Diagnostic Testing for Mitochondrial Disease

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Disclosures

- None

Objectives

- Review traditional diagnostic pathways
- Discuss newer testing that has become available in recent years
- Review new approaches to attempt to shorten time to diagnosis and increase precision

Traditional approach to diagnosis

- Different pathways had been taken for pediatric and adult diagnosis
- Adult diagnosis has typically been more difficult due to the more subtle presentation compared to childhood presentations
- Infants and children can have more pronounced clinical and biochemical findings in blood
- Spectrum of laboratory findings that become less pronounced as patients are diagnosed at later ages

Infant diagnostics

- Infants have been identified with biochemical testing in blood
  - Lactate/pyruvate can be very elevated
  - Plasma amino acids (alanine/lysine)
  - Sometimes unusual metabolites in other testing:
    - Acylcarnitine profile
    - Urine organic acids
    - Ammonia
  - Confirmatory testing:
    - Tissue sampling (skin/muscle biopsy)
    - Genetic confirmation if possible

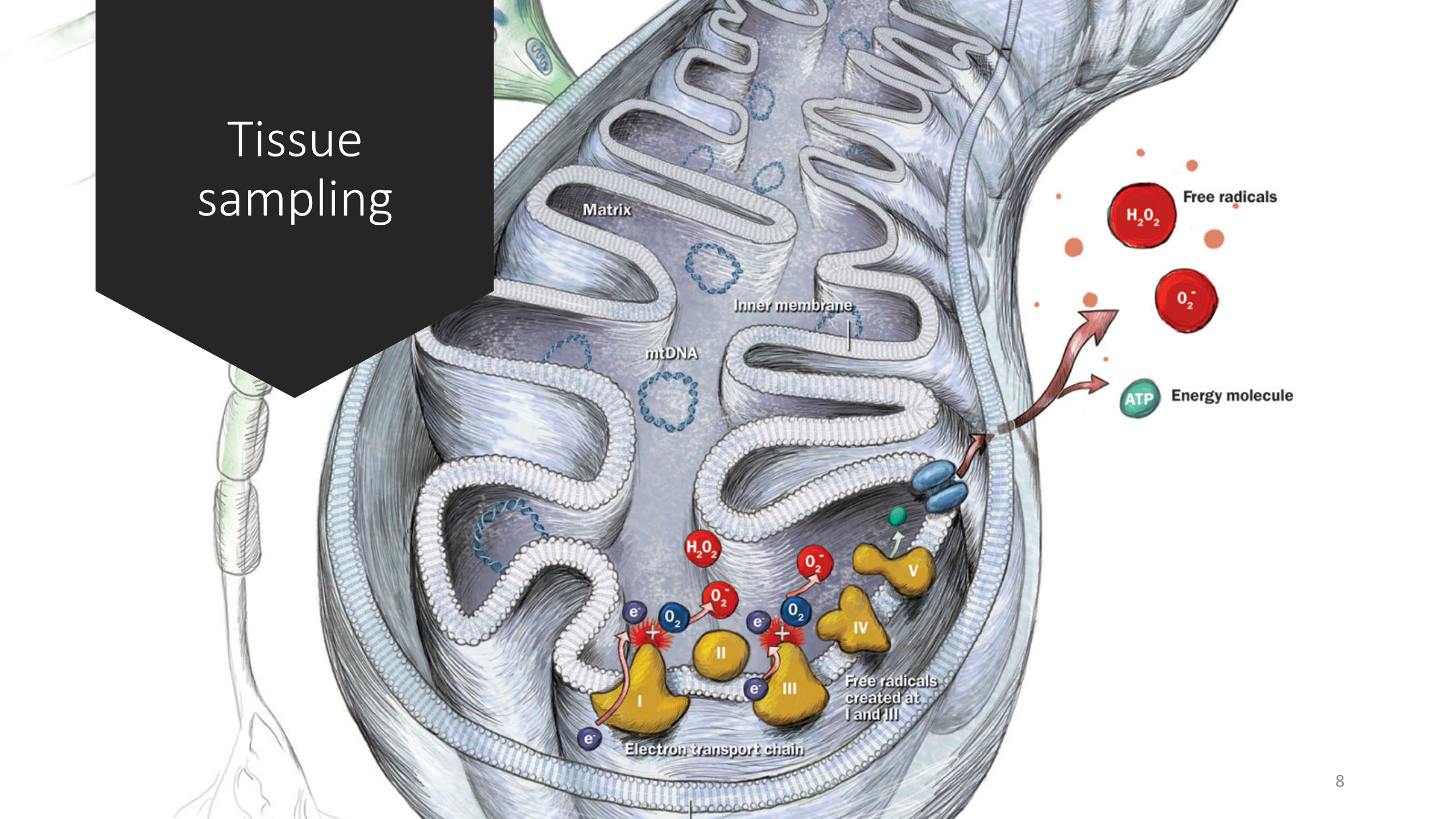
Childhood/Adolescent diagnostics

- Children and adolescents have been identified with biochemical testing in blood
  - Lactate/pyruvate typically not as elevated as in infants
  - Plasma amino acids (alanine/lysine)
  - Sometimes unusual metabolites in other testing:
    - Acylcarnitine profile
    - Urine organic acids
    - Ammonia
  - Confirmatory testing:
    - Tissue sampling (skin/muscle biopsy)
    - Genetic confirmation if possible

Adult diagnosis

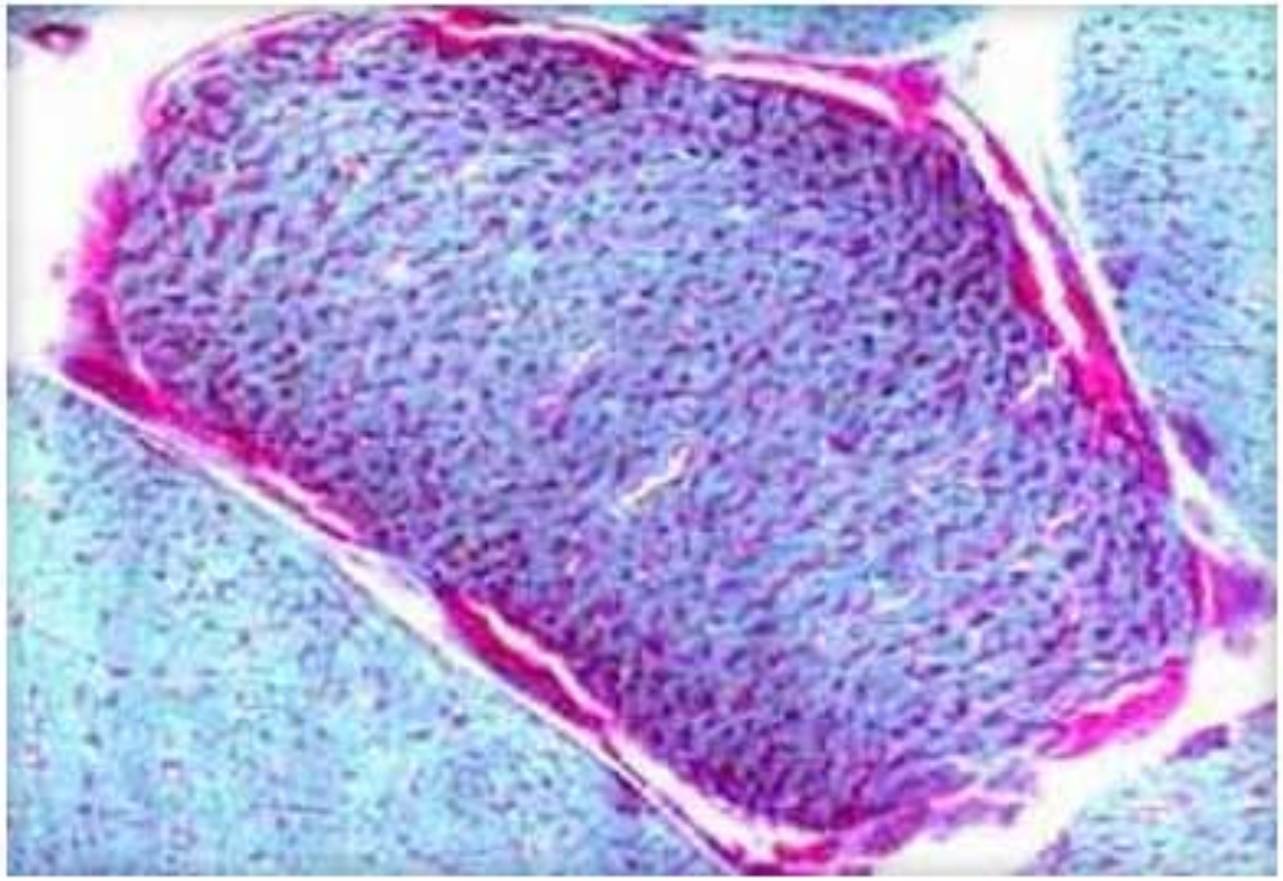
- Adults typically do not have biochemical abnormalities in blood
  - This may have been a contributing factor as to why it was thought that adults could not have mito
  - We now know that adults under stress may show biochemical abnormalities
  - Tissue sampling has been able to confirm a diagnosis in adults without biochemical abnormalities

