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Cardiomyopathy of Friedreich's Ataxia: Use of Mouse Models to Understand Human Disease and Guide Therapeutic Development

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Abstract

Friedreich's ataxia is a multisystem disorder of mitochondrial function affecting primarily the heart and brain. Patients experience a severe cardiomyopathy that can progress to heart failure and death. Although the gene defect is known, the precise function of the deficient mitochondrial protein, frataxin, is not known and limits therapeutic development. Animal models have been valuable for understanding the basic events of this disease. A significant need exists to focus greater attention on the heart disease in Friedreich's ataxia, to understand its long-term outcome, and to develop new therapeutic strategies using existing medications and approaches. This review discusses some key features of the cardiomyopathy in Friedreich's ataxia and potential therapeutic developments.

Keywords

Cardiomyopathy; Friedreich's ataxia; Mitochondria; Therapy

Introduction

Friedreich's ataxia (FA) is the most common inherited ataxia in man. It is a relentlessly progressive cardio- and neurodegenerative disease typically beginning in early to mid childhood that within 10–15 years of onset leads to loss of motor skills and, ultimately, inability to stand or walk [31]. Patients experience a primary neurodegeneration of the dorsal root ganglia leading to the hallmark clinical findings of progressive ataxia [14, 69], debilitating scoliosis, and severe cardiomyopathy that causes death in the third to fifth decade of life [58]. Significant other problems include diabetes in 10% of patients and severe scoliosis requiring surgical correction. No cure exists.

Genetics of FA

The incidence of FA is approximately 1 in 30,000 people (prevalence of 1:50,000). It occurs with equal frequency in males and females [78] and has a carrier frequency of 1:60–1:120 [20, 21, 72, 84]. Genetically, FA is passed on as an autosomal recessive trait caused predominantly by a GAA triplet expansion in the first intron of the human frataxin gene (*FRDA*) on chromosome 9q21.11 (reviewed in [57, 58]). Patients frequently have 600–1,200

repeats of this GAA triplet, predicted functionally to “silence” the human *FRDA* gene through the formation of triple-stranded DNA [76] as a result of the GAA repeats forming non-Watson–Crick pairings with the bases of the DNA double helix [23]. The result is transcriptional inhibition and partial silencing of the *FRDA* locus, with severely reduced expression of the protein, frataxin. Thus, patients with FA have levels of frataxin ranging from 5% to approximately 25% of normal. The heterozygous parents are not affected. A correlation exists between the GAA repeat number and the onset and severity of clinical symptoms, with higher repeat numbers related to more severe disease and cardiomyopathy [22, 55, 64].

Clinical Cardiac Findings in FA

The cardiomyopathy in FA is a significant problem, affecting virtually all hearts to a variable degree [31, 35, 50]. The heart becomes hypertrophic, typically maintaining adequate systolic function [41] until shortly before death [64]. Arrhythmias are common and frequently intractable. Cardiac magnetic resonance imaging (MRI) demonstrates the severe hypertrophy very well, as shown in Fig. 1, but echocardiography also is excellent for long-term evaluation of left ventricular wall thickness and function (Fig. 2). Both ventricles are affected, and arrhythmias are common, especially those of atrial origin. Although the primary cause of death in FA is heart failure [30], surprisingly little has been written about the natural history and pathology of the FA heart, with most publications focusing instead on the dramatic neuropathology. Since 1966, fewer than 200 publications have been written on the heart in FA, compared with more than 2,500 publications reporting on the neurologic findings of this disease.

The cardiomyopathy of FA most often presents symptoms in the second to third decade of life, although a significant range exists. For example, a hypertrophic heart can be the initial finding, resulting even in transplantation before the diagnosis of FA [43], whereas other patients may not manifest cardiac symptoms. Findings have shown significantly less myocardial energy generation in FA patients than in control subjects. The degree of reduction correlates with the degree of hypertrophy [6] and may even precede its development [48]. Use of the antioxidant and coenzyme Q mimetic, idebenone, may help to improve myocardial energy deficiency and decrease hypertrophy both by acting as an antioxidant and by enhancing electron transport within mitochondria [51, 74]. This has been the case in short-term (1 year) studies. However, longer-term studies using idebenone have shown a reduction in cardiac mass but not a decrease in arrhythmias or progression of heart failure [59, 87], and there is no research on the clinical relevance of these changes. Antioxidant therapies have not shown any benefit or improvement in terms of neurologic or cardiac symptoms [40, 49]. However, a key limitation of antioxidant therapies is that they frequently have a very short half-life and thus are not at therapeutic levels. Therefore, measures to extend the half-life of these agents may improve their efficacy.

Multiple authors have noted that the cardiac phenotype is quite variable [33, 41] and that cardiac MRI of left ventricle (LV) mass correlates with the GAA repeat number and longer duration of disease [64]. Hypertrophy may progress to dilation in time, giving the appearance by echocardiography that ventricular hypertrophy has improved [33]. Earlier echocardiography and radionuclide studies demonstrated good systolic function of both ventricles but impaired diastolic filling [19, 56]. These findings are consistent with the histologic appearance of fibrosis in the LV wall and suggest that there may be diastolic dysfunction in the FA heart. Virtually all hearts are affected in FA, and subtle electrocardiographic (ECG) findings are frequently among the earliest findings of cardiomyopathy. In Fig. 3a and b, ECGs from two patients demonstrate the nonspecific T-

wave changes often seen in the lateral chest leads. However, ECG is not diagnostic of FA and has not shown utility for prognosis in this disease.

At the microscopic level, changes in the LV include cardiomyocyte hypertrophy, focal necrosis, and diffuse fibrosis [35]. Electron microscopy shows mitochondrial proliferation, loss of contractile sarcomeres, and electron dense particles within mitochondria that may represent iron deposition [52]. This is strikingly similar to what is found in the mouse heart with frataxin (FXN) deficiency (Fig. 9). Recent studies of patients with FA using cardiac MRI and adenosine have shown that the heart has a significantly decreased myocardial perfusion reserve index, which parallels the onset of metabolic syndrome [65]. Furthermore, the impaired perfusion reserve does not correlate with degree of hypertrophy or fibrosis, suggesting that this may be an important tool for identifying potential therapeutic targets to prevent the development or progression of heart failure.

Basic Findings and Mouse Models

The frataxin protein is an essential and highly conserved protein expressed in most eukaryotic organisms (reviewed by Puccio and Koenig [61]). For example, more than 90% homology exists between the mature frataxin proteins of man and mouse (Fig. 4). The FXN protein is targeted to mitochondria and appears to function in mitochondrial iron homeostasis [36], notably in the de novo biosynthesis of iron–sulfur cluster proteins [83, 85, 92] and probably heme [71, 91]. The 210 amino acid precursor FXN protein (23.1 kDa) contains a mitochondrial targeting sequence at the amino terminus that is processed in two steps by the mitochondrial matrix processing peptidase as it is imported into the matrix [9], yielding a 130 amino acid, 14.2-kDa protein [12, 77]. In its absence, free iron accumulates in mitochondria, with loss of activity of Fe–S-containing proteins [45, 73] as well as energy production [47, 48]. From a therapeutic standpoint, this is important: if the cell ever encounters frataxin protein or mRNA, it is handled appropriately [60]. Currently, treatment options logically include antioxidants and iron chelation [38, 70]. Although early clinical trials have shown mild biochemical improvement [32], they have not been clinically effective. It also is probable that significant and permanent damage has already occurred by the time the diagnosis of FA is made, and recent studies have supported earlier intervention [4].

