

- Good morning, everyone. My name is Amy Gray, and I'm the Chief Executive Officer of the CMTA, and we're just thrilled that you're here to join us for what we believe will be an incredibly informative, educational, and engaging day. We have over 400 people registered for our conference today, people from all across the US and around the world, internationally, as far away as Australia, Egypt, and across Europe. So welcome to all of our international guests as well. One of the aspects that makes these conferences so special is the opportunity to connect with fellow CMTers. And although we're not meeting in person, we're meeting virtually, our teams work really hard to make this as engaging and interactive as possible. So we have some sessions planned where you can connect with one another over the breaks and over lunch. So we really encourage you to participate in those, and we'll be giving directions on how to click through the links to join those activities as the day goes on. We have an absolutely incredible lineup of speakers for you today, and I want to thank each of our speakers for giving us their Saturday, but not just their Saturday because I think what we are so appreciative of is each of these speakers is so engaged all throughout the year and supporting our CMT community and our CMT patients. So we want to thank them for the work that they do year round to support the CMT community and our mission. I also want to thank Laurel Richardson, our Director of Community Outreach, for putting such an incredible agenda together today, incredible lineup of speakers. She's an absolutely amazing ambassador to the CMTA, and we have a number of staff online as well today that you'll get to meet throughout the day, just an incredible team that's here to support you and help you and provide resources in your journey with CMTA. The conferences, as you can imagine, are made possible through the support of individuals and corporations, and through that support, we're able to host activities. So I want to thank our corporate sponsors today that made this possible: Far Next, Cydan, Allard, Psychogenics, and Stealth. Thank you for your support and your funding to make this conference possible today.

- So I'm Alex Fay. I'm a pediatric neurologist. I work in San Francisco and Oakland, and I work with Matt Hall, our genetic counselor, who you'll hear from in a few moments. I start with this painting here on the left side of the screen because it's one of the few artistic depictions I'm aware of. It's thought to show an individual with Charcot-Marie-Tooth disease, and I'd be pleased to see others if you know of any. Here's what I want to talk about in the next 30 minutes or so: a quote unquote "typical" case of CMT, and, I think as you'll see, there is no typical; a brief comment on the history and the name; some discussion of how we diagnose CMT; genetic testing, which Matt will take a deeper dive into; management, what we can do now; and then what the future holds. And I think that's a very exciting part. And I'm going to be focused on the peripheral nervous system. CMT, of course, is a peripheral nerve disease, so we're dealing with the part of the nervous system that starts where the nerves leave the spinal cord and ends just before they touch the muscle and the skin. And so this is maybe as typical a story as you can find for a child who might come to my clinic with concerns for a peripheral neuropathy of some kind. This is a nine-year-old girl with increasing falls. She was developed normally up until now. She walked at 12 months of age, which is completely normal, but over the past six months to a year, she started to trip more when she tries to run and is

having more difficulty keeping up with other kids in school. And her father is especially concerned because he started to have similar symptoms when he was about her age, and he's walking, but he uses ankle foot orthotics now. And he's starting to see some of the changes in his daughter's feet that he noticed in his own starting in childhood. So that's our case, and I will come back to her. But before I do, I want to just comment on what's with the name Charcot-Marie-Tooth. So this is a painting from 1887. This guy at the middle is Dr. Charcot, considered the father of modern neurology, and he held these big Tuesday teaching sessions at his hospital in Paris. And if you look three people to the right, this is Dr. Marie, and the two of them wrote this paper in 1886 about a form of muscle atrophy that's progressive, often familial, starting in the feet and legs and reaching later the hands. And in the same year, a Dr. Tooth in London published a similar description of several cases, and so the name Charcot-Marie-Tooth eventually stuck as a description for this presumably genetic condition starting in the feet and moving up the legs to the hands. But how do we define CMT these days? So the definitions have gotten more precise over time. Another word, another term for CMT is hereditary motor and sensory neuropathy, or HMSN, but CMT has really been the more widely used term for this condition, which is a gradually progressive, usually symmetric, length dependent condition that affects the motor and the sensory peripheral nerves. It's a rare condition but not that rare. So prevalence is estimated somewhere in the range of one in 10,000 to one in 1,000. Onset is usually in the first two decades. Progression is typically slow and begins with problems in the feet and the ankles. But as you'll see in my talk, CMT can start in many different ways, and there is no typical CMT. Some other early signs of peripheral neuropathy can include numbness or tingling in the feet or hands, pain in the feet or hands, injuries without knowing it due to loss of sensation, delays in walking, as we didn't see in our nine-year-old patient, but some children with CMT will walk later than normal, frequent falls, slower walking or running compared to peers, and then changes in the foot structure that can happen early on, like high arches and hammertoes. So what happens if you or your child has symptoms of neuropathy? Usually, the pediatrician is the first person you talk to, and it may not be clear what's going on initially. So the pediatrician may not be sure if this is a neuropathy or something else and might refer a child to physical therapy, to an orthopedic surgeon, to a geneticist, or maybe to a neurologist. But hopefully, you eventually make your way to a pediatric neurologist or a physiatrist or rehabilitation medicine specialist, since these are the two types of physicians that usually have the most experience with CMT. And what happens in the neurologist's office? So we collect information about symptoms and any other medical problems. We ask about family history. We do a head to toe exam, a neurological exam that focuses on strength and sensation, reflexes, balance, coordination, gait. And then based on that, we try to give you an impression of what we think might be the problem and what tests could be helpful to confirm the diagnosis. And the further tests might include an electrical study called a nerve conduction study, possibly blood tests for other causes of neuropathy, and maybe genetic testing. So if we come back to our nine-year-old girl with increasing falls, as I mentioned, she has a family history in her father. Her exam does show high arches in her feet and hammertoes, and she has weakness in her finger muscles and her ankle and toe muscles. She has some sensory loss that is worse than in

