Spinal Muscular Atrophy: Classification, Diagnosis, Management, Pathogenesis, and Future Research Directions

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Spinal muscular atrophy is an autosomal recessive neurodegenerative disorder that affects the motor neurons responsible for movement of the proximal muscles of the trunk and body. To date, the disease can be classified into 3 main categories based on severity and age of onset. During the October 18th symposium held in Pittsburgh, Pennsylvania, researchers met to (1) describe current diagnostic strategies, (2) discuss recent thoughts on pathogenesis, (3) review current therapies and clinical trials, and (4) define future research directions. In her opening remarks, Dr Story Landis, director of the National Institute of Neurological Disorders and Stroke, emphasized the degree to which the Neurobiology of Disease in Children conference series has broadened awareness of the many rare diseases affecting children, not only through the advancement of research but also by educating practitioners about diagnostic strategies. Dr Landis also discussed the role this conference may play in fostering research that seeks to develop a single mechanism of therapy for spinal muscular atrophy. She also discussed the current funding situation at the National Institutes of Health and addressed the crucial function of volunteer research organizations that sponsor research in further improving management of this condition. This article summarizes the presentations and includes the verbatim edited transcript of question-and-answer sessions.

Keywords: spinal muscular atrophy; clinical features; pathogenesis; future research directions

Clinical Neurology of Spinal Muscular Atrophy

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Historical Descriptions of Spinal Muscular Atrophy

Victor Dubowitz, MD, PhD, FRCP, DHC, Hammersmith University, London, United Kingdom

Dr Dubowitz reviewed the history of spinal muscular atrophy, an autosomal recessive disorder leading to degeneration of the anterior horn cells of the spinal cord. Classically, a well-defined severe form exists and is referred to as Werdnig-Hoffmann disease. In addition, Kugelberg-Welander described a mild form of the disease, and the differences in severity between these 2 extremes have allowed for observation of a clinically intermediate form. The more severe form typically presents at birth or in the first months of life with severe paralysis and marked weakness of the limbs and trunk. These infants display the hallmark signs of spinal muscular atrophy, including degeneration of the intercostal muscles, a bell-shaped chest, and abdominal breathing (due to the fact that this
is the only neuromuscular disease known to spare the diaphragm). Patients with the intermediate form have been described clinically as being able to sit unsupported, although never able to stand or walk; those with the mild form have been documented with late onset of the disease, allowing them the ability to walk. Histological findings have revealed spinal muscular atrophy is caused by the denervation and group atrophy of large muscle fibers, thus explaining the varying severities.

Werndig and Hoffmann are credited with first describing infantile spinal muscular atrophy (the severe form) in papers published in 1891 and 1893, the latter a follow-up in which Hoffmann described 7 cases of the disease from 4 different families. Dr Dubowitz noted the importance of recognizing the complex history surrounding the early descriptions of spinal muscular atrophy. He also acknowledged Thomson and Bruce for their work, which cited a case of progressive spinal muscular atrophy in a child with a spinal lesion and hypermobile joints. It is now known that children with intermediate spinal muscular atrophy have hypermobile joints and their fingers can usually be hyperextended by 90° at the metacarpal pharyngeal joints. Dr Dubowitz credited Beevor, at Queen Square Neurological Institute in London in 1902, with possibly describing the first true case of type 1 or severe spinal muscular atrophy. Beevor described what is considered today to be a prepathological state of spinal muscular atrophy and, unknowingly, what is considered clinically the truly severe form of the disease today.

Description of the mild form arose as the result of Kugelberg and Welander’s efforts to separate and define spinal muscular atrophy from muscular dystrophy. Their original paper was published in the American journal Archives of Neurology and Psychiatry (Chicago) in 1956 and described 12 cases of spinal muscular atrophy. The mild form was similarly cited by Wohlfart, a Danish neurologist, in 1955 in Acta Psychiatrica Neurologica. Credit can now be given to both groups of investigators and physicians who pooled data on this less severe form of spinal muscular atrophy characterized by proximal wasting and weakness affecting the lower limbs more than the upper, with typical onset in adolescence (although in some of their cases, the disease actually started around 2 or 3 years of age).

In 1960, Dr Dubowitz began to define the intermediate form of spinal muscular atrophy, again a result of the attempt to differentiate spinal muscular atrophy patients from those in a muscular dystrophy ward at Queen Mary’s Hospital for Children. After discovery of the genes for spinal muscular atrophy in 1990, Dr Dubowitz pushed for a classification of the disease based upon clinical presentation; this classification system will be discussed in further detail in the section covered by Dr Barry Russman. Despite the various descriptions of this disease reiterated at different times in history, we are continuing to learn more about spinal muscular atrophy through the common genetic basis linking all 3 forms.

**Clinical Classification and Disease Heterogeneity**

*Barry Russman, MD, Oregon Health & Science University, Portland, Oregon*

Dr Russman briefly discussed the classification system now used for patients with spinal muscular atrophy and the heterogeneity of the disease. A thorough patient history and physical examination are considered essential tools for proper diagnosis of spinal muscular atrophy. For example, Dr Russman noted that the most unique clinical finding for this condition, polymyinoclonus (muscle trembling of the fingers), is commonly seen in many spinal muscular atrophy patients but was only first described in 1971. It can now be used as a classification and diagnostic tool. The current classification system relies heavily upon adjectives to describe the varying types of spinal muscular atrophy, and this allows for extreme variability in the meaning each adjective may have for the clinician. This makes it very difficult to determine a sound prognosis and treatment plan for each patient. Thus, the Maximum Function Achieved schema was adopted as a means to clarify what a child’s prognosis would be according to his or her (motor) function at the age of disease onset. While clarifying the functional component of the Maximum Function Achieved classification system, Dr Russman and colleagues concluded that function is gained before it is lost and that all spinal muscular atrophy patients experience a net loss of function over time.

Various classification systems using function as the primary adjective have been published, including studies by Hausmanowa-Petrusewicz and those by Zerres. However, the system created by Dr Dubowitz in 1995 and published in Neuromuscular Diseases, in which the forms of spinal muscular atrophy severity were categorized by function and assigned numeric values such as 1.1, 1.5, 1.9, and 2.1, has been a popular and practical diagnostic tool. Because spinal muscular atrophy patients differ in their functional ability, some of those with the type 2 form of the disease (or spinal muscular atrophy 2.0) have a life expectancy of 30 years, while patients with the type 2 form who are strong sitters and may receive a numeric ranking higher than 2.0 should expect to live to age 50. The current prognosis for patients with spinal muscular atrophy type 3 has been established, and they should expect to live a normal life span. The variability is the same for spinal muscular atrophy type 3 patients, patients with the mild Kugelberg-Welander form of the disease. In the coming years and in the next few presentations, we will see how genotype/phenotype correlation may serve as a true aid for classification and prognosis.

Dr Russman also briefly discussed several of the various diseases that share in genieity with spinal muscular atrophy.
Kennedy syndrome was discussed as a heterogeneous spinal muscular atrophy disease in which patients have weakness and a normal DNA test result; on examination, electromyography (EMG) and muscle biopsy show evidence of denervation, despite intact axons and intact myelin. The late onset and gynecomastia associated with the disease are usually strong indicators for diagnosis. Another disease mentioned was Fazio-Londe disease, a very rare condition characterized by the progressive paralysis of muscles innervated by cranial nerves. The largest class of heterogeneous diseases includes the hexosaminidase A deficiencies. These patients typically have cerebellar abnormalities in addition to anterior horn cell abnormalities, and their muscles are weaker distally than proximally, and DNA test results are negative. EMGs typically show denervation with intact peripheral nerves in these patients. The key diagnostic factors for these patients include distal weakness and respiratory distress, due to the fact that they are able to use only half of the diaphragm. This tends to be fatal. However, the disease may occur without involvement of the diaphragm or respiratory distress, and this finding has been linked to chromosome 12q24, the same chromosomal area to which the diaphragmatic form has been linked. The disease may also manifest with atrophy seen in the scapuloperoneal area first and then eventually progress to involve the hands and proximal lower extremities. Dr Russman also described another disease involving degeneration of the anterior horn cells that presented with facial weakness, and this was evident upon EMG and muscle biopsy. Lastly, a disease heterogeneous with spinal muscular atrophy was mentioned as having spinal muscular atrophy–associated problems not linked to motor neuron degeneration but to brain and cerebellar atrophy, with congenital fracturing. Although this disorder as well as muscular atrophy–associated problems not linked to motor neuron degeneration but to brain and cerebellar atrophy, with congenital fracturing. Although this disorder as well as those described above are not linked to chromosome 5q, they are all considered to be heterogeneous forms of the disease.

Modern Diagnosis and Management Part I

Tom Prior, PhD, Ohio State University, Columbus, Ohio

Dr Prior first described the diagnostic testing currently being used to identify approximately 95% of the individuals with spinal muscular atrophy regardless of severity (type 1, 2, or 3). All are lacking exon 7 on the SMN1 gene, which was formerly referred to as the telomeric version of the SMN2 gene. Thus, standard testing used in most laboratories in the country involves separating the 2 genes using polymerase chain reaction (PCR) analysis to amplify SMN1 so deletions can be identified. The specificity of this test is approximately 99%; however, some individuals are asymptomatic and lack SMN1; therefore, the diagnostic sensitivity is approximately 95% specific.

The nondeletion patients (the additional 5%) most commonly experience an insertion of 11 bases. The common point mutations associated with the various forms of spinal muscular atrophy include a 2-base deletion, a missense mutation in the 5’ end of the gene or missense mutations occurring in the area of exon 6 and exon 7, and single-base changes in the areas of exons 6 and 7. All mutations typically cause some alteration in the normal folding of the SMN protein.

Dr Prior also spoke about the importance of carrier testing for spinal muscular atrophy, a disease with a carrier frequency of 1 in 40 individuals, with no apparent population inconsistencies. The test is performed using PCR analysis to identify the asymptomatic carriers with a 50% reduction of SMN1, as opposed to the affected individuals who are missing the gene entirely. Although this carrier frequency is quite high, it is still possible to find carrier individuals without an affected relative. Of importance is the idea that carrier testing can be used diagnostically for the 5% of children without the homozygous deletion. Using the Hardy-Weinberg rule, this 5% of children will be compound heterozygotes; in other words, they will present with spinal muscular atrophy due to a deletion and an additional abnormality, specifically a point mutation in the other SMN1 gene. Thus, a 2-tier test can be run on these individuals. If testing results for the homozygous deletion are negative yet the clinician strongly believes the child is presenting with spinal muscular atrophy, they will likely present with the same 50% reduction as the carriers but in addition will have a gene sequence performed because the SMN1 gene is small. For these individuals, the most common abnormality is a point mutation involving the conversion of serine to isoleucine. Additionally, linkage data show that markers have been lost on transmission between the parent and affected individual, and it is important to note that approximately 2% to 3% of mutations in spinal muscular atrophy have been documented as occurring de novo.

