Hi, my name is Amy Goldstein, I’m the Director of the Neurogenetics and Metabolism Program here at Children’s Hospital in Pittsburgh and today I’m going to talk to you about current diagnosis strategies and treatment options for mitochondrial disease. First I have a few disclosures, I am a member of the Board of Trustees of the United Mitochondrial Disease Foundation, I also do Grand Round speaking for them and go around the country delivering this talk to primary care pediatricians and other clinical care practitioners in order to help educate people about mitochondrial disease and finally I am on the Board of the Mitochondrial Medicine Society where I’m the current Secretary and Treasurer.

So today I’m going to run through some of the neurologic symptoms that children and adults can have when they have mitochondrial disease, I’ll talk briefly about the evaluation and then I’m going to spend the majority of the talk on the genetics and molecular testing especially because this has just skyrocketed in the past two years. I’ll then review the classification system and then review molecular testing in a patient cohort so we can see how this plays out in real practice. And then I would also like to talk to you about the North American Mitochondrial Disease Consortium which are clinical centers of excellence for mitochondrial disease and it will be a way for us to learn more about the natural history, the epidemiology and a way to be organized for clinical trials in the future.

So first we’ll talk about the neurologic red flag symptoms. So many, many people will have neurologic symptoms and it doesn’t necessarily mean that they have mitochondrial disease but this is a nice table and a brief review that was published by Simi Parikh several years ago that helps review
some of these key symptoms. So I’ll just go from the top down. For stroke we look for nonvascular
distribution and the MRI ABC mapping might show a mixture of hyper and hypo-intensity. In the
basal ganglia of the brain we look for bilateral symmetric lesions sometimes referred to as Leigh’s
syndrome. Also children can have brain stem lesions and sometimes these will show up during an
intercurrent illness. Encephalopathy and hepatopathy, this combination of brain symptoms and liver
symptoms is quite characteristic of mitochondrial disease, especially if it’s precipitated by a valproic
acid exposure or Depakote, a commonly used anticonvulsant and this is associated with fulminant
hepatic failure in some of the specific diseases that we’ll speak about later.

In terms of epilepsy again epilepsy is a very, very common symptoms of childhood one that any
child neurologist will see much of in their career, however we look for unusual presentations of
epilepsy, a child that comes in with Epilepsy Partialis Continua or an ongoing simple partial seizure
of one side, especially if it’s associated with a brain lesion; myoclonus or myoclonic seizures and
then status epilepticus, and status especially if it’s the very first seizure that the child has and they
present in status at a very stormy beginning of their epilepsy history. Cognitive decline, especially if
there is regression with illness, any common childhood illness like a viral or bacterial infection such
as strep throat and not just a child who is lethargic or a little mopey but a child that literally will lose
skills, have a regression and then they need to relearn those skills after the illness is resolved.

Ataxia is a very common mitochondrial symptom especially when it is associated with epilepsy or
other systemic symptoms, especially if it looks like it’s a progressive course; neuroimaging might
show cerebellar atrophy or white matter lesions or basal ganglia lesions. Ocular signs can very important and we work here closely with a neuro ophthalmologist and the pediatric ophthalmologist to help us further evaluate the eyes, we look for optic nerve atrophy, ophthalmoplegia and ptosis or retinopathy and some of the diseases have some very specific findings that I’ll talk about later; and then a sensorineural hearing loss especially if it’s presenting at an early age and associated with other systemic symptoms. So again that’s just a red flag review of neurologic symptoms as they can present in the setting of mitochondrial disease. Some of these might be the very first symptoms but commonly they are after some of the more systemic but maybe more benign symptoms will appear such as failure to thrive, or a little developmental delay or a little hypotonia and then you’ll have a more red flag symptom.

Other systemic clinical symptoms can occur and I’m going to take you around this slide. So we look for symptoms that involve the heart, the heart is a very strong muscle but also has its own nerves and so we look for conduction disorders, especially Wolff-Parkinson-White syndrome, again not just an isolation but in a setting of other systemic symptoms especially the ophthalmoplegia and ptosis, other ocular symptoms that I had mentioned, or skeletal weakness. And then a cardiomyopathy and we feel that somewhere between 5 and 10% of all idiopathic cardiomyopathy is probably attributable to mitochondrial disease.

We talked about the eye and the liver already, in terms of the kidney, Fanconi syndrome or a glomerulonephropathy, steroid resistant nephrotic syndrome can be a symptoms if mitochondrial
disease. In terms of the pancreas we see many people with diabetes mellitus especially adults that are not typical for developing diabetes in their older age, it’s not associated with obesity or metabolic syndrome. We also see exocrine pancreatic failure in some of the mitochondrial diseases. In blood we can see marrow syndrome such as Pearson’s marrow syndrome, we discussed the inner ear and sensorineural hearing loss already, in the colon we can see profound dysmotility and pseudo-obstruction, and then we’ve talked about the brain and the muscle already.