Animal models mimicking FA have been invaluable both for the study of the basic biology and for the clinical implications of this disease. Although highly useful, each of these models also has significant limitations. Two basic strategies have been used to recapitulate this disease in the mouse. The first strategy accomplishes a conditional ablation of the mouse *FRDA* gene by the Puccio lab, in which exon 4 of the *FRDA* gene is deleted using Cre-mediated excision in a tissue-specific fashion [62]. This causes complete absence of the FXN protein only in the target tissues. This is necessary because complete ablation of the *FRDA* gene in the mouse is embryonically lethal, demonstrating the importance of early frataxin expression [13]. However, when the *FRDA* gene is ablated in heart and brain or in heart alone (skeletal muscle expression also is ablated but has no phenotype), then significant phenotypes are generated that recapitulate many features of FA. For example, mice in which the *FRDA* gene is ablated in brain (neural tissues) and heart demonstrate a severe neurologic phenotype, cardiomyopathy (dilated), and a very short life span [62], with less than 20% surviving beyond 30 days of life. These mice grow poorly (Fig. 5a, b) compared with their heterozygous littermates, demonstrating ataxia and loss of proprioception in behavioral studies such as the rotarod. As with FA in the human heart, clear evidence points to iron deposition in the mouse hearts, as shown by Perls' stain (Fig. 6a, b), and to severe fibrosis (Fig. 6c, d). Analysis of hearts from the *FRDA* KO animals shows that they have statistically higher apoptosis counts than age-matched wild-type

controls, indicating ongoing cardiomyocyte loss (Fig. 7a–c). In contrast, mice in which the *FRDA* gene is ablated only in sarcomeric tissues (heart and skeletal muscle) predictably do not have ataxia but experience a cardiac phenotype of hypertrophic cardiomyopathy very reminiscent of the human heart disease [90]. Thus, this strategy mimics the phenotype of the human disease well but not the correct genotype. The mice also are lacking any FXN protein in the affected tissues (“knocked out”), whereas humans have residual low-level expression of FXN protein and thus a more variable presentation. These mice are especially useful for studying the histology of FA and for developing protein or enzyme replacement therapies such as with cell-penetrant peptide fusion proteins [68] (e.g., transactivator of transcription (TAT)-frataxin).

The cardiac pathology for these conditional knockout mice frequently is very similar to that for the hearts of patients with FA. In addition to iron deposition in the myocardium mentioned earlier, they also show a cardiomyopathy on echocardiography with evidence of diastolic dysfunction. For example, Fig. 8 shows the echocardiograms of a normal littermate mouse (Fig. 8a, b) and a conditional *FRDA* knockout mouse (Fig. 8c, d). The M-mode of the normal mouse (Fig. 8a) shows good LV function and a normal mitral inflow pattern based on the E-A waveform. In panel Fig. 8c, the M-mode demonstrates a dilated, poorly contractive LV with a slower heart rate and reversal of the E-A waveforms (Fig. 8d). Examination of the cardiomyocyte ultrastructure in these mice by electron microscopy is particularly informative. The normal mouse heart shown in Fig. 9a demonstrates normally shaped mitochondria tightly packed between rows of sarcomeres. The alignment of the sarcomeres is highly regular and robust. In contrast, Fig. 9b demonstrates the severe disorganization of the cardiomyocyte from an *FRDA* conditional knockout mouse heart. In this case, very few sarcomeres remain, and mitochondria have proliferated extensively. There is little evidence of the tight relationship between energy-generating mitochondria and the site of ATP utilization (sarcomere) seen in the normal mouse cardiomyocyte. This parallels exactly what is found in the human heart [52] and has strong clinical implications for patients with FA. Ultimately, LV contractile function decreases due to loss of contractile proteins and disruption of the normal mitochondria-to-sarcomere relationship.

In the second strategy, mice have been generated that have a “knock-in” of the pathologic human *FRDA* gene with GAA repeats into the mouse by both the Pook lab [2] and the Pandolfo lab [53]. In these models, the native mouse *FRDA* genes have been ablated (KO), leaving expression of frataxin dependent only on the human *FRDA* gene, which also contains the triplet expansion region. The phenotype is less severe in these animals, but the genotype more accurately mimics the human disease. These animals have been especially useful for studying therapeutic approaches that increase transcription of the *FRDA* gene containing the triplet expansion, such as histone deacetylase (HDAC) inhibitors [63].

Therapy for Mitochondrial Disorders

Repair of mitochondrial disorders such as FA is necessarily more complex than replacement of a cytosolic gene product and must consider not only the need to target and cross multiple membranes in mitochondria but also the fact that many enzymes in mitochondria are hydrophobic and not readily soluble. Additionally, many of the mitochondrial gene defects cause severe neurologic symptoms as the primary or most prominent phenotype, and drug delivery across the blood–brain barrier is notoriously difficult [3]. As a result, therapies for mitochondrial defects, such as with coenzyme Q for OXPHOS diseases, currently focus primarily on using small molecules to enhance flux through the electron transport chain [29] or on altering the precursor pool of substrate to avoid the defective metabolic pathway such as changing dietary intake of fatty acids to avoid medium chain lipids in medium-chain acyl-dehydrogenase deficiency [25]. To date, success with gene therapy has been very limited

[46], and enzyme replacement therapy (ERT) for mitochondrial defects in the intact animal has had no success, much less correction of a defect across the blood–brain barrier.

Background on ERT

Currently, ERT, or protein replacement therapy, is a well-established therapy for human diseases. The most common example is insulin injections for the treatment of diabetes. Other examples would be Pompe's disease [10] with recombinant human acid α -glucosidase and Gaucher disease with imiglucerase [26]. Multiple pharmaceutical houses, such as Genzyme, Shire, BioMarin, and Eli Lilly, produce proteins from bacterial or human cell lines for ERT. Surprisingly, ERT for mitochondrial disorders has not had significant success, most likely due to the dual difficulty of achieving cell penetration by a recombinant protein and import of the protein into the mitochondrial matrix. Thus, no cures currently exist for mitochondrial disorders, with most therapies providing “work-around” solutions to the metabolic defect. The combination of ERT with the power of cell-penetrating peptides such as TAT to deliver a therapeutic protein to mitochondria is a logical and exciting possibility for addressing mitochondrial disorders [66].

