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Unanswered Questions in Friedreich Ataxia

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Abstract

During the past 15 years, the pace of research advancement in Friedreich ataxia has been rapid. The abnormal gene has been discovered and its gene product characterized, leading to the development of new evidence-based therapies. Still, various unsettled issues remain that affect clinical trials. These include the level of frataxin deficiency needed to cause disease, the mechanism by which frataxin-deficient mitochondrial dysfunction leads to symptomatology, and the reason selected cells are most affected in Friedreich ataxia. In this review, we summarize these questions and propose testable hypotheses for their resolution.

Keywords

antioxidant; cerebellum; dorsal root ganglion; mitochondrion

Friedreich ataxia is an autosomal recessive genetic disorder caused by homozygous mutations in the *FXN* gene, with 97% of patients carrying GAA repeat expansions in the first intron of both alleles of *FXN*.¹ This expansion results in decreased expression of the mitochondrial matrix protein frataxin, which functions in iron-sulfur-cluster assembly.² Decreased frataxin expression impairs the function of mitochondrial iron-sulfur-cluster-containing enzymes within the respiratory chain and leads to accumulation of iron in the mitochondrial matrix.^{3–4} Cells derived from patients with Friedreich ataxia exhibit increased susceptibility to oxidative stress, and mitochondrial dysfunction and reactive oxygen species production are proposed to be key features of the pathophysiology of the condition.^{5–8}

The pathology and clinical presentation of Friedreich ataxia are unique. Patients exhibit progressive limb and gait ataxia, sensory loss (particularly in proprioception and vibratory

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Conflicts of Interest

The authors report no conflicts of interest.

Authors' Roles

Dr Tennekoon made the presentation on which the article was based. Dr Lynch wrote the first draft. Dr Tennekoon, Dr Wilson, and Mr Deutsch provided further ideas and critically revised the work.

sense), weakness, and dysarthria.⁹ Cognition is typically spared, and weakness is a modest component of the clinical difficulties early in the disease. Cardiomyopathy, diabetes mellitus, hearing loss, scoliosis, and visual loss can occur. The classical neuropathology of Friedreich ataxia at autopsy readily explains the typical phenotype (Table 1). Patients have loss of large dorsal root ganglion cells and atrophy of their axons in the dorsal columns, loss of the dorsal spinocerebellar tract, corticospinal tract degeneration, degeneration of primary sensory nuclei (cochlear nucleus, lateral geniculate nucleus), and loss of the dentate nucleus of the cerebellum. Like the unique symptomatology of Friedreich ataxia, this neuropathology is distinct from other neurodegenerative disorders, although it has substantial overlap with inherited vitamin E deficiency.^{10–11}

With the discovery of the disease-related mutation and its gene product, one would imagine that the relation of the neuropathology to the gene product, its function, and its pathophysiology would be readily apparent. However, many questions remain, some of which directly influence the obstacles faced in drug development and clinical trials for Friedreich ataxia. In this review, we explore these remaining questions and associated obstacles.

Question 1: What Level of Frataxin Deficiency is Needed to Cause Disease?

Although the exact function of frataxin in mitochondria is still not completely understood (see other articles in this volume), its overall role in disease appears clear: in the 97% of patients with Friedreich ataxia who carry 2 GAA expansions, relative absence of frataxin, from decreased transcription, gives rise directly to the pathophysiological sequelae of Friedreich ataxia. Consequently, therapies that increase frataxin should be the most directly beneficial for the disorder if timed appropriately.^{12–14} However, to measure response to therapy, one must measure frataxin levels directly in accessible tissues. Such assays have been designed, and the results reveal a series of surprises.^{15–18}

Frataxin levels have been measured in large cohorts in a variety of tissues, including muscle, peripheral blood mononuclear cells, whole blood, buccal cells, and platelets. The results are similar in each of these tissues. Levels of frataxin are roughly 5% to 20% of control values in patients with Friedreich ataxia, and there is a correlation between clinical severity and GAA repeat length with frataxin level. However, this is a surprisingly small decrease: in many inherited metabolic disorders, enzymatic levels fall to less than 5% of normal before causing disease.^{19–20} In addition, while carriers of Friedreich ataxia have intermediate levels, some mildly affected patients fall into the carrier range.

