cid
- DNA is the “blueprint” or “instruction manual” for the body
  - Meaning our DNA is the code that provides instructions to make our body
    - For example, it tells our body to give us certain color eyes, hair, height and many more things!
- It is inherited (meaning passed down from our parents)
- 4 “building blocks” or “letters” or bases – A, C ,T ,G
- DNA is broken into sections called “genes”
- If DNA is like our “instruction manual”, think of genes like a single step in the instruction manual





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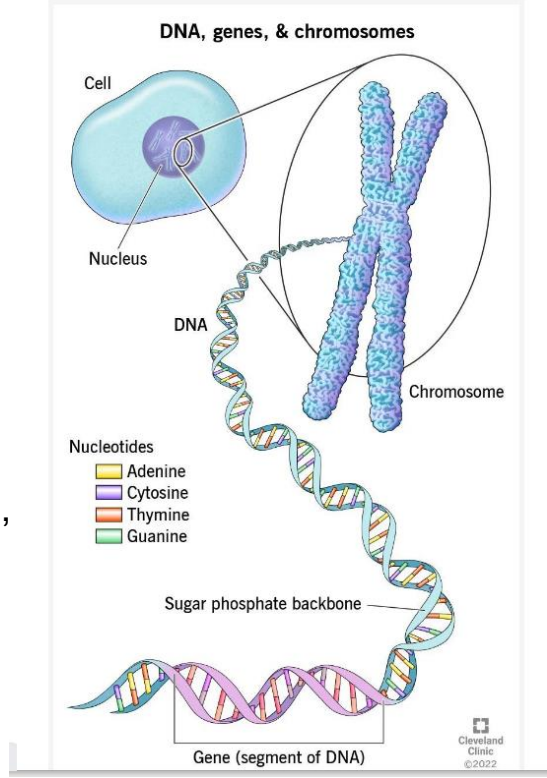
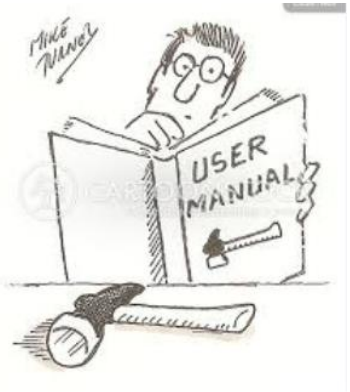
Genetics Background

- Changes in DNA are called “Variants”
- Classified in one of three broad categories
  - **Benign –** does not cause symptoms, is not reported out
  - **Variant of uncertain significance (VUS or VOUS) –** unclear if causes symptoms or not. Most VUSes are reclassified as benign (~80% PMID: 37878314)
  - **Pathogenic –** would cause symptoms if inherited in the appropriate manner
    - (ie autosomal recessive disorders need two pathogenic variants, 1 from Mom, 1 from Dad)
    - (autosomal dominant disorders need one pathogenic variant)
    - *mtDNA disorders are more complicated





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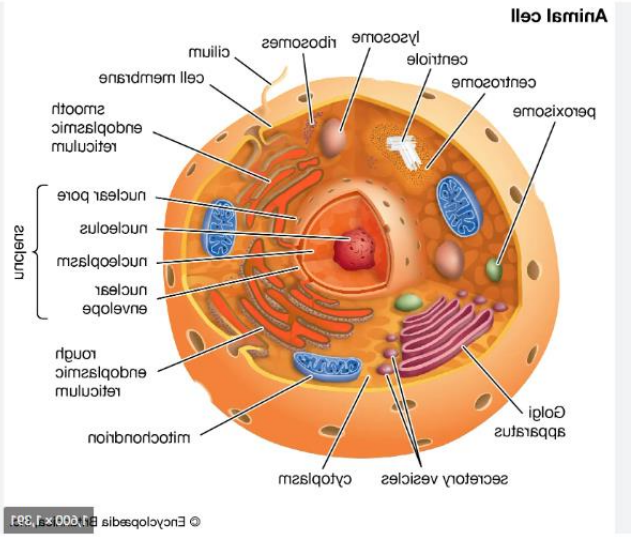
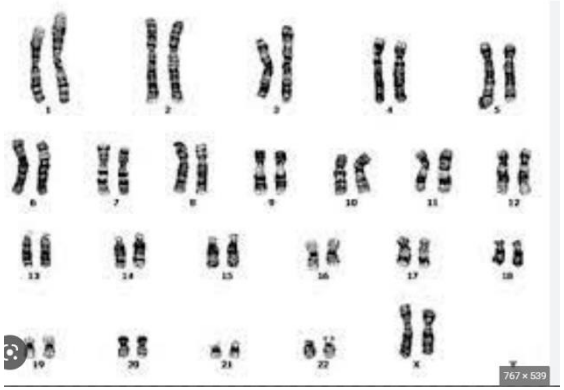
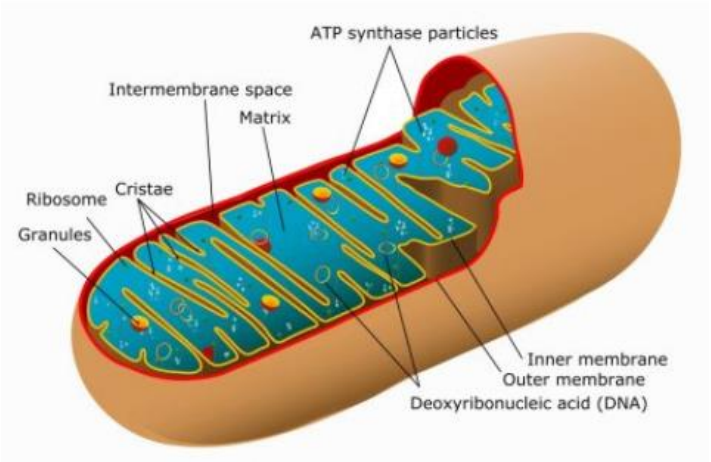
Mitochondrial Genetics Background



