

Molecular insights into Friedreich's ataxia and antioxidant-based therapies

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Friedreich's ataxia (FRDA) is an autosomal recessive neurodegenerative disease causing limb and gait ataxia and cardiomyopathy. The disease gene encodes a mitochondrial protein of unknown function, frataxin. The loss of functional frataxin is caused by a large GAA trinucleotide expansion in the first intron of the gene, thus impairing gene transcription. The lack of frataxin appears to result primarily in disabled recruitment of early antioxidant defenses, resulting in oxidative insult to the highly sensitive iron-sulfur proteins aconitase and three mitochondrial respiratory chain complexes (I–III). Accordingly, antioxidant-based therapy appears promising in counteracting the course of the disease.

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Friedreich's ataxia is a degenerative disorder characterized by a progressive cerebellar ataxia with a lack of deep-tendon reflexes, a pyramidal weakness and dysarthria. Increased heart wall thickness is observed in all patients, and 70% of the patients actually develop a life-threatening hypertrophic cardiomyopathy [1]. Moreover, 10% of the patients also presented with diabetes mellitus and 20% of the patients had carbohydrate intolerance. Friedreich's ataxia is a common autosomal recessive disorder with a frequency of 1/50 000 live births. The disease gene has been mapped on chromosome 9q13 [2] and identified by positional cloning [3]. This gene encodes a 210 amino-acid protein called frataxin. The mutation responsible for the disease consists of a GAA repeat expansion in the first intron of the frataxin gene present in 98% of the patients and results in a loss of function of frataxin because of impaired gene transcription [4].

A role for frataxin in iron metabolism?

The precise role of human frataxin is still unknown, but studying a yeast homologue, YFH1, has provided the first clue to understanding its function. Indeed, the yeast frataxin homologue had been previously identified as a mutant unable to grow on iron-limited medium. Deletion of *YFH1* results in strains unable to carry out oxidative phosphorylation, because of a mitochondrial respiratory chain deficiency. In some conditions, these strains also lack mtDNA [5,6]. Moreover, a doubling of iron content was observed in *Ayf1* cells at the expense of cytosolic iron as a tenfold increase in iron content was observed in the mitochondria. Finally, it has been shown that YFH1 and frataxin proteins localize to mitochondria [7]. Taken together, these results suggested that frataxin is involved in mitochondrial iron metabolism.

At the same time, a very specific enzyme deficiency, combined complex I, II and III deficiency, was detected

in endomyocardial biopsies of two patients with Friedreich's ataxia [8]. These three complexes have in common the presence of iron-sulfur clusters (ISC) associated with at least one of their subunits. Another ISC-containing protein, aconitase, was also found to be severely deficient. This additional result denoted a generalized iron-sulfur protein (ISP) deficiency in these patients and established that Friedreich's ataxia should be considered a true mitochondrial disorder. These data have been subsequently confirmed by analyses performed on post-mortem brain and heart samples [9]. In tissue homogenates, the measured aconitase activity actually represents the activity of two different aconitases, mitochondrial aconitase, which is part of the tricarboxylic acid cycle, and cytosolic aconitase. Therefore, this generalized deficiency of ISC-containing proteins (mitochondrial and cytosolic aconitases, complex I, II and III) does not result from different mutations in different genes, but from either abnormal assembly or injury of the iron-sulfur cluster in the proteins. This ISC deficiency was also observed in the yeast mutant, as complex II, complex III and aconitase were also severely deficient in the *Ayf1* strains compared with the wild-type strains [8]. These results, in both humans and yeast, clearly show that the abnormal function in Friedreich's ataxia involves ISC.