Unlike most autosomal recessive disorders, approximately 5% of the carriers identified with affected children have 2 copies of SMN1, and in normal carrier testing situations, this must be taken into account. This finding is also important because it has recently been found from the carrier studies that 2% or 3% of individuals in the normal population have 3 copies of SMN1. With monosomal testing, Dr Prior’s laboratory has recently been able to show proof for the concept of a 2-copy carrier after managing to separate chromosomes 5s. In looking at the spinal muscular atrophy population, we have found that approximately 90% of spinal muscular atrophy type 1 patients very rarely have more than 2 copies of SMN2; however, perhaps 97% of spinal muscular atrophy type 3 patients have 3 or more copies of SMN2. This correlation has held up very well, and it may give way to a very strong phenotype/genotype association. Approximately 75% of patients with spinal muscular atrophy type 2 or type 3 have 3 copies of SMN2, 20% to 25% have 4 copies, and a
few very rare patient cases have been seen in which individuals have 5 copies. In all, the clinical data show that, with additional copies of SMN2, a milder phenotype is observed, or patients even present as asymptomatic. However, it is still too early to rule out modifiers, as there have been cases in which modifiers are all that can explain the outcome, but it is still arguable that SMN2 may be a sensitive phenotypic marker for spinal muscular atrophy.

Lastly, Dr Prior covered the issue of newborn screening and the importance of making an early diagnosis while the motor neuron counts in newborns are still high and the opportunity of offering a therapeutic treatment would be most effective and beneficial. The methodology currently being tweaked for newborn screening involves the use of a LightCycler TaqMan Assay PCR analysis, which is more time-efficient than gel electrophoresis assays. The control gene used in Dr Prior’s laboratory was RNase P, which ensured that carriers would give a normal result, the goal of these screenings being to detect the affected individuals. For this type of testing, it is ideal to run a large number of cycles so as to maximize the quantity of DNA obtained from the blood spots used in newborn screenings and to minimize the costs. The incidence rate of 1 in 10,000 should be the driving factor to robotize the newborn screenings.

Modern Diagnosis and Management Part II

Susan Iannaccone, MD, FAAN, University of Texas Southwestern Medical Center, Dallas, Texas

Dr Iannaccone presented empirical experience and current data regarding the treatment plans being developed for childhood spinal muscular atrophy. This includes children with spinal muscular atrophy type 1, type 2, or type 3, or what is sometimes referred to as nonsitters, sitters, and walkers, respectively. As has already been stressed, the prognosis and management of spinal muscular atrophy depend on recognition of the patient’s disease severity; however, the number 1 cause of morbidity and mortality in spinal muscular atrophy patients is respiratory failure, the complication of severe restrictive lung disease, which is progressive. The bell-shaped chest in the type 1 spinal muscular atrophy baby, referred to by Dr Dubowitz, results from the discrepancy between paralysis of the intercostal muscles and maintained function of the diaphragm. Upon inspiration, the ribs collapse, creating atelectasis. Spinal muscular atrophy type 1 patients, in particular infants with this form, are at a much higher risk for respiratory failure than type 3 patients, whereas restrictive lung disease may be insidious in the intermediate spinal muscular atrophy patients.

Sleep-disordered breathing is typically noticed in these children before symptoms appear and is now treatable with noninvasive ventilation. In type 1 spinal muscular atrophy, infants’ failure to thrive is typically seen, usually as a result of bulbar weakness and aspiration or reflux. Failure to thrive should be treated immediately and proactively because this condition rapidly exacerbates any preexisting weaknesses the child has, while at the same time reducing the child’s reserve against any intercurrent illnesses. Patients with type 2 spinal muscular atrophy typically present with constipation as a result of hypotonia of the abdominal musculature and immobility, which can be treated with added fiber to the diet and water. Type 3 patients tend to be very thin due to the high caloric requirements needed to help them maintain mobility and, as with the school-age type 2 patients, should be monitored for maintenance of proper diets.

In addition, many children with spinal muscular atrophy suffer from several typical orthopedic problems. Some children are born with a congenital clubfoot deformity, and those patients who survive are at increased risk of developing scoliosis and contractures of the lower extremities. Some are also born with congenital scoliosis, making management very difficult. Lastly, there are psychosocial complications that arise in treating children who have spinal muscular atrophy. It has been determined that most have normal or high IQ scores, which presents both a challenge and an opportunity for these patients. Depression is not typically seen, perhaps because of the plateau phase seen in the progression of spinal muscular atrophy; however, there are many family stressors that present, especially those financial in nature. As always, infection of any sort can place these patients at increased risk for respiratory failure, and we are reminded that early intervention and vigilance are key.

In treating these patients, the goal is to promote lung capacity because cross-sectional data have shown that those with the type 1 form in particular have collapsing of their ribs and are not experiencing the normal lung growth seen in the first 2 to 3 years of life. With the new noninvasive ventilation readily available, a new awareness of sleep-disordered breathing, and a multidisciplinary approach, we are now better maintaining the health of these patients. Use of the new ventilators with smaller masks increases comfort and decreases incidence of skin breakdown, and, in addition to the use of new bilevel positive airway pressure machines (now also more portable and easy to use, with preset rates and pressures), has allowed us to monitor these children at home. The current pulse oximeters also allow us to monitor the sleeping patterns of these children at home and then download the information recorded from these machines to analyze at a later time. An increase in the number of polysomnogram clinics across the country that now focus on children allows for annual monitoring, and children placed on nocturnal ventilation can have their rates and pressures tracked and altered appropriately as they grow. Both the noninvasive ventilators and new measures for sleep-disordered breathing are helping us achieve our goal of keeping these children healthy and out of the hospital.
Multidisciplinary care is most needed for coordinating the surgeries and postoperative care common to children with spinal muscular atrophy, including the Nissen fundoplication, gastrostomy, and scoliosis corrections. Airway clearance should be dealt with aggressively before and after surgery. Caloric supplementation and therapy to regain motility after surgery also should be carefully addressed. With this approach, treatment can be more clearly delivered, and patients can return to a better quality of life more quickly.

**Questions and Answers**

**Dr Kissel:** Dr Russman, do you bother ordering SMN deletion studies in some of these atypical phenotype spinal muscular atrophy patients that you see, for example, somebody with a distal presentation?

**Dr Russman:** Do we order the DNA tests for SMN if the child has anything but proximal muscle weakness? Is that the question?

**Dr Kissel:** Yes, if you see somebody with the distal form of spinal muscular atrophy which you described, do you bother testing for the SMN deletion?

**Dr Russman:** I do not order that test. I think it’s unnecessary. I think you can determine clinically that it’s distal weakness. I probably will get an EMG to see that there is evidence of denervation with intact peripheral nerves, and I might not even go on to a biopsy.

**Dr Dubowitz:** I would like to disagree completely.

**Dr Kissel:** That is why I asked the question.

**Dr Dubowitz:** It is the exception that proves the rule. In our unit about 2 years ago, there was a newborn diagnosed with congenital myopathy because the EMG was myopathic. The child had a biopsy that looked like the one in Beevor, with a myopathic pattern of variation, and the creatine kinase was approximately 300, which of course can occur in the first weeks. And as we were discussing the pathology, I then asked, “Well, let’s see a picture of the child.” When they showed a picture, I noted, “Well, that’s spinal muscular atrophy, look, and do the SMN,” and it had a deletion. I think the longer you’re in neuromuscular disorders, the more you realize everything is up for grabs. And now that the congenital dystrophy genes can cause adult limb-girdle dystrophy and vice versa, I think in any suspicious case that is a bit atypical, if you do not look for these things, then you will be surprised.

**Dr Russman:** The scenario Dr Dubowitz just gave us was quite different from the way I answered Dr John Kissel’s question. If you are not sure about a child or baby, first of all, let me just say the EMG, as you know, in newborns, is very unreliable. Dr Dubowitz just said useless, and I’ll agree, although I have a scenario, Victor, in a 2-month-old in which it was incredibly helpful. But if I have a 6-month-old with proximal muscle weakness and I see slight tremor in the hands, then I can diagnose spinal muscular atrophy because it is that simple. If I notice distal muscle weakness, primarily in a 6-month-old, and I am very confident that distal weakness with decreased reflexes, I will not order the SMN gene test.

**Audience Member:** Dr Prior, you mentioned having both SMN alleles or both SMN genes on 1 chromosome, and you said that this condition can be detected through research technology. Is that technology available clinically?

**Dr Prior:** No, it is not.

**Audience Member:** As a follow-up to that, in that instance, is the family at a dead end in terms of additional advice?

**Dr Prior:** When a parent with a normal gene has an affected child with a homozygous deletion, this does reduce the parent’s chance of being a carrier because there are only 2 possibilities, and I think they are fairly equivalent. First, the 2-copy chromosome, which means the affected child is carrying a de novo mutation. Linkage testing can be done for those families because occasionally the linkage will show the de novo mutations. Markers in the parent drop the effect, and that can be done in routine diagnostic labs; however, performing the monosomal testing is not routine.

**Dr Alexander:** Dr Prior presented stunning data on the frequency of the carriers with spinal muscular atrophy of 1 in 40. Did anybody consider why such a high frequency exists? For example, when you think of cystic fibrosis patients, we think that the frequency is probably due to survival from the plague. But what can explain or serve as the reasoning for why such a high carrier rate exists for spinal muscular atrophy?

**Dr Prior:** The question is why do we have or is there a heterozygote advantage in spinal muscular atrophy? What you’re referring to with cystic fibrosis, I’m going to hand over to Dr Burghes, but the nature of the gene area being unstable may also contribute to a high frequency of recombinational events and conversion events, which just may make it more unstable. It does have a de novo mutation rate, and I know Dr Burghes would like to comment.

**Dr Burghes:** I want to review an old principle; the Hardy-Weinberg principle on disease with a 1 in 10 000 frequency would give you a carrier rate of about 1 in 50 to 1 in 40, so it’s actually not unexpected at all. The frequency of carriers for the frequency of disease is exactly correct. The other thing is the 2% mutation rate; if you go back and calculate this, everyone in this room should have spinal muscular atrophy, OK? So it doesn’t quite work. The surprise is the 2% de novo mutation rate, which means there must be a reversion rate the other way around that balances exactly—2% forward, 2% back, if you do the math on it.

**Audience Member:** People have talked about the diaphragm being relatively spared in spinal muscular atrophy. How spared is the diaphragm? Is it completely normal? Is it relatively spared? Has anybody looked at the histology of the diaphragm in spinal muscular atrophy patients?

**Dr Dubowitz:** When you look at the diaphragm clinically, it seems to be completely spared, and it’s essentially the only functional respiratory muscle. John Scott and Susan Iannaccone, I believe, also showed that very clearly with the in-drawing of the chest and the expansion of the diaphragm. I’m sure that if you look at the diaphragm histologically, one would find some evidence of denervation, as you do in all muscles, irrespective of whether it’s proximal or distal, but clinically, there’s no doubt at all that the...
diaphragm is functional. And the original reason why we were unable to be included with the special committee that met in 1990 is they believed clinical observation was not enough; special observation, such as ultrasound, was needed.

Dr Russman: Let me disagree before your next question. Just remember all forms of spinal muscular atrophy present as a slowly progressive disease. It is not static. If you watch children with spinal muscular atrophy type 1 who have no intercostal movement initially and only diaphragmatic movement, eventually that will fail also. I remember reading a few studies that showed diaphragmatic involvement in the children who eventually passed away. It does become involved; it just happens to be the last muscle that is lost. I don't know if you feel that way, Victor.

