Summary – Genetics of Mitochondrial Disease

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Introduction Nancy Slate is the Research Coordinator for Dr. Sims' Partners DNA and Tissue Repository for Molecular Studies in Disorders of Energy Metabolism at MGH in Boston. She also coordinates research studies and manages the IRB protocols for Dr. Vamsi Mootha at the Center for Human Genetic Research (also at MGH). In addition, Ms. Slate works with Dr. Sims in the Mitochondrial Clinic at MGH as a Mito counselor. Her discussion will try to address the complex issues of genetics and Mitochondrial Disease. The slides which accompany this discussion and are available at the Mito website are attributed to Monica Dandapani from the *Genetic Counseling Teaching Aid Handbook (2008).*

How Mitochondrial Diseases are Inherited To discuss inheritance, we must start by looking at cells, all of which have many different functions.

Slide 1 shows two kinds of DNA in a cell. The Nuclear DNA is organized on chromosomes in the nucleus while the mitochondrial DNA is represented by lots of little circles located in the cytoplasm. Each cell has only one nucleus but many mitochondria even though this slide shows only one mitochondria for demonstration purposes.

Slide 2 shows the nuclear chromosomes numbers 1-22 arranged largest to smallest. These types of chromosomes are called autosomes. When we think of autosomal dominant or autosomal recessive inheritance nuclear inheritance or mendelian inheritance these are the chromosomes that we refer to. The last pair of chromosomes are the sex chromosomes. The sex chromosomes are X and Y; XX being female, XY being male. Usually a person inherits 2 copies of each chromosomes and, therefore, 2 copies of genes, one copy from the mother and one copy from the father. When most people talk about genes they usually are referring to nuclear genes.

We also know that some people may have less than 46 chromosomes, others more than 46. The genes on the chromosomes make the proteins that work in different parts of the cell including mitochondria.

Slide 3 shows mitochondrial DNA or mtDNA which is a ring structure containing 37 genes.; however, we know that there are about 1000 nuclear genes that help the mitochondria function.

One of the jobs that the mitochondria does is to synthesizes ATP from ADP and a phosphate in a process called oxidative phosphorylation or OXPHOS. This is done in steps. Food is taken in and broken down through the citric acid cycle for carbohydrates and beta oxidation for fatty acids. The energy from these processes releases electrons to the electron transport chain or the respiratory chain. The ETC is composed of 5 complexes and each complex has subunits. C. 1,3,4, and 5 have both nuclear encoded and mtDNA encoded subunits. C. 2 composed of 4 subunits is totally nuclear encoded.

- Complex 1 has ~ 46 subunits - 7 mtDNA and ~ 39 nDNA encoded
- Complex 2 only has 4 subunits, all nuclear encoded
- Complex 3 has 11 subunits - 1 mtDNA and 10 nDNA encoded
- Complex 4 has 13 subunits - 3 mtDNA and 10 nDNA encoded
- Complex 5 has ~ 18 subunits - 4 mtDNA and ~14 nDNA encoded

These combinations allow for different types of inheritance of both mtDNA and nDNA.

As electrons flow along the chain the energy produced pumps protons from the matrix, the innermost mitochondrial space, through c.1,3,4 to the intermembrane space. A chemical gradient is established which allows ATP synthesis to occur at c.5 which uses the energy of the protons flowing back into the matrix to attach a phosphate to ADP to produce ATP which the cell uses for energy.

When we speak about mitochondrial disorders, we or talking about disorders of energy metabolism. The electron transport chain is one of the ways that the body can make energy.

So, to summarize, there are 2 kinds of DNA, Nuclear and Mitochondrial and inheritance of each kind is different depending on whether the damaged gene is in nuclear (n) or mitochondrial (mt) DNA.

Inheritance patterns Mito disease may be inherited or sporadic. Sporadic occurrences may be point mutations newly arisen in a person or they may be caused by deletions.

There are theories about the causes, but these are still part of the ongoing research into mitochondrial diseases. In most medical research we proceed from a person's symptoms then eventually look at the cellular level (as with cardiac disease, someone has a heart attack and eventually we look at the heart cells). But with mitochondrial disease, we are working backwards: we know lots about cellular activity but are still trying to learn what this means for patients and their symptoms.

Our focus today is on inherited forms of Mitochondrial Disease. In these forms, nuclear DNA or mitochondrial DNA changes are passed down from parents to children.

Slide 4 shows us an example of *Mendelian inheritance* of Nuclear DNA (nDNA). The chromosome (with the red X) shows a mutation that has made a gene not work on that chromosome. When we look at a cell with a nonworking gene, a protein is not being made.

Slide 5 shows *dominant inheritance* where the pair depicted on the left has one gene that is not working but the pair on the right has both genes working. In a dominant condition both genes are needed for function so it takes only one dose of a nonworking gene to cause a problem. On the right side of the slide, both parents give a working copy to offspring, so no condition is passed on. There is a 50% risk with each pregnancy that a dominant trait will be passed on. A parent with a dominant condition usually shows symptoms; however, other factors such as variable expressivity or reduced penetrance may mask the effect. When one takes a family history you would expect to see some family members in every generation with the condition in some manifestation.