And then in the middle of the slide you’ll see this depiction of a mitochondria and the mitochondria as we all remember makes ACP that’s it’s job is to make energy for every cell to run. And it does that through a process of making these subunits from DNA and the DNA is going to come from two places, either the mitochondria’s own mitochondrial DNA which is the circular unit here, or the nuclear DNA which will code for proteins that need to be then imported into the mitochondria and then these two together, the nuclear subunits and the mitochondrial subunits will come together to form the electron transport chain and then that will help undergo oxidative phosphorylation and make ATP.

So in terms of the evaluation of a patient who has mitochondrial disease we do the typical history looking at the family history, especially looking at the maternal side for any suspicious mitochondrial like symptoms. We do the physical exam, and then there are certain metabolic labs that can be helpful. If they are normal it does not necessarily rule out mitochondrial disease but these can be helpful in terms of screening and we can look at blood or urine or cerebral spinal fluid. We
like to look at lactate, pyruvate and ammonia, serum amino acids, in particular the alanine or the alanine to lycine ratio, or other markers of sick mitochondria. The same thing in the urine, organic acids a big marker is 3 methylglutaconic acid or ethylmalonic acid and then we like to look at the carnitine and acylcarnitine profile, the carnitine might be low and the acylcarnitine profile may show either a primary fatty acid oxidation disorder or some secondary problems in fatty acid oxidation because that process is also taking place in the mitochondria. Again none of these biomarkers are 100% sensitive or specific for mitochondrial disease so if they are negative it does not rule it out and if they are positive it could still be secondary to another problem. Then we can do some molecular testing either testing for mitochondrial DNA mutations or looking at the nuclear DNA mutations and I’m going to spend a lot of time discussing this in a few minutes.

And then we can do tissue biopsies and even though that has been the primary mode of diagnosing patients in the past 5 to 10 years again with the advent of better molecular testing, especially of the nuclear DNA genes that are involved, we’ve tried to back off of doing the invasive testing especially thinking about muscle biopsy as a gold standard. That is no longer true. So we can do several different tissue biopsies. We can look at the skin, we can look at the muscle, we can look at the liver. We like looking at tissue that’s very symptomatic, so if a child is presenting with a primary hepatopathy and an encephalopathy a liver biopsy may be the best way that we can diagnose mitochondrial disease by doing some specific testing.
What do we do with this tissue? We can look at the histopathology, especially with muscle or liver, electromicroscopy can be helpful in looking at the mitochondria to see if they are enlarged or if they have abnormal cristae. We can look at mitochondrial DNA sequencing in these tissues of muscle and liver. We can look at the electron transport chain and see if any of the complexes of that chain are specifically deficient or if there is an overall deficiency in all of the complexes. We can also pyruvate dehydrogenase at specific enzymes that can present similarly to mitochondrial disease and in fact is a very frequent cause of Leigh’s syndrome. And then we can look at mitochondrial DNA copy number for depletion syndrome, and I’ll talk about the depletion syndromes in a little bit.

So again going back to that initial cartoon that I showed a few slides ago we have the mitochondrial DNA and then we have the nuclear DNA components and those have to combine for our electron transport or the respiratory chain subunits and those terms are interchangeable, respiratory chain is the same thing as electron transport chain and then these subunits will undergo oxidative phosphorylation to generate ATP. There are 37 mitochondrial encoded genes that will make 13 proteins that are all part of the respiratory chain and then additional 24 RNAs that are needed for that protein synthesis. And then when we look at the nuclear DNA there are over 1500 genes that are nuclear encoded that are very important in the mitochondria for mitochondria functioning and this is not catalogued in the MitoCarta which was catalogued at the Broad Institute in Cambridge, Massachusetts in the Mootha Lab by Vamsi Mootha. So far we have found mutations in all 37 mitochondrial encoded genes and more than 200 nuclear genes and this is still growing at a rate of
about 10 new genes per year being discovered where the mutations are causing pathology and primary mitochondrial disease.

