Friedreich's Ataxia

Subjects: Pathology
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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.

Keywords: 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.

	Pre-Clinical Studies in FRDA			**Clinical Trials in FRDA**		
Compound	**Model**	**Doses/ Treatment**	**Ref.**	**Nº Subjects**	**Doses/ Treatment**	**Ref.**
	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]
Resveratrol	Human fibroblast	25 µM to 125 µM once	[70]	40 patients (estimated)	2 g daily for 24 weeks. Capsules.	ClinicalTrials.gov Id: NCT03933163
	MSC	25 µM to 125 µM once				
	iPSC-derived neurons	10 µM to 50 µM once				
Sulforaphane (SFN)	Mouse NSC34 motor neurons	5 µM for 24 h 10 µM for 24 h	[79]			
	Human fibroblasts					
	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	50 nM for 24 h 50 nM for 24 h	[82]	103 patients	150 mg daily for 48 weeks. Capsules.	[83]
	Human Fibroblast					
Melatonin				Case report: 1 FRDA patient	5 mg and 10 mg	[88]

References

1. Delatycki, M.B.; Corben, L.A. Clinical features of Friedreich ataxia. J. Child Neurol. 2012, 27, 1133–1137.
2. Cady, R.B.; Bobechko, W.P. Incidence, Natural History, and Treatment of Scoliosis in Friedreich's Ataxia. J. Pediatric Orthop. 1984, 4, 673–676.
3. Cnop, M.; Mulder, H.; Igoillo-Esteve, M. Diabetes in Friedreich ataxia. J. Neurochem. 2013, 126 (Suppl. 1), 94–102.
4. Harding, A.E.; Hewer, R.L. The heart disease of Friedreich's ataxia: A clinical and electrocardiographic study of 115 patients, with an analysis of serial electrocardiographic changes in 30 cases. Q. J. Med. 1983, 52, 489–502.
5. Pandolfo, M. Friedreich ataxia: The clinical picture. J. Neurol. 2009, 256 (Suppl. 1), 3–8.
6. Sharma, R.; De Biase, I.; Gomez, M.; Delatycki, M.B.; Ashizawa, T.; Bidichandani, S.I. Friedreich ataxia in carriers of unstable borderline GAA triplet-repeat alleles. Ann. Neurol. 2004, 56, 898–901.
7. Rotig, A.; de Lonlay, P.; 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.
8. Martelli, A.; Puccio, H. Dysregulation of cellular iron metabolism in Friedreich ataxia: From primary iron-sulfur cluster deficit to mitochondrial iron accumulation. Front. Pharmacol. 2014, 5, 130.
9. Garcia-Gimenez, J.L.; Gimeno, A.; Gonzalez-Cabo, P.; Dasi, F.; Bolinches-Amoros, A.; Molla, B.; Palau, F.; Pallardo, F.V. Differential expression of PGC-1alpha and metabolic sensors suggest age-dependent induction of mitochondrial biogenesis in Friedreich ataxia fibroblasts. PLoS ONE 2011, 6, e20666.
10. Bolinches-Amoros, A.; Molla, B.; Pla-Martin, D.; Palau, F.; Gonzalez-Cabo, P. Mitochondrial dysfunction induced by frataxin deficiency is associated with cellular senescence and abnormal calcium metabolism. Front. Cell. Neurosci. 2014, 8, 124.