her feet than in her upper legs or her hands. Her reflexes, which we check with the reflex hammer, are normal in her arms but reduced or absent in the legs, and then she takes high steps when walking because she has a foot drop on both sides. And so the next step that I would recommend for her is the nerve conduction study to make sure that this is a peripheral nerve problem. The nerve conduction study can tell us how well the peripheral nerves are working, both the motor and sensory nerves. And what this test involves is stimulating the nerves with this metal probe you see here and measuring whether the nerve signal gets through to the other end of the nerve and how fast it gets through. And so we usually stimulate the nerve at two points at least, and we get these two peaks on our computer screen. One comes earlier from stimulation closer to the end of the nerve, and one comes a little bit later stimulation, stimulating higher up in the nerve. And basically, if the nerve fiber itself, the axon where the electrical conduction occurs, is damaged, we get small signals like you see in panel B here. And sometimes the coating around the nerve cable, the myelin sheath, which is made up of Schwann cells and helps to insulate the nerves, if that's damaged, then the nerve conduction speed slows down, and these two peaks get further apart. And so this gives us the two main types of CMT, demyelinating, often called CMT1, and axonal, often called CMT2. And so in our nine-year-old young lady, her nerve conduction studies show this pattern in B. Her motor and sensory amplitudes are very small. Her conduction velocities are relatively normal. And so this suggests a problem with the yellow cable in the middle of the nerve rather than the myelin coating around the nerve, so an axonal neuropathy. So CMT is not the only cause of neuropathy in childhood. So in her case with the family history, CMT or a genetic condition is going to be most likely, but there are a lot of other things that can cause injuries to nerve in childhood. If a child is so unfortunate as to need chemotherapy early in childhood, this can lead to nerve damage. So can vitamin deficiencies. These are all very rare with a normal diet, but they can certainly appear. Diabetes, thyroid disease, autoimmune diseases, particularly CIDP, or chronic inflammatory demyelinating polyneuropathy, infections around the world. Leprosy is a not uncommon cause, and then there are some very rare situations where Lyme disease, chickenpox, and other conditions can cause a neuropathy in children. And then of course, there are other genetic disorders beyond CMT. And when we want to make a diagnosis of CMT, it's I think it was we're discovering CMT is actually many, many dozens, if not hundreds, of diseases. We've identified at least 100 genes that can cause a CMT-type clinical picture, and these can be dominant, recessive, axonal, demyelinating forms, even mitochondrial forms, and so figuring out which form our nine-year-old patient has is not so easy. Sometimes we can get clues from seeing if other organ systems are involved, although we consider a CMT to be primarily a condition affecting the peripheral nerves. There are some cases where the nerves in the back of the eye, the optic nerves are affected. Sometimes there's hearing loss. Sometimes the nerves to the vocal cords are affected, causing hoarse voice. There can be muscle stiffness or spasticity that can look like a spinal cord problem. In some cases, a child may have intellectual disability or abnormal findings on the brain MRI, suggesting a central nervous system involvement, and there are even forms that affect the kidneys and white blood cells. So some practical points on genetics. The most common form of CMT is called CMT1A. This is a demyelinating form affecting the myelin

