

Making a Difference in Duchenne Muscular Dystrophy & Spinal Muscular Atrophy Pediatricians at the Front Line of Early Diagnosis & Improved Survival

Katherine Mathews: I'm going to start here on recognizing and avoiding missed opportunities to treat patients with Duchenne muscular dystrophy, spinal muscular dystrophy, and other neuromuscular diseases.

I am going to start by doing an introduction to neuromuscular diseases in general; review the importance of early diagnosis and recognition; provide a few hints for evaluating motor function; and then talk about some resources that are readily available to primary care physicians who are on the front line of these diseases. First, any time I talk about this: I'm a neurologist and I localize, that's what we do. We're taught strokes, we localize. The motor system includes everything involved in the initiation and carrying out of a movement. It starts with the brain; it goes to the spinal cord, anterior horn cell, peripheral nerve, neuromuscular junction, and muscle. When we talk about neuromuscular diseases, we generally are referring to anterior horn cell and below. When we talk about neuromuscular diseases, we typically are not including those that primarily start in the brain, where the motor problem is primarily at the level of the brain.

[Let's vote.] "Signs of neuromuscular disease might include which of the following?"
(Music playing)

"Weakness" was the correct answer and weakness is a common characteristic across all neuromuscular diseases. Spasticity, microcephaly, hemiparesis and chorea all suggest a central cause, a brain-related cause of weakness. So again, the focus is on anatomic location and the diseases that are associated with those various anatomic locations. Motor neurons/anterior horn cells: spinal muscular atrophy is the most common disorder associated in childhood. Peripheral

nerve includes the peripheral neuropathies, Charcot-Marie-Tooth probably being the most common in childhood. Acquired causes like Guillain-Barre might also be an example. Neuromuscular junction: autoimmune myasthenia gravis or congenital myasthenic syndromes, genetic causes of myasthenia gravis. Muscle: congenital myopathies and all muscular dystrophies. So those are not the entire list of all possibilities but are common examples.

I'm talking about all these anatomic localizations as if they are silos and they live alone but in reality, biology is never so simple. When we talk about a muscle disease it doesn't mean that it cannot have central nervous system involvement. Duchenne, for example, often has cognitive impairment as part of it. You must recognize that we talk about the anatomic localization, but biology is more complicated.

You guys have the toughest job. We have the easy job because you guys have already identified the patient, you've sent them to us and said they are weak, and it is our job then to figure out why they are weak. But honestly, that is easier than what you have, and this is part of the reason why. You see thousands of kids. You see children with developmental delay for a lot of different causes and, as shown on this slide, neuromuscular diseases are not the most common. You are not going to see a lot of these. So, what we are hoping to do tonight is to help you recognize the rare disease when it comes through your door.

A couple of things that we know about the diagnosis and neuromuscular disease: there is often a delay in the diagnosis in childhood. Parents typically are the first ones to notice and they don't typically discuss them with their primary care physician when they first notice them. They may be afraid; they may have made up excuses (e.g., "this is just a family story.") The American Academy of Pediatrics strongly recommends surveillance as something that happens at every well-child visit. You have to ask the questions. And we know that without doing formal

screening or using formal screening tools, just sort of your clinical judgment alone, you will miss motor delay in about two-third of cases. This is an old paper, but I think the findings still hold up. So, surveillance, asking at every visit, screening using screening tools for motor diagnosis, and recognition are critical to making an early diagnosis. And I hope I will show you some tools that you can use today.

Let's vote again. "Which of the following is not true about the early diagnosis of inherited neuromuscular diseases?" (Music playing)

Okay, so "D," the "diagnosis of inherited neuromuscular diseases is emotionally stressful for parents, so should be postponed for as long as possible" is not true. Parents—and there have been polls of parents—want to know sooner. The time period between when they know something is wrong and the time that somebody listens to them and starts coming up with a diagnosis is very stressful for parents. We don't try to delay it as long as possible. So, a little bit more detail in diagnostic delay in the two main diseases we are going to be talking about today. These are MD STARnet data. In the first column is the main parameter, and that's what I am going to focus on, or the thing that was looked at. The second column is the mean age at which this occurred. So, based on chart review, parents first recognized earliest signs of symptoms about 2-1/2, they first brought it up with their healthcare [provider] or they first had a healthcare provider do any sort of intervention or evaluation at 3-1/2 and the first neurology visit and then diagnosis was at 4-1/2. Altogether there was about a two-and-a-half-year delay from the time that parents first recognized that there might be something wrong with their child and the time that they saw a neurologist and had a first CK. That is a very difficult and long time and it has not changed over the last 40 or 50 years. Every country, every place we look, it is about the

same. We can't necessarily have parents report their concerns earlier, but we can shorten the time between first healthcare evaluation and first diagnostic test.

We see a similar pattern in SMA. As Dr. Finkel will tell you later, SMA is divided into types based on motor milestones and roughly by age of presentation. In the most severe kind that presents in infancy, there is about a four-month delay in diagnosis. In the older onset types there was up to about a year or a little more delay in diagnosis from the time the parents first recognized symptoms to the time the child was given a diagnosis of spinal muscular atrophy. And again, as you will hear, all these delays result in loss of potential response to treatment.

I'll just mention one of these, the second bullet point: these delays postpone initiation of treatment that has become rather effective in some diseases. We have several recent FDA-approved therapies, and there are more on the way in the diseases we are talking about and in other neuromuscular diseases. Reducing that diagnostic odyssey and relieving caregiver stress is an important reason for making a diagnosis. Genetic counseling: we all have too many families who have three children with SMA or three children with Duchenne. Then there is participation in treatment trials to improve ongoing treatment.

