Summary – Interpreting Common Lab Tests for Mitochondrial Disease

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Introduction Dr. Haas is an expert in the area of mitochondrial disease and has over 40 years of experience working with Mito patients and families. Dr. Haas is the founding physician of the Mitochondrial and Metabolic Disease Center at UCSD. The Center endeavors to provide diagnosis and care both locally as well as across the US.

Mitochondrial Genetics When thinking about the genetics of mitochondrial disease the nucleus is very important. The accompanying slides show pictures of mitochondria with the nucleus in the middle and the nucleolus in the center of the nucleus. Kidney cells provide a good example of how mitochondria work, as these cells are very active and have a high energy demand. 46 chromosomes are located inside the nucleus of human cells, and these in turn are made up of super coiled DNA. DNA is the "code" which makes proteins and controls the cell function. This complicated system is typically disrupted in people with mitochondrial disease.

It is believed that in the evolution of organisms, mitochondria came from bacteria. The mitochondrial DNA in humans comes from the ova (the mother's egg), while the 1500+ nuclear mitochondrial genes come from both parents. In other words, mitochondrial disease is not always maternally inherited.

Classification of Mitochondrial Disease

Mitochondrial disease affects all organ systems, especially those which have high energy demands. For example, the brain and central nervous system (CNS) have very high energy demands; consequently, mitochondrial dysfunction may precipitate symptoms such as stroke, seizures, ataxia and migraine headaches. Sensory effects or deficiencies in vision or hearing are also common. Because of the heart's high energy demands, approximately 15% of Mito patients have cardiac issues such as cardiomyopathy or conduction defects. The most common condition related to mitochondrial dysfunction in the endocrine system is diabetes; in fact, adult onset diabetes has been found to have a mitochondrial component. Finally, GI symptoms are also very common. Because of these various expressions of Mito, multiple specialty clinics may follow and treat Mito patients. In fact, one clue to Mito diagnosis is when a patient has more than one organ system involved in their disease process.

What do mitochondria do? Major mitochondrial functions are:

- make ATP, a molecule that stores energy to be used later by the body. This process is called oxidative phosphorylation;
- metabolize fats, carbohydrates and amino acids and then interconvert them;
- synthesize proteins;
- reproduce themselves (An essential component of reproducing themselves is called programmed cell death - it is important that old cells are replaced. Mitochondrial disease can cause the death of cells when this is not needed.)
- make free radicals (which is ok to fight infections, but an excess is not good);

- create an immune response;

Mitochondrial disease is an "ox-phos" disease, or a disease of energy metabolism related to impairment of oxidative phosphorylation. A nuclear gene defect is the cause of about 80% of childhood disease while mtDNA defects are responsible for about 60% of adult Mito disease.

Difficulty in Diagnosing Mito

Because of the variety of presenting symptoms as well as the different genetic causes, mitochondrial disease remains difficult to diagnose. Often patients who present with various symptoms "look normal" while others may have a variety of more obvious issues. Dr. Haas's slides demonstrate cellular changes as well as images from MRI in patients with Mito.

Heteroplasmy

The concept of heteroplasmy is important in mtDNA caused disease. This means that within the cell there is a mix of mutated DNA as well as normal (wild type) DNA. The severity of disease depends on the percent of the mix of these two types as well as the location of the defect. Though it is unpredictable, muscle, brain and endocrine systems are most typically affected. A mother could be carrying some mutated genes but show little or no disease, yet her child could present with a higher percent mix and therefore have more disease symptoms. The slides show cells from a patient with MELAS that demonstrates heteroplasmy in cells.

Genetic mitochondrial disease can present as acute/sub-acute or chronic. Acute/subacute conditions may include severe metabolic crisis, encephalopathy, arrhythmia, heart block, blindness or stroke. Chronic manifestations may include growth retardation, developmental delays, strabismus, diabetes, irritable bowel syndrome, cardiomyopathy, neuropathy, ataxia, hypotonia, weakness, exercise intolerance, and/or dementia. The severity of symptoms is often linked with the onset of disease; most experts believe that infantile or early childhood onset is associated with greater disease severity.

