Summaary - BLOOD TESTS FOR MITOCHONDRIAL DISEASE
Dr. Steve Sommers, PhD, MEDOMICS

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On September 16, 2010 we introduce Dr. Steve Sommer, MD and PhD to discuss his company, MEDomics, at MEDomics.com. MEDomics involves using blood tests for diagnosing mitochondrial disease instead of the painful, invasive, and costly test using muscle biopsies. Search for blood tests for mitochondrial disease in the search box on the website.

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A little bit of a background on Steve Sommer. In 1979 he created the Founding Fellows of the American College of Molecular Genetics. They are responsible for the first exam for molecular genetics. His job title of molecular geneticist stands for a DNA diagnostician. He has been doing DNA diagnoses for over 20 years and was a staff member at the MAYO clinic. In 1996 he moved to Pasadena to the molecular genetics/molecular diagnosis department. There he created a DNA diagnostic lab in 1997 and in 2008 started MEDomics to apply DNA sequencing to clinical diagnosis of mitochondrial disease. PCR (polymerase chain reaction) became available in 1985 and the newest technique of NextGen sequencing became available in 2005; these two form the basis of the "diagnostic revolution". Dr. Sommer himself describes his career and MEDomics as "living and breathing mitochondrial medicine".

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MEDomics and Dr. Steve Sommer use MitoDX to analyze the mitochondrial genome at a whole new level called NextGen sequencing. There is an increasing frequency of mitochondrial disease in children, and has been growing since the beginning of mitochondrial medicine about 20 years ago. Now, we are able to diagnose mitochondrial much better and more exactly than before. The reality is that every minute a baby is born that will be diagnosed with Mito before age 10.

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Think of mitochondrial disease as an elephant. Half of the elephant is due to mutations in the DNA that are within the mitochondria. Mitochondria have their own specific piece of DNA that includes 37 genes and 16,500 nucleotides. These genes are what mitoDX addresses and sequences. The other half of the elephant includes genes in the regular nuclear chromosome, that is, genes found in cells' nuclei that are important in mitochondrial function.

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MitoDX uses NextGen sequencing to sequence ALL nucleotide bases of the mitochondrial genome thousands of times. This allows a diagnosis of mitochondrial disease to be made with a blood sample much more thoroughly and reliably. However, in some cases, it still does not find the mutation that can provide a diagnosis of mitochondrial disease.

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Massively parallel sequencing uses different technology to allow the mitochondrial genome to be enumerated so many times that heteroplasmy can be detected at extremely low levels, as low as 1%.

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The big picture of the mitochondrial genome is that some of us have only one type of DNA of mitochondrial DNA within our cells in a given tissue. However, some others have two or more types. Because some of the genomes may differ by one nucleotide or more, there is a possibility for this difference to cause a problem. The percentage of abnormal mitochondrial DNA is key. If, in a cell or tissue, there are 30%, 40%, or 70% mitochondrial genome abnormalities, that may produce an energy bottleneck. If there is an energy stress, there will probably be dysfunction within that tissue. The abnormality percentage threshold depends on the tissue.

When MEDomics sequences all 16,500 nucleotides we ask whether there is any heteroplasmy in any one of those nucleotides. The lab technicians look one by one. If more than 99% of the nucleotides are of one kind, they call the nucleotide homoplasmy. If less than 99% are identical, it is called heteroplasmy. MEDomics technology can find heteroplasmic changes down to 1%.

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Saliva, blood, or muscle tissue can be used to extract DNA. They then do a PCR amplification. MEDomics does something called library construction and emotion pcr amplification. They use technology called slide fire system solid sequencer, priced at $600,000. This makes it possible to generate an incredible amount of sequencing in fact, there is so much data that it takes enormous computing power to process. There is then a bioinformatics group that does the data analysis using software developed over the last year and a half. Following the analysis, Dr. Sommer looks at the data and puts it into an understandable clinical perspective and sends the report to the physician. Sommer provides an interpretation in a report only one a half pages long, similar to a miniature master's thesis. Dr. Sommer calls this process "genostics" instead of diagnostics because diagnostics provide physician with little bits of data that they are the gatekeepers are. In this process, so much information is analyzed that MEDomics' technicians such as Sommer are the physician's partner in

the endeavor because the physician would not be able to keep up with all of the data for each patient.

