Tools for Testing Mitochondrial Disorders: The Latest Advances in Genetics and Genomics

- What is genomic sequencing and how does it change testing for mitochondrial disorders?
- Is NextGen testing appropriate for all people with suspected mitochondrial disease?
- How can DNA sequencing change information available about family inheritance of mitochondrial diseases?
- Do advances in genomic sequencing impact treatment options for Mito patients?

Richard G. Boles, M.D.
Associate Professor of Pediatrics, Keck School of Medicine at USC
Division of Medical Genetics, Children’s Hospital Los Angeles
Medical Director, Courtagen Life Sciences, Inc.
“Any sufficiently advanced technology is indistinguishable from magic.”

Clarke’s Third Law
Potential Conflicts of Interest

I wear many hats

Medical Director of Courtagen Life Sciences Inc.
- Test development
- Test interpretation
- Marketing

Researcher with NIH and foundation funding
- Studying sequence variation in mitochondrial genes that predispose towards functional disease
- Treatment protocols

Clinician treating patients
- Functional disease (CVS, autism, etc.)
- Other mitochondrial, metabolic, and genetic disorders
- General pediatrics
What Are Mitochondria?
What Are Mitochondria?

Ask the Wookieepedia!
What Are Mitochondria?

Ask the Wookieepedia!

Midi-chlorians were intelligent microscopic life forms that lived symbiotically inside the cells of all living things.

"Without the midi-chlorians, life could not exist, and we would have no knowledge of the Force. They continually speak to us, telling us the will of the Force." - Qui-Gon Jinn
What Are Mitochondria?

Don’t they look similar?
What Is Mitochondrial Disease?
What Is Mitochondrial Disease?

Genetic defects affecting the body’s ability to make ATP (energy) are termed “mitochondrial disorders”

Mutations can be in the nuclear DNA (chromosomes) or the mitochondrial DNA (mtDNA)
What Is Mitochondrial Disease?

These conditions are genetic, although many families have only one affected person. Even when familial, with every relative affected in a very different manner, the connections are difficult to see.

Signs and symptoms come and go to different parts of the body depending on the energy flux of each tissue in each minute. Patients are often not believed, or thought to be “psychiatric”.

In addition to the 37 genes on the mtDNA, there are at least another 1,088 genes in the nucleus that encode proteins which are imported into the mitochondria.

Most patients do NOT have a real diagnosis!
Simplified Map of Energy Metabolism
mtDNA

- 16.6 kb
- 37 genes
  - 13 proteins
  - 22 t-RNAs
  - 2 r-RNAs
- 1 kb control region
Mitochondrial Genetics
The Basics

▷ 13 proteins are encoded by the mtDNA and translated on intra-mitochondrial ribosomes.
  • Inheritance of mutations in these genes is maternal (AKA mitochondrial, cytoplasmic), although some are new mutations.

▷ Of about a thousand proteins in the mitochondria, all of the others are coded on the nuclear DNA, translated on cytoplasmic ribosomes, and imported into mitochondria.
  • Inheritance of mutations in these genes is generally autosomal recessive (as in most other metabolic disorders), although some are autosomal dominant, X-linked, and trinucleotide repeats,
How Do We Get an Exact Diagnosis?
Mitochondrial Medicine

Diagnosis

- Molecular diagnostics
  - Standard mtDNA analysis
    - PCR for common point mutations (3243A>G, 8344A>G, 8993T>G or C)
    - PCR or Southern blotting for large rearrangements
  - However: the above only test for a small minority of known mutations. They are negative in the vast majority (94% in one study) of children with suspected mito disease.
  - Full mtDNA sequencing (for cases with maternal inheritance and negative standard mtDNA testing)
  - Nuclear DNA testing is useful in certain phenotypes (COX deficiency, MNGIE, mtDNA depletion or multiple deletions, AR/AD CPEO/KSS, etc.)
Sanger Sequencing - ABI 3730XL
Sequencing Costs Have Decreased Substantially

Sequencing Cost / 30x Genome

Moore’s Law

$100,000,000
$10,000,000
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$10,000
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Mitochondrial Medicine

Diagnosis – The Emerging Standard

- mtDNA sequencing – full molecule
- Mito-exome sequencing – all 1,088 MitoCarta genes
Next Generation Sequencing – Illumina MiSeq
Next Generation Sequencing: Parallel Sequencing by Synthesis

- Bind single DNA molecules to surface
- Amplify on surface

~1000 molecules per ~1 um cluster
Our Solution: Two Tests

- **Mitochondrial Disease is caused by mutations in both genomes:**
  - Mitochondria DNA – thousands of copies, 37 genes, high mutation rate
  - Nuclear DNA – two copies, 1,088 genes, low mutation rate

**mtSEEK Test**
- Analyse 37 genes in the Mitochondrial Genome

**nucSEEK Test**
- Analyse 1,100 genes in the Cell nuclear DNA associated with Mitochondrial function
Next Generation Sequencing – Illumina MiSeq
Bioinformatics – Simplifying the Data

• Sequences are compared to the human genome reference sequence and differences between the reference and the test sequence are identified.
• Each sequence will produce about 3,000 variants
• The data are filtered for:
  • coverage (how many times each base was measured) - removing variants that have not been sequenced enough times to provide confidence in the result
  • variants located in the intron regions
  • variants that are synonymous – change a nucleotide, but do not result in an amino acid change
producing about 300 variants per nucSEEK sequence
• Final filter: all common variants present in 1% or greater of the population are removed – leaving about 30 variants per sequence that may have some association with disease.
Bioinformatics – Interpreting the Data