Dr Dubowitz: I think you may be right, but do not say that to the parents because they want hope, and they want to know that the diaphragm is functioning. And on this note, I have to tell you a very quick anecdote. Some years ago, I was watching the Jerry Lewis Band Show over Labor Day, and they often show spinal muscular atrophy children because they're so bright and responsive. And there was a doctor presenting a child with type 2 spinal muscular atrophy who said, "This is a progressive disease, and they get progressively weaker, and ultimately, they go into this profound respiratory distress," and the mother says, "But she's getting better, doctor." And the doctor says, "As I was saying, this is a progressive disease," and I felt like shaking the television set; "For God's sake, listen to what the mother is telling you." This type of information will come from the parents, and they will relay to the clinician that the child is doing more than he or she had done before. And some of them achieve certain additional functions, but on the whole, it's relatively stable, and over a period of 30 or 40 years, certainly there is a little bit of change.

Audience Member: The reason I bring up the question is that in the mouse models we have looked at, the diaphragm muscles are extremely, profoundly affected. How difficult would it be to obtain some diaphragmatic tissue from a spinal muscular atrophy patient and look at it histologically?

Dr Russman: As I mentioned, it does deteriorate, and on autopsy, it does show that they have deteriorated. You're right. I'm not sure we can use the mouse model to correlate it with the human model, but the children do get worse. And I think that Dr Dubowitz is referring to the 3- or 4-year-old child who, we did show in our studies, about 40% actually did improve function before they got worse, and this was validated. Our data were validated when the Germans published their paper in 2001, showing that a certain percentage of patients did show improved function before they got worse. Just like the honeymoon period in Duchenne—Duchenne children usually, many times will get better between 4 and 6 or 7 years of age, before they start to deteriorate. I think the window is larger in spinal muscular atrophy than it is in Duchenne, but they do all get worse.

Audience Member: I would like to add 2 things to Susan's list regarding proactive preventive care. First, I would like to suggest smoking cessation programs for families with affected children, and second, it is important to have spinal muscular atrophy kids fully immunized, including the influenza vaccine.

Dr Maria: What thought has there been regarding incorporation of the SMN2 effects on prognosis, in terms of classification? Families are going to want to have a sense of the big picture and what effect SMN2 is having in the way of the severity of disease. What is the current thought, in terms of updating the classification system with some of that data?

Dr Prior: We acquire a lot of SMN2 levels due to the fact that we are creating a lot of the treatment plans for patients. My own feeling is that it is still a research test, and we obtain a baseline SMN2 on all patients on the valproic acid studies and other studies and not perform the test unless a physician requests it, and I do not obtain an SMN2 on an individual. I still think the classification is clinical, and I think that the SMN2 correlation can be supported. However, I would hate to experience an exception, particularly in a newborn, and give the family a false comfort in saying the case may not be as severe and “not to worry because there are 3 copies of SMN2.” So I run a very conservative approach to this disease, and it has been a research test at our lab, as a baseline SMN2 is obtained on every single patient that's enrolled in any of the patient treatments.

Dr Russman: That is an incredibly important question. Why? Because Athena Diagnostics advertises that it will do copy counts. And just as Dr Prior stated, I am very concerned about that. I do not think the correlation is strong enough yet to use the phenotype/genotype concepts, as opposed to the clinical concepts, for determining prognosis. So I'm against obtaining copy counts in patients with spinal muscular atrophy on that basis. On the other hand, if they're involved in clinical trials, it's mandatory that copy counts are obtained.

Dr Dubowitz: I agree based on the fact that throughout the history of neuromuscular disorders, we continue to be surprised. In fact, I've started a new column in the journal as of December, in Neuromuscular Disorders, called “Clinical Casebook.” But if you have a clinical case, and the geneticist tells you something completely different, I think it's very important to be confidently aware of this, that there are exceptions, and I don't think we can extrapolate from one to the other. You still need to look at the clinical picture.

Dr DeVivo: Why is this neurogenic disease proximal rather than distal?

Dr Dubowitz: In 1957, I thought I had the answer to that question when I started doing histochemistry of muscle because I thought that we had different distribution. This is a huge question, as to why so many neuromuscular diseases are proximal. The simple answer is usually that there is a greater load on these muscles as opposed to the more distal muscles, but then why are certain diseases distal and not proximal? I don't know.

Audience Member: In these spinal muscular atrophy 1 patients who get put on bilevel positive airway pressure early, can one estimate or get a sense of how long that prevents them from being placed on full-time mechanical ventilation?

Dr Iannaccone: The question is, in the spinal muscular atrophy type 1 patients that we put on noninvasive ventilation, are there any data on how long it is before they need full-time ventilation? In my experience, it’s a matter of months, and I don't think using noninvasive ventilation has provided long-term survival for any spinal muscular atrophy 1 patients. However, I do think it has provided some improved quality
of life, just like the gastrostomy tube can improve quality of life. There may be initial resistance against this technology on the part of the family, but those who opt for it find that it has helped and has prolonged life (we are speaking in terms of weeks). But it can prevent lung infections and keep the child out of the hospital, whereas invariably, the patients or the families who opt for no treatment will end up in the hospital eventually. I recently saw a child who was diagnosed with spinal muscular atrophy immediately after birth. I never saw the child before she went home from the newborn nursery on hospice. But now at 3 months of age, she is still alive, although suffering from pneumonia, which scared the family, and they ended up in the hospital. Thus, the situation was slightly more problematic from a management standpoint. No, it does not provide long-term survival, but I think it does improve quality of life.

Audience Member: As a follow-up to that, do you find that most parents who opt for that do not accept mechanical ventilation long-term?

Dr Iannaccone: Yes, in my experience, that’s true.

Dr Russman: I would like to bring up 1 point that has not yet been mentioned, which is hip dislocation in children with spinal muscular atrophy. For those who are involved in the care of spinal muscular atrophy patients, this will become an issue. And the answer is, unless the patient is experiencing pain, they do not need to be relocated. It is fine to have a hip dislocation. The straight back is most important, although I can’t prove that with data; that is based on empirical data and experience.

Audience Member: So could you tell me what the status is of the effort to increase the number of diseases for newborn screening, and what is the likelihood that spinal muscular atrophy will be on that list?

Dr Prior: Well, I think that one will find that there is still quite a vast amount of variability in what’s offered on the menu from state to state. With the advent of the tandem mass spectrometer, I know a lot of states now have increased their menus up to 35 or 40 disorders, and many of these are very, very rare, and not all of them have cures or potential treatments. For me, I think the big question with newborn screening for spinal muscular atrophy would be, are we able to incorporate DNA testing rather than metabolite protein testing and able to roboticize it, thus enabling us to drive the cost down? Tandem mass spectroscopy is very easy to multiplex or add new things on; the expense is fairly cheap, or at least it’s not high. With spinal muscular atrophy, the challenge will be to roboticize it, get the DNA out of it, do an assay that can be done in 4 to 6 hours, and then be able to drive the cost down. We cost-assessed the TaqMan assay, and we felt that it was probably somewhere around $25 a person, and that’s still on the high end for newborn screening. My goal would be to use a Luminac system and aim for $4 or $5 a screening. I think we need to do a pilot, and we have talked to various states about running a pilot study and collecting data, in terms of how often do we see spinal muscular atrophy and what is its frequency. I think the vast amount of data that we need can be acquired, so I’m really looking forward to getting some pilot information for those of you who have strong feelings regarding newborn screening.

Audience Member: I believe you have funding from the National Institute of Child Health & Human Development. Do you think they would be willing to help fund such a pilot study?

Dr Prior: The Institute of Child Health & Human Development funded this proposal, and again, it varies from state to state with IRB approval. Actually, people from Minnesota, such as Carol Renaldo and others, may be involved in the first pilot study we conduct, and we’ve also talked to Susan Iannaccone about Texas. There are also differences from state to state in the availability of blood spots for testing.

Audience Member: I cannot add too much more. I was going to mention the Institute of Child Health & Human Development’s commitment to this, and I feel that they are truly committed to adding spinal muscular atrophy to the newborn screening procedure. I think your work and work like that is essential in order to develop the necessary methodology and the cost-effective approach to newborn screening that will facilitate this being done. The economic aspect of implementing spinal muscular atrophy testing as part of the newborn screening is going to be one of the biggest hurdles to get over. The argument as to whether you have a treatable disease or not to justify doing newborn screening, I think, is gradually melting away. And there are a lot of issues that can be used to argue that point, not the least of which is cystic fibrosis, where we know that early diagnosis and effective intervention and management can alter survival very much, as Susan has discussed with us.

Dr Iannaccone: I would like to add family studies to that list. Audience Member: And family studies, I think, carry the day in terms of that argument, as such. So you don’t need something like phenylketonuria and the dietary treatment that initiated newborn screening, but rather improved standard of care once you make the diagnosis early, as such. Thus, I think it is really developing the assays in a cost-effective manner that will allow them to be applied in a large population that will allow it to be implemented.

Audience Member: I would like to readdress the issue of newborn screening, in terms of respiratory care. I’m one of the few pulmonologists who are in the audience, and I just wanted to address the person’s question and Susan’s comments. I agree with you, Susan, that there is a population of spinal muscular atrophy type 1 patients, technically, the type zeros, whom from a respiratory perspective, even with noninvasive ventilation, will not survive. But I think there is a population of spinal muscular atrophy type 1 patients for whom noninvasive ventilation has made a difference, and those patients have survived much longer. I have a cohort of 20 patients with spinal muscular atrophy type 1, and of those, 4 have died, and the vast majority are over 3 years of age. I’m convinced this has to do with the noninvasive ventilation because we’re able to get them through that viral illness that would be life-threatening because they’re on bilevel positive airway pressure. And their parents know the aggressive airway clearance techniques, and they’re very proactive in taking care of this. And so that sort of relates back to the newborn screening piece. In addition to standards of care, there has been a tremendous amount of progress in the
Molecular Mechanisms in Spinal Muscular Atrophy

Moderator: Christine J. DiDonato, PhD, Northwestern University, Chicago, Illinois

Dr Sumner presented an introduction to the molecular mechanisms of spinal muscular atrophy. She began by summarizing what is known about the molecular biology of this disorder and then spoke about the important questions that still remain in understanding spinal muscular atrophy pathogenesis. Lastly, she discussed the development of spinal muscular atrophy therapeutics using histone deacetylase inhibitors as an example. Three elements of spinal muscular atrophy must be explained by any molecular model of the disorder. First, proximal muscles are more involved than distal, and legs are more involved than arms, which are more involved than the face; second, the phenotypic severity of spinal muscular atrophy is variable; and third, the course of spinal muscular atrophy differs from the steady progression seen in other motor neuron diseases.

Spinal muscular atrophy is caused by a mutation of the survival motor neuron gene (SMN) on chromosome 5. Humans possess 2 or more copies of SMN: SMN1, which codes for a so-called full-length transcript with all of the gene's exons, and variable copy numbers of SMN2, which has a base pair difference in the splice enhancer region of exon 7. Because of this difference, some transcripts from SMN2 lack exon 7 and thus code for a truncated SMN protein, which is believed to be unstable and rapidly degraded. SMN2 produces some full-length protein, but this is less than is produced by SMN1, so spinal muscular atrophy is believed to be the result of SMN protein deficiency. An inverse correlation between the number of SMN2 copies a patient has and the severity of disease has been demonstrated in humans, with individuals with 5 SMN2 copies showing no symptoms. This has also been shown in a mouse spinal muscular atrophy model, where mice with 2 SMN2 copies have severe disease and die by 1 week of age, whereas mice with 8 copies of this gene are completely protected. A complete lack of SMN protein is incompatible with life, but it is not known why SMN protein deficiency selectively affects motor neurons. This may be related to the relative amounts of the various serine/arginine-residue proteins, which regulate splicing, in the various cell types of the body.