What is the explanation for such a small decrease? There are a variety of potentially artifactual reasons why only a modest decrease in frataxin is seen experimentally. First, frataxin measurements are being made from samples of unaffected tissues; frataxin deficiency in affected tissues may be much greater. Indeed, it has been hypothesized that the level of frataxin drops over time in affected tissues (but not unaffected) because of progressive expansion of GAA repeats in affected tissues.^{21–22} In addition, comparisons to metabolic disorders may be inherently unfair: many metabolic deficiencies can be compensated for through alternate pathways, while at present few clear redundancies have been found in the iron-sulfur-cluster assembly functions of frataxin.

However, such discrepancies in the expected levels of frataxin can also generate new scientific hypotheses. First, is it possible that other genetic modifiers outside of expanded GAA repeats are required for Friedreich ataxia, or produce disease in carriers? At present, no person with 2 abnormal *FXN* alleles and no clinical Friedreich ataxia phenotype has been identified. Furthermore, no clinical manifestations have been identified in carriers, though a question remains as to whether carriers are slightly more likely to have diabetes or

scoliosis.^{23–24} Still, among those patients who have 2 expanded *FXN* alleles, it remains possible and perhaps probable that modifiers exist that alter frataxin levels, either globally or in a tissue-specific manner. For example, DNA methylation and histone deacetylase activation appear to be 2 epigenetic processes involved in regulating frataxin levels.^{13,25}

Another possibility for a confounding of the clinical measures of frataxin is the possibility that the measurements are not made at the correct time. Measurements to date have been made with samples collected randomly. While potential circadian rhythms could confound any measurement, another possibility is that the relevant levels of frataxin are not those found in quiescent states, but rather in states in which frataxin is upregulated in response to cellular or oxidative stress. The expanded GAA repeat in *FXN* could readily interfere with rapid increases in transcription above basal levels. Unfortunately, although this hypothesis is plausible and interesting, it is at present not supported or denied by objective data. Another uninvestigated temporal variability in frataxin measurement is developmental. All studies done to date have measured frataxin levels after presentation; no study has investigated the developmental time course in preclinical individuals. Levels of frataxin earlier in development might establish a pathophysiology that is self-propagating. In addition, as there is some variability in the GAA repeat length among cells, continued cellular division over time will select for the cells with the most normal levels of frataxin. This has been used to explain the statistical rise in frataxin levels in buccal cells with age. Thus a variety of scientifically interesting and testable ideas could give rise to the paradoxically high levels of frataxin.

The exact quantitative role of frataxin deficiency in Friedreich ataxia must also account for another group of patients: those with point mutations. Patients with point mutations in *FXN* have an increased risk of atypical features: visual loss, hearing loss, and retained reflexes (which could reflect either increased spasticity or decreased neuropathy).^{26–29} Most point mutations, such as those at RNA splice sites, the start codon, large deletions, and nonsense mutations, are predicted to lead to production of no functional protein. Most remaining missense mutations are found in the core of the protein, and are likely to lead to protein instability. Such mutations are thus likely to be associated with more severe phenotypes, including diabetes mellitus, hearing loss, and vision loss, simply on the basis that they produce less functional frataxin. However, 4 specific point mutations (G130V, I154F, W155R, and R165C/P) are found outside the core of the protein and could produce normal levels of stable protein. In studies examining frataxin levels in patients with point-mutation alleles, there is some evidence that this is true. However, the forms of frataxin with these specific point mutations have different functional properties. Interestingly, these individuals, particularly those with G130V and R165C/P mutations, may have very different phenotypes, with at times sparing of sensory nerves and a pathologic retention of reflexes consistent with spasticity.^{29–30} Thus, frataxin levels and frataxin function in different cells can both be important in defining the pathophysiological phenotype.

Does Reactive Oxygen Species Production Happen in Friedreich Ataxia?