Nuclear DNA



mitochondrial DNA (mtDNA)



- Mitochondrial disease can be caused by changes in either the nuclear DNA OR the mitochondrial DNA
- These genes provide “instructions” create the components that help the mitochondrial make energy

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Mitochondrial Genetics Background

Nuclear DNA

- 23 pairs of chromosomes, so only 2 copies of each gene
  - one copy from Mom, one copy from Dad
- 20,000 genes
- Multiple different inheritance patterns

Mitochondrial DNA

- Multiple copies
- Variants might be present in some tissues but not others*
- 37 genes
- Maternally inherited

Note:

- Both nuclear DNA and mitochondrial DNA can have *de novo* variants
- *De novo* literally means “from the beginning”
- A *de novo* variant means the child has the variant but parent(s) do not
- Everyone has de novo variants, they play a role in making us unique from relatives
  - If de novo variants occur in genes that don’t tolerate change well, they often cause symptoms.
- Take home point – PMD has multiple different inheritance patterns

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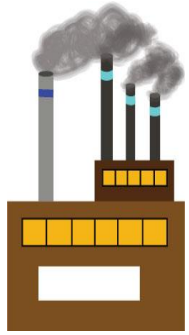
How do mitochondrial make energy?

Complex I

Complex III

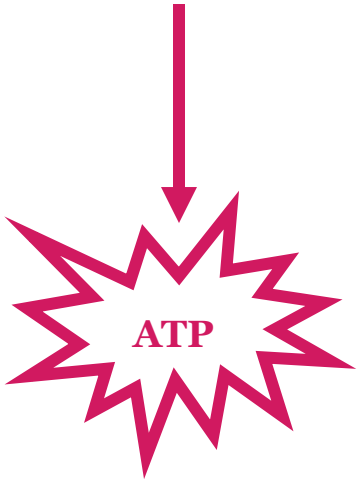
Complex IV

Complex V / ATP Synthase



Complex II

- **These "complexes" are made from instructions provided by DNA, including mtDNA!**



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How do mitochondrial make energy?

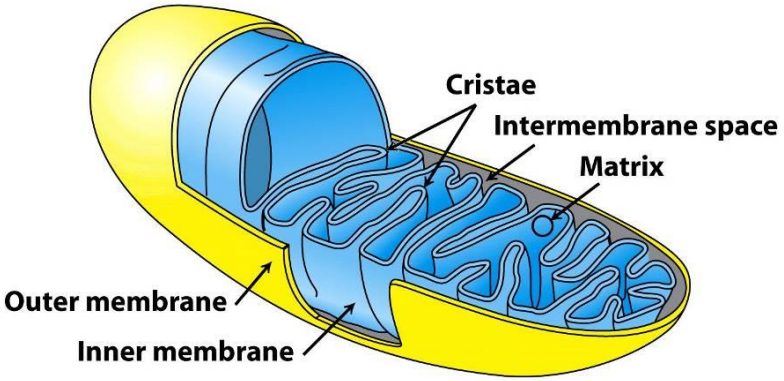

Figure 18-2b
Biochemistry, Sixth Edition
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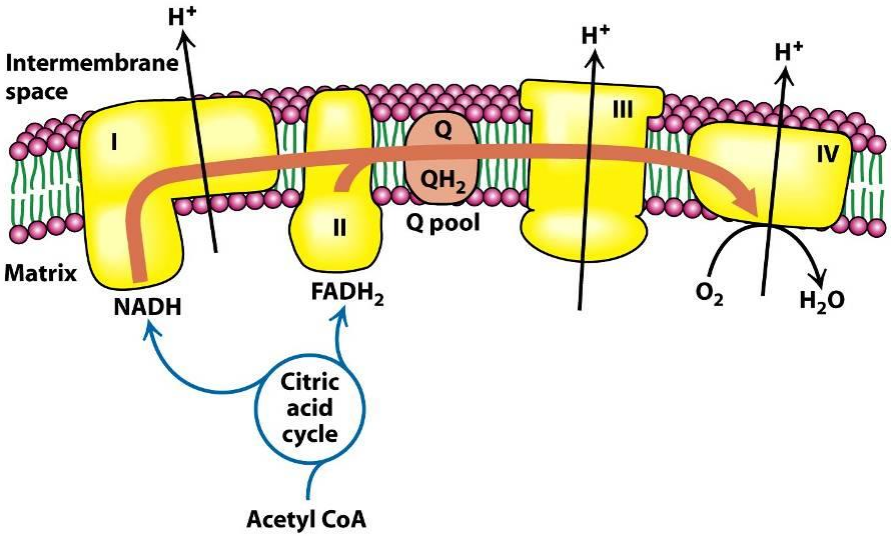