Background on Cell-Penetrant Peptides

The cell-penetrant peptide, TAT, is a protein transduction domain that has been used to transduce multiple cargos such as proteins into cells and tissues of animals and cells in culture. As a short cationic peptide derived from the larger TAT protein of HIV, TAT has cell-penetrant properties [17, 28, 79]. Cell-penetrant peptides typically are small cationic peptides that can transport a cargo of molecules such as proteins, peptides, or oligonucleotides into cells that otherwise cannot absorb large molecular weight compounds. Various transduction peptides such as polyarginine, synB5, antennapedia, herpes simplex virus type 1 protein, and many others have been used to transport complex proteins into cells and have shown promise as therapeutic strategies for several disease conditions [18, 54, 75, 86]. Delivery of a cargo by a cell-penetrant peptide has been accomplished for multiple organelles such as mitochondria (Fig. 10) [17], lysosomes [93], and the nucleus [39], which are capable of delivering a cargo across the placenta [16]. Finally, TAT has already been used to replace missing cytosolic enzymes such as purine nucleoside phosphorylase [86] in animal models of disease and in a cell culture mitochondrial model of human disease (i.e., lipoamide dehydrogenase deficiency) [67]. Additionally, it is not necessary to restore all the missing protein to restore adequate amounts of enzymatic activity in mitochondria [88]. This would be key for the human disease, in which patients have reduced expression of the frataxin protein compared with some of the current animal models in which the gene product has been ablated [62, 90]. Thus, cell-penetrant peptides offer an exciting potential to deliver protein replacement for many inherited and acquired cardiac diseases [88]. This is important because delivery of drugs and therapeutic compounds is primarily limited by their ability to penetrate the cell membrane.

The bioavailability of compounds targeted to intracellular sites also depends on the conflicting requirements of their being sufficiently polar for administration and distribution yet nonpolar enough to diffuse through the nonpolar lipid bilayer of the cell [3]. In addition, the molecular weight of most drugs that can easily traverse the lipid membrane is approximately 500 Da [44]. Thus, most successful compounds have narrow physical characteristics. Many promising drugs fail because they fall outside this range, and efforts to make them available may be toxic. In addition to this, many sites of action for presumed therapeutic compounds, such as enzymes or regulatory proteins, require processing and targeting of the compound once inside the cell. This step alone represents a significant hurdle to the development of many strategies for repairing defects within a cell. Already,

TAT has been used to replace missing cytosolic enzymes such as purine nucleoside phosphorylase in animal models of disease [86]. Thus, cell-penetrant peptides offer an exciting potential to overcome many of these problems for ERT.

We [16, 17] and other investigators [67] have shown that TAT can transduce the mitochondrial membranes to deliver a cargo. Figure 11 demonstrates the hypothesis and evidence of a cell-penetrant peptide, TAT, attached via a mitochondrial targeting signal from mitochondrial malate dehydrogenase (mMDH) to enhanced green fluorescent protein (eGFP). This protein is able to move across the cell membrane and localize in the mitochondrial matrix independently of the mitochondrial import apparatus where it is processed appropriately. The mechanism whereby this occurs is not well understood but appears to involve sodium channels in the mitochondrial membranes [68]. This is in contrast to the more complex multistep import of the natively expressed and imported mitochondrial protein shown in Fig. 12. In this case, the nuclear encoded protein is first translated in the cytosol, recognized by the mitochondrial receptor, imported via the ATP requiring import apparatus, and processed and folded in the matrix. Irrespective of the mechanism whereby a replacement protein reaches mitochondria, there are three key points regarding ERT for mitochondrial defects:

1. The native mitochondrial targeting sequence may be necessary for maximal restoration of enzymatic function. Rapoport et al. [66, 67] showed that it was necessary to have the lipoamide dehydrogenase (LAD) mitochondrial targeting sequence for maximal restoration of LAD activity in patients with the mitochondrial disorder: LAD using TAT-LAD [5]. Deleting the mitochondrial targeting sequence (TAT- Δ -LAD) restored a significantly lesser amount of LAD activity within mitochondria. This is a subtle but important point to consider: TAT can move both ways across a membrane and thus pull the therapeutic cargo out of mitochondria. By including the mitochondrial targeting sequence, the matrix-processing peptidases recognize the sequence and clip it. The cargo is left in the matrix, whereas the TAT peptide can transduce out of the mitochondrion (Fig. 11a). Repeated dosing should therefore result in accumulating amounts of cargo in mitochondria over time.
2. To restore activity of the native complex, TAT is capable of transducing single components of very large enzyme complexes. Proteins are normally imported into mitochondria in denatured form [24], and mitochondria are thus well equipped to refold proteins into an active configuration for incorporation with larger enzyme complexes. Because cell-penetrant peptides depend on the fusion protein being sufficiently denatured to make the penetrant peptide available for initiation of transduction, protein transduction technology is perfectly suited for ERT in mitochondria.
3. It is not necessary to restore all the missing protein to restore adequate amounts of enzymatic activity in mitochondria. Small amounts of normal protein may be adequate to repair the defect. This concept also is important for organs into which it is typically difficult to deliver drugs, such as the brain or placenta. Findings have shown that TAT crosses the blood-brain barrier and placenta quite well [7, 16, 79]. This opens the possibility of initiating ERT for mitochondrial defects before birth or for neurologic disorders that manifest well after birth.

Treatment Considerations

The cardiac output of affected patients experiencing FA often may reflect the characteristics of a restrictive cardiomyopathy with a greater dependence on heart rate to maintain adequate output. If this is the case, there will predictably be an increase in LV filling pressures, with

resultant atrial arrhythmias commonly seen in advanced FA. Use of after-load-reducing agents, such as losartan or angiotensin-converting enzyme (ACE) inhibitors, may be beneficial in long-term treatment of this heart disease. It also may decrease the physiologic incentive for cardiac hypertrophy and energy utilization by ventricular cardiomyocytes. In contrast, those agents that slow heart rate and decrease oxygen utilization, such as carvedilol or other β -adrenergic blocking agents, may not be well tolerated. For example, if diastolic filling is impaired due to a restrictive characteristic of this cardiomyopathy, then slowing of the heart rate would predictably decrease cardiac output. The literature is mixed with regard to the use of β -adrenergic blocking in cardiomyopathies of childhood. Isolated reports describe the use of high-dose β -adrenergic blocking agents in FA as either beneficial or of little value [40, 42]. For example, carvedilol is beneficial in adult-onset ischemic cardiomyopathy but has little effect on cardiomyopathies of childhood, in which the etiology of the heart failure is much different and variable [82].

Currently, our treatment options are very limited and logically include antioxidants, such as idebenone, and iron chelation, such as with deferiprone [38, 70]. In addition, management of heart failure symptoms often requires conventional therapies with afterload reduction, salt restriction, and diuresis. Although early clinical trials have shown modest biochemical improvement [32], these therapies have not resulted in substantial sustained clinical improvement because they are designed to control downstream events resulting from the loss of FXN, and the cellular damage is progressive. It also is probable that significant and permanent damage has already occurred by the time the diagnosis of FA is made, making these therapeutic approaches less effective. Recent studies have supported earlier intervention. For example, according to the findings of Boddaert et al. [4], iron chelation with a membrane-permeant chelator that shuttles iron to transferrin reduced the brain iron load and had the greatest effect in the youngest patients. If confirmed, this is a strong argument to begin therapy for FA as early as possible and supports the drive toward newborn screening for FA (see commentary by Isaya [37]). Expansion of newborn screening to include diseases such as FA has been advocated in a recent publication describing recommendations for uniform newborn screening [89]. Work is underway at Mayo Clinic to develop a protein-based assay to screen newborns for FA (supported by the Friedreich's Ataxia Research Alliance). However, despite concerted clinical efforts with antioxidant and iron chelation strategies, no therapy has been substantially effective for this disease.

