

Summary: Genetics, Genomics, & Mitochondrial DNA Testing

By Dr. Richard Boles, Director of Metabolic & Mitochondrial Disorders Clinic at Children's Hospital, Los Angeles, Associate Professor of Pediatrics at the Keck School of Medicine at USC, and the Medical Director of Courtagen Life Sciences, Inc

His discussion, summarized here, is accompanied by slides ([Tools for Testing Mitochondrial Disorders: The Latest Advances in Genetics & Genomics](#)) as well as a short video. Dr. Boles answers questions about this new field of DNA sequencing and genetic testing, including:

What are Mitochondria? The Mitochondria can be considered the body's "life force" because they are necessary for energy production.

What is Mitochondrial Disease? Genetic defects affecting the body's ability to make ATP (energy) are termed Mitochondrial Disorders. Mitochondrial disorders include a very large category of disorders; while hundreds of mitochondrial defects have been described, there are probably thousands more not yet classified.

What about mitochondrial DNA and nuclear DNA? Of about 1,100 genes that encode the proteins that make up a mitochondria, only 37 genes are encoded by mitochondrial DNA (mtDNA). All of the remaining genes are encoded by the DNA found in the nucleus (nuclear DNA, chromosomes). Even though mtDNA has only 37 genes compared to almost 1,100 nuclear-encoded mitochondrial genes, because of the rapid mutation rate of mtDNA, many patients have mtDNA mutations. Thus, there both the 37-gene mtDNA and the 1,100 nuclear-encoded mitochondrial genes need to be sequenced in many cases in order to make a precise diagnosis of mitochondrial disease.

While mutations causing disease can be found in the nuclear DNA (nDNA) (chromosomes) or the mitochondrial DNA (mtDNA), regardless of the site of the genetic defect, the fundamental component of mitochondrial disease is improper energy production and energy metabolism.

These conditions are genetic, although many families have only one affected person. Even when familial, with every relative affected in a different manner, the connections are difficult to see. Signs and symptoms may come and go in different parts of the body, depending on the energy demands of each tissue type or organ from moment to moment. Unfortunately, due to the variability of symptoms and the complexity of the diagnosis, patients are sometimes dismissed or labeled "psychiatric." Add to this complexity the fact that there are over a thousand genes that code for a mitochondria, and one can see how difficult it can be to make and confirm a diagnosis of mitochondrial disease. Consequently, there are still many patients with mitochondrial disease that still do not have a genetically defined diagnosis. We are, however, finally at a time and place where a much higher percentage of patients are able to obtain a "real" genetic diagnosis. *(See slides for maps of energy metabolism which show just how complicated it is to make energy, and therefore, to formulate a diagnosis.)*

Mitochondrial Genetics (The Basics) Of the approximate 1,100 proteins in the mitochondria, 37 are encoded on the mtDNA, which is located inside the mitochondria. Thirty-seven genes may not seem like many BUT, because they were derived from bacteria, they have a very high mutation rate. These 37 genes code for 13 proteins, which are translated on intra-mitochondrial ribosomes. All of these proteins are components of the electron transport chain. The inheritance of mutations in these genes is maternal, as they come only from the mother.

All of the other ~1,100 mitochondrial proteins are coded on the nuclear DNA, translated on cytoplasmic ribosomes, and imported into mitochondria. These 1,100 proteins include most of the components of the electron transport chain, Krebs cycle enzymes, other metabolic enzymes, import mechanisms, transporters, the machinery that replicates, reads, and proof-reads mtDNA, regulatory factors, and many others. Inheritance of mutations in these genes is generally autosomal recessive (as in most other metabolic disorders), although some are autosomal dominant, X-linked, or inherited in other manners.

It is primarily because of the genetic complexity that the diagnosis of mitochondrial disorders is so complex.

How do we get an exact Diagnosis? Mitochondrial Medicine: Diagnosis

Molecular Diagnosis (slide #18 shows what was available about 2 years ago)

- *Standard mtDNA Analysis: PCR (Common Point Analysis)* only tests for small minority of known mutations. Tends to be negative in the vast majority (94% according to one study) of children with suspected Mito disease.
- *Full mtDNA Sequencing:* For cases with maternal inheritance & negative standard mtDNA testing.
- *Nuclear DNA Testing:* Useful in certain phenotypes (see slides).