Figure 18-17
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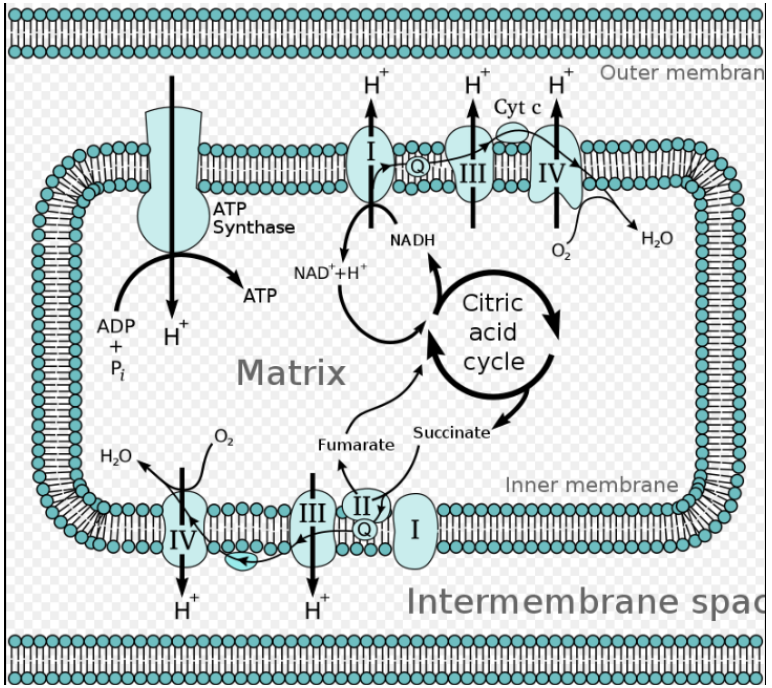

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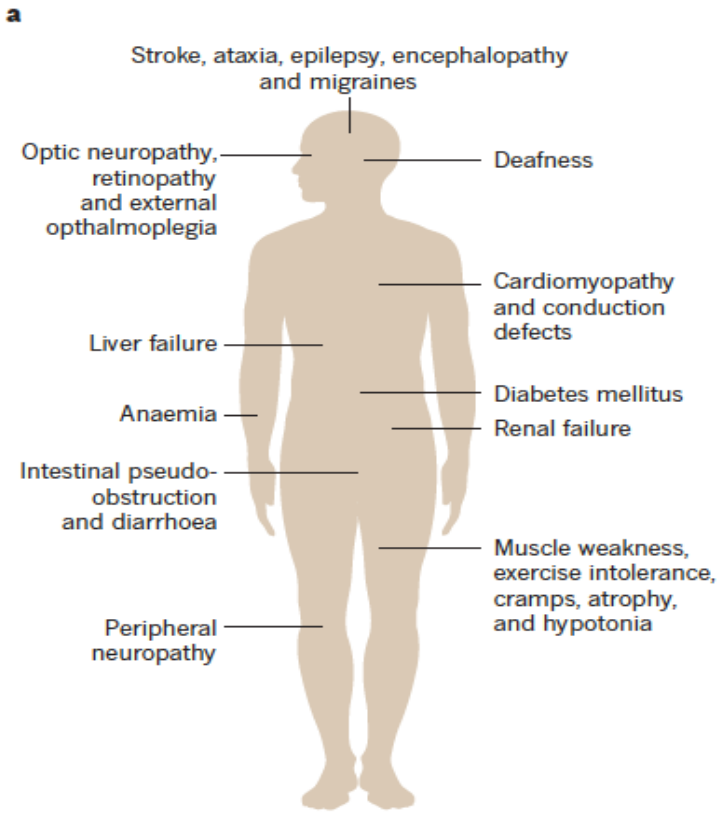
Important Terminology

- **Primary mitochondrial disease** = PMD
- ETC = **Electron transport chain** which is often used interchangeably with respiratory chain, or **oxidative phosphorylation** (OxPhos)
  - These are the "energy factories", complexes I-V
  - From a technical perspective, ETC = complexes I-IV, OxPhos = complexes I-V
- **Phenotype** = constellation of symptoms
- **Variant** = a change in the DNA
  - 3 broad classifications, benign, "Variant of uncertain significance", or pathogenic
- **Genome** – the complete sent / sequence of DNA (usually referred to as "nuclear DNA genome" or "mitochondrial DNA genome"
- **Whole exome sequencing –** a genetic test that sequences the protein coding region of the entire nuclear genome (which is only about 1-2% of the DNA, but where 80-85% of disease causing mutations are expected to be found)
- **Whole genome sequencing –** a genetic testing that sequences the entire nuclear genome (and often the mitochondrial genome as well). While it covers the entire genome, some areas are trickier to interpret than other (in other words, still not a "perfectly comprehensive" test)

What is a “PMD”?

- Primary mitochondrial disease = PMD
- The most common inborn error of metabolism (1/4,300 people have PMD; PMID: 25652200)
- Clinically heterogenous (aka variable) group of disorders caused by pathogenic variants in genes affecting oxidative phosphorylation
  - >400 genes (PMID: 36813310)
  - Two genomes (nDNA and mtDNA)
- “Phenotype” is challenging to describe
  - Any age of onset
  - Varying rates of progression vs stability
  - Can be multisystemic (MELAS) or isolated organ system (LHON)
  - “Red flag symptoms” +/- abnormal biochemical labs



Vafai and Mootha, *Nature*, 2012

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“Classic” PMD Syndromes

“Classic” PMD syndromes are descriptions of people’s symptoms, often with biochemical tests or muscle biopsy evidence suggesting a PMD

These diagnoses were identified / created well before the human genome project.