sheath around the nerve cable, and about 70% of patients with CMT1 have CMT1A, which is caused by a duplication event in the PMP22 gene on chromosome 17, and about 40% of these cases happen out of the blue, so there's no family history. So it's a good lesson that a genetic disease doesn't mean that anyone else in the family has that condition. They can show up even if no one in the family has ever heard of CMT. So CMT1, the demyelinating form, has a prevalence of around 15 in 100,000 in the US, and CMT2, the axonal form, like our nine-year-old girl has, is about half as common, and the X-linked form is a little less common than that. But, of course, there are many, many patients with rare forms and many patients who still don't have a specific genetic diagnosis. So we have some different genetic techniques we can take. These include panel testing, looking at sets of genes that we know are associated with CMT, and many companies offer these. And these have about a 30% success rate, so much better than it was 10 years ago but still a lot of room for improvement. Another approach is what's called whole exome sequencing. This is looking at all the protein coding parts of all the genes, about 25,000 genes in the human genome, and this can now be done relatively quickly, too. And the diagnostic rate here is variable, depending on which publication you look at, but I've seen ranges from as low as 11% and as high as 50%. And then there's this emerging technique of whole genome sequencing still not widely available, but this looks at really almost every possible genetic change that can happen in our DNA. And so this is something we're looking towards in the future, not quite there yet. But this, I hope, will help us get a higher diagnosis rates for our patients with CMT. After you make a diagnosis of CMT, what does life look like in the next couple of years? And the simple answer is that it's complicated. This is a publication from 2017 looking at about 200 children with CMT of different forms, and they're rated on this CMT peds rating scale where a higher number means more symptoms. And so you can see over two years that some of the children here stay about the same, others get to a higher count pretty quickly, and some actually improve a little bit. And so looking at an individual child with CMT, you really can't predict the future. You can only talk in terms of averages, and we can say in general, looking at this study, that patients with the axonal form of CMT, CMT2, tend to progress a little bit faster than those with CMT1, but that's just an average. There are certainly patients with CMT2 who stay about the same over two years. So we don't have a crystal ball, even when we have a genetic diagnosis. So I've started with an introduction to a quote unquote "typical" case of CMT, but CMT can show up in lots of other ways, like this three-year old patient of mine who I met just after his third birthday. He was born healthy and until six months was doing fine, but then some delays in his motor development and low muscle tone were noted. He was having trouble holding his head up. At 14 months, he still had many of the same problems, still trouble holding his head up, was not sitting independently yet, and could not bear weight on his legs at an age when most children are already taking their first steps. And he had a few words, and then, by 21 months of age, his reflexes were hard to pick up. He was clearly delayed across multiple developmental domains functioning at about a nine month old level. And I met him when he was about three, three and a half, and I did a nerve conduction study, and I could not get any responses in either his motor or his sensory nerves. And suspecting a peripheral nerve problem, we sent off genetic testing and found a mutation in the PMP22 gene which, as I