The 294–amino acid SMN protein is encoded by 8 exons and oligomerizes with other proteins called Gemins to form a very stable SMN complex. It is possible that exon 7 codes for domains that are important for SMN oligomerization and that excision or mutation of exon 7 produces proteins that cannot oligomerize and are thus targeted for degradation. SMN is localized in the cell cytoplasm but also exists in nuclear structures called gems. The number of gems, like SMN2 copy number, is inversely linked to disease severity. SMN has recently been found in neuronal axons and in the growth cones of axons. Its abundance and activity decrease during development, so disease onset may occur when SMN protein levels fall below a specific threshold. SMN's main known function is in the assembly of small nuclear ribonucleoproteins, which are an essential part of the spliceosome and are crucial in every cell. This function may be particularly important in motor neurons and lead to spinal muscular atrophy, or SMN may have another role in motor neurons, such as in axonal RNA trafficking and processing.

Animal models of spinal muscular atrophy have been developed in worms, flies, fish, and mice. These models have confirmed that spinal muscular atrophy is caused by SMN protein deficiency, can reveal genes that modify spinal muscular atrophy phenotype, and serve as a great model on which to screen potential therapeutics. Dr Sumner summarized some of the important questions that remain to be answered in spinal muscular atrophy. These include the following:

- Why does SMN protein deficiency cause selective cellular death of motor neurons?
- Does SMN have a specific motor neuron function, or do motor neurons require more of this protein's general function than other cells of the body?
- Is spinal muscular atrophy really a cell autonomous disease affecting only motor neurons, or does it affect muscles and other cells as well? This question may have implications in the development of therapeutics.
- Do glia have any potential role in this disease?
- Is spinal muscular atrophy a developmental or degenerative disease? Understanding when this disease really begins is vital for determining the appropriate timeline of therapeutic intervention.

There are several potential approaches to therapeutic intervention in spinal muscular atrophy. Several methods used in other neurodegenerative diseases, including gene therapy, neuroprotection, and cell replacement, may be
important in spinal muscular atrophy. Additionally, because all spinal muscular atrophy patients have at least 1 copy of SMN2, a great deal of research is focused on ways to activate this gene to prevent spinal muscular atrophy or reduce disease severity. Lastly, developing ways to promote inclusion of exon 7 to stabilize existing SMN protein is another promising approach.

One potential way to activate SMN2 is through the use of histone deacetylase inhibitors. Acetylation of histone proteins can promote increased access to genes and, thus, increased gene expression. Histone deacetylases mediate deacetylation, and inhibiting histone deacetylases promotes increased gene expression. Patient-derived fibroblasts treated with valproic acid, a well-known antiepileptic and histone deacetylase inhibitor, showed increased levels of full-length SMN transcript and increased levels of full-length protein in vitro. Valproic acid treatment also resulted in an increased gem number. A histone deacetylase inhibitor given to mice after disease development supported in vitro findings. It resulted in increased levels of full-length SMN transcripts and increased amounts of SMN protein. Mouse survival was also improved by 20%, and this correlated with improved motor behavior and improved muscle pathology. However, currently available histone deacetylase inhibitors are not very potent, so more effective drugs must be pursued.

**SMN Function in the Cell**

*Stephen Kolb, MD, PhD, University of Pennsylvania, Philadelphia, Pennsylvania*

Dr Kolb focused on the role of SMN in the cell and specifically on the functions of the SMN complex. He began by reiterating that the function of SMN that leads to spinal muscular atrophy disease is not yet known but that what is known about the protein and its genetics forms the foundation for answering this question. The SMN protein exists in cells as part of multiprotein oligomers called gems, which are found in the cytoplasm of cells as well as in discrete nuclear bodies. As more is discovered about the elements that comprise these complexes, it has become clear that the SMN complex is involved in RNA processing. The SMN complex binds to ribonucleic acid (RNA) binding proteins (Sm proteins) and to small noncoding RNA, and the interaction between these is necessary for the assembly of small nuclear ribonucleoproteins, which comprise the spliceosome. It is believed that SMN protein is necessary to facilitate this interaction.

SMN and other elements of the SMN complex are ubiquitously present in structures called gems in the cell cytoplasm. The small nuclear ribonucleoproteins formed from the interaction between small noncoding RNA and RNA-binding proteins are found primarily in the nucleus but are also found within gems in the cytoplasm. Because of this, it is hypothesized that the SMN complex performs its role in small nuclear ribonucleoprotein synthesis in the cytoplasm but then travels with these into the nucleus, where they are released and function as part of the spliceosome. Spliceosomes contain a ring structure of 5 different small nuclear ribonucleoproteins called U small nuclear ribonucleoproteins, each with a similar uridine-rich Sm site.

The stepwise assembly of small nuclear ribonucleoproteins begins when arginine-rich domains of cytoplasmic Sm proteins are methylated and are bound to the SMN complex. After this binding, small noncoding RNAs are recruited to the complex and are bound to the methylated Sm proteins. To be functional, the SMN complex must bind RNA (small noncoding RNA) and must bind small nuclear ribonucleoproteins. The protein-binding role of SMN is mediated through interaction with the methylated arginine-rich sites of the Sm proteins, and because these are present in a variety of RNA-binding proteins, the SMN complex may have a role in the assembly of other ribonucleoproteins in the cell as well. Various work has shown that the SMN complex recognizes both the Sm site and a 3′ terminal stem loop structure, and that these are bound by Gemin 5. Although Sm proteins bind spontaneously to small nuclear ribonucleoproteins in solution, the SMN complex serves the error-reducing role of mediating the stepwise assembly of small nuclear ribonucleoproteins, which ensures that all downstream splicing is reliable and correct. Dr Kolb ended by summarizing a technique developed to quantify small nuclear ribonucleoprotein assembly. When applied to a spinal muscular atrophy 1–derived patient cell line, this showed that the SMN components were unchanged but that SMN expression and small nuclear ribonucleoprotein assembly levels were similarly decreased. He suggested these findings about the function of the SMN complex in cells provide new targets for potential therapies for spinal muscular atrophy.

**SMN and Neurodevelopment**

*Christine Beattie, PhD, Ohio State University, Columbus, Ohio*

Dr Beattie spoke about the zebrafish spinal muscular atrophy model system and its usefulness in learning about the biological basis of the disease, specifically, its role in investigating what happens to motor neurons in spinal muscular atrophy and the particular role of the SMN complex in motor neurons. Zebrafish are external breeders, so embryos can be harvested very early in development. They contain large amounts of body muscle, which is not highly derived and retains its stereotypic organization throughout development. The initiation of motor axon outgrowth and innervation of muscle begins as early as 24 hours into development. Muscles are organized into 30 segments, and at the first stage of development, each segment is innervated by 3 motor neurons in a stereotypical manner. The zebrafish embryo is transparent during early development, so its spinal cord and primary motor neurons can be visualized and manipulated as necessary.
To model the decreased SMN protein levels observed in spinal muscular atrophy without knocking out the gene completely, antisense nucleotides called morpholinos that bind to RNA and block translation were injected into the embryos very early in development. This resulted in approximately a 77% decrease in SMN protein levels. Phenotypically, this produced dramatic defects in motor axon outgrowth but no other recognizable defects in development. In vivo imaging of these axons over time reveals inappropriate axonal branching or truncation of motor axons with complete sparing of sensory and interneurons, as well as no obvious defects in muscle patterning and formation of synapses. No cell death was observed during the first 3 days of development, which suggests that faulty projections rather than motor neuron death are the main manifestation of low SMN levels early in development. To delineate the specific need for SMN in motor neurons, individual motor neurons in wild-type zebrafish embryos were injected with morpholinos. Similar problems in axonal projects were observed, suggesting that SMN is necessary within the cell for normal motor neuron pathfinding. SMN may assemble small nuclear ribonucleoprotein complexes that transport RNAs important for axon formation and pathfinding down the growth cone.

To study the long-term consequences of reduced SMN protein levels, Dr Beattie and colleagues quantified axonal defects in 400 morpholino-injected zebrafish as severe (15%-16%), moderate (22%), mild (36%), and no defect (26%). Adding full-length human RNA of the SMN gene significantly rescued the phenotype; the severe group was completely eliminated, and 80% showed no defect. The delta 7 SMN gene form produced no statistically significant rescue. A cytoplasmic targeting motif VDQNQKE, which is part of exon 7 and allows targeting of RNAs to the cells, showed rescue effects comparable to the full-length transcript. This sequence must have a specific role, which, when missing, produces motor neuron defects.

Because SMN is known to bind to Sm proteins, this function was explored in more detail. The ability to rescue was not concretely related to Sm binding ability, suggesting that another function of SMN besides small nuclear ribonucleoprotein assembly is responsible for the phenotypic defects of motor neurons caused by lower SMN protein levels. SMN protein, therefore, appears to have an independent function in motor neurons.

Animal Models of Spinal Muscular Atrophy

Christine J. DiDonato, PhD, Northwestern University, Chicago, Illinois

Dr DiDonato focused on the use of population screening in various organisms and the power these models provide us for understanding and better defining the genetic component of SMA, in addition to lending insights to chemical screening technologies for therapeutic development. Specifically, Dr DiDonato spoke about the Drosophila spinal muscular atrophy model system and its usefulness for the study of SMN activity during the larval stages of development. Interestingly, the mature spinal muscular atrophy mutant Drosophila have been found to have electrophysiological abnormalities at the neuromuscular junction. And the characterization of SMN mutant Drosophila throughout the various larval stages has shown 2 problems exist, one with the remote RNA localization and the other with remote translocation. The failure of both processes ultimately leads to the degeneration of the mutant larvae.

With the characterization of the SMN mutant larval phenotype, the opportunity has arisen to perform suppressor screening tests in search of genes that may alleviate the various spinal muscular atrophy phenotypes that have been previously described. Additionally, the use of Drosophila provides an excellent medium for the direct screening of chemical compounds to serve as promising therapeutics.

