# Friedreich’s Ataxia

Subjects: Pathology

Contributor: José LUIS García-Gimenez

Friedreich’s ataxia (FRDA, MIM 229300) is an autosomal recessive neurodegenerative disease and it is the most prevalent hereditary ataxia in the Caucasian population, with a prevalence of around 2–4 in 100,000 individuals. FRDA is classically characterized by progressive gait ataxia, dysarthria, dysphagia, oculomotor dysfunction, loss of deep tendon reflexes, signs of pyramidal tract involvement, scoliosis, visual loss, and poor hearing, and in some cases, cardiomyopathy, diabetes mellitus.
FRDA is caused by an unstable GAA expansion located in intron 1 of the FXN gene (9q21.11) that encodes for frataxin (Fxn). The function of Fxn is not completely known, but the most widely accepted theory is that it plays a role in the biogenesis of iron-sulfur clusters required for the correct function of several proteins.

FRDA thioredoxin glutaredoxin

## 1. Introduction

This rare childhood-onset disease is characterized by progressive spinocerebellar neurodegeneration, peripheral sensory neuropathy, vestibular and cerebellar pathology, and pyramidal disabilities in the last stages. All these disease-related alterations cause symptoms of gait and limb ataxia, lower limb areflexia and dysarthria in these patients[1]. Other non-neurological features of FRDA are scoliosis, diabetes and cardiac complications[2][3][4], which are the main cause of death in these patients, mostly in early adulthood. Most FRDA patients are homozygous for the GAA·TTC triplet repeat expansion in the *FXN* gene localized in chromosome 9q21.11 producing decreased protein levels of the protein product frataxin (FXN)[5][6]. Regarding the molecular characteristics of FRDA, there are well known alterations consisting of mitochondrial respiratory chain dysfunction[7], accumulation of mitochondrial iron[8], decreased mitochondrial DNA levels and adenosine triphosphate (ATP) generation, increased oxidative stress and unbalanced antioxidant response[9], as well as alterations in calcium homeostasis[10] and lipid metabolism[11][12].

Enzymatic antioxidant systems include superoxide dismutase Copper-Zinc superoxide dismutase (CuZnSOD) and Manganese superoxide dismutase (MnSOD), catalase, glutathione peroxidases, peroxiredoxins, and the TRX and GLRX systems, among others. Superoxide dismutase and catalase have previously been described as being altered in FRDA [64,65] and we have also found a deficiency in the expression of cytosolic CuZnSOD and mitochondrial MnSOD[9] [60], which is in agreement with previous studies demonstrating that the up-regulation of MnSOD fails to occur in FRDA fibroblasts when they are exposed to iron[13][14]. However, despite the critical importance of the thioredoxin superfamily for cellular metabolism described above, there is little information on the

specific role of TRX and GLRX systems in FRDA, and, therefore, we consider it to be of special relevance to elucidate their function in the molecular physiopathology of the disease.

The principal function of frataxin is still unknown; however, the involvement of the FXN protein in iron-sulphur clusters (Fe-S clusters), heme group biosynthesis[15], and mitochondriogenesis[15] has been reported, although only the role of the FXN protein in Fe-S cluster biogenesis seems to be more convincing and extensively proved[16]. The generation of iron-sulphur clusters and their insertion in apoproteins is a complex process that involves many players located in mitochondria and cytosol and divided into three sequential steps. In the first step, the [2Fe-2S] cluster is assembled on the scaffold protein iron-sulfur cluster assembly protein (ISCU2) from inorganic iron and sulfur. During this step, it has been proposed that frataxin interacts with ISCU2 and five other additional ISC proteins that form the ISC assembly complex. Furthermore, in the first studies, it was suggested that the FXN protein donated iron to the cluster [2Fe-2S][17][18]. Posterior experiments proposed frataxin as an allosteric regulator for sulfur transfer to the Fe-S cluster[19][20][21], although currently the main mechanism accepted is a preloading of ISCU2 with iron[22]. Dysregulation of FXN protein function in ISC assembly can produce several alterations in cells, including those produced by deficits in Fe-S cluster-containing mitochondrial enzymes, such as aconitase and succinate dehydrogenase. In fact, low levels of aconitase, an enzyme from the tricarboxylic acid cycle (TCA cycle), and mitochondrial respiratory complexes I, II, and III have been determined in frataxin-deficient animal and cellular models[10]. These alterations lead to metabolic changes that decrease ATP generation in the mitochondrion. In addition, studies on FRDA patients[7] and cellular[9] and animal models[7][23][24] showed mitochondrial dysfunction and lower ATP levels. Another important enzyme that contains a Fe-S cluster is ferrochelatase (FECH), which catalyzes the last step of heme group biosynthesis, where the iron atom is incorporated into protoporphyrin IX. Previously, an iron atom should be provided to FECH through a process that is not yet known. The involvement of FXN in this process has been reported by in vitro analysis and one study in yeast that showed an FXN protein interaction with ferrochelatase[25]. Nevertheless, the role of frataxin in heme group biosynthesis is controversial and the last suggested model of mitochondrial heme metabolism did not include this protein[26]. These results are in agreement with a recent analysis in erythroid progenitor cells from FRDA patients, in which heme synthesis was not altered during erythroid differentiation[27].

