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Childhood Ataxia: Clinical Features, Pathogenesis, Key Unanswered Questions, and Future Directions

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Abstract

Childhood ataxia is characterized by impaired balance and coordination primarily due to cerebellar dysfunction. Friedreich ataxia, a form of childhood ataxia, is the most common multisystem autosomal recessive disease. Most of these patients are homozygous for the GAA repeat expansion located on the first intron of the frataxin gene on chromosome 9. Mutations in the frataxin gene impair mitochondrial function, increase reactive oxygen species, and trigger redistribution of iron in the mitochondria and cytosol. Targeted therapies for Friedreich ataxia are undergoing testing. In addition, a centralized database, patient registry, and natural history study have been launched to support clinical trials in Friedreich ataxia. The 2011 Neurobiology of Disease in Children symposium, held in conjunction with the 40th annual Child Neurology Society meeting, aimed to (1) describe clinical features surrounding Friedreich ataxia, including cardiomyopathy and genetics; (2) discuss recent advances in the understanding of the pathogenesis of Friedreich ataxia and developments of clinical trials; (3) review new investigations of characteristic symptoms; (4) establish clinical and biochemical overlaps in neurodegenerative diseases and possible directions for future basic, translational, and clinical studies.

Keywords

cardiomyopathy; cerebellar ataxia; childhood ataxia; frataxin; Friedreich ataxia; GAA repeat expansion; hypertrophy; iron accumulation; iron-sulfur clusters; mitochondria; oxidative stress

CLINICAL ISSUES

Moderator: David Lynch, MD, PhD, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

Overview of Childhood Ataxia

Brent Fogel, MD, PhD, University of California, Los Angeles, Los Angeles, California

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Dr Fogel's talk centered around an overview of childhood ataxia, beginning with a general definition of ataxia and moving deeper into specific causes and classifications of childhood ataxia — chiefly cerebellar ataxias. Ataxia can be defined as a loss of balance and coordination. Ataxia does not result from muscle weakness, but rather from a malfunction in one or more of the following: sensory pathways, particularly proprioception, vestibular system, and the cerebellum. Symptoms of ataxia all reflect poor coordination of voluntary movement — speech, swallowing, and gait: dysarthria, dysphagia, and dysmetria.

Throughout his presentation, Dr Fogel focused primarily on cerebellar ataxia, which results from degeneration of the cerebellum. This degeneration prevents the cerebellum from integrating all of the sensory, visual, and environmental inputs and therefore blocks the ability to predict the effects of these motor commands, resulting in a lack of coordination. There are a number of different acquired, idiopathic, and genetic causes of cerebellar ataxia. Dr Fogel mainly focused on the genetic causes. With this said, hereditary cerebellar ataxias can be categorized into 2 types of inheritance: autosomal dominant and autosomal recessive. Both types share a common prevalence rate of about 4 cases per 100 000 people worldwide.

Autosomal dominant cerebellar ataxias, more commonly referred to as the spinocerebellar ataxias, are caused by the transmission of a single mutant allele to the next generation. Typical age of onset for spinocerebellar ataxias ranges from 20 to 50 years, and the phenotype is typically slowly progressive, clinically heterogeneous ataxia. Currently, there are close to 20 identifiable genes for spinocerebellar ataxias, with spinocerebellar ataxia type 3 being the most common and spinocerebellar ataxia type 1, spinocerebellar ataxia type 2, spinocerebellar ataxia type 6, and spinocerebellar ataxia type 7 following behind. These disorders share a similar molecular pathogenesis in that they are repeat expansion disorders with polynucleotide expansions of CAG or polyglutamine, if they are coding. Furthermore, the concept of anticipation, the idea that polynucleotide expansion disorders are unstable in the DNA, therefore possessing the ability to expand from one generation to the next, must be taken into account with these repeat expansion disorders (especially in children).

Contrary to autosomal dominant cerebellar ataxias, autosomal recessive cerebellar ataxias result from the transmission of 2 mutant alleles to the next generation. Currently, there are 25 identifiable genes that cause these disorders, and they typically show up in children, since the onset is usually before 20 years of age. Phenotypically, these disorders are slowly progressive, symmetrical cerebellar ataxias and are often associated with a sensory or a sensory-motor polyneuropathy. Furthermore, these disorders typically involve other organ systems located outside of the central nervous system. The disorders that comprise the autosomal recessive cerebellar ataxias can be categorized by various classification schemes. To this end, Dr Fogel presented a clinically-based classification: (I) Friedreich ataxia-like, (II) Friedreich ataxia-like with cerebellar atrophy, and (III) early-onset ataxia with cerebellar atrophy¹ (summarized in Table 1).

(I) Friedreich ataxia-phenocopy is made up of 4 distinct entities. First, Friedreich ataxia, the representative disorder for this class as well as the most common autosomal recessive disorder, results from a mutation in the frataxin gene. Clinically, patients will present with spinocerebellar ataxia with limb, gait, and truncal ataxia featuring degeneration of the peripheral sensory nerves, posterior columns, and lateral corticospinal tracts. Second, ataxia with vitamin E deficiency is caused by a mutation in the alpha-tocopherol transfer protein, which is involved in vitamin E metabolism and transport. The clinical presentation is similar to Friedreich ataxia but with pigmentary retinopathy. Third, abetalipoproteinemia is a mutation of the microsomal triglyceride transfer protein, which is involved in lipoprotein metabolism. This disorder also presents with pigmentary retinopathy and lipid malabsorption. Lastly, Refsum disease is a peroxisomal disorder caused by a mutation in the

phytanoyl-CoA hydroxylase gene and less frequently by a mutation of peroxin-7. Again, the clinical features of this disorder are the same as Friedreich ataxia but with anosmia.

Disorders that are similar to Friedreich ataxia but initially manifest with cerebellar atrophy make up the second class of autosomal recessive cerebellar ataxias. The representative disorder DNA polymerase-gamma ataxia results from a mutation of DNA polymerase-gamma, which is the sole mitochondrial DNA polymerase. This disorder presents with a variety of features: myoclonus, diminished cognitive features, psychiatric symptoms, migraine headaches, and epilepsy. Other members of this class include late-onset Tay-Sachs disease and cerebrotendinous xanthomatosis. First, late-onset Tay-Sachs disease is a mutation in the hexosaminidase A gene impairing ganglioside metabolism. Distinguishing clinical features include extrapyramidal signs and mood disturbances. Next, cerebrotendinous xanthomatosis is caused by a mutation in the sterol 27-hydrolyase gene and presents with early cataracts and tendon xanthomas.

The third and most extensive class of autosomal recessive cerebellar ataxias is the early onset ataxias typically seen before the age of 5 years and associated with cerebellar atrophy. Members of this class all have a similar phenotype consisting of oculomotor apraxia, extrapyramidal signs, and cognitive impairment. First, ataxia telangiectasia, the representative disorder, is caused by a mutation in the ataxia telangiectasia mutated (*ATM*) gene, which is a tyrosine kinase involved in DNA damage repair. This is the second most common autosomal recessive disorder, second only to Friedreich ataxia. Furthermore, onset is typically before 3 years of age and the condition is rapidly progressive. In addition to this class' similar phenotype, ataxia telangiectasia clinically presents with oculocutaneous telangiectasias, immunodeficiency, radiosensitivity, and an increased cancer risk.

In addition, 2 more members of this class are ataxia with oculomotor apraxia type 1 and type 2. Type 1 results from a mutation in the aprataxin gene, whereas type 2 from the senataxin gene; both genes are involved in DNA repair. Furthermore, cayman ataxia is caused by a mutation in the caytaxin gene, and the clinical features of this disorder are pure cerebellar ataxia associated with psychiatric symptoms. Other members of this class include Marinesco-Sjögren syndrome, autosomal recessive cerebellar ataxia type 2, dysequilibrium syndrome, and peroxin-associated ataxia.

How should a pediatrician evaluate a child for suspected autosomal recessive cerebellar ataxia? Essentially, the key is establishing the phenotype by gathering a detailed clinical and complete family history, comprehensive neurological examination, and magnetic resonance imaging (MRI) of the brain. Furthermore, it is important to rule out acquired causes of ataxia since these conditions are potentially reversible. Beyond these preliminary assessments, genetic testing for Friedreich ataxia should be considered. While conducting this gene test, however, one should avoid large gene panels, as many of the genes are extremely rare. Lastly, if the gene testing for Friedreich ataxia is negative, it is advised to examine serum biomarkers because this is less expensive than genetic testing and can give valuable diagnostic clues. Throughout the evaluation, it is also important to refer to a neurogeneticist or ataxia specialist, if needed.

In summary, cerebellar ataxias are caused by a variety of acquired, idiopathic, and genetic etiologies. In terms of genetic etiologies, 2 modes of inheritance were expanded upon: autosomal dominant and recessive cerebellar ataxias. The autosomal recessive disorders are primarily due to loss of function in major metabolic and cellular pathways. When evaluating a patient for autosomal recessive cerebellar ataxias, it is crucial to use a systematic approach to make a definitive molecular diagnosis.

Clinical Features of Friedreich Ataxia

Martin Delatycki, MBBS, PhD, FRACP, Bruce Lefroy Centre for Genetic Health Research, Victoria, Australia; Presentation presented by Dr Louise Corben

Dr Delatycki's presentation discussed the history of Friedreich ataxia and described its clinical features. During the 1860s, Nikolaus Friedreich, who was a professor of medicine at the University of Heidelberg, discovered the disease known today as Friedreich ataxia. Throughout a series of 5 reports, Friedreich described the disease after seeing 2 siblings who had symptoms of ataxia but came from asymptomatic parents. Initially, Friedreich did not indicate the absence of reflexes, a hallmark feature of the disease, since deep tendon reflexes were not discovered until 1875 by his student, Wilhelm Heinrich Erb.²

Friedreich ataxia is a multisystem autosomal recessive cerebellar ataxia. In terms of molecular genetics of the disease, the clinical features of Friedreich ataxia result from a mutation of the frataxin gene located on chromosome 9q13. Because this gene normally functions in regulatory mitochondrial iron metabolism, the absence of normal frataxin causes oxidative stress and cell damage. Furthermore, 98% of the people with Friedreich ataxia are homozygous for a GAA triplet repeat expansion in the first intron of the frataxin gene, and the other 2% have point mutations. With this disease being the most common inherited ataxia, the prevalence rate is 1:30 000 to 50 000 people within the Caucasian population. The incidence of Friedreich ataxia in Asian and African American populations is much lower. Approximately 1:85 people carry a mutant copy of the frataxin gene.

Friedreich ataxia is a devastating disease. In addition to it being a debilitating disease, many patients are wheelchair-bound by 19 years of age, and the average life expectancy is only 40 years of age. The typical age of onset of disease is 10 years. Interestingly, there is an inverse relationship between the age of onset and the size of the GAA repeat expansion. The size of the GAA repeat expansion directly correlates with the severity of disease. The first clinical feature of Friedreich ataxia is often progressive limb ataxia. As an illustration, the parents of a child typically report to the physician that the child is becoming increasingly clumsy with physical activity. On examination there is an absence of lower limb deep tendon reflexes and decreased vibration and proprioception; the ataxia has both sensory and cerebellar features. Moreover, patients may have prominent Babinski sign, scoliosis, foot deformities, diabetes, visual disturbance, dysarthria, dysphasia, eye movement abnormalities, hearing and cognitive deficits, sexual problems, sleep apnea, urological disturbances, psychological issues, and cardiomyopathy.

In 1996, Campuzano and team members identified the molecular basis for Friedreich ataxia. Prior to this, however, Friedreich ataxia was diagnosed by a diagnostic criteria first proposed by Geoffroy and colleagues and later by Harding in 1981.² As described in Dr Delatycki's review published in the *Journal of Medical Genetics*, the diagnostic criteria proposed by Geoffroy and colleagues in 1976 consist of primary criteria that are essential for diagnosis, and secondary criteria. First, the primary criteria consist of onset of disease before the end of puberty (20 years of age), progressive ataxia of gait, dysarthria, loss of joint position or vibration sense, absent tendon reflexes in legs, and muscle weakness. Next, the secondary criteria are extensor plantar responses, pes cavus, scoliosis, and cardiomyopathy.

Later in 1981, Harding expanded on this diagnostic criterion, proposing that the age of onset be before 25 years of age, and the patient needed to have progressive unremitting ataxia of limbs and of gait as well as absence of patellar and ankle jerks. In addition, patients needed to have dysarthria and extensor plantar responses. If these secondary criteria were absent, then the patient must have an affected sibling and should undergo electrical studies.²

As previously noted, the hallmark clinical feature in Friedreich ataxia is ataxia. Interestingly, Friedreich ataxia presents with ataxia of many different systems of the body rather than solely gait or upper limb ataxia. In addition to ataxia, children will present with scoliosis, which is a major source of morbidity in terms of managing cardiac and respiratory function, as well as posture. In a study conducted by Labelle and colleagues, all 56 patients enrolled in the study had a spinal curve of greater than or equal to 10%. Furthermore, these children often have significant double curves and thoracic and lumbar scoliosis. Dr Delatycki emphasized the importance of actively and aggressively managing this clinical feature early in the disease process, as scoliosis is an inevitable consequence of the disease.

Another clinical feature associated with Friedreich ataxia is foot deformity. Although pes cavus is not a major source of morbidity, equinovarus deformity can cause significant morbidity. This deformation can be alleviated by physical therapy, splinting, or Botox. Furthermore, in some cases, tendon-lengthening surgery can be beneficial and can correct the deformity. On a different note, patients with Friedreich ataxia have decreased cognitive function. To this end, the intelligence level of a patient is characterized by concrete thinking, poor competence in forming concepts, and visuospatial reasoning with reduced speed in processing information. As mentioned in a study conducted by Dr Delatycki's colleague, in terms of motor cognition, patients with Friedreich ataxia had significantly difficulty with accommodating unexpected movement, initiating movement, and reacting to incongruent stimuli.

Other clinical features of Friedreich ataxia include dysarthria and dysphagia. Dysarthria, ataxic speech, is present in more than 90% of patients. The range of dysarthria is mild to moderate, and the severity of dysarthria correlates with the duration of disease. On a similar note, dysphagia also progresses with the duration of the disease. In addition, dysphagia can cause aspiration with or without pneumonia, which can lead to mortality.

Less common symptoms of Friedreich ataxia include visual and hearing impairment as well as diabetes mellitus. In terms of visual impairment, Durr and colleagues conducted a large study concluding that 13% of the patients had visual loss. Furthermore, visual quality of life was reduced in these patients, which had a significant impact on daily activities requiring the use of intact vision. In addition, patients with Friedreich ataxia have impaired hearing. In one of Dr Rance's publications, he noted that people with Friedreich ataxia have a form of auditory neuropathy. Furthermore, Dr Rance found that patients had abnormal speech understanding when tested with background noise, which is a major problem because many of these patients are in school environments where background noise is pervasive. Lastly, 10% to 30% of patients with Friedreich ataxia have diabetes mellitus due to combined insulin deficiency and insulin resistance. Therefore, it is important to test patients for glucose tolerance and insulin resistance.

Because Friedreich ataxia is a devastating disease, it is no surprise that patients are often faced with increased rates of depression and mood disturbances. Seeking psychological support is crucial and highly encouraged. Unfortunately, there is no cure for Friedreich ataxia, and there is still no treatment to slow its progression. Therefore, further research is needed to manage and care for these patients.