Because of this diagnostic delay, the CDC funded an initiative between 2010 and 2011 and the childmuscleweakness.org website was launched in 2012. It was designed to be a real time reference tool and educational tool. I use it a lot with trainees. It includes a lot of different elements and I am going to show you some of them, but it includes a motor development assessment, a motor delay algorithm, a number of short quick real time videos, discussion of how you interpret a CK level, how do you talk to parents, what are the first words parents use in describing delay. And we've seen a steady increase in use since 2012 based on Google analytic

analysis. It is a useful site, people are using it and I hope that you will find it useful the next time you have somebody come in that you need to work up.

I mentioned the video library, and I just took a screenshot of some of the pictures that you can see on it. It has both normal and abnormal children and it is narrated by an Australian physical therapist, who has a fabulous accent, so it is easy to listen to. And then there are also downloadable PDFs designed for primary care providers and I listed some of the guidelines that are available to download.

There are developmental screening tools that go through particular milestones. I gave the milestone gait at 12+ months. It talks about what you should look for, how to see it, and gives developmental norms that are supported by the literature. There are those for each of the major age ranges and developmental milestones.

The American Academy of Pediatrics was very much involved in childmuscleweakness.org and published guidance for the evaluation of motor delay shortly after it came out and the two documents are very much in sync. The AAP guidance is developmental surveillance at every visit and the publication from *Pediatrics* 2013 lists tables of motor milestones. Formal screening at 9, 18, 30, and 48 months typically by a parent report tool, and there is reference to how to choose a parent report tool if you don't already have one that you use. And then there is a nice differential diagnosis table. So that is another nice resource for referring to.

Briefly, the steps to early diagnosis are: listen to what the parents say, observe for signs of weakness, evaluate motor development, and check CK. The mnemonic is "developmental delay, do a CK." And then refer to a specialist, if appropriate, then surveillance, and then take a developmental history. I think these things are things you know. Is he keeping up with peers?

Is he falling? What is the course and progression? Is he continuing to get better? Is he losing skills? Is there a family history of neuromuscular disease? In one study that looked at this, 80% of parents' concerns were correct and accurate. If parents present very vague concerns, follow up with questions.

Going back to localizing the problem, when you go to do your exam you need to determine: is this a peripheral problem or a central problem? Is this a neuromuscular problem, peripheral, or is it brain spinal cord? Chest size may be a clue and you can read here for yourself. Facial movement: children with neuromuscular diseases may have relatively expressionless face; often have a high arched pallet. Those are not the case usually in cerebral palsy. Tongue fasciculation, little wiggle under the tongue, may be present in SMA; those are very difficult to recognize with confidence in a baby. Reduced tone may be present in either. You're not going to have increased tone and scissoring in a neuromuscular condition. Reflexes tend to be absent or decreased in neuromuscular diseases while increased in central diseases. You will see several videos that will show you what children with neuromuscular disease look like when walking.

Other things to look for on exam: look at muscle bulk; look at the posture, and the resting posture in an infant. Does the child have hyperlordosis, are they arched, do they have scapular winging, do they have other abnormalities of movement, of motor posture? And then watch them walk, don't just watch the kid on the mom's lap, or on your exam table and never see them move. Watch them get up from the floor, watch them walk, watch them run.

And this is just a still shot from the video library of a child in the middle of a Gowers' maneuver.

You are going to determine if this is likely to be central or peripheral. If you think it is peripheral and it is a concern, they're not meeting milestones, measure a CK early on. The AAP guidelines also recommend measuring a TSH at the same time. Based on your CK, the history, the clinical evaluation, you will decide whether urgent referral is needed. If you don't need urgent referral, early access referral, and close follow-up, don't see them back in six months. If it is a young infant, see them back in a month. If it is an older child, see them back in three months, but closely follow up for their motor milestones.

Red flags at any age, regression in milestones, loss of skills, tongue fasciculation, and then some sort of key milestones. Head lag should not be present at five months; they should be sitting by seven months. They should be walking and rising to stand readily by 1.5 years, jumping at 2.5 years, and doing stairs with alternating feet at 3.5. If you see one of the red flags in this or from the childmuscleweakness.org website tell the neurologist you are referring to, they will get them in earlier.

A few clinical pearls: developmental progress does not exclude an underlying neuromuscular condition, children often have slow progress, and they don't necessarily regress early on. You're trying to identify them early. Neuromuscular diseases can involve the brain, normal CK does not eliminate a neuromuscular condition, so many of these disorders have normal CK. If it is abnormal and highly abnormal then you must refer immediately. If it is normal, that does not mean you are off the hook.

AST and ALT also come from the liver, so if you are screening for toxicity from a medication and you find a shockingly high AST don't assume it is necessarily from the liver, do a CK also. A negative family history is seen in many of the children with neuromuscular

conditions, so that does not rule out your diagnosis. And all weak children are hypotonic, but not all hypotonic children are weak, so consider your differential diagnosis.

So, a three-year-old boy was seen for a well child check. The mother reports he is making steady progress, but he is slower than peers on the playground. You review his developmental records and see that he walked at 16 months and did not start talking until around 18 months. He has global developmental delay perhaps, borderline. Prenatal and perinatal histories are unremarkable, he is healthy, not on any medications, and there is no family history of neurologic disease. His growth is normal, his general exam is normal. He is not able to run, but walking appeared normal. When you put him on the floor, he puts his hand on his knee when he arises from sitting on the floor. He has firm calf muscles; reflexes are 2+ and symmetric.

Let's vote. "What is the next best step in his diagnostic evaluation?" (Music playing)

CK is the correct answer. In summary, developmental surveillance and screening can result in early diagnosis of rare neuromuscular diseases. Physical examination findings help to localize the cause of weakness. Elevated CK is a clue to some kinds of neuromuscular disease, and early recognition matters. Management and treatment options are available and result in better outcomes. I would like to thank all the many colleagues who worked with me in childmuscleweakness.org, the CDC for supporting that work, and the NIH for ongoing support.