Since the discovery of frataxin and the observation that mitochondrial iron accumulation occurs in Friedreich ataxia, the understanding of the downstream pathways has been dominated by the concept that frataxin deficiency leads to reactive oxygen species production, which leads in turn to cellular dysfunction and cellular death in Friedreich ataxia. However, years later the presence of reactive oxygen species production remains equivocal. Multiple in vivo spectroscopy studies have demonstrated impaired generation of adenosine triphosphate in muscle, consistent with proposed deficiencies in the mitochondrial electron transport chain.^{31–33} Still, the byproduct of abnormal adenosine triphosphate production, reactive oxygen species generation, has not been consistently shown.^{34–38} Initial

studies showed elevations in malondialdehyde and 8OH-2-deoxy guanosine, but such findings have not been reproduced consistently. More recently, reactive oxygen species-mediated DNA damage has been observed, but whether it is a direct consequence of frataxin deficiency remains unknown.³⁹

Why is reactive oxygen species generation not more readily observed? One possibility is that as patients become more affected, the failure to produce adenosine triphosphate becomes dominant and few reactive oxygen species are produced. If production of adenosine triphosphate were to be increased (for example by a therapeutic intervention), patients with Friedreich ataxia might be at more risk for reactive oxygen species production. Another possibility is that reactive oxygen species production is tissue selective, and the “signal” from pathologic production is lost in “noise” of normal reactive oxygen species production in intracellular signaling. This would be particularly true in studies of patients who take a large number of global antioxidants, which might mask production from all tissues.

Is it important to understand the answer to this question? Certainly so, from the perspective of therapeutic intervention. Some of the agents being developed for Friedreich ataxia have been proposed to have purely antioxidant properties rather than having both antioxidant properties and the ability to act directly in the electron transport chain. If reactive oxygen species production is actually normal in Friedreich ataxia, then pure antioxidants are destined to fail as therapeutics for the disorder. In addition, many of the simplest biomarkers would logically be based on reactive oxygen species production, and validating such biomarkers would improve the ability to assess drugs in phase 2 trials, but if little disease mediated reactive oxygen species generation occurs in Friedreich ataxia, then such measures are useless. Consequently, settling this issue is important for future drug development in Friedreich ataxia.

Why Does Friedreich Ataxia Affect Selected Neurons Far More Than Others?

The question of selective vulnerability appears in the understanding of almost all neurological disorders, including Alzheimer disease, Parkinson disease, and Huntington disease. While a variety of explanations can be offered for sporadic, nongenetic disorders, the most perplexing situations have arisen with genetic disorders. Frataxin, like huntingtin (the protein mutated in Huntington disease), is a widely expressed protein.⁴⁰ Although there are little data on the detailed anatomical mapping of frataxin expression in the nervous system, simple anatomic distribution of frataxin does not clearly match the differential cell loss characteristic of Friedreich ataxia.⁴⁰ Consequently, the unique neurodegenerative pattern of Friedreich ataxia can only be explained by more complex ideas.

One possibility for resolving this dilemma is that neurologists may have overestimated the neuronal selectivity of the disorder. With the improved definition of Friedreich ataxia by genetic criteria and the ability to perform more detailed clinical testing, the classical perspective of Friedreich ataxia as a relatively exclusive dorsal root ganglion-related disease is evolving. Magnetic resonance imaging now readily quantifies the slowly progressive changes in the dentate nucleus and its downstream axons in the superior cerebellar peduncle, and novel imaging suggests a more diffuse axonal process.^{41–43} Clinically, cognitive abnormalities are appearing in individuals at more advanced stages of the disorder.⁴⁴ Lastly, neuronal pathology not associated with cell death, specifically a grumose degeneration of Purkinje cell axons in the dentate nucleus in Friedreich ataxia, has now been defined.⁴⁵ These data suggest that the clinical phenotype may have more global features not appreciated before such detailed testing.