Dr DiDonato also spoke about the use of the spinal muscular atrophy mouse model, which has provided a great deal of understanding regarding the disease pathology and pathophysiology of spinal muscular atrophy. Several mouse models have been generated to correlate with the 3 distinct forms of spinal muscular atrophy: mild, intermediate, and severe. Two transgenic animals have been created as a model for spinal muscular atrophy type 1. The animal expressing a high copy number of the SMN2 gene appears phenotypically normal, whereas animals with the low copy number of the SMN2 gene show decreasing levels of SMN. Interestingly, this model has shown that expression of increased levels of SMN from SMN2 can rescue the severe spinal muscular atrophy phenotype without toxic side effects. Otherwise these animals have a lifespan of approximately 5 days. For type 2 spinal muscular atrophy, a model was generated in which SMN cDNA lacking exon 7, also known as delta 7, was placed under the control of an endogenous human SMN promoter to create transgenic animals with an increased survival of 16 days. Combined with SMN2 normal mice to look at the various transcripts that could be generated, low levels of SMN protein were found. Pathologically, with dystrophin staining, the skeletal muscles of these animals show a normal distribution of muscle fibers; however, the fiber size is diminished, and the fiber shape is angular. The type 3 transgenic model lives into adulthood, allowing for long-term study of the disease pathogenesis. These animals have 1 copy of the SMN gene and 1 point mutation described previously by Dr Prior and contain the SMN2 gene that provides for the full-length SMN transcript. Phenotypically, these type 3 mice are no different from their littermates, but 3 and a half months later when these animals have reached adulthood, there is a 29% difference between the spinal motor neurons of the mutant mice compared to a normal mouse.
The animal models discussed above cannot be used to determine the levels of SMN protein required for normal postnatal health and development of motor neurons due to the fact that these models use cDNA. A different approach is needed to address the following question for the express purpose of future clinical trials: How much SMN is enough? Although an exact amount has yet to be determined, a technique has been established requiring the modification of the mouse SMN gene to induce alternative splicing. The murine SMN gene closely resembles human SMN1 in that the first nucleotide is a c-nucleotide on exon 7; however, the SMN1 gene exists as a single copy in the mouse and does not undergo alternative splicing in the mouse. With each mutation induced into the SMN gene, the PGKneo cassette, a selective marker for embryonic stem cell transfections, was maintained. To create animal models the mouse and does not undergo alternative splicing in the mouse. With each mutation induced into the SMN gene, the PGKneo cassette, a selective marker for embryonic stem cell transfections, was maintained. To create animal models of spinal muscular atrophy, a C to T and a 2B mutation were used. The induced mutations did induce alternative splicing, and the extent varied depending on the modification to exon 7 of the SMN gene. As a result, the new alleles can then be used to create varying SMN transcript content from 100% of exon 7 containing transcripts to 0%, based on the mutation introduced throughout the exon. Additionally, these 2 new alleles, the C-T/neo and 2B/neo alleles, can now be used in rescue experiments.

Questions and Answers

Audience Member: Dr Beattie, how long do the morpholino oligomers that you inject into the embryos have their effect?
Dr Beattie: Right. The question is when the morpholinos are transient. With our system, we actually can knock it down to about 14 days or for 2 weeks. Thus, between 14 days and 17 days, we see the protein levels rise back up, and I didn't show that Western, but in some morpholinos, the protein levels come back up after 4 or 5 days, but this one in particular is actually quite persistent. But you're right; when we created our survival curves, we did so using a 30-day time period. In the middle of that curve, at about 14, 17 days, the protein levels start to rise back up. Now this may be why we see the plateau in our survival curves, or it may be that the damage is done and nothing gets better; we really don't know that yet. I should say that we are very aware of the transient nature of these morpholinos, and through collaboration first with Tom Prior and then with Cecilia Momens at the Fred Hutchinson Research Center, we screened for point mutations in the SMN gene in the zebrafish genome, and we now have at our facility 3 point mutations. As you've heard, however, these are going to be embryonic lethal; we already know that from our crosses. Dr Liu at my lab is making the fish SMN2 transgenic with the same construct that Dr Umrao Monani used to make the mouse. We now have those constructs that have been injected, and those fish are growing. We will then make the fish version of the mouse version of the human disease, and that may answer some of those questions.

Audience Member: Dr Sumner, on 1 of your slides, you discussed whether spinal muscular atrophy is a degenerative disease or a developmental disease. Maybe you'd like to elaborate because everything I think I've heard so far is that the animals do deteriorate, degenerate, and certainly, our experience with humans is their disease does progress—it gets worse. Perhaps you can just elaborate upon what you mean by a degenerative disease versus a developmental disease.
Dr Sumner: When we speak of degenerative diseases, we think of everything as forming normally, and then some event triggers later degeneration, whereas in the developmental disease, there is actually some impairment to the development. I think whether spinal muscular atrophy is primarily a developmental disease or a degenerative disease, or both, remains somewhat of a mystery, and the way I conceptualize it is that it may be a bit of both. I think Christine Beattie's work really raises this issue, that perhaps the motor unit never does form completely normally, and that may be quite a subtle abnormality, but that puts the motor unit at risk for subsequent degeneration. And that's sort of how I conceptualize it, but I'm sure others have their own ideas about it.

Audience Member: This question is based on the minor debate as to whether there is as much motor neuron loss. And so Dr Beattie, I wanted to know more about 1 of your slides, on which I believe you noted that the TUNEL reaction was indicative of apoptosis, is that right?
Dr Beattie: Correct, that was at 3 days of development. I would predict, looking later in the development, that we would see some cell death, as you see in the mouse models.

Audience Member: How much do we actually see in the mouse models?
Dr DiDonato: There is motor neuron loss, and no one that I am aware of has done TUNEL staining throughout development to determine the amount of motor neuron death throughout.

Audience Member: I think part of the debate, certainly in some of the human material, is whether there is true loss of motor neurons or whether there are changes that occur in the motor neurons that change their phenotypic characteristics in such a way that you can no longer recognize the motor neuron as a motor neuron; it blends into the cellular elements of the background, in the ventral root and as such. Thus, I think this has some implications in terms of potential for the rescue of motor neurons.

Audience Member: I think it would be interesting to take a look at that in the mouse models—if you characterized it and performed a study based upon size, alpha motor neurons versus gamma motor neurons in terms of alpha motor neuron loss, and asked the question, is there an increase in terms of size for gamma.

Dr DiDonato: I think it would be interesting to take a look at that in the mouse models—if you characterized it and performed a study based upon size, alpha motor neurons versus gamma motor neurons in terms of alpha motor neuron loss, and asked the question, is there an increase in terms of size for gamma.

Dr Sumner: In my recent analysis of spinal cords from mice at about 13 days of age, in which we stained all neurons in the ventral core, I would say that there is some loss of motor neurons, presumably about 25% lost. It could be that the motor neurons were never there; we didn't test that, but at that time, there is about 25% deficiency. In addition, we also measured the size of the motor neuron cell bodies, and that was also small. So there's been work in the past that
has suggested that the size of the motor neuron cell body is determined in part by the size of the motor unit, and so it may be that remaining anterior horn cells that are present are small because the motor unit is actually small.

Dr Burghes: I concur with those numbers; it’s about 30%. Dr Kolb, I want to understand 1 thing. I was under the assumption that Gemin 5 was localized to the cytoplasm and not in the nucleus, and so does it release the RNA? Does it bind the RNA in the cytoplasm and then release it in the nucleus?

Dr Kolb: The subcellular distribution of Gemin 5 is the same as SMN, in that it is primarily in the cytoplasm, but also in these gems.

Dr Burghes: So it’s identical.

Dr Kolb: It is, well, in HeLa cells, in the cell cultures that I know of. I have not, as you know, looked in detail in primary neuronal cultures to see if these match up. Some cells don’t have gems; not all cells have gems, and you can do things to cells to change the number and frequency of gems, and it doesn’t necessarily correlate with the amount of SMN there. So, what are they? Whether they are an active component of the SMN complex or whether they are a storage depot, much like other known nuclear bodies in the cell, is not completely clear, but in our model, the Gemin 5 binds to RNA in the cytoplasm. In fact, the small nuclear ribonucleoprotein assembly occurs in the cytoplasm, possibly in conjunction with an import process into the nucleus, and it is at that point that the small nuclear ribonucleoprotein, the formed small nuclear ribonucleoprotein, is released into the nucleus. The details of how exactly that happens, obviously, we don’t know. We don’t have a molecular basis for that.

Dr Burghes: Charlotte, you implied that the action of the drug is through SMN. Do you alter the motor neuron pathology?

Dr Beattie: [Interposing] Arthur is referring to his own work that has shown that some of the other drugs, other histone deacetylase inhibitors given to mice, have not shown much induction of SMN, and yet still they see an improvement in the phenotype in the mice. So all we can note is that trichostatin A (TSA) is associated with increased SMN levels, and this is associated with improved small nuclear ribonucleoprotein assembly. With those biochemical effects, we see an improvement of survival. Of course, it remains very possible that the improvement has nothing to do with SMN induction, and in fact, there is evidence from the muscle world that histone deacetylase inhibitors do have primary effects on muscle. There was a paper published in Nature Medicine this month, I believe, which shows that TSA, the drug we used, actually improves mdx mice and that TSA was active. Mdx mice serve as the muscular dystrophy mouse model, and this drug actually improved myofiber size and number, perhaps through activating satellite cells, which can regenerate new muscle fibers, within myofibers. Thus it is possible that what we are seeing is really a primary effect on muscle, unrelated to SMN induction. The primary pathological improvement we saw was in muscle, an improvement in myofiber size and improvement in myofiber number. We did not see an increase in anterior horn cell body number, although we do believe that we were seeing a slight improvement in the size of the anterior horn cell. This suggests again that perhaps individually, while we are not changing the number of anterior horn cells, perhaps we are improving their motor unit size such that they are innervating more myofibers. That may be 1 interpretation. Unfortunately, what we really did not look at and should have was the anatomy of the neuromuscular junction. Therefore, all I can comment on is anterior horn cell body number and the muscle, nothing in between, but we’re working on that.

Audience Member: Steve, regarding the gems, how do you explain the function of this gene?

Dr Kolb: I think it is a false choice to declare that SMN function in spinal muscular atrophy is either a basic biological function or a motor neuron–specific function. I think that there is probably a parsimonious explanation for this. In terms of the downstream events, the consequence in terms of the transcriptome of a motor neuron is interesting to see in these different cell types. When you decrease SMN, you have a morphological change in the cell, and it almost doesn’t matter what cell it is. The reason that a cell has a phenotype is because of the tightly regulated transcriptome, the splicing isoforms that the cell chooses to make. I think the hypothesis would be that by decreasing the fidelity or, not even the level, but just the fidelity of small nuclear ribonucleoproteins, for example, that you might have downstream consequences in the transcriptome, and possibly in a way, that would only affect motor neurons. This is just a hypothesis. To obtain the evidence, the experiment would need to be done, but it’s very technically difficult to do and would involve comparing the entire transcriptome of the motor neuron to that of a motor neuron in spinal muscular atrophy. I would also include the surrounding cells, the glial cells, and the muscle while you’re at it—and not only compare the levels of gene expression, but the relative amounts of different splicing variants of different genes. Neurology is already teaching us that cell splicing variants in, say, potassium channels can cause disease; therefore, it’s not unreasonable to think that a general, subtle deficit in the cell’s ability to regulate this massive splicing challenge could end up in a cell-selective phenotype.

Audience Member: Steve, does delta 7 affect the SMN exon 7 mutant?

Dr Kolb: The form of SMN delta 7 expressed from the SMN2 gene does not incorporate into gems, and in fact, in the absence of artificially expressing it to very high degrees, it is very difficult to detect. As I think Christine mentioned, this form, in our thinking at least, is readily recognized as being a nonfunctional part of the SMN protein and as a result is degraded. Regarding the localization, I can’t really answer. I know it is in the cytoplasm for a short day.

Audience Member: This question is addressed to anyone. Is messenger RNA the only type of RNA associated with the SMN protein in the growth cone or the nerve terminal? And if it is the only RNA form, is the SMN protein important simply for RNA translation?