mentioned earlier, is associated with the most common form of CMT, CMT1A, but can also, in cases of point mutations, cause a very early onset severe form of CMT called Dejerine-Sottas, or congenital hypomyelinating neuropathy. And this is a problem with the formation of the myelin sheath around the nerves. When we think about neuropathies in neonates, we have to mention spinal muscular atrophy, which is a very severe disease in its infantile form, typically fatal by two years of age, though there are milder forms that show up a little bit later. And we now have three treatments for this condition, which is amazing because when I was going through my medical training, we had really not much to offer for these children. And now we have these three treatments that can stop progression and even allow for nearly normal development in children with a previously fatal condition. And this is really the biggest story in pediatric neurology in recent years, one of the biggest medical stories, and it gives me hope that if we can treat this really severe peripheral nerve condition, that we can definitely do the same for CMT in time. So this severe form of CMT, Dejerine-Sottas, typically shows up in children with low muscle tone, respiratory and feeding problems, very abnormal nerve conduction tests, even abnormal brain MRI, and can be caused by mutations in many different genes. So moving onto an older age group, this is a 14-year-old boy I met in my residency who I saw when he was coming in with his second strange episode where he would get weak and numb on one side of his body. Now on the left, the last episode was on the right. And when I examined him, he looked a lot like the adults that I would see with strokes. He had slurred speech, his left face, arm, and leg were weak, but he also had some unusual features. I couldn't get his reflexes, and he had decreased vibration sensation in his toes and also high foot arches and contractures at the ankles, so his ankles were really stiff. He also had an abnormal brain MRI, but he recovered completely within 48 hours. He had these unusual bright areas in his brain, which are not quite typical for stroke but are typical for the condition that he ended up having, which is X-linked CMT. And we made this diagnosis by identifying a point mutation in the connexin 32 gene. And we found out later his brother and mother both carry the same mutation, and they also have findings of peripheral neuropathy. And so X-linked CMT is a little less common than CMT1 in CMT2 but still not that uncommon. And in a lot of ways, patients with X-linked CMT have very similar symptoms to other types of CMT, but they can also have these stroke-like episodes and even pretty severe episodes of confusion when they go to high altitude. Fortunately, these episodes tend to resolve completely within a few hours to days. They may often have hearing loss and tremor, and again, these are caused by mutations in a gene on the X chromosome called connexin 32. And then to mix things up a little further, in terms of other ways that CMT can show up, this is a 17-year-old patient of mine who was working on his car for eight hours one day. Completely healthy before then, but after working on his car for eight hours, he could not move his left arm anymore. And he was numb along the left lateral forearm and didn't actually come in to see a doctor for a couple of weeks, and by that time, he had already lost some muscle bulk in his left shoulder and biceps and was weak in his thumb and had decreased reflexes. I did a nerve conduction study on him and actually found problems not just in the left shoulder area and the brachial plexus, which are the nerves that go from the neck down to the arm, but even on the unaffected side, he had a carpal tunnel syndrome on the

right, and his ulnar nerves at the elbows pictured here on the left side of your screen were affected in both of his arms. So basically, we uncovered some things on the nerve conduction study that were not apparent based on his symptoms. And based on the nerve conduction study, I suspected a particular form of CMT called HNPP, or hereditary neuropathy with liability to pressure palsies. And the genetic testing confirmed this with deletion of the entire coding sequence of the PMP22 gene, one of his two copies. And so this is a unique form of CMT that causes episodic symptoms. So the nerves are very prone to compression at the carpal tunnel and the wrist and at the ulnar nerve at the elbow and the peroneal nerve at the knees. So people who cross their legs a lot when they're sitting are prone to a neuropathy at the peroneal nerve. And in all of us, that can cause some numbness and tingling that goes away in a couple of minutes. For people with HNPP, it can lead to weakness that lasts for days or even weeks. And there are characteristic nerve conduction findings, MRI findings, and genetics usually shows a deletion of one of the two copies of PMP22. So coming back to our nine-year-old patient, genetic testing in her showed a mutation in the mitofusin-2 gene, which confirmed a diagnosis of CMT2A. This is the most common form of CMT2. But we have a diagnosis, so now what? So there are things we can do to help patients with CMT currently, and I refer all of the children I take care of with CMT to physical and occupational therapists. I try to get them to keep moving in whatever way they enjoy to help build and maintain muscle strength and balance. We try to avoid things that worsen neuropathy. So not becoming diabetic, avoiding these rare vitamin deficiencies, caution with chemotherapies if a child is so unfortunate as to need such treatment, ankle foot orthotics like on the right can be very helpful for decreasing tripping and falls, as can other adaptive devices. But really, what we all want is for something that can stop the progression or even reverse CMT. And so there are fortunately a lot of researchers and clinicians around the world who are working on many, many different approaches to treating CMT. Sometimes, that involves replacing a gene that's missing or inactivating a gene that's causing a toxic effect in the nerves, sometimes promoting nerve growth or maintenance, and other times preventing nerve damage. And I'm just going to mention a couple of these. I don't have time to go through all of them, but there's so much exciting research going on. One of the most exciting developments in science in the last 10 years is CRISPR, and you may have heard about the 2020 Nobel Prize for chemistry being awarded to these two incredible researchers, one in France and one close by to me here in Berkeley. And what they discovered was this genetic system for cutting DNA in very specific places. And what this allows for is potentially editing our genetic material to delete, repair, or replace a genetic problem that's causing a disease. And this is exactly the approach that two of my collaborators in San Francisco are taking, Bruce Conklin and Luke Judge. They're focused on patients with CMT2, several specific forms, and what they're doing is they collect a blood sample. They're able to turn the white blood cells into a type of stem cell called induced pluripotent stem cells, and then they can turn those stem cells into neurons that they grow in a dish, and then they use this CRISPR technology to treat the neurons in the dish and try to make them healthy. And then the next step will be to use this same treatment on an animal model like a mouse and then eventually bring these to patients. And so these are how normal neurons look in a dish. In a patient with a CMT2E, you can see these

green clumps of material are not normal, and those cause the nerves to not work very well. And if they use their CRISPR treatment, they can make the nerves look normal again. And so they're actually working on these next steps of treating mice with CMT and seeing if this will be a viable treatment in the coming years for some of our patients with this form of CMT2.