Cardiomyopathy in Friedreich Ataxia

Mark Payne, MD, Indiana University School of Medicine, Indianapolis, Indiana

Dr Payne discussed heart disease in Friedreich ataxia while focusing on 3 objectives: identifying important clinical issues regarding the heart in a patient who has Friedreich ataxia; identifying important basic questions and findings for the heart in Friedreich ataxia;

and briefly touching on future research directions. In general, heart failure occurs when the heart is unable to meet the metabolic demands of the body, regardless of the cause. Quantifying heart failure is challenging because it is based solely on clinical findings rather than a laboratory diagnosis. Heart failure occurs either as a result of a structural defect or a malfunction in the primary pump, which is the cause of heart failure in a patient with Friedreich ataxia.

Nearly 100% of children and adults who have Friedreich ataxia will have some level of cardiomyopathy. In most patients, the heart becomes very thick, which can be described as hypertrophic cardiomyopathy. Systolic function is preserved until late in the course of disease, but diastolic function is quite impaired. As a result, it is difficult to fill and perfuse the heart with blood. Furthermore, the patient will have impaired diastolic measures and impaired coronary reserve. Also, the patient will show scar formation and cardiomyocyte drop-out. Anecdotally, these patients do not tolerate fluid shifts and will often develop cardiac arrhythmias.

Even though heart failure is the most common cause of death in patients with Friedreich ataxia, not much is known about heart function in this disease. However, it is known that heart failure in Friedreich ataxia results from dysfunction of the mitochondria (the powerhouse of the cell and control center of life and death programs) rather than of the contractile proteins in the heart. Consequently, patients with Friedreich ataxia are treated differently than patients with inherited cardiomyopathies such as hypertrophic obstructive cardiomyopathy. Iron-sulfur clusters within mitochondrial and cytosolic proteins are controlled by frataxin, which is absent in Friedreich ataxia. Since the operative iron-sulfur clusters are not able to function correctly, electrons are unable to easily move down the electron transport chain, leading to free electrons that participate in formation of lipid peroxidation, hydroxyl free radicals, and peroxynitrite. Unfortunately, as with all mitochondrial disorders, there is no definitive therapy at this time, making this the most common cause of death in patients with Friedreich ataxia.

In terms of the pathology of a Friedreich ataxia heart, both ventricles of the heart are affected and astoundingly thick. Rather than being 1 centimeter in thickness like a normal heart, a heart in Friedreich ataxia can be as thick as 4 centimeters on the lateral walls. Furthermore, on examination of a cross-section of a diseased heart, there is extensive fibrosis in between the contractile fibers, and a vast amount of iron deposition within the heart. In addition to the heart having mitochondrial proliferation that extends out of bounds, when viewing a heart on electron microscopy, the entire cardiomyocyte is filled with misshapen mitochondria, some of which contain electron dense granules.

Dr Payne presented electrocardiograms from his patients to point out that nearly all patients with this disease have an abnormal electrocardiogram. For example, the T-wave repolarization on the electrocardiogram will present very flat. Furthermore, Dr Payne noted that the electrocardiogram is sensitive in detecting cardiomyopathy, but it is not specific in characterizing the severity of the cardiomyopathy. Therefore, the main tool for following the condition of a heart in Friedreich ataxia is echocardiography. When reviewing a patient's echocardiogram, the walls of the heart will appear very thick. Dr Payne also showed images of cardiac magnetic resonance imaging (cardiac MRI), the most accurate way to measure the left ventricular mass and its function, pointing out the thickness of the left ventricular wall. Relating back to Dr Delatycki's talk and the correlation between the size of the GAA repeat and severity of the disease, Dr Payne noted that longer GAA repeats also associate with greater left ventricular hypertrophy.

What is there to offer in terms of therapies for the heart in Friedreich ataxia? Simply put, not much. First, the diastolic function of the heart needs to be addressed. There is not much to offer in this regard, but clinical trials need to be conducted using calcium channel blockers, as these may be beneficial. Next, the loss of cardiomyocyte function has to be addressed. There has been a long history of trying multiple drugs such as idebenone, antioxidants, deferiprone, and erythropoietin, but none of these agents have had long-term benefit in terms of neurologic or cardiac function.

Because cardiomyopathy in Friedreich ataxia has gained more attention within the past few years, some novel therapies have been proposed. First, Dr Payne discussed protein replacement therapy. In a murine mouse model, frataxin replacement therapy improved survival. In addition, Dr Payne briefly touched on the work being done with ubiquitin inhibitors and histone deacetylase inhibitors, which both have the same effect of maintaining a functional level of frataxin within the cell.

A number of important clinical questions remain. Why does Friedreich ataxia present so late in childhood? What is the minimal level of frataxin needed to be functional and effective? Lastly, what is the clinical basis for cardiac failure and treatment? All these questions are under study.

Genetics of Friedreich Ataxia and Issues in Study of Rare Diseases

Jennifer Farmer, MS, CGC, Friedreich's Ataxia Research Alliance, Downingtown, Pennsylvania

Ms Farmer discussed additional details of the genetics of Friedreich ataxia but also focused on the implications of clinical research studies and the role of advocacy groups. The incidence of Friedreich ataxia is close to 1 in 50 000 individuals, and the carrier frequency is estimated to be 1 in 100 individuals. When 2 carriers have a child, there is a 25% chance the child will develop Friedreich ataxia. Although the prevalence of the disease in the United States is about 6000 individuals, about 15 000 individuals worldwide have the disease. Furthermore, Friedreich ataxia is more prevalent in certain populations. Specifically, Friedreich ataxia can be found in the Americas, Europe, Australia, Asia, the Middle East, North Africa, and India. In contrast, the prevalence of Friedreich ataxia is lower in China, Japan, or in Africa, possibly because of a founder mutation and how the disease has spread.

The gene responsible for Friedreich ataxia was identified in 1996. In addition to this gene being located on chromosome 9, there is a common mutation in the gene, which is a GAA repeat expansion. The repeat expansion is unique in that it is located in an intron rather than in an exon. This is significant because this mutation causes abnormal DNA structures and changes in the heterochromatin that reduce the amount of protein product frataxin made in the cell, affecting normal mitochondrial function. Furthermore, a person unaffected by Friedreich ataxia normally has 3 to 33 GAA repeats. In contrast, individuals with Friedreich ataxia will have anywhere from 60 to 1500 GAA repeats on both alleles, making them homozygous; this is true for 95% of patients. The other 5% of individuals are compound heterozygous, meaning that they have a triplet repeat on one allele and a point mutation on the other.

Interestingly, there is a correlation between the size of the GAA repeat expansion, age of onset of disease, and the amount of frataxin produced. Patients who are homozygous for the GAA repeat expansion have 2 alleles, and the smaller of the 2 alleles allows physicians to predict the age of onset, because it allows a greater amount of frataxin to be produced. To this end, frataxin assays have been developed for clinical diagnosis and research. By using this assay, physicians are able to see the amount of frataxin that the patient produces,

allowing them to predict disease severity. To follow up on this, Ms Farmer presented a graph that showed the age of onset versus the number of GAA repeats in the smaller allele. Analysis of this graph reveals that most patients with Friedreich ataxia have less than 20% of normal frataxin levels.

Identification of the gene responsible for Friedreich ataxia has allowed for the development of molecular genetic diagnostic tests, which can detect about 98% of individuals who are symptomatic. This molecular genetic testing is done by first looking for GAA repeat expansions and then sequencing the gene for point mutations, if needed. Development of the molecular genetic test in combination with the frataxin assay has expanded the phenotype of Friedreich ataxia and provided better diagnosis for atypical patients. To this end, these patients will have a smaller GAA repeat expansion sizes, usually between 100 and 300 repeats.

Despite advances in clinical diagnosis using molecular tools, some issues regarding clinical studies remain. For instance, since Friedreich ataxia is a rare disease, there are challenges related to small cohort sizes, patients being spread across a wide geographical area, and a limited number of clinicians who are knowledgeable about the condition. Furthermore, Friedreich ataxia has a diverse phenotype, and the rate of progression can be variable, even though the GAA repeat helps predict the age of onset. Both of these clinical issues have made it hard for clinicians to specify how a particular patient will progress.

Ms Farmer ended her discussion by talking about the important role of the advocacy group Friedreich's Ataxia Research Alliance. First, the Friedreich's Ataxia Research Alliance understands the importance of expanding the scientific community. To this end, conferences are held each year, bringing together both experienced and new investigators in order to inform people about the disease. Ms Farmer stressed the importance of working together to build relationships and partnerships between the scientific and medical community, government, industry, and the patient community, since Friedreich ataxia is a rare disease. Furthermore, the Friedreich's Ataxia Research Alliance focuses on developing translational tools (biorepositories with DNA, RNA, cells, and tissues) that will advance the drug discovery in a speedy fashion while also reducing redundancy. Lastly, the Friedreich's Ataxia Research Alliance makes sure there is clinical infrastructure in place to support clinical trials, for there is a patient registry ensuring adequate recruitment for each study. These clinical trials are crucial because they will lead to new therapies that will be able to better treat patients with Friedreich ataxia.

Questions and Answers

Dr Maria: Since pediatricians see children at the very early stages of the disease, what are some of the early clinical hallmarks that we need to be paying attention to? Dr Corben: In terms of early presentation for Friedreich ataxia, typically ataxia is the first presentation. For example, the child is clumsy or falling over. With this being said, however, some children have presented with cardiac symptoms or scoliosis first. Therefore, it is important to be alert to the fact that it may not necessarily just be ataxia or absent reflexes.

Dr Lynch: Most young children present with ataxia with the absence of reflexes and a normal MRI scan. With this said, however, I would say that most of the diagnoses, as I recall, would not be 5-year-olds who were symptomatic but 10-year-olds who were symptomatic when they were 5 years old who went through a long diagnostic odyssey in the attempt to get there. Surprisingly, this has not shortened in length even with genetics. In summary, clearly the loss of reflexes and a truly ataxic gait with a sensory ataxia are the hallmark early findings that physicians should be on the lookout for.

Dr Maria: Since heart failure is the dominant cause of death in the disease, is there any experience with transplantation? What are the ethics of transplantation in Friedreich ataxia?

Dr Payne: First, at the time of diagnosis, send these patients to a cardiologist, since these patients will develop cardiomyopathy. Second, these patients do progress to the point where heart failure becomes life-threatening. The considerations for heart transplant are not trivial. The bottom line is that if transplanted, these patients will outlive the natural history of the transplant, so from this standpoint, transplant is worthwhile. With that being said, however, very few centers are actually willing to transplant these patients, since these patients are perceived as having substantial mental cognition impairment, and the United Network for Organ Sharing (UNOS) is reluctant to supply organs for this. Since UNOS is applying pressure to many centers to drop their transplant program, I think most transplant centers are reluctant to pursue transplants, unfortunately. In conclusion, I strongly support the idea of heart transplant for these patients, as it is a viable option for the heart failure in this particular disease.

Dr Lynch: There have been several individuals in Canada and at least a couple in Europe who have been transplanted for the cardiomyopathy of Friedreich ataxia, but I do not know of any in the United States.

Dr Payne: There are only 3 reports in the literature, but none of these reports are from the United States.

Audience member: What are the problems with using alpha-fetoprotein as a screening tool for ataxia telangiectasia? What is the youngest age this is reliable?

Dr Crawford: The normal alpha-fetoprotein level is 2000 to 3000 ng/mL in newborns. At about 8 months of age, this level starts to come down to normal. With this said, if a child has an abnormally high alpha-fetoprotein after 1 year of age, this level will decrease if it is not ataxia telangiectasia and continue to increase if it is ataxia telangiectasia. I would not use alpha-fetoprotein as a screening tool before 1 year of age.

Audience member: What is the opinion about scoliosis surgery for these patients?

Dr Payne: These patients will most likely need scoliosis surgery, which is a risky surgery. Therefore, a team approach comprised of the neurologist, cardiologist, anesthesiologist, and orthopedic surgeon needs to be taken since this is a multisystem disease.

Audience member: What kind of treatment can be used to treat the cardiomyopathy feature of Friedreich ataxia?

Dr Payne: I do not have a therapy since there are no clinical studies looking at this. Further clinical studies are needed to look at specific drugs, beta-blockers being one of these, that can be used to treat both systolic and diastolic function rather than giving drugs based on previous studies done on adults.

Audience member: First, what allows mice to survive the energetically demanding time early in development but not once adolescence starts? Second, if mice are treated early with electron transport modifiers, such as idebenone, can this slow the progression of the secondary changes?

Dr Payne: I think that understanding why the infant and child do so well but then begin to manifest symptoms at the start of adolescence is a key basic and clinical question that needs to be asked. I do know that if I treat the mice early in life with powerful antioxidants I see no effect. I have not used idebenone, but others that have may be able to comment on that. One

limitation with the mouse model is that they do not reproduce what is genotypically or phenotypically seen in humans. People are knocked down whereas the mice are knocked out. When a humanized Friedreich ataxia gene gets put back into the mouse model, the phenotype is not great, but it is good for testing drugs that affect gene expression, which is why we use them.

Dr Lynch: Just to follow on that, idebenone has been used in the mouse model, and it causes a small but statistically significant increase in the lifespan.

Audience member: How do you approach a patient that comes to you with a large ataxia panel already performed?

Dr Fogel: Most commonly, patients come in with test results that have a variance of unknown significance. This can be challenging to interpret, since this unknown significance can be due to the fact that it has never been seen clinically before. With this said, it is important to remember that just because they are of unknown significance does not mean they are not pathogenic since every variant that has ever been reported to be pathogenic was at one time of unknown significance. Therefore, it is important to take a systematic approach when evaluating these results, and there are a number of ways to do this. The most detailed way would be to look bioinformatically — see if the variant has been reported in normal populations that do not have a clinical phenotype, and also see if bioinformatically it would be predicted to alter protein structure or function.

PROGRESS TO DATE IN FRIEDREICH ATAXIA

Moderator: Christopher W. Gomez, MD, PhD, University of Chicago, Chicago, Illinois

New Pathways

Massimo Pandolfo, MD, Free University of Brussels, Brussels, Belgium

To identify potential targets for treatment, the pathogenesis of Friedreich ataxia needs to be understood. Pandolfo presented the established pathway of a cell that is deficient for frataxin. Cabantchik and colleagues in Israel induced the expression of an inhibitor RNA by adding tetracycline, which caused a reduction in the level of frataxin. Results showed impaired mitochondrial function as seen by a drop of mitochondrial membrane potential, increased reactive oxygen species (which is a sign of oxidative stress), and iron redistribution with increased iron level in the mitochondria and decreased iron level in the cytosol.³

The function of frataxin explains the 3 consequences when the level of frataxin is decreased: mitochondrial dysfunction, production of free radicals, and changes in iron metabolism. A number of functions have been proposed for frataxin. Evidence in the past few years has shown that the primary function of frataxin is involved in iron-sulfur cluster synthesis, which occurs mainly in the mitochondria. A few years after the discovery of the frataxin gene, Rustin and colleagues from France found that iron-sulfur cluster enzyme activities were decreased in endomyocardial biopsies of young patients who presented with hypertrophic cardiomyopathy, one of the first signs of Friedreich ataxia.