They have many different genetic causes to them

- MELAS
  - (Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes)
- MERRF
  - (Myoclonic Epilepsy with Ragged-Red Fibers)
- MIDD
  - (Maternally Inherited Deafness and Diabetes)
- Leigh Syndrome
- Kearns Sayre Syndrome
- CPEO
  - (Chronic Progressive External Ophthalmoplegia)
- LHON
  - (Leber’s Hereditary Optic Neuropathy)

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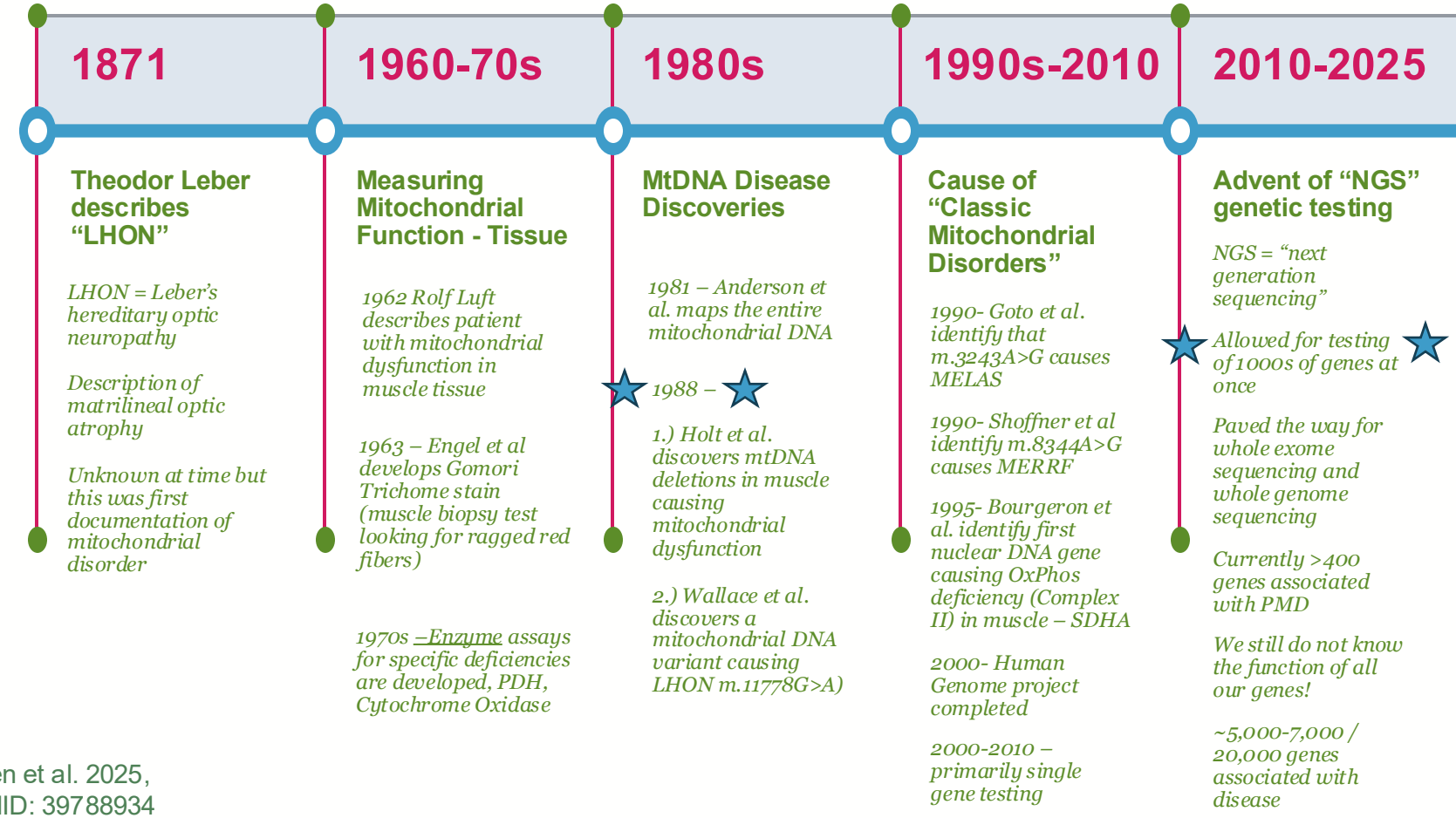
Red Flag Features of PMD

In additional to “classic syndromes” (ie MELAS, KSS, Leigh Syndrome, LHON etc.)

- Neuro: **Leigh Syndrome** (bilateral BG / brainstem lesions), **strokes in non-vascular pattern**, epilepsy, movement disorders, ataxia
- Ears: *Sensorineural Hearing Loss*
- Eyes: **Ptosis, ophthalmoplegia,** retinitis pigmentosa, optic atrophy
- Respiratory: Respiratory failure
- Cardiovascular: Cardiomyopathy, cardiac conduction defects
- Gastrointestinal: **Infantile liver failure,** intestinal pseudo-obstruction
- Renal: Renal tubulopathy, **focal segmental glomerular sclerosis**
- Musculoskeletal: Myopathy, exercise intolerance, muscle weakness
- Endocrine: Diabetes, hypogonadotropic hypogonadism, hypoparathyroidism, premature ovarian insufficiency
- Metabolic: Lactic acidosis **(particularly congenital lactic acidosis)**
- Constitutional: short stature, failure to thrive, cachexia, **cervical lipomas**

PMID: 38069070

History of PMD



Wen et al. 2025,
PMID: 39788934

How is mitochondrial disease diagnosed?