Transplantation also has been offered as a therapy for the severe heart failure found in FA, but it is not commonly performed. Approximately five transplantation cases have been reported in the literature, with the first dating to 2001 [43]. As reported, all the patients did well with their transplant. Several showed significant improvement in their motor skills and strength, suggesting that part of the symptoms of neuromuscular disease may have resulted from severe congestive heart failure (CHF) instead [80]. Although FA typically is considered a hypertrophic heart disease, several of the patients exhibited dilated cardiomyopathy with severe CHF (New York Heart Association class 3 or worse) at the time of transplantation. This also has been described by earlier authors [1, 11] and may represent a terminal stage of the cardiomyopathy [8, 33]. Interestingly, progression of neuromuscular findings, such as ataxia, was arrested for prolonged periods for several of these patients after transplantation [43, 80]. No significant deleterious effects of immunosuppression have been noted beyond prednisone-induced diabetes [80]. Key considerations for transplantation in FA include the life span of the patient, which typically will exceed the life of the transplant [81], and the degree of associated comorbidities in FA, such as severe scoliosis causing respiratory compromise. Other centers have since followed suit and currently offer transplantation for select patients with FA as well as other neuromuscular disorders.

Summary

Friedreich's ataxia is a prime example of the multisystem effects that mitochondrial diseases can present with in clinical medicine. Thus, cardiomyopathy may be a presenting finding (murmur at examination), with the diagnosis of ataxia following some years later. More commonly, ataxia is the finding at presentation. Treatment of FA is confounded by the elusive function of the frataxin protein and the need to deliver a therapy into mitochondria. It also is hindered by the lack of clinical understanding of the heart disease in FA. Particularly lacking are long-term studies. New discovery certainly is rapidly advancing our understanding of the basic biology of FA, and the lessons learned have great potential for application to other mitochondrial diseases. Thus, a great need exists to understand the natural history and outcome of the heart disease in FA. But given the relatively small amount of literature on the heart in FA compared with that for the neurologic and histologic phenotype, greater emphasis on clinical studies and trials examining this mitochondrial cardiomyopathy are urgently needed to advance the development of therapies.

Multiple lines of investigation being aggressively pursued to develop innovative strategies likely will have application to other mitochondrial disorders as well. Two strategies worthy of note directly attack the problem in FA. The goal of the first strategy is to improve the expression of the frataxin protein by increasing ability of the DNA transcription machinery to read through the diseased *FRDA* gene. Work on this strategy has been conducted by Joel Gottesfeld, who reported earlier that histone deacetylase (HDAC) inhibitors would increase the frataxin mRNA transcript and could increase FXN protein levels of cells in culture [34]. When HDAC inhibitors were applied to the FA mice with expression of the diseased human *FRDA* gene (the "knock-in" mouse described earlier), an increase in FXN protein occurred [63]. This strategy also has significant potential for application to other triplet expansion diseases.

Applying the second strategy, our laboratory has pursued an enzyme-replacement approach, which genetically fuses the missing protein, FXN, to a cell-penetrant peptide, TAT, to deliver the protein into the cell and mitochondria. We reported earlier that TAT was capable of pulling exogenous proteins across cell and mitochondrial membranes to deliver a cargo to the matrix of mitochondria in both isolated cells and tissues of the intact mouse [15, 17, 68]. In fact, TAT even crosses the placenta to deliver proteins to mitochondria of the fetus [16], thus offering the potential for initiating therapies for mitochondrial defects before birth. Studies underway use the conditional ablation knockout mice from the Puccio lab to explore this approach. Early results are highly promising (data in preparation), and other investigators also have recently reported that TAT can deliver therapeutic proteins in mouse or human models of disease [27, 67, 86].

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References

1. Alboliras ET, Shub C, Gomez MR, Edwards WD, Hagler DJ, Reeder GS, Seward JB, Tajik AJ. Spectrum of cardiac involvement in Friedreich's ataxia: clinical, electrocardiographic, and echocardiographic observations. *Am J Cardiol*. 1986; 58:518–524. [PubMed: 2944367]
2. Al-Mahdawi S, Pinto RM, Ismail O, Varshney D, Lympieri S, Sandi C, Trabzuni D, Pook M. The Friedreich ataxia GAA repeat expansion mutation induces comparable epigenetic changes in human

- and transgenic mouse brain and heart tissues. *Hum Mol Genet.* 2008; 17:735–746. [PubMed: 18045775]
3. Begley DJ. The blood–brain barrier: principles for targeting peptides and drugs to the central nervous system. *J Pharm Pharmacol.* 1996; 48:136–146. [PubMed: 8935161]
 4. Boddaert N, Le Quan Sang KH, Rotig A, Leroy-Willig A, Gallet S, Brunelle F, Sidi D, Thalabard JC, Munnich A, Cabantchik ZI. Selective iron chelation in Friedreich ataxia: biologic and clinical implications. *Blood.* 2007; 110:401–408. [PubMed: 17379741]
 5. Brautigam CA, Chuang JL, Tomchick DR, Machius M, Chuang DT. Crystal structure of human dihydrolipoamide dehydrogenase: NAD⁺/NADH binding and the structural basis of disease-causing mutations. *J Mol Biol.* 2005; 350:543–552. [PubMed: 15946682]
 6. Bunse M, Bit-Avragim N, Riefflin A, Perrot A, Schmidt O, Kreuz FR, Dietz R, Jung WI, Osterziel KJ. Cardiac energetics correlates to myocardial hypertrophy in Friedreich's ataxia. *Ann Neurol.* 2003; 53:121–123. [PubMed: 12509856]
 7. Cao G, Pei W, Ge H, Liang Q, Luo Y, Sharp FR, Lu A, Ran R, Graham SH, Chen J. In vivo delivery of a bcl-xl fusion protein containing the TAT protein transduction domain protects against ischemic brain injury and neuronal apoptosis. *J Neurosci.* 2002; 22:5423–5431. [PubMed: 12097494]
 8. Casazza F, Morpurgo M. The varying evolution of Friedreich's ataxia cardiomyopathy. *Am J Cardiol.* 1996; 77:895–898. [PubMed: 8623752]
 9. Cavadini P, Adamec J, Taroni F, Gakh O, Isaya G. Two-step processing of human frataxin by mitochondrial processing peptidase: precursor and intermediate forms are cleaved at different rates. *J Biol Chem.* 2000; 275:41469–41475. [PubMed: 11020385]
 10. Chakrapani A, Vellodi A, Robinson P, Jones S, Wraith JE. Treatment of infantile Pompe disease with alglucosidase alpha: the UK experience. *J Inherit Metab Dis.* 2010; 33:747–750. [PubMed: 20865334]
 11. Child JS, Perloff JK, Bach PM, Wolfe AD, Perlman S, Kark RA. Cardiac involvement in Friedreich's ataxia: a clinical study of 75 patients. *J Am Coll Cardiol.* 1986; 7:1370–1378. [PubMed: 2940284]
 12. Condo I, Ventura N, Malisan F, Rufini A, Tomassini B, Testi R. In vivo maturation of human frataxin. *Hum Mol Genet.* 2007; 16:1534–1540. [PubMed: 17468497]
 13. Cossee M, Puccio H, Gansmuller A, Koutnikova H, Dierich A, LeMeur M, Fischbeck K, Dolle P, Koenig M. Inactivation of the Friedreich ataxia mouse gene leads to early embryonic lethality without iron accumulation. *Hum Mol Genet.* 2000; 9:1219–1226. [PubMed: 10767347]
 14. De Biase I, Rasmussen A, Endres D, Al-Mahdawi S, Monticelli A, Coccozza S, Pook M, Bidichandani SI. Progressive GAA expansions in dorsal root ganglia of Friedreich's ataxia patients. *Ann Neurol.* 2007; 61:55–60. [PubMed: 17262846]
 15. Del Gaizo Moore V, Payne RM. Transactivator of transcription fusion protein transduction causes membrane inversion. *J Biol Chem.* 2004; 279:32541–32544. [PubMed: 15169776]
 16. Del Gaizo V, Payne RM. A novel TAT-mitochondrial signal sequence fusion protein is processed, stays in mitochondria, and crosses the placenta. *Mol Ther.* 2003; 7:720–730. [PubMed: 12788645]
 17. Del Gaizo-Moore V, MacKenzie JA, Payne RM. Targeting proteins to mitochondria using TAT. *Mol Genet Metab.* 2003; 80:170–180. [PubMed: 14567966]
 18. Drin G, Cottin S, Blanc E, Rees AR, Tamsamani J. Studies on the internalization mechanism of cationic cell-penetrating peptides. *J Biol Chem.* 2003; 278:31192–31201. [PubMed: 12783857]
 19. Dutka DP, Donnelly JE, Palka P, Lange A, Nunez DJ, Niho-yannopoulos P. Echocardiographic characterization of cardiomyopathy in Friedreich's ataxia with tissue Doppler echocardiographically derived myocardial velocity gradients. *Circulation.* 2000; 102:1276–1282. [PubMed: 10982543]
 20. Epplen C, Epplen JT, Frank G, Mitterski B, Santos EJ, Schols L. Differential stability of the (GAA)_n tract in the Friedreich ataxia (STM7) gene. *Hum Genet.* 1997; 99:834–836. [PubMed: 9187683]
 21. Filla A, De Michele G, Marconi R, Bucci L, Carillo C, Castellano AE, Iorio L, Kniahynicki C, Rossi F, Campanella G. Prevalence of hereditary ataxias and spastic paraplegias in Molise, a region of Italy. *J Neurol.* 1992; 239:351–353. [PubMed: 1512613]