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At this point, Dr. Sommer would like to skip over the bioinformatics pipe line to discuss the clinical analysis of the first 21 cases referred by mitochondrial doctors to be diagnosed using MitoDX. It is important to remember that this involves looking at only half the elephant, the mitochondrial genome. Our mitochondrial DNA is derived from our mothers. This is very different from the DNA found in the nucleus that is half inherited from each parent. One of the hallmarks of mitochondrial genome disease is that there is a tendency that all the children of a mother will be affected, however, it is often the case that one might be severely affected, the others mildly affected. Mitochondrial disease will affect each one differently. Unless a physician is incredibly skilled, it is easy to miss the possible differences but similar diagnosis of each of the siblings. Another characteristic is that children of a mother will have some overlapping symptoms but have their own differences.

In the 21 cases, 8 of them had heteroplasmic mutations that were very likely or likely to be the cause of mitochondrial disease.

In another 7 they found no evidence of mitochondrial genome disease. This tells the physician to look elsewhere, to nuclear genes or other genetic defects or environmental factors.

The last 6 had heteroplasmic mutations but they were unlikely to be the cause of the symptoms being experienced.

This concludes that 12 out of 21 cases did not find anything likely to contribute to the mitochondrial disease. This in itself is incredibly helpful when being able to rule out another possible cause of mysterious symptoms. This only narrows the search even more.

Using this type of testing, blood is better than muscle to diagnose the disease. In some cases, the physician may have enough to make the diagnosis of mitochondrial disease based solely off of this particular test. However, some others, given these results, may want to reevaluate the case. Either way, MitoDX prevents unnecessary muscle biopsies.

The last group of slides includes 4 patients: Two with definite mutations, one with no mitochondrial disease but mutations, another with no mitochondrial disease and no mutations. There are few facts changed for privacy but this does not change the substance of the findings.

In a few months, MEDomics will be offering a test called MITONUCLEOME DNA GENETICS, which will evaluate hundreds of nuclear mutations that are known to cause mitochondrial disease.

QUESTION & ANSWERS:

Has there been a correlation study between muscle biopsy results and blood sample results?

That work is ongoing. There is no perfect correlation that is part of the art of medicine. MitoDX provides a level of scrutiny not possible before but it has to be put in the big picture of mitochondrial disease diagnosis.

I have a possible diagnosis of mitochondrial disease but a muscle biopsy came back negative. Will this test pick something else up that the biopsy missed?

YES. However,you need to have a discussion with your physician about taking the next step. If you want a second opinion there are resources such as Children's Hospital in LA website that provides a list of professionals. MEDomics does not consult with patients directly. Also, the mitochondrial medicine society provides a partial list of doctors.

How do you approach getting this test with doctors?

You can bring the MEDomics website to the physician's attention, MEDomics.com.

Is there any way the test can determine if you're a carrier? Should you have son tested if he does not have symptoms but your nephew has mitochondrial disease?

For this type of concern, it's best to speak to your physician. MEDomics looks at all the bases so sometimes a child has symptoms and one of the mutations is not found because other mutations can cause the same symptoms. We look at ALL the nucleotides. It would be beneficial because one could confirm or refute a diagnosis using NextGen sequencing.

If a person has spare frozen muscle tissue but could also provide a blood sample, which is preferable?

Both of them would be ideal, especially frozen muscle tissue. Does the possible mitochondrial diagnosis affect the muscle? The most common organ that is affected is the brain but that is hard to test without sticking needles in people's brains which we certainly do not want to do. So, the muscle is used as a relatively accessible tissue but not applicable to all patients. MitoDX can be especially helpful in families where there's

not a major symptom in the muscle. Yes, both samples are good. If you already have muscle saved, use it if the muscle is affected by mitochondrial disease.

On MEDomics.com, is there a list of doctors or centers that are using MEDomics testing right now?

No. Los Angeles Children's Hospital and University of California San Diego are good places that do this testing. Cleveland Clinic and the University of Ontario, Canada Hospital are also other clinics that offer this testing.

My son has mitochondrial disease and I carry the same mutation. We had muscle biopsies done and confirmed the case of a mutation in the mitochondrial DNA. Would your test diagnose more mutations? Would it also determine if there are other nuclear DNA that can contribute to our version of mito? I believe there is something else going on, what can your test show or pick up on?