11. Turchi, R.; Tortolici, F.; Guidobaldi, G.; Iacovelli, F.; Falconi, M.; Rufini, S.; Faraonio, R.; Casagrande, V.; Federici, M.; De Angelis, L.; et al. Frataxin deficiency induces lipid accumulation and affects thermogenesis in brown adipose tissue. Cell Death Dis. 2020, 11, 51.
12. Navarro, J.A.; Ohmann, E.; Sanchez, D.; Botella, J.A.; Liebisch, G.; Molto, M.D.; Ganfornina, M.D.; Schmitz, G.; Schneuwly, S. Altered lipid metabolism in a Drosophila model of Friedreich's ataxia. Hum. Mol. Genet. 2010, 19, 2828–2840.
13. Chantrel-Groussard, K.; Geromel, V.; Puccio, H.; Koenig, M.; Munnich, A.; Rotig, A.; Rustin, P. Disabled early recruitment of antioxidant defenses in Friedreich's ataxia. Hum. Mol. Genet. 2001, 10, 2061–2067.
14. Jiralerspong, S.; Ge, B.; Hudson, T.J.; Pandolfo, M. Manganese superoxide dismutase induction by iron is impaired in Friedreich ataxia cells. FEBS Lett. 2001, 509, 101–105.
15. Jasoliya, M.J.; McMackin, M.Z.; Henderson, C.K.; Perlman, S.L.; Cortopassi, G.A. Frataxin deficiency impairs mitochondrial biogenesis in cells, mice and humans. Hum. Mol. Genet. 2017, 26, 2627–2633.
16. Chiabrando, D.; Bertino, F.; Tolosano, E. Hereditary Ataxia: A Focus on Heme Metabolism and Fe-S Cluster Biogenesis. Int. J. Mol. Sci. 2020, 21, 3760.
17. Yoon, T.; Cowan, J.A. Iron-sulfur cluster biosynthesis. Characterization of frataxin as an iron donor for assembly of [2Fe-2S] clusters in ISU-type proteins. J. Am. Chem. Soc. 2003, 125, 6078–6084.
18. Layer, G.; Ollagnier-de Choudens, S.; Sanakis, Y.; Fontecave, M. Iron-sulfur cluster biosynthesis: Characterization of Escherichia coli CYaY as an iron donor for the assembly of [2Fe-2S] clusters in the scaffold IscU. J. Biol. Chem. 2006, 281, 16256–16263.
19. Parent, A.; Elduque, X.; Cornu, D.; Belot, L.; Le Caer, J.P.; Grandas, A.; Toledano, M.B.; D'Autreaux, B. Mammalian frataxin directly enhances sulfur transfer of NFS1 persulfide to both ISCU and free thiols. Nat. Commun. 2015, 6, 5686.
20. Gervason, S.; Larkem, D.; Mansour, A.B.; Botzanowski, T.; Muller, C.S.; Pecqueur, L.; Le Pavec, G.; Delaunay-Moisan, A.; Brun, O.; Agramunt, J.; et al. Physiologically relevant reconstitution of iron-sulfur cluster biosynthesis uncovers persulfide-processing functions of ferredoxin-2 and frataxin. Nat. Commun. 2019, 10, 3566.
21. Fox, N.G.; Yu, X.; Feng, X.; Bailey, H.J.; Martelli, A.; Nabhan, J.F.; Strain-Damerell, C.; Bulawa, C.; Yue, W.W.; Han, S. Structure of the human frataxin-bound iron-sulfur cluster assembly complex provides insight into its activation mechanism. Nat. Commun. 2019, 10, 2210.
22. Roland Lill; Sven-A. Freibert; Mechanisms of Mitochondrial Iron-Sulfur Protein Biogenesis. *Annual Review of Biochemistry* **2020**, *89*, 471-499, 10.1146/annurev-biochem-013118-111540.
23. Llorens, J.V.; Navarro, J.A.; Martinez-Sebastian, M.J.; Baylies, M.K.; Schneuwly, S.; Botella, J.A.; Molto, M.D. Causative role of oxidative stress in a Drosophila model of Friedreich ataxia. FASEB J. 2007, 21, 333–344.

