Summary - Molecular Diagnostic Testing for Mitochondrial Disorders
Dr. Darius Adams

Medical Director -- Goryeb Children's Hospital

Genetics and Metabolism Division

Personalized Genomic Medicine Program at Atlantic Health System

Traditional approaches to diagnosing mitochondrial diseases have largely been based on biochemical analysis with different routes taken for pediatric and adult patients (slide 4).

- The adult pathway has typically been more difficult due to the more varied and subtle presentation compared to childhood presentations. The spectrum of laboratory findings is also less pronounced in adult patients, further adding to the challenges of diagnosing mitochondrial disease in this population.
- Infants and children can have more pronounced clinical and biochemical findings in the blood coupled with a very rapid onset and progression of symptoms that can lead to early deaths. Until the 1980s-1990s, this rapid onset and progression lead to the belief that mitochondrial disease was a childhood disorder. Mitochondrial disease is now known to exist across the lifespan.

Traditional Infant Diagnostics (slide 5)

Infants have been identified with biochemical testing in blood and urine.

- Lactate/pyruvate can be elevated (should be ordered together for best information)
- Plasma amino acids may reveal abnormalities in the alanine to lysine ratio
- Sometimes unusual metabolites in other tests are evident:
- Acylcarnitine profile
- Urine organic acids
- Ammonia

Traditionally, when abnormalities are found in the above testing, tissue sampling would be the next step via skin or muscle biopsy to look at enzymatic function to make a diagnosis. Genetic testing would be done after the biopsy to see if details could be added to the diagnosis. The expense of genetic testing has made this option difficult to obtain, so most Mito patients were diagnosed biochemically with the biopsy.

Childhood and adolescent diagnostics have also been traditionally identified with biochemical testing in blood and urine samples (slide 6).

- Lactate/pyruvate typically not as elevated as in infants
- Plasma amino acids may reveal abnormalities in the alanine:lysine ratio
- There may be unusual metabolites in other tests:
- Acylcarnitine profile
- Urine organic acids
- Ammonia

Diagnosis relies more heavily on tissue sampling/biopsy with genetic confirmation if possible.

Adult diagnosis is more challenging because adults typically do not have biochemical abnormalities in the blood (slide 7). This fact may have been a contributing factor as to the early belief that adults could not have Mito. We now know that, under stress, adults may show biochemical abnormalities. In some cases, clinical assessment with tissue sampling (biopsies) has been able to confirm a diagnosis in the absence of biochemical abnormalities.

Tissue Sampling studies the cells' electron transport chain, typically within a small sample of muscle and/or skin (biopsy). The sample is snap frozen in liquid nitrogen or examined immediately as a fresh muscle analysis. Most samples are frozen and sent to facilities better equipped to complete the needed testing. Complexes I-V take place in the mitochondria and depict the vital processes needed to make energy for the cell. Deficiencies in these complexes can be uncovered with enzymatic testing. There is not a dramatic difference between frozen and fresh in terms of Complexes I-IV (slide 8). A fresh sample does allow for testing of a few additional enzymes, including testing for Complex V. Pyruvate dehydrogenase and pyruvate carboxylase enzymatic deficiencies can be picked up with biochemical panels. Enzyme testing is always a tricky process. At times, the sample is not good enough to run a quality enzyme analysis. The lab uses control enzymes to be certain that the results are reliable.

Pathology -- one of the initial routes to assess or screen for mitochondrial disorders. Most institutions do have histologists on staff who can analyze samples for abnormalities. Unfortunately, histology is rarely helpful in mitochondrial diagnosis, but if classical changes, like ragged red fibers as pictured (slide 9), are found, more evidence for a diagnosis is provided.

The Genomic Era

The technology to test hundreds or even thousands of genes with a single blood or tissue sample now exists (slide 10). Disease-causing DNA mutations can be found both within the:

- mitochondrial DNA (mtDNA) -- 37 genes, and
- nuclear DNA (nDNA) -- encodes for proteins and enzymes which are then transported into the mitochondria to make ATP. Most mitochondrial diseases are caused by nuclear DNA mutations, which surprised many clinicians in the '80s and '90s.

Next (or Current) Generation Sequencing

Panel testing -- mitochondrial gene panels that include both nuclear and mitochondrial genes that have been associated with mitochondrial disorders.

Exome analysis -- can test all genes (up to 20,000 genes). It's a powerful tool, but has some limitations.