Sometimes there can be two things going on. Usually it's one thing because these genetic diseases are rare but it raises the question of: Is there something that explains EVERYTHING? As you understand, if there's something on your father's side, that's not going to show up in the mitochondrial DNA. It would be nuclear. It doesn't seem certain the picture is clear in your particular case; MitoDX can provide a clearer picture of what's going on. However, to determine whether it is the right next step for you, consult with a mitochondrial doctor and make that decision together.

How is your test different from the DNA sequencing that was being performed beforehand?

There are two types of DNA sequencing tests that have been available in the past. The first is sequencing for a particular mutation. Then there are a few labs that started offering mitochondrial genome sequencing. But what they did was sequence the 16,500 bases once, maybe twice. They could pick up the major sequences or something that had a very high heteroplasmy. MEDomics sequences it thousands of times thanks to NextGen sequencing. We pick up changes that are as low as 1% of the total mitochondrial genome. We can quantitate much better the data that is found. There are other mutations that might be involved that would have been missed because it was below the threshold. That's where MitoDX comes in and eliminates that problem.

What are the chances that identical twins will have the same mutations in the mitochondrial genome?

Almost always the answer is that their source of mitochondrial disease will be the same. The one's diagnosis should apply to the other. Even in identical twins one might have severe symptoms and the other different/less severe symptoms. One aspect of mitochondrial genome disease, which you wouldn't see this with nuclear mitochondrial mutations, is that you can have identical twins with significantly different symptom severity and some different symptoms to one or the other. And a mito doctor might see that and it would "smell" like mitochondrial genome disease as opposed to mitochondrial nuclear DNA disease.

If someone has mitochondrial disease and there is a nuclear dominance of Warburg syndrome in our family, is there a possibility that it could be a mutation in the nuclear genome as well?

There are labs that perform testing to that syndrome and the symptoms of Warburg syndrome would be different than that of mitochondrial disease. There is a useful website called GENETEST that lists the diagnostic labs in the US on a gene-by-gene basis. You can search for a given gene or disease and you can find the diagnostic labs that have specific testing for it. www.Genetest.org.

Is there a blood test becoming available in the next couple months for the nuclear genome?

Soon, in the next few months. The name of that test will be MitonucleomeDX. The OME stands for the whole and this test would simultaneously test for hundreds of nuclear genes including all of those that have been identified with mitochondrial disease. Until then, there is testing provided by other labs for individual genes. Doctor Shoffner's lab in Atlanta, Baylor College of Medicine, provide a gene-by-gene test.

Does your test differ from a muscle biopsy and/or mtDNA studies and does it make a more conclusive statement?

Yes. First of all, the transgenomic test uses something that's not quite as rigorous as our sequencing. Ours uses sequencing and massively parallel sequencing to provide a greater sensitivity from blood. It is important to talk to a professional familiar with your particular case. We can provide information to the doctor, the doctor who has the transgenomic report and knows the details of your clinical situation. That's the person who has the information to determine whether MitoDX will be helpful. Perhaps there's no uncertainty in that person's mind, then there's no point in doing the MitoDX. If there are remaining questions, MitoDX could very well help. Just a reminder, the mitochondrial genome is half of the elephant. The other half is the nuclear genome, and

as I think at least one of the questions asked, one can "smell" often the presence of mitochondrial genome disease and mitochondrial nuclear disease. It's NOT perfect that that's the art of clinical genetics and metabolic disease diagnosis.

Now it's possible to look at EVERYTHING in the genome, which wasn't possible before. Something coming within the next ten years is genomics rational nutrition battery of tests that will show the connection between biochemical differences of each person relative to metabolism and 99% of the drugs we take through food. All the things that are not part of our biochemistry are drugs. We differ at many places in the genome, and, sometimes due to these differences, meds cause problems. We are working on being able to have ALL genes analyzed so that we can look at the whole elephant.

How important is it to have nuclear genome testing done?

Everything depends on the details. Sometimes biochemical testing can narrow things down, sometimes it can't. If symptoms still cannot be explained and treatments are not working, other testing could possibly help. As with mitochondrial genome testing, if there's some reasonable suspicion you have mitochondrial genome disease, the test would be helpful.

How can my doctor contact you?

Contact us through MEDomics website at MEDomics.com

Is there anything special on the MEDomics website that may be especially useful for patients?

On our media page you can listen to soundbites on LA CBS, Hawaii CBS, Denver NBS that provide an explanation to people that may be confused by mitochondrial disease. Often times it is difficult to provide good information and show how a diagnosis can result in treatment that can make a huge difference in a person's life and health to people not affected by mitochondrial disease.