Iron homeostasis and iron-sulfur cluster biosynthesis are closely related processes. Indeed, impaired FeS-dependent activities and an activation of IRP1 (iron regulatory protein 1) have been described in the liver of frataxin-deficient mice, increasing iron import and availability by promoting gene expression of the iron-response element (IRE) containing promoter genes[28]. Iron accumulation in the spleen, liver, and heart has been described in FRDA patients[29] and animal models[30][31], thus suggesting altered iron metabolism in FRDA. However, controversial studies about iron accumulation in neural tissues can be found in the literature[32][33][34][35]. The implication of iron accumulation in the physiopathology of FRDA is not yet clarified, and further analyses are needed to address this issue, especially regarding neural degeneration in FRDA. However, the newly described process of ferroptosis has provided a possible mechanism for neuronal death, since it explains many of the pathological characteristics of neuronal degeneration in FRDA. Ferroptosis is a regulated cell death that is distinct from other cell death processes, such as apoptosis, classical autophagy, and necrosis. Ferroptosis is characterized by an overwhelming, iron-dependent accumulation of lethal lipid hydroperoxides[36]. It has been suggested that the

initiation of ferroptosis might be directly triggered by an increase in free iron levels, for example by a dysregulation of ferritinophagy, a selective autophagy of ferritin[37]. Iron increase or accumulation induces the Fenton reaction which promotes the production of ROS, and together with the lipoxygenase activity of 15-LOX (ALOX15), oxidizes polyunsaturated fatty acids phospholipids (PUFA-PLs) which activate the ferroptosis pathway[38][39]. In addition, inhibition of glutathione peroxidase enzyme 4 (GPX4)[40][41] or GSH unavailability or defects in its restoration[36][42] produce lipid hydroperoxide accumulation that triggers ferroptosis. Importantly, the implication of TRX1 and TRXRD in ferroptosis has also been described[43][44]. Increased ROS, lower reduced GSH concentrations and enhanced sensitivity to oxidants compared with control neurons have also been observed in these FRDA cell models[45][46]. Part of ROS production occurs in the mitochondria as a consequence of the malfunction of respiratory complex I[47]. Importantly, through the mitochondrial one-carbon metabolism, NADPH production is severely compromised when the function of Complex I is affected[48], as occurs in blood cells from FRDA patients[49][50]. The compromised levels of NADPH may affect cellular thiol-based redox regulation because the classical thioredoxin system is composed of TRX, TRXRD and NADPH, which are required as electron donors for TRXRD[51] and glutathione reductase to replenish GSH levels, which are used by glutaredoxins[52] and GPX4 to reduce lipoperoxides[53][54].

In relation to this, FRDA neurons have shown higher lipoperoxide levels, increased ROS, lower reduced GSH concentration, and enhanced sensitivity to oxidants compared with control neurons[45][46]. Neurons from a YG8R mouse model also showed a mitochondrial energy imbalance, as a consequence of an inhibition of mitochondrial Complex I and increased lipid peroxidation, which contribute to cell death[55]. Furthermore, patients with FRDA present a disturbance of GSH homeostasis[56][57][58], lipoperoxidation and thiol oxidation[59][60]. Together with iron accumulation, all these results suggest the occurrence of ferroptosis in FRDA.