- Not perfect as it does not detect deletions, duplications, or gene expansion.
- Exome looks at exons (the coding regions of the gene), usually ~ 2 bp into introns.
- Introns are not examined, which may impact disease.
- Coverage can vary and is not quite as accurate as doing more focused analysis.
- Some suggest doing exome testing early on in the diagnostic journey to shorten the diagnostic process.
- Sanger and Next Generation sequencing comparison in detail (slide 12)
    - Sanger -- traditional method, looking at one gene at a time
- very expensive and time consuming, requiring manual intervention
- high precision -- looks at both exons and introns
- need to have diagnosis more pinned down to know which genes to study
    - Next Generation -- simultaneous analysis of thousands of genes
- Cost-effective, efficient, fast, and automated
- high coverage needed for accuracy

Diagnostic Exome (slide 13)

- Can analyze ~20,000 nuclear and mitochondrial genes with one blood sample that offers large quantities of data and has given rise to Bioinformatics (the process by which we have referenced genomes that allow clinicians to rule out benign changes that are found). Any individual can have thousands of benign, or harmless, gene changes that would be difficult to manually evaluate to confirm that they are benign. Bioinformatics and computer technology cut down on the processing time for these Variants of Unknown Significance (VUS), which do tend to be benign.
- Papers have shown reduction of time to diagnosis with complex cases
- Precision -- ~1,200 nuclear and mitochondrial genes are involved with mitochondrial function (slides 14-15). When able to detect changes, analysis increases the precision of diagnosis, which may help to better tailor therapies on an individual basis. Understanding mitochondrial disease subtypes will help find more effective treatments, instead of just offering more general supportive therapies. This precision will also continue to become more important in the future as gene therapy is used to treat genetic disorders.

-- Ability to discover new or rare forms of mitochondrial disease

- Autosomal recessive mitochondrial genes
- Autosomal dominant mitochondrial genes
- X-linked

-- Potential cost savings

-- Exome studies have a pick-up rate of up to ~40% for some labs. (This refers to diagnostic yield when testing patients with complex presentation. This number increases to even 90-95% when other biomarkers are present, such as elevated lactate pyruvate ratios.)

-- Saves patients from more invasive testing, such as a muscle biopsy.

- Bioinformatics -- growing database that catalogs normal genomic variation (slide 16). Uses super computers to catalog the discovered variation in genes.
  -- Well over 30,000 reference genomes have been sequenced.

  -- Can be used to check against a genome of interest to rapidly remove more common normal variants.

- Panel testing vs. exome analysis coverage (slides 17-19)
  -- Panel testing is focused on genes of interest. Same-day testing at the office visit with patient responsibility limited to $100 with some insurances is offered by some labs. Insurance companies tend to cover this more focused gene testing. Focused testing does allow for a closer look at specific genes for deletions, duplications, gene expansions, and methylation anomalies

  -- Whole Exome – Broad-based, usually requiring prior authorization, so testing cannot typically 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 day of visit.

  -- Both types of testing are not covered by all insurance types yet and still cost several thousand dollars. Denial of coverage does not appear to be solely a cost issue as some insurance companies label as “experimental” and may not fully understand this newer technology.

- Combined testing -- still has a role
  -- Genomic data is not everything, although progress continues to that end goal. Some specialists have opined that if they don't see a molecular change, disease does not exist in a patient.

  -- Biochemical testing is still helpful.

  -- Need to consider phenotype -- or clinical presentation of symptoms.

  -- Many centers use phenotype, biochemical results, and targeted gene testing to work toward a diagnosis.

New Diagnostic Pathway (slide 20-21)
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.

Benefits:

- Potentially increased precision
- Lower cost and may be safer
    -- General anesthesia can be an issue in Mito patients
    -- Anesthesia + surgery can cost over $10,000
    -- 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
- Genetically confirmed diagnosis is needed to enter all current mitochondrial disease studies to better evaluate the efficacy of treatment drugs on the various subtypes of this disease.

Genomics is still relatively new and clinicians still have much to learn (slide 22). Variants of Uncertain Significance (VUS) are a common finding. VUSs are not known to be pathogenic (disease-causing) or benign based on current data. The VUS needs to relate to current clinical findings. Testing other family members may help determine the significance of the VUS, especially when other family members have similar findings. As bioinformatics becomes more robust, official diagnosis rates may increase.

Conclusion

As genomic testing improves:

- increase in precision of diagnosis
- allow for more targeted therapies in the future as opposed to the more generalized therapies currently in use
- more focused care of individuals
- Emergence of new diagnostic pathways can shorten time to diagnosis and can lower over costs.