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Abstract

Friedreich ataxia is the most common human ataxia and results from inadequate production of the frataxin protein, most often the result of a triplet expansion in the nuclear *FXN* gene. The gene cannot be transcribed to generate the messenger ribonucleic acid for frataxin. Frataxin is an iron-binding protein targeted to the mitochondrial matrix. In its absence, multiple iron-sulfur-dependent proteins in mitochondria and the cytosol lack proper assembly, destroying mitochondrial and nuclear function. Mitochondrial oxidant stress may also participate in ongoing cellular injury. Although progressive and debilitating ataxia is the most prominent clinical finding, hypertrophic cardiomyopathy with heart failure is the most common cause of early death in this disease. There is no cure. In this review the authors cover recent basic and clinical findings regarding the heart in Friedreich ataxia, offer recommendations for clinical management of the cardiomyopathy in this disease, and point out new research directions to advance the field.

Keywords

cardiomyopathy, frataxin, Friedreich ataxia, heart, mitochondria

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Many inherited diseases of the nervous system involve mitochondrial dysfunction.^{1,2} These can result either from gene defects in mitochondrial-encoded proteins or in nuclear-encoded gene products that are targeted to mitochondria. Among the many other organ systems also affected by these mitochondrial disorders, the heart is key in terms of life span and quality of life. Because the heart is heavily reliant on energy generation by mitochondria, any disruption in oxidative phosphorylation will ultimately be associated with cardiac failure as well. In this regard, Friedreich ataxia is a classic mitochondrial disorder that affects multiple organ systems, but is most notable for its impact on neurologic function and on the heart. There is no cure for this disease.

Basic Findings

Gene Defect

As the most common inherited ataxia in humans, Friedreich ataxia is a progressive cardio- and neurodegenerative disease that is typically diagnosed in mid-childhood. The incidence of Friedreich ataxia is approximately 1 in 30 000 people (prevalence of 1:50 000) with equal frequency between sexes,³ and the condition is associated with a carrier frequency of 1:60 to 1:120.⁴⁻⁷ Inheritance is autosomal recessive and is predominantly caused by a triplet nucleotide repeat expansion in the first intron of the human frataxin gene (*FXN*) on chromosome 9q21.11 (reviewed by Patel and Isaya and by Pandolfo^{8,9}).

There is a correlation between triplet repeat number and the onset and severity of clinical symptoms, with higher repeat numbers, 600 to 1200 repeats, being associated with more severe disease and cardiomyopathy.¹⁰⁻¹² More recently, the degree of DNA methylation at the *FXN* locus around the triplet expansion has been correlated with frataxin expression and clinical outcome in Friedreich ataxia, and may explain some of the variability in clinical phenotype relative to the triplet expansion.^{13,14} Patients experience a loss of motor skills and, ultimately, inability to stand or walk within 10 years to 15 years of onset.¹⁵ This primary neurodegeneration of the dorsal root ganglia leads to the hallmark clinical findings of progressive ataxia^{16,17} and debilitating scoliosis, and often accompanies the onset of severe hypertrophic cardiomyopathy.

Frataxin is an essential and highly conserved protein expressed in most eukaryotic organisms that appears to

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function in mitochondrial iron homeostasis, notably the *de novo* biosynthesis of iron-sulfur cluster proteins¹⁸ and heme biosynthesis.^{19,20} The frataxin precursor protein is 210 amino acids in length (23.1 kDa) and contains an 80 amino acid mitochondrial targeting sequence at the amino terminus that is removed in 2 steps by the mitochondrial matrix processing peptidase²¹ on import into the mitochondria.²² The final 130 amino acid frataxin has a predicted M_r of 14.2 kDa,^{23,24} and no other posttranslational modifications have been identified. Frataxin has been shown to bind iron along an acid ridge.²⁵ Although the exact function of frataxin has not been defined, recent studies suggest that frataxin acts as an allosteric activator with Fe^{2+} in the formation of iron-sulfur clusters by forming a protein complex that includes ISD11, ISCU, FXN, and NFS1.²⁶⁻²⁸ Frataxin is predicted to induce a conformational change in the complex, enabling direct sulfur transfer from cysteine for iron-sulfur cluster assembly. The absence of frataxin is associated with severe loss of activity in iron-sulfur-containing proteins,²⁹ such as aconitase, and loss of energy production.^{30,31}