24. Lin, H.; Magrane, J.; Rattelle, A.; Stepanova, A.; Galkin, A.; Clark, E.M.; Dong, Y.N.; Halawani, S.M.; Lynch, D.R. Early cerebellar deficits in mitochondrial biogenesis and respiratory chain complexes in the KIKO mouse model of Friedreich ataxia. Dis. Models Mech. 2017, 10, 1343–1352.

25. Taejin Yoon; J.A. Cowan; Frataxin-mediated Iron Delivery to Ferrochelatase in the Final Step of Heme Biosynthesis. *Journal of Biological Chemistry* **2004**, *279*, 25943-25946, 10.1074/jbc.c400107200.

26. Amy E. Medlock; Mesafint T. Shiferaw; Jason R. Marcero; Ajay A. Vashisht; James A. Wohlschlegel; John D. Phillips; Harry A. Dailey; Identification of the Mitochondrial Heme Metabolism Complex. *PLOS ONE* **2015**, *10*, e0135896, 10.1371/journal.pone.0135896.

27. Hannes Steinkellner; Himanshu Narayan Singh; Martina U. Muckenthaler; Hans Goldenberg; Rajeswari R. Moganty; Barbara Scheiber-Mojdehkar; Brigitte Sturm; No changes in heme synthesis in human Friedreich´s ataxia erythroid progenitor cells. *Gene* **2017**, *621*, 5-11, 10.1016/j.gene.2017.04.014.

28. Alain Martelli; Stéphane Schmucker; Laurence Reutenauer; Jacques R.R. Mathieu; Carole Peyssonnaux; Zoubida Karim; Hervé Puy; Bruno Galy; Matthias W. Hentze; Hélène Puccio; et al. Iron Regulatory Protein 1 Sustains Mitochondrial Iron Loading and Function in Frataxin Deficiency. *Cell Metabolism* **2015**, *21*, 311-323, 10.1016/j.cmet.2015.01.010.

29. J.L. Bradley; J.C. Blake; S. Chamberlain; P.K. Thomas; J.M. Cooper; A.H.V. Schapira; Clinical, biochemical and molecular genetic correlations in Friedreich's ataxia. *Human Molecular Genetics* **2000**, *9*, 275-282, 10.1093/hmg/9.2.275.

30. 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.

31. Huang, M.L.; Becker, E.M.; Whitnall, M.; Suryo Rahmanto, Y.; Ponka, P.; Richardson, D.R. 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.

32. Koeppen, A.H.; Michael, S.C.; Knutson, M.D.; Haile, D.J.; Qian, J.; Levi, S.; Santambrogio, P.; Garrick, M.D.; Lamarche, J.B. The dentate nucleus in Friedreich’s ataxia: The role of iron-responsive proteins. Acta Neuropathol. 2007, 114, 163–173.

33. Harding, I.H.; Raniga, P.; Delatycki, M.B.; Stagnitti, M.R.; Corben, L.A.; Storey, E.; Georgiou-Karistianis, N.; Egan, G.F. Tissue atrophy and elevated iron concentration in the extrapyramidal motor system in Friedreich ataxia: The IMAGE-FRDA study. J. Neurol. Neurosurg. Psychiatry 2016, 87, 1261–1263.

34. Koeppen, A.H.; Morral, J.A.; Davis, A.N.; Qian, J.; Petrocine, S.V.; Knutson, M.D.; Gibson, W.M.; Cusack, M.J.; Li, D. The dorsal root ganglion in Friedreich’s ataxia. Acta Neuropathol. 2009, 118, 763–776.

35. Llorens, J.V.; Soriano, S.; Calap-Quintana, P.; Gonzalez-Cabo, P.; Molto, M.D. The Role of Iron in Friedreich’s Ataxia: Insights From Studies in Human Tissues and Cellular and Animal Models. Front. Neurosci. 2019, 13, 75.

36. Scott J. Dixon; Kathryn M. Lemberg; Michael R. Lamprecht; Rachid Skouta; Eleina M. Zaitsev; Caroline E. Gleason; Darpan N. Patel; Andras J. Bauer; Alexandra M. Cantley; Wan Seok Yang; et al. Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death. *Cell* **2012**, *149*, 1060-1072, 10.1016/j.cell.2012.03.042.

37. Brent R. Stockwell; José Pedro Friedmann Angeli; Hülya Bayir; Ashley I. Bush; Marcus Conrad; Scott J. Dixon; Simone Fulda; Sergio Gascón; Stavroula K. Hatzios; Valerian E. Kagan; et al. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell* **2017**, *171*, 273-285, 10.1016/j.cell.2017.09.021.

38. Kagan, V.E.; Mao, G.; Qu, F.; Angeli, J.P.; Doll, S.; Croix, C.S.; Dar, H.H.; Liu, B.; Tyurin, V.A.; Ritov, V.B.; et al. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. Nat. Chem. Biol. 2017, 13, 81–90.

39. Shah, R.; Shchepinov, M.S.; Pratt, D.A. Resolving the Role of Lipoxygenases in the Initiation and Execution of Ferroptosis. ACS Cent. Sci. 2018, 4, 387–396.

40. Gaschler, M.M.; Stockwell, B.R. Lipid peroxidation in cell death. Biochem. Biophys. Res. Commun. 2017, 482, 419–425.

41. Yang, W.S.; Kim, K.J.; Gaschler, M.M.; Patel, M.; Shchepinov, M.S.; Stockwell, B.R. Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. Proc. Natl. Acad. Sci. USA 2016, 113, E4966–E4975.

