Good evening everybody and welcome to this month’s CMTA webinar. Genetic Testing and Why it is Important. Hopefully we’ll have a good evening with Windows here. Tom Winder is with Invitae. He has been involved with genetic diagnosis for over 15 years and he’s going to be able to really help us understand a little bit more about this process and what it means to us all here – those of us with CMT. Before we get started, I just want to let you know that I’ll be monitoring during the broadcast. On the right-hand side you have an area there for questions, so please feel free to ask any questions that you’d like. Tom was kind enough to say that he will answer questions as we go along, so we’ll have time during the broadcast and a little bit of time afterward to answer any questions that you might have, and that’s always a really good way to do it. If something comes up on a slide here, please raise your hand, and we’ll do our best to get to it right away. If you have any technical questions throughout, you can either use the chat window or the question window. I’ll do my best to help you through any technical issues that you’re having to get started. With that, I’m going to turn the program over to Tom, and I hope you all enjoy what he has to say.

Thank you, Bob. Thanks for the invitation to present here, and I look forward to it. I’m sure we’ll have some good questions. I just want to mention as a way of disclosure, I am an employee of Invitae Corporation. It’s a genetic diagnostic company that performs fee-for-service commercial testing for CMT as well as many other genetic disorders.

The agenda that I want to talk about is really kind of meant to be general and getting more specific as we go. What is genetic testing and why is it important? Also, I’ll do a CMT overview, which to this audience, might not be that important, because I’m sure you know more about it than I do. I’ll talk about clinical perspective, history of CMT because it is quite interesting, and genetics of CMT. It is a complex field and there’s a lot known. Then we’ll talk about Invitae’s testing options for Charcot-Marie-Tooth and then hopefully at the end we’ll have some questions.

What is genetic testing? What do we mean by that? Genetic testing tries to identify in an individual or in biological family members certain gene variants that can cause disease or at least increase the risk or chance of causing disease. You may think that technique has won this battle, that Mother Nature and genetic testing are a simple matter nowadays. However, this is really not the case at all, and we still face many challenges. Charcot-Marie-Tooth genetic testing serves as a very good example of many of these challenges that still exist.

Genetic variation does more than just cause disease. The differences that we notice among individuals, even among close family members, like siblings, result from the unique collection of genetic variation that each of us has. There is one key exception, and that is, of course, identical twins. They have the exact genetic composition, therefore, they appear to us of being identical. However, environment works on those individuals over time, and they may develop behavioral differences and so forth. They
cut their hair different, but they are genetically identical. It’s always interesting to see identical twins and realize the uniqueness of that.

When we talk about genetic testing, we only mean testing for highly heritable conditions as purely genetic or very nearly purely genetic. If there’s a continuum that we can draw, and it shows that genes in the environment have varying amounts of influence on our outward traits—or phenotype. There are traits that result from environmental effects only—our genetic makeup has little or no influence on these traits. For example, if we fail to eat enough vitamin C, we get scurvy. That’s an environmental effect. Similarly, if we’re infected by certain bacteria or viruses or other infectious agents, we come down with a sickness regardless of what gene variants we possess. In the middle of this spectrum, there are traits that are more equally impacted by genes and environment. For example, a man may have heart disease running in his family, but if he takes really good care of himself with diet and exercise, he can potentially avoid the condition.

There are traits that are influenced solely by our genes. For example, when a baby is born with too many copies of chromosome 21 in every cell, he or she will have Down syndrome. Similarly, when someone inherits disease-causing variant in the peripheral myelin protein 22 gene or the myelin protein 0 gene, he or she will have Charcot-Marie-Tooth. Something else to keep in mind is that the laboratories are only good for testing for these single gene traits or conditions. That is diseases that are known to be caused by a single gene or by a small number of genes. The truth is, though, that most common human diseases, for example, coronary artery disease, high blood pressure, obesity, diabetes mellitus—things like this—are influenced by many genes plus by the environment. At this time, the diagnostic field is simply not good at unraveling all these multigene interactions.

