Summary – Muscle Biopsy Testing for Mitochondrial Disease
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Why are muscle biopsies done? Understand first that mitochondrial disease is not one disease, but rather is a group of diseases which are characterized by problems of decreased energy production. Patients (with suspected mitochondrial disease) who come to a physician seeking care generally fall into two categories: classical clinical symptoms which are fairly clear - these patients can fit into one of the subsets of mitochondrial disease (MELAS, for example). A blood test can confirm the diagnosis, along with newer genetic testing which can further confirm the diagnosis.
Most patients, however, have a range of symptoms that affect everything from the autonomic system to the cardiac system. These symptoms may all be linked but do not initially or easily fall into a clear mitochondrial disorder. When faced with such an array of complex symptoms, it is difficult to diagnose a distinct disorder. The gold standard for diagnosing mitochondrial disease has been the muscle biopsy.

What is a muscle biopsy? To test for mitochondrial disease we need to look at tissue that is energy dependent. Muscle tissue is certainly energy dependent and more easily accessible than cardiac or brain tissue! During a muscle biopsy, a small sample of muscle tissue is removed and inspected; histological examination looks at the structure of the muscle and its characteristics, especially looking for "ragged red fibers". However, histology can be normal in some mito patients. The energy production pathway can also be examined through study of the enzymes (enzymology) It is important to note that muscle biopsies are not absolute - there are false positives as well as false negatives. In addition, some conditions such as Alzheimer's and Parkinson's Diseases result in a "secondary" mitochondrial disease.

Muscle biopsies can be "fresh" or "frozen." Since there only a few centers across the United States who can do these biopsies (fresh), many are done as "frozen" biopsies. Even then, having a muscle biopsy often entails travel and expenses for the Mito patient and his/her family. Fresh biopsies are considered better because they are more likely to pick up the underlying disease. For example, at the Cleveland Clinic, 20% of the cases would not have been found had a frozen biopsy been done rather than the fresh biopsy. The results are still not absolute and the cost factors for the histological and enzyme studies can be significant. Typically, most insurance policies will pay for the muscle biopsy, ($5,000-10,000) but some may not; additional tests may be added to the muscle biopsy which could increase costs (costs have been reported to be as high as $50,000). Co-pays and out-of-network costs also need to be considered.

Muscle biopsies are invasive procedures and when done on children require general anesthesia. Also, muscle biopsies do not differentiate between primary and secondary mitochondrial disease. Primary disease is a disorder of the gene that produces the protein - the mitochondrial protein here does not work. In secondary disease a gene has mutated that is not related to protein and energy production but somehow effects energy production.

Buccal Swabs Research is currently underway to develop another means of diagnosing mitochondrial disorders. This method uses buccal swabs - that is, taking a small tissue sample from the inside of the cheek using a cotton swab-like instrument and then doing an assay on the tissue sample. The initial results are very promising. Of the 26 patients who had also had muscle biopsies, 23 of the buccal swab tissue assays directly coordinated with the biopsy results. The other three happened to all come from the same laboratory so it is possible that there was a problem at the lab rather than faulty assays. So far, the buccal swab assays have been done for Complex 1 and Complex 4 only. There are five (5) complexes to be considered in Mito Disease. A complex is simply a group of chemicals of the electron transport chain which act together to produce energy. This research is still a small study but will be expanding soon to be a full fledged clinical trial. In her own practice, Dr. Kendall generally does not use muscle biopsies for her patients for these reasons: 1) high cost, 2) intrusiveness of procedure 3) results are not absolute.

Diagnosis through genes Probably the most promising trend in mitochondrial diagnosis is identifying the genes involved in the disorder. The definitive Mito diagnosis can be made by finding the actual gene mutation that causes the mito disease. When you look at patient populations, for example those with Complex 1 disorders, there is a whole range of symptoms and all are linked to a genetic cause. It is important to know both the cause of the symptoms as well as what the disease means to the individual patient who may have the various symptoms. As more clinical trials are done, we will be able to target specific treatments for specific symptoms based on the genetic cause of the disease.

Genetic diagnosis is important for treatment. For example, if we had 50 patients with Complex 1 (all with various symptoms), we might do a drug trial and find that only 2 patients respond favorably to drug X. On the surface, this would seem to mean that drug x is not a good drug to use. However, if we had a genetic diagnosis, we might find that the 2 patients who had positive responses to drug x had exactly the same gene mutation. This is how genetic diagnosis can guide treatment.

In the past we have only been able to look at mitochondrial genes, those passed on through the maternal side since there are a limited number (13) of genes there. Most cases of Mito disease, however, are caused by nuclear mitochondria which come from both parents. Currently, laboratory research is being conducted at companies such as MEDomics to develop panels that screen for all of these nuclear genes. The results from this research could be available in a little as 6 months to a year, so that diagnosis can be made through blood tests and based on genetic markers rather than muscle biopsies.

Summary In the past, muscle biopsies have been the gold standard for Mitochondrial diagnosis mostly because of our limited ability to screen for nuclear genes. Soon, however, we will have the ability to screen for these. Also, with the development of more conclusive enzyme testing, fewer muscle biopsies will be used for diagnosis. Mitochondrial medicine is evolving. Ten years ago, the test for Cystic Fibrosis was the

sweat test; but now we know where the CF gene is so we go straight there and look for it for diagnosis. Emerging trends in mitochondrial medicine will give us greater understanding of the functionality of the complexes and may even make muscle biopsies obsolete over time.

If a muscle biopsy is done, patients should read the release form carefully to be sure that they "own" or control the tissue sample and do not relinquish control to the laboratory. Of noteworthy importance, the Mayo Clinic is developing a "Mito bank" in order to collect and catalog blood and tissue samples from mito patients in order to expedite future research. For more information, visit their website at: http://mayoresearch.mayo.edu/mitochondrial-disease-biobank/

For all patients, knowledge is power; the most important thing you can do is find out all you can about your (or family members) disorder. You must be comfortable with what you are told to do, ie, with your treatment plan.