Slide 6 shows *Nuclear recessive inheritance* (most mitochondrial conditions are thought to be autosomal recessive recessive). A recessive condition needs two doses or both genes not working to show symptoms. Both parents carry a copy of the nonworking gene. There are 4 possible outcomes (see slide): 25% risk of no disease, 50% risk of being a carrier, 25% risk of condition resulting. The family history in this case would show a sibship with clinical symptoms.

Slide 7 In Nuclear DNA transmission we can also see *X linked transmission*. If the change is on the X of the mother's chromosomes the risk is to male offspring who receive that X from their mother; the female here is the carrier and she may not show any signs of disease.

XX female, no disease X(X) female, may show no symptoms but is a carrier with nonworking X

XY male, no disease, no carrier (X)Y male, has disease

In X linked inheritance, the risk is that 50% of males will have disease, and 50% of females will be carriers. A family history will show males, on the maternal side, with clinical symptoms.

In summary, there are three kinds of inheritance patterns for Nuclear DNA mitochondrial Diseases: *autosomal dominant, autosomal recessive and X linked*.

Matralineal Inheritance(mtDNA)

Slide 8 shows an egg and sperm. The egg has ½ of the nuclear DNA and 100% of the mtDNA while the sperm has ½ nuclear DNA and no mtDNA in the head. The tail of the sperm has the mtDNA. When the egg and sperm unite the tail falls off and the offspring inherit the maternal mtDNA. So, the female passes on the mtDNA, but in varying amounts. If a male has an mtDNA condition, he will not pass it on to his children.

Slide 9 shows *homoplasmy* - the cell is getting ready to divide. Mitochondria make copies of their DNA and then split in half in a process called fission. The mitochondria is randomly distributed between the two cells. This slide shows mitochondria with no changed mtDNA.

Slide 10 shows *heteroplasmy* - there is a mix of functioning and nonfunctioning mtDNA. Symptoms may occur when parts of the body receive these nonworking cells. The "threshold effect" happens when a system or organ/ tissue needs more energy and the body is unable to supply enough ATP. Some tissues need more energy (ie, GI tract, muscles) than others (ie, skin) and therefore have a lower threshold and will show clinical symptoms sooner.

With mtDNA inheritance, all maternal offspring will inherit the mtDNA, but depending on the *heteroplasmy* (how the different cells get distributed), will determine disease and/or symptoms.

Inheritance Pattern Presentation: Children versus Adults Both the literature and experience indicate that there is a difference in how children and adult are affected by the genetic transfer of nonworking mitochondria. It appears that the more severe the metabolic marker, the earlier the symptoms will appear, and that they will probably be more severe. Examples are Leigh's Disease (inheritance can be through mtDNA or nDNA) which effects children early and severely and is both progressive and neurodegenerative; depletion syndromes like congenital myopia, infantile myopathy and liver failure are other early childhood conditions. GI dysmotility, seizure disorders are also examples of childhood presentations and are usually inherited by autosomal recessive means. Using something called mitomap.org may be helpful to track mtDNA changes. Many mitochondrial conditions that are mtDNA inherited can present in childhood or adolescence (like MELAS), but also can go unnoticed until adulthood when some stressor like the flu, dehydration or metabolic crisis may unmask the condition. In adult onset Mitochondrial disease, symptoms occur much later in life and usually have a more chronic course. Often, we only know conditions exist by taking very careful family histories after the fact of disease or symptom occurrence.

New Testing Methods & Emerging Trends-Before having any Mito testing done, one should ask these questions about what it is you want to find out from the testing...Why are you getting tested?

- Prenatal or family planning
- Knowledge to try to find the gene
- Molecular diagnosis to avoid a muscle biopsy
- Insurance coverage

Regardless of the reasons (and it is good to find a molecular cause if possible), the treatment should not change because treatment should always be aimed at treating the symptoms. One of the newest testing methods is called MitoDx and is done by Dr. Sommer's Medomics. Because mtDNA has heteroplasmy, you need to know that there is enough mtDNA showing up in a sample to pick up disease, and that is why blood testing may not always pick up a nonworking gene if the threshold is too low. Medomics' Next generation sequencing may be able to solve this problem by providing more coverage. Their new Mitonucleome dx clinically available test looks at 174 nDNA genes and

mtDNA genes. This is the most comprehensive commercial test available at the moment. But it still is essential to ask what you are trying to find out with these tests.

As far as research goes, Dr. Mootha's research has identified 1100 genes that have some function related to mitochondria. The goal is to learn more about these genes in the hope of developing clinical tests and new treatment strategies. Mitochondrial Disease is still a very new disease - the term Mitochondrial medicine was first introduced in the 1980's, so as more time passes, we will know much more both for diagnosis as well as treatment.

Summary - Knowing inheritance patterns can help us understand mito disease in families and newer tests like next generation sequencing may help us pinpoint the exact gene that is not working, but we still need to continue to look for treatment options for all patients with mitochondrial disease regardless of causation.