This is the mitochondrial respiratory chain and again I’ve said that the respiratory chain and the electronic transport chain are the same thing. This is a very large cartoon and sometimes this is shown in a more simplified way but this is Complex I, this is Complex II, this is Complex III, this is Complex IV and this is Complex V, and Complex V is a little motor that will generate ATP at the end of the cycle. And as you can see this spans over the intramitochondrial membrane, that’s where this electron transport chain sits and down here is a little chart that shows how many subunits total go into each one of these Complexes. Complex I is the largest being made up of 46 subunits and again that’s here, and then we have a breakdown so we can see that 7 of these are encoded by the mitochondrial DNA and 39 of them are encoded by the nuclear DNA. You’ll see that Complex II is extremely small and it’s all nuclear encoded. Complex III again has 11 subunits, Complex IV has 13 subunits and Complex V has 17 subunits. So if we are going to ask the question what’s the most common Complex deficiency that we see, it’s Complex I because that’s the largest subunit. But again most of these are not mitochondrial encoded, most of these are nuclear encoded.

This is the mitochondrial DNA and again it’s not a very large piece of DNA, it’s not even 17,000 base pairs, so it’s very small compared to our nuclear DNA, but this is a current map of all the mutations that we see in their positions and what diseases are associated with that particular area, and these are the names of the genes and if we go around the circle we can see again many, many
mutations, sometimes causing a syndrome of cardiomyopathy and encephalomyopathy, sometimes the mutations is associated with a known disease entity, some of these have acronyms that will go through, this one is called MELAS which stands for Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-Like Episode. Another common one is LHON or Leber’s Hereditary Optic Neuropathy which is a cause of blindness. And again each one of these disorders is caused by a specific point mutation, a change in the DNA which causes a change in the protein which causes a defect in the subunit and then a malfunction in the subunit and a malfunction in oxidative phosphorylation. Not only will this cause a decrease in ATP production but one of the other very important functions of the mitochondria is to take care of our reactive oxygen species and the oxidative stress that is going on in every cell and so if you can’t take care of those then you’ll have a buildup, a toxic buildup of these reactive oxygen species. And this is where antioxidants may play a role in therapy but that’s also part of the pathology of this disease is that reactive oxygen species causing cellular damage.

One other thing I want to point out before we move on is that if you see this section here it’s labeled Typical Sporadic Deletion in KSS/PEO, which is Progressive External Ophthalmoplegia/ Pearson Marrow Syndrome. So if you are missing 25% of your mitochondrial DNA and these are the typical break points that we see, it’s a 5 kilobase deletion, this is typical of that disorder. And again it usually sporadic, you usually don’t inherit this from your mother in her mitochondrial DNA, we usually don’t see this associated with a family history as opposed to all of these point mutations where there can be a very strong maternal family history. The material family history is extremely
important in the disorders that are associated with mitochondrial DNA mutations, but those are a minority of the syndromes that we see in childhood. Most childhood diseases are nuclear encoded. So we are going to start talking about the nuclear genes.

This is an article from New England Journal that was published last year and this was a current update of all of the genes that we know cause primary mitochondrial disease and again these in red are the ones that are associated with a mitochondrial DNA and all of the others are associated with nuclear DNA, and you can see how they’ve broken this up very nicely into the location of where these genes and their proteins actually are playing a role. And so some of these have a submitochondrial location, some of them are located in the matrix, some in the outer membrane, some in the intermembrane space and then of course the ones that are important for the electron transport chain are located in the inner membrane.

So as I’ve been saying the genetic testing for mitochondrial disease has evolved very rapidly and I just want to take us through a brief history of what we’ve known about the genes and the gene mutations and disease. So starting in about 1988 the first gene mutation was discovered causing Leber’s Hereditary Optic Neuropathy by Doug Wallace and then since the ‘90s and then for about 15 years we could do mitochondrial DNA common mutations for the diseases such as MELAS, MERRF, NARP and Leber’s Hereditary Optic Neuropathy. I’ve mentioned MELAS before, MERRF is Myoclonic Epilepsy with Ragged Red Fibers, NARP is Neuropathy Ataxia and Retinitis Pigmentosa and I’ve already discussed Leber’s Hereditary Optic Neuropathy.
The other test we could then do is the mitochondrial DNA deletions or duplications and the most common deletion again was that 25% missing piece of the circle associated with Kearn Sayre Syndrome and Pearson Marrow Syndrome. And then what evolved over this time period were single gene tests as the genes were discovered, POLG, Polymerase Gamma 1, SURF1, SCO1 and SCO2 and again these genes are all associated with some common pathology and some pretty well known phenotypes at this point in time but there were a handful, literally a handful of genes available up until 2005 and again today less than 10 years later we now have over 200 nuclear genes that are known to cause disease. And then if we had families that had more than 1 affected family member of course we could do some nuclear gene linkage analysis studies to help find the mutation.