Now that we have some idea of what we mean by genetic testing, let’s ask the question “Why is it important?” First, gene testing can confirm a clinical diagnosis beyond any doubt. Second, once a genetic diagnosis is made, other similar conditions can be ruled out. Genetic testing can lead to early diagnosis, possibly even before all the clinical signs declare themselves, and this obviously would enable very early interventions, and that’s very important today because in many disorders, for example cystic fibrosis, early, aggressive treatment results in longer lives. Also, a genetic diagnosis will often allow a clinician to better predict a patient’s prognosis and progression. Genetic diagnosis can help a couple make decisions about future family plans and about screening in other family members. That can be very important. Next, a genetic diagnosis is often required for enrollment into clinical trials or research programs, and some diseases therapeutic drugs are even designed to treat only specific mutations within a gene. For example, when dystrophin causes Duchenne muscular dystrophy. Next, a genetic diagnosis can end or prevent a diagnostic odyssey. Long years and years and years of families taking their children to various experts and specialists. It gets very, very expensive and drawn out and a lot of hope is expended in this process, so if a test is available, it can lead to earlier diagnosis. Finally, genetic diagnosis may help direct treatments and similarly can discontinue ineffective treatments and focus on ones that are expected to actually be effective.

A common question is, “Can my family history tell me the same thing as a genetic test?” The answer’s not easy, but in general, family history is a very important part of the diagnostic process used by
clinicians, particularly neurologists, neurogeneticists, and genetic counselors. The family history helps choose the right genetic test options. It can rule out certain types of inheritance patterns and so forth. I have even heard genetic counselors say the best genetic test is a good family history, so a good family history can go a long way. Later on, I can help point you to a family tree online tool that you can actually use if you’re interested in putting together your family history.

In the next section, I’ll talk a little bit about the clinical perspectives of CMT. In terms of how common or how rare Charcot-Marie-Tooth is, in terms of a genetic disease, it’s quite common. Statistics vary, but somewhere around 1 in every 25 people is affected. If you compare this to another well-known genetic disease, cystic fibrosis, where about 1 in 3500 births in the US are affected, it really is a common genetic disorder. However, in terms of all medical conditions, CMT is rare. A common medical condition is something like coronary artery disease – on the order of 8% of men in the population with have something like that over time. In terms of disease burden, it’s rare. In terms of a genetic inherited condition, it’s quite common.

CMT is also referred to as hereditary motor and sensory neuropathy. Clinically, the main features are weakness and wasting of the distal limbs, pes cavus or high-arch feet, absent or reduced tendon reflexes. Other symptoms are neuropathic pain, skeletal deformities, and respiratory problems.

Question: I was curious if you have identical twins, does it mean if one has CMT, the other will have CMT? Would the severity of the CMT be the same amongst identical twins?

Answer: With identical twins, you would certainly expect both will have CMT. If one has it, the other will have it. For something like CMT, which is considered a purely genetic condition – there’s not a lot of environmental effect, then even with identical twins, the clinical course would be expected to be very, very similar. There could be slight differences, and those would be expected to be from things like nutrition. Things that would vary between the two.

I just want to point out that the nerve conduction velocity test is routinely used to differentiate between these two major types of CMT – axonal versus demyelinating. I’ll talk more about those later. CMT can affect both adults and children. It’s an inherited condition, and treatments include physical and occupational therapy, braces, surgical corrections, and the use of meds to control neuropathic pain.

One thing to think about is what else looks like Charcot-Marie-Tooth? This is the kind of thing that neurologists would consider when seeing a patient for the differential diagnosis. By far the most common condition that causes peripheral neuropathy is diabetic neuropathy. In one study, 4400 patients and half had peripheral neuropathy. These patients were diabetics and half of them had peripheral neuropathy. If you consider that around 10% of the population has diabetes – that equates to a lot of people in the US with peripheral neuropathy due to diabetes – so this is much more common
than Charcot-Marie-Tooth disease. Another type that’s not CMT is inflammatory demyelinating polyneuropathy. This is another CMT-like condition; however, it is an autoimmune disorder. Finally, long-term alcohol abuse, neurotoxic medications and infection can cause CMT-like conditions.