Dr Beattie: Michael Sendtner’s group has probably looked at this question to the greatest extent. I think a number of groups are looking at it now, as to whether the SMN complex associates with different RNAs. What Michael
Sendtner has shown is that SMN does complex with HNRPR, which itself binds RNAs, messenger RNAs, and he has also shown that in motor neurons from the spinal muscular atrophy severe mice, there’s less β-actin in the growth cone. Proving that there is less β-actin in the growth cone because there is less RNA being transported due to a deficiency of a complex has not been directly shown yet. This is what people are working on right now in a number of different labs; they are asking what RNAs are in the motor axon growth cones or any other neuronal growth cone, for that matter, because we do not know. A number of people are working on that now and, in addition, are attempting to understand which transcripts are present. It has been done in injury models. Jeff Twist designed an injury model and asked the question, “Which RNAs are now upregulated in the growth cone or present in the growth cone?” It is being looked into. The development hasn’t been completed yet, but hopefully some labs are working on finding out which RNAs are in the growth cones and does SMN complex with them, meaning is it relevant for their transport. That is the general area we are in right now.

Audience Member: Dr DiDonato, I have a clinical question.

Dr DiDonato: Yes, what we have done is due to the fact that the animals we’ve generated are in a Black background and they’ve just recently been born. Therefore, I can’t really make any predictions upon that. What I can tell you is that we can create a gradation from 0 to 100% in 10% to 15% increments. And at least in the embryos, the embryos for the 2B null and the C-T null are phenotypically normal.

Audience Member: Dr DiDonato, I have a clinical question. In your low copy number SMN knockout mice that die early, did they die simply of malnutrition because they cannot feed, or is there a respiratory ailment? And if so, is there sparing of the diaphragm?

Dr DiDonato: In the animals without any copies of the SMN2 transgene, or when you ablate SMN, those animals die at or begin to degenerate and die at the morulus stage. Thus, they do not even implant into the uterine wall. And I’m going to let Arthur answer as to when you have 1 copy of SMN1.

Dr Burghes: First of all, in 2-copy number SMN2, severe mice die at 5.16 days. If one actually takes embryos at 18.5 days and measures them, they are already smaller. So the size reduction actually occurs prior to birth. Now the question is whether they die of malnutrition or whether it’s respiratory. We’re not very good mouse doctors; we’re pretty bad mouse doctors. So we’re not absolutely sure what they die of, except that they are very malnourished. I do want to add 1 thing. If you take the mice that live to 14 days, one can force feed them, and we have done this with Pedialyte. It may not be the right thing, and we need to do this again, but what we found was that those mice do not live longer after we basically feed them with gavage feeds.

Dr Sumner: When do you start that?

Dr Burghes: We start them at 4 days or 2 days old.

Audience Member: Those mice, I think, die from a combination of malnutrition and respiratory distress, and the reason why I say that is because if you open up their stomachs at the end stage of disease, you don’t find any food or any milk in the stomach at all. Also, if you look at the intercostal muscles and the diaphragm muscles, you find severe wasting, and the mice display distressed breathing, which makes me think that it’s a combination of malnutrition as well as respiratory distress. Does that answer your question, Victor?

Audience Member: Dr Beattie, in general, you mentioned that there is variability in the axonal phenotype of the morpholino-injected animals. Do you have any speculations or thoughts on the variability, despite the same level of knockdown that you see?

Dr Beattie: I believe so. One possibility is that the morpholinos are mosaic. We have injected these into the 1-cell stage embryo, but we can’t say that it is reaching every single cell and every single motor axon at every level in the same amount. However, our genetic mutants, which will be on-board quite soon, should answer that question. The idea that not every axon is affected, that everything is not affected at the same and in the same way, which might be more realistic, may be a part of the disease. It really remains to be seen, but the mosaicism is raised as a side effect of the morpholinos.

**Translational Research**

**Moderator: Kathryn Swoboda, MD, University of Utah, Salt Lake City, Utah**

**Search for Active Compounds**

Christopher E. Henderson, PhD, Columbia University, New York, New York

Dr Henderson began by explaining the therapeutic challenges that spinal muscular atrophy presents. It is known that a mutation in the SMN genes and a consequent reduction in functional SMN lead to motor neuron death. Every step of the molecular and cellular pathways that leads to this death of spinal neurons is a potential target for therapeutic intervention. Unfortunately, our mechanistic knowledge of these pathways is not very clear. The development of pharmaceuticals, and especially of neuroactive compounds, depends on identifying a receptor or kinase that is important in the disease mechanism and then targeting this kinase through high-throughput screening of compounds. Because no such target has yet been uncovered in spinal muscular atrophy, this approach is currently impossible. Instead, Dr Henderson introduced the idea of targeting a cell model instead of a specific protein through phenotypic screening.

The first strategy for slowing the cellular pathway leading to the cell death observed in spinal muscular atrophy...
is increasing the availability of SMN. This can be done by searching for agents that increase SMN2 transcription or those that promote correct splicing of exon 7, which is responsible for decreased SMN functionality. The regulatory elements of SMN2 have been expressed in cell lines and can be modified to increase SMN2 transcription. Intervention at this level targets the disease trigger and is thus very attractive. However, several challenges exist with this approach. First, it is difficult to ensure that any compound exclusively targets SMN2 gene transcription; second, it is unknown whether upregulation of SMN2 in a patient after the disease has been declared will make a significant difference in the disease process and patient phenotype; and third, compounds that regulate SMN2 expression in 1 cell type may not be effective in another, and this will present an important challenge in translating such compounds into patients.

Another strategy for interrupting the spinal muscular atrophy disease process is to increase correct splicing of exon 7 of the SMN2 gene. It has been shown that exclusion of exon 7 is responsible for the incomplete ability of SMN2 to compensate for the SMN1 mutation seen in spinal muscular atrophy patients. Because exon 7 is incorrectly spliced in 9 of 10 cases of spinal muscular atrophy, a 10-fold increase in correct SMN transcription may potentially be achieved by targeting this, versus the 3-fold increases reported when attempting to upregulate SMN transcription. Using sequence-targeted splicing agents to effect correct inclusion of exon 7 also has the potential advantage of high specificity.

An important aspect of screening for active compounds is the availability of an accurate cell or animal model. Dr Henderson described the applicability and feasibility of a motor neuron model. Developing motor neurons send axons to their target muscles during fetal development. Approximately half of these cells undergo developmental cell death, and half survive and continue to develop. In disorders such as amyotrophic lateral sclerosis or spinal muscular atrophy, these neurons later experience abnormal degeneration or death. Although these 2 events of neuronal survival and developmental cell death are probably not identical, understanding the associated mechanisms may provide clues regarding “leads” in the search for active compounds to treat abnormal motor neuron death in spinal muscular atrophy. Motor neurons can be purified and cultured from the embryonic spinal cord, and these cultures are a good model on which to test genetic or pharmaceutical treatments. Cell death in this primary motor neuron model is stimulated by removing known trophic factors, and a company called Trophos has used flash cytometry to identify compounds that block the cell death cascade and lead to survival in the absence of trophic factors. One such compound, TRO19622, which protects primary motor neurons from cell death by binding to the mitochondrial pore protein VDAC, has also been shown to increase axonal growth. This compound has also been shown to be neuroprotective in vivo in the spinal muscular atrophy and amyotrophic lateral sclerosis mouse models, to cross the blood-brain barrier, and to be safe in humans. A phase 2/3 clinical trial in amyotrophic lateral sclerosis and spinal muscular atrophy patients is now planned. The mechanism of action of TRO19622 was identified by screening a library of expressed proteins.

Although identification of this compound shows what promise the primary motor neuron culture technique has as a model for screening compounds, it is limited in that it is a model of naturally occurring cell death, rather than the SMN deficiency, that causes neuronal death in spinal muscular atrophy. One approach to addressing this issue is extracting motor neurons from spinal muscular atrophy mice, which have the same response to trophic factors and similar dendrite length as those extracted from healthy mice but show a 20% to 25% reduction in axonal length and an even greater reduction in the size of the growth cone. However, this difference is too small to screen for, and so the challenge becomes one of developing a more robust phenotype. Dr Henderson mentioned that extracting the motor neurons when disease becomes evident (at several weeks old) in spinal muscular atrophy mice may provide more mature, and thus, more affected neurons, but there is no real way to identify maturity. Alternately, SMN levels could be decreased even more, but this approach may be so drastic that the mechanisms of death in these cells no longer accurately represent what is seen in patients. The most promising approach may be to retrieve embryonic stem cells from spinal muscular atrophy patients or spinal muscular atrophy mice and to differentiate these into motor neurons. Regardless of the approach, Dr Henderson stressed the importance of a collaborative, interdisciplinary effort in translating active compounds into the clinical setting.

Use of Animal Models in Preclinical Testing

Arthur Burghes, PhD, Ohio State University, Columbus, Ohio

Dr Burghes spoke about the animal models he and his colleagues at the Ohio State Center for Motor Neuron Disease have developed and how they are applying these models to the search for therapies. The main goal in developing a mouse model of spinal muscular atrophy was to replicate the genetic mechanism of the disease. This was done by removing the mouse SMN gene and replacing it with human SMN2; human SMN2 was used because elements in the intron sequences of the gene may vary by species, and these sequences may have important interactions with active compounds. Mice with just 1 copy of the human SMN2 gene usually do not live through embryogenesis, and 2-copy mice survive an average of 5.1 days. There are also “escapers” that have 1 copy but also a delta 7 insert and can survive for approximately 14 days, and these are the mice.
commonly used for drug testing. The mouse model is highly relevant for the testing of possible therapeutics for spinal muscular atrophy because the gene splicing of SMN2 is the same in the mouse and human.

Various targets for changing the phenotype of spinal muscular atrophy mice include increasing SMN copy number, altering the splicing of the SMN2 gene, and using read-through compounds to bypass the stop codon in exon 7 to encourage correct translation. Dr Burghes suggested that a 3-copy mouse would be a valuable contribution to the field. Also, studying the phenotypic effect of various missense mutations or intragenic suppressors on axons in the mice may be useful in validating this model as a good assay for the SMN function that is crucial in spinal muscular atrophy.

Dr Burghes was careful to note that, although the various mouse models are a very good tool for the screening of possible therapeutics, mouse and human are very different, so effectiveness in one is not guaranteed in the other. However, the mouse model can show where and when therapeutics will most effectively alter phenotype, and although this may not be the same in human spinal muscular atrophy, this knowledge will be an excellent starting point in human therapeutic trials. In recent mouse trials, SMN copy number appears to have a very strong effect on phenotype if raised in neural tissue but a negligible effect if raised in skeletal muscle. It also had an effect when applied early. Several compounds tested on these mice show promise, but they have short half lives and must be administered systematically in high doses. Phenylbutyrate, in particular, appeared to increase life span and delay the onset of body mass loss if administered before motor neuron loss in the spinal cord. Overall, Dr Burghes concluded that intermediate spinal muscular atrophy mice can be used effectively to test the efficacy of drug and gene therapy.