A “confirmed” PMD diagnosis now requires genetic confirmation:

Why?

- **The symptoms of PMD can be very non-specific**
  - Previously -“3 organ system involvement” raised concern for mitochondrial disease
  - Fatigue, GI issues, headaches = 3 organ systems
    - Differential for these symptoms is exceptionally broad and includes non-genetic causes like chronic dehydration
  - But these symptoms can certainly be seen in mitochondrial disease
  - How do we narrow down potential causes more specifically?
  - ***Genetic testing***

Why Genetic Testing for diagnosis?

Genetic testing evaluates “germline DNA”

- This is the DNA we are born with
- Divided into two pools, nuclear DNA and mtDNA

Germline DNA is not expected to change over time *** (caveat)

Muscle biopsy data on other hand:

- very sensitive to handling and collection procedures
- can look different in different tissues
- can vary from biopsy to biopsy
- therefore, confirmation has shifted from a biopsy driven paradigm to a genetic testing driven paradigm
  - Also genetic testing is non-invasive

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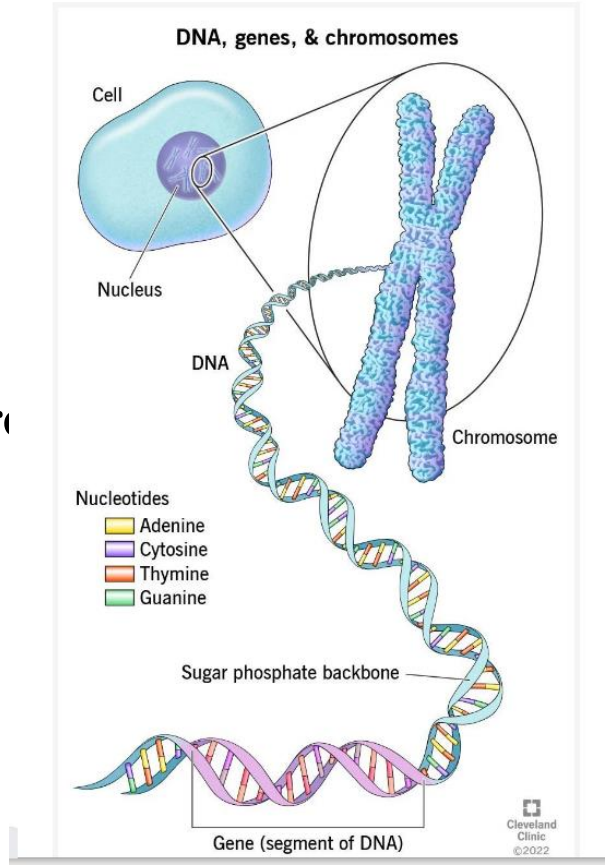
What is DNA?

- 4 bases – A, C ,T ,G
  - These are called nucleotides
  - A-T
  - C-G
    - Pairing is called a “base pair” or bp
    - Size of stretch of DNA is often referr in
    - Mbp (mega base pair – 1 million) or
    - About 3 billion basepairs in human nuclear DNA
- “blueprint” for the body
- “double helix



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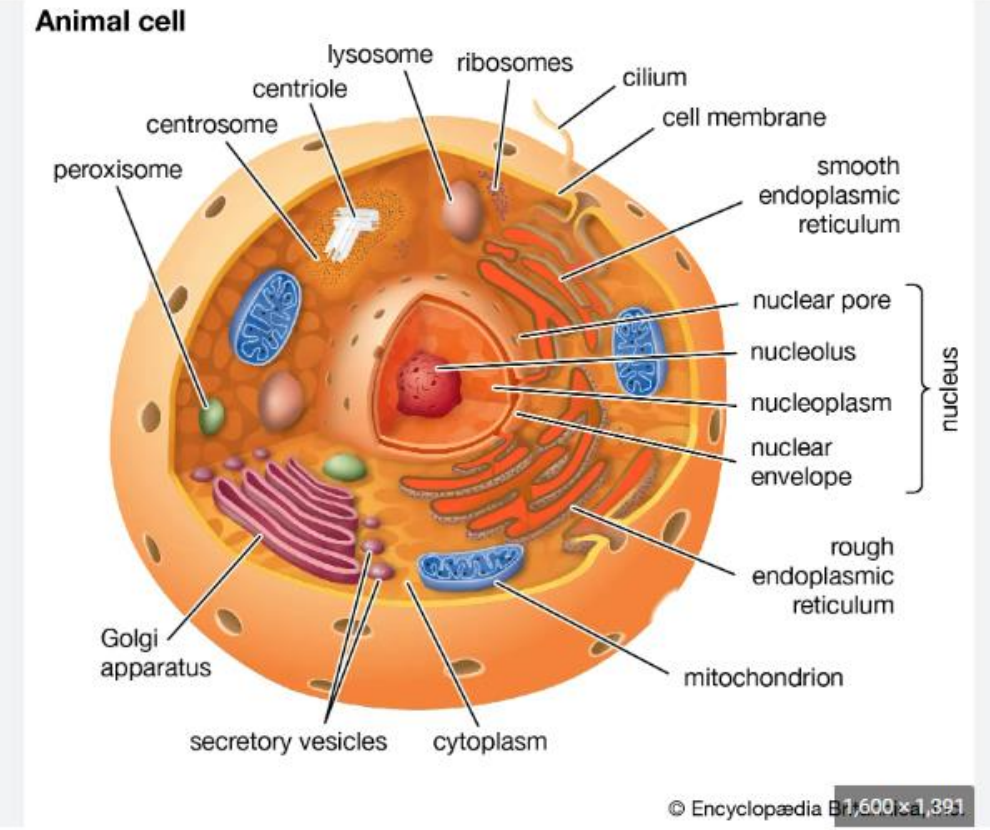
Anatomy of cell