42. Jennifer Yinuo Cao; Aunoy Poddar; Leslie Magtanong; Jennifer H. Lumb; Trevor R. Mileur; Michael A. Reid; Cole M. Dovey; Jin Wang; Jason W. Locasale; Everett Stone; et al. A Genome-wide Haploid Genetic Screen Identifies Regulators of Glutathione Abundance and Ferroptosis Sensitivity. *Cell Reports* **2019**, *26*, 1544-1556.e8, 10.1016/j.celrep.2019.01.043.

43. Llabani, E.; Hicklin, R.W.; Lee, H.Y.; Motika, S.E.; Crawford, L.A.; Weerapana, E.; Hergenrother, P.J. Diverse compounds from pleuromutilin lead to a thioredoxin inhibitor and inducer of ferroptosis. Nat. Chem. 2019, 11, 521–532.

44. Behnisch-Cornwell, S.; Bandaru, S.S.M.; Napierkowski, M.; Wolff, L.; Zubair, M.; Urbainsky, C.; Lillig, C.; Schulzke, C.; Bednarski, P.J. Pentathiepins: A Novel Class of Glutathione Peroxidase 1 Inhibitors that Induce Oxidative Stress, Loss of Mitochondrial Membrane Potential and Apoptosis in Human Cancer Cells. ChemMedChem 2020.

45. Codazzi, F.; Hu, A.; Rai, M.; Donatello, S.; Salerno Scarzella, F.; Mangiameli, E.; Pelizzoni, I.; Grohovaz, F.; Pandolfo, M. Friedreich ataxia-induced pluripotent stem cell-derived neurons show a cellular phenotype that is corrected by a benzamide HDAC inhibitor. Hum. Mol. Genet. 2016, 25, 4847–4855.

46. Lupoli, F.; Vannocci, T.; Longo, G.; Niccolai, N.; Pastore, A. The role of oxidative stress in Friedreich's ataxia. FEBS Lett. 2018, 592, 718–727.

47. A.D. Vinogradov; Vera G. Grivennikova; Oxidation of NADH and ROS production by respiratory complex I. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* **2016**, *1857*, 863-871, 10.1016/j.bbabio.2015.11.004.

48. Eduardo Balsa; Elizabeth A. Perry; Christopher F. Bennett; Mark Jedrychowski; Steven P. Gygi; John G. Doench; Pere Puigserver; Defective NADPH production in mitochondrial disease complex I causes inflammation and cell death. *Nature Communications* **2020**, *11*, 2714, 10.1038/s41467-020-16423-1.

49. Heidari, M.M.; Houshmand, M.; Hosseinkhani, S.; Nafissi, S.; Khatami, M. Complex I and ATP content deficiency in lymphocytes from Friedreich's ataxia. Can. J. Neurol. Sci. 2009, 36, 26–31.

50. Salehi, M.H.; Kamalidehghan, B.; Houshmand, M.; Yong Meng, G.; Sadeghizadeh, M.; Aryani, O.; Nafissi, S. Gene expression profiling of mitochondrial oxidative phosphorylation (OXPHOS) complex I in Friedreich ataxia (FRDA) patients. PLoS ONE 2014, 9, e94069.

51. Anton A. Turanov; Sebastian Kehr; Stefano M. Marino; Min-Hyuk Yoo; Bradley A. Carlson; Dolph L. Hatfield; Vadim N. Gladyshev; Mammalian thioredoxin reductase 1: roles in redox homoeostasis and characterization of cellular targets. *Biochemical Journal* **2010**, *430*, 285-293, 10.1042/bj20091378.

52. Arne Holmgren; Antioxidant Function of Thioredoxin and Glutaredoxin Systems. *Antioxidants & Redox Signaling* **2000**, *2*, 811-820, 10.1089/ars.2000.2.4-811.

53. Cozza, G.; Rossetto, M.; Bosello-Travain, V.; Maiorino, M.; Roveri, A.; Toppo, S.; Zaccarin, M.; Zennaro, L.; Ursini, F. Glutathione peroxidase 4-catalyzed reduction of lipid hydroperoxides in membranes: The polar head of membrane phospholipids binds the enzyme and addresses the fatty acid hydroperoxide group toward the redox center. Free Radic. Biol. Med. 2017, 112, 1–11.