The history of this disorder is quite interesting and involves some important people through history and important events as well. It was first described clinically in 1866 in France by three neurologists – two French and one British gentleman – named Charcot, Marie, and Tooth. Charcot is considered the father of modern neurology. If you read about him, he’s a really interesting guy. For that time, he had very futuristic thinking and looking for ways to treat patients. He trained many neurologists at the time and he was quite big into psychoanalysis and hypnosis as well. He taught the likes of Sigmund Freud in France. The three saw patients at this teaching hospital in Paris. Even today, this hospital is one of the largest teaching hospitals and most well-respected teaching hospitals in all of Europe. It’s quite interesting that this disorder was first described there. Furthermore, the history is important and interesting because it’s really tied in to the whole revolution in human genetics.

The timeline is that this disorder was first described in 1866, but it wasn’t until 1992 when the first CMT gene was identified when the duplication of the PMP22 gene was found and was found to be the cause of CMT type 1A. A year later, the MPV gene was identified as causative for CMT1 Deutsche. What’s interesting is the span of time between the description of the phenotype in 1866 and the first gene discovery. A lot of years went by. There were large amounts of genes being discovered in the early 2000s. That’s because the human genome project was completed in 2001. What this did was allow researchers a better road map of the human genome to be able to find these problem genes.

Another spike of gene discovery occurred in more like 2010 and 2013. It’s still going on. This time in history corresponds to the advent of high throughput DNA sequencing methods, often called next generation sequencing, used to do gene discovery. The history of CMT is really tied to a lot of medical genetics history and landmarks – very interesting.

The Genetics of CMT

We’ll really consider ways that CMT is inherited within a family. Genetic testing and genetics with CMT still poses some challenges. One complicating factor is that three different forms of inheritance are possible. In some families, it’s not clear what the inheritance pattern is. Usually because there’s only one affected individual.

The three types of inheritance that are possible are autosomal recessive, sometimes called horizontal inheritance, because it’s not passed from an affected individual down through the pedigree. It’ll appear in one generation among siblings usually.

The next type is autosomal dominant, sometimes called vertical transmission. That simply means that it’s going downward through the pedigree and several generations can be affected.
The third type is x-linked or sex-linked. This is vertical type without male-to-male transmission. It’s on the X chromosome being passed from a carrier mom and then to an affected son. If it’s passed to the daughter, she’s generally unaffected or less affected, but she can pass it on to sons and daughters as well whereas autosomal dominant, you can have male-to-male transmission.

These three types of inheritances are possible in CMT. To further complicate matters, many different genes are known to cause CMT that follow each of these inheritance patterns. Even if an inheritance pattern is clearly seen in a family, clearly autosomal dominant, that doesn’t pinpoint the exact gene.

The CMT types are very confusing. It’s been described as an alphanumeric soup with all the letters and numbers. . . Part of the problem, I think, is that the types refer to both inheritance patterns and to the nature of the nerve damage – back to the axonal versus demyelinating.

A purple nerve is like an insulating electrical wire on which the copper wire on the inside conducts current and the plastic coating insulates the wire. On the nerve, the axon conducts impulses and myelin, which is really just a mixture of proteins and lipids, coats and insulates the axon. The Schwann cells surround the axon and produce this myelin – protein/lipid mixture which is the insulating component. Nerve conduction velocity can be used in the clinic to differentiate between axonal CMT where the axon itself is damaged – in those cases nerve conduction velocities are normal or just mildly slowed, or it can tell a physician if it’s a demyelinating CMT in which the myelin coating is damaged and nerve conduction velocities are very slow. Of course, because it’s CMT, it’s got to be complicated, there are intermediate types as well.

The most common type of CMT is autosomal dominant demyelinating CMT, or CMT1. The most common genes in this type are PMP22 (CMT1A) and MPV (CMT1B).

CMT2 refers to axonal CMT with autosomal dominant inheritance. The most common gene for this is MFM2 (CMT2A2).

CMT4 refers to autosomal recessive inherited types, regardless of whether they’re axonal or demyelinating. An example is GDAP1 gene (CMT4A). This categorization isn’t used as much today.
The last major type of CMT is CMTX, by far the most common type. Xylem CMT, which is the GJB1 gene and is CMTX1. This gene previously was referred to as Connexin 32.

Question: Does it ever skip a generation and if it does, is that where it ends or does it just skip and continue on?