Iron-sulfur clusters are complexes of iron and sulfur atoms with variable geometries. The cube geometry is used by a number of proteins as prosthetic groups to function properly. Some proteins in the mitochondria that require iron-sulfur clusters are involved in energy metabolism, such as aconitase in the citric acid cycle. Iron-sulfur clusters are found in the cytosol involving the metabolism of amino acids, nucleic acids, and iron. In the nucleus,

iron-sulfur clusters possibly function in DNA repair.⁴ With the various activities that involve iron-sulfur clusters, changes in frataxin levels can lead to cell dysfunction.

Mitochondrial iron-sulfur biogenesis occurs through a multiprotein complex where iron-sulfur cluster S, a cysteine desulfurase, obtains sulfur from cysteine and iron transferred by a carrier. Assembly occurs on the iron-sulfur cluster assembly scaffold protein ISCU and the nascent iron-sulfur clusters are transferred to the apoproteins by several chaperones.⁵ Barondeau and colleagues found that frataxin is a component of the multiprotein complex, but rather than contributing directly to enzymatic activity, frataxin accelerates the activity of the complex. In the absence of frataxin, the complex is at an off-state and produces a minimal amount of iron-sulfur clusters, but with the presence of frataxin, the complex is at an on-state and activates increased amount of iron-sulfur cluster synthesis.⁶

Experimental designs of iron-sulfur cluster biogenesis deficiency in the mitochondria results in mitochondrial iron accumulation, decreased energy production, increased oxidative stress, and altered iron metabolism. These abnormalities are also seen in disorders related to metabolic myopathy. The pathways that are activated due to iron-sulfur cluster biogenesis deficiency are relevant to the pathogenesis of Friedreich ataxia. The iron metabolism pathway is atypical in Friedreich ataxia by the redistribution of iron within the cell and is largely dependent on the alteration of the iron regulatory protein-1. Iron regulatory protein-1 and iron regulatory protein-2 are localized in the cytosol and exist in 2 different states. When iron levels are low in the cytosol, the 2 proteins act as RNA-binding proteins and bind to specific sequence motifs called iron responsive elements in messenger RNA. The iron regulatory proteins binding to iron-responsive elements in the 3' untranslated regions protect the TrF transcripts from degradation and cleavage. When the proteins are localized at iron responsive elements in the 5' untranslated regions, the translation of the messenger RNA will be blocked. The rate of translation for proteins in the iron metabolic pathway varies by the binding location of the iron regulatory proteins. For instance, translation of ferritin protein occurs less, whereas translation of the transferrin receptor increases so the cell can increase the intake of iron.⁷ Alteration of the iron regulatory protein-1 determines the function of the protein. In the absence of an iron-sulfur cluster, iron regulatory protein-1 functions as an iron regulatory protein, whereas the presence of an iron-sulfur cluster causes iron regulatory protein-1 to act as the enzyme, aconitase. One consequence of a low level of frataxin is the lack of iron-sulfur clusters in the iron regulatory protein-1, which will activate cellular iron influx and decrease the expression of genes that use iron. The influx of iron is imported into the mitochondria by mitoferrins, which results in the accumulation of iron in the mitochondria and low levels of iron in the cytosol.

Pandolfo hypothesized that the regulation of the anomaly in iron metabolism would alter pathways found in cells deficient in frataxin. In the 1990s, Pandolfo and Kaplan generated the GAA knock-in mouse model, which can either be homozygous or heterozygous for the GAA repeat, respectively referred to as the KIKI and KIKO mouse model. The KIKO mouse model has a level of frataxin that is 25% to 30% of the level in normal mice and exhibits little motor phenotype and some biochemical phenotype, which means that minute alternations of frataxin levels cause biological changes. The skeletal muscles have decreased expression of slow-twitch fibers and increased lipid synthesis that resemble changes in insulin-resistant muscle. Testing on KIKO mice showed reduced sensitivity to insulin, which provides a link between low levels of frataxin and diabetes in patients with Friedreich ataxia. Similar in patients, β -cells in the KIKO mice are abnormal with endoplasmic reticulum stress and mitochondrial abnormalities. Studies on gene expression, mouse models, and human tissue show that the pathways with frataxin are altered.

Pandolfo discussed a new pathway that involves decreased cytosolic iron causing suppression of iron-sulfur cluster synthesis and indirectly affecting frataxin. The cofactor peroxisome proliferator-activated receptor- γ coactivator (PGC)-1 α is responsible for the coactivation of transcription factors related to mitochondria biogenesis and lipid metabolism. Pandolfo collaborated with Coppola to investigate how peroxisome proliferator-activated receptor- γ coactivator-1 α is involved with mitochondrial abnormalities and insulin sensitivity. Decreasing the expression of frataxin in muscle cells decreased transcriptional expression of peroxisome proliferator-activated receptor- γ coactivator-1 α , which then affects the expression of the genes regulated by peroxisome proliferator-activated receptor- γ coactivator-1 α . Another finding was the increased expression of a transcription factor called sterol regulatory element-binding protein-1, which activates lipid synthesis.⁸ Most frataxin-deficient cells except for cardiomyocytes have downregulated expression of peroxisome proliferator-activated receptor- γ coactivator-1 α . Peroxisome proliferator-activated receptor- γ coactivator-1 α silenced by siRNA results in downregulation of frataxin. By stimulating expression of peroxisome proliferator-activated receptor- γ coactivator-1 α through the AMP kinase pathway, frataxin levels increased and restored mitochondria function, such as expression of antioxidant enzymes, which in turn reduces the number of reactive oxygen species in the cell.

Changes in iron metabolism affect the pathways of energy metabolism, lipid metabolism, mitochondrial biogenesis, and antioxidant response. Use of iron chelators such as deferiprone or deferoxamine mesylate reduces iron levels in the cytosol, downregulates the frataxin gene, and increases iron regulatory proteins. A type of loop is created; low levels of frataxin decrease cytosolic iron, and on the other side, low levels of cytosolic iron decrease frataxin. Low levels of iron activate the hypoxia-inducible factor pathway. In normal conditions, these transcription factors in the cytosol are degraded if enough oxygen is present in the cell. The enzymes that identify these transcription factors for degradation are iron-dependent. Depletion of cytosolic iron prevents hypoxia-inducible factor degradation, which stay activated. Hypoxia-inducible factor-1 α stimulates the expression of microRNA, which suppresses the expression of an iron-sulfur cluster assembly enzyme.

Hypoxia-inducible factor-2 α decreases when cytosolic iron is reduced because the increased level of iron regulatory proteins without iron-sulfur clusters binds to the 5' iron-responsive elements on the hypoxia-inducible factor-2 α . The targets of this protein are skeletal muscle fiber genes, which has decreased expression in Friedreich ataxia.

AMP kinase activates mitochondrial biogenesis through peroxisome proliferator-activated receptor- γ coactivator-1 α and is sensitive to energy levels in the cell. If this pathway is inhibited there is a deficiency in mitophagy, which is degradation of mitochondria. Abnormal proliferation of mitochondria with reduced membrane potential has been observed in some cells affected by frataxin deficiency. Pandolfo hypothesized that decreased mitophagy and mitochondrial proliferation may be involved in Friedreich ataxia. In preliminary data of a short time frame, the quantities of peroxisome proliferator-activated receptor- γ coactivator-1 α remain the same, but more studies need to be conducted to investigate whether peroxisome proliferator-activated receptor- γ coactivator-1 α has been modified.

When alterations occur in pathways involved with frataxin, multiple consequences occur. Continuation of the studies of pathways affected in Friedreich ataxia will lead to a better understanding of the disease mechanisms.

Epigenetic Controls

Joel Gottesfeld, PhD, The Scripps Research Institute, La Jolla, California

To understand how Friedreich ataxia may be a disease related to chromatin, Dr Gottesfeld provided information on chromatin biochemistry. Euchromatin, an area of active genes, is a form of chromatin enriched in histones that have lysine residues acetylated at particular amino acids. Inactive genes are in condensed chromatin known as heterochromatin that are depleted in acetylated histones and enriched with different methylation markers from euchromatin. Festenstein described that from methods developed by Herman and colleagues, the insertion of DNA with GAA repeats on a transgene results in gene silencing through the formation of heterochromatin.⁹ The formation of heterochromatin is dependent on the levels of a protein named HP1, which normally has a trimethylation of histone H3 at lysine 9. In 2005, Gottesfeld and colleagues studied the chromatin structure of the frataxin gene in lymphocytes from individuals affected with Friedreich ataxia and unaffected individuals. Pathogenic alleles in affected cells displayed characteristics of heterochromatin: hypoacetylation and high levels of trimethylation of histone H3 at lysine 9. The proposed model presented an active frataxin gene with high levels of acetylation, whereas the inactive frataxin gene has low levels of acetylation and low levels of the trimethylation of histone H3 at lysine 9. This trimethylation marker recruits HP1, which acts as a “molecular glue” to condense chromatin and render it transcriptionally inactive.

Gene activation is regulated by 2 classes of enzymes, histone acetyltransferases and histone deacetylases, which induce the active state or inactive state. Investigation of histone deacetylases to reactive the silent frataxin gene resulted in the class of molecules called *ortho*-aminobenzamides.¹⁰ Colleagues in Gottesfeld’s research team conducted a structure activity relationship study on *ortho*-aminobenzamides and determined similarities in pimelic diphenylamide (106), a histone deacetylase inhibitor that chelates the zinc ion in the active site of histone deacetylase enzymes, specifically histone deacetylase 3. When compound 106 was removed from culture medium of lymphoblasts, acetylation persists for several hours, and during this time course, levels of frataxin protein increased.¹¹ Compound 106 was injected into the KIKI mouse model, which is homozygous for GAA repeats in the first intron of the frataxin gene, and the brains were harvested several hours post-injection. The molecule crossed the blood-brain barrier and high levels of histone acetylation were recorded. Compound 106 increased the low level of frataxin in the KIKI mouse model to normal levels, similar to those found in wildtype mice as seen by restoration of frataxin messenger RNA expression.¹²

In 2010, Gottesfeld reported the derivation of Friedreich ataxia induced pluripotent stem cells. The cells were fully characterized through immunostaining, pluripotency messenger RNA expression, global gene expression analysis, teratoma formation, and normal karyotype retention. The Friedreich ataxia induced pluripotent stem cells showed signs of frataxin gene silencing through decreased levels of frataxin mRNA and frataxin protein. Chromatin analysis revealed the Friedreich ataxia induced pluripotent stem cells displayed characteristics of heterochromatin similar to the lymphoblasts treated with compound 106. The Friedreich ataxia induced pluripotent stem cells were differentiated into neurons. Fluorescence Activated Cell Sorting analysis for the neural stem cell marker nestin was positive. In dissociated cell culture after 2 to 3 weeks, these cells were stained for beta-2-microtubulin, an early neuronal marker, and Fluorescence Activated Cell Sorting analysis showed 88% purity.¹³

Although the Friedreich ataxia induced pluripotent stem cell-derived neurons are not large sensory neurons of the dorsal root ganglion, further unpublished studies by Gottesfeld and colleagues investigate whether these neurons recapitulate neuronal gene expression and have signatures of Friedreich ataxia. Through mRNA analysis by quantitative reverse transcription polymerase chain reaction, the trend seen from neural stem cells to immature neurons shows that pluripotency mRNAs are reduced upon neuronal differentiation and

neuronal mRNAs are increased. Levels of frataxin mRNA and frataxin protein are reduced in the Friedreich ataxia induced pluripotent stem cell-derived neurons compared with unaffected induced pluripotent stem cell-derived neurons. Heterochromatin-mediated gene silencing occurs in the Friedreich ataxia induced pluripotent stem cell-derived neurons, as concluded from the histone hypoacetylation around the GAA repeats and trimethylation of histone H3 at lysine 9.

A different histone deacetylase inhibitor, called histone deacetylase 3-selective inhibitor 109, increases frataxin gene expression and frataxin protein levels in these neurons, whereas histone deacetylase 1 and histone deacetylase 2 selective inhibitors that are known to not increase frataxin expression had no effect on the neurons. Through the experiments conducted with histone deacetylase 3-selective inhibitor 109, histone acetylation was induced and trimethylation of histone H3 at lysine 9 was reduced in Friedreich ataxia induced pluripotent stem cell-derived neurons. Gene activation occurs at the frataxin gene locus and affects other genes as well. The levels of frataxin mRNA and frataxin protein in the neurons from individuals with Friedreich ataxia were raised to levels similar to heterozygotes, which would be a therapeutically beneficial level of frataxin gene activation, since carriers are unaffected by Friedreich ataxia.

An expansion of the GAA repeats occurs when fibroblasts are dedifferentiated into induced pluripotent stem cells, but no expansion occurs when the induced pluripotent stem cells are differentiated into neural stem cells or neurons. Treatment of the Friedreich ataxia induced pluripotent stem cell-derived neurons resulted in increase of frataxin mRNA, but no further GAA repeat expansion. With regard to mitochondrial function, analysis of mitochondrial enzymes revealed no significant difference between Friedreich ataxia induced pluripotent stem cell-derived neurons and unaffected induced pluripotent stem cell-derived neurons. The mitochondrial membrane potential is reduced in Friedreich ataxia induced pluripotent stem cell-derived neurons, which is a measure of mitochondrial function. From fluorescence assay with a tetramethylrhodamine dye that measures mitochondrial membrane potential, a significant drop in value is seen in Friedreich ataxia neurons compared with unaffected neurons. After a 3-day treatment of histone deacetylase 3-selective inhibitor 109, the mitochondrial membrane potentials in Friedreich ataxia neurons are restored to levels seen in unaffected neurons.

Histone deacetylase 3-selective inhibitor 109 has 2 drawbacks: poor metabolic stability and poor penetration through the blood-brain barrier. The *ortho*-aminobenzamide portion of histone deacetylase 3-selective inhibitor 109 is essential for activity, but reacts with the carbonyl of the amide to form a benzimidazole, which is inert as a histone deacetylase inhibitor. This reaction occurs under acidic pH conditions such as those found in the stomach or in serum. The insertion of an unsaturated linkage adjacent to the amide prevents benzimidazole formation. The other caveat of 15% brain penetration can be solved by removing one of the amide linkages via substitution to remove that polar group.¹⁴ The challenge of including both changes to the single molecule was overcome by using the copper (I)-mediated cycloaddition reaction to produce a triazole to couple building blocks that had either an unsaturated double bond or ether. This version of the molecule was tested in neurons by Gottesfeld and colleagues. RepliGen Corporation (Waltham, Massachusetts) tested another version with a different heterocycle in the linker region in the KIKI mouse model. Both versions of the histone deacetylase 3-selective inhibitor 109 restore frataxin transcription in the Friedreich induced pluripotent stem cell-derived neurons and in the KIKI mouse model.

To study the effects of histone deacetylase inhibitors on humans, RepliGen licensed the agents that were studied by Gottesfeld and colleagues in 2007 and developed histone

deacetylase 3-selective inhibitor 109 (RG2833), which has improved pharmacology properties. After issues in toxicity with canines were resolved, a full clinical development plan was submitted at the end of 2011 to the United States Food and Drug Administration and was granted orphan drug designation. A pilot repeat dose toxicity study on a back-up compound called 963 in canines found no adverse reactions at a therapeutic dose. As of early 2012, the Italian Health Ministry and Ethics Committee has approved RG2833 for phase I trials in humans at San Luigi Hospital in Turin, Italy.