Sanger Sequencing (slide # 19)

- Sequencing costs have decreased substantially (see graph shown on slides). Ten years ago (around 2002) it cost hundreds of millions of dollars to sequence one person's DNA, whereas now it costs about \$2000.
- Slide #20 shows a sample of DNA sequencing.

The Emerging Standard (Today) "Expanded DNA Testing"

- mtDNA Sequencing - the full molecule; Mito-exome Sequencing, all 1,088 MitoCarta genes
- "Next Generation Sequencing" (See sides #22-24)
- The value of the test is only as good as the value of the interpretation. Advanced interpretation is now possible due to sophisticated computer analysis.
- In addition, technology is now less expensive but more sensitive. The difficult part is evaluating over a thousand variants per patient to find possibly one real mutation causing disease.

Our Solution -two (2) tests

Slide # 25 is a Courtagen slide and demonstrates how new mitochondrial testing offered by Courtagen Life Sciences, Inc. can now provide much more sensitive DNA sequencing

Mito disease is caused by mutations in both genomes:

- Mitochondrial DNA contains thousands of copies, 37 genes, and a high mutation rate;

- Nuclear DNA (from both parents -two copies) consists of at least 1,088 genes and has a low mutation rate.

- Now analyzing and sequencing the 37 genes in the Mito genome (mtSEEKPDx™ Test) **and** 1,100 genes in the cell Nuclear DNA associated with mito function (nucSEEKPDx™ Test) is possible. Together these test ALL known proteins in the mitochondria. The vast majority of all mitochondrial proteins have been identified.

Bioinformatics -Simplify the data The power of the new technology is in the ability to first sequence the genes, then to compare against references. Sequences are compared to known reference sequences and differences are identified. Finally, a process of filtering data results in about 30 variants per sequence/person that may have some association with disease. The machine actually identifies about 1,000 variants among the 1,100 genes, and then implements software to further filter down to the approximately 30 rare variants in each patient. These sequence variants are then evaluated based on a number of parameters (suspected change to protein function, evolutionary conservation, frequency in the human population, suspected mode of inheritance, clinical manifestations, and the perceived accuracy of the laboratory data). Obviously, it is the power of the computer that allows us to now perform not just the sequencing but the filtering process also. In the end, the ultimate question is “Does this change cause disease?”

Interpretation, which is essential, is ultimately performed by the physician who acts as part of the diagnostic team (not necessarily the physician caring for the patient). What do we know about this gene where the protein function changes have occurred? Is there one mutation, such that the patient could be a carrier or affected with dominant disease, or are there two mutations causing recessive disease? This interpretation by the physician is a critical step, is difficult work and takes much thought. In addition, it is here that the clinical history specific to the patient is important. In other words, a diagnosis cannot be made on the genetic data alone, as the patient’s clinical (symptoms, family history, biochemical labs) information is necessary to interpret the genetic findings.

One other aspect to consider is that most of the studies have been done in the US or Europe so the data base actually only defines groups of people representing a specific and limited geographic group. Knowing about the ethnicity of groups of people or where they are from will become important as this field expands so that comparisons in the database can be made. What is "normal" for one group of people may not be for another.

The data will be mined over time to compare sequence changes with clinical findings. For example, Dr. Boles has previously published some common genetic changes that predispose towards the development of cyclic vomiting. The thousands of variants found among all patients tested with cyclic vomiting will be compared on a computer with control (normal) DNA sequences, to identify additional variants related to cyclic vomiting. Thus patients tested by this technology in which such a variant was found would learn about this development at a later time, possibly months or years after the test was performed. Courtagen is setting up the infrastructure to mine the sequences on patients with the following conditions:

- Seizures
- Autism
- Chronic pain
- Chronic fatigue or exercise intolerance
- Intestinal pseudoobstruction
- Intestinal failure (on TPN)
- Hypoglycemia
- Myopathy

- Cerebral degeneration (loss of mental abilities)
- Mental retardation (static abilities)
- Cyclic vomiting
- Mood disorder (depression, bipolar)
- Cardiomyopathy

Additional conditions will likely be added as well over time.