- Sequence variants are evaluated for predicted pathology = the likelihood that a variant adversely affects protein function:
  - prevalence – how frequent the variant is in humans
  - conservation – how common mutation is in other species
  - protein function – predicted by 3 computer algorithms
- Suspected mode of inheritance: dominant, recessive, unknown versus the number of probable mutations found.
- The patient’s phenotype – clinical manifestations.
- Any laboratory or other data provided.
Major Human mtDNA Haplogroups

www.mitomap.org
## mtSEEK Validation Study

Algorithm calls on single nucleotide variants of interest

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Number of heteroplasmic single nucleotide variants per sample

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\[ P = 0.03 \]

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nucSEEKPDX™ Sample Report

Assay: nucSEEKPDX™ Comprehensive Sequence Analysis of Nuclear Mitochondrial Genes

Richard G. Boles, M.D., Medical Director
David Novell, M.D., Laboratory Medical Director

Assay: nucSEEKPDX™ Comprehensive Sequence Analysis of Nuclear Mitochondrial Genes
Richard G. Boles, M.D., Medical Director

Table 1: Sequence Variants

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List of all lns is (insertions and deletions):
- deletion chr8: 439369890 CCGC > C
- insertion chr1: 127/122015 G > UG
- insertion chr2: 120254737 G > CTGATC
- insertion chr17: 1132340 T > TGGCGTTCAGAGGAGC
- deletion chr1: 43122231 CTT > C

LIMITATIONS
Next-Generation Sequencing for clinical diagnosis is still a relatively new frontier of medicine. Our test may exceed the scope of previously described data published by researchers. While this expanded coverage has increased our breadth of sequencing, variants may be found that have an unclear clinical significance. In these cases, our Interpretation team will research the variant and the change it causes to offer our best deduction for the clinical significance of the variant. As further research is published, and the particular variant's significance is substantially altered in our assessment, we may provide the patient with an appendix to their initial report.

Due to the limitations of Next-Generation sequencing, large insertions and deletions may not be detected. This test was developed and its performance characteristics have been determined by Courtagen Life Sciences. The test has not been cleared by the US Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform high complexity clinical laboratory testing.

Richard G. Boles, M.D.
Medical Director
Courtagen Life Sciences, Inc.
### Table 1: Sequence Variants

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Competitive Features

- Saliva ("Spit and Send")
- More genes: 1,088 versus ~100-500
- Phenocopies: Over 1,300 genes
- Better coverage: > 95% of genes have excellent coverage
- Pseudogene proof: 450 mito genes = 1,513 copies
- Parallel validation and control samples on every run
- Includes deletion testing on mtSEEKPDx
- Turnaround Time: 6 weeks versus many months
- Validated algorithms: Control samples
- Easy-to-understand interpretations and recommendations
- Phenotype-based interpretation: Data mining
- Special commitment towards functional disease
- Courtagen will handle obtaining the authorization
- Testing/interpretation is limited to the listed disorder
Potential Advantages of Sequencing

- Establish/prove an exact diagnosis
  - Justify existing mitochondrial treatments/precautions
  - Limit further diagnostic testing
  - Finally, an answer

- Determine the mode of inheritance

- Help guide therapy
  - Which cofactors are likely to work?
  - Suggest new/different therapies

- An investment in further knowledge
  - Delayed diagnoses/recommendations

- We shall find out!
Mito-Exome Limitations

- ~150 mitochondrial genes are yet to be discovered
- Phenocopies: 200 does not cover all
- 95% coverage is still not 100%
- Promoter and other regulatory mutations
- BioInformatic/interpretation is not perfect
- Some genes might have unrecognized dominant mutations
- Some patients have polygenic disease
What Do I Need To Get Started?

1. A collection kit will be sent by mail for the saliva sample. Results are not affected by diet, treatment, or time.

2. An order for the test from any physician (e.g. “nucSEEK on Juan Garcia”). It’s the law.

3. Authorization from your insurance company. Rules vary by state; Courtagen is here to help you.
What Test?
Who to Test?

- 37 gene mtDNA?
- 1,088 gene MitoCarta genes?
- Both?
- Neither?

- My child only?
- Mom too?
- Siblings?
Maternal Inheritance
Mitochondrial Genetics
Most Likely Mode of Inheritance

- **Infantile onset, severe**
  - Autosomal recessive
  - X-linked recessive
  - Maternal

- **Late onset, less severe**
  - Maternal
  - Autosomal dominant
Mitochondria and Autism

- Autistic spectrum disorders (ASD) are common in children with mitochondrial disease.
- Markers of abnormal mitochondrial function are common in patients with ASD.
  - A minority have frank mitochondrial disease.
  - One small study (10 patients and 10 controls) published in JAMA in 2010 revealed that “children with autism were more likely to have mitochondrial dysfunction, mtDNA overreplication, and mtDNA deletions than typically developing children.”
- ASD and mitochondrial disorders have many of the same co-morbidities, including bowel conditions.
- The relationship between ASD and mitochondria is complex and not well understood.
In children, mitochondrial disease is more common than cancer and muscular dystrophy, combined!

Thank You!
Mitochondrial disease is a “call to arms”