22. Filla A, De Michele G, Cavalcanti F, Pianese L, Monticelli A, Campanella G, Coccozza S. The relationship between tri-nucleotide (GAA) repeat length and clinical features in Friedreich ataxia. *Am J Hum Genet.* 1996; 59:554–560. [PubMed: 8751856]
23. Gacy AM, Goellner GM, Spiro C, Chen X, Gupta G, Bradbury EM, Dyer RB, Mikesell MJ, Yao JZ, Johnson AJ, Richter A, Melancon SB, McMurray CT. GAA instability in Friedreich's ataxia shares a common DNA-directed and intraallelic mechanism with other trinucleotide diseases. *Mol Cell.* 1998; 1:583–593. [PubMed: 9660942]
24. Gaume B, Klaus C, Ungermann C, Guiard B, Neupert W, Brunner M. Unfolding of preproteins upon import into mitochondria. *EMBO J.* 1998; 17:6497–6507. [PubMed: 9822595]
25. Gillingham MB, Scott B, Elliott D, Harding CO. Metabolic control during exercise with and without medium-chain triglycerides (MCT) in children with long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) or trifunctional protein (TFP) deficiency. *Mol Genet Metab.* 2006; 89:58–63. [PubMed: 16876451]
26. Goker-Alpan O. Optimal therapy in Gaucher disease. *Ther Clin Risk Manag.* 2010; 6:315–323. [PubMed: 20668714]
27. Gustafsson AB, Sayen MR, Williams SD, Crow MT, Gottlieb RA. TAT protein transduction into isolated perfused hearts: TAT-apoptosis repressor with caspase recruitment domain is cardioprotective. *Circulation.* 2002; 106:735–739. [PubMed: 12163436]
28. Gustafsson AB, Gottlieb RA, Granville DJ. TAT-mediated protein transduction: delivering biologically active proteins to the heart. *Methods Mol Med.* 2005; 112:81–90. [PubMed: 16010012]
29. Haas RH. The evidence basis for coenzyme Q therapy in oxidative phosphorylation disease. *Mitochondrion.* 2007; 7(Suppl):S136–S145. [PubMed: 17485245]
30. Hanley A, Corrigan R, Mohammad S, MacMahon B. Friedreich's ataxia cardiomyopathy: case based discussion and management issues. *Ir Med J.* 2010; 103:117–118. [PubMed: 20486316]
31. Harding AE. Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. *Brain.* 1981; 104:589–620. [PubMed: 7272714]
32. Hart PE, Lodi R, Rajagopalan B, Bradley JL, Crilley JG, Turner C, Blamire AM, Manners D, Styles P, Schapira AH, Cooper JM. Antioxidant treatment of patients with Friedreich ataxia: four-year follow-up. *Arch Neurol.* 2005; 62:621–626. [PubMed: 15824263]
33. Hawley RJ, Gottdiener JS. Five-year follow-up of Friedreich's ataxia cardiomyopathy. *Arch Intern Med.* 1986; 146:483–488. [PubMed: 3954519]
34. Herman D, Jenssen K, Burnett R, Soragni E, Perlman SL, Gottesfeld JM. Histone deacetylase inhibitors reverse gene silencing in Friedreich's ataxia. *Nat Chem Biol.* 2006; 2:551–558. [PubMed: 16921367]
35. Hewer R. The heart in Friedreich's ataxia. *Br Heart J.* 1969; 31:5–14. [PubMed: 5774037]
36. Huang ML, Becker EM, Whitnall M, Rahmanto YS, Ponka P, Richardson DR. Elucidation of the mechanism of mitochondrial iron loading in Friedreich's ataxia by analysis of a mouse mutant. *Proc Natl Acad Sci USA.* 2009; 106:16381–16386. [PubMed: 19805308]
37. Isaya G. Ironing out a therapy for Friedreich ataxia. *Blood.* 2007; 110:1–2.
38. Jauslin ML, Meier T, Smith RA, Murphy MP. Mitochondria-targeted antioxidants protect Friedreich ataxia fibroblasts from endogenous oxidative stress more effectively than untargeted antioxidants. *Faseb J.* 2003; 17:1972–1974. [PubMed: 12923074]
39. Jensen KD, Nori A, Tijerina M, Kopeckova P, Kopecek J. Cytoplasmic delivery and nuclear targeting of synthetic macromolecules. *J Control Release.* 2003; 87:89–105. [PubMed: 12618026]
40. Kearney M, Orrell RW, Fahey M, Pandolfo M. Antioxidants and other pharmacological treatments for Friedreich ataxia. *Cochrane Database Syst Rev.* 2009; CD007791. [PubMed: 19821439]
41. Kipps A, Alexander M, Colan SD, Gauvreau K, Smoot L, Crawford L, Darras BT, Blume ED. The longitudinal course of cardiomyopathy in Friedreich's ataxia during childhood. *Pediatr Cardiol.* 2009; 30:306–310. [PubMed: 18716706]
42. Kosutic J, Zamurovic D. High-dose beta-blocker hypertrophic cardiomyopathy therapy in a patient with Friedreich ataxia. *Pediatr Cardiol.* 2005; 26:727–730. [PubMed: 16235005]