Mitochondrial Function

With help from human patients and animal models, recent investigations have uncovered a great deal of information leading to a better understanding of the mechanisms underlying mitochondrial dysfunction in Friedreich ataxia. The earliest functional studies of frataxin deficiency demonstrated impaired activity of the iron-sulfur cluster proteins of the electron transport chain, including complex I, II, and III.²⁹ This study also discovered that mitochondrial aconitase—the only iron-sulfur cluster-containing protein of the tricarboxylic acid cycle—also displayed impaired activity. This seminal work revealed Friedreich ataxia as a mitochondrial disorder and provided the basis for understanding frataxin's role in mitochondrial iron homeostasis. In addition to its role in iron-sulfur cluster assembly, frataxin was shown to play an active role as a citrate-dependent iron chaperone involved in aconitase activation.³² Consistent with impaired electron transport chain activity in Friedreich ataxia, phosphorus magnetic resonance spectroscopy studies showed reduced adenosine triphosphate (ATP) production in patient skeletal muscle and heart.^{30,33} Furthermore, the level of energy deficit in studied patients strongly correlated with the degree of cardiac hypertrophy, thus highlighting the importance of impaired energy homeostasis in Friedreich ataxia cardiomyopathy. Most mitochondrial and biochemical defects identified in human patients have also been recapitulated in mouse models of Friedreich ataxia,^{34,35} which have provided valuable systems for testing potential therapeutic interventions.^{36,37}

Although iron-sulfur cluster enzyme deficiency and impaired energy generation is widely regarded as the major pathogenic mechanism underlying Friedreich ataxia cardiomyopathy, there are also important arguments for disrupted mitochondrial and cellular iron homeostasis as late-onset factors of disease progression. Iron deposition in cardiomyocytes often accompanies myocardial hypertrophy in Friedreich

ataxia,³⁸ suggesting a role for iron toxicity-mediated oxidative tissue damage. However, the myocardial iron-positive granules only become evident on postmortem tissue analysis, which limits an accurate interpretation of a role for iron dysregulation in disease progression. A detailed analysis of the neuron-specific enolase and muscle creatine kinase mouse models of Friedreich ataxia demonstrated that cardiac hypertrophy and mitochondrial iron-sulfur cluster protein defects precede any evidence of myocardial iron deposition, suggesting that iron accumulation may be a secondary disease phenotype.³⁴ Nonetheless, the muscle creatine kinase mouse models do eventually develop mitochondrial iron deposition along with marked alteration in expression of genes involved in mitochondrial and cellular iron import and storage.¹⁹ Attempting to limit cardiac iron deposition in the muscle creatine kinase mouse models via a mitochondrial permeable iron chelator seemed to limit cardiac iron loading and hypertrophy.³⁶ Another study of iron chelation therapy in a frataxin-knockdown cell line found an increase in aconitase activity as well as improvements in mitochondrial membrane potential and ATP production.³⁹ Together, these studies indicate that a dysregulation of mitochondrial iron metabolism may ultimately be important in the progression of Friedreich ataxia cardiomyopathy.