**Human Trials**

*Kathryn Swoboda, MD, University of Utah, Salt Lake City, Utah*

Dr Swoboda presented the clinical trials under way in the field of spinal muscular atrophy and discussed some of the challenges that spinal muscular atrophy human trials present, including the difficulty of establishing reliable outcome measures. More aggressive clinical care for patients with the type 1 form of spinal muscular atrophy is substantially increasing their mean survival, and fortunately this is no longer a valid outcome measure. Additionally, because motor denervation in spinal muscular atrophy is slowly progressive, many patients may have progressive disease but remain functionally stable through compensation. This and irreversible complications such as scoliosis and contractures all complicate the task of identifying stable outcome measures for improved or decreased strength and motor abilities of patients. Another question in clinical trials is whether to control for the number of SMN2 copies a patient has or just for spinal muscular atrophy type. This is valid because copy number is known to affect the clinical severity of the disease and because many of the active compounds under investigation target SMN2 expression.

In studying the natural history and prevalence of spinal muscular atrophy, several additional challenges arise. First, although the prevalence of spinal muscular atrophy type 2 appears highest, the incidence is actually highest for type 1. Second, in the first few years of life, the peripheral nerves and central nervous system are actively myelinated, leading to improvements in motor function. This process is simultaneous with the denervation caused by spinal muscular atrophy and complicates the clinical picture, which shows gain and then loss of skills. Natural history studies have shown that postnatal denervation is a significant factor in spinal muscular atrophy but that many type 1 patients have decreased tone and evidence of weakness at birth, suggesting a prenatal neuronal death component. Denervation progresses with age, and its severity correlates with disease type. Alternately, strength appears to be stable with functional decline, so it can serve as a baseline against which to track improvement.

Dr Sumner briefly touched on an electrophysiologic technique, measuring ulnar compound motor action potentials as a method to evaluate changes in motor unit number. This technique is noninvasive, can be performed at any age, shows significant decline in a type 2 population by 30 months, and correlates with functional data as measured by the Hammersmith Functional Motor Skill Scale.

Despite all of these challenges, several groups are carrying out human trials in spinal muscular atrophy, and their trials are serving to identify the ideal study set-up for future drug trials and to test the effectiveness of some promising compounds. The clinical trials in spinal muscular atrophy are currently focused on (1) histone deacetylase inhibitors and neuroprotective agents and (2) albuterol and carnitine. Dr Swoboda briefly mentioned the various groups in the United States and in Europe involved in spinal muscular atrophy trials of acetyl-L-carnitine, riluzole, valproic acid, creatine, gabapentin (in adults), and hydroxyurea. The main focus of the trials currently under way is identifying sensitive and reliable outcome measures and study structures, and progress is being made in these fields.

For example, Dr Swoboda’s group is working on a phase 1/2 open-label valproic acid and carnitine study. Valproic acid has a very well-known side-effect profile in other populations, so it was known to be acceptably safe in patients older than 2 years. Forty-two patients were enrolled in the preliminary study (including those with type 1, 2, and 3) and were evaluated at 3 months, 6 months, and 1 year. Adverse events included weight gain, which in spinal muscular atrophy patients can lead to functional decline, mild bone marrow suppression, some anemia, and lactic acidosis in one 3-year-old patient with the type 1 form of the disease. Overall, this study showed promise, with a small (mean, 4.7 points on the
be carried out. An SMN1 nerve conduction study and a creatine kinase test should be repeated, and an EMG muscular atrophy patients are diagnosed by this test. If an test identifies a homozygous deletion, and 95% of spinal muscular atrophy is confirmed if the copy count is also appropriate if.

Dr Wang then explained the specific care recommendations of each Standard of Care Committee group. Pulmonary care recommendations are made based on the functional level of the patient (nonsitters, sitters, or walkers). The most important general principle for the treatment of respiratory problems in spinal muscular atrophy is early discussion about care options and an agreement with parents about the level of chronic and acute care that should and can be provided. Pulmonary care should include appropriate and complete evaluation of pulmonary function, chronic care that maintains an open airway and addresses hypoventilation, and acute care that provides airway clearance, assisted cough, and postural drainage.

The main gastrointestinal and nutritional requirements of spinal muscular atrophy patients include feeding and swallowing, gastrointestinal dysfunction, growth and nutrition, and problems of acute illness and acute care. Feeding and swallowing problems are more common in nonsitters and are caused by fatigue. These problems may cause choking and heighten the risk for aspiration, so evaluation by an occupational therapist or feeding specialist is an important part of spinal muscular atrophy care. To manage feeding problems, food consistency or positioning during feeding can be changed. In severe cases, tube feeding may be necessary. The committee advocates a Nissen fundoplication for every patient with a gastrostomy tube, even if they show no signs of reflux. Problems of gastrointestinal dysfunction include constipation or silent aspiration, both caused by low gastrointestinal motility and tone. Nutrition should be a major priority for spinal muscular atrophy patients, and growth should be carefully monitored, with supplementation where necessary.

The orthopedic challenges of spinal muscular atrophy are profound weakness, which can manifest in reduced functional motor strength, limited mobility, decreased range of motion, contractures, scoliosis, and decreased bone density. Interventions include splinting, elastic arm slings, use of lightweight toys for infants, and the electronic activities activation device. Appropriate rehabilitation recommendations are made on the basis of each patient’s functional level, but the overall goal is to achieve maximal independent living with maximized mobility and reduced injury.

Standards of Clinical Care

Ching H. Wang, MD, PhD, Stanford University, Stanford, California

Dr Wang described the current standard of clinical care for spinal muscular atrophy, beginning by describing the problems that arise in caring for patients with the disease. These include the lack of a community standard for clinical care; the variability of the clinical phenotype in spinal muscular atrophy; the multisystem nature of the disease, which requires a multidisciplinary approach; variation in the availability of resources for care; physician value differences; and the challenge that variation in care presents in identifying outcome measures for clinical trials. A 12-member Standard of Care Committee was formed in 1994 to address these issues and to reach a care consensus, with a focus on pulmonary, gastrointestinal and nutrition, orthopedic rehabilitation, diagnostic, and palliative medicine. The committee continues to review literature and its recommendations but has arrived at the following standard of clinical care.

When a patient presents with possible spinal muscular atrophy, the most important diagnostic test to be performed should be a gene deletion test for SMN1. The diagnosis of spinal muscular atrophy is confirmed if the test identifies a homozygous deletion, and 95% of spinal muscular atrophy patients are diagnosed by this test. If an SMN homozygous gene deletion is not identified, the clinical examination should be repeated, and an EMG nerve conduction study and a creatine kinase test should be carried out. An SMN1 copy count is also appropriate if proximal weakness is greater than distal weakness and creatine kinase levels are normal. Once a diagnosis is established, the neurologist should spend time educating the family about the clinical classification of spinal muscular atrophy, its pathogenesis, and its prognosis, but also about the various specialties that may be involved in the care of the patient. Families should be given information about available family support groups and clinical trials. Dr Wang noted that physicians should avoid the compassionate use of trial drugs in favor of systematic studies.

Lastly, families should be provided with genetic counseling, either by the neurologist or through a referral to a geneticist. The phenotype/genotype correlation of SMN2 copy number and spinal muscular atrophy type should be explained conservatively, especially in prenatal diagnosis, because copy number is not always predictive of disease severity. Siblings and relatives should undergo genetic testing, and lastly, family planning issues such as the risk of recurrence, neonatal diagnosis, preimplantation diagnosis, and neonatal screening should be discussed.
Questions and Answers

Audience Member: Is it going to be a recommendation of the Standard of Care Committee that the SMN2 copy number should be shared with patients and their families?

Dr Wang: It has not recommended against it, but it's been recommended that it be used cautiously.

Dr Burghes: I do want to make 1 point on the use of SMN2 copies and hypermorphs in general, in the genetic disease. It's actually extremely hard to get direct correlation that's really tight because anything that alters the amount of SMN coming from SMN2 will cause the assay not to be a 100% read-out. So I think it's unrealistic to expect an SMN2 copy number; you're not even assaying whether it was an intact gene. It's really important how much SMN it produces, and you're not measuring that, so you'd have to change the assay to be able to measure the amount of SMN coming from it in spinal cord, preferably.

Dr Swoboda: Dr DeVivo?

Dr DeVivo: Kathy, 1 question for you and 1 for Arthur. The literature gives us dominant data for adults, for motor unit number estimates and compound muscle action potentials, but it's hard to find good data in infants and children. Do you know whether there are some normative data for infants and children? And if so, are they significantly higher than normal values for adults?

Dr Swoboda: So what I'm measuring is the maximum ulnar compound muscle action potential, and what's published in the pediatric literature, there are no normals, but what's published is generally a single placement. So I think it's a little different when you're looking at the typical ulnar compound muscle action potential; for instance, a typical ulnar compound muscle action potential for a 3-month-old would be around 3. However, a maximum is going to be 5. And the way we've gotten our normal data is we do have our Institutional Review Board protocol to study this in some of the normal siblings of patients with spinal muscular atrophy, and that's been very helpful. But other parts of that data must come from patients with other disease, like kids with cerebral palsy, where you don’t really know that they're a normal control population, exactly. So I think that's still a challenging area, but I think we have a lot of good data from normal siblings that will increase over time; and then Arthur's question?

Dr DeVivo: On 1 of your slides, you had, in the lower right, looking at the slide, the percent synapses in collateral sprouts, if I remember correctly. It was higher in the…

Dr Burghes: [Interposing] Mild mice.

Dr DeVivo: Compared to the normals, the wild-types.

Dr Burghes: Yeah.

Dr DeVivo: Could you tell me what exactly that means?

Dr Burghes: Basically, what that means, in the mild spinal muscular atrophy mice, which are the HG SMN1, there is considerable sprouting, a lot more than you would find in a normal mouse.

Dr DeVivo: In the surviving motor neurons, they have increased number of synapses?

Dr Burghes: Yes, correct.

Dr DiDonato: When Paul Zeras published a paper on the natural history of spinal muscular atrophy, he did a correlation of SMN2 copy number, and he talked, in a very large cohort, about children who were able to be ambulatory. I think it was type 3 patients who were able to be ambulatory for 20, 30, maybe 40 years plus, and I can't remember the details of that. Do you have anecdotal information about those children who were able to walk for a period of time? Did they maintain their walking, and can we take some of this information and possibly use it toward thinking about treatments so we can keep kids up until 3 years of age, before they lose their motor neurons or have a dramatic improvement in the outcome of those able to walk?

Dr Swoboda: Well, I think that is older data. Dr Zeras’ study published 10 years ago of the 3 as presenting with weakness before age 3 years and 50% losing ambulation by age 12, but I'm not sure that’s the case anymore with more aggressive therapy. I'm not sure. Susan might have a comment on this, but I think the type 3 population is the most difficult population because they’re so highly variable. I studied older type 3s. We have a 50-year-old type 3 who's still walking, who presented in her teenage years, and I can tell you what her compound muscle action potential is; it's still 9, 9 mV. It's still well within normal range, but if you look at her motor unit number estimate values, in type 3s, the motor unit number estimate or the number of fibers going down to that muscle, that estimate is much more sensitive. Most of the type 3s have normal compound muscle action potential values, and I've done several sib pairs in the type 3s, and they match their sibling pairs who don't have spinal muscular atrophy, yet it's the motor unit number estimate that, once it falls below 50% normal, that's when they begin to show weakness. We have been prospectively following some siblings who are not yet showing symptoms but have a sibling with type 3 and are deleted to see what predictive value these tests have.