43. Leonard H, Forsyth R. Friedreich's ataxia presenting after cardiac transplantation. *Arch Dis Child*. 2001; 84:167–168. [PubMed: 11159298]
44. Levin VA. Relationship of octanol/water partition coefficient and molecular weight to rat brain capillary permeability. *J Med Chem*. 1980; 23:682–684. [PubMed: 7392035]
45. Li K, Besse EK, Ha D, Kovtunovych G, Rouault TA. Iron-dependent regulation of frataxin expression: implications for treatment of Friedreich ataxia. *Hum Mol Genet*. 2008; 17:2265–2273. [PubMed: 18424449]
46. Lim F, Palomo GM, Mauritz C, Gimenez-Cassina A, Illana B, Wandosell F, Diaz-Nido J. Functional recovery in a Friedreich's ataxia mouse model by frataxin gene transfer using an HSV-1 amplicon vector. *Mol Ther*. 2007; 15:1072–1078. [PubMed: 17375064]
47. Lodi R, Cooper JM, Bradley JL, Manners D, Styles P, Taylor DJ, Schapira AH. Deficit of *in vivo* mitochondrial ATP production in patients with Friedreich ataxia. *Proc Natl Acad Sci USA*. 1999; 96:11492–11495. [PubMed: 10500204]
48. Lodi R, Rajagopalan B, Blamire AM, Cooper JM, Davies CH, Bradley JL, Styles P, Schapira AH. Cardiac energetics are abnormal in Friedreich ataxia patients in the absence of cardiac dysfunction and hypertrophy: an *in vivo* 31P magnetic resonance spectroscopy study. *Cardiovasc Res*. 2001; 52:111–119. [PubMed: 11557239]
49. Lynch DR, Perlman SL, Meier T. A phase 3, double-blind, placebo-controlled trial of idebenone in Friedreich ataxia. *Arch Neurol*. 2010; 67:941–947. [PubMed: 20697044]
50. Malo S, Latour Y, Cote M, Geoffroy G, Lemieux B, Barbeau A. Electrocardiographic and vectocardiographic findings in Friedreich's ataxia: the Canadian journal of neurological sciences. *J Can Sci Neurol*. 1976; 3:323–328.
51. Mariotti C, Solari A, Torta D, Marano L, Fiorentini C, Di DS. Idebenone treatment in Friedreich patients: one-year-long randomized placebo-controlled trial. *Neurology*. 2003; 60:1676–1679. [PubMed: 12771264]
52. Michael S, Petrocine SV, Qian J, Lamarche JB, Knutson MD, Garrick MD, Koeppen AH. Iron and iron-responsive proteins in the cardiomyopathy of Friedreich's ataxia. *Cerebellum*. 2006; 5:257–267. [PubMed: 17134988]
53. Miranda CJ, Santos MM, Ohshima K, Smith J, Li L, Bunting M, Cossee M, Koenig M, Sequeiros J, Kaplan J, Pandolfo M. Frataxin knockin mouse. *FEBS Lett*. 2002; 512:291–297. [PubMed: 11852098]
54. Mitchell DJ, Kim DT, Steinman L, Fathman CG, Rothbard JB. Polyarginine enters cells more efficiently than other polycationic homopolymers. *J Pept Res*. 2000; 56:318–325. [PubMed: 11095185]
55. Montermini L, Richter A, Morgan K, Justice CM, Julien D, Castellotti B, Mercier J, Poirier J, Capozzoli F, Bouchard JP, Lemieux B, Mathieu J, Vanasse M, Seni MH, Graham G, Andermann F, Andermann E, Melancon SB, Keats BJ, Di Donato S, Pandolfo M. Phenotypic variability in Friedreich ataxia: role of the associated GAA triplet repeat expansion. *Ann Neurol*. 1997; 41:675–682. [PubMed: 9153531]
56. Palagi B, Picozzi R, Casazza F, Possa M, Magri G, Zoccarato O, Graziano E, Ferrari F, Morpurgo M. Biventricular function in Friedreich's ataxia: a radionuclide angiographic study. *Br Heart J*. 1988; 59:692–695. [PubMed: 3395528]
57. Pandolfo M. Friedreich ataxia. *Arch Neurol*. 2008; 65:1296–1303. [PubMed: 18852343]
58. Patel PI, Isaya G. Friedreich ataxia: from GAA triplet-repeat expansion to frataxin deficiency. *Am J Hum Genet*. 2001; 69:15–24. [PubMed: 11391483]
59. Pineda M, Arpa J, Montero R, Aracil A, Dominguez F, Galvan M, Mas A, Martorell L, Sierra C, Brandi N, Garcia-Arumi E, Rissech M, Velasco D, Costa JA, Artuch R. Idebenone treatment in paediatric and adult patients with Friedreich ataxia: long-term follow-up. *Eur J Paediatr Neurol*. 2008; 12:470–475. [PubMed: 18234531]
60. Pook MA, Al Mahdawi S, Carroll CJ, Cossee M, Puccio H, Lawrence L, Clark P, Lowrie MB, Bradley JL, Cooper JM, Koenig M, Chamberlain S. Rescue of the Friedreich's ataxia knockout mouse by human YAC transgenesis. *Neurogenetics*. 2001; 3:185–193. [PubMed: 11714098]
61. Puccio H, Koenig M. Recent advances in the molecular pathogenesis of Friedreich ataxia. *Hum Mol Genet*. 2000; 9:887–892. [PubMed: 10767311]