Still, such abnormalities cannot entirely explain the selective cell death of Friedreich ataxia. First, most of these new abnormalities are noted later in the disease, raising the question of the degree to which they are primary. More importantly, some of the selective neuropathology is reproduced in mouse models. A neuron-specific knockout of frataxin in mouse causes severe neurodegenerative changes like those of Friedreich ataxia, focused on dorsal root ganglion neurons and cerebellum, but also causes spongiform change in the cortex, a feature not seen in Friedreich ataxia.⁴⁶ Other models also recreate only partially the features of Friedreich ataxia. In mice expressing only an expanded copy of the human *FXN* gene, thus producing a partial knockdown of frataxin, the animals develop a late neurodegenerative disease with primarily degeneration of sensory nerves and the cerebellum;⁴⁷ while the animals are not severely affected, the pathology mimics that of late-onset Friedreich ataxia, (but lacks the severe, large-dorsal root ganglion-neuron selectivity of early onset Friedreich ataxia).^{48–49} A similar but more severe pattern emerges with mice in which *FXN* is deleted postnatally using an inducible promoter.⁵⁰ This shows that even though the mouse models do not fully recreate Friedreich ataxia, much of the selective neuropathology can be produced by lowering frataxin levels and thus reflects neuronally selective features of frataxin dependence.

Three neuronally selective components outside of frataxin distribution could potentially contribute to the cellular selectivity of Friedreich ataxia: (1) the relative level of oxidative phosphorylation in specific neurons; (2) the relative redundancy of frataxin function within specific neurons; and (3) the relative frataxin dependence of non-metabolic processes contributing to the survival of specific neurons. The selective pathology of Friedreich ataxia is frequently attributed to the high energy requirements of the affected cells: dorsal root ganglion neurons, upper motor neurons, cardiomyocytes, retinal ganglion cells, and auditory nerves, with a specific comparison to the overlap with other mitochondrial illnesses. Indeed, the dorsal root ganglion neurons and the upper motor neurons are among the longest in the body and thus require the most energy for carrying out axonal transport. However, lower motor neurons are equally active and relatively spared in Friedreich ataxia, and the dentate nucleus neurons are not particularly energy-requiring. In addition, the link to other mitochondrial disorders falls apart in the visual system. In most mitochondrial disorders, the photoreceptors, not the retinal ganglion cells, are most affected.^{51–54} Thus, a purely mitochondrial view does not explain the neuropathology of Friedreich ataxia.

Another possibility is the relative redundancy of different iron-sulfur-cluster-synthesizing pathway components. While no exact frataxin homologue is known at present, other components of the iron-sulfur-cluster synthetic machinery could conceivably modulate the relative dependence of cells on frataxin function. For example, knockout of the iron regulatory protein 2 in mice selectively causes motor neuron degeneration, reflecting the relative absence of compensatory mechanisms, while iron regulatory protein 1 knockout mice have limitations in iron-sulfur-cluster synthesis restricted to brown fat and kidney.^{55–57} Similar compensatory mechanisms may exist for frataxin deficiency, leading to cellular abnormalities in selected neurons.

A final possibility is that the most vulnerable neurons in Friedreich ataxia share another susceptibility not yet understood. To aid in identifying such susceptibility, it is helpful to review the temporal course of neuroanatomical and clinical abnormalities in Friedreich ataxia, and the specific anatomical features of the proprioceptive inputs to the cerebellum. At onset of clinical disease, patients frequently have absent reflexes and sensory nerve action potentials in their legs with no change over the next several years; large-fiber deficiency appears to be from early loss of peripheral sensory neurons in a nonprogressive manner.⁵⁸ In addition, the spine is already small, reflecting a significant atrophy of the spinal cord, again likely a direct result of early loss of primary sensory neurons and

deterioration of posterior columns.⁵⁹ Although most autopsy studies are performed on patients well after onset of symptoms, a recent report shows severe atrophy of the spinal cord in an individual who was clinically asymptomatic.⁶⁰ Thus, much of the sensory-pathway loss precedes clinical presentation. Strangely, however, patients have not completely lost sensation at that time, suggesting that the loss may be longstanding and compensated for early in the disease.