Tissue sampling



Pathology

- In addition to enzyme analysis, histology can be performed
- Unfortunately histology is rarely helpful
- If you do find classical changes it can provide more evidence for a diagnosis



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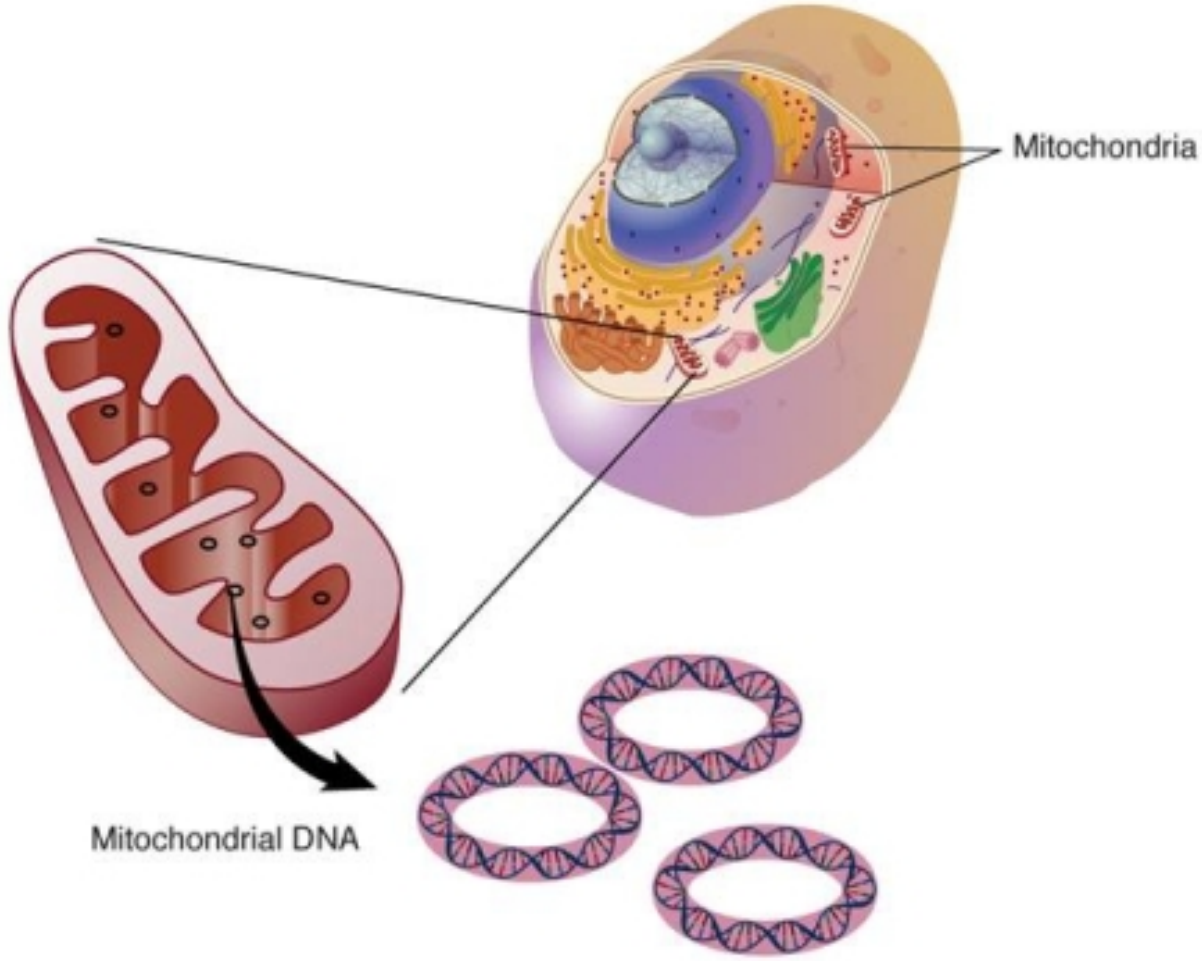
The Genomic Era