## 2. Activation of the Thioredoxin family by NRF2 Activators as Therapeutic Options in FRDA

As shown above, it is well established that oxidative stress plays a key role in the pathophysiology of FRDA both by means of unbalanced antioxidant enzymatic and non-enzymatic responses. Therefore, for many years, antioxidants have been evaluated as potential therapeutic agents for FRDA, and some authors have recently reviewed potential therapies based on antioxidant strategies[61]. Among the different therapies evaluated, overexpression of NRF2 seems to be a promising approach to promote antioxidant response in FRDA, and hence, several trials using omaveloxolone or resveratrol in order to overexpress NRF2 and stimulate ARE activation have been proposed[61].

Targeted therapies to stimulate the expression of TRX have a wide array of beneficial effects in neurodegenerative disorders and other hyperinflammatory diseases in which the expression or function of these proteins are altered. Preclinical and clinical studies using recombinant TRX (rhTRX) are currently underway, although there are also natural substances (including active principles from plants) which can induce the expression of thioredoxin family proteins[62]. Yodoi et al. reviewed the most promising strategies to deliver TRX as a therapeutic agent, including (i) topical application, (ii) oral delivery, and (iii) TRX-overexpression using exogenous stimuli[62]. Topical applications may have little relevance for neurological diseases, but oral delivery and TRX-overexpression can be considered

feasible therapeutic strategies in neurodegenerative disorders such as FRDA. Nevertheless, it is more plausible to use an indirect strategy to induce TRX superfamily overexpression. Thus, as described in the previous section, it would be possible to activate TRX by upregulating the stability, expression, and activation of NRF2. In this regard, a recent review by La Rosa, Bertini and Piemonte described the pharmacological interventions aimed at restoring the NRF2 signaling network in FRDA[63]. Among the several molecules described to stimulate NRF2 overexpression, resveratrol was found to increase both NRF2 stability and mRNA overexpression of NRF2[64][65] and, in turn, TRX1[66][67].

Resveratrol has been proposed as a potential antioxidant treatment in FRDA and as an inducer of frataxin expression. In FRDA mouse models and cells from FRDA patients (i.e., fibroblasts and lymphoblasts), resveratrol treatment demonstrated an ability to increase the transcription of a stably transfected frataxin-green fluorescent protein[68]. However, these results were not reproduced in peripheral blood mononuclear cells obtained from FRDA patients[69] nor in induced pluripotent stem cell (hPSC)-derived neurons from patients with FRDA[70]. Moreover, an open-label trial using low-dose (1 g daily) and high-dose resveratrol (5 g daily) in FRDA patients, despite suggesting clinical benefits for high-dose resveratrol, did not demonstrate an increase in frataxin levels in FRDA patients[69]. Interestingly, in a model of ischemia-reperfusion of liver, trans-resveratrol demonstrated the ability to maintain TRX redox activity by diminishing TXNIP protein expression and, more importantly, the ability to inhibit the secretion of the TRX1 protein[71]. The same results were observed in an in vivo model of old mice with or without 3-month resveratrol treatment[72]. These results suggest that the expression of TRX can ameliorate the symptoms of FRDA probably by improving some of the mechanisms we have described in the previous section, despite not increasing the expression of frataxin levels.

Compared with resveratrol, sulforaphane (SFN) more potently activates NRF2 to induce the expression of the antioxidant system[73] [175]. SFN is an isothiocyanate derived from glucoraphanin, which is mainly found in cruciferous vegetables such as broccoli, Brussels sprouts, cabbages and cauliflower. Its potential to increase the expression and activity of NRF2 and TRX1 has been demonstrated by ARE transcription activation in murine retina[74]. Interestingly, Jazwa et al. showed that intra-peritoneal injection of SFN can cross the blood–brain barrier in the MPTP mouse model of Parkinson's disease, being detected in the brain 15 min after injection[75]. Besides its potential to increase the expression of NRF2 and TRX1 in some cellular models such as retinal cells[74] and human hepatoma cells[76], SFN also demonstrated its ability to increase the expression of TRXRD, and together with selenium helps to increase the activity of TRXRD[76]. The reactivation of TRXRD may serve to re-establish the pool of reduced TRX and maintain antioxidant homeostasis in cells, which, in turn, may contribute to the release of NRF2 from KEAP1[77], thereby activating the transcriptional function of NRF2. It is noteworthy that Chiang et al. also found that SFN can increase the expression of both NRF2 and its inhibitor KEAP1 in a SK-N-MC neuroblastoma cell line, which could be explained as a feedback mechanism to prevent NRF2 overexpression and its downstream antioxidant defense genes[78].