(Applause)

Craig McDonald: It is really a pleasure to be here. I'm going to start with a case here: a 2-year + 10-month old with early motor delay, slight language delay, ambulated at 17 months of age. Our laboratory values show a CK value of 28,000. AST and ALT values are eight times elevated above the normal range and the LDH is elevated six times above the normal value. So oftentimes a child like this, because of the elevated AST, ALT, and LDH, is inappropriately

referred to a pediatric GI specialist for evaluation of liver disease. I can't tell you how many times we see patients who have gone through a rather extensive workup. If we look at this child in terms of his rising off the floor, you can see this very slight push off the knee, a very early Gowers' sign. Let's look at that once more as he is getting off the floor, just a very slight push off the knee. This is a very early Gowers' sign. The child was referred to pediatric neurology. The way in which Duchenne is diagnosed in this day and age is with complete gene sequencing of the dystrophin gene with deletion testing by technique such as MLPA. In this case, it showed a deletion of exons 48 through 50 of the dystrophin gene consistent with a diagnosis of Duchenne muscular dystrophy. Duchenne is an X-linked disorder, as manifested in males. Females carry the disease; males will display the disease. It is relentlessly progressive, a fatal disease. If you look at those muscle biopsies you can see the loss of muscle fibers over time. The clinical course is characterized by progressive quadriparesis, weakness, loss of motor milestones, and loss of upper limb function. These patients also have very severe restricted lung disease and cardiomyopathy as well, which leads to early death.

If we look at the cause of Duchenne dystrophy, it is due to a genetic mutation of the dystrophin gene. These patients have for the most part out-of-frame mutations and they have complete absence of the dystrophin protein. You can see it on the right there, it is located on the intracellular side of the muscle membrane and it links the actin cytoskeleton within the muscle cell to this series of transmembrane glycoproteins, so it is an important structural protein. Here it is again, you can see it there: the dystrophin protein on the intracellular side of the sarcolemma membrane again linking the actin filaments to these transmembrane series of glycoproteins.

So, what does dystrophin do? It works as a shock absorber preventing contraction-induced injury to muscle fibers. So, it helps stabilize muscle fibers, prevent injury during

contraction and if dystrophin is missing as seen on the left, there is absence of linkage between that actin skeleton on the intracellular side of the muscle membrane and the rest of those structural dystrophin-associated proteins. This causes tears in the muscle membrane. You get leakage of calcium into the intracellular side of the sarcolemma membrane and subsequently inflammation, development of free radicals, oxygen deprivation. The hallmark of a dystrophy is really that of fibrosis, replacement of muscle fibers by fat and connective tissue, and eventually muscle cell death.

So again, to review the pathomechanism of the disease: a gene abnormality of the dystrophin gene causes an absence of dystrophin which leads to a structural defect in the muscle membrane. The membrane has instability and then the muscle fiber will undergo apoptosis, necrosis, activation of inflammatory pathways such as NF-kB, and the satellite cells will be activated which are important in muscle fiber regeneration. The muscle fibers go through cycles of degeneration, regeneration, and degeneration and regeneration. Eventually the fibers will undergo fibrosis and ultimately fiber death. So, if we look here at the upper histology you can see the tremendous loss of muscle fibers over time in children as they age with Duchenne muscular dystrophy. The hallmark of the dystrophy is progressive loss of muscle fibers. You can see the 9-year-old has very extensive loss of muscle fibers, some fatty infiltration, and a lot of scar tissue in the muscle. That picture on the right is a post-mortem histological view of the muscle tissue. There literally isn't much muscle fiber left. If you look at these post-mortem photographs, in the lower left you can see that is the elbow and literally that yellow muscle there is the biceps muscle, and that picture on the right is literally that patient's post-mortem picture of the gastrocnemius muscle.

The creatinine kinase will be markedly higher. It can be as much as 10 to 50 times higher in younger patients with Duchenne dystrophy. So there on the right you see the CK values oftentimes on the order of 10,000 to 30,000. The CK values will decrease over time, so by the time the child is 10 to 12-year-old oftentimes the CKs are down to perhaps the 2,500 range. But they are usually 50 times the normal in very young patients with Duchenne muscular dystrophy.

One of the very earliest clinical signs is that of neck flexor weakness and here is that 2-year + 10-month old, you can see him lying supine on the exam table. He literally has less than antigravity neck flexion and that is the first muscle group to show weakness in a Duchenne patient. So, lay your patient supine and check for that head lag as a sign of neck flexor weakness.

This is the so-called Gowers' sign due to hip extensor weakness. Children, as they are getting up off the floor, their hip extensors are weak and in order to transition from a sitting to a standing position they have to push off the knee in order to achieve a standing position. That is the so-called Gowers' sign that we see in Duchenne muscular dystrophy.

As was mentioned, the liver function tests are oftentimes elevated in muscular dystrophy, ALT, AST, and LDH also spill from the muscle and so this can sometimes prompt an inappropriate focus on hepatic dysfunction. If you see patients, particularly those with developmental delay who have elevated liver transaminases, you really do need to check a CK.

Here is a 9-year-old patient with Duchenne dystrophy rising from the floor. He will get wide-based here. He will tripod. He pushes off the knee in order to stand. Here he is unable to transition from a sitting position without the use of the arms. He is unable to hop, unable to...here he is trying to hop on one leg, he is unable to jump. Here he is ambulating with a very lordotic posture and then trying to do just even a single climb in terms of stair climbing. His

stepping is very much impaired. These are some of the tests we evaluate in this so-called Morse or ambulatory assessment, which is a clinical endpoint in Duchenne dystrophy.