Answer: It can appear to skip a generation and the cause of that would be a girl inherits it from her carrier mom or from an affected dad and is subclinical – really doesn’t show any signs, although she has the mutated gene in every cell, so she can pass it on. The reason she’s not affected is that, women have two X chromosomes, and each cell will silence one of the X chromosomes in varying ratios. It would just be by chance that a woman who carries a GJB1 mutation had an uneven silencing of the mutated X chromosome, and, therefore, her clinical condition would be very, very mild or even completely normal. That certainly happens. It’s well established in medical genetics this concept, which is named after the person that first described it – Lyonization. Dr. Lyon discovered this process of differential mapolation of the extra X chromosome. That can certainly happen, but it doesn’t go away, so the woman who has the mutation but is unaffected can still pass it on, and it can then pass on to a boy, the boy will be affected, pass down to a girl, the girl will be variably affected as well.

Question: If a parent has one type of CMT, is it possible for a child to have a different type?

Answer: yes, there can be variability in the presentation, but the type is specific really to the gene, so that’s not going to change. However, the clinical presentation can be variable even within a family. X-link inheritance has some crazy things and it can appear that generations get skipped, but really they’re not.

It is a complex naming system for these subtypes. It is probably one of the more confusing ones in medical genetics, so if you’re confused by the naming system, don’t feel bad at all. Just try to keep in mind inheritance pattern and is it demyelinating type or is it axonal type. Then I think you can speak intelligently about it.

I want to show what Invitae has available for clinical testing and also just give a little highlight of what our company is. We’re a new company kind of taking a different approach to genetic testing trying to simplify things ultimately, hopefully to make genetic testing more available to more people. We believe that if we can do that, then genetic information is better utilized. We share the data in public databases. It’s of more use to researchers and clinicians alike. So, we’re really trying to do things that way. If some of you pay attention to biotech news and that sort of thing, you know that there’s this thing called direct-to consumer genetic testing, 23andme is an example. We’re not that. We want to make things understandable to the patients, but we still require that genetic tests coming to us go through a physician and reports go back to a physician and genetic counselor and so forth. We really
believe that the healthcare professional is fundamental in the whole process of understanding genetic diagnosis and ordering tests all the way through to the returning results.

The company started 4 or 5 years ago with a focus on diagnosis of inherited cancers and has more recently expanded into other areas. My team works in the area of neuromuscular disorders. Right now, primarily Charcot-Marie-Tooth diagnosis, but also hereditary spastic paraplegia, dystonia, and most common, Duchenne muscular dystrophy. We are still expanding into other forms of dystrophies and congenital myopathies and several other areas.

A current CMT test covers 32 genes. They are well established in the literature as causative for Charcot-Marie-Tooth disease. There are more than 32 genes reported to cause CMT; however, for at least some of these additional genes, the scientific evidence is not really convincing that they’re disease causing, and, in fact, in some cases, if you read the clinical description, it doesn’t sound like the patients really have CMT. They have some sort of neuropathy but it really doesn’t fall under the category of CMT. We think right now that there’s between 32 and 40 well-established causative genes for CMT. Our assay today covers 32 of them. We do routinely update our test by adding additional genes that are in these well-documented causative genes, and that will happen again probably in the spring. Our ordering options are quite flexible. A clinician can choose the overall comprehensive CMT test that covers all 32 genes or if the clinician is really sure that it’s X-linked (there are four x-linked genes), they can just order that panel. There are particular reasons for doing that kind of thing rather than ordering the broader panels.

Scientists that do this diagnostic testing for a career are always wondering how well did their test perform. There are many ways to look at that, but one way is just to calculate the percentage of the tests that have positive results. Some people call it the detection rate – or a positive detection rate. For CMT testing, as you can imagine, with so many different genes, so many difficult inheritance patterns, there is not a well-established detection rate. There are a lot of reasons for that, but it’s all due to these variables. One study may have looked at 25 genes in their test panel, another study may have used 15 genes. It’s really hard to compare and come up with a standard detection rate. There have been some pretty large studies done. Supporta in 2011 found a detection rate of 67%, which is quite high for any genetic test. It was a large number. There were 787 positive results, and the most prominent types were just as we would expect, the CMT1A and the GJB1 X-linked type. There was an even larger study done in 2014 that found a positive rate of 18.5%. There were over 3000 positive results, so a very large number of patients were tested.

Thus for Invitae, our positive rate is kind of in between 27.5% and it is much smaller numbers. It’s 110 positive tests. Again, the positive rate depends on a lot of things: How well the patients are characterized; did the doctor have a really good sense that it’s CMT or are we just taking a chance at it, and they just want to rule it out. It’s a difficult thing to come up with, but those are some results.