Increased sensitivity to oxidative stress is also likely to contribute to cardiac disease progression in Friedreich ataxia. Reactive oxygen species are a physiological byproduct of aerobic mitochondrial respiration that, in excessive quantities, can damage cellular macromolecules, including DNA, protein, and lipid membranes, and may ultimately lead to apoptotic cell death. Although healthy cells have robust mechanisms for mitigating reactive oxygen species, evidence indicates that frataxin-deficient cells are less capable of coping with oxidative insults.^{40,41} This increased sensitivity, or lower threshold, for oxidative stress may be causally related to disrupted iron use, but may also represent an independent factor in disease development. Earlier work demonstrated that fibroblasts in patients with Friedreich ataxia are hypersensitive to hydrogen peroxide and that this sensitivity is partially rescued through iron chelation.⁴⁰ An additional investigation also noted that a key antioxidant protective mechanism, mitochondrial manganese superoxide dismutase, failed to be induced in both Friedreich ataxia patient fibroblasts and in the heart of mouse models.⁴¹ The increased sensitivity to reactive oxygen species in Friedreich ataxia has also been supported through studies in yeast and human lymphoblasts, indicating impairments of the glutathione defense to reactive oxygen species.⁴² Although there may be an increased vulnerability to oxidative damage because of impaired endogenous antioxidant defenses, research in animal models suggests that oxidative stress, by itself, is not a notable sequelae of frataxin deficiency.⁴³ Thus, it is important to distinguish between increased basal levels of oxidative stress and an increased sensitivity to oxidative stress, as this has been a topic of considerable controversy in Friedreich ataxia research.⁴³ Nevertheless, the continuous bioenergetic and mechanical demands of the human heart may be sufficient to exceed

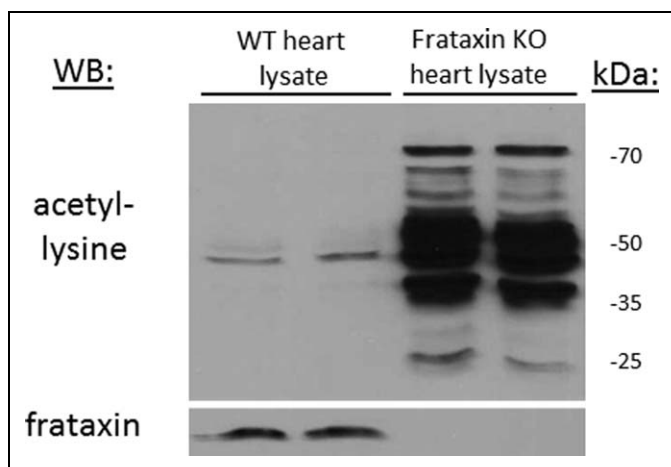


Figure 1. Protein acetylation in normal wild-type (WT) and frataxin knockout (KO) hearts. Total heart lysates were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and then probed with anti-acetyl-lysine antibody. The anti-frataxin antibody shows complete absence of frataxin protein in the frataxin KO hearts. WB = western blot; kDa = kilodaltons.

this lower threshold for oxidant stress and promote the development of cardiac pathology.

Last, increasing evidence suggests that frataxin-deficient cells and tissues may experience impaired lipid metabolism. A recent study of *Drosophila* models of Friedreich ataxia demonstrated a global accumulation of lipids as well as specific accumulation of lipid droplets in glial cells.⁴⁴ Consistent with these findings, a recent gene expression study of peripheral blood lymphocytes derived from human Friedreich ataxia patients and heterozygous carriers for the GAA repeat expansion provided evidence for altered lipid homeostasis.⁴⁵ These results emphasize that altered lipid biology may be a systemic factor of Friedreich ataxia pathogenesis, a notion that has critical implications for both cardiac and mitochondrial function in Friedreich ataxia. The β -oxidation of fatty acids occurs in the mitochondrial matrix, where it serves to generate acetyl-coenzyme A for further oxidation through the tricarboxylic acid cycle. Fat is the predominant source of carbon fuel for the heart, which derives as much as 70% of the ATP necessary for contraction from fatty acid oxidation.^{46,47} Importantly, that a prolonged energetic shift away from fatty acid oxidation is a well-known characteristic in the evolution of heart failure,⁴⁸ suggesting that this may contribute to the cardiomyopathy of Friedreich ataxia.