Here is a patient again rising from the floor. This will be a very classic Gowers' sign. He will tripod, his legs will get very wide-based, and he has to push off the knee in order to get to that standing position, and that is the so-called Gowers' maneuver.

Here he is trying to run down the hallway, a 10-meter corridor. Here he does it in a much-slowed fashion. You can see the waddling gait pattern. He is unable to really run with both feet off the ground; you can see how tired he is even after running 10 meters.

You can see the tremendous burden of the disease as the disease progresses with loss of head control, trunk control. Here he is in the home environment, his father having to assist him with transfers. This is almost a deadweight transfer to his wheelchair. Here is the father transferring him back into the bed. He has very poor trunk posture. You can see the contractures of his knees and ankles and then because of his pulmonary involvement, he has restrictive lung disease and he is using BiPAP or bi-level ventilation at nighttime.

There are a number of approaches to treating Duchenne muscular dystrophy in this day and age. Really six main categories of therapeutic targets are being used, one being replacement of the dystrophin protein or perhaps upregulation of a replacement protein, utrophin, decreasing inflammation and fibrosis being probably really the mainstay for Duchenne dystrophy. Other therapeutics focus on increasing muscle mass, increasing regeneration, correcting blood flow regulation, correcting perturbations in calcium handling, and addressing the bioenergetics or the mitochondrial dysfunction that occurs due to the calcium leak into the muscle membrane.

Let's vote here. The question: "All the following are common findings in Duchenne muscular dystrophy except?" (Music playing)

So again, “CK elevated to twice the normal value.” Usually in Duchenne dystrophy CK is elevated really in the order of 10 to 50 times normal or even greater, but again a normal CK doesn’t necessarily rule out other forms of muscle disease or other dystrophies.

So why do we use steroids in Duchenne muscular dystrophy? They really target the inflammatory pathways and particularly NF-kB which is chronically activated in Duchenne muscular dystrophy. Essentially this is the main pathway, the NF-kB pathway, which is targeted. There are miRNAs which become elevated in Duchenne muscular dystrophy and increase with disease severity. Inflammatory cytokines induce these miRNAs. The use of steroids such as prednisone or deflazacort block the expression of these miRNA and they can alleviate the pathogenesis in Duchenne muscular dystrophy.

So again, with NF-kB being chronically activated the two mainstays are treatment with either prednisone or prednisolone or recently the FDA approved deflazacort in Duchenne muscular dystrophy.

There are a number of contemporary treatments that have affected the natural history of disease progression and survival in Duchenne dystrophy, steroids being one. Secondly, the aggressive management of spine deformity; steroids can help alleviate the proportion of patients who develop severe spine deformity or scoliosis. Pulmonary management with airway clearance strategies and mechanical cough assistance devices, use of nocturnal or nighttime non-invasive ventilation, and then also cardiac management with early afterload reduction with medication such as ACE inhibitors and recognition and management of heart failure have all had a positive impact on survival in Duchenne dystrophy.

These are data from an article we published just this year, in February, in the *Lancet* looking at the effect of steroids long-term on a variety of motor milestones in Duchenne

muscular dystrophy. The data on the left act show the difference in the age at loss of ambulation in Duchenne patients treated with steroids shown in the red, this is the proportion of patients who continue ambulating versus the blue which are patients that were never treated with steroids, and there is a prolongation of the age at loss of ambulation by about three and half years with the use of steroids. And really a variety of motor milestones are slowed in terms of the age at which those motor milestones are lost with steroid treatment.

Strikingly, the data on the right show that even continuing steroids long-term has a tremendous impact on the progression of the disease. So, this is the age at which patients ultimately lose distal hand function, the red being the steroid treated patients and the blue data being the Kaplan Meier survival curve for those who had never been treated with steroids. The median age at loss of hand function was about 31 years of age in the steroid-treated patients and was about 23 years of age in the non-steroid treated patients. So not only do we treat with steroids usually after the diagnosis is made, oftentimes steroids will be started before the age of 5 at the time at which the diagnosis is made, but oftentimes steroids are continued really throughout the course of the disease.

Now if we look at pulmonary function and pulmonary decline there is a linear decline in pulmonary function as measured by the forced vital capacity that occurs in Duchenne patients over time and the data show that steroids can have a positive effect of this, and this is important because when patients actually reach certain critical thresholds of forced vital capacity with disease progression, there are really quite critical management interventions that are implemented. Nocturnal hypoventilation usually triggers the use of bi-level ventilation at nighttime when the forced vital capacity approaches a critical threshold of about 50% and when

patients approach a 30% forced vital capacity level, they will usually require continuous ventilation throughout the day and night.

These are data from a recent article that we have published, which show the difference in progression of forced vital capacity loss in patients treated with steroids, which is the red bars, showing their absolute forced vital capacity over time, versus the patients who were never treated with steroid in blue. What you can see here is that the actual peak obtained forced vital capacity in the steroid-treated Duchenne patients is higher and the age at which they eventually progress to a very low forced vital capacity of 1 liter or below is prolonged by steroid use.

In fact, when we treat these patients early on, and prolong ambulation, that seems to also have an impact on the course of their disease in terms of pulmonary parameters. These data show the relationship between the age at loss of ambulation and the age of onset of a 1 liter forced vital capacity in Duchenne patients. The red bar shows patients that have lost ambulation before 10 years of age. The blue bar is patients who have lost ambulation at 13 years of age or later and the time at which they transition to a 1 liter forced vital capacity. So, if we can prolong ambulation it results in prolonging future loss of pulmonary function. And this is important because if we can prolong the time it takes a patient to reach that critical threshold of a vital capacity of 1 liter that can improve survival. The odds ratio for risk of death in these Duchenne patients who progress below that 1 liter forced vital capacity, the odds ratio for death, was four-fold higher. Steroids really do have an impact on disease survival as well.