We’ve been talking about 35 or 40 genes, but the important thing is only a small number of these genes account for the vast majority of cases. Studies repeatedly show that four genes with a PMP22, MPV, mitufusin and GJV1 are involved in somewhere around 90% to 92% of all cases and the remaining 10%
of cases had mutations in one of these many, many rare genes remaining 35 genes. So in the past, when DNA sequencing was still a fairly expensive proposition, the logical approach to diagnose the patient would be to test them just for those four genes, particularly if there was a sign of X-link inheritance, you could test just the one gene. Things are changed now with the less expensive testing methods and the clinicians just want to go straight to the larger panel more often than not and not just with the four genes.

Finally, I just want to point out that a negative test for CMT does not rule out the diagnosis. It might seem counterintuitive, but the negative test in an affected individual is not a rare occurrence in genetic testing. The reasons for this are many-fold. One is we have not yet identified all the disease-causing genes, certainly not in CMT. There are certainly more genes to be discovered. Another reason is there is still the possibility or the presence in a particular patient of a mutation type that goes undetected by the current test methods. There could be gene variants in an untranslated part of the gene that’s not tested that somehow interferes with regulation of the gene and causes disease, so there are still limitations. This is why I always recommend genetic testing, to be able to talk to a genetic counselor that really understands the limitations that come with this.

Again, I mentioned that we’re really trying to simplify things. We want to make genetic testing more acceptable to people, and we think that the whole process can be simplified, and one part of this is to make the billing transparent and straightforward. I think individuals who pay for genetic testing out of their own pockets will appreciate that, and I think eventually the insurance companies are going to get on board and understand that this is the way to go. We’ve adopted this three-pricing method that applies to all our tests, so we hope it’s going to make things easier for patients and insurance companies to know up front what the cost will be. I have had some medical issues, and, wow, you go to the doctor and you have no idea what things they’re going to tell us. Particularly in genetic testing when insurance doesn’t pay for things very well, this is an important thing to know.

Generally for the clinician, the ordering process is simplified. They can order one large panel. They can order any subpanels, or they have the flexibility to make custom tests for any particular patient’s needs so they can go across panels and choose individual genes that they might think are important to the patient.

Lastly, I just wanted to point out the Family History tool that is available on our website. The link is https://www.invitae.com/en/familyhistory/. This is intended for anybody to use. You can construct your own family tree, putting in medical information. It’s free of charge. It’s not going to direct you to order a test or anything like that. It allows you to save your information and go back to it and add to it and so forth. This is the same history tool that clinicians, particularly genetic counselors, use when they’re taking family history from their patients and goes into their electronic medical records for use in the future. If anybody has an interest, feel free to give that a shot.

QUESTIONS
Q Two of the mother’s daughters are showing signs of CMT, but the insurance company has turned her down for genetic testing. She’s trying to figure out if there are certain things you can do to get genetic testing and/or how much it would cost.

A We offer that out-of-pocket rate for patients. The other thing to remember is that the MDA (muscular dystrophy assoc) don’t just take care of people with muscular dystrophy. CMT is one of their disorders as is ALS and some other things. Depending on which MDA clinic you get into, they have certain amounts of funds to do some diagnostic testing. There are experts that staff the clinics and are involved a lot in diagnostics. It might be something to think about if you’re near an MDA clinic.

Q If you suspect CMT in the family, is it reasonable to have just one family member get testing and make that leap of faith to everyone else.

A That’s generally what a genetic counselor would consider. Real world experience, they will have tested whoever has the best insurance and then once the diagnosis is made, they’re going to know the gene and particular indication, and that is a much less expensive proposition to target just that site in other individuals. That could be done after the first individual is diagnosed with that information then have other family members tested.

Q There is a person who has a father who had CMT and passed away. He doesn’t know what type of CMT his father had. Is it smart to get tested for it or just wait until symptoms approach?