- 30 minutes to go, doc.

- All right, thank you. So what about the most common form of CMT, CMT1A? This is a Goldilocks problem. So as I mentioned earlier, the CMT1A is caused by having an extra copy of the PMP22 gene. But then my 17-year-old patient has another condition caused by having one less copy of PMP22, it causes HNPP, and there's an even more severe form like my four-year-old patient has where you have zero copies of PMP22. So to treat this extra copy of PMP22, the answer can't be to just get rid of PMP22 all together. You're going to make things worse. So is there some strategy that can be used to turn down PMP22 levels a little bit but not too much? And the answer is yes. There are several research groups around the world that are working on, I think very promising approaches. One is this three drug combination that's in clinical trials that can turn down the levels of PMP22. Another is a technique called a gene therapy where this gene called neurotrophin-3 that's helpful for maintaining function of peripheral nerves in Schwann cells is introduced into the nerves using a safe virus, and that's in clinical trials as well. And then there's some promising developments using antisense oligonucleotides. This is the same type of technology used in one of the treatments for spinal muscular atrophy that I mentioned earlier, and this is showing some promise in rodent models of CMT1A and hopefully it will be something that can help patients in the future. There's also gene therapy being used for several forms of CMT where the problem is that a patient is missing an important gene for nerve function. And these are just examples of several trials that are ongoing, either at the preclinical level or actually patients are receiving treatment for some of these. So stay tuned for results from those studies. So the take home points I hope that I've shown you in the last few minutes that CMT, there is no typical picture of CMT. Our nine-year-old patient is about as close as you can get, but as I showed you, there are many other different types of symptoms that patients with CMT can experience. The list of CMT associated genes is continuing to grow, and we've passed 100, so genetic diagnosis is complex. But I also think it's really important to get a specific genetic diagnosis because this is what opens the way to some of these exciting treatments that are in development. We can do a lot of things to make life a little easier now, but hopefully targeted treatments over time are going to really be transformative for individuals living with CMT. And so my hope is that the young woman in this painting from Andrew Wyeth in the 1940s, I think there's a lot more that we can do for her today. But my hope is that in the future, she will be, instead of scooting across the field on her arms and legs, that she would be able to get up and walk to the house in the distance here. And so with that, I'm going to finish my talk and pass the baton to Matt Hall to talk to you about genetic counseling and CMT.

- [Laurel] Thank you, Dr. Fay. Hi, my name is Matt. I'm a genetic counselor working with Dr. Fay. Thank you guys for having me. It looks

like we've got about 10 minutes to sort of go through the basic genetics of CMT, and I'm going to try to hold myself to that so that we have time to answer questions. There's already a couple of questions in the chat that I want to get to that I think are hard to answer through the chat. Am I showing the right screen?

- Yes. Yep, it looks good.

- Okay, perfect.

- Thanks Matt.