Friedreich ataxia is a chromatin disease where GAA repeats induce heterochromatin-mediated gene silencing through histone modifications. Novel histone deacetylase inhibitor prevents frataxin gene silencing by increasing histone acetylation on pathogenic frataxin alleles in both human Friedrich ataxia cells and in the mouse model. Friedrich ataxia induced pluripotent stem cells mimic frataxin gene silencing, and these cells can be differentiated into neurons in vitro. Friedrich ataxia neuronal cells respond to histone deacetylase inhibitors. RepliGen has improved those histone deacetylase inhibitors in terms of brain penetration and metabolic stability. These compounds react in Friedrich ataxia neurons and the mouse model.

Therapeutic Developments

Rob Wilson, MD, PhD, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania

Dr Wilson framed the presentation on therapeutic developments in Friedrich ataxia around 3 biochemical defects: decreased frataxin gene, mitochondrial iron accumulation, and oxidative stress.

Decreased expression of the frataxin gene results from a GAA repeat expansion typically occurring in the first intron.¹⁵ Evidence shows the expression is primarily mediated through chromatin condensation of the DNA around histones. In Friedrich ataxia, the exon sequences are intact, which is not true in many other genetic disorders. Methods to induce re-expression of the frataxin gene can be effective treatments. Histone deacetylase inhibitors can reverse gene silencing, and the treatment has moved onto clinical trials.¹⁶

Erythropoietin, a hormone secreted by the kidneys to signal erythropoiesis, can potentially increase frataxin expression. Because of the increased levels of iron required during erythropoiesis, upregulation of iron metabolism-related genes, which include frataxin, is required. Edison Pharmaceuticals (Mountain View, California) is conducting preclinical trials on erythropoietin and analogs to pinpoint the property that increases frataxin expression without causing erythrocytosis. Delatycki and colleagues found that resveratrol, a compound studied for antioxidant effects, increased frataxin expression in cells in culture and in mouse models with GAA repeat expansions. The study is in clinical trial as an open-label trial, with 30 patients receiving 1.0 gram of resveratrol per day for 12 weeks, then 5.0 grams per day for another 12 weeks. Payne and colleagues have developed a protein-based therapy by attaching the TAT peptide from HIV to frataxin, which enables frataxin to cross membranes. The TAT-frataxin crosses into the mitochondrial matrix and increases the level of frataxin in cells that are in culture. The Hélène Puccio conditional knockout mouse models were injected with the TAT-frataxin, and life expectancy was increased by 50% or more and had corrections in the cardiomyopathy. High-throughput screening searched for activators of the frataxin gene. Napieralla has developed an assay with access to a large library of compounds and is collaborating with medicinal chemists.

Another therapeutic approach focuses on mitochondrial iron accumulation. Frataxin participates in a complex that initiates iron-sulfur cluster assembly and serves as a positive regulator of the complex. Pandolfo had discussed in his presentation that the decrease in

level of frataxin causes iron-sulfur cluster defects, which are associated with mitochondrial iron accumulation through biochemical feedback loops. Rouault has hypothesized that mitochondria require intact iron-sulfur clusters to sense iron in the mitochondria in order to signal the cell about the level of iron. Mitochondrial iron accumulation is secondary to the iron-sulfur cluster defect. The formations of planar structures or cubes are held together by cysteine residues and serve as prosthetic groups in enzymes and cellular reactions. Rotig and colleagues found that the amount of aconitase, which contains iron-sulfur clusters, is dramatically reduced in heart muscle biopsies of 2 patients who have Friedreich ataxia. Aconitase intraconverts citrate and isocitrate in the citric acid cycle and has an iron-sulfur cluster at the active site, which is susceptible to disruption.¹⁷ The approach of using lipid-soluble chelators allows crossing through 3 membranes into the mitochondrial matrix and the ability to bind heavy metals. Deferiprone, which is being studied by ApoPharma in Toronto, is a small lipid-soluble molecule that chelates iron. The compound is in phase 2 trial with a placebo, low dose, medium dose, and high dose. The high-dose portion of the trial was ended due to toxicity. The overall results of the trial are mixed. Deferiprone can pose health risks, but has been approved for thalassemia in the United States. Patients on deferiprone require weekly complete blood count tests to monitor neutrophil levels, and 1% of these patients get agranulocytosis. Pandolfo showed that high-dose deferiprone depletes cytosolic iron and worsens frataxin expression. Other chelators are in preclinical development. Chelators for the treatment of Friedreich ataxia need to be lipid-soluble and sufficiently weak as chelators to not compete with the iron needed by different enzymes.

The third biochemical defect that guides the approach of therapeutic development is oxidative stress. Defects in iron-sulfur cluster assembly lead to increased superoxide formation. Mitochondria iron accumulation leads to what is known as the Fenton reaction in the mitochondrial matrix. In the electron transport chain, electrons are transferred from NADH by electron carriers and complexes, which require iron-sulfur clusters. Under normal respiration, 2% to 4% of the electrons do not complete the electron transport chain. When complex I is at a reduced state, oxygen binds with the electrons and becomes superoxide so oxidative stress begins as reductive stress. With defects in the iron-sulfur cluster assembly, complex I is in a highly reduced state, which causes increased production of superoxides. The ability to convert between the ferrous (2+) state and ferric (3+) state allows iron to transfer electrons along the electron transport chain. Any excess accumulation of iron in the mitochondrial matrix results in ferrous iron donating electrons to hydrogen peroxide to form hydroxyl radicals, which is highly reactive and damaging to cells. In particular, a free radical extracts a hydrogen atom from a lipid, which reacts with oxygen to form a lipid peroxyl radical. The lipid peroxyl radical extracts hydrogen from another lipid in this chain reaction. Polyunsaturated fatty acids are susceptible to hydrogen extraction due to the double bonds and are prevalently located in the inner mitochondrial membrane.

Antioxidants are being used as treatment for oxidative stress. In the United States, idebenone has reached phase 3 trial as a double-blind, multi-dose, placebo-controlled trial, and the results did not reach the primary endpoint, which is noted as an improvement in the Friedreich ataxia rating scale. Although earlier studies have found shrinking of the heart, this does not necessarily translate to improve function. Idebenone, a coenzyme Q10 analogue, has a para-benzoquinone group with a short tail. Edison Pharmaceuticals (Mountain View, California) is developing EPI-A0001 and EPI-743. EPI-A0001 has a para-benzoquinone head group and recently completed an early phase 2 trial, which was a small study of placebo, low dose, and high dose. The results were statistically significant, but a longer investigation is needed. Currently in preclinical trials, EPI-743 has been given to patients with late-stage Friedreich ataxia, but the measurements are inconclusive. Pioglitazone, a drug for diabetes, is pleiotropic. The drug activates genes that are involved in mitochondrial biogenesis and antioxidant defenses and is in clinical trials in France for Friedreich ataxia.

EGb761 is a free radical scavenger from ginkgo biloba and is in clinical trials. Hecht is designing antioxidants specific for the defects in the electron transport chain of Friedreich ataxia and has found to be effective in cell culture. Ox1, an indole compound, acts as an antioxidant and may be able to weakly chelate iron. Retrotope has developed a polyunsaturated fatty acid with hydrogen molecules that are susceptible to radical reactions with deuterium. Deuterated polyunsaturated fatty acid stops the chain reaction of reactive oxygen species and has been developed into a dietary supplement.

The “hypothetical vicious cycle” in Friedreich ataxia refers to decreased iron-sulfur clusters leading to accumulation of iron, causing increased reactive oxygen species, which may further disrupt iron-sulfur cluster synthesis. The therapeutic treatments discussed could affect the cycle at different points. Combination therapy is a possible approach to reduce individual treatment doses and minimize side effects with a synergism of the positive effects. Wilson provided an image from the Friedreich’s Ataxia Research Alliance website that shows the stages of clinical trials with updated details and updates.¹⁸

Clinical Trial Results

Susan Perlman, MD, University of California, Los Angeles, Los Angeles, California

Dr Perlman addressed the issues behind the reason for the lack of proven disease-modifying therapies for Friedreich ataxia. Although agents are being developed and tested, the lack of statistically significant results is due to various reasons: ineffective therapy targets, modifications of the drugs needed, lack of sensitive measurements, and the need for long-term studies.

The number of Friedreich ataxia clinical trials has been exponentially rising since 1997. The tools that have made these advancements possible are the understanding of the disease mechanisms, identification of candidate drugs, documentation of natural history baseline, validated rating scales, development of biomarkers, and quality of life measures. A common data elements project conducted by the National Institutes of Health will establish common guidelines to contributors in the field of Friedreich ataxia. Recruitment has been difficult in the studies of this disease, but there have been improvements through the establishment of clinical research centers, patient registry, and operational databases. Future directional uses of telemedicine, electronic monitoring, and international collaboration will provide advancements in the field.

For candidate drugs to be used in human trials, the advantages and disadvantages must be assessed. The mechanisms of these agents must be supported by rational through basic sciences. Evidence should be shown in animal or pilot human studies to prove the efficacy of the agent’s effects, with benefits outweighing risks. The availability of agents is influenced by the cost, safety profile, and participation of the pharmaceutical companies. To conduct the clinical trials, funding must be provided. Dr Perlman reviewed the types of clinical trials. Friedreich ataxia studies will have difficulty reaching phase 3 or phase 4 because these trials require a large number of study subjects. At the time of the Neurobiology of Diseases in Children symposium, current studies in Friedreich ataxia include 13 completed studies (12 drug trials and 1 quality of life study). The pilot study with varenicline for the symptomatic treatment of Friedreich ataxia was the only study terminated. Six clinical trials, which are in phases 1, 2, or 3, are actively recruiting. Three out of these 6 clinical trials are open-label trials.¹⁹ Limitations in patient numbers, amount of money, and amount of time leads to open-label trials, which are informative, but difficult in terms of showing a disease-modifying effect. The Friedreich’s Ataxia Research Alliance website includes information about studies and resources on patient registry, newborn screening project, and autopsy and tissue donation program.²⁰

In 2006, the safety study of idebenone to treat Friedreich ataxia was published as a phase 1A and phase 1B study. Current studies on idebenone have reached phase 2 and phase 3. Other recent studies include the safety and tolerability of deferiprone and safety and efficacy of A0001. In 2011, a phase 2 trial was completed for studying carbamylated erythropoietin as a means to increase frataxin protein without increasing blood count. Development of new drugs can take up to 15 years and cost over \$500 million. Collaborative efforts are required for funding the studies, especially those in phase 2 and phase 3. The “vicious cycle in Friedreich ataxia” of frataxin protein deficiency through decreases in iron-sulfur clusters, increases of iron levels, and increases of free radicals are targets for the development of treatment therapies. Advancements in studying Friedreich ataxia can lead to insight in other neurodegenerative disorders.

Dr Perlman provided an overview of various studies. A study in Great Britain with coenzyme Q10 and vitamin E has shown improvement in cardiac and skeletal muscle biogenetics, led to changes in the International Cooperative Ataxia Rating Scale, and led to changes in disease progression.²¹ A recent publication emphasized the role of decreased serum coenzyme Q10 in subjects as a predictor of positive clinical response to coenzyme Q10 and vitamin E therapy.²² A different antioxidant, idebenone, is in early pilot studies and there is suggested cardiac, but not neurologic, benefit.²³ The phase 2 study showed benefit in one of the secondary outcome measures, which were neurologic measures.²⁴ The phase 3 study did not reproduce the phase 2 data, which raises the question as to whether this is the correct target or if other lipid-soluble antioxidants should be studied instead.²⁵ Some symptomatic benefits have been reported from antioxidant therapy; some patients taking coenzyme Q10 or idebenone report feeling less fatigued, but the long-term disease-modifying benefit remains unclear.

Despite these results, a study organized by Lynch found that many patients with Friedreich ataxia are taking nonprescription antioxidants, which is a confounder to formal trials.²⁶ Other confounders are related to ethics. A patient may be denied a lipid-soluble antioxidant since he or she is on a placebo for the span of the study. Other studies may exclude the use of these agents because the potential of interference with results. Restoration of iron hemostasis with deferiprone at a high dose was stopped early because of neurologic side effects.²⁷ The study is pending for phase 3, whereas deferiprone was approved in 2010 as a treatment for thalassemia, so this agent is available to a portion of the population.

Aside from antioxidant agents, other agents are being studied to specifically increase frataxin levels. The research studies require a public-private partnership of funding. Exercise studies are non-drug trials being conducted for Friedreich ataxia. A publication in 2010 reviewed exercise capacity and idebenone intervention in children and adolescents with Friedreich ataxia. The study found no evidence that idebenone improved exercise capacity relative to placebo.²⁸ Exercise has been advised for patients with neuromuscular diseases. In 1986 at the Sixth International Congress of Neuromuscular Disease, Perlman described an exercise study where out of the 20 patients participating, all 10 patients with Friedreich ataxia were more likely to improve or stabilize after a 2-year home exercise program. A 2009 exercise training study conducted in Germany consisted of a 4-week intensive coordination training for 16 patients with progressive ataxia due to cerebellar degeneration or degeneration of afferent pathways. The study found significant improvements in motor performance and reduction of ataxia symptoms. Patients with predominant cerebellar ataxia showed greater improvement than patients with afferent ataxia in terms of movement speed, lateral sway, and coordination of limbs. With continuous training, both groups showed stabilizing improvements.²⁹

Dr Perlman presented a map showing the distribution of families with Friedreich ataxia patients in the United States. The map also includes the Friedreich ataxia research centers, and some patients must travel far to get to a center. A trial accessible to all patients with Friedreich ataxia should be designed. Registries should include more patients with Friedreich ataxia. Clinical trials should be designed to gain participation from these patients. Being knowledgeable about clinical trial design can help more efficiently develop new treatment agents. Any challenges that could arise must be addressed proactively. Out of the number of existing trials, participation in one trial may disqualify the patient from another trial. Clinicians have the obligation to connect patients with the proper research that would be most beneficial to the patients.

Questions and Answers

Dr Gomez: Is there a trend against antioxidants that could be a possible mainline therapy? Through clinical reasoning, ataxia of typical Friedreich type is so similar to ataxia of vitamin E deficiency.

Dr Gottesfeld: It is too soon to tell. Although idebenone has reasonable drug-like properties compared with coenzyme Q10, it's not particularly an effective drug in terms of its absorption and stability in the bloodstream. A0001 solves both of those problems and had a positive result in the phase 2 trial, although very preliminary.

Audience member: Is there any evidence that heterozygotes are at increased risk for any neurologic symptoms or cardiac symptoms during their life?

Dr Pandolfo: There seems to not be any such evidence. The metabolic studies, at least the one that we carried out, indicated some metabolic parameters may be intermediate in heterozygous carriers between controls and patients. For instance, the disposition index is an indicator of better cell function in respect of insulin sensitivity. It's clearly decreased in the group of patients with Friedreich ataxia compared to controls, and the mean of carriers is in between, but the clinical relevance is unclear.

Audience member: What is the role of Friedreich ataxia investigators? Is there an effort to prioritize or is it left to those who will take the initiative and have the funds to decide the primary targets?

Dr Lynch: This has been a collaborative field. Ms Farmer and I meet regularly on average once a month with a pharmaceutical company or another group. We can inform them about the disorder so they can prioritize or design their trials. We have to recruit a lot of patients. We have learned how to be more efficient, sometimes by brute force, and all of us are actively involved in discussions with companies.

Dr Payne: Do rigorous exercise programs affect the rate of scoliosis development and do they have a positive benefit on cardiac physiology in Friedreich ataxia?