Retrieved from:
https://www.britannica.com/science/cell-biology

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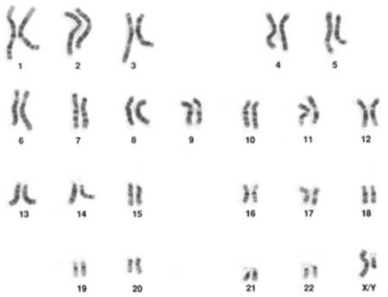
DNA Discussion Cont.

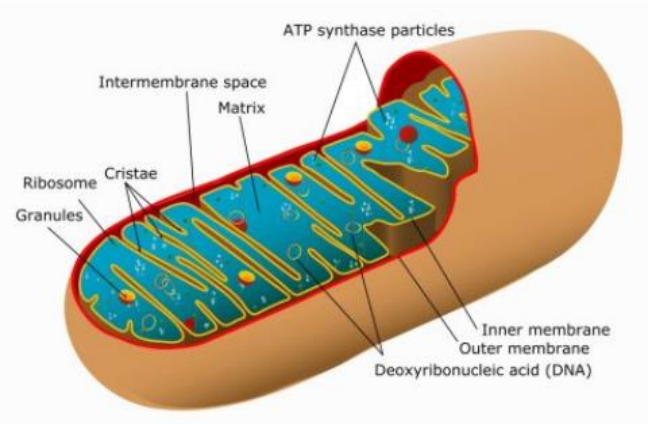
Nuclear DNA

- In nucleus of cell
- 23 pairs of chromosomes
  - One from Mom, one from Dad – i.e only 2 copies
- 20,000 genes
  - Relationship of only ~6,000 / 20,000 genes to symptoms is known
- Many inheritance patterns



Mitochondrial DNA

- Maternally inherited
- Many copies
  - "Heteroplasmy / Homoplasmy"
- **Might look different in different tissues *****



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Heteroplasmy vs Homoplasmy

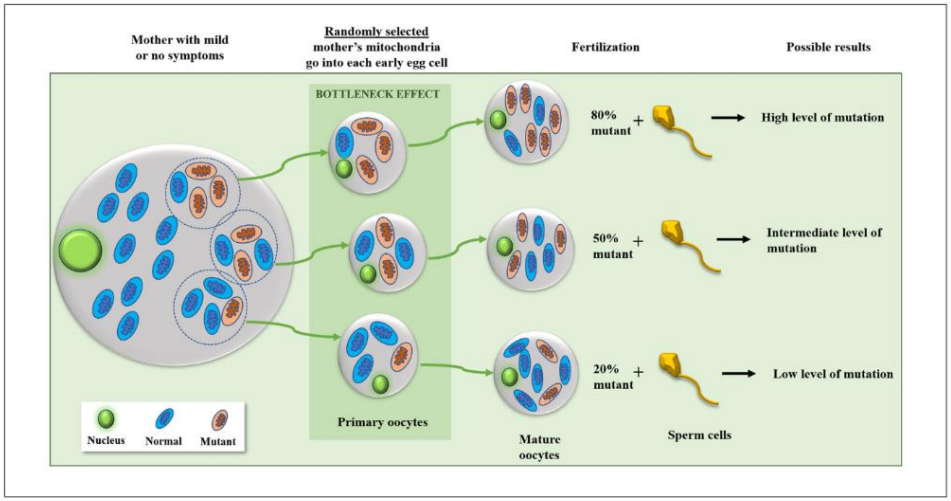
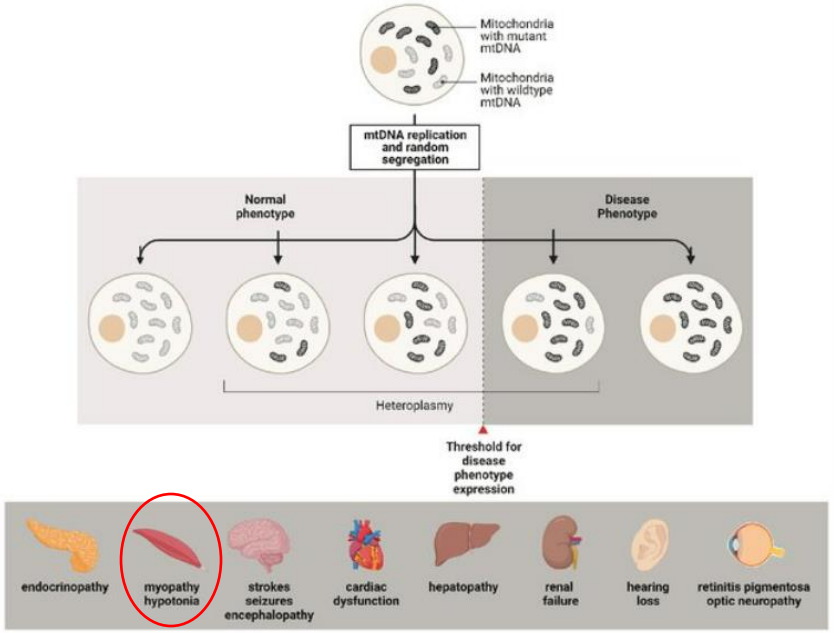


Figure 4. Mitochondrial bottleneck during oogenesis causes various levels of heteroplasmy in the offspring.