- Sure. So thank you guys again for having me. So I have started working with Dr. Fay in neuromuscular just before COVID started, but I also covered a maternity leave for Carly Siskind at Stanford, probably about five years ago now, working with her team and the Inherited Neuropathy Consortium, so I think it's exciting to be able to present some of the data that was collected at that time. And then also, if I look familiar, that could be why. I may have met you there. So the Inherited Neuropathy Consortium is an international group of studies that all collect clinical presentation data on different types of CMT. As far as I know, it's the largest cohort of CMT and genetic neuropathy that's been published. It skews a little bit more towards rare forms of CMT, but it gives a good general idea of what's the most common types, and so I'll go over the most common types and what goes on with their - what inheritance pattern goes along with those most common types. So like Dr. Fay said, CMT1A is the most common. This should have an A at the end. Sorry about that. In the international cohort, it was about 55%, but in a lot of cohorts, it's about - it reaches 90%. So it can be quite quite a wide range. And the other, I think, interesting thing to note about these different subtypes is that this really just shows that the vast majority of CMT is inherited in a dominant manner. So CMT4 is recessive and only counts for about 2%, CMTX is X linked but still dominantish, and it's only about 7%, so the vast majority is dominant. So that's what we'll spend most of our time talking about. And then within the people in that cohort who got a genetic diagnosis, which is around 1,000 people, still a vast majority were CMT1A, followed by CMTX, 2A, and then 1B, and then other accounted for 11%. And each of those genes counted for usually less than 1%, but there's one that's about 2%. So I wanted to go off of a little bit of what Dr. Fay said about demyelinating versus axonal. I think one of the important things to understand about that is that there are two different cells that are composing a neuron. The Schwann cell sends out arms that wrap around the axon of a neuron, and so there are two different cell types, and so that have different functions, and, therefore, a different kind of profile of gene expression. And that kind of explains why the genetics of the two can overlap sometimes, but it's often pretty separate. In the cohort, in the Inherited Neuropathy Consortium, one of the questions about going through genetic testing is what is the chance of actually finding a genetic cause, and they found that it was much more likely to receive a genetic diagnosis if you had a demyelinating form or CMT1 versus an axonal form, or CMT2. So about 90% got a diagnosis with demyelinating forms, and only about 43% for CMT2. Another sort of interesting thing that I'd like to add to genetics is that the presentation for each person with the same genetic change isn't



necessarily the same. So there are two concepts. One is variable expressivity, meaning that not everybody with the same genetic change has the same symptoms, and the other one is penetrance. So that means that not everyone with the same genetic change has symptoms at the same time. So as we go through the most common types, I've attempted to display some penetrance information in a meaningful way. So even with the most common form, CMT1A, where there's a whole extra copy of the peripheral myelin protein 22 gene, 75% are symptomatic by 10, certainly 85% by 20, but there are still about 15% of patients who still don't have symptoms by their 30s or 40s, or that's when they're starting to have symptoms. This is a protein that is involved in myelin, and myelin essentially is the material in the Schwann cells that helps insulate the axon. So you can think of the axon of a neuron being sort of the copper wire and then of an electric signal, and then the myelin sort of being the insulation to help it move efficiently. It's inherited in an autosomal dominant manner, CMT1A. So autosomal just means that it's not a sex chromosome. So, you know, typically, chromosomes come in pairs where one from each pair comes from Mom and one from each pair comes from Dad. The first 22 don't have a relationship to sex or gender, but the last two determine physical sex. So typically, males have one X and one Y, and typically, females have two Xs. So autosomal means that it's located on any of the other chromosomes that are not related to sex. What does the actual inheritance pattern look like in a family? So the majority of the time, there is an affected parent, and an affected parent has a 50-50 chance of passing down the extra copy of the gene to each child. So there's one copy of the chromosome that has just one copy of the gene, which is normal, but then the other copy of the gene has a whole extra, or the other copy of the chromosome has a whole extra copy of the gene. So if that's passed down, then that child will have CMT1A. The other sort of concept to note with dominant inheritance and especially CMT1A is that there are de novo mutations or de novo duplications. So what that means is that sometimes, you know, depending on the cohort between like 10 and 40% of the time, cases happen with parents who don't have the the extra copy of the gene, but it's something that occurs in one egg or one sperm, and that's known as a de novo, or sometimes people call it a spontaneous change. That I think sometimes people often feel guilty about because they wonder if there's something that they could have done differently to stop a de novo change from happening. But we all have about 85 on average de novo changes from our parents, so these kinds of mutations are things that are a normal process. It's just that when they happen in an important place, then that they can cause an issue. Then the second most common is CMTX. So this one is interesting because it is on the X chromosome but both males and females tend to be affected. So because males only have one copy of the X chromosome, they don't have an extra copy of the gene to compensate. And in this particular gene, GJB1, the types of changes that happen aren't a whole extra copy like PMP22. They're actually changes in the gene that stop the protein from having a normal shape or function. And so females have a whole extra copy. It's like this backup copy, but what complicates it more is that that backup copy isn't enough for females to not have symptoms. So certainly, males are much more likely to have symptoms much earlier, and the vast majority of females have symptoms at some point. Even if they don't have any clinical complaints, they may have some identifiable slowing on the EMG. And so this also was a question in the chat. This is a demyelinating form as well. And then