Dr Perlman: With the use of exercise, a couple of well-done studies show that exercise seems to improve coordination and balance in certain people with ataxia. For scoliosis, exercise does not slow the rate of development of scoliosis, but it does reduce pain and get rid of some mechanical disadvantages of a spine with scoliosis. Some children won't progress to the point where they will need surgery, so exercise does have a role. It is hard to recruit for clinical trials for drugs, and it may be even harder to recruit for exercise studies as well.

Dr Gomez: Payne, what is the effect on the heart with exercise?

Dr Payne: This is a question still being studied. I'm curious as to whether or not exercise slows progression of cardiomyopathy in the patient with Friedreich ataxia and whether it slows progression of the scoliosis.

Dr Payne: Are we missing timing of treatment by starting some drugs at the time that patients are symptomatic rather than identifying them early?

Dr Perlman: Early identification and treating patients with agents may not work if they already have symptoms for 5 years. Some families that have one child with Friedreich ataxia decide to test everyone else despite the concern about testing presymptomatic children. The consortium is not quite at a point to think about preclinical disease-modifying therapy because we haven't really decided whether any of the agents are going to be disease-modifying, but it is a future goal.

Dr. Gottesfeld: If drug treatment is started too late, then you won't see an effect, but without an effect, you can't start on a presymptomatic patient. Patients do show progress even with treatment at a late stage so there is ongoing pathology. A new approach could have earlier-stage patients treated with weaker agents to show the effects. If some of the agents show an effect on moderately affected individuals, then we would have the clearance to pursue treatment for presymptomatic patients.

Dr Landis: In reviewing some of the failed clinical trials, for example, minocycline in amyotrophic lateral sclerosis and failed translational UO1 projects, we recognize that a critical factor is whether preclinical studies on animals were done with sufficient rigor. There have been a number of articles recently in the scientific journals about this issue. We are beginning to be very attentive to this, so that rigorous preclinical studies should be done for the new therapeutics and can prevent prioritization from occurring among studies.

Dr Pandolfo: Although that is true, we do not have the ideal animal model for Friedreich ataxia. The animal models reproduce some parts of the disease to varying degrees, and they can be used to test certain treatments. The right model needs to be used to test a certain treatment. For example, the condition of knockout animals may not show sign of oxidative stress so antioxidants cannot be tested on these animals. Treatments to regulate frataxin in the presence of the expansion cannot be done since it is complicated, but the ability to test these treatments is evolving. Better animal models and studies conducted correctly with these animal models is still a major need in the field studying Friedreich ataxia.

Audience member: How will histone deacetylase inhibitors be administered to patients? Is there documented improvement in the function of the cerebellum in those mice, and how would it be measured?

Dr Gottesfeld: To answer the first question, RepliGen Corporation has developed an oral dosing formulation as a pill and is in full preclinical studies in canines. Brain penetration does occur after oral dosing.

Dr Pandolfo: These drugs have been tested on 2 animal models. One is the knock-in model. They have a very discrete phenotype, which is biochemical. These drugs correct the biochemical phenotype. For instance in gene expression profiles, frataxin deficiency is mostly corrected in the brain, including the cerebellum. The other animal model is a knock-out mouse model that develops some mild but identifiable abnormalities, but within 6 months on these drugs, there was improvement in a number of parameters.

Audience member: Dr Perlman, do you have any specific recommendations on how to shorten the trials? Is there a survival advantage for Friedreich ataxia heterozygotes as there

is with diseases such as cystic fibrosis? Is there information about why Friedreich ataxia is prevalent?

Dr Perlman: Carriers of one defective Friedreich ataxia gene show some changes in expression of other genes as seen on microarrays. Some of those genes are anti-aging genes. One of my patients, after reading this literature, suggested the possibility of families with known carriers may consist of family members with fewer diseases of aging or longer life expectancies. An epidemiologic study would take thousands of patients to show anything significant, and recruitment would be difficult. To make clinical trials more efficient and cost-effective, distinguishing between effective drugs from ineffective drugs could be done if biomarkers are correlated with the disease pathophysiology. With nerve stress and nerve damage, a minimum of 6 months is required to observe any change to the brain, which can limit the duration of clinical trials.

Dr Wilson: The Friedreich's Ataxia Research Alliance has funded studies to develop improved biomarkers, in order to shorten phase 2 trials. Through Dr Lynch's efforts with the Friedreich's Ataxia Research Alliance, development is underway to improve performance measures and ataxia scales, which are clinical endpoints for phase 3 trial.

Dr Crawford: What is the value of frataxin as a biomarker for a surrogate endpoint to be able to get early readouts on your gene-modifying therapies?

Dr Pandolfo: Frataxin is a biomarker. If the therapy is able to normalize frataxin levels in patients, then we still don't know in clinical terms if the disease will be stabilized or how long it will take. The frataxin level is a valuable biomarker and will be used in a number of trials. If a drug increases frataxin level such as histone deacetylase inhibitors or erythropoietin, then changes in frataxin will be the first biomarker used. For other drugs that act on downstream mechanisms, we can check that they do not further decrease levels of frataxin.

NEW INVESTIGATIONS IN FRIEDREICH ATAXIA

Co-director and Moderator: Susan Perlman, MD, University of California Los Angeles, Los Angeles, California

Clinical Measures

David Lynch, MD, PhD, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

Development of a drug for Friedreich ataxia requires a target. The level of frataxin can be affected through transcription or by slowing frataxin breakdown. A drug can target a downstream function such as iron accumulation, reactive oxygen species production, or mitochondrial function. Organ function can be another viable target.

Every clinical research study needs to be measureable. It is important to note the location of the measurements and if the measurements are relevant to the biological or clinical question. Western blots and mRNA levels can measure the level of frataxin. Iron accumulation is measured in the dentate nucleus of the brain using magnetic resonance imaging T2* (MRI-T2*), but the iron is speculated to be extracellular. Data of reactive oxygen species in blood or urine is inconclusive. Measurements of neurological function and cardiac function need to be relevant to the question being studied. Understanding measures is important for ensuring that biological functions are measured in clinical trials. Every person, particularly a patient with Friedreich ataxia, has variability from day-to-day; thus, measurements need to be reproducible.

Dr Lynch described the measures in clinical trials. Phase 1 trials are safety trials. Phase 2A trials are proving the principle being tested, such as histone deacetylase inhibitors increasing the level of frataxin in tissue. Phase 2B trials prove the concept; for example, analysis of insulin-resistant markers might change over the course of therapy, which alone may not be clinically relevant. Phase 3 trials is the registration phase, where measurements are reproducible, accurate, and clinically relevant to patients. When the first idebenone trial was proposed in 1999, no prior measures existed to validate the trial. A natural history study was required, which followed patients over time to track whether those measurements correctly exhibited disease progression. The Muscular Dystrophy Association and the Friedreich's Ataxia Research Alliance initially funded the startup of a clinical network. The participating centers follow a cohort of about 600 individuals and have been able to propose and validate different types of measures. The long-term natural history study consists of some patients participating in their eighth year.

When an individual enrolls in the study, initial measures are validated, but it is important to conduct a long-term follow-up and perform the next measures. Information about the patient is acquired such as demographics, genetics, and medical history. The clinical exam-based measures include the Friedreich Ataxia Rating Scale plus subscores, functional staging, and Friedreich Ataxia Rating Scale Activities of Daily Living. Performance measures are timed or quantifiable tests that measure a specific domain of neurologic function. For example, the 9-Hole Peg Test requires patients to place pegs in a pegboard under timed conditions to measure upper limb function. The Timed 25-Foot Walk measures the ability of walking. The PATA test measures speech, whereas the low-contrast letter acuity tests visual function. Information for various biomarkers is also collected.

Clinical exam-based measures are conducted in the clinic setting and may seem less relevant to daily life than other types of measures. Performance measure simulate skills found in daily activities and are under timed conditions. For drug-approval registration to the U.S. Food and Drug Administration, clinical exam-based measures and performance measures are allowed, but biomarkers are rarely accepted as registration endpoints. Even when biomarkers are from biologically relevant tissue, vast amounts of correlative data are required. The goal of the natural history study is to obtain 10-year longitudinal data that match the cross-sectional data. Three types of measurements are being conducted worldwide, which are quantified versions the neurological exam. Subramony developed the Friedreich Ataxia Rating Scale. The International Cooperative Ataxia Rating Scale includes eye movement measurements. Scale for the Assessment and Rating of Ataxia is frequently used in Europe.

The advantages of these 3 types of measurements are validity and familiarity. The rating scales are valid through testing large populations across the world, patients were tracked over time, and changes were proportional. Regulatory agencies are familiar with these rating scales. The disadvantages of these rating scales are reproducibility and sensitivity. The factors of interrater reproducibility and variability with patients from day-to-day affect the measurements. With the scales, patients may change on an average of 3 points per year, so in turn, longer trials should be piloted to reach a level that pharmaceutical companies denote as an acceptable level of change divided by changes in standard deviation. In that case, a trial would include 50 to 150 patients per arm and last for 2 years. The scales, although not sensitive, are acceptable for registration.

In contrast, performance measures are different from rating scales, since performance measures focus on one domain of neurologic function. A statistical sum of the performance measures results in a functional composite, which was originally developed for multiple sclerosis studies. Each number is specific so it is highly sensitive in many situations, and the

data are easy to collect. There are no “major floor or ceiling effects” when each measurement is sensitive to one component of the disease. However, most regulatory agencies are not familiar with the types of performance measures, and performance measures are less helpful when used individually. Evidence has shown that performance measures reflect real-world function. Physical therapists consider pegboards as the best objective test for arm function. Delatycki has shown that Timed 25-Foot Walk best reflects home-based measures of ambulation. Specific loss of visual abilities correlates with visual quality of life.

For drug approval through the Food and Drug Administration, a clinical trial can be designed around a type of measurement that was discussed or a different approach of focusing on a subdomain. For example, in the area of neurologic stabilization or improvement, the goal for a trial could be improving vision in patients with Friedreich ataxia and the target is measured by the low-contrast letter acuity test. Improvements of cardiac health, which include decrease in mortality, decreased progression of congestive heart failure, and decreases in arrhythmias, could be possible goals if predictability can be measured. In principle, exercise tolerance could be an approvable outcome if patients with varying progressions of Friedreich ataxia are capable of completing a specifically designed single task. Theoretically, improvement in diabetes is a possible outcome, but only 10% to 20% of patients with Friedreich ataxia have diabetes that is modifiable. Biological tests can measure nerve cell loss, which is mainly for phase 2 trials; for example, ocular coherence tomography looks at the retina. Patient-driven outcomes are important neurological measures in Friedreich ataxia. Subjective questionnaires gather information from patients but are age-dependent. The Friedreich Ataxia Impact Scale compiles questions related to the disease through a psychometric design, but is long and not validated against the Friedreich Ataxia Rating Scale or performance measures.

In the long-term natural history study, the focus is currently on whether mitochondrial enzymes change, but the data are inconsistent so far. Reactive oxygen species production is not easily detected. RNA profiling can establish new biochemical markers. Muscle spectroscopy shows abnormal adenosine triphosphate production in patients with Friedreich ataxia, but a protocol needs to be designed that is suitable for a phase 3 trial. Validation of measures means that phase 3 trials are implemented, but there is a lack of biomarker identification in clinical trials. The type of measure used is based on the experiment, the subgroup of patients, the biological agent, and the target age.

Insulin Resistance

Steve Willi, MD, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania

Diabetes mellitus is a lifelong disease marked by high levels of sugar in the blood, which can be caused by the lack of insulin, insulin resistance, or both. The criteria for the diagnosis of diabetes are random plasma glucose greater than 200 mg/dL, fasting glucose greater than 126 mg/dL, hemoglobin A1c greater than 6.5%, 2-hour glucose greater than 200 mg/dL during an oral glucose tolerance test, or symptoms of diabetes, which include polyuria, polydipsia, and unexplained weight loss.³⁰ The major classification of diabetes mellitus is type 1 and type 2, where type 1 is insulin-dependent and type 2 is non-insulin-dependent. Other classifications of diabetes fall under the categories of gestational diabetes, maturity onset diabetes of youth, and diabetes secondary to other conditions, due to genetic syndromes, and due to pancreatic disease.

The pathogenesis of type 1 diabetes is a chronic autoimmune inflammation of the islet cells. The disease has a genetic predisposition with possible unknown environmental triggers. Cellular infiltration of lymphocytes and macrophages occurs with autoantibodies to islet cell

antigens. The onset of other autoimmune disease typically occurs with type 1 diabetes. Although the α -cells and δ -cells are unaffected, β -cells are destroyed over time. The β -cell function gradually declines to approximately 20 percent before the onset of type 1 diabetes. In contrast, the pathogenesis of type 2 diabetes is more complex than type 1 diabetes. A number of metabolic processes are affected, which can lead to hyperglycemia. Insulin production is decreased in the pancreas, which correlates with the decrease of glucose uptake in muscle and liver. The progression of type 2 diabetes begins with insulin resistance, where there is suboptimal insulin-stimulated glucose uptake by tissue. β -cell dysfunction develops and is measured by qualitative and quantitative reduction in insulin secretion. The disease progresses to an impaired glucose tolerance or pre-diabetic state where compensatory hyperinsulinemia with normoglycemia develops into hyperglycemia. Type 2 diabetes is characterized by impaired insulin action combined with impaired insulin secretion with subsequent hyperglycemia. In comparison, type 1 diabetes is an absolute insulin deficiency whereas type 2 diabetes is insulin resistance with relative insulin deficiency. The development of type 1 diabetes occurs quickly and typically in young people. Type 2 diabetes onset is associated with increasing age and obesity.

The pathogenesis of Friedreich ataxia results in mitochondrial disease due to the variable number of GAA repeats, which affects the function of various tissues. The tissues that are most relevant to diabetes would be the pancreas as well as the skeletal muscles. The development of diabetes in Friedreich ataxia shows both insulin resistance and defects in insulin secretion by pancreatic β -cells. Impairment of either of these pathways attributes to mitochondrial dysfunction. Previous studies have suggested that both insulin resistance and β -cell dysfunction are responsible for the blood sugar abnormalities in Friedreich ataxia.³¹ The study of diabetes development in Friedreich ataxia started in the 1960s, with reports of diabetic ketoacidosis presenting in Friedreich ataxia. In large case series, an estimated 10% to 40% of patients with Friedreich ataxia develop glucose intolerance and diabetes. Several studies have examined the pathophysiology of diabetes. A study back in 1981 demonstrated a compensatory increase in insulin production as a response to insulin resistance. In another study, insulin resistance was documented with an abnormal drop in the level of glucose measured by an insulin tolerance test, although this was replaced by other techniques.

Several tests are conducted to evaluate the risk of diabetes developing in individuals. Many population-based studies use the fasting glucose and insulin value. The homeostatic model assessment model estimates insulin production and insulin function. A more updated model, called the quantitative insulin sensitivity check index model, is appropriate for population-based studies. In the clinic, oral glucose tolerance testing shows β -cell function and insulin resistance from a dose of oral glucose and measures blood sugar changes over time. The normal response is a peak of plasma glucose of less than 140 mg/dL and a return to near baseline by 120 minutes, whereas in diabetes, the elevated blood sugar does not fall back to baseline. The rise of plasma glucose for impaired glucose tolerance would peak between 140 to 200 mg/dL, and plasma glucose levels returns close to normal.