- We have entered an era where we can test hundreds or even thousands of genes with a single sample
- There is DNA within mitochondria and mitochondrial genes in the cell nucleus



Next (Current) Generation Sequencing

- Panel testing on blood:
  - Mitochondrial panels that include nuclear and mitochondrial genes
- Exome analysis on blood
  - Not perfect
  - Does not detect
    - del/dup
    - Gene expansion
    - Looks at exons, usually ~2 bp in to introns
    - Coverage varies

Sanger vs. Next Generation sequencing

	Sanger sequencing	*Next generation sequencing*
Strategy	Separate reaction for the sequencing of all exons of a single gene	One single reaction for the simultaneous analysis of different genes
Use	Identification of unknown mutations by sequencing of whole genes	Analysis of unidentified mutations in heterogeneous disease
Benefit	High precision	Highly cost-effective and efficient by simultaneous and fast analysis
Disadvantage	Expensive and time-consuming due to limited automation and necessity of many different reactions	Interpretation of the abundance of data challenging High coverage needed for accuracy

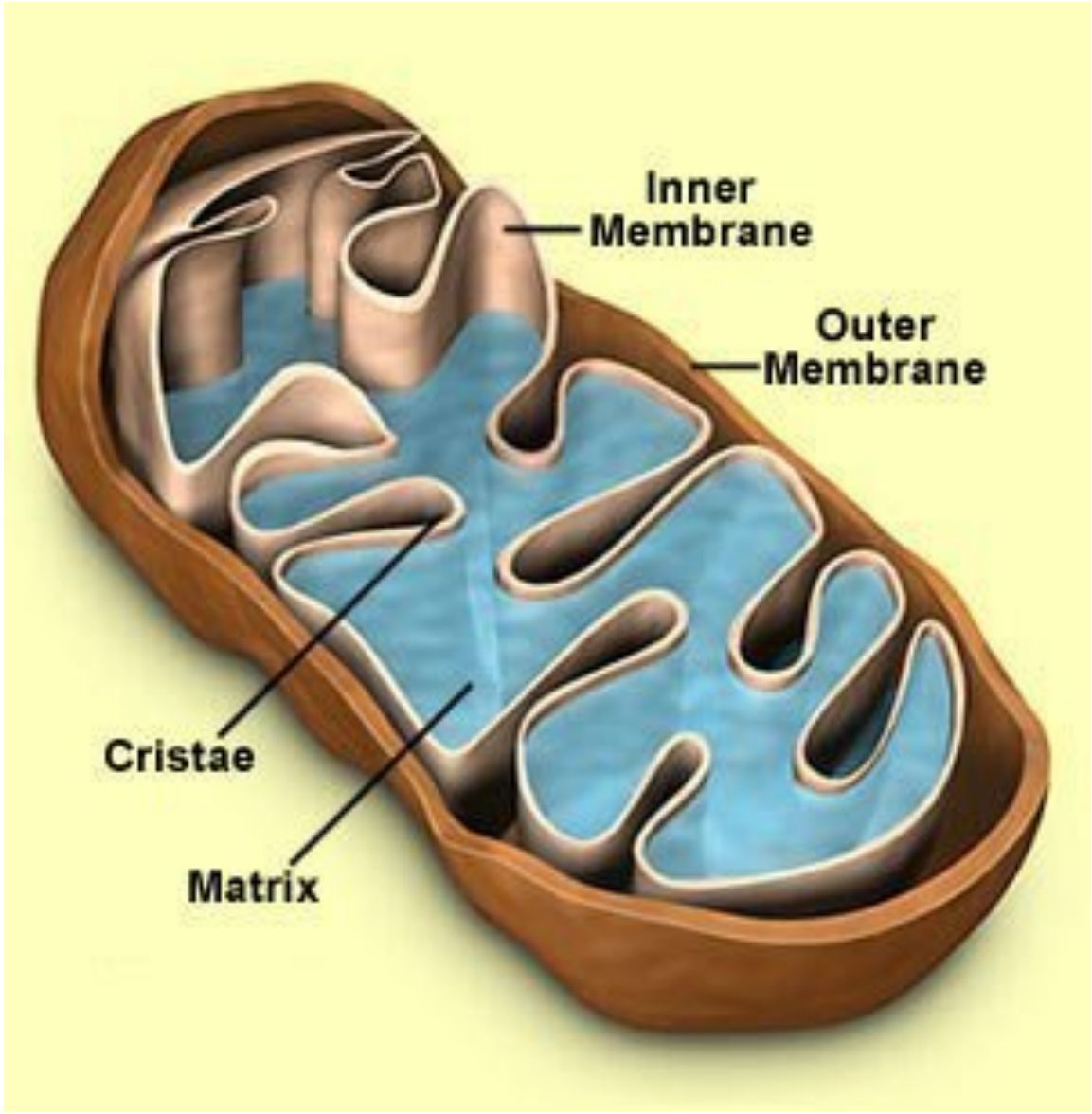
NGS: Next generation sequencing

The Diagnostic Exome

- Several papers have shown reduction in time to diagnosis in complex cases
- Can analyze ~20,000 nuclear genes and mtDNA genes on one blood sample
- If able to detect changes, increases precision of diagnosis
- Potential cost savings
- Exome has a pick up rate up to ~40% (for some labs)

Why do we need more precision?

- ~1200 genes involved in mitochondrial function
- Combination of:
  - Nuclear DNA
  - Mitochondrial DNA
    - 37 genes



Reference: http://www.kathleensworld.com/mitochon.html

Precision

- Critical to potential future therapies
- Nuclear Gene discovery for rare forms
  - Autosomal recessive mitochondrial genes
  - Autosomal dominant mitochondrial genes
  - X-linked
- Will allow us to tailor therapies more on an individual basis

Bioinformatics

- Growing database that catalogs normal genomic variation
- Well over 30,000 reference genomes have been sequenced
- Can be used to check against a genome of interest to rapidly removed more common normal variants

Panel testing vs. Exome analysis Coverage

- Insurance has generally been more willing to cover panels vs. whole exome analysis
- Panel testing
  - Availability of deletion/duplication analysis
  - With some labs, can do testing the day of the visit with the patient with patient responsibility limited to $100 for some insurance
  - Focused on genes of interest