When SFN treatment was evaluated in frataxin-silenced motor neuron-like cells[79], neural stem cells isolated from the KIKO FRDA mouse model[80] and also in FRDA fibroblasts[81], this antioxidant was able to revert the cellular

phenotypic defects, providing neuroprotection in the neuronal models. Unfortunately, despite these findings, SFN has not yet been evaluated in clinical trials for FRDA.

Omaveloxolone is another inductor of NRF2 expression able to reverse the FRDA phenotype in different pre-clinical models. Omaveloxolone protects the cells against oxidative stress, avoids lipid peroxidation, decreases the mitochondrial ROS generation, and increments reduced glutathione levels[82]. Recently, results from a clinical trial with this drug have been published pointing out that omaveloxolone significantly improves neurological function and is generally safe and well tolerated[83].

Finally, melatonin has been defined as a principal regulator of Nrf2 signaling and improves oxidative stress state (reviewed in[84]). Moreover, melatonin has been described as an endoplasmic reticulum stress mediator, promoting TRX1 activity by inhibiting the TXINP/NLRP3 pathway[85]. Despite the fact that melatonin has been described as a possible treatment in other neurodegenerative diseases[86], in FRDA, only one case report has been described. In this case, the authors of the study administrate 5 and 10 mg of melatonin to an FRDA patient to treat REM (rapid eye movement phase of sleep) Sleep Behavior Disorder; however, they did not find any benefit after melatonin treatment[87].

The activation of thioredoxin superfamily proteins through NRF2 activators (such as omaveloxolone, resveratrol, sulforaphane and melatonin) can represent promising therapeutic options in FRDA, and, as such, they have been or are already being subject of pre-clinical and clinical trials (Table 1). The reason is that the activation of NRF2 and in turn TRXs and GRXs may contribute to decreased oxidative stress in FRDA cells, to improve the metabolism of iron-sulfur clusters required for appropriate mitochondrial metabolism, to decrease iron-catalyzed mitochondrial damage and also to inhibit ferroptosis, all of them related with the molecular pathogenesis in FRDA. We consider that further efforts exploring therapeutic candidates overexpressing NRF2 and thioredoxin family proteins may increase the therapeutic strategies for this neuromuscular disease.

**Table 1.** Pre-clinical and clinical antioxidant therapies in FRDA.

| Compound | Pre-Clinical Studies in FRDA | | | Clinical Trials in FRDA | | |
|---|---|---|---|---|---|---|
| | Model | Doses/ Treatment | Ref. | Nº Subjects | Doses/ Treatment | Ref. |
| Resveratrol | YG8R mouse | 200 mg/kg daily for 3 days. Subcutaneous injection. | [68] | 27 FRDA patients: 13 low dosis and 14 high dosis | 0.5 g or 2.5 g twice daily for 12 weeks. Capsules. | [69] |

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|---|---|---|---|---|---|---|
| Sulforaphane (SFN) | Human fibroblast<br>MSC<br>iPSC-derived neurons | 25 µM to 125 µM once<br>25 µM to 125 µM once<br>10 µM to 50 µM once | [70] | 40 patients (estimated) | 2 g daily for 24 weeks. Capsules. | ClinicalTrials.gov Id: NCT03933163 |
| | Mouse NSC34 motor neurons<br>Human fibroblasts | 5 µM for 24 h<br>10 µM for 24 h | [79] | | | |
| | Neural stem cells KIKO mouse | 5 µM for 2, 6, and 24 h | [80] | | | |
| | Human fibroblast | 10 µM for 2, 6, and 24 h | [81] | | | |
| Omaveloxolone | Cerebellar Granule Neurons KIKO and YG8R mice<br>Human Fibroblast | 50 nM for 24 h<br>50 nM for 24 h | [82] | 103 patients | 150 mg daily for 48 weeks.<br>Capsules. | [83] |
| Melatonin | | | | Case report: 1 FRDA | 5 mg and 10 mg | [88] |

patient

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