In support of this, we have recently shown that there is pronounced hyperacetylation of cardiac mitochondrial proteins in both the neuron-specific enolase and muscle creatine kinase mouse models of Friedreich ataxia, and this is caused by inhibition of the mitochondrial NAD⁺-dependent deacetylase sirtuin-3 (SIRT3).⁴⁹ An example of this is shown in Figure 1, where total heart proteins from 2 wild-type mice (WT heart lysate) and 2 frataxin knockout mice (Frataxin KO heart lysate) have been probed for acetyl-lysine modification on Western blotting. Heart proteins from the frataxin knockout animals

have far higher levels of mitochondrial protein acetylation. This is important because the SIRT3 deacetylase is an important regulator of fatty acid oxidation and overall oxidative metabolism in mitochondria via deacetylation of key proteins.⁵⁰ These results potentially provide a mechanism for impaired lipid metabolism in Friedreich ataxia hearts. However, further work is necessary to determine what role, if any, impaired lipid homeostasis plays in Friedreich ataxia-associated cardiomyopathy.

Clinical Findings

Heart Failure in Friedreich Ataxia

Heart failure from cardiomyopathy is the primary mode of death in ~60% of patients with Friedreich ataxia. In a recent retrospective review, Tsou and colleagues identified congestive heart failure in approximately 30% of patients dying from Friedreich ataxia, and slightly less than 20% of patients had severe arrhythmias contributing to, or causing, death.⁵¹ Patients with Friedreich ataxia develop a severe cardiomyopathy leading to death from heart failure in the third to fifth decade of life.⁹ The cardiomyopathy associated with Friedreich ataxia is hypertrophic (very thick ventricular walls) and the heart typically maintains adequate systolic function⁵² until shortly before death.¹² The hypertrophy derives from a striking proliferation of mitochondria within the cardiomyocytes, and a marked loss of contractile fibers.^{37,38} Thus, measures to decrease contractile force, such as negative inotropic medications (eg, β -blockers, or disopyramide), may not be effective in Friedreich ataxia because the cell biology is very different from that of hypertrophic obstructive cardiomyopathy, where these drugs have proven useful for controlling defects in overexpression of contractile proteins.⁵³ Dynamic obstruction of the left ventricular outflow tract is rarely seen in Friedreich ataxia-associated cardiomyopathy. Although mitochondrial protein mass is significantly increased, energy generation in heart is compromised as noted above.^{31,33} One direct consequence of this abnormality is that these mitochondria may not be capable of metabolizing fatty acids well, and thus may be more dependent on glycogen stores and glucose for energy generation.

Patients with Friedreich ataxia also have an impaired myocardial perfusion reserve index, even prior to onset of overt cardiomyopathy, which is associated with microvascular disease and fibrosis in the heart (Figure 2).⁵⁴ Thus, patients with Friedreich ataxia may experience chest pain or symptoms of decreased coronary reserve in the absence of epicardial coronary disease.⁵⁵ Arrhythmias are also common in patients with Friedreich ataxia, particularly atrial arrhythmias, and contribute to mortality.⁵¹ The electrocardiogram is abnormal in more than 90% of cases and most often demonstrates T-wave abnormalities, such as inversion or flattening, in the left chest leads (Figure 3). In addition, the left ventricular volume is decreased in patients with Friedreich ataxia,⁵⁶ and there is diastolic dysfunction⁵⁷ because of scarring and hypertrophy. The diastolic dysfunction emphasizes the need to control