- Whole exome
  - Broad based
  - Typically requires prior authorization, so testing can't be obtained the day of visit
  - At least one lab is now offering to coordinate insurance authorization with patient responsibility limited to $100 for some insurance and can send sample day of visit

However…

- Unfortunately, this kind of testing can’t be done with all insurance types yet
- Doesn’t appear to be a cost issue in all cases
- Insurance companies may label it as “experimental”
- May not understand exactly what it is or don’t want to know
- Gene panels and exome analysis still several thousand dollars

Combined testing

- Genomic data isn’t everything
  - That day is not yet here, although we have certainly made progress
  - Some other specialist colleagues have opined that if they don’t see a molecular change, disease does not exist in a patient
- Biochemical testing is still helpful
- Still need to consider phenotype
- Targeted testing:
  - Deletions
  - Duplications
  - Gene expansions
  - Methylation anomalies

New Diagnostic Pathway

- Clinical evaluation
- Start with biochemical testing
  - Lactate/pyruvate
  - Plasma amino acid
  - Ammonia
  - Acylcarnitine profile
  - Urine organic acids
- Mitochondrial Next Generation Sequencing Panel (6-8 weeks)
- If Mito panel is negative, reflex to larger panel or exome analysis (8-10 weeks)
- If negative, perform muscle biopsy

New Diagnostic Pathway

- Primary benefit is potentially increased precision
- Lower cost and may be safer
  - General anesthesia can be an issue in Mito patients
  - Anesthesia + Surgery can cost $10k
  - Add an overnight stay and costs are higher
- Overall processing time is improving
- Allows for detection of rare forms and possibly related disorders
- Opens up options for more targeted treatment in some cases

Genomics is relatively new…

- Since genomic testing has only been around for a few years we still have a lot to learn
- Finding a lot of variants of uncertain significance
  - Not known to be pathogenic or benign bases on current data
  - Need to relate to current clinical finding
  - Sometimes family testing can help determine significance
- As bioinformatics becomes more robust, we may be able to increase official diagnostic rates

Conclusion

- As we improve our ability to perform genomic testing:
  - Increase precision of diagnosis
  - Will allow for more targeted therapies in the future
  - More focused care of individuals
- Emergence of new diagnostic pathways
  - Can shorten time to diagnosis
  - Can lower overall costs