The intravenous glucose tolerance test is used in clinical research to calculate the insulin sensitivity index and acute insulin response to glucose, and the disposition index is calculated from these 2 values. Measurements of glucose and insulin are tracked over time. At time = 0, a glucose injection results in a rapid rise of glucose level to over 200 mg/dL. The response of insulin that is produced by the patient is referred to as the first phase of insulin release and is the response to the rapid rise of glucose. With the decline in glucose, an injection of insulin is augmented at approximately 20 minutes. By using the rate of decline in glucose levels in relation to the ambient insulin levels, calculations derive the minimal model, which is used to determine the insulin sensitivity index and β -cell response. The data are used in clinical research to assess the ability of a person to produce insulin in

the acute insulin response to glucose, which addresses the adequacy of insulin secretion. The insulin sensitivity index quantifies the capacity of insulin to promote glucose disposal. The disposition index is the product of the acute insulin response and the insulin sensitivity index. The acute insulin response and the insulin sensitivity index assume a hyperbolic relationship in humans such that insulin sensitivity decreases and the secretion by the pancreas increases. Patients who are at higher risks of diabetes will have a lower disposition index, which essentially represents the ability of the β -cells to compensate for insulin resistance. Studies have shown a heritability of greater than 50 percent in several populations and the disposition index can be a predictor of type 2 diabetes risk and onset.

As part of a clinical trial to evaluate the effect of an investigational agent in Friedreich ataxia, the glucose metabolism was assessed in 31 nondiabetic patients with Friedreich ataxia. The relation between various clinical and biochemical markers of the disease and insulin dynamics were examined. In graphing insulin sensitivity against acute insulin response to glucose, most patients with Friedreich ataxia had decreased disposition indices falling in the 23rd percentile of the entire population, which is consistent with increased risk of diabetes. An association was found between younger age of onset and decreasing disposition index, which validates that severity of disease is reflected in the disposition index. When looking at minimum GAA repeats and disposition index, an inverse relationship was found such that the disposition index was higher in individuals with lower number of GAA repeats, a reflection of disease severity. The preliminary results of the study suggest the disposition index is significantly decreased in the non-diabetic group of patients with Friedreich ataxia.

In patients with Friedreich ataxia who were compared with a control group matched for age and body mass index, insulin sensitivity index was lower. A commensurate increase in insulin production resulted in disposition indices that were not quite significantly different than the disposition indices in the control group. Patients with Friedreich ataxia had a degree of insulin resistance that was fairly significant, and the compensatory rise in β -cell function was a good response to the challenges presented. In reference to the medication, the measures did not demonstrate a tangible change in the predisposition to the development of diabetes as measured by the disposition index.

Cardiac Approaches

Subha Raman, MD, MSEE, Ohio State University, Columbus, Ohio

Cardiac disease in Friedreich ataxia is nearly universal by histopathology. The clinical severity varies; patients may experience a volume overload during surgery. A comprehensive natural history has only recently been published, in 2011. A retrospective case-control analysis of patients with Friedreich ataxia revealed median age of death was 30 years old (59% of the deaths were due to definite cardiac causes and 3% due to probable cardiac causes, so cardiac-related deaths are a major source of mortality).³²

The electrocardiogram in patients with Friedreich ataxia is usually abnormal so it does not provide specific information. The echocardiogram is not as predictive either. Cardiovascular magnetic resonance imaging detects phenotypic trait, such as hypertrophy. For example, changes can be detected efficiently through magnetic resonance imaging if the intervention is targeting left ventricular hypertrophy. Left ventricular hypertrophy has normative values, whereas concentric remodeling due to thick muscle walls has filling volumes that are relatively small.³³ Concentric remodeling and left ventricular hypertrophy both show similar cumulative hazard for adverse cardiac events.³⁴

New therapeutic approaches require identifications of new targets. Cardiovascular magnetic resonance imaging can detect microvascular disease in the heart through first-pass perfusion imaging. This procedure acquires serial cross-sections through the heart during administration of evasive dilators to test the microvascular reserve. These images are compared with images taken under resting conditions. Qualitatively, the subendocardium with microvascular disease has absent perfusion compared with the subendocardium at resting conditions. The data can be quantified to a graph of signal intensity versus time, which is called the myocardial perfusion reserve index. Cardiovascular magnetic resonance imaging has the ability to characterize myocardial fibrosis and tissue fibrosis through the fact that fibrotic myocytes and normal myocytes have measurable differences in T1 characteristics. Fibrosis is substrate for diastolic dysfunction, which is true for a variety of cardiovascular diseases. This information is applicable to Friedreich ataxia if the primary mode of cardiac dysfunction is due to impaired filling or diastolic dysfunction. It is important to recognize that fibrosis is also a substrate for arrhythmia.³⁵

Dr Raman and colleagues conducted a prospective study at Ohio State University with a cohort of patients with Friedreich ataxia ranging from 18 to 57 years old with no antecedent cardiac disease. The minimum GAA repeat lengths ranged from 41 to 1500. Most of the patients had nonspecific electrocardiographic abnormality. None of the patients had diabetes, but they had metabolic abnormalities. All except 4 of the patients had at least one metabolic syndrome factor, and 8 had 3 or more. A strong correlation exists between relative wall thickness in concentric remodeling and the minimum GAA repeat length. The impaired myocardial perfusion reserve index decreases with increasing number of metabolic syndrome factors. Myocardial fibrosis was present in more than half of the patients with Friedreich ataxia that was not present in the control group.³⁶ Raman presented 2 cardiovascular magnetic resonance studies of an 18-year-old patient with concentric remodeling and a 37-year-old patient with advanced cardiomyopathy. She showed functional images and late gadolinium-enhanced images. In the concentric remodeling, the muscle was thick relative to the size of the ventricle, but no significant fibrosis. Images of advanced cardiomyopathy showed poor contractile function of the heart with white regions in the muscle as evidence of fibrosis. The most concerning part was the left ventricular thrombus that developed in the akinetic scarred region of the left ventricle. Relative negative findings were poorly correlated with overall left ventricular mass, which is the marker for left ventricular hypertrophy, and GAA repeat length. Cardiovascular disease occurs independently of the severity of neurological disease.

To correlate myocardial disease and Friedreich ataxia, metabolic pathways are affected. With a frataxin mutation, β -cell apoptosis and skeletal muscle lipogenesis lead to metabolic syndrome features. Changes in metabolism decrease capillary density, which contributes to impaired myocardial perfusion reserve. In a different pathway, a frataxin mutation leads to mitochondrial iron accumulation. Iron accumulation results in mitochondrial dysfunction, which decreases flow-mediated vasodilation and results in impaired myocardial perfusion reserve. With chronic subendocardial ischemia, abnormal remodeling occurs. In review, decreased levels of frataxin lead to metabolic abnormalities or microvascular disease, which causes structural and functional changes in the heart and may result in arrhythmias, heart failure, and death.

Although therapeutic designs specific to Friedreich ataxia and cardiovascular diseases are yet to be developed, therapeutic options are available to treat specific conditions. Available therapeutic options for microvascular disease are calcium channel blockers, nitrates, and beta-blockers. Dr Payne had shown that a small ventricle with an impaired stroke volume might not respond well with beta-blockers because cardiac output is being maintained at an elevated heart rate. Therapeutic options for metabolic abnormalities are statins, fibrates, and

treatments for glucose intolerance or diabetes mellitus. Statins could be used, although low-density lipoprotein levels were not significantly elevated in the studied cohort. Fibrates can target high triglycerides and low high-density lipoproteins. Agents such as metformin can treat glucose intolerance in patients. Agents for fibrosis or cardiac remodeling include angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and aldosterone antagonists.

Aldosterone antagonists have recently shown effectiveness in patients with Duchenne muscular dystrophy. Raman and colleagues analyzed the effects of aldosterone antagonists at a cellular level on “het” mice, which were deficient for dystrophin and haplosufficient for utrophin with skeletal myopathy and cardiomyopathy. Muscle physiology measurements showed that treatment significantly reduced the amount of fibrosis at 8 weeks of life; when treatment was started earlier, results showed minute detectable fibrosis. The results of cardiovascular magnetic resonance imaging on the mice with treatment and measurements of ejection fraction were normal. Measurements of strain, which measure deformation at a regional level, were significantly improved when treatment was started prior to 8 weeks of life. By understanding the mechanisms of cardiomyopathy in Friedreich ataxia, more refined and targeted treatments will be developed.³⁷

A novel approach involves metabolic modulators such as ranolazine, which is a sodium channel inhibitor. Ranolazine improves diastolic function, improves microvascular function, and reduces ischemia. The agent is particularly appealing to study for cardiomyopathy in Friedreich ataxia because it has potential benefit at the myocardial energetic level.³⁸ Ranolazine has potential advantages in increasing insulin secretion in response to glucose load.³⁹

In addition to randomized trials for these therapeutic agents, lifestyle changes such as diet and exercise can improve cardiovascular health in patients with Friedreich ataxia. These approaches have minimal potential risk and may yield significant benefit.

In collaboration with the National Institute of Neurological Disorders and Stroke, guidelines have been developed for cardiac outcomes common data to minimize burden of data collection and maximize uniformity of data acquisition. Typical data acquired in the clinic are from electrocardiograms, echocardiograms, and Holter monitor. Periodic testing should include fasting lipid profile and capillary blood glucose. Cardiovascular magnetic resonance imaging has increased in availability and this data will be informative. “Stage B cardiomyopathy,” a term used in cardiovascular disease and treatment, has been linked to improved outcomes, although applicability has not yet been shown in Friedreich ataxia. For example, testing on a patient with structural heart disease at stage B identifies concentric remodeling, impaired myocardial perfusion reserve, and fibrosis.⁴⁰ These preclinical markers predict subsequent sequelae, such as heart failure. Development of targeted therapeutics can prevent disease progression.

Speech Perception and Communication Deficits in Children with Friedreich Ataxia

Gary Rance, PhD, University of Melbourne, Melbourne, Victoria

Dr Rance’s talk focused on speech perception and hearing difficulties in children with Friedreich ataxia. Nearly all children and adults with Friedreich ataxia have some degree of auditory neuropathy-type hearing loss. In this situation, patients have normal sound detection and normal peripheral function, meaning that their ears functionally work fine. The peripheral function can be measured in terms of pre-neural response coming from the cochlear microphonic and the otoacoustic emissions, which are sounds produced by a healthy cochlea. Furthermore, because patients with Friedreich ataxia have normal sound

detection, results from an audiogram are perfectly normal. In contrast, patients will have abnormal neurotransmission through the cochlear nerve and the auditory brain stem, producing abnormalities in the auditory brainstem response, which is a pattern of the electrical activity that occurs within the first 10 milliseconds after sensing an audible stimulus.

As an example, Dr Rance reviewed auditory brainstem responses from non-affected and affected subjects with Friedreich ataxia. First, in a non-affected subject's auditory brainstem response, there is a clear sequence of peaks and troughs along the waveform, which is expected. Next, Dr Rance showed what an auditory brainstem response looks like for a patient that is in the early stages of the disease process. Rather than having the normal sequence of peaks and troughs along the wave form, these patients will have some of the same wave forms present, but the amplitudes will be much smaller, particularly in the late brain stem regions. Lastly, an auditory brainstem response for a patient later in the disease course is entirely absent. To this end, there is full potential at the start of the auditory brainstem response due to the cochlear microphonic, which means that the sound presented is loud enough to generate activity at the cochlear level so there should be something measurable within the brainstem region. However, this is not the case, indicating that something may be going wrong in terms of neural transmission since these are patients late in the course of disease. Dr Rance has 2 ideas as to what may be going on. First, he believes it could be due to the fact that there are too few neuronal elements available to produce a response that can be measured from the scalp, or it could be due to a desynchronization of the electrical activity as it passes up through the auditory brainstem, leading to disrupted timing of varying degrees.

Unfortunately, there are some consequences as a result of the absence of the auditory brainstem response. There is disrupted neural synchrony in the auditory pathway. Subsequently, this leads to a loss of timing cues in the neural representation of an auditory signal. This is particularly important in terms of a functional sense when looking at the neural representation of complex signals such as speech. Interestingly, the degree of auditory deficit does correlate with overall disease progression in Friedreich ataxia.

The remainder of Dr Rance's talk centered on a current study that he has been carrying out over the last few months in his clinic. There are 3 aims to this study. The first aim is to measure speech perception ability in both quiet listening conditions and background noise conditions in a group of children who have Friedreich ataxia. The second aim is to look at binaural speech perception ability, focusing on the ability to combine signals that are presented to both ears at the same time. Lastly, the study looks at the effects of Friedreich ataxia on everyday listening and communication to gain a deeper understanding of these patients' day-to-day hearing experiences.

This study consists of 9 children ranging from 9 to 18 years of age with Friedreich ataxia as well as a group of control subjects who match the patients in terms of age, gender, and hearing level. In terms of these patients' scores on the Friedreich Ataxia Rating Scale, there are some mildly affected subjects with a score of 31 and more advanced patients with a score up to 108. Furthermore, all of the children had normal sound detection, so they could all hear sounds perfectly normally.

Results for the first aim came from a monaural speech perception test, which is a test that presents sounds or a series of words to one ear at a time. The results were analyzed from a graph comparing the consonant-nucleus-consonant phoneme score and the sound-to-noise ratio measured in decibels. The consonant-nucleus-consonant phoneme score measures the number of phonemes (speech sounds) that the child perceives and imitates correctly,

whereas the sound-to-noise ratio measures the listening ability in environments ranging from completely quiet with no competing signals to environments in which the sound-to-noise ratio are the exact same. In terms of the control group, subjects fell within the typical range for quiet environments and those environments where speech and background noise were at the same level. On the other hand, subjects with Friedreich ataxia scored on the lower end of the normal range in quiet environments and scored significantly lower than the normal range when sound and background noise were indistinguishable. This finding shows that children with Friedreich ataxia truly have a problem in terms of hearing perception when they are trying to distinguish sounds from background noise.

The Listening in Spatialized Noise Test is used to look at binaural speech perception. This test measures the listener's ability to integrate speech signals between 2 ears at the same time. All of the testing is done using headphones in which the sound stimuli are modified to simulate a 3D listening environment. Furthermore, the parameter of this test is called the spatial difference, which is the effect of the simulated sound direction or the difference between a speaker located directly in front of the patient compared with one on the righthand side. A sound coming from directly in front of the patient reaches both ears at the same time of equivalent loudness, whereas a sound coming from the side of the patient reaches the first ear 200 μ sec before the other. Interestingly, this timing difference is useful in normal listeners, as it allows them to compare the signal that is incident on the 2 sides and use the difference to extract what they are listening for from within the background noise. However, patients with Friedreich ataxia have an issue with binaural integration because there is neural dyssynchrony and they are not able to distinguish the sound from the background noise.

To look at everyday listening and communication of these patients, the third aim, the Abbreviated Profile of Hearing Aid Benefit was used. This test looks at the degree to which the child experiences auditory difficulties in day-to-day life. There are 4 aspects of auditory function that this test focuses on: everyday communication, background noise, reverberation, and sound aversion. Across the 4 parameters, the control children reported they have difficulty hearing in about 10% of situations, consistent with previous studies conducted on adults. Results for patients with Friedreich ataxia were much worse than controls. For example, patients reported they have a significant amount of trouble with general communication 20% of the time, unlike controls who reported difficulty 10% of the time. In summary, these findings show that Friedreich ataxia does cause functional deficits in hearing and communication in children.