Starting from about 2006 to 2011 we could do not just looking for these point mutations but full out mitochondrial DNA sequencing so we were then picking up rare homoplasmic variance sometimes associated with disease but many times not necessarily associated with disease and so it became a question without even the cause of our patient’s disorder. We still had deletion duplication studies, we could then do mitochondrial DNA copy number or copy number analysis on tissue, specifically muscle and liver. And so we would actually try to figure out how much in terms of percent, how much of the mitochondrial DNA was present with 100% being normal and then we could find out if there was a depletion with a decrease in this number, less than 50% of the mitochondrial copy number, or less than 30% of the copy number. There are also times where we see a proliferation where we see more than 100% of the copy number being present.
And then there was evolution of these targeted nuclear panels. So for instance if you did a skin biopsy or muscle biopsy and you knew your patient had Complex I deficiency you could then send a panel of about 10 genes that were specific for Complex 1 deficiency. And again these are the nuclear genes. Or if we found a mitochondrial depletion in the copy number analysis then we could do a depletion gene panel. And again that all changed starting in 2012 because not only did we have nuclear genes that we could do one at a time or maybe 10 at a time doing traditional Sanger sequencing but now all of a sudden we had the ability to do massive parallel sequencing or what’s also called next generation sequencing or even targeted exome sequencing, this is all the new genetic lingo that we are using where we can do 100 genes, 400 genes, even more than 1,000 genes, that MitoCarta inventory is now available to look in blood for all those nuclear mutations. And these are all targets of specifically mitochondrial proteins. And of course we know whole exome sequencing is now available that’s not specific to these mitochondrial genes but any gene in the body we can send whole exome sequencing and find out if there is a mutation and link it back to possibly mitochondrial disease. And what is around the corner is probably whole genome sequencing, not just looking at the exomes but the entire nuclear DNA.

So if we look at this from a historic timeframe and again this is updated as of a year ago we are now up to 37 out of 37 of these genes but it evolved pretty nicely again starting in 1998 and then over the next few decades we discovered all of those genes. And then again beginning in about the mid-90s
we started rapidly detecting nuclear gene changes that are responsible for mitochondrial disease and as I said we are now over 200 genes and counting.

So we are going to talk a little bit more about the nuclear genes. And this is a slide again a paper that published in 2010 but it breaks up very nicely where these nuclear genes are located and then it gives us a hint as to how they could be causing some mitochondrial dysfunction. So this is a much more simplified version of our electron transport chain with Complex I, Complex II, coenzyme Q10 which is a shuttle of electrons between these two Complexes. We have Complex III, cytochrome C, another shuttle, Complex IV and Complex V, and all of the subunits, the nuclear encoded and the mitochondrial encoded for Complex I. Again this is very large so there are many here. Those of Complex II, those of Complex III, some of these you will see are assembly factors so not just the actual subunit but how these subunits all come together to make one unified Complex, the assembly proteins are very important.

We also then have mitochondrial DNA maintenance genes and these are very important. The ones that will help make sure that the mitochondrial DNA is maintained and then can replicate and then can translate, undergo transcription and translation, and then make protein. We also have some genes that are very important in the membrane function and membrane stabilization, iron, sulphur assembly and then channels, channels that come through this membrane and allow the import of anything that’s needed whether we are talking about an ion or a protein or some other native substance. Each one of them has to have its own channel and transporter.
Okay, so the classifications if we look at that last slide the different areas are a gene that codes for structural protein or an assembly factor of those Complexes. Intragenomic communication again just basically means any nuclear genes that then will help control the mitochondrial replication, maintenance and repair, the mitochondrial DNA does not have any genes that will help take care of it. In fact there is only one gene, POLG which is a nuclear gene that helps with replication and making sure that there are no mistakes that are made, it’s the only polymerase that is active in mitochondrial DNA synthesis.

Then we have other genes that are important in mitochondrial DNA, protein synthesis, there is also this concept of mitochondrial dynamics. Mitochondria are not these static organelles that we see in the textbooks, they actually will undergo fission and fusion coming together and breaking apart and coming back together. And they are proteins that are important in fission and fusion and diseases that are associated with any mutation in those proteins. Then we have again proteins that help maintain this lipid milieu of the inner mitochondrial membrane and then the solute transporter carriers across that membrane.

So I’m going to go through these very quickly but by category. So first we are going to talk about these Complexes, and again the genes are listed under there. But then this is again just a very brief sketch of some of the genes that are active for Complex I, Complex II, Complex III, Complex IV and Complex V, and I just want to point out a few things about clinically we see Leigh Syndrome in
some of these, so again Leigh Syndrome is not tied down to just one Complex, it’s a common end point of several Complex deficiencies or gene mutations in the nuclear gene panel order in the mitochondrial genome. Again, very similar we see these symptoms that I’ve already mentioned, cardiomyopathy, hepatopathy, myopathy, encephalomyopathy, the brain and the muscle are involved. Something that’s very interesting, some of the Complex II nuclear genes are not responsible for a primary mitochondrial disease as we know it such as Leigh Syndrome and these other more common symptoms but rather SDHB, C and D are associated with this hereditary paraganglioma and pheochromocytoma syndrome spectrum. Complex III genes have been responsible especially this BCSIL has been responsible for GRACILE Syndrome and Bjornstad Syndrome, but again we go back to our very common symptoms that we are used to seeing for mitochondrial disease. Complex IV can cause Leigh Syndrome, hypertonia and encephalocardiomyopathy. And Complex V has also been associated with cardiomyopathy or epilepsy.