Hypotheses regarding frataxin functions revisited

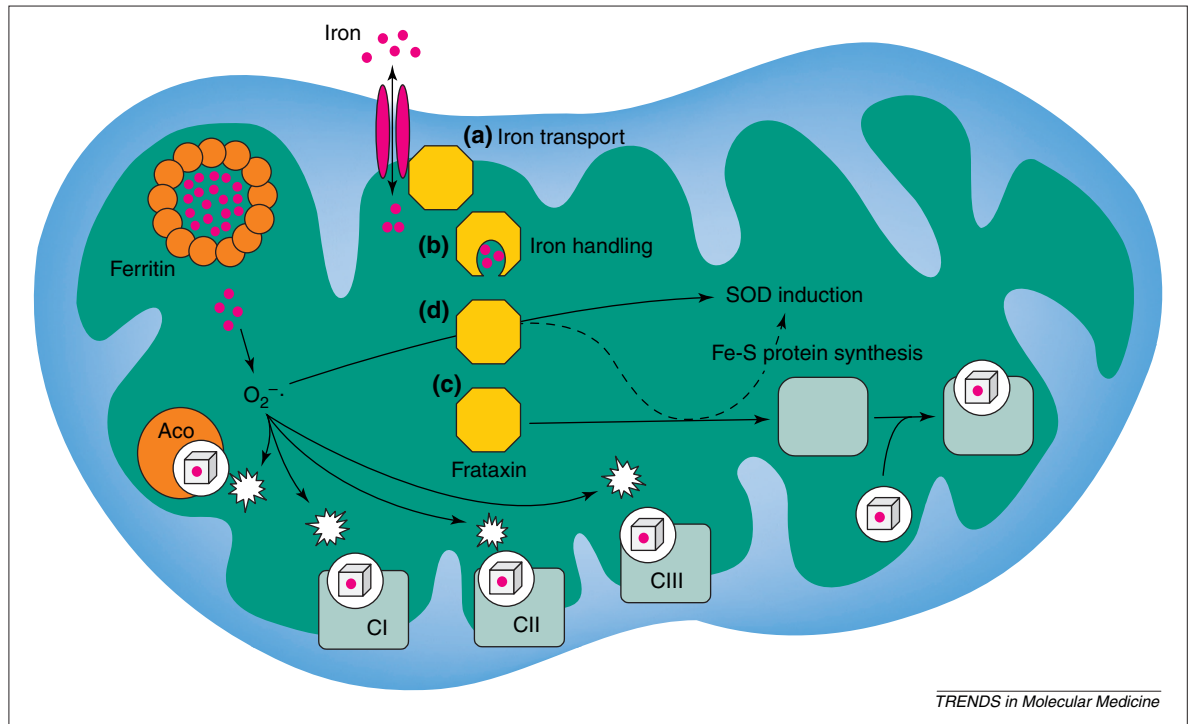
Although the consequences of a lack of function of frataxin have now been described in detail, and several multi-organism approaches have established that the frataxin protein is mitochondrially located, in the matrix at or near the inner membrane, the function of frataxin remains a matter of debate (Fig. 1).

The idea that frataxin might be a protein involved in the control of mitochondrial iron transport stems from the observation that the yeast YFH1 mutant accumulates iron in the mitochondrial matrix, presumably at the expense of cytosolic iron. This hypothesis implies that frataxin stimulates iron transport out of the mitochondria and that lack of this function results in intramitochondrial iron accumulation [5].

According to a second hypothesis, frataxin plays a role in mitochondrial iron storage. This mainly stems from the observation that the addition of ferrous iron to the purified yeast YFH1 results in the assembly of a YFH1 multimer that can sequester up to 3000 atoms of iron [10]. This gives frataxin a potential role in mitochondrial-iron bio-availability. Moreover, it has

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Fig. 1. Hypothetical frataxin functions. (a) frataxin might be a protein involved in mitochondrial iron transport. (b) frataxin might play a role in mitochondrial iron handling. (c) frataxin might be involved in iron-sulfur cluster assembly. (d) frataxin might be involved in the protection against free radicals. Abbreviations: aco: aconitase; CI, CII, CIII, complexes I, II and III of the respiratory chain; SOD: superoxide dismutase.



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been shown that human frataxin expressed in *E. coli* and *S. cerevisiae* assembles into a polymer that can bind iron, suggesting that human frataxin also acts as an iron storage protein [11]. Interestingly, a mitochondrial ferritin was recently identified in both mouse and human [11].

A third hypothesis suggests that frataxin is directly involved in ISC assembly or ISP synthesis, as a quite specific ISC deficiency is observed in heart of FRDA patients and mouse models [8,13]. In keeping with this, the phylogenetic distribution of frataxin might also indicate a role in iron-sulfur cluster protein assembly [14]. Moreover, the presence of frataxin was found to be necessary for ICS assembly into yeast ferredoxin suggesting a direct role of frataxin at the level of ICS biogenesis [15].

Recently, it has been shown that frataxin is involved in the protection against oxidative insult resulting from respiratory chain impairment [16]. This might be through either mitochondrial antioxidant defence induction, or control of the synthesis of a key superoxide dismutase (SOD) signaling component, or the sensing of free iron in the mitochondrial matrix. Indeed, it has been shown that cultured skin fibroblasts from Friedreich's ataxia patients and cardiomyocytes of frataxin knockout (KO) mice are unable to induce antioxidant defenses, namely SOD activity in response to superoxide overproduction resulting from the inhibition of the respiratory chain [16]. This conclusion has been strengthened by the subsequent observation that the oxidative injury caused by iron supplementation also failed to induce SOD in cells from Friedreich's ataxia patients [16]. Understanding of the mechanism by which absent or decreased levels of frataxin result in

disabled early antioxidant defenses will require delineation of the poorly known SOD signaling pathways in human cells. Finally, it has also been suggested that frataxin could be an activator of oxidative phosphorylation [18], although this could not be confirmed by another group [19].