just looking at the inheritance pattern. I'm not going as fast as I wanted to. CMTX is on the X chromosome. So males have one copy. Females have two copies. So if a male has it, he'll pass it down to all his females because then they'll have two Xs. And if a female has it, it's 50-50 chance of passing down to each regardless of sex. One of the reasons why females have lower and much more variable age of onset is because we think because of X inactivation, the best example I can show that are cats, the orange and black cats. This particular color gene is on the X chromosome, so pretty much any cat with this pattern of fur is a female with two X chromosomes, and every cell with two X chromosomes turns one off randomly so that it balances out the amount or dose of each of those genes. And so just like with cats randomly turning off one X chromosome with something you can actually see, you can imagine that with females who have CMTX, if their cells randomly turn off the affected copy more that has a mutation, then there'll be more mildly affected versus the opposite. If the normal gene gets turned off at a higher rate by random chance, then they'll be more mild. This has not been able to be replicated in blood, but we think that it's still something that happens in those actual tissues. So I'm running out of time, so I'm going to skip ahead a little bit past CMT2A and the MPZ. Those are both autosomal dominant. What I will say about CMT2A is that it does occasionally inherit in a recessive manner, so that's interesting. With CMT1B, it's autosomal dominant. And the interesting thing about this is that the actual mutation in the gene can tell you with more certainty than most genes when the onset is. So infantile onset tends to be certain mutations within the gene, and childhood and adult onset tend to be other mutations in the same gene. And then there are also recessive inheritance patterns, meaning that both parents are carriers, and only if they both pass down their affected copy will the child be affected. Another thing I'd like to talk about a little bit is variant interpretation. So Dr. Fay alluded to this a little bit as well, which is that you can get variants of unknown significance on genetic testing. And really, some variants we know are benign, and we've seen them in people without CMT or other genetic conditions, but the spectrum varies. So there are things that aren't common enough to be known for sure to be benign but are likely benign; variant of unknown significance, which haven't been seen in either population enough to really know; likely pathogenic; and then pathogenic. So those are all the types of results you can get from genetic testing. And then what test to actually do sort of depends on some personal choices and what insurance will cover and where you're at in the diagnostic process. So we're definitely moving away from single gene testing, but I do remember the time when we started with PMP22 testing and then would see what happened next. Now, I think we're just starting with gene panels on people, and those end up being negative or normal. We don't find anything, then we move on to whole exome sequencing. But with all of these tests, it's possible to have completely normal results, which don't necessarily mean that there's not a genetic change somewhere. For example, whole exome sequencing looks at all the genes, but all the genes only account for about 2% of the DNA. And then the other thing about whole exome sequencing as well is that the interpretation leaves a lot to be desired. So we're really good at collecting all the sequencing, and there was a question in the chat about this. "What's the reliability of whole exome sequencing?" As long as it was done in a clinical lab and not a research lab, which almost all

testing is, the variant call is very reliable, but the interpretation might change over time. So if it's a variant of unknown significance right now, it might eventually change to be a benign variant or eventually change to be a pathogenic variant as more data's collected as more people do the test. So with whole exome sequencing, you can just get an answer right at the get go, but the chance to get an answer and a chance to get an unknown significance variant are about equal. And then whole genome sequencing just also is even more difficult to interpret than whole exome sequencing. And with whole exome sequencing, you can also get a gene of unknown significance, meaning that we don't even really know for sure if the gene itself is associated with CMT. Another thing that we like-

- 10 minutes to go, Matt.

- Oh, what did you say?

- 10 minutes to go. Okay. Well, those are supposed to be questions, right?

- I think ideally, there will be some minutes for questions, but if you have some wrap up, that's fine, too.

- Yeah, I'm pretty much done. So I always underestimate the amount of time I'm going to take. And then even though this is technically a pediatric session, I do think that recurrence risk is on the minds of parents of pediatric cases, and there is some consideration of whether or not to have more kids. And there are reproductive genetics options, and I like to stress all of these equally. You know, there's definitely no pressure from genetics to intervene. So, you know, just kind of go with whatever risk is associated with that particular subtype is fair. Also depends on your own experience, mostly, with what you want to do. You can also do prenatal testing on a pregnancy to find out during a pregnancy if the familial variant was inherited, and then you can also do in vitro fertilization and actually screen embryos before pregnancy for certain genetic mutations. And so obviously, if these are all very personal choices, and I think that they're all fair options for people, depending on what their own experience has been and what their own personal beliefs are. So that does bring me to just sort of wrapping up. So the take home points are that mutations themselves don't skip a generation, but sometimes they can look like they do. If there's someone who's mildly affected in the family or in CMTX has skewed X inactivation, genetic testing can sometimes just give an answer, and other times it can create more questions like is this gene actually associated with CMT? Is this particular mutation pathogenic or disease causing or not? And then there's a lot of overlap in the presentation of genes. So we're moving away from doing just a single gene test and doing larger panels and then following that up with whole exome sequencing if it's something that we can get covered by insurance, so that's always, always an issue. And then the hope is that there are going to be therapies on the horizon that are specific to the gene that's causing the condition. So I think that's a motivating factor certainly to try to get to the bottom of the etiology of every case. And I think that as clinical trials do become available, there'll be a justification for insurance to do a better job covering the