54. Su, L.J.; Zhang, J.H.; Gomez, H.; Murugan, R.; Hong, X.; Xu, D.; Jiang, F.; Peng, Z.Y. Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. Oxidative Med. Cell. Longev. 2019, 2019, 5080843.

55. R Abeti; M H Parkinson; I P Hargreaves; P R Angelova; C Sandi; M A Pook; Paola Giunti; A Y Abramov; 'Mitochondrial energy imbalance and lipid peroxidation cause cell death in Friedreich's ataxia'. *Cell Death & Disease* **2016**, *7*, e2237-e2237, 10.1038/cddis.2016.111.

56. Calap-Quintana, P.; Soriano, S.; Llorens, J.V.; Al-Ramahi, I.; Botas, J.; Molto, M.D.; Martinez-Sebastian, M.J. TORC1 Inhibition by Rapamycin Promotes Antioxidant Defences in a Drosophila Model of Friedreich's Ataxia. PLoS ONE 2015, 10, e0132376.

57. Shan, Y.; Schoenfeld, R.A.; Hayashi, G.; Napoli, E.; Akiyama, T.; Iodi Carstens, M.; Carstens, E.E.; Pook, M.A.; Cortopassi, G.A. Frataxin deficiency leads to defects in expression of antioxidants and Nrf2 expression in dorsal root ganglia of the Friedreich's ataxia YG8R mouse model. Antioxid. Redox Signal. 2013, 19, 1481–1493.

58. Auchere, F.; Santos, R.; Planamente, S.; Lesuisse, E.; Camadro, J.M. Glutathione-dependent redox status of frataxin-deficient cells in a yeast model of Friedreich's ataxia. Hum. Mol. Genet. 2008, 17, 2790–2802.

59. Piemonte, F.; Pastore, A.; Tozzi, G.; Tagliacozzi, D.; Santorelli, F.M.; Carrozzo, R.; Casali, C.; Damiano, M.; Federici, G.; Bertini, E. Glutathione in blood of patients with Friedreich's ataxia. Eur. J. Clin. Investig. 2001, 31, 1007–1011.

60. Bulteau, A.L.; Planamente, S.; Jornea, L.; Dur, A.; Lesuisse, E.; Camadro, J.M.; Auchere, F. Changes in mitochondrial glutathione levels and protein thiol oxidation in yfh1 yeast cells and the lymphoblasts of patients with Friedreich's ataxia. Biochim. Biophys. Acta 2012, 1822, 212–225.

61. Laura R. Rodríguez; Tamara Lapeña; Pablo Calap-Quintana; María Dolores Moltó; Pilar González-Cabo; Juan Antonio Navarro; Antioxidant Therapies and Oxidative Stress in Friedreich´s Ataxia: The Right Path or Just a Diversion?. *Antioxidants* **2020**, *9*, 664, 10.3390/antiox9080664.

62. Masao Saitoh; Hideki Nishitoh; Makiko Fujii; Kohsuke Takeda; Kei Tobiume; Yasuhiro Sawada; Masahiro Kawabata; Kohei Miyazono; Hidenori Ichijo; Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK)

1. *The EMBO Journal* **1998**, *17*, 2596-2606, 10.1093/emboj/17.9.2596.

63. Piergiorgio La Rosa; Enrico Bertini; Fiorella Piemonte; The NRF2 Signaling Network Defines Clinical Biomarkers and Therapeutic Opportunity in Friedreich's Ataxia. *International Journal of Molecular Sciences* **2020**, *21*, 916, 10.3390/ijms21030916.

64. Kode, A.; Rajendrasozhan, S.; Caito, S.; Yang, S.R.; Megson, I.L.; Rahman, I. Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells. Am. J. Physiol. Lung Cell. Mol. Physiol. 2008, 294, L478–L488.

65. Ungvari, Z.; Bagi, Z.; Feher, A.; Recchia, F.A.; Sonntag, W.E.; Pearson, K.; de Cabo, R.; Csiszar, A. Resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2. Am. J. Physiol. Heart Circ. Physiol. 2010, 299, H18–H24.

66. Kaga, S.; Zhan, L.; Matsumoto, M.; Maulik, N. Resveratrol enhances neovascularization in the infarcted rat myocardium through the induction of thioredoxin-1, heme oxygenase-1 and vascular endothelial growth factor. J. Mol. Cell. Cardiol. 2005, 39, 813–822.