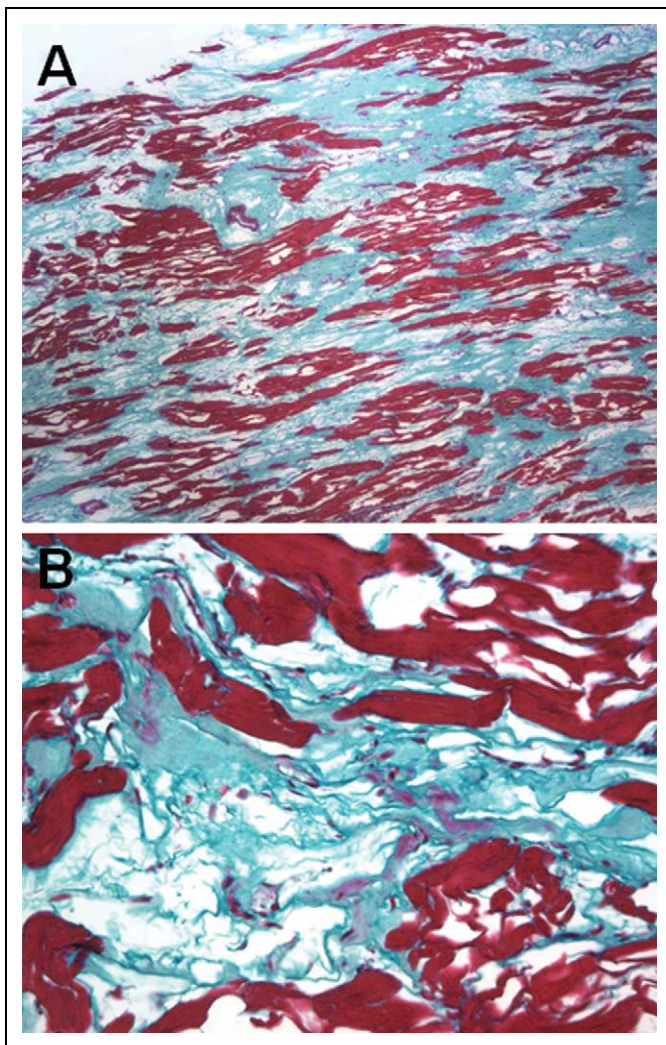


Figure 2. Masson Trichrome stain of heart from 2 patients with Friedreich ataxia. (A) Low-power magnification of the first patient shows the extensive fibrosis (green) and loss of muscle (red). (B) Higher-power magnification of the second heart showing extensive fibrosis.

arrhythmias in these patients because the atrial contribution to left ventricular filling and cardiac output will be important.

Although the primary cause of death in Friedreich ataxia is heart failure,^{51,58} surprisingly little has been written about the natural history and pathology of the frataxin-deficient heart, with most publications focusing instead on the dramatic neuropathology. A search of Medline shows that between 1991 and 2011, approximately 2462 articles have been published in which Friedreich ataxia was either the primary or substantial topic of the article (Figure 4). In the 6 years prior to the identification of the human *FXN* gene and description of the mutation(s) causing Friedreich ataxia,⁵⁹ there was an average of 31.5 ± 9 articles per year on Friedreich ataxia. With identification of the disease gene in 1996 and the formation of the patient advocacy group Friedreich's Ataxia Research Alliance in 1998, publications on Friedreich ataxia increased dramatically to 82 ± 11.5 articles/yr over the intervening 15 years

to 2011, thus emphasizing the power of organized patient advocacy groups. The number of publications designated as a review on Friedreich ataxia also increased across this time span, from $13\% \pm 4.1\%$ prior to 1996 to $24\% \pm 7.9\%$ ($P = .0046$, 95% 2-tailed confidence interval), reflecting the greater increase in primary research publications on which to base a review. Surprisingly, the number of publications in which the heart is either a primary focus, or major component of the publication, has not changed significantly during the past 10 years. Given that the metabolic derangements resulting from loss of frataxin cause a detectable cardiomyopathy in more than 92% of patients,^{60,61} and the heart is the primary cause of death in more than 60% of patients,⁵⁷ it is imperative to direct basic and clinical research to the cardiovascular system to improve life span and heart function in Friedreich ataxia.