testing. So sorry that I talk so fast. It feels like that. If you have questions about a specific case, too, I'm happy for you to email me whatever direct testing results you have or if you don't have any genetic testing results and just are wanting advice on where to have your PCP start. I imagine that most of you aren't in California, but yeah. So thank you, guys.

- Matt, yeah. Jonah, before you start with a couple of questions, I just want to remind everyone that we do have a hard stop at 11:10, and I will be putting the link to go back to the main Zoom hub for the fireside chat. I'll be putting that in our chat feature down here. It looks like we have time for a question or two. And if your question is not answered, we will take those questions and get those answers for you. So we will go through the chat, make sure we grab those questions. We actually get a print out from Zoom. So Jonah, do you want to start with a couple of questions?

- Sure, and Doc and Matt, I'll let you determine who's best to field each one. But first one is, "How common are the stroke-like episodes in CMTX? Do they start to appear at a certain age? What should the response be, intervention to these episodes if they appear, or do they resolve on their own?"

- Yeah, so I don't think we have an exact percentage of how often they happen. They're not uncommon. Fortunately, they don't tend to happen all that often, even in the patients who do have them. And fortunately, they do go away, but they look really scary at the time, and so the key is if you or a child with CMTX has one of these episodes, don't just assume that that's what it is. I don't have to worry about it. You do want to make sure that it's not something more worrisome. And so these episodes are emergencies, especially the first couple of times that they happen, and typically, a child will need to be monitored overnight, maybe for a couple of days until the symptoms get better, until other things like strokes and bleeds and things like that have been ruled out. So definitely important to be careful with these episodes, even though when we figure out what they are, they typically don't cause any long-term problems.

- Awesome. Okay. "With so many options for genetic testings, NGS, whole exome, etc., can you offer recommendations about which to select? Does it depend on the clinical presentation and the EMG/NCV test results?"

- You want to take this one, Matt?

- Sure, yeah. So I think that for a long time, it depended on much more heavily on all of those results. Now that the cost to do a large genetic test and a small genetic test or at least a large panel of genes and a small panel of genes is relatively the same, it's a lot less important to have those things available before you go through the first round of genetic testing. So a lot of panels will just include all of the dominant, all of the demyelinating, all the axonal, all of the recessive all in one test, just to see what comes back. If that comes back completely negative, I think those other evaluations can be helpful to sort of determine if someone like Dr. Fay would be able to tell if they

really think that it looks like a genetic form that might be more rare. And so it can help you decide how much you want to push for whole exome sequencing or if there's a large out of pocket expense for whole exome sequencing it can help you decide if you want to go that extra step. But I would say that for the first round of genetic testing, it's a lot less expensive than it used to be, and it covers most of the genes that are associated with CMT right now for most of the panels. So it's less important than I think it used to be.

- Now, Jonah, you're muted.

- Jonah, you're muted.

- You're muted, Jonah.

- I was like, "Why is everyone talking to me?" Okay. "With all the great approaches that we have, I wonder if a child can participate in the gene therapy trial, AAV vector. Is he, she able to participate in the trials in CRISPR in the future? Thanks."

- So usually when there's a clinical trial, there are exclusion criteria which mean that if one of these things applies to you or your child, they can't participate in a trial. And usually, that includes that you've previously gotten another treatment for the disease. So for example, if a child in the future were to receive a gene therapy, probably, they would not be able to join in the next type of clinical trial after that because typically in a trial, we want to study children who are approximately have the same risk factors coming in and where we don't have to account for differences like what other treatments have they received in the past. So usually, patients in a clinical trial will not have been a part of previous clinical trials where they've gotten a treatment for that disease.

- So I know there were more questions. As Laurel said, you're going to get them answered. We promise you that. We will follow up with you and get them answered. We just want to thank Matt and the doc for joining us this morning and for a really great presentation. I learned a good amount, so thank you both for the work that you're doing.