Next I want to talk about coenzyme Q10 synthesis and again CO-Q10 is one of the supplements that we may put a patient on, but it’s a very important part of this electron transport chain helping to shuttle electrons from Complex II to Complex III, and the reason that these are important is because these disorders may actually be treatable with coenzyme-Q10 and these patients usually have a very dramatic response to that treatment. And six major phenotypes have been associated with CO-Q10 and it’s surprising how different some of these are from each other. We can have a very early form which is an encephalomyopathy form with seizures and ataxia, a multisystem infantile form with encephalopathy, cardiomyopathy and renal failure, a cerebellar form with ataxia and cerebellar
atrophy, Leigh Syndrome with growth retardation and isolated myopathy and again a steroid resistant nephrotic syndrome which I mentioned earlier. And this patient may not have any other symptoms so it’s important for a nephrologist or a primary care pediatrician to know about that syndrome and to either check for the gene or see if the patient dramatically responds to CO-Q10. There are probably other genes involved in CO-Q10 synthesis and these are the ones that we know of currently.

Okay, now we are going to switch down to this area, the mitochondrial DNA maintenance and replication genes. And a problem in these genes is going to cause that mitochondrial depletion syndrome and again these are associated with a reduction in mitochondrial DNA copy number. If the mitochondrial DNA cannot replicate there will be a reduction of the copy number, it will be low and it will be associated with a depletion syndrome. And there are three clinical categories in this area, myopathic and these are the genes that are associated with that form; and encephalomyopathic again brain and muscle, and their associated genes; and then a hepatocerebral form where the liver and the brain are both affected, and the genes that are associated with that phenotype.

So some of the symptoms that we see in mitochondrial depletion syndromes and again these are usually multisystemic, many of them can affect the brain, the ear with sensorineural hearing loss, the liver and some of these are associated with primary liver failure and not any other systemic symptoms with some of these genes. The muscle, the peripheral nerve, the intestine especially thymidine phosphorylase causing a syndrome named MNGIE which I’ll speak about in a minute,
and then the kidney can also have some isolated dysfunction with this RRM2B, so again multisystemic presentation for the depletion syndromes. And if we look at this based on age babies will present with lactic acidosis, failure to thrive and hypotonia, and over time we see some of the more adult presentations are cognitive impairment or dementia, psychiatric symptoms and GI symptoms. And then in this middle range of childhood/juvenile we see epilepsy, migraine headaches and liver involvement.

POLG or polymerase gamma 1 is the most common cause of mitochondrial depletion syndrome. As I mentioned already it’s the only polymerase that is helpful in working for mitochondrial maintenance and repair. And this has 6 known syndromes that are associated with mutations in POLG, Alpers Syndrome, or Alpers-Hunterlocher Syndrome which is associated with fulminant hepatic failure especially on exposure to valproic acid for epilepsy; this myoclonic epilepsy myopathy sensory ataxia or MEMSA phenotype, which we see more in the older children, teenagers, young adults, ataxia neuropathy spectrum which is mainly seen in adults; childhood myocerebrohepatopathy spectrum which is extremely rare, and in fact more rare than Alpers Syndrome; and then we see two forms of progressive external ophthalmoplegia and autosomal recessive form and autosomal dominant form. And so what’s unique about the 6 categories is that POLG is usually inherited in a compound heterozygote state where you have one mutation from mom, one mutation from dad, they are mainly autosomal recessive diseases except for this autosomal dominant form of progressive ophthalmoplegia where you only need one mutation and usually one of the parents has this disorder.
This is the polymerase gamma gene as we see all the exons and so far these are all the mutations that have been reported in this disorder and this is kept up to date on a very frequent basis at this website. You can go to this website if you send the POLG genes and you have several mutations come back you can go to this website, plug in the mutations and read about what case reports have been common in the literature and this is constantly being updated because sometimes the genes that we think are pathogenic are reclassified and vice-versa, the genes that we are not sure, maybe there are first seen as a variant of unknown significance we find enough patients with that mutation and it may be reclassified.