The nucSEEKPDx™ assay also sequences about 100 non-mitochondrial genes that cause disease that can LOOK like mitochondrial disease, including Angelman, Rett, peroxisomal disorder, congenital defects of glycosylation, disorders of creatine synthesis, some other metabolic disorders, neurotransmitter conditions, channelopathies, glucose and creatine transporter defects, and many others. By doing so, this test can replace a spinal tap and comprehensive metabolic work-up, in most settings.

Courtagen Life Sciences, Inc (See slides # 30 +). Courtagen Life Sciences, Inc. validates the studies and does significant data mining so that the interpretation is as accurate as possible. One of the slides shows a sample report that might be sent to an MD which demonstrates how the MD can interpret the results and what can be done for treatment. A link to the NIH website is also provided.

How is a patient tested? Patient samples are collected via a "spit & send" method. A kit is sent to the patient who then literally spits saliva into kit and sends it off in a package that is provided. There are very few restrictions to this type of saliva sampling (the only requirement is that the patient not have eaten for about 20 minutes prior to the test). No blood testing or fasting is required. Through Courtagen, test results are available in about six to eight weeks, and include a simplified explanation and interpretation of the results, along with clinical recommendations when appropriate. For patients unable to provide saliva via a "spit" (for example, an autistic child), a blood sample can be used.

The patient's treating/referring physician must order the test (by email, phone call, etc). (Federal law requires that a doctor order all diagnostic tests). Insurance coverage of nextgen sequencing still varies from state to state. In addition, patients and their physicians often question how to proceed when children or multiple family members appear to be affected. This may depend on the inheritance pattern. Usually, if mitochondrial disease first manifests in infancy and is severe, it is most likely related to recessive inheritance pattern (consequently, nuclear DNA would be tested first). When symptoms occur later in childhood or are relatively mild, frequently the mitochondrial DNA will be tested first. If two siblings are affected, then typically the one more severely affected is tested first.

How Courtagen helps navigate the "minefield" of insurance coverage:

Courtagen Life Sciences, Inc. will:

- take on the burden of preauthorization testing protocol since it varies state by state as well as by insurance company
- set up contracts whenever possible with insurance companies for both pre and post testing
- establish programs to cap out of pocket costs for patients for non-covered items (this too varies by state)

Test results are sent to the physician who then can share with the patient/family. The genetic samples are kept since this is a new field and discoveries are made every day. If a new disease-related gene is discovered down the road, then a patient's sample can be run again and compared

against new data.

Questions that remain Though this expanded DNA testing does not totally eliminate the usefulness of muscle biopsy, it significantly reduces the number of patients for whom biopsy might be useful. For example, someone who has a severe muscular disorder might still need/want a muscle biopsy, but for others, genetic testing generally will replace the need for a biopsy.

There is still some disagreement on how or when one can be sure the Mito disorder has been caused by a single gene mutation or by multiple mutations - even those at NIH cannot agree. There is also discussion about how much laboratory/clinical data should be gathered prior to genetic testing. Dr. Boles recommends a middle path: gather some clinical data, then proceed with genetic testing, and do more clinical testing as needed after genetic studies have been done. Also, he believes that as time goes on and the database is expanded, more data will be available for disorders caused by multiple mutations (polygenic disease).

Summary All the mitochondrial genes are sequenced (about 1,100) as well as several look-alike conditions. Some other companies may be able to sequence all the genes in the body (exome sequencing), but they do not filter, interpret, and mine the data as sensitively, carefully, or persistently. The sensitivity to find mutations in mitochondrial genes also is substantially lower in exome sequencing. The Courtagen method endeavors to very carefully identify and interpret all genes related to mitochondrial disorders.

The system is still not perfected, but work continues. Some patients have multiple genes and mutations that cause disease that may not have been seen before. As these are collected and added to the database, they will help further diagnosis and understanding.

There are several potential advantages of this kind of sequencing; among them are

- establish/prove an exact diagnosis (justify early treatment & precautions, limit further testing, & get answers)
- determine mode of inheritance
- help guide therapy (what cofactors are likely to work, suggest new treatments)
- an investment in further knowledge

Though today there is no cure for mitochondrial disorders; however, better diagnosis leads to better treatment.