Clinical Implications

The cardiomyopathy of Friedreich ataxia is both progressive and deceptive. Several studies have shown that earlier presentation of Friedreich ataxia symptoms, which correlates with higher GAA repeat numbers, is associated with a more aggressive progression of hypertrophic cardiomyopathy.^{12,57,62} This can be misleading in that cardiac systolic function, as measured by ejection fraction using echocardiography, is often in a low normal range, even though the left ventricle is markedly thickened. As noted above, however, diastolic function is impaired because of left ventricular hypertrophy,⁵¹ as well as the interstitial scarring and fibrosis that are hallmarks of this cardiomyopathy, as shown in Figure 2.^{38,63} Because of the physical limitations in activity and capabilities of these patients because of their ataxia, it is difficult to quantify progression of heart failure based on lifestyle or activity metrics, such as the New York Heart Association classification of heart failure. This becomes important when evaluating patients with Friedreich ataxia for procedures that will impose a significant cardiovascular stress, such as correction of scoliosis. The thickness of the left ventricle means that both diastolic perfusion of the heart and coronary reserve⁵⁴ will be impaired, and thus these hearts may not tolerate lowered blood pressures frequently associated with certain surgeries, such as spinal fusion, for correction of scoliosis.

As noted above, previous studies have shown that the mitochondria in these hearts do not have a normal ability to generate energy,³³ which may reflect both an impaired ability to burn fats and defects in the electron transport chain.⁴⁹ Thus, maintaining adequate serum glucose levels may be important to avoid depressed cardiovascular function and injury. Last, extensive scarring and diastolic dysfunction of the myocardium can limit the heart's ability to alter stroke volume and cardiac output. Thus, these patients may not tolerate large fluid shifts associated with major surgeries and can develop acute heart failure without additional support, such as dialysis or inotropic support. Careful monitoring of fluid balance and cardiovascular function is essential in patients undergoing stressful events,

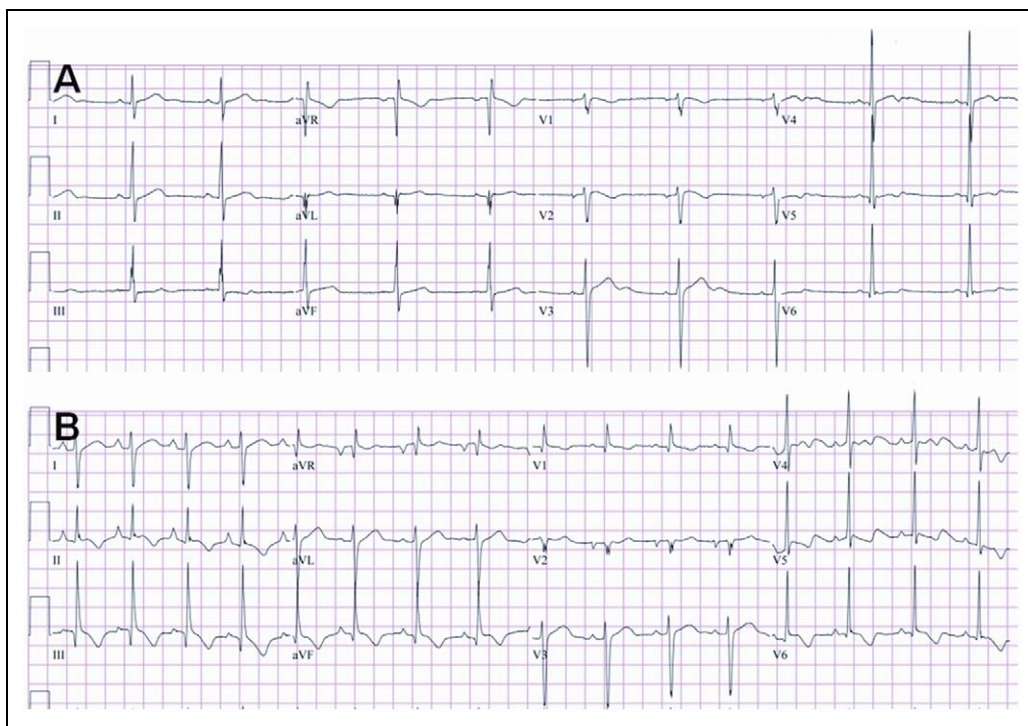


Figure 3. Electrocardiograms in Friedreich ataxia. (A) 18-year-old man with Friedreich ataxia. (B) 15-year-old girl with Friedreich ataxia. Note the abnormalities in the T waves that are especially prominent in the chest leads, V1-V6, indicative of cardiomyopathy. This is not specific to Friedreich ataxia nor is it prognostic.