To sum up, Friedreich ataxia does affect the perception of complex signals, especially speech. Furthermore, this has a significant clinical impact on everyday listening and communication among these patients, especially because many are school-aged children in environments where they are unable to distinguish sounds from background noise. In addition, it seems that the measurement of binaural integration may offer insights into central auditory pathway function and be a potential biomarker for drug trials.

New Dilemmas in Friedreich Ataxia Diagnosis

Grace Yoon, MD, The Hospital for Sick Children, Toronto, Ontario, Canada

Dr Yoon addressed new dilemmas in diagnosing Friedreich ataxia focusing on presymptomatic genetic testing. Presymptomatic genetic testing can be defined as genetic testing of an at-risk individual who has no clinical symptoms of the disease. Universally, the primary justification for embarking on presymptomatic genetic testing in children and adolescents should be a timely medical benefit and/or a substantial psychosocial benefit. There is an increasing body of literature on the various ethical issues involved in

presymptomatic genetic testing, and Dr Yoon discussed 4 important publications from various genetic societies and genetic bodies that summarize currently accepted recommendations regarding presymptomatic genetic testing.

The first publication Dr Yoon discussed was from the 1995 publication of *The American Journal of Human Genetics*.⁴¹ First, this publication highlighted some of the ethical, legal, and psychosocial implications of genetic testing in healthy children. Next, this publication gave a nice overview of how the role of parental authority has changed over the years. To this end, as late as the early 1900s, children were considered property of their parents, specifically their father, and any decisions regarding the child was exclusively the right of the parent with no consideration for the child. Obviously, this has changed over the years, but this publication points out that the ethical principles of justice, nonmaleficence, autonomy, and beneficence need to be considered when addressing issues of genetic testing.

A second publication by the Canadian Pediatric Society and the Canadian College of Medical Genetics from 2003 issued a position statement on presymptomatic testing in healthy children. This publication reviewed the literature in regard to the problems associated with presymptomatic genetic testing in terms of psychosocial, ethical, and legal difficulties. In addition, this publication commented on the problem of presymptomatic genetic testing in children who were being considered for adoption. Another publication discussed by Dr Yoon was from the Human Genetics Society of Australasia in 2008, which laid out the foundation of the criteria that should be used when considering genetic testing for emancipated minors or adolescents that did not apply to the traditional models of genetic testing at the time. Lastly, Dr Yoon commented on a publication from the European Society of Human Genetics that reviewed all of the literature of the ethical, legal, and psychosocial implications of genetic testing in asymptomatic minors, opinions of clinical geneticists, as well as other stakeholders.

Since Friedreich ataxia is a disease without any definitive treatments, the guidelines for presymptomatic genetic testing are different than those used for a disease that can be treated. Each case is unique and needs to be managed on a case-by-case basis, because the needs of each family and child can be quite different. Prior to presymptomatic genetic testing, it is recommended that the parents and child be educated and counseled on the psychological and social risks of the test as well as the benefits of presymptomatic genetic identification from both the parents' and child's perspective. Although an early presymptomatic diagnosis could be beneficial to the parents in terms of family planning, gaining resources, and connecting with support services, it is important to consider what an early presymptomatic diagnosis could do to the child's future. For instance, insurance companies, educational institutions, and future employers may discriminate against the child because this child will inevitably develop Friedreich ataxia.

At the 2011 Friedreich's Ataxia Research Alliance International Scientific Conference held in Strasbourg, France, the genetics working group came up with multiple reasons for not offering and for offering presymptomatic genetic testing for this disease. Reasons for not offering the test included the need to respect a minor's autonomy and freedom to choose whether to undergo genetic testing when he or she reaches adulthood. The other reason for not offering presymptomatic genetic testing is because there is no effective treatment for Friedreich ataxia at this time. In contrast, there are reasons for offering presymptomatic genetic testing. First, by allowing genetic testing before symptoms begin, there is a potential benefit of surveillance for cardiac manifestations of the disease, most specifically cardiomyopathy. Next, an early diagnosis will allow the family to plan for the child's future needs. For instance, because the parents will know that the child will eventually need wheelchair access, they are able to make modifications to the home to better the child's life.

Lastly, presymptomatic genetic testing would allow the affected child the ability to participate in clinical trials of therapeutic agents at an early stage of the disease course.

Dr Yoon presented a case to introduce the idea that siblings of a suspected or affected child with Friedreich ataxia also deserve special consideration. The case involved an 11-year-old who presented with classic symptoms of Friedreich ataxia and her 4-year-old brother who was completely healthy with no developmental or neurological concerns. Of course the 11-year-old would be diagnosed with Friedreich ataxia without any problem since she presented with ataxia, absent reflexes, and extensor plantar responses, but what about the 4-year-old brother? Until recently, this child would have been overlooked since he showed no symptoms. Because clinicians now know there is a large range in the age of onset, clinical symptoms, and that the GAA repeat expansion is unstable between generations, it has become evident that siblings of children affected with Friedreich ataxia also need to be given special attention, as they may have inherited 2 frataxin mutations even though they have not developed clinical symptoms.

Initially, the 4-year-old developed flu-like symptoms. Fifteen days later, he lost consciousness after vomiting and was pronounced dead on arrival at the hospital. Extensive investigations were conducted for the usual causes of cardiomyopathy, metabolic diseases, and infection, which all came back negative. The results from the sister's genetic test for Friedreich ataxia confirmed her diagnosis, and on postmortem testing, the 4-year-old boy was found to be homozygous for 800 GAA repeat expansions in the frataxin gene. In terms of the child's cardiac autopsy, the gross autopsy showed dilation and hypertrophy of the heart while the microanalysis showed an impressive amount of fibrosis and lymphocytic infiltration. Furthermore, the autopsy showed that the spinal cord had a loss in myelin and axons in the dorsal root ganglia and that the medulla had a loss of neurons in the gracile and cuneate nuclei.

This case raises multiple implications for clinical practice. First, this case shows that there is evidence of neurodegeneration in a presymptomatic patient from a neurological perspective. Next, this case raises the question of whether Friedreich ataxia should be diagnosed by the clinical phenotype in patients who are at a genetic risk for the disease. Another question this case raises is whether cardiac screening should be implemented for patients at risk for Friedreich ataxia.

In conclusion, many areas need to be addressed in the future. The first thing that Dr Yoon thinks needs to be done is make a call for evidence-based studies, which look at the genetic and counseling needs of patients with Friedreich ataxia and their families. Furthermore, more attention needs to be drawn to advancing the studies of biomarkers and frataxin assays because these may better define the early disease stages. Finally, advances in therapeutic trials for Friedreich ataxia need to be made, as this will largely make a difference in terms of deciding whether to routinely implement presymptomatic genetic testing in the Friedreich ataxia at-risk population.

Questions and Answers

Audience member: In a general sense, do you recommend doing echocardiography on all younger siblings of children with Friedreich ataxia?

Dr Yoon: Given my experience, I personally would, but there are other people who will not.

Dr Maria: One of the things I had not appreciated was the oxidative stress and inflammatory aspects of the disease. First, how much does oxidative stress play a part in the pathogenesis of this disease, and does it play a role in diabetes? Second, has anybody looked at vascular

resistance reactivity or vascular stiffness since these are more sensitive measures of oxidative stress than cardiac size or wall thickness?

Dr Lynch: I can take the biochemical side of the reactive oxygen species. In terms of animals, such as mice, they die without overt reactive oxygen species production, and the studies in humans are equivocal as to whether we can demonstrate it. We can certainly demonstrate abnormal adenosine triphosphate production, and to quote one of Dr Wilson's analogies, if your car is not running very well, you do not make a whole lot of exhaust. So if you do not run anything through the oxidative phosphorylation you may not produce any adenosine triphosphate, which could lead to false negatives.

Dr Willi: I would like to comment on diabetes since that was briefly mentioned. Only in the past 8 to 10 years has the importance of mitochondrial function as it relates to type 2 diabetes been appreciated. There have been a number of high-impact studies demonstrating that as mitochondrial dysfunction occurs over time the mitochondrial function diminishes and that it can be measured in the muscles as well as intramyocellular fat deposition, which is also progressive over time. I do not know of any studies that have looked at reactive oxygen species specifically as it relates to diabetes.

Audience member: I was wondering if you have any information regarding the status of the cerebellum from the autopsy of the child?

Dr Yoon: Unfortunately, I do not. It was not as complete of an autopsy as we would have liked.

Audience member: It is known that there is an association between auditory processing problems and dyslexia. Furthermore, it is known that many of these children have cognitive problems. I was wondering if these children also had dyslexia or if people are looking into that?

Dr Rance: First, none of the children in the group had dyslexia. Next, we also screened the subjects for cognitive issues, and none of them had any. Furthermore, we screened the subjects for dyspraxia since this is an important factor to consider when assessing patients with Friedreich ataxia. Once again, none of the subject had dyspraxia.

Dr Maria: Just another quick question regarding the case presented — was the child examined or was it just that the parents said the child was asymptomatic?

Dr Yoon: No, the child previously had well-child visits.

Executive Summary of the Day

Gihan Tennekoon, MD, PhD, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

Dr Tennekoon gave a closing talk highlighting important points discussed throughout the day and reviewed certain points of emphasis. As previously mentioned, Friedreich ataxia is an autosomal recessive inherited disease. Friedreich ataxia is due to an expansion of the GAA repeats in the frataxin gene located on chromosome 9. Pandolfo and Koenig discovered this gene, and since then, genetic testing for Friedreich ataxia has been developed. In addition to expanding the understanding of how the disease works, genetic testing has allowed an expansion with regard to the disease's clinical phenotype. To this end, it is now known that young infants and adults can get Friedreich ataxia.

The levels of frataxin seen in this disease are puzzling, and much more research needs to be done to have a more solid understanding of the levels. In classic Friedreich ataxia, the frataxin level is less than 30% of the control amount of protein. People with point mutations of the disease have this same level of frataxin, and they have the same clinical picture as classic patients. On the other hand, patients with late-onset Friedreich ataxia have frataxin levels near that of the carriers. It is interesting that late-onset patients and carriers have the same amount of frataxin, since late-onset patients show clinical symptoms while carriers do not.

The next issue that Dr Tennekoon addressed was with regard to selective vulnerability. The central nervous system is composed of the visual system, the corticospinal tract, and the spinocerebellar tract. It is not understood why there are phenotypic differences given that there is selective vulnerability in the central nervous system. Also, it is not known why the dorsal root ganglia and Clarke's column are affected given that frataxin is expressed in every cell in the nervous system. Both the dorsal root ganglia and Clarke's column are formed early in development, which could hint at a problem with the formation of cells and the columns early in the developmental process. Dr Tennekoon believes there is early selective injury to the developing nervous system due to reactive oxygen species from mitochondrial dysfunction.

Another issue that needs more understanding is with regard to why the frataxin protein is not being made. As Dr Pandolfo discussed, it seems that heterochromatin formation is the reason why the gene is not transcribed, and this is where histone deacetylase inhibitors work very well. It is known that the frataxin protein is needed for iron-cluster formation. Without the protein, there seems to be an accumulation in the mitochondria, but the cell is not able to sense this accumulation of iron. In fact, the cell actually thinks there is less iron than what is needed. As a consequence, the cell compensates for this by making changes in transcription and translation of genes.

In conclusion, there are several questions that remain unanswered with regard to Friedreich ataxia. More research needs to be conducted to figure out the correlation between the frataxin levels and disease onset. As mentioned earlier, late-onset patients have 60% to 65% of the amount of protein found in control subjects, the same amount asymptomatic carriers have. With this said, there does not seem to be a distinct correlation between the amount of protein and disease onset. The second issue that needs further attention is the idea of selective vulnerability in the nervous system. To this end, not all of the cells in the brain are affected, for there are certain tracks that are more affected than others. Currently, it is not known why this is the case. Hopefully within the next few years we will have an answer to this question.

Future Directions: Panel Discussion/Questions

Moderator: Katrina Gwinn, MD, National Institutes of Health, National Institute of Neurological Disorders and Stroke

Dr Crawford: I would like to mention a couple of things that came up throughout the day, and I would also like to comment on the parallels and similarities in terms of ataxia telangiectasia. Children with ataxia telangiectasia walk at the normal age, 12 to 14 months, but they wobble from the very beginning. One of the features that many mothers will say is that their child cannot stand in one place because they are always falling off their center. Remarkably, this does not change much as the disease progresses until the age of 5. About 80% of the children who have been evaluated by a child neurologist get a diagnosis of ataxic cerebral palsy.

After 5 years of age, children with ataxia telangiectasia start to decline. During this time, children will manifest not only instability of gait but also an array of different abnormalities, including oculomotor problems, oculomotor apraxia, gait instability, and difficulty with dysarthria. Furthermore, these children have an odd posture — they will hold an eccentric position for a long time. Interesting, around 12 to 14 years of age, they start to plateau in terms of disease progression and do not decline any further. Therefore, there is an initial subnormal state, a decline phase, and then a rather prolonged plateau stage in which they do not worsen except for extrapyramidal movements (they will gain extra movements that get in the way). With this said, ataxia telangiectasia has a distinct phenotype.

I want to end by talking about another theme that is true about ataxia telangiectasia and other neurodegenerative disorders, and it may also be true for Friedreich ataxia. I am talking about the theme of natural history. We think of natural history as being the pre-programmed degeneration of the brain that will inevitably happen. What we are seeing in ataxia telangiectasia, however, is that the nervous system is brought down since they are sick children with immune problems, infections, and potential cancers. This leads me to the idea that the brain is in fact an organ in the body. To this end, the brain is fascinating, and it is intertwined in the body and affected by what is happening within the body. Therefore, when something is going on in the brain or in the body or when there are recurring infections and oxidative stress from being sick, these all have the effect of dragging down the nervous system and making the brain worse.

This raises the possibility that fastidious care in other ways may have a pure brain effect, and we may be able to change what is occurring in the brain.

Dr Mathews: When do you reliably see that ataxia telangiectasia is the reason for the child's bloodshot eyes?

Dr Crawford: Everybody knows about the characteristic bloodshot eyes of ataxia telangiectasia. With this said, however, these children do not have bloodshot eyes when they go see the doctor, since bloodshot eyes do not show up until they start to decline at age 5. This is the reason a lot of children will be misdiagnosed as having ataxic cerebral palsy, since these children lack bloodshot eyes.

Dr Gomez: Can you comment on the role of the ataxia telangiectasia mutated protein Western blot? Also, can you comment on the importance of screening the parents for carcinoma?

Dr Crawford: Western blot is a way that research laboratories can go in and look to see if in fact the protein is missing. Since many of the mutations are protein nulls you can look for their absence. In regard to your second question, it is known that heterozygotes have an enhanced cancer risk, but a modest one at that. There was recently a lot of discussion about breast cancer being more common, but we are now taking it further down and realizing that certain mutations like missense mutations appear to be a higher risk for both breast cancers and certain lymphoreticular malignancies. There is reason to tell an adult to do breast exams if they are at risk for solid tumors, but it is not encouraged to X-ray screen them since that may be a pathogenic factor.

Audience member: Can you comment briefly on what the ataxia telangiectasia mutated protein is?

Dr Crawford: Ataxia telangiectasia mutated protein is the most interesting molecule around since it is at the very top of the cascade cell surveillance. With this said, if you disturb a cell by heating, cooling, watering, salting, or radiating it, ataxia telangiectasia mutated protein

is a nascent kinase that will then break open and start to kinase everything in its surroundings.