Okay next I want to talk about MNGIE and MNGIE is Mitochondrial Neuro Gastro Intestinal Encephalomyopathy. These patients present with ptosis and ophthalmoplegia, GI dysmotility to the point of sometimes pseudoobstruction, cachexia, these patients cannot gain weight and in fact look pretty wasted, a peripheral neuropathy and a leukoencephalopathy, a very diffuse white matter disease. And this is usually disease of young adults, we usually do not see this in young children although dysmotility can be a common symptom in young children so we do test for this quite a bit. It’s an extremely rare disorder, less than 100 patients worldwide, however there is now a clinical treatment trial to look at bone marrow transplant as a cure for this disease.

Okay, now I’m going to talk about these mitochondrial DNA expression genes and this is a very busy slide but I would just like to point out some of the diversity again in this category. We can see
cortical dysgenesis, right, agenesis of the corpus callosum and some dysmorphology. In this one, DARS2, we can see multiple respiratory chain deficiencies, leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation. We can see pontocerebellar hypoplasia and epilepsy, we can see something that looks like Charcot-Marie-Tooth Syndrome, we can see cardiomyopathy, and again a cystic leukoencephalopathy, polymicrogyria. So it used to be thought that if someone has dysmorphology or a dysplastic brain it could not be mitochondrial disease and we are seeing that that is just not true. Some of these translational mitochondrial DNA translational defects again coming from nuclear genes are causing these syndromes.

Next I want to talk about membrane function with some of these very important genes and these genes are also responsible for fission and fusion of the mitochondria and a mutation in some of these genes are associated with optic atrophy or Charcot-Marie-Tooth disease with this mitofusin 2 or autosomal dominant hereditary spastic paraparesis. There are phospholipid defects in the membrane and there is a gene called acylglycerol kinase that causes Sengers Syndrome and has also been now associated with a mitochondrial depletion syndrome specifically a myopathic form. TAZ, this Tafazzin gene is associated with Barth Syndrome and again this is also because of a defect in cardiolipin in that mitochondrial membrane. And then SERAC 1 mutations cause a syndrome called MEGDEL. And this is a cartoon depicting some of these phospholipids in the mitochondrial membrane, our acylglycerol kinase is here and our cardiolipin is here and we can see that a disruption along this membrane would certainly thrust the dynamics of this membrane causing some
solute transporter problems and possibly causing a problem in this ANT transporter which will help get ATP out of the mitochondria where it needs to function.

And then we have the chaperonin or the solute transporter genes and I’ll just mention one of these, the SLC25A22 which is responsible for Otohara Syndrome which is one of the early infantile epileptic encephalopathies also associated with cortical dysgenesis.

So I’ve just told you about all these different genes and what the syndromes are and the question is how does this work? If I have a patient in my office and I want to send these nuclear genes how often am I going to actually detect mitochondrial disease in one of these patients? So when the MitoCarta gene inventory was first derived and then they wanted to put this into practice they took a group of mitochondrial disease patients, suspected patients, and then ran them through this MitoExome, or the MitoCarta nuclear panel. Again over 1,000 genes were tested. And so of these patients they were able to find known disease in 26% of them, candidate genes in another 30% and unfortunately 45% of them were still unknown. No known mutations were found in any of the genes that were screened. So we still have a lot to learn. But the good news is we were able to diagnose possibly over 50% if these candidate genes can be proven to be pathogenic.

So some of the known OXPHOS genes that were found, we’ve talked about them or they’ve been in some of the tables that I talked about including the MNGIE genes, and then they did find one mitochondrial deletion that had been missed on previous testing. The potential new genes and these
are all the candidate genes but they’ve been able to confirm two of them, this NDUFB3 by doing some special fiberglass testing to prove that it was causing a complex deficiency and then the acylglycerol kinase gene which I have already discussed has been associated with membrane stability and Sengers Syndrome by two separate patients that both had a myopathic presentation were included in this group. And so I’m going to talk about these two patients.

So patient number 41 died at 18 years of age, had a cardioskeletal myopathy, had cataracts, failure to thrive, fatigue, had multiple complex deficiencies in muscle biopsy and the muscle mitochondrial DNA copy number was extremely reduced at 4%. So again the depletion syndrome. Patient 42 was a product of a consanguineous marriage which increases the chance that we are dealing with an autosomal receptive disease, died at 4 days of age from respiratory distress, circulatory failure, gestational pulmonary hypertension and cataracts, again had multiple complex deficiencies of I, III and IV, and again had reduced mitochondrial DNA copy number indicating a depletion syndrome.