The difficulty of diagnosing mitochondrial disease led to a group of physicians getting together in 2007 to help primary care physicians with this diagnosis by publishing an article in **Pediatrics,** *Practical Approaches for the Primary Care Physician.* Since there are no biomarkers for mitochondrial disease, making the diagnosis is complicated. Medical history, clinical findings, and specific biochemical laboratory abnormalities are all part of the diagnostic process. Tissue biopsy and evidence of abnormal electron chain transport or DNA mutations are also part of this process.

Testing for Mito

The approach to a mitochondrial disease diagnosis then involves these:

- physical Exam
- clinical symptoms (evidence of multiple organ system involvement)
- family history
- organ evaluation (MRI, EKG)

- metabolic tests (blood, urine, cerebrospinal)
- molecular genetics (DNA)
- tissue biopsy (skin, muscle, liver, heart)
- biochemistry (ox-phos studies, electron transport chain analysis)

Other tests on blood, urine, cerebrospinal fluid may include CPK, lactate & pyruvate, ammonia, plasma amino acids, plasma acetyl-carnitine profile, plasma carnitine, urine organic acids, & DNA studies.

There are a number of tests available to help diagnose Mito, and these are all listed on the slides accompanying this discussion. Among these tests are:

- Tissue (i.e. looking at muscle under microscope)
- DNA testing of blood (mitochondrial DNA, however, is best done on tissue samples rather than blood)
- Electron transport chain studies on fresh muscle tissue
- Energy assays on muscle tissue
- Protein studies

While all of these tests can be complicated, it is also important to identify if a patient has Mito and its extent, so that physicians can attempt to treat, predict the course of disease and screen additional family members as appropriate. Hopefully newer treatments will be developed to treat specific Mito diseases/symptoms.

Another issue in making a diagnosis is that muscle biopsy in children is often negative - in other words the muscle tissue looks normal even when an underlying mitochondrial defect is present. Currently DNA testing options are expensive, although right now can be very expensive but hopefully within the next 10 years this will become more affordable.

Heteroplasmy can make tissue DNA diagnosis difficult because the amount of normal DNA and damaged DNA in any tissue sample may differ. While blood cells have a small amount of heteroplasmy, urine is a little better when testing for DNA. Saliva has also been used recently and seems to provide a good sample for DNA testing.

Muscle biopsy even using an electron microscope can also be difficult because often some muscle cells will look normal. Special electron chain assay tests are being attempted; however, discrepancies in laboratory findings do exist. The kind of tissue best suited for diagnosis depends on whether the Mito defect is nuclear DNA (blood is better) or mtDNA (muscle or electron transport chain studies are better).

Finally, a common lab finding in cells are the ragged red fibers in muscle tissue, as shown in the example on the slides.

In summary, diagnosis of a mitochondrial disorder must include the full clinical picture as well as a variety of biochemical markers and defects. Additional articles written by Dr. Haas and others (referenced in the slides), discuss diagnosis at length and categorize symptoms so that Mito can be classified into several different categories:

definite disease, probable disease, possible disease, and unlikely disease. Ideally, patients fit into these categories depending on their symptoms.

The Future

Research in the field of mitochondrial medicine is progressing at a rapid rate and hopefully will lead to better diagnosis and treatment. We now know that mitochondria move back and forth between a network and being fragmented; these networks form connections then break - called fusion and fission. This occurs inside the cell all the time. It is not a static state as once believed. For example, it is now known that a specific blindness in Mito disease is caused by a fusion problem. Autism may also be one of these diseases caused by a disruption in this fission/fusion action.
Much research still needs to be done but much has been done and hopefully both testing and treatments will become more available to those with mitochondrial disease.

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