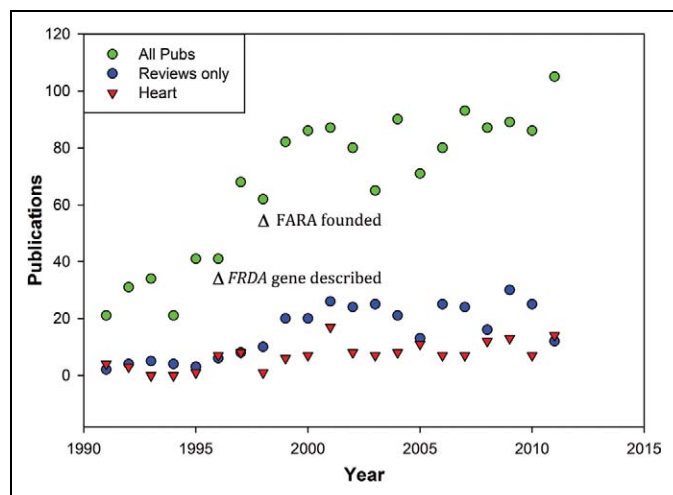


Figure 4. Publications on Friedreich ataxia, 1991–2011. ● = All publications (Pubs) on Friedreich ataxia. ● = Review publications on Friedreich ataxia. ▼ = Cardiac publications in Friedreich ataxia. Time points of Friedreich ataxia gene (*FRDA*) identification and foundation of the Friedreich’s Ataxia Research Alliance (FARA) are marked by Δ.

such as scoliosis surgery or hydration therapy in the emergency room setting.

Conclusion

Although significant advances have been achieved in understanding Friedreich ataxia, many questions remain in terms

of therapeutic development and clinical management. These are both urgent and may have application to other rare diseases. Key among these has been why it takes so long for the symptoms of Friedreich ataxia to appear when the expression of the gene defect began in utero. For example, patients with Friedreich ataxia are typically diagnosed at 10 ± 7.4 years of age,⁶⁴ yet the gene defect was present and can be detected at birth. Complete loss of frataxin is lethal to the embryo,⁶⁵ suggesting that despite a detectable decrease in protein early on, the expression of frataxin is sufficient to allow normal organ formation and function during human development. Clearly, patients also have heart and neurologic involvement prior to diagnosis and onset of symptoms, as evidenced by case reports of children diagnosed after death.⁶⁶ These questions become important from the standpoint of basic understanding and therapy development: (1) Are there different mechanisms regulating transcription of the *FXN* gene in the developing embryo that may be more efficient and allow expression of greater amounts of frataxin? Examples of this may include epigenetic control of expression, such as with chromatin remodeling and/or DNA methylation state. Understanding these embryonic mechanisms in the context of triplet expansion diseases may lead to greater advances in therapeutic development for Friedreich ataxia and other diseases. (2) Does the onset of symptoms herald the loss of cell mass (because of cell death) rather than cell dysfunction? If so, then recovery of organ or tissue function later in life may be impossible using current therapies because the loss of heart and neural cells is too great. This

would emphasize earlier identification of patients, such as with newborn screening, to allow earlier therapeutic intervention. Thus, even current therapeutic approaches that are not successful in established patients with Friedreich ataxia may have protective benefit if started prior to loss of cells and onset of symptoms.

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Author Contributions

RMP wrote the paper and generated Figures 3 and 4. GRW wrote and edited the paper and generated Figure 1.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

The use of laboratory mice in this study was approved by the Indiana University School of Medicine's Institutional Animal Care and Use Committee (IACUC) and Laboratory Animal Resource Center (LARC). All conducted experiments conform to the American Veterinary Medical Association's Panel on Euthanasia.

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