And then when they looked at these mutations they found patient 41 was a compound heterozygote for AGK and then patient 42 expectedly was homozygous and had the same mutation inherited from each parent and again the AGK was verified by finding two unrelated patients with very similar phenotypes, multiple complex deficiency, weakness, mitochondrial reduction in copy number with a depletion syndrome and this gene is now proven to be a cause of myopathic depletion syndrome.
So next I’m going to switch gears entirely and talk a little bit about therapeutic. And this is a slide that I just feel so demonstrative of all the multiple areas that we can intervene in terms of therapy, the mitochondria are very complicated. And we talked about the respiratory chain here, so certainly one way of treatment is to help increase the respiratory chain’s efficiency, but we can also help by reducing this reactive oxygen species which I mentioned earlier with many, many different compounds that help scavenge this reactive oxygen species or help stimulate cellular antioxidant pathways. We can modulate calcium flux, calcium homeostasis is another important job of the mitochondria and then we can target antiapoptotic mechanisms and the mitochondrial protein pore.

So the news about therapy is not good, there was a very large Cochrane Collaboration review several years ago that was undertaken by Patrick Chinnery who was at a mitochondrial center in Newcastle in the UK and he reviewed all of the publications that had been done for mitochondrial disease therapy. In 2003 he found 678 abstracts and only 6 of them passed scrutiny in terms of being very well designed, randomized control trials. In 2012 there were more than double that number of abstracts and only 12 of them passed scrutiny and 8 of these were new. The bottom line is that there is no proven effective therapy for mitochondrial disease and we know several, you know many of these patients have a fatal course of this disorder. What he found though again was that there were very few randomized control trials, but they were very difficult to compare to each other because the groups themselves were either heterogeneous or there were different dosing of the compounds that were used, or there were different outcome measures used as end points, and most importantly there
is an unknown natural history for many of these disorders so it’s very hard to know if a drug is changing the course when we don’t know what the natural course should be.

So CO-Q10 has been tested and there is the one trial high dose CO-Q and they had 30 patients with DNA proven disease that were given 600 mg twice a day versus a placebo, no clinical improvement. Three studies of creatine with 38 patients, most had a primary DNA proven disease, two studies showed no improvement, one study showed improved hand grip but there were no meaningful outcomes, no improvement in quality of life and there was one study with a combination creatine, CO-Q10 and alpha-lipoid acid which again helped improve strength but it was such low sample size that quality of life measures were not undertaken. There have been 4 studies of DCA and DCA is used to help reduce lactic acidosis, and there were improvement in select biomarkers only, for instance it can lower the exercise lactate spike that is commonly seen, however when this was tried for MELAS there were concerns about peripheral nerve toxicity and the study was actually terminated early. We may still go back and do a DCA trial for PDH deficiency and we are awaiting to hear about funding and that will be a multicenter trial. There have been two other studies, one for dimethyl glycine, one of a whey-based cysteine and again no clinical improvements.

So there have also been studies of this drug called Idebenone which is very similar to CO-Q10 and this was a very long study and this was for Leber’s hereditary optic neuropathy and there were improvements of visual acuity and then post hoc another group actually realized that this was very mutation specific, that one mutation of Leber’s did better than the other two groups and that
prolonged treatment with Idebenone was required to see any changes, so now this is back in trial. It has not been proven, the study has to be repeated.

Certainly in our treatments we can certainly tell people to avoid what are known mitochondrial poisons. We very commonly have people avoid Depakote. If I have a patient that I’m treating for epilepsy and especially if they are young and especially if I don’t know the etiology of their seizures and if there is any concern about delay I will certainly avoid Depakote unless we have another etiology for their disease. We tell our patients especially the older patients and the adults to avoid alcohol, to try to avoid MSG, tobacco, aminoglycoside antibiotics which are associated with deafness and that is due to a mitochondrial DNA point mutation. And then many of our patients have had trouble with Propofol and so we have our anesthesiologists avoid that if it’s going to be given in a prolonged fashion.

One of the other treatments that’s emerging is exercise and there have been several studies which I’ll just run through quickly showing that aerobic conditioning and strength training can not only improve your exercise capacity and fatigue issues but has actually been shown to help improve the mutation load that people might have and help grow better, healthier mitochondria. And again these are just several of those papers showing that aerobic training is very important and as we’ve seen mitochondrial DNA shifting and improvement in exercise parameters.
L-arginine has been a treatment that’s evolved very nicely for the treatment of MELAS and specifically for the stroke. So we are currently using that if someone who has MELAS comes into the hospital having an acute stroke we will give them IV Arginine and then we will send them home on oral Arginine and so far it does look like it’s very helpful in terms of preventing future strokes and these are still in clinical trials, mainly out of the group in Texas at Baylor.