These are data from the muscular dystrophy network showing the loss of gross motor skills in relation to a healthy typically developing population, and Duchenne patients not only are they delayed in their development early on, but if you look at their scaled scores versus healthy typically developing children, their scaled gross motor scores go down over time. So not only do

they have early gross motor delay, but they increasingly fall further and further behind their age-matched typically developing peer groups. So again, these patients will fall further and further behind. Dr. Connolly showed that treating these patients with steroids early on, between 6 months of age and up to 30 months of age, resulted in some tendency towards improvement in their Bayley gross motor skills in relation to a historical control group. So, the trend has been to treat Duchenne muscular dystrophy patients earlier and earlier in the course of the disease, but generally most patients will be treated with steroids as soon as the diagnosis is made and before the age of 5 years of age.

Again, there are other designer steroids, other medications that also impact these inflammatory pathways, such as vamorolone and edasalonexent, which are our new emerging medications with potentially better side-effect profiles than steroids that are also under active investigation for young patients with Duchenne muscular dystrophy.

I would like to just turn now to some of the combination treatments, the other key treatments which involve replacement of dystrophin with other pharmacologic approaches. There are a number of therapeutics which target dystrophin restoration, Antisense oligonucleotides and other RNA based therapies called PMOs, PPMOs and more recently microdystrophin gene therapy.

Let's go ahead and vote. "What is the most common recommended age to start glucocorticoids in Duchenne muscular dystrophy?" (Music playing)

So again, it is shortly after the age of diagnosis, often prior to age 5. I would like to just briefly talk about some of the newer based therapeutics which really make the early identification and treatment of Duchenne dystrophy patients critical. One which is approved in Europe by the European Medicines Agency, Ataluren, enables the ribosome to bypass a

nonsense mutation which is present in about 13% of Duchenne patients. There is a stop codon mutation which stops translation of the normal protein, and the medication can allow for read-through of the stop codon mutation and the protein can be normally assembled. This is an orally bioavailable compound highly specific for stop codon mutation or read through codons which target the nonsense mutation without affecting the normal termination codon. There are some data that were published recently in the *Lancet* showing that in a subgroup of patients where the treatment can actually be adequately evaluated in a one year trial, Ataluren slowed the loss of an ambulatory measure of the six-minute walk distance, so didn't improve the ambulatory measure, but it slowed the relative loss of that ambulatory measure over time in relation to a placebo group.

There is something we refer to as a reading frame rule, where out-of-frame mutations will result in disruption of the open reading frame, ultimately complete loss of functional dystrophin and a Duchenne phenotype with an out-of frame-mutation. An in-frame mutation will preserve the open reading frame of the gene and result in a partially functional protein or low levels of protein. The majority of Duchenne patients and also another form of muscular dystrophy, Becker muscular dystrophy will follow this rule.

So essentially if you have this as a situation where there is a deletion, there are 79 exons in the gene, there is a deletion of exon 45, again causing an out-of-frame mutation, a non-functional dystrophin protein, so this would be a mutation that would cause a Duchenne phenotype, as opposed to another mutation which is actually an in-frame mutation of exon 45 and exon 46, which causes a shortened protein but a functional protein with the binding domains of both sides of the protein being intact and that gives rise to a more mild Becker protein. There are medications which will bind specifically to RNA targets, messenger RNA targets, during

translation of proteins. These are chemically modified nucleic acid molecules which had been used to target specific gene mutations in Duchenne muscular dystrophy. The idea here is for instance a particular medication which targets exon 51 could be used in a subset of patients with very specific gene mutations.

So here is a normal dystrophin messenger RNA, the ribosome comes down, translates a normal dystrophin protein, and then in the case of an exon 48 to 50 deletion that disrupts the reading frame you eventually essentially stop translation of the protein and you end up with a shortened, but nonfunctional protein which disintegrates and that would then lead to a Duchenne muscular dystrophy phenotype. So there again is the unstable truncated protein. And then in the case, again, of a medication that targets exon 51, you can essentially bind down to exon 51, bring that mutation back into an in-frame mutation, a shortened protein but essentially a functional protein is created. So that is the idea of exon skipping: essentially taking an out-of-frame mutation and making it into an in-frame mutation and creating a shortened but functional dystrophin protein.

It has been estimated that an exon skipping approach could target upwards of 70% of all Duchenne patients, and there is a variety of medications which are currently under investigation. One is FDA-approved, it is Eteplirsen. This is a PMO chemistry which again has been used to target exon 51 to bring patients back into frame, and here is muscle biopsy specimens showing Eteplirsen-treated patients with low levels of dystrophin on the left in comparison to untreated control patients and fold changes of dystrophin in these patients who have been treated anywhere from upwards to 11-fold to 15-fold increases in dystrophin expression out to 3 years of age with treatment.

We now have emerging data that show the very low levels of dystrophin produced by these medications could be clinically relevant to a patient and it may be that even less than 3% of dystrophin levels with protein measurement by Western Blot could be clinically relevant to a patient and have an impact on the natural history of disease progression in Duchenne dystrophy. These were the data that were produced with eteplirsen or Exondys 51. Again, there were increasing levels of dystrophin which occurred over 3 years of age and after patients were treated for longer and longer periods of time. There was slowing of the progression of the disease, this measured by the six-minute walk test, the forced vital capacity, and there was also a larger percentage of patients treated with this medication who continued ambulating even well past 15 to 16 years of age. Because of these low levels of dystrophin, the medications were ultimately approved by the FDA.