Dr Hirano: I would like to comment on my favorite organelle: the mitochondria. As I listened to all of these talks about Friedreich ataxia, I made constant comparisons to the other mitochondrial ataxias. I realized that there is overlap between the clinical and biochemical aspects in regard to things like hypertrophic cardiomyopathy since this is very common to mitochondrial diseases in general. However, there are differences in terms of the brainstem changes and the brainstem auditory evoked response changes. For instance, in mitochondrial encephalomyopathies, it is a cochlear problem rather than a brainstem dysfunction. So there is certainly overlap in some areas, which is why these patients are sometimes mistaken for other mitochondrial diseases rather than Friedreich ataxia. With this said, it is important to think about maternally inherited mitochondrial disorders when you encounter a patient with ataxia since these disorders can also present with ataxia.

It was mentioned in Dr Fogel's talk that there are autosomal causes of mitochondrial ataxia like DNA polymerase subunit gamma, and I want to highlight a group of mitochondrial diseases that is actually treatable. Coenzyme Q10 deficiency is a mitochondrial disease that is treatable. Coenzyme Q10 is synthesized within the mitochondria, and it is a critical molecule in the respiratory chambers. A person can have primary defects of coenzyme Q10 deficiency due to defects in biosynthesis, or a person can have secondary defects. To this end, one of the causes of primary coenzyme Q10 deficiency is a mutation in aarF domain containing kinase 3 gene, which presents as an autosomal recessive ataxia. With regard to secondary coenzyme Q10 deficiency, mutations in genes like aprataxin will cause this secondary defect. It is important to know that both primary and secondary forms can be improved with coenzyme Q10 supplementation. Furthermore, it is important to know that you cannot measure blood to diagnose this condition since blood levels can be affected by oral intake of coenzyme Q10. With this said, it is necessary to do a muscle biopsy to diagnose these defects.

Audience member: Can the muscle biopsy be mailed to you for testing?

Dr Hirano: Yes, if you mail us the muscle biopsy we can test it, or there are several other groups that can.

Audience member: Does staining the muscle biopsy with coenzyme Q10 help us predict the results?

Dr Hirano: Some of the patients have increased lipids, so you may see a lipid accumulation by oil red O; however, they are generally normal.

Audience member: Is there any phenotypic clue that could lead to it being primary or secondary defects?

Dr Hirano: There are multiple phenotypes associated with coenzyme Q10 deficiencies in infants and young children. Often times, it presents as steroid resistant nephrotic syndrome. Therefore, this is one phenotype that neurologists should be alert to. Furthermore, examples of primary coenzyme Q10 deficiencies include encephalopathy or sometimes Leigh's disease with steroid resistant nephrotic syndrome. Lastly, the most common phenotype seen in slightly older children or adults is the cerebellar ataxia sometimes associated with epilepsy, cognitive problems, pes cavus, or neuropathy. In summary, if you think you have a patient with a genetically undefined ataxia, it is worth considering a muscle biopsy since it could be a treatable condition.

Audience member: How sensitive is measuring coenzyme Q10 and leukocytes? I know it is not 100% sensitive, but is it a useful screening tool before proceeding to a muscle biopsy if you are concerned about coenzyme Q10-associated ataxia?

Dr Hirano: There are not enough data at this time to answer that confidently. There is some suggestion that you can use leukocytes or isolated mononuclear cells to detect coenzyme Q10 deficiency, but it has not been done on patients enough to be sure. Therefore, we still prefer muscle biopsy.

Audience member: Do any of these patients have metabolic stroke?

Dr Hirano: Yes, one of the original patients we studied was a 33-month-old child who presented at 1 year of age with nephrotic syndrome and later had stroke-like episodes. Therefore, he had a brain and kidney disease. Interestingly, his younger sister, who was 9 months old at the time, started to develop proteinuria, as well. We began to treat the 33-month-old with coenzyme Q10 supplementation, and he improved and regained developmental milestones. In terms of kidney failure, the patient already had a kidney transplant so that obviously did not improve. Also, his younger sister, who had nephrotic syndrome, only improved in terms of her proteinuria. The proteinuria did not go away completely, but it did not develop into renal failure. Furthermore, she did not develop neurological complications. In summary, stroke-like episodes have occurred, and they can be prevented by coenzyme Q10 supplementation.

Dr Gwinn: I would now like to shift gears and ask the panelists if they would like to react to anything Dr Tennekoon presented?

Dr Wilmot: In terms of the developmental issue that Dr Tennekoon brought up, I think that a lot of neurologists do believe that it is a developmental type of an issue. This is evident in the case presented today by Dr Yoon since there was early involvement of the dorsal columns and dorsal root ganglia. However, more research needs to be done in this area in order to have a solid answer.

Dr Gomez: I would like to add one thing. If the pediatric form is a developmental basis then there still has to be an account for a generative form as well.

Audience member: Would the association between developmental diseases and degenerative diseases be relevant to the discussion?

Dr Gwinn: There are a number of examples in the human nervous system such as Gautier disease and the typical onset of Parkinson's disease. With regard to the relationship between Down syndrome and Alzheimer's disease, patients have an extra copy of amyloid beta (A4) precursor protein (APP) gene, but the onset of Alzheimer's occurs later in life. Organ systems are affected as people grow old so development and degeneration are linked.

Panel member: In a similar scenario, general myasthenic syndromes can cause intrauterine defects, arthrogyrposis, and mutations in the same gene that give rise to a late-onset disorder.

Dr Crawford: The word "developmental" can be confusing because with malformation sequence, deficits become more obvious with age. Disruption in the formation of the brain due to issues with signaling can cause progressive abnormality. For example, children with ataxia telangiectasia will have profound impairment for the first 5 years of life, but after the fifth year of life, the decline of development is at a milder rate than previous years. Early

progression does not necessarily predict the rate of progression later on in life so there is variability from year to year.

Dr Pandolfo: A specific vulnerability applies at a certain time and at a certain place. During development, the stages or processes that are particularly vulnerable to that genetic defect would present early and referred to changes in development. Later processes and stages are also affected by that genetic defect. It's just the matter of the vulnerability and time it occurs. For instance, idiopathic epilepsies are not degenerative but there are vulnerabilities that are related to the specific time and specific cell type. A number of us are studying the subtle defects in differentiation of induced pluripotent stem cells. Their ability to differentiate is normal but the subtle defects may be prominent in certain cell types during development.

Audience member: Is there any evidence that retrograde transport, NT-3, or trophic support are abnormal?

Dr Pandolfo: From the pathology literature, the vulnerable cells in the dorsal root ganglia could be NT-3 responding TrkC carrying neurons. Friedreich ataxia has not been studied systematically, although we had attempted to look at the KIKO mouse model about 13 years ago, which may have been the wrong model for that study. Corticospinal degeneration seems to be learned dependent in patients.

Audience member: Another example for the discussion of development and degenerative diseases is Rett syndrome, since development occurs normally, but patients show regression with age.

Audience member: Is there a secondary cerebral folate deficiency present in Friedreich ataxia as seen in other mitochondrial disorders? Has folinic acid or cerebrospinal fluid neurotransmitters been studied in Friedreich ataxia or seen to improve ataxia?

Panel member: Folate deficiency was first noted in patients with Kearns-Sayre syndrome. Mild improvements in ataxia have been detected. For example, Mercedes Pineda in Spain has reported ataxia improvements in patients with Kearns-Sayre syndrome. We have not tested for DNA polymerase gamma in patients with Friedreich ataxia.

Audience member: In reference to the high carrier rate for Friedreich ataxia and the epigenetic mechanisms, is there a possibility of an environmental postgenetic predisposition?

Panel member: Carriers have been studied. Detailed electrophysiology and other techniques have been conducted to analyze for subtle conduction defects and other anomalies, but not much has been found.

Panel member: There could still be a defect. The question should be focused on why aren't carriers affected? If this a reduction of function type of situation, then why is there such a threshold effect especially with late-onset cases? Some patients have 25% frataxin protein level at age 18 while their parents at age 95 with all kinds of oxidative stressors will show no abnormal decrease in protein level. This type of situation has not been studied in detail. I anecdotally ask about family members especially if the patient has late onset, but have never heard of older family members with movement issues. Patients with large expansions on alleles may have further expansion in the affected tissue. A combination of enriching for viable cells versus further somatic mutation may impact the difference between a heterozygote and person who has low amounts of frataxin.

Dr Wilson: A simplistic answer is that the normal level of frataxin exceeds the amount required for normal function. To comment about the environmental factors, Dr Lynch's work with sibling pairs have shown a high discordance in Friedreich Ataxia Rating Scale scores even though the siblings have similar numbers of GAA repeats. Drawing from Dr Perlman's observations about exercise, the sibling who is more active seems to be in better condition than the sibling who is less active. Different theories exist about the benefits of exercise, such as the number of mitochondria in the muscles increase due to exercise. To address about the high carrier frequency, I can think of several formal possibilities: heterozygote advantage, very high new mutation rate, and the founder effect. A very high new mutation rate is exemplified by spinal muscular atrophy, where the addition of disease alleles to the gene pool tries to make up for the alleles that are lost.

Ms Farmer: I wanted to add to Dr Wilson's comment about discordance between siblings. In the clinic, it seems that being thinner and having a smaller body habitus will predictor that the patient will be in worse condition than the sibling. For example, a sibling with a stocky build tends to have less cardiomyopathy, less scoliosis, and slower rate of neurologic degeneration than his or her sibling who is a smaller individual.

Dr Gwinn: This seems like anecdotal data that could be explored through registry data.

Dr Maria: My question is related to early clinical diagnosis since it takes a long time to reach a diagnosis. If early diagnosis is possible, could general pediatricians be able to detect irregularities through deep tendon reflexes? Many children with ataxic cerebral palsy are not areflexic, but their reflexes are certainly depressed. Is there a period of time where a child with Friedreich ataxia has hyperreflexia before having areflexia?

Panel member: I think you see both types. With older onset, the children tend to have a couple of reflexes, but these reflexes drop out. Many parents whose patients come to me say that other clinicians have never found any reflexes in their child. I am unsure of the parents' reports since there may have been an earlier period of reflexes or how often they had seen the same provider.

Dr Lynch: Parents frequently report that reflexes were not found in their children and the information is reliable, but not perfect. A few patients had mild epilepsy early in life so I can pull neurologic records and found that reflexes were rarely found. I have suspicions that a substantial number of patients never had reflexes, referring mainly to the knees and ankles.

Panel member: Scoliosis and areflexia are reported before neurological problems. I've had patients who were diagnosed with Friedreich ataxia by their orthopedists who were able to realize this after they ordered diagnostic testing after attempts to check reflexes.

Dr Mathews: With only 5 to 10 minutes to exam a patient, pediatricians often do not see the patients off the exam table to observe them walking or moving in the hallway, which may shorten the path toward a diagnosis. This can be help for a number of disorders. A current campaign is educating primary care providers on key landmarks related to that.

Audience member: Mild motor immaturities, hypotonia, and hyporeflexia are common in children under 5 years old. I don't think reflexes are a practical way to ascertain early onset of Friedreich ataxia. With Dr Hirano's comment about seizures, what proportion of the severe spinocerebellar syndromes is associated with refractory seizures and the presence of refractory seizures? How does that change your treatment of those patients?

Dr Hirano: The refractory seizures are mainly seen in children with Alport syndrome, the infantile hepatocerebral disease. In children with ataxia and seizures, the seizures are usually not refractory and treatable with standard anticonvulsive therapies.

Dr Gomez: In the next 5 years, there will be a large bioinformatics database of ataxia-causing genes with the availability of whole genome sequencing, and clinicians will have to sort out which variants are pathogenic.

Panel member: The challenge in neurology will be narrowing the field of polymorphisms of unknown significance in genes that have little known phenotype. It will be interesting to see what happens in the next 10 years.

Dr Crawford: To endorse and extrapolate further on Dr Perlman's comment on ethics of small trials, there are difficulties in conducting safety studies in small numbers of children. The problem that is occurring is the studies being conducted without controls and being published in review articles. Once published, this information will affect development of other agents and will take much more information to prove to patients that the initial publication was not accurate.

Dr Gwinn: In closing of the panel discussion, we hope to see movement forward in the clinical trials within the next couple of years.

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Table 1**Molecular Characterization of the Autosomal Recessive Ataxias**

	Abbr.	Gene	Protein	Protein Function
Friedreich ataxia-like				
Friedreich ataxia	FRDA	<i>FXN</i>	Frataxin	Mitochondrial metabolism
Ataxia with vitamin E deficiency	AVED	<i>TTPA</i>	α-tocopherol transfer protein	Vitamin E homoeostasis
Abetalipoproteinemia	ABL	<i>MTP</i>	Microsomal triglyceride transfer protein	Lipoprotein metabolism
Refsum’s disease	RD	<i>PHYH</i>	Phytanoyl-CoA hydroxylase	Fatty-acid oxidation
		<i>PEX7</i>	Peroxin-7	Peroxisomal protein importation
Friedreich ataxia-like with cerebellar atrophy				
Late-onset Tay-Sachs	LOTS	<i>HEXA</i>	Hexosaminidase A	Ganglioside metabolism
Cerebrotendinous xanthomatosis	CTX	<i>CYP27</i>	Sterol 27-hydroxylase	Bile-acid metabolism
DNA polymerase γ disorders	POLG	<i>POLG</i>	DNA polymerase γ	Mitochondrial DNA repair and replication
Spinocerebellar ataxia with axonal neuropathy	SCAN1	<i>TDP1</i>	Tyrosyl-DNA-phosphodiesterase 1	DNA repair
Autosomal recessive cerebellar ataxia, type 1	ARCA1	<i>SYNE1</i>	Synaptic nuclear envelope protein-1	Cerebellar architecture
ANO10 ataxia	ANO10	<i>ANO10</i>	Anoctamin 10	Predicted Ca ²⁺ activated chloride channel
Early-onset ataxia with cerebellar atrophy				
Ataxia telangiectasia	AT	<i>ATM</i>	Ataxia telangiectasia mutated	DNA repair
Ataxia telangiectasia-like disorder	ATLD	<i>MRE11</i>	Meiotic recombination 11	DNA repair
Ataxia with oculomotor apraxia, type 1	AOA1	<i>APT</i>	Aprataxin	DNA repair
Ataxia with oculomotor apraxia, type 2	AOA2	<i>SETX</i>	Senataxin	DNA repair, transcription, RNA processing
Autosomal recessive ataxia of Charlevoix-Saguenay	ARSACS	<i>SACS</i>	Sacsin	Protein folding and/or quality control
Infantile-onset spinocerebellar ataxia	IOSCA	<i>C10orf2</i>	Twinkle, twinkly	Mitochondrial DNA metabolism
Cayman ataxia	CA	<i>ATCAY</i>	Caytaxin	Glutamate signaling
Marinesco-Sjögren syndrome	MSS	<i>SIL1</i>	BiP-associated protein	Protein folding and/or quality control
Autosomal recessive cerebellar ataxia, type 2	ARCA2	<i>ADCK3</i>	aarF domain-containing kinase-3	CoQ10 synthesis, mitochondrial metabolism
Dysequilibrium syndrome	DES	<i>VLDLR</i>	Very-low-density lipoprotein receptor	Intracellular signaling
		<i>CA8</i>	Carbonic anhydrase VIII	Intracellular calcium signaling
Peroxin-associated ataxia	PEX-associated	<i>PEX2</i>	Peroxin 2	Peroxisomal metabolism
		<i>PEX10</i>	Peroxin 10	
		<i>PEX16</i>	Peroxin 16	

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