Whatever the exact primary role of frataxin might be, at some point the consequence of a frataxin defect is an accumulation of free iron in mitochondria. The free iron overload in turn enhances the oxidative stress through Fenton chemistry (iron-triggered oxygen reactive species production) and further alters the mitochondrial functions. Iron-sulfur clusters, in respiratory chain complexes I, II, III and mitochondrial aconitase, which are known to represent critical targets for oxygen free radicals, would rapidly lose their activity in Friedreich's ataxia patients.

Mouse models of Friedreich's ataxia

Research from Michel Koenig's laboratory has generated several mouse models for Friedreich's ataxia [13,20]. They first established that inactivation of the Friedreich's ataxia mouse gene leads to early embryonic lethality without iron accumulation [20]. Subsequently, two mouse models were obtained by conditional KO of the frataxin gene: a neuron and heart-specific KO mouse model was created using the neuron-specific enolase promoter (NSE) and a muscle-specific KO using the muscle creatine kinase promoter (MCK). NSE mutants developed ataxia with an average onset at 12 days accompanied by progressive loss of proprioception. Both mice also presented with cardiac hypertrophy that develops into a dilated cardiomyopathy, with earlier onset in the NSE mouse. Histological examination of hearts of the two mutants

displayed abnormalities suggestive of a mitochondrial dysfunction. Finally, the two mutants presented a respiratory chain defect consistent with a loss of ISC at seven weeks of age for the MCK and in NSE mice after death. It is important to note that iron accumulation was only observed at ten weeks of age in the MCK mouse, whereas the iron content was normal at seven weeks when significant ISC deficiency could be already detected. In NSE mice, no iron deposit could be observed even after death. This leads to the conclusion that ISP deficiency and cardiomyopathy occur before any significant accumulation of mitochondrial iron [13].

Possible therapeutic approaches

The improved understanding of the pathogenesis of Friedreich's ataxia allowed the development of possible therapeutic approaches for this disease. Because iron accumulation was initially thought to play a primary role in the pathogenesis, there has been an initial enthusiasm for trialing iron chelators. However, it was rapidly realized that most available chelators reduced intracellular iron, but did not significantly remove mitochondrial iron. In addition, major side effects might be associated with the use of chelators [21]. Therefore, in the absence of gene therapy, it seemed reasonable to attempt to prevent ISC damage presumably resulting from superoxide injury, by using antioxidant compounds. The potential protective effect of various substances against iron-induced ISC damage was first assayed *in vitro* using human heart homogenates. With this aim, the effect of iron on lipid peroxidation, complex II activity and aconitase activity in human heart homogenates was studied in detail [22].

Reduced iron (Fe^{2+}), tested as ferrous chloride, but not oxidized iron (Fe^{3+}), resulted in rapid lipoperoxidation and a decrease of complex II activity in heart homogenates, indicating that the toxic effect of iron depends on its redox status. For this reason, reducing agents such as ascorbate or glutathione might be harmful for patients with iron overload, as these drugs are likely to reduce free iron *in vivo* [23]. As far as soluble matrix enzymes are concerned, ferrous ions (Fe^{2+}) alone failed to cause any damage to aconitase activity. But addition of a chelator, EDTA or desferioxamine, well known to protect membrane components from iron-induced peroxidation, led to a significant loss of aconitase activity. Thus, iron chelators changed the nature of the targeted enzymes rather than affording protection against iron toxicity and could be detrimental as well. The protective effect of antioxidant quinones, ubiquinone (coenzyme Q10) and idebenone, a short-chain analogue of ubiquinone, was next studied in human heart homogenates [22]. Idebenone is known to cross the blood-brain barrier [24], and is already available as a drug, Mnesis. Complex II activity in heart homogenates was severely damaged after incubation with reduced iron (Fe^{2+}), but low concentration of either coenzyme Q10 or idebenone