These are clinical photographs of my three youngest patients that I have been treating with this medication, eteplirsen, for over 3 years of age now. One of them is nearly 10 years of age, on the far right, an 8 ½ -year-old in the middle, and a 7 ½ year-old on the far left. You can see this looks like a very different phenotype than what seen previously with the typical disease progression with Duchenne dystrophy. These patients seem to be doing very well, particularly if treated early in the course of the disease.

There is another medication under investigation which targets exon 53, so again this is early examples of precision medicine targeting very specific gene deletion abnormalities. Here is another medication again targeting exon 53, this is NS pharma drug Viltolarsen which has been shown to produce up to 10-fold to 20-fold changes in dystrophin levels and these patients are showing some improvement relative to historical controls in a variety of motor parameters such as the timed Gowers' sign, the stand from supine, velocity on the left, the timed climbing

stairs on the right, time to walk or run 10 meters on the left, and the Duchenne specific evaluation of the North Star ambulatory assessment on the right. This looks to be very promising as well.

And then finally there are new emerging next generation exon skipping drugs called peptide conjugated PMO drugs which are more effective at penetrating the muscle cell. They again act in a sequence specific binding to these RNA targets. They are very specific to certain mutation subtypes and the preclinical data to date seem to show that there is much improved dystrophin expression in skeletal muscle, and there is even dystrophin in this being seen in the cardiac muscle as well as the smooth muscle as well. This is a very exciting advance which may be present.

And just to finish with microdystrophin gene therapy, this is where an effective but small copy of the dystrophin gene is attached onto a virus and essentially an AAV virus is administered in a single dose to dystrophic humans. This is under human investigation. I'm going to show you a video here of a dystrophic dog species treated with this microdystrophin gene therapy. This is the dog, unable to jump over the stick because of weakness, a Duchenne muscular dystrophy dog. Here is the dog species after a single dose of AAV gene therapy treatment and it is quite impressive. There have been four patients treated at Nationwide Children's and other patients have been treated at other centers in the United States with other constructs, so Nationwide Children's has a microdystrophin trial underway with Dr. Mendell. Florida Gainesville with Barry Byrne has a different AAV microdystrophin construct under investigation. And then Duke with Dr. Edward Smith has another AAV microdystrophin construct which is being actively investigated. This is really a very exciting therapeutic

advancement which we hope will have a tremendous impact on the natural history of progression of this disease.

Obviously, this is early in investigation, the safety is unknown, the durability of the effect is unknown, there is potential that some patients may have antibody titers which could affect their eligibility, and we don't know yet whether the AAV will transduce the satellite cells. We don't know what the possibility will be in terms of re-dosing, but this is a very exciting advance.

I would like to conclude now by just summarizing: if you have developmental delay do a CK. It is quite important and Duchenne patients will have markedly elevated CKs, the CK is a very good screening test to evaluate a patient suspected of Duchenne muscular dystrophy. But again, a normal CK doesn't necessarily rule out other forms of muscular dystrophy or muscle disease. Check for neck flexor weakness, check for a Gowers' sign. Steroids are increasingly prescribed by neurologists at the time of diagnosis and they've had tremendous effect on disease progression over time, they've had a positive effect on survival as well.

Then I just would like to leave you with the thought that really there are very exciting precision medicine therapeutics, which offers great hope for meaningful disease treatment and disease-modifying management, greater function and longer lifespan in Duchenne muscular dystrophy.

Richard Finkel: I'm going to share with you some thoughts about spinal muscular atrophy. I realize—that many of you don't actively treat a child with SMA, so why is this important? Well I hope I'm going to convince you why and that you'll be ready when you do see that patient.

I'm going to talk briefly about what we mean by SMA, the diagnostic testing, treatment guidelines, the importance of early diagnosis, treatment strategies, and now newborn screening.

So SMA is fairly common. It is 1 in 11,000 live births and the carrier frequency is about 1 to 40 or 1 in 60, so in this room there are three or four people who are carriers for SMA. The prevalence is about 25,000 individuals living with SMA in the United States. The cause is a deficiency of a protein called the survival motor neuron, or SMN protein, and due to a deficiency of this protein which is on a genetic basis, it is due to some mutations in the SMN1 gene, which I will show you shortly. But a deficiency of this protein leads to premature motor neuron degeneration and that in turn leads to progressive muscle atrophy and weakness and loss of function.

Now it is important to keep in mind that these babies are typically normal at birth and they develop weakness subsequently and there is a spectrum. And we break it down into three categories: Type 1 which are the little babies, the most severe form; Type 2 an intermediate form. So, here is a little baby, and this is one of those snapshot pictures; when you see that you say okay this looks like SMA and I'll show you some more examples subsequently. And here is a cute little boy who is Type 2, who can sit but has never walked. Type 3 is children that walk, but they don't walk quite normally and they're at risk for loss of ambulation.

So just very quickly, the first 100 years after the original description of SMA by Werdnig and Hoffman in the late 19th century was really to try to understand the disease and that there were these three groups, three conditions, and it took us a while to figure out that in fact that it is all due to the same genetic abnormality in this SMN gene. The discovery of the SMN causative gene as shown here in the left-hand panel was not that long ago, 1995. Shortly after that there was a robust amount of scientific investigation, the development of animal models because SMA does not occur normally in any other species besides humans. So, it led to the development of

transgenic mice and the NIH sponsored specific funding to do drug discovery efforts and that led to the pipeline and some of the drugs that I am going to talk about subsequently.

Importantly, also was the development of standard of care guidelines in 2007 and then recently updated this year and what I hope to convince you is that while we have these exciting drugs, including the first drug that was approved for treatment of SMA just about two years ago, at Christmastime in 2016, we still need to focus on adequate standard of care and support for these very fragile infants and babies.