62. Puccio H, Simon D, Cossee M, Criqui-Filipe P, Tiziano F, Melki J, Hindelang C, Matyas R, Rustin P, Koenig M. Mouse models for Friedreich ataxia exhibit cardiomyopathy, sensory nerve defect, and Fe-S enzyme deficiency followed by intramitochondrial iron deposits. *Nat Genet.* 2001; 27:181–186. [PubMed: 11175786]
63. Rai M, Soragni E, Jenssen K, Burnett R, Herman D, Coppola G, Geschwind DH, Gottesfeld JM, Pandolfo M. HDAC inhibitors correct frataxin deficiency in a Friedreich ataxia mouse model. *PLoS One.* 2008; 3:e1958. [PubMed: 18463734]
64. Rajagopalan B, Francis JM, Cooke F, Korlipara LV, Blamire AM, Schapira AH, Madan J, Neubauer S, Cooper JM. Analysis of the factors influencing the cardiac phenotype in Friedreich's ataxia. *Mov Disord.* 2010; 25:846–852. [PubMed: 20461801]
65. Raman SV, Phatak K, Hoyle JC, Pennell ML, McCarthy B, Tran T, Prior TW, Olesik JW, Lutton A, Rankin C, Kissel JT, Al-Dahhak R. Impaired myocardial perfusion reserve and fibrosis in Friedreich ataxia: a mitochondrial cardiomyopathy with metabolic syndrome. *Eur Heart J.* 2010 (in press).
66. Rapoport M, Lorberboum-Galski H. TAT-based drug delivery system: new directions in protein delivery for new hopes? *Expert Opin Drug Deliv.* 2009; 6:453–463. [PubMed: 19413454]
67. Rapoport M, Saada A, Elpeleg O, Lorberboum-Galski H. TAT-mediated delivery of LAD restores pyruvate dehydrogenase complex activity in the mitochondria of patients with LAD deficiency. *Mol Ther.* 2008; 16:691–697. [PubMed: 18362926]
68. Rayapureddi JP, Tomamichel WJ, Walton ST, Payne RM. TAT fusion protein transduction into isolated mitochondria is accelerated by sodium channel inhibitors. *Biochemistry.* 2010 (in press).
69. Reddy PL, Grewal RP. Friedreich's ataxia: a clinical and genetic analysis. *Clin Neurol Neurosurg.* 2007; 109:200–202. [PubMed: 17049722]
70. Richardson DR. Friedreich's ataxia: iron chelators that target the mitochondrion as a therapeutic strategy? *Expert Opin Investig Drugs.* 2003; 12:235–245.
71. Richardson DR, Huang ML, Whitnall M, Becker EM, Ponka P, Rahmanto YS. The ins and outs of mitochondrial iron-loading: the metabolic defect in Friedreich's ataxia. *J Mol Med.* 2010; 88:323–329. [PubMed: 19997898]
72. Romeo G, Menozzi P, Ferlini A, Fadda S, Di Donato S, Uziel G, Lucci B, Capodaglio L, Filla A, Campanella G. Incidence of Friedreich ataxia in Italy estimated from consanguineous marriages. *Am J Hum Genet.* 1983; 35:523–529. [PubMed: 6859045]
73. Rotig A, de LP, Chretien D, Foury F, Koenig M, Sidi D, Munnich A, Rustin P. Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia. *Nat Genet.* 1997; 17:215–217. [PubMed: 9326946]
74. Rustin P, Rotig A, Munnich A, Sidi D. Heart hypertrophy and function are improved by idebenone in Friedreich's ataxia. *Free Radic Res.* 2002; 36:467–469. [PubMed: 12069112]
75. Ryser HJ, Drummond I, Shen WC. The cellular uptake of horseradish peroxidase and its poly(lysine) conjugate by cultured fibroblasts is qualitatively similar despite a 900-fold difference in rate. *J Cell Physiol.* 1982; 113:167–178. [PubMed: 6127344]
76. Sakamoto N, Ohshima K, Montermini L, Pandolfo M, Wells RD. Sticky DNA, a self-associated complex formed at long GAA*TTC repeats in intron 1 of the frataxin gene, inhibits transcription. *J Biol Chem.* 2001; 276:27171–27177. [PubMed: 11340071]
77. Schmucker S, Argentini M, Carelle-Calmels N, Martelli A, Puccio H. The in vivo mitochondrial two-step maturation of human frataxin. *Hum Mol Genet.* 2008; 17:3521–3531. [PubMed: 18725397]
78. Schols L, Amoiridis G, Przuntek H, Frank G, Epplen JT, Epplen C. Friedreich's ataxia: revision of the phenotype according to molecular genetics. *Brain.* 1997; 120:2131–2140. [PubMed: 9448568]
79. Schwarze SR, Ho A, Vocero-Akbani A, Dowdy SF. In vivo protein transduction: delivery of a biologically active protein into the mouse. *Science.* 1999; 285:1569–1572. [PubMed: 10477521]
80. Sedlak TL, Chandavimol M, Straatman L. Cardiac transplantation: a temporary solution for Friedreich's ataxia-induced dilated cardiomyopathy. *J Heart Lung Transplant.* 2004; 23:1304–1306. [PubMed: 15539131]

81. Segovia J, Alonso-Pulpon L, Burgos R, Silva L, Serrano S, Castedo E, Jimenez J, Fuentes R, Canas A, Ugarte J. Heart transplantation in Friedreich's ataxia and other neuromuscular diseases. *J Heart Lung Transplant*. 2001; 20:169. [PubMed: 11250265]
82. Shaddy RE, Boucek MM, Hsu DT, Boucek RJ, Canter CE, Mahony L, Ross RD, Pahl E, Blume ED, Dodd DA, Rosenthal DN, Burr J, LaSalle B, Holubkov R, Lukas MA, Tani LY. Carvedilol for children and adolescents with heart failure: a randomized controlled trial. *JAMA*. 2007; 298:1171–1179. [PubMed: 17848651]
83. Shan Y, Napoli E, Cortopassi G. Mitochondrial frataxin interacts with ISD11 of the NFS1/ISCU complex and multiple mitochondrial chaperones. *Hum Mol Genet*. 2007; 16:929–941. [PubMed: 17331979]
84. Skre H. Friedreich's ataxia in Western Norway. *Clin Genet*. 1975; 7:287–298. [PubMed: 1126051]
85. Stemmler TL, Lesuisse E, Pain D, Dancis A. Frataxin and mitochondrial FeS cluster biogenesis. *J Biol Chem*. 2010; 285:26737–26743. [PubMed: 20522547]
86. Toro A, Grunebaum E. TAT-mediated intracellular delivery of purine nucleoside phosphorylase corrects its deficiency in mice. *J Clin Invest*. 2006; 116:2717–2726. [PubMed: 16964310]
87. Velasco-Sanchez D, Aracil A, Montero R, Mas A, Jimenez L, O'Callaghan M, Tondo M, Capdevila A, Blanch J, Artuch R, Pineda M. Combined therapy with idebenone and deferiprone in patients with Friedreich's ataxia. *Cerebellum*. 2010; 10:1–8. [PubMed: 20865357]
88. Vyas PM, Payne RM. TAT opens the door. *Mol Ther*. 2008; 16:647–648. [PubMed: 18362922]
89. Watson MS, Mann MY, Lloyd-Puryear MA, Rinaldo P. Newborn screening: toward a uniform screening panel and system. *Genet Med*. 2006; 8:1S–11S. [PubMed: 16783161]
90. Whitnall M, Rahmanto YS, Sutak R, Xu X, Becker EM, Mikhael MR, Ponka P, Richardson DR. The MCK mouse heart model of Friedreich's ataxia: alterations in iron-regulated proteins and cardiac hypertrophy are limited by iron chelation. *Proc Natl Acad Sci USA*. 2008; 105:9757–9762. [PubMed: 18621680]
91. Yoon T, Cowan JA. Frataxin-mediated iron delivery to ferrochelatase in the final step of heme biosynthesis. *J Biol Chem*. 2004; 279:25943–25946. [PubMed: 15123683]
92. Zhang Y, Lyver ER, Knight SA, Pain D, Lesuisse E, Dancis A. Mrs3p, Mrs4p, and frataxin provide iron for Fe-S cluster synthesis in mitochondria. *J Biol Chem*. 2006; 281:22493–22502. [PubMed: 16769722]
93. Zhang XY, Dinh A, Cronin J, Li SC, Reiser J. Cellular uptake and lysosomal delivery of galactocerebrosidase tagged with the HIV TAT protein transduction domain. *J Neurochem*. 2008; 104:1055–1064. [PubMed: 17986221]

Fig. 1. Cardiac MRI in the sagittal plane of a young male with Friedreich's ataxia and severe hypertrophic cardiomyopathy. Note the thickened LV wall, small LV chamber, and ascending aorta

Fig. 2. Echocardiography in the short-axis plane of a hypertrophic cardiomyopathy from a young man with Friedreich's ataxia. Note the severely thickened LV posterior wall (markings are 1 cm)