One other unusual feature of the neurodegeneration in Friedreich ataxia is the direct connectivity of the major neurons involved, particularly the connection of the proprioceptive dorsal root ganglion axon to the cell bodies in the nucleus dorsalis of Clarke. The proprioceptive-neuron-to-nucleus dorsalis of Clarke-cell synapse is uncommon in that there is a one-to-one relationship between the proprioceptive axon and the nucleus dorsalis of Clarke cell.^{61–63} The trophic relationship during the developmentally critical period is sufficiently strong that in cats the early loss of proprioceptive axons leads to death of nucleus dorsalis of Clarke cells. If extrapolated to humans, this would suggest that, in addition to the progressive aspect of Friedreich ataxia, some of the deficit may be developmental in origin. Interestingly, inherited vitamin E deficiency, which shares a phenotype and the loss of the large dorsal root ganglion neurons with Friedreich ataxia, does not clearly include loss of the dorsal spinocerebellar tract.⁶⁴ As this most likely represents a postnatal disorder (as maternal vitamin E should be sufficient before birth), this contrast demonstrates another potential developmental link to the selective neuropathology of Friedreich ataxia.

Furthermore, this is consistent with basic neurodevelopmental paradigms, in which peripheral primary sensory and motor systems are controlled by late prenatal apoptotic events. Motor neurons, sympathetic neurons and sensory neurons all survive programmed cell death based on retrograde transport of peripheral growth factors.⁶⁵ The large dorsal root ganglion neurons require a specific growth factor, neutotrophin-3 (NT-3).^{66–67} If axonal transport (an energy-requiring process) of neutotrophin-3 required full frataxin expression to completely save large dorsal root ganglion neurons, then a subset of such neurons might be lost early, giving rise to secondary loss of cells of the nucleus dorsalis of Clarke early and developmental contributions to Friedreich ataxia. Neurotrophic tyrosine kinase receptor, type 3 (TrkC) is a marker for large proprioceptive neurons of the dorsal root ganglion, and selectively binds neutotrophin-3.⁶⁸ Interference of neutotrophin-3 / Neurotrophic tyrosine kinase receptor, type 3 signaling early in development may affect the survival and/or development of large sensory neurons. If decreased frataxin expression affects axonal transport, exogenous NT-3 could be used to protect and/or rescue function.

Is there a developmental component to Friedreich ataxia? Complete frataxin knockout gives rise to early prenatal death showing that frataxin has developmental roles.⁶⁹ However, there are no direct data addressing the questions raised here. Most likely, the selective neuropathology of Friedreich ataxia reflects a variety of the issues noted: metabolic demand, developmental features, and relative redundancy. The relative roles may be graded, with the developmental components being least significant in later-onset patients; such individuals are more likely to have preserved reflexes and even on occasion almost normal sensory potentials.⁷⁰ However, determining the answers to the cause of selective neuronal loss is crucial for therapeutics in Friedreich ataxia. If large dorsal root ganglion neurons are absent at presentation, then therapies directed to save them specifically—for example, targeted gene therapy—will be unsuccessful. In addition, if much of the damage is preclinical, some of the events later in the disease may be preordained, and instead reflect neuron-related cell death pathways that are not even necessarily dependent on frataxin expression.

Conclusions

Few disorders of the rarity of Friedreich ataxia have been associated with such rapid research advances. With the gene mutation identified slightly more than 15 years ago, few could have hoped for so many agents to be undergoing development. Still, understanding why some succeed and some fail, and creating even better interventions, will require resolution of some of the questions raised here. The next few years may be as exciting as the previous 15 and lead us closer to a complete cure for Friedreich ataxia.

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Table 1**Correlation of Neuropathology and Clinical Features in FRDA**

Symptom/Sign	Anatomic Localization
Ataxia	Proprioceptive Neurons of DRG
	Dorsal spinocerebellar tract
	Dentate nucleus of cerebellum
Dysarthria	Dentate nucleus of cerebellum
Loss of proprioception	Proprioceptive Neurons of DRG
Loss of other sensory modalities	Other non-proprioceptive neurons of DRG
Vision loss	Lateral geniculate
Hearing loss	Cochlear nuclei
Weakness	Corticospinal tracts
	Metabolic myopathy
Spasticity	Corticospinal tracts

Abbreviations: DRG, dorsal root ganglion; FRDA, Friedreich ataxia