This chart is a little confusing but I'm just going to try to quickly go through it. This is to highlight that Type 1, which are the babies like I showed you, they present before 6 months of age and they make up over half of the total population of SMA. They never sit and without supportive care they typically die by 2 years of age. That intermediate form, the little boy that was sitting, present typically between 6 and 18 months of age and they make up about a quarter of the overall population, these are the babies that do sit but as they grow up, they never walk independently, and they will live into adulthood. We're now focusing on transition of these patients. We expect that they will be able to live a fruitful life, not necessarily independently, but what we've also recognized is that these children are cognitively normal. It is a very cruel disease because you have progressive motor neuron degeneration and weakness and yet cognitively, they are intact. Type 3 makes up only about 13% of the total group. It presents after 18 months of age and as I showed you these are children that can walk but may lose that and they have normal survival. This is really a spectrum and we artificially break it down into these three groups.

I want to turn to the genetics briefly. There is the SMN1 gene, as shown here, and it has 8 exons or coding regions. And then there is the SMN2 gene which normally is just sitting

silently in the background and why is this important? Well, for a couple of reasons. One is if you're missing SMN1 you're totally dependent on the SMN2 gene. And the second point is that these two genes are not exactly identical, there is one nucleotide difference as shown here, the c.850 C>T change, and what that does is it largely eliminates exon 7, so it is excluded from the RNA and that means that the protein that is generated from the SMN2 gene is often a deficient protein that is non-functional.

There is the SMN1 gene and if you have it you're fine, you don't need SMN2 but if you are missing SMN1 or there are mutations, then you're totally dependent on this backup SMN2 gene. The two genes make the same protein, as shown here. And this just shows in a little more detail what I just said. The SMN1 gene makes an abundant amount of normal protein, and there is only a trickle amount that comes from the SMN2 gene because exon 7 is largely excluded. You can see number 7 is missing here because of that one little change; it causes a change in the splicing. I will show you why this is important in a moment.

This would be an example of a child that has SMA because they are missing a functional copy of the SMN1 gene and they are reliant on this backup SMN2 gene. You can think of SMA as a protein deficiency disease. There is one gene that causes all three types of SMA, but what we've learned is that the more copies of this backup SMN2 gene, the relatively more protein you make; it is not normal, but the more you make, the milder the phenotype.

Now there is a complex diagnostic algorithm that has been published and this is really for the neurologists to work through in a way that tries to take the child, where there is a clinical suspicion of SMA, and get to the right diagnosis. When I was in medical school, we were doing muscle biopsies, we were doing EMGs. We don't do any of that these days in most cases. We go right to genetic testing. When you see a child and you suspect that they have a neuromuscular

disease, do the CK. Now in this case the CK is normal, it is slightly elevated, but consider that they still may have a neuromuscular condition. In this case refer them to a neuromuscular specialist neurologist for further characterization.

Let me show you some videos that hopefully you will burn into your mind because I think these are quite instructive. On the left is a normal twin and I'm just going to pull him up to sit and he's having a good old time. You can see excellent head control at six months and I'm going to lift him up and you can see he doesn't slip through, he has good weight bearing, and he has good lateral neck control when I tip him around. Okay. And I'm going to lie him down prone and he lifts his head up and he gets ready and he is going to try to roll over. So that is little Christopher. These are used with the permission of the parents of course. Now I want you to compare that to Charlotte, his twin sister, who has SMA. Notice the very poor head control. I have to support her, and it is very wobbly. I can't really get her to have good head control. And I'm trying to just sort of get her secure there and not get her too fussy. In a moment I'm just going to try to lift her up and you'll see how she slips through at the shoulder girdle. And then you can see the very poor weight-bearing effort. Unfortunately, this is a typical baby with SMA Type 1, Werdnig Hoffman disease, and this little girl died two months later of a respiratory illness.

Now this is not long after the early presentation and the diagnosis and I want you to compare it to the child over here, it is a separate child who is really end-stage. This is a child 32 months of age, this is a video. But you can see there is virtually no movement, but the child can follow command. With his eyes he can blink, and he can...there is a slight bit of movement but virtually quadriplegic. We do have a defined natural history of SMA and we can see this

progressive decline in motor function. We know to anticipate needs for nutritional support such as feeding tubes and ventilator support.

This is a child with Type 2 and I'm asking this little boy to lift his arms up over his head, so you can see that he sits very nicely but he is not able to achieve much.

And then here is a girl with Type 3 and she is running, and she has a Trendelenburg hip waddle and here she is going up the stairs and she is pushing off her thigh as if it is a Gowers'. You can see it is challenging for her, and I see her now about three years after this was taken and she is no longer able to climb stairs. She is still walking. In the right-hand panel, she is getting up from the ground and as you saw from Dr. McDonald and Dr. Matthews, she exhibits a Gowers' sign, which means there is proximal weakness; it is not specific for Duchenne.

Alright, here we go. "Which statement below is correct about how the patient with SMA presents?" (Music playing)

Alright. Look at that. So not only are you staying here, but you're listening, that's great.

(Laughing)

Okay, let's move on. When to suspect SMA? The babies present as a floppy baby, the frog leg posture, everything you saw with little Charlotte, the poor head control, the slipped through poor weight bearing. All that testing takes about five to ten seconds in the office. The child with Type 2 SMA presents with a plateau in motor development and some hypotonia, joint laxity, poor weight bearing, absent reflexes, and they may have some fasciculation of the tongue as well, and then you saw in the Type 3 there is just an abnormal gait and often the reflexes are absent as well.

There are standard of care guidelines. The important point here is that the patient and the family and the primary care physician are at the center and the specialists serve really as

consultants and we now look at having a multi-disciplinary clinic to try to pull together the different coordinated care.