Folinic acid has been a useful treatment, several of the patients with mitochondrial disease have been found to have a secondary cerebral folate deficiency, it’s been associated with mutations in polymerase gamma, it’s been associated with KSS and so we will do a spinal tap, look for the folate levels, the marker is 5 MTHS and if that’s low then we will start them on folinic acid. The symptoms are irritability, sleep problems and white matter disease.

Again I mention MNGIE and stem cell transplant or bone marrow transplant and so far 24 patients have undergone transplant and the induction methods and the chemotherapy have been changing over time for this disease so hopefully more patients will be able to tolerate this procedure. The biggest problem in patients with MNGIE is that they are extremely malnourished because of the massive GI symptomatology that they have and so therefore it’s very important to consider this early in the course before they are so malnourished that they are not going to survive the transplant. And a prospective trial is underway at Columbia University in New York.
So in conclusion, mitochondrial disease is very heterogeneous although there are certain red flags that we can look for. The diagnostic testing has evolved and it’s changed over time so that 5, 10 years ago muscle biopsy was the gold standard and now we are leaning more towards sending blood for these nuclear panels and mitochondrial DNA sequencing. Again the molecular testing has evolved from single gene or small panels to this massive parallel sequencing, so it’s much easier, more time effective, more cost effective to do these panels and look at multiple genes at the same time. And we also need a central repository so that we know how many patients have the same mutation, how can we make sure that variance of unknown significance can be matched up with each other so that we can declare that a gene mutation is in fact pathogenic with similar pathology. And the central repository is coming together with help of the United Mitochondrial Disease Foundation, the North American Mitochondrial Disease Consortium and the NIH, and then something called MSeqDR which is a worldwide genetic collaborative effort putting all the mutations in the same database.

Clinicians need tests that are cost effective, that are reliable, that are meaningful for their patients and their families and will help end this diagnostic odyssey. Many times one of the most troubling things for the family is to still be at this years later with their child who is very sick, 3 years later, 5 years later still without a definite diagnosis. And so doing the gene tests if we can get an answer can be very, very gratifying for the family, it will help confirm the diagnosis, there is an ability to then offer genetic testing. If those parents want to have more children or other family members are concerned about their risks it opens up education, support groups and then of course the clinical registry and natural history studies through the consortium.
We have a potential to offer therapy based on specific molecular pathways and right now there is a study of FE743 which is a vitamin E analog and the study is open only for patients with a molecular diagnosis who have Leigh Syndrome, but you have to have a nuclear or mitochondrial DNA proven gene mutation in order to get into that study. And what we are seeing coming down the pike is lots of effort in drug discovery and treatment based on the specific mechanisms and that’s why I took you through that classification system. We need better biomarkers, we need to find outcome measures to follow these patients over time and to know what outcome measures are going to be important to patients, especially if we are going to put them through a clinical trial and again these clinical trials are evolving through NAMDC.

So I keep mentioning NAMDC, I want you to know what it is. It is again the North American Mitochondrial Disease Consortium. There are many sites throughout the U.S. and Canada. In Pittsburgh we are a site, I believe Boston will be becoming a site very soon, but they are in New York. Again the main headquarters is Columbia University. There are sites in Akron, Buffalo, Cleveland, Dallas, Texas, Gainesville, Florida, Houston, Texas, Hamilton, Ontario, Palo Alto, California, Philadelphia, Rochester Minnesota, San Diego, Washington, DC and Denver. And so what we are hoping for is that patients that are diagnosed with a mitochondrial disease will go to one of these centers and be enrolled in NAMDC so that we have a patient registry. There are several other things that we are trying to do through NAMDC again, the natural history studies, there is currently one for Alpers, KSS that are soon to be up and running, there are several clinical trials.
We’d also like to do pilot studies, there is also a bio-bank associated with Mayo Clinic so if our patients have had a skin biopsy or muscle biopsy or someone dies and they have an autopsy and the family would like to donate tissue the bio-bank is the perfect place for that, so that investigators can then use patient specimens in order for drug discovery. And then of course our clinical registry which will help us understand the epidemiology much better about this disease. There is also a clinical fellowship program associated with NAMDC so if there are any young doctors that would like to have further training specifically in mitochondrial disease we would like to invite you to apply for the fellowship and more information can be found online. And finally, as a joint effort between the clinicians, investigators and the patients in the United Mitochondrial Disease Foundation there is also another very active mitochondrial organization called Mito Action and they also have a very active website, very good following and lots of education online and at support group meetings.

And I’d like to thank my patients who have mitochondrial disease who have taught me everything that I know about mitochondrial disease and more, and with that I’d like to close and please complete your post-test – the post-talk questions to see if hopefully I’ve taught you something through this lecture. Thank you for your attention.