Dr Maria: I have 2 questions, 1 more basic and 1 more clinical. So on the basic question, we’ve heard about concentration in the growth cone, in terms of SMN, and on the issue of timing, of when expression of the gene is important. Do we know anything about whether the deficit affects growth cone extension, you know, filopodia, adhesion points, et cetera, or is it more of a stabilization of the growth cone? What do we know about SMN and its functional effect on growth cones? Is it extension or stabilization of the growth cone?

Dr Burghes: Well, the simple answer is very, very little. What I will say is when you cross the spinal muscular atrophy mice onto Tom Jessell's HB9 GFP line and you actually follow all the SMN points out, they look very, very normal in the spinal muscular atrophy mice. It's very hard to see any difference in the rate at which the growth cone goes or in where it's actually tracking to.

Now the motor neurons in the mice are much more complicated, so it’s hard to say that when you get to the fine ends, when it fans out to the muscle, that every one of those contacts is normal. But the 1 thing we see there
is in the very severe mice; at E18.5, we can find a considerable number of unoccupied synapses. So the first thing I would say is that if you take the fish and the culture model, they're in a slightly different environment. In other words, something in the mouse compensates for that growth defect that you can see and capture on the fish. It doesn't mean the assay of function in fish or in culture is incorrect. It means that there's something else in the background with the mouse, and that is 1 of the reasons you have to go between species with each of these things. So that'd be the first answer. The second answer to your question about what is happening with translation, and what the growth cone looks like, basically, we have no idea at the present time. There are some studies going on, but I would say that it's open for study.

Dr Maria: What are the areas in the standard of care recommendations that are still very controversial and that might be important to focus on going forward?

Dr Wang: The devil is in the details, and if you came to our Spinal Muscular Atrophy Standard of Care meeting in Stanford, you would have known that—Mary right there could bear witness to this. The pulmonary group was meeting until midnight, and they were still debating on certain things. What I showed here basically is just an outline. The example could be a bronchodilator—which type could be helpful? They were going into that kind of debate, and there are certain things that I highlighted, some of the more obvious controversial points, such as the SMN2 gene copies, things like that that people debate about and so forth. The group had to make a decision on what to recommend and what not to recommend. Mary, do you want to address some of that?

Dr Swoboda: I'll say 1 very hot topic was the newborn screening issue. People still feel very uncomfortable diagnosing a type 3 early on. I'm perfectly comfortable. I'm a big proponent. But there are many physicians who don't want to know if a type 3 patient is not going to present until the teenage years or maybe never. So I think that was 1 area. Mary?

Dr Schroth: Ching presented what we all agree on, so the things that we don't agree on aren't going to get into this paper. This document really is a baseline. It's a place to start from. It's not the end-all of how we do our standard of care; that's still in development across the country. And actually, this also involved a lot of Europeans, which was very interesting because they have a very different approach because they have different resources. For the pulmonary side, you know the thing that was, to us, very obvious, and you say it's common sense, but I'll tell you, not every child who has spinal muscular atrophy 1 or 2 gets a cough machine right away. And to us, it's common knowledge. We thought that was like a no-brainer; every kid with spinal muscular atrophy 1 and 2 should have a cough machine, and we think that's a very basic standard of care issue, but I'll bet in this room, very few of you have your spinal muscular atrophy 1s, unless you're very into a lot of this kind of care, on cough machines. The medications are very controversial. Do you use bronchodilators or not? Do you do inhaled steroids, which a lot of our patients are on? Do you do prednisone when they get sick? Those are all things that we didn't have agreement on, and there are no data, zero, from the literature. So it's continuing to pursue that information and trying to do some clinical trials. And as you know from today, it's very difficult to do clinical trials on type 1s because we're trying to figure out how to help them survive long enough. So we spent a lot of time in the pulmonary group talking about the various things, and we're just showing you what we agreed on. So things we didn't agree on will be mentioned, maybe mentioned in the article, but this is a process. This is not the end.

Dr Wang: Also, I wanted to address that this document is not aimed at only the neurologists. It's going to be aiming at many different practice disciplines and primary care physicians. So while some of them may look intuitive to us, it will be totally novel to a primary care physician.

Audience Member: I was not able to attend this meeting, but I did participate in the consensus committee for Duchenne dystrophy, and 1 of the most controversial subjects that we discussed there was the polysomnogram, and do you need a polysomnogram to diagnose sleep-disordered breathing or not. There were about 30 people on that committee, and we were divided right down the middle, between yes and no. And all of the guys that voted yes were directors of polysomnogram laboratories, so I briefly referred this morning to the fact that there are only 365 nights in the year, and there are a limited number of beds in pediatric polysomnogram labs. So we cannot do polysomnograms on every patient; it's just not logistically feasible. Do you use nocturnal oximetry? Can you do it at home? Does it have to be done in a lab or a hospital or a hospital room? I think that is 1 big area of controversy right now.

Dr Hoffman: I apologize. This is not a molecular genetics question either, a little bit more onto controversy about the pH study. And I guess I'm thinking if there are areas that are real controversies, hopefully, when something comes out, it'll list the potential benefit if you do this or potential complication if you do this. For the pH study, I had a child once I was following with Down syndrome and severe hypotonia, and the surgeons wanted a pH study before they would put in a gastrostomy tube, and so he aspirated during the pH study and went home with a tracheotomy. I don't know, Dr Wang, if you think that there is an increased risk of reflux all the way up to the trachea with the pH study; but even if it's not a recommendation not to do one, I would hope that the potential that doing one might cause an aspiration would be listed, versus if you do the fundoplication. I know kids do have discomfort from fundoplication; there's a potential of having that, but spelling it out for people, even clinically, they can use that to make their decisions.
Dr Wang: The gastrointestinal group actually came up with the recommendation, saying that a pH probe is not useful, and that's going to appear in this documentation.

Audience Member: I was wondering about the comment on the impact on progression and treatment of scoliosis. When you pick up scoliosis in these kids, OK, it's fine that it's there, but it's just an observation that it's there. What do you do about it once you find it?

Dr Wang: Addressing the scoliosis issues goes from monitoring to management, and we had a group of orthopedists along with the rehab people, and their recommendation, there were some monitoring procedures that they need to do, and then what procedures the orthopedics would like to institute once they do it, how they do it, and that kind of thing. There will be some statement about that. I cannot address all the details, but advance routine monitoring and then, the management, in terms of surgical or bracing, will all be addressed in this document.

Executive Summary

Jill Jarecki, PhD, Research Director, Families of Spinal Muscular Atrophy, Libertyville, Illinois; Cynthia Joyce, Executive Director, Spinal Muscular Atrophy Foundation, New York, New York; Jill Heemskerk, PhD, Director, NINDS Program, Bethesda, Maryland

Dr Jarecki, Dr Heemskerk, and Ms Joyce presented an executive summary of the symposium, with the aim of identifying questions that have yet to be answered in spinal muscular atrophy research. They summarized the key points of each presentation and focused on translational research questions, clinical trial challenges, and clinical care issues, as these topics relate to current research trends in the field.

As we know, SMN is a ubiquitously expressed protein involved in small nuclear ribonucleoprotein and messenger RNA processing that may also have a role in axonal function. The exact mechanism by which SMN deficiency effects a spinal muscular atrophy phenotype and why motor neurons are uniquely susceptible to such a deficiency are unknown. Effective animal models have been developed and are being used to investigate the promise of various active compounds, such as histone deacetylase inhibitors. With this background, the questions that remain in the field of translational research include the following:

- Do we need cell-specific therapies? Should active compounds be specifically screened for activity in motor neurons?
- Does increasing SMN levels postnatally show therapeutic benefits such as increasing levels during the embryonic period, as been shown to in the spinal muscular atrophy mouse model?
- Besides increasing SMN levels, what other therapeutic approaches (such as neuroprotective factors, etc) have potential as spinal muscular atrophy treatments?

A major challenge in designing spinal muscular atrophy clinical trials is the variability of the disease phenotype. It is difficult to include type 1, type 2, and type 3 patients in 1 trial because of the slower clinical progression of type 2 and type 3 patients and their different clinical needs. Another problem is the difficulty of capturing the acute progressive phase of the disease when many motor neurons die quickly and the difficulty of assessing disease progression in the context of the normal development of a growing child. Some of the questions that remain in clinical trial design are as follows:

- How will the gain in function through normal growth and development affect the design of spinal muscular atrophy clinical trials?
- How can we design trials that protect the health of fragile type 1 spinal muscular atrophy babies?

Cynthia Joyce presented some of the efforts aimed at addressing the above questions. Clinicians, basic researchers, and patient advocacy groups are collaborating to establish a solid understanding of the natural history of spinal muscular atrophy. A great deal of work is also focused on validating outcome measures and identifying new outcome measures for clinical trials. One approach is the development of biomarkers to measure disease progression; potential quantitative markers of disease progression include SMN protein levels and SMN transcription levels in the blood. Another issue in spinal muscular atrophy is patient recruitment and continued participation in clinical trials. An international patient registry has been established by the University of Indiana Genetics Department to refer patients with interest in participating in clinical trials. Ms Joyce stressed the need for more spinal muscular atrophy clinical trial centers throughout the country and for an integrated approach to clinical care.

Future Directions Panel Discussion

Moderator: Jill Heemskerk, PhD, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

Panel Members: Wilson W. Bryan, MD, Medical Officer, Food and Drug Administration, Rockville, Maryland; Daryl DeVivo, MD, Columbia University, New York, New York; John T. Kissel, MD, Ohio State University, Columbus, Ohio; Sakamuri V. Reddy, PhD, Medical University of South Carolina, Charleston, South Carolina; Mary Schroth, MD, University of Wisconsin Hospital, Madison, Wisconsin

The panel discussion focused on answering the questions posed by Dr Heemskerk, Dr Jarecki, and Ms Joyce in the executive summary, on identifying future directions needed to improve the clinical care of children with spinal muscular atrophy, and on the research necessary to find effective therapies for this disease. The following topics were discussed as important issues for future research.
Translational Research
Screening for potential treatments is an important part of research in this field. To design promising clinical trials, target therapeutics that are likely to work must be identified by screening.

Clinical Trials
How can we decrease “compassionate use” of off-label drugs and instead encourage physicians to refer patients to ongoing clinical trials? Spinal muscular atrophy presents a finite patient population, so it is very difficult to fill clinical trials with naïve patients, those who have not been treated. A way to improve communication about available trials is necessary to improve our understanding of the actual benefits of potential medications. One suggestion for improving patient enrollment and retention in clinical trials is to ensure that each patient is treated at some point during the trial.

Identifying sensitive outcome measures, such as molecular correlates or disease progression or reliable biomarkers, is necessary in the field and would allow for smaller clinical trials. It is not necessary that these have clinical meaning, but instead, they must reflect therapeutic efficacy while preserving reliability, sensitivity, and resistance to bias.

Clinical Care
How should a clinical care team be put together, and how can patients be managed long-term? This is a prohibitively expensive but very important aspect of caring for spinal muscular atrophy patients. It was suggested the key clinical care centers would benefit from pairing with industry. However, this must be done carefully to protect patients’ interests. The creation of a database for natural history information of spinal muscular atrophy patients from all clinical trial centers was suggested as a beneficial endeavor.

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