67. Thirunavukkarasu, M.; Penumathsa, S.V.; Koneru, S.; Juhasz, B.; Zhan, L.; Otani, H.; Bagchi, D.; Das, D.K.; Maulik, N. Resveratrol alleviates cardiac dysfunction in streptozotocin-induced diabetes: Role of nitric oxide, thioredoxin, and heme oxygenase. Free Radic. Biol. Med. 2007, 43, 720–729.

68. Lingli Li; Lucille Voullaire; Chiranjeevi Sandi; Mark A. Pook; Panos A. Ioannou; Martin B. Delatycki; Joseph P. Sarsero; Pharmacological Screening Using an FXN-EGFP Cellular Genomic Reporter Assay for the Therapy of Friedreich Ataxia. *PLOS ONE* **2013**, *8*, e55940, 10.1371/journal.pone.0055940.

69. Eppie M Yiu; Geneieve Tai; Roger E. Peverill; Katherine J. Lee; Kevin D. Croft; Trevor A. Mori; Barbara Scheiber-Mojdehkar; Brigitte Sturm; Monika Praschberger; Adam P. Vogel; et al. An open-label trial in Friedreich ataxia suggests clinical benefit with high-dose resveratrol, without effect on frataxin levels. *Journal of Neurology* **2015**, *262*, 1344-1353, 10.1007/s00415-015-7719-2.

70. Pauline Georges; Maria-Gabriela Boza-Moran; Jacqueline Gide; Georges Arielle Pêche; Benjamin Forêt; Aurélien Bayot; Pierre Rustin; Marc Peschanski; Cécile Martinat; Laetitia Aubry; et al. Induced pluripotent stem cells-derived neurons from patients with Friedreich ataxia exhibit differential sensitivity to resveratrol and nicotinamide. *Scientific Reports* **2019**, *9*, 1-7, 10.1038/s41598-019-49870-y.

71. Valérie Nivet-Antoine; Charles-Henry Cottart; Herve Lemaréchal; Michel Vamy; Isabelle Margaill; Jean-Louis Beaudeux; Minique Bonnefont-Rousselot; Didier Borderie; trans-Resveratrol downregulates Txnip overexpression occurring during liver ischemia-reperfusion. *Biochimie* **2010**, *92*, 1766-1771, 10.1016/j.biochi.2010.07.018.

72. Tatiana Bedarida; Stephanie Baron; Françoise Vibert; Audrey Ayer; Daniel Henrion; Elizabeth Thioulouse; Carmen Marchiol; Jean-Louis Beaudeux; Charles-Henry Cottart; Valerie Nivet-Antoine; et al. Resveratrol Decreases TXNIP mRNA and Protein Nuclear Expressions With an Arterial Function Improvement in Old Mice. *The Journals of Gerontology: Series A* **2015**, *71*, 720-729, 10.1093/gerona/glv071.

73. Christine A. Houghton; Robert G. Fassett; Jeff S. Coombes; Sulforaphane and Other Nutrigenomic Nrf2 Activators: Can the Clinician's Expectation Be Matched by the Reality?. *Oxidative Medicine and Cellular Longevity* **2016**, *2016*, 1-17, 10.1155/2016/7857186.

74. Masaki Tanito; Hiroshi Masutani; Yong-Chul Kim; Mai Nishikawa; Akihiro Ohira; Junji Yodoi; Sulforaphane Induces Thioredoxin through the Antioxidant-Responsive Element and Attenuates Retinal Light Damage in Mice. *Investigative Opthalmology & Visual Science* **2005**, *46*, 979-987, 10.1167/iovs.04-1120.

75. Agnieszka Jazwa; Ana I. Rojo; Nadia G. Innamorato; Marlen Hesse; Javier Fernández-Ruiz; Antonio Cuadrado; Pharmacological Targeting of the Transcription Factor Nrf2 at the Basal Ganglia Provides Disease Modifying Therapy for Experimental Parkinsonism. *Antioxidants & Redox Signaling* **2011**, *14*, 2347-2360, 10.1089/ars.2010.3731.

76. Korry J Hintze; Karl A. Wald; Huawei Zeng; Elizabeth H. Jeffery; John W. Finley; Thioredoxin reductase in human hepatoma cells is transcriptionally regulated by sulforaphane and other electrophiles via an antioxidant response element.. *The Journal of Nutrition* **2003**, *133*, 2721-2727, 10.1093/jn/133.9.2721.