A That’s a tough question. You can do the testing but a negative result really doesn’t tell you anything because you don’t know if it would have been diagnosed in the father. One thing I think a genetic counselor would say is try to find out when the father was becoming symptomatic and maybe look at the patient, are they showing signs around that age. I would recommend talking to a genetic counselor about that. I just want to point out I’m a little uncomfortable answering questions that are very clinical. I’m a laboratory scientist, so I don’t want to take anybody down the wrong road. I really have a lot of respect for the clinicians, genetic counselors, and neurogeneticists. They’re the experts.

Q Is CMT1 caused by elongation of one or two strands of DNA?

A I think what that means is the duplication of PMP22. I guess technically you’d call it an elongation of the strand. It’s essentially a duplication of an entire coding part of the gene, so now instead of copies – one in each chromosome 17 – now there’s three copies. That overproduction of that protein actually results in disruption of the myelin on the axon. It’s the classic PMP22 mutation.

The other type of mutation that occurs there is the deletion of one of the PMP22 genes, and that results in a slightly different condition – hereditary neuropathy with pressure palsies.

Q If I was tested in 1995, with all the advancements in genetic testing, is it smart to go ahead and get re-tested or are those results still valid?

A The only people I think should be retested should be ones who had negative or equivocal tests back then. The thinking is that in 1990, they maybe knew about a small number of genes (3 or 4) and
now we know about 32-40. If you had a negative test back then, there’s a chance that your causative
gene has been discovered and a new test would uncover that. If you had a positive result, particularly
the PMP22 gene, MPV gene, GJV1 – no, there’s no need to re-do that test.

Q There’s an individual here who has a 21-year-old son who has been diagnosed with CMT that is
unlike any other this clinician has seen. They really want to know what to look for. For a person that
has been diagnosed with CMT but there’s no specific time that the clinician has given them. Is that a
good candidate for genetic testing?

A Exactly. There’s always slightly different clinical presentations occurring. It may very well be
caused by one of the known genes. That’s really a good candidate for testing. Since there aren’t clues in
the clinical presentation, I think the counselor would probably recommend doing the larger panel. In
this day and age, what geneticists will do is test an individual like this for specific disease panel, CMT
test. If that comes back negative, they’ll do something called an axonal test. That’s almost research but
not quite. In this test, they look at all protein-coating genes through all our genome. Really, they’re
trying to find a new or novel gene that’s responsible for the disease. It’s quite a bit more expensive than
a CMT panel test, but it’s used very commonly nowadays, and it’s seen as the next step past your
disease-targeted panel test, so there are options.

Q There is a question here about prenatal testing. Is there prenatal testing available?

A We don’t at this time. I’m sure other laboratories are doing prenatal CMT testing. There’s no
reason it wouldn’t be possible. There are issues with prenatal testing. One is that it’s much, much
better to know the family mutation. If you’re doing prenatal testing as a diagnostic thing, in other
words, trying to find the family mutation, that’s risky business. If a parent has CMT and they know the
mutation, then prenatal testing is not a big issue at all.

Q We have another question here about location. What is the procedural option if you wanted to
talk to your physician about getting tested?

A We do have genetic counselors. We don’t actually have physicians, so the tests are ordered by
your physician. A small blood tube is sent to the laboratory in San Francisco, analyzed there, recorded
there, and then it electronically goes back to your physician for them to interpret with you. We do have
genetic counselors. They’re mostly there to help clinicians help understand results, help them choose
ordering, and that sort of thing.

Q Just about any physician then could order the test?

A That’s correct. Personally, I believe that for something like this, Charcot-Marie-Tooth, any
neurogenetic disorder, it’s really best to go to a neurologist. CMT is probably common enough that a
general neurologist is going to have a pretty good idea of what they’re doing. If you’re talking about
some really rare genetic conditions, then you probably want to go with a subspecialist, but at least get
to a neurologist, academic medical centers always have excellent care for this type of disorder. The
other thing that’s important is not just the physician, but since you’re doing a genetic test, it’s always a
good idea to have a genetic counseling service. That’s one thing Invitae can help with. If you don’t have access to a genetic counselor, you can come to our company, make a phone call, talk to client services, and they’ll be able to find genetic counseling service in your area.

Closing remark:

There was one other question that I was forwarded early on in which they basically are asking where you can go to find a clearing house for genetic testing availability. There’s something called genetic testing registry – I think this is an NIH funded thing –


Another one is NextGXDX.com – that’s a site that actually lists prices and you and your physician can actually see what’s available there.