Fig. 3. Electrocardiogram (ECG) from two patients with Friedreich's ataxia. Note the nonspecific T-wave changes in the chest leads (V1–V6) in both (a) and (b). There is T-wave inversion in V5 and V6 of patient (a), and right ventricular hypertrophy is present in a

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Human      MWTLGKRAVAGLLASPSPAQAQTLTRVPPAEALAPLCGPRGLRTDIDATCTPPRASSNQR 60
Mouse      MWAFGGRAAVGLLPRTASRASAWVGMPRWEPITCGRGLHVTVMAGAT-RHAHLNLH 58
           ***: * ** , *** , : : : * : , ** * *****: . : : * , * : * :
           ↑ Mature aa 81

Human      GLNQIWNVKQSVYLMNLEKSGTLGHPGSLDETTYERLAEETLDSLAEFFEDLADKPYTF 120
Mouse      YL-QILNIKQSVCVVHLENLGTLDNPSSLDETAYERLAEETLDSLAEFFEDLADKPYTL 117
           * ** * : **** * : : ** : *** , * , *****: *****: *****: *****:

Human      EDYDVSFGSGVLTVKLGGDLGTYVINKQTPNKQIWLSSPSSGPKRYDWTGKNWVYSHDGV 180
Mouse      EDYDVSFGDGVLTIKLGGDLGTYVINKQTPNKQIWLSSPSSGPKRYDWTGKNWVYSHDGV 177
           *****: , *****: *****: *****: *****: *****: *****: *****: *****:

Human      SLHELLAAELTKALKTKLDLSSLAYSGKDA 210
Mouse      SLHELLARELTKALNTKLDLSSLAYSGKGT 207
           *****: *****: *****: *****: *****: *****: *****: *****:

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Fig. 4.

Sequence homology between human and mouse frataxin protein. The native mitochondrial targeting sequence extends to amino acid 80 in the human and is removed at import into mitochondria. Both mouse and human have more than 90% sequence homology between the mature frataxin proteins from residue 81 to terminus

Fig. 5. Growth of the *FRDA* knockout mouse (*FRDA* KO). **a** *FRDA*-conditional KO mouse generated using the neuron-specific enolase promoter driving Cre expression in brain and heart (designated as NSE-KO). The mouse is 28 days old. For comparison, a wild-type littermate at 28 days is shown. **b** Growth of the *FRDA*-conditional KO mouse compared with the growth of the age-matched control littermates. Growth of the KO animals plateaus at 12 days of life

Fig. 6. Histology of the *FRDA* knockout mouse (*FRDA* KO) heart. **a, b** Perl's stain for iron deposition of 28-day-old wild-type heart and 28-day-old *FRDA* (NSE-Cre promoter) KO heart. **b** Note the intense blue stain of iron deposition in the *FRDA* KO heart (*arrow*) ($\times 40$ magnification for both). **c, d** Fast Green Sirius Red staining of 28-day-old wild-type heart **c** and 28-day-old *FRDA* KO (NSE-Cre promoter) heart. **d** Note intense red staining of scar tissue in **d** from the *FRDA* KO heart ($\times 10$ magnification for both)

Fig. 7.

Cardiomyocyte apoptosis in the *FRDA* knockout mouse (*FRDA* KO) heart. **a, b** Two different mice. The *FRDA*-conditional KO mouse was generated using the neuron-specific enolase promoter driving Cre expression in the brain and heart. The mice are 28 days old. **a, b** Anti-caspase 3 staining of cardiomyocytes (*arrow* points to dark brown stain). **c** Apoptosis counts quantified in wild-type and *FRDA* KO hearts. Values are expressed as cardiomyocyte apoptosis mean counts per high-powered field $\pm$ standard error of the mean (SEM). WT = 9, KO = 10. $**P < 0.05$. The *FRDA* KO hearts have statistically greater numbers of activated caspase-3-positive cells

Fig. 8.

Echocardiography of the *FRDA* knockout mouse (*FRDA* KO) heart and wild-type control heart. Littermate mice were 28 days of age and underwent echocardiography using a VisualSonics high-frequency ultrasound machine using 1–2% isoflurane inhaled anesthetic. **a, b** M-mode ultrasound of wild-type mouse in short axis demonstrating vigorous contraction and high heart rate (407 bpm). **b** Normal mitral inflow from the same animal with normal E-A slope. **c, d** A 28-day-old *FRDA* KO mouse showing poor cardiac function (M-mode in short axis) **c** and very low heart rate (197 bpm). **d** Mitral inflow from the same animal with reversal of the E-A slope indicating poor diastolic function of the left ventricle

Fig. 9.

Electron microscopy of the *FRDA* knockout mouse (*FRDA* KO) heart and wild-type control heart. Littermate mice were 28 days of age at the time of the study. **a** Wild-type mouse showing normal mitochondria (“m”) in rows between abundant, well-ordered sarcomeres (“s”). **b** Conditional *FRDA* KO mouse with ablation of the *FRDA* locus in the heart and brain (NSE-Cre promoter). Note extreme proliferation of enlarged mitochondria in **b**. There is a severe loss of sarcomeres (“s”). Bar = 1,000 nm in both **a** and **b**

Fig. 10.

Transduction of a TAT-fusion protein into tissues. A TAT-mMDH-eGFP protein was expressed and purified from bacterial culture, then injected into a wild-type mouse intraperitoneally. The mouse was killed 24 h later, and heart and brain were imaged using confocal microscopy. In both the heart and brain, a strong green fluorescent signal is present indicating the presence of eGFP in tissues. The control tissues were taken from a noninjected mouse and show minimal autofluorescence

Fig. 11.

A TAT-fusion protein transduction into mitochondria. **a** The positively charged peptide of TAT interacts with negative charges of the cell membrane (not shown) and the mitochondrial phospholipid membrane to initiate transduction of the TAT-mMDH-eGFP fusion protein across both mitochondrial membranes. Inside the matrix, the mitochondrial-processing peptidase (MPP) clips the mMDH sequence, leaving the GFP cargo trapped in the matrix while the TAT-mMDH peptide diffuses out. The transduction into mitochondria is independent of membrane potential and ATP status, and is not receptor mediated. The *asterisk* indicates the contact site. **b** Full-length TAT-mMDH-eGFP (*cartoon A*) was incubated with isolated mitochondrial matrix proteins and separated by 12% SDS-PAGE followed by blotting to membrane and probing for GFP by Western blotting using anti-GFP antibody (Promega). *Cartoons A, B, and C* show the progressive predicted sizes after digestion with the contents of the mitochondria and are present on the Western blot at the predicted sizes

Fig. 12.

Translation and import process of a native mitochondrial protein. The nuclear expressed mRNA is translated in the cytosol by ribosomes to generate a mitochondrial protein with a transit peptide (mitochondrial-targeting sequence). This transit peptide is recognized by the mitochondrial receptor and imported via the energy-requiring apparatus to the mitochondrial matrix, where the MPP recognizes the transit peptide and clips it. The mature protein then is generated. A mitochondrial import reaction for ^{35}S -radiolabeled rat mMDH is shown on the *left* with precursor, intermediate, and mature bands after import into mitochondria. Compare this multistep import process with TAT-mMDH-eGFP transduction in Fig. 11