77. Marcus Cebula; Edward E. Schmidt; Elias Arnér; TrxR1 as a Potent Regulator of the Nrf2-Keap1 Response System. *Antioxidants & Redox Signaling* **2015**, *23*, 823-853, 10.1089/ars.2015.6378.

78. S. Chiang; M.L.H. Huang; Des R. Richardson; Treatment of dilated cardiomyopathy in a mouse model of Friedreich's ataxia using N-acetylcysteine and identification of alterations in microRNA expression that could be involved in its pathogenesis. *Pharmacological Research* **2020**, *159*, 104994, 10.1016/j.phrs.2020.104994.

79. Sara Petrillo; Emanuela Piermarini; Anna Pastore; Gessica Vasco; Tommaso Schirinzi; Rosalba Carrozzo; Enrico Bertini; Fiorella Piemonte; Nrf2-Inducers Counteract Neurodegeneration in Frataxin-Silenced Motor Neurons:

Disclosing New Therapeutic Targets for Friedreich's Ataxia. *International Journal of Molecular Sciences* **2017**, *18*, 2173, 10.3390/ijms18102173.

80. Piergiorgio La Rosa; Marta Russo; Jessica D'Amico; Sara Petrillo; Katia Aquilano; Daniele Lettieri-Barbato; Riccardo Turchi; Enrico S. Bertini; Fiorella Piemonte; Nrf2 Induction Re-establishes a Proper Neuronal Differentiation Program in Friedreich's Ataxia Neural Stem Cells. *Frontiers in Cellular Neuroscience* **2019**, *13*, 356, 10.3389/fncel.2019.00356.
81. Sara Petrillo; Jessica D'Amico; Piergiorgio La Rosa; Enrico Bertini; Fiorella Piemonte; Targeting NRF2 for the Treatment of Friedreich's Ataxia: A Comparison among Drugs. *International Journal of Molecular Sciences* **2019**, *20*, 5211, 10.3390/ijms20205211.
82. Rosella Abeti; Annalisa Baccaro; Noemi Esteras; Paola Giunti; Novel Nrf2-Inducer Prevents Mitochondrial Defects and Oxidative Stress in Friedreich's Ataxia Models. *Frontiers in Cellular Neuroscience* **2018**, *12*, 188, 10.3389/fncel.2018.00188.
83. David R. Lynch; Melanie P. Chin; Martin Delatycki; S.H. Subramony; J. Chad Hoyle; Sylvia Boesch; Wolfgang Nachbauer; Caterina Mariotti; Katherine D. Mathews; Paola Giunti; et al. Safety and Efficacy of Omaveloxolone in Friedreich's Ataxia (MOXIe Study): A Multicentre, Randomised, Double-Blind, Placebo-Controlled Trial. *SSRN Electronic Journal* **2020**, *null*, null, 10.2139/ssrn.3576913.
84. Zahra Ahmadi; Milad Ashrafizadeh; Melatonin as a potential modulator of Nrf2. *Fundamental & Clinical Pharmacology* **2019**, *34*, 11-19, 10.1111/fcp.12498.
85. Sushweta Mahalanobish; Sayanta Dutta; Sukanya Saha; Parames C. Sil; Melatonin induced suppression of ER stress and mitochondrial dysfunction inhibited NLRP3 inflammasome activation in COPD mice. *Food and Chemical Toxicology* **2020**, *144*, 111588, 10.1016/j.fct.2020.111588.
86. Dongmei Chen; Tao Zhang; Tae Ho Lee; Cellular Mechanisms of Melatonin: Insight from Neurodegenerative Diseases. *Biomolecules* **2020**, *10*, 1158, 10.3390/biom10081158.
87. S Nune; R Donald; V Shakkottai; F Hassan; 1242 A Case of Friedreich's Ataxia and REM Sleep Behavior Disorder in a Teenager causing Suboptimal Non-Invasive Positive Pressure (NIPPV) Compliance. *Sleep* **2017**, *40*, A462-A462, 10.1093/sleepj/zsx052.032.
88. S Nune; R Donald; V Shakkottai; F Hassan; 1242 A Case of Friedreich's Ataxia and REM Sleep Behavior Disorder in a Teenager causing Suboptimal Non-Invasive Positive Pressure (NIPPV) Compliance. *Sleep* **2017**, *40*, A462-A462, 10.1093/sleepj/zsx052.032.