When we talk about supportive care and medications for SMA of course immunizations are important, the RSV prophylaxis with palivizumab or SYNAGIS in the first two years of life and it is hard to get insurance to cover that sometimes but we're usually successful. Respiratory support with nebulized bronchodilators and short-term mucolytics. GI support is really important with MiraLAX, Senna's and GERD treatment such as with ranitidine. Bone health and then I'm going to focus a little more on specific targeted treatments.

As you can tell a lot of this is being handled at the primary care physician level and we make recommendations in our notes and we try to coordinate the care with the primary care team.

Okay, time to vote. "Which statement below is correct regarding treatment for SMA?"

(Music playing)

The point we are trying to make here is that early diagnosis is important and I'm going to try to convince you with some clinical trial data now that show you that those babies who were treated early after diagnosis do better than those who were treated later and have a longer and larger burden of disease.

We're going to go back to our cartoon here about the genetics of SMA and the treatment strategies are two-fold. One is to try to replace the missing SMN1 gene and that would be a gene replacement therapy. The second is to modulate the SMN2 gene to try to shift this more from 90% excluding exon 7 more towards a 50/50 balance as shown here. By doing so you're going to generate more of the deficient SMN protein, as shown by the little cartoon.

I'm going to turn now to the one drug that has been approved for treatment of SMA. It is called nusinersen, and it has been available for almost two years. What is interesting about this drug is that it works by modulating the splicing as I mentioned and the drug itself, as you'll note here, is a little string of oligonucleotides, so it is 18 letters of DNA-like material. Instead of trying to skip over an exon as in Duchenne, what this is trying to do is modulate splicing to include exon 7 in the messenger RNA transcript and by doing so it allows exon 7 and exon 8 to join and you're going to make more of this normal protein.

So how well does this drug work? Well there was an open-label phase 2 study, which I participated in, and I'm going to show you some videos that I hope you'll find convincing. So first off there was improvement in survival compared to a natural history control and much to our surprise, or at least my surprise, these babies showed improvement in two different motor function scales. The natural history predicted a slow decline whereas we were seeing that there was improvement in these babies.

So here is one baby who was at 7 weeks of age. You can see typical frog leg posture, the paradoxical or belly breathing pattern. And here he is at 14 months of age. Now this drug is given by repeated intrathecal injections, so it is a little bit challenging, but remarkably these babies have tolerated the treatments well and you can see how finally we're able to coax him to roll over. So that is really a transformational type of motor skill because babies with SMA Type 1 never roll over. So right here you can see now he's not rolling over at a normal age, it is 14 months, but he is already making remarkable gains.

Let me show you some further gains he has made. Now, so this is an important skill, but not captured in our clinical trial, this was just in the clinic.

And here he is more recently, this was about a year ago and he is continuing to make progress. You can see he has a TLSO brace on and he is certainly not walking normally, but the point here is this is a boy who genetically was predicted to have died before age 2 and here he is almost 4 years of age continuing to make gains.

I want to shift now to a pre-symptomatic study. So here is a baby that has been treated pre-symptomatically whose sibling died at 7 months of age of Type 1 SMA and I think you can see in just a few quick seconds how this baby is doing so well. Here he is at 16 months, really wanting nothing to do with me that day, but he is walking. The little girl on the right is too cute to not include. She is ready to go to school at 22 months. Both of these children not only are walking but walking at a normal age. And comparing the data from the different studies, these were symptomatic treated patients, these were the pre-symptomatic patients that are improving in their motor milestones over time and I think you can see the early and more robust response in the pre-symptomatic patients compared to the slow steady improvement in the symptomatic patients.

I want to finish with just a brief mention about the gene therapy study. It is called...the drug itself is called AVXS-101, and it is trying to replace the missing SMN1 gene as I mentioned. What we've learned from this, the first Phase 1 study that was published last year, is that there is improved survival, motor function, pulmonary function, and feeding; and importantly, that the babies who are treated early after diagnosis responded most favorably. That is shown here, and I will just have you focus on the right-hand panel and you can see the babies who are younger that they shot up on this motor function scale and they reached a ceiling effect, so they couldn't get any better than by 3 to 6 months of age. Whereas the babies who were treated later seem...they improved, but not quite as readily. This is a group treated with a lower

dose, but this high dose group is quite spectacular, and that is being hopefully confirmed in some current studies.

I'm just going to finish with a word on newborn screening and to let you know that the RUSP, which is the committee that reviews suggestions to add something to the newborn screening platform, approved SMA just in July and 11 states in the U.S. are now actively screening or having pilot studies for SMA and this is also true in many countries in Europe. So, this is coming to a state near you and we anticipate that in the near future babies will be identified pre-symptomatically. Let's finish with a case of a 12-month-old girl who was seen for a well child check. The father shares his concern that she is no longer able to bounce on her legs. She was hospitalized at 9 months of age with RSV and needed C-PAP support for two days and since then she seems to have plateaued and made no gains in her motor skills. Her chart notes indicate normal prior developmental milestones including sitting independently at 6 months of age and her first words at 10 months. Review of the family history was positive for cerebral palsy in a cousin. The vital signs show a plateau in weight gain since 9 months of age with normal height and head circumference growth.

Here is your final chance. "What would you look for on your exam as you're looking at this child in your office?" (Music playing)

Okay, so hypotonia, head lag, tongue fasciculation. And it would hypo or areflexia, not hyperreflexia. Okay, so with that the take home points, is that SMA is...has changed. Now that we have these treatments it is a treatable disease. Nusinersen is now a treatment standard, gene therapy is likely to be an option shortly. There are other drugs in the pipeline. We are seeing a changing phenotype. Standard of care remains critically important, early identification of these patients and treatment clearly makes a difference and newborn screening is coming.

With that I'm going to stop and just some of my acknowledgements. I thank you for your attention.