Images retrieved from: PMID: 35843711, PMID: 38047549

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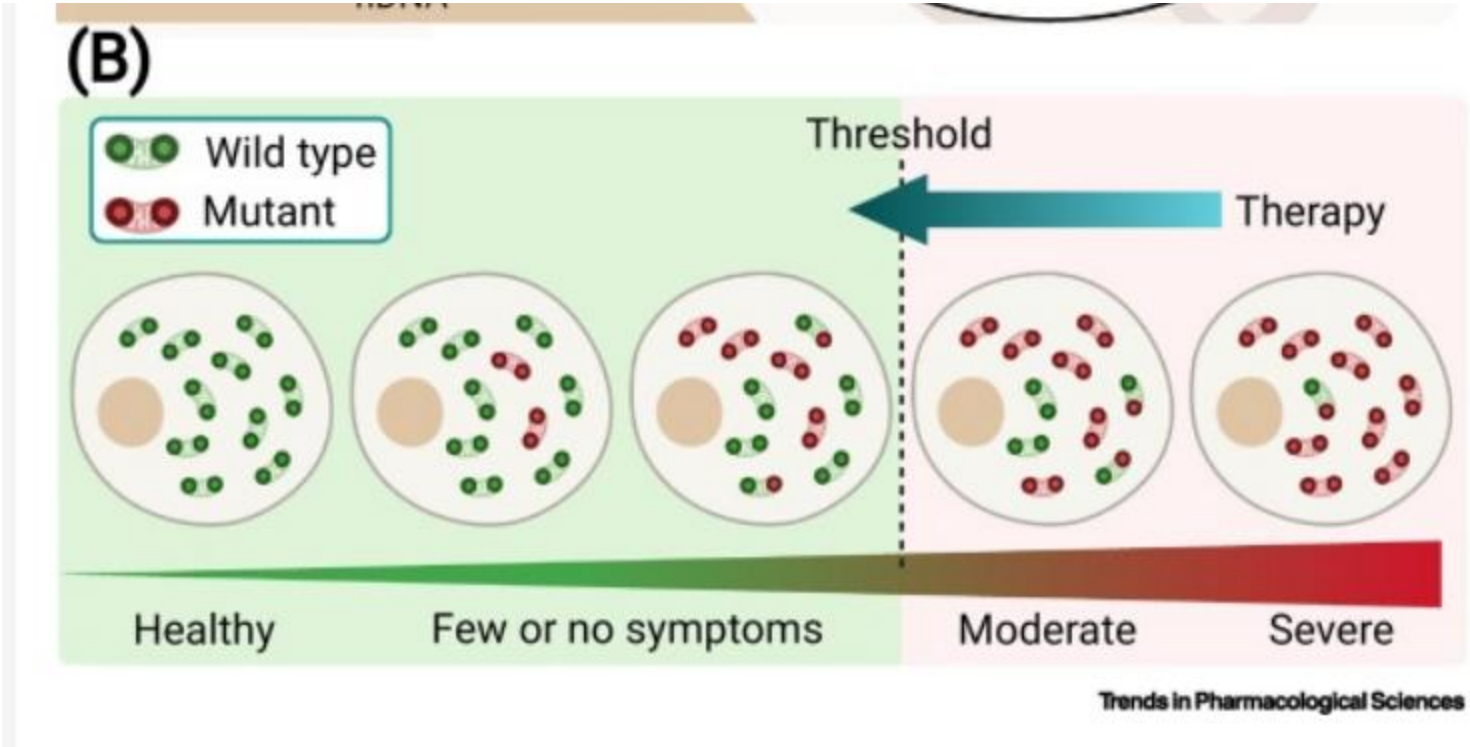
Threshold Effect

- Note: Threshold effect can vary between variants
- **One of <u>main</u> reasons for muscle biopsy is to make sure there is not an mtDNA variant that exceeds threshold effect level in muscle**



Spinazzola et al., 2024

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Practical Example:

Note: this case example is reflective of many cases but is not specific to any one person.

- 35-year-old man with history of double vision and ptosis (drooping eyelids) goes to eye doctor
- Eye doctor examines him and says he has “ophthalmoplegia” weakness of the eye muscles limiting eye movements
- Genetic testing in cheek swab (testing nuclear DNA and mitochondrial DNA) is sent, but is negative
- Muscle biopsy is performed and this person carries a pathogenic (single) mitochondrial DNA deletion in his muscle @ 40% heteroplasmy – diagnosis confirmed

Common Genetic Testing and Sample types

Non-invasive Testing (nearly always "first tier test"):

- Whole Exome Sequencing + mitochondrial DNA sequencing
- Whole genome sequencing (often includes mtDNA, but may vary)
- Panel testing of nuclear DNA genes associated with PMD and mtDNA genes

- These test are commonly performed in **blood, saliva, buccal swab**

Mitochondrial DNA Testing (additional tissues):

If mtDNA in buccal, blood, or saliva is negative or unrevealing, may move on to:

- Urine
- Muscle biopsy – To completely exclude an mtDNA disorder, technically a muscle biopsy is needed
- **However – balance of recommending invasive procedure weighed with degree of suspicion for a primary mitochondrial disease.**
  - **A muscle biopsy may not be indicated for all people undergoing a mitochondrial disease evaluation**
  - **These discussions are best to have with your doctor**

Muscle Biopsy Overview:

Tests Commonly Performed on Muscle Biopsy

- Muscle Histology
  - Looks at muscle fibers, screens for muscular dystrophies, inflammatory muscle diseases etc.
  - This is where ragged red fibers and COX negative fibers are identified
  - Electron microscopy (EM) may be performed but different institutions have different policies regarding when to perform EM

- **Mitochondrial DNA Sequencing and Deletion Analysis**
  - Only way to completely exclude mtDNA variants
  - Balance of risk benefit
- *Mitochondrial Respiratory Chain Enzyme Analysis
  - Blue Native Gel Electrophoresis
- Mitochondrial DNA Content
- Coenzyme Q10 Determination

*previous “gold-standard” for mitochondrial disease diagnosis. Now used as supportive evidence

What are ragged red fibers?

- Mitochondrial stain red on Gomori trichrome stain
- An RRF shows accumulation of mitochondria at the surface of the muscle cell
- The proliferation and accumulation of mitochondria suggests an “energy problem” or mitochondrial dysfunction

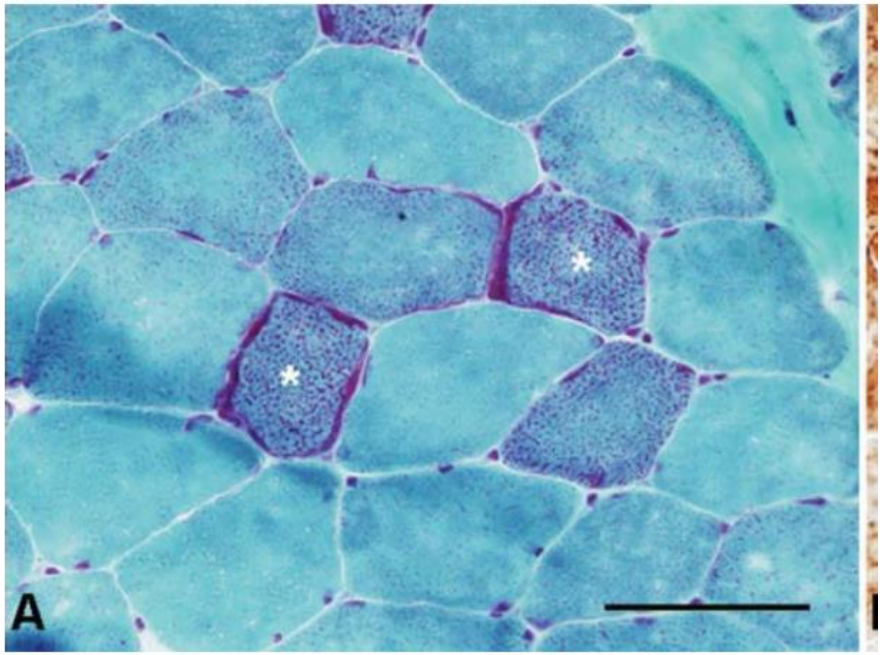

FIGURE 1. Biopsy of the vastus lateralis. **(A)** Ragged-red fibers (asterix) are present in trichrome stain and **(B)** positive in cytochrome *c* oxidase stain (bar = 50 μm). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

PMID: 20544923

<u>Differential diagnosis:</u>

- Can be acquired with age
- Inclusion body myositis
- PMID: 7818253

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Confirming Diagnosis: Common tissue testing

Tissue	Histology	mtDNA Sequencing	ETC	BNG	Complex I WB	mtDNA Content	CoQ10
Blood	-	+	-	-	-	-	+
Buccal	-	+	-	-	-	-	-
Urine	-	+	-	-	-	-	-
Fibroblast	-	+	+	+	+	-	-
Liver	+	+	+	+	-	+	-
Heart	+	+	+	+	-	-	
Muscle	+	+	+	+	-	+	+

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Why are fewer clinics offering muscle biopsy?

1.) **Hard to collect**

- Requires partners in general surgery and in pathology for appropriate collection, handling and shipping
- Muscle tissue is very sensitive
- Ideally muscle is collected from specific muscle – vastus lateralis

2a.) **Very few labs perform the testing on a clinical basis in the United States**

2b.) **Insurance approval is hard to obtain**

3.) **Tests are hard to interpret and can be misleading** (ie “false positives”)

- Complex I deficiency is reported in Down Syndrome (PMID: 21338338), but Down Syndrome is not considered a primary mitochondrial disease (Trisomy 21 – many genes involved, many of which are not mito genes)

- People acquire RRFs and COX- fibers with age

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Summary

- Mitochondrial Disease diagnoses require genetic testing for confirmation
  - Commonly done through blood, buccal, saliva samples on whole exome sequencing, genome sequencing or “dual genome panels”

- Muscle biopsies have shifted from the “gold standard” to a supportive role
  - *** only way to completely exclude mtDNA variants
  - Can be useful for providing support for variants of uncertain significance

- Not all institutions may have the ability to offer muscle biopsy
  - Often has specific processing and handling requirements
  - Muscle biopsy may not be indicated in certain instances
    - While generally considered “low risk procedure”, it still requires general anesthesia and is a surgical procedure

Questions?

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