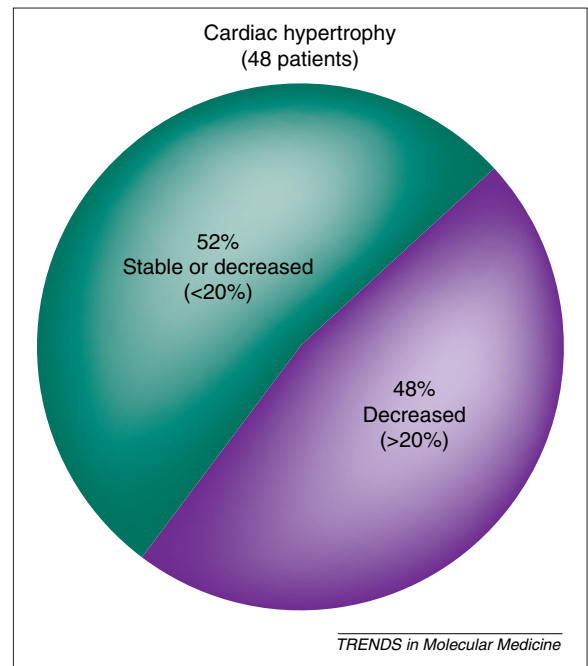


Fig. 2. The effect of idebenone oral treatment (5mg/kg/day) on the heart left ventricular mass index in Friedreich's ataxia. 48% of the patients presented a >20% reduction of the left ventricular mass whereas the others remain stable or presented a <20% reduction of the left ventricular mass [24].

reduced *in situ* by the respiratory chain, protected complex II activity against iron injury [25]. The same protecting effect was observed for membrane lipids.

Considering that the *in vitro* model of iron-induced injury mimics the damage caused to heart in Friedreich's ataxia patients, it was assumed that quinones, in particular idebenone, might improve heart and possibly brain function of Friedreich's ataxia patients.

Clinical studies with idebenone

Three patients were initially treated with idebenone (5mg/kg/day). This resulted in a substantial improvement of heart hypertrophy. The septal thickness actually decreased by ~30%, the left ventricular wall thickness decreased by 10–20% and the left ventricular mass index decreased by 20–30% after four months of treatment in all three patients [22]. Next, a larger uncontrolled trial performed on 48 patients fully confirmed these promising results. After six months of idebenone treatment, 48% of the patients presented a >20% reduction of the left ventricular mass (Fig. 2), which is highly significant, as reduction of heart hypertrophy has never been reported in the natural course of the disease. Cardiac hypertrophy was largely stabilized in the other half. In none of them did the hypertrophy significantly increase over the six-month period of the trial. These data remain constant after more than two years of treatment (D. Sidi, unpublished). However, after one year of treatment, ataxia and deep-tendon reflexes did not change significantly. Nevertheless, most of the patients and their families reported a decreased fatigability, an improvement of delicate movements

(handwriting, drawing, control of the wheelchair command), an improvement in the use of the voice and decreased swallowing difficulties [26]. Response to idebenone treatment could not be correlated either with the number of GAA repeats in the smallest allele of the frataxin gene or with the age of the patients, which indicates that idebenone might be efficient at any age during the course of the disease. Finally, the cardiomyopathy improvement was not related to the severity of the cardiac hypertrophy before treatment, as no significant correlation was found between the left ventricular mass index change and the initial left ventricular mass index. Therefore, the variable efficiency of the drug among affected individuals remains unexplained. Noticeably, no significant side effects of the drug were noted over the one-year period. Increasing the drug from 5 to 10 or 15 mg/kg/day has proven useful for some patients [25]. Noticeably, a very short term (six weeks), double-blind placebo-controlled study on nine patients failed to detect improvement [27]. However, two additional preliminary studies also reported improvement in patients. The first double-blind placebo-controlled trial was performed on 22 adult patients, who showed a decrease of heart septal thickness after six months of idebenone supplementation [28]. The second open trial was performed on eight paediatric patients and resulted in an improvement of the neurological condition of the patients, especially in fine manipulation, nystagmus and eye movements [29].

An alternative antioxidant therapy using ubiquinone (400 mg/day) plus vitamin E (2100 IU/day) has been recently evaluated, but both the neurological and echocardiographic exams did not show any consistent benefits of the therapy after six months [30]. Nevertheless, after three months of treatment, a partial reversal of surrogate biochemical markers (heart phosphocreatine and skeletal muscle ATP [31]) might denote improved cellular function and certainly supports the evaluation of additional antioxidant therapies in Friedreich's ataxia.

Concluding remarks

Results from most of the various trials on idebenone treatment of Friedreich's patients so far indicated a positive effect of this drug on heart hypertrophy. Most patients also reported a much better general condition and less fatigability, which might be in part a result of better heart function. However, although improvements in strength and delicate movements were reported, the neurological condition of the patients was not significantly modified as ataxia and deep-tendon reflexes did not change. Intensive efforts to identify neurological tissue-targeted antioxidants should improve Friedreich's ataxia treatment. Nevertheless, the fact that the progressive course of the disease might reflect progressive damages to an increasing number of mitochondrial components is an incentive to give idebenone to patients as early as possible.

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