

## Friedreich ataxia: an overview

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### Abstract

**Friedreich ataxia, an autosomal recessive neurodegenerative disease, is the most common of the inherited ataxias. The recent discovery of the gene that is mutated in this condition, *FRDA*, has led to rapid advances in the understanding of the pathogenesis of Friedreich ataxia. About 98% of mutant alleles have an expansion of a GAA trinucleotide repeat in intron 1 of the gene. This leads to reduced levels of the protein, frataxin. There is mounting evidence to suggest that Friedreich ataxia is the result of accumulation of iron in mitochondria leading to excess production of free radicals, which then results in cellular damage and death. Currently there is no known treatment that alters the natural course of the disease. The discovery of the *FRDA* gene and its possible function has raised hope that rational therapeutic strategies will be developed.**

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### History

The disease which is now called Friedreich ataxia (FRDA) was described in five papers by Nicholaus Friedreich over the period 1863-1877.<sup>1-5</sup> In this series of reports Friedreich described the condition which now bears his name in nine members of three families. His initial reports noted that the age of onset was around puberty and ataxia, dysarthria, sensory loss, muscle weakness, scoliosis, foot deformity, and cardiac symptoms were present. It was not until the final two reports<sup>4,5</sup> that the absence of deep tendon reflexes was noted as these were only first described by Friedreich's student, Erb, in 1875.<sup>6</sup> A major cause of published debate centred on the presence of lower limb reflexes. Some authors said that the diagnosis of FRDA could not be made where knee and ankle jerks could be elicited,<sup>7</sup> while others opposed this view.<sup>8</sup>

The eponymous description of the disease was proposed by Brousse in 1882.<sup>6</sup> Many early publications are confused by the presence of cases, which are now recognised not to have been FRDA. In particular, many cases that were almost certainly Charcot-Marie-Tooth disease or syphilis were included in these reports. The Roussy-Levy form of Charcot-

Marie-Tooth disease type I has often been confused with FRDA because of the presence of ataxia and tremor. However, the Roussy-Levy syndrome is dominantly inherited. Another condition that has caused confusion because of the overlap with FRDA has been spastic paraparesis. Bell and Charnichael<sup>9</sup> concluded that spastic ataxia is a subset of FRDA, a point Harding<sup>6</sup> disagreed with.

### Incidence and prevalence

FRDA is the commonest inherited ataxia.<sup>10</sup> Before the availability of molecular diagnosis, FRDA was estimated to affect about 1:50 000 people with an estimated carrier prevalence of about 1:110.<sup>11-13</sup> More recent studies based on molecular data suggest a higher prevalence. On the basis of examining the *FRDA* gene in 178 healthy subjects in Germany, a carrier rate of 1:60-1:90 was estimated.<sup>14</sup> Another study estimated the carrier rate to be 1:85 with a disease prevalence of 1:29 000.<sup>15</sup> The incidence of FRDA in Asians and in those of African descent is very low.<sup>16</sup>

### Genetics of FRDA

Friedreich noted the familial nature of FRDA,<sup>1</sup> although the exact mode of inheritance was confused by what were, in retrospect, misdiagnoses including Charcot-Marie-Tooth disease and autosomal dominant spinocerebellar ataxia.<sup>10</sup> Segregation analysis confirmed autosomal recessive inheritance.<sup>6, 17</sup> The high rate of consanguinity in affected families was also consistent with this mode of inheritance.<sup>6, 9, 17, 18</sup>

Pseudo-dominant inheritance has been described in a number of families and is not surprising given the carrier rate of about 1:100.<sup>11, 19</sup>

### Clinical features

The main clinical features of FRDA are those listed in the diagnostic criteria in table 1. The incidence of these in various studies is shown in table 2. The cardinal clinical features are progressive gait and limb ataxia, absent lower limb reflexes, extensor plantar responses, dysarthria, and reduction in or loss of vibration sense and proprioception (sensory modalities mediated by posterior column neurones). Cardiomyopathy, scoliosis, and foot deformity are common but non-essential features. The use of strict diagnostic criteria was essential to ensure that patients used to study the natural history of FRDA, as well as for molecular studies, defi-

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Table 1 Diagnostic criteria for FRDA proposed by Geoffroy et al<sup>1</sup> and Harding.<sup>6</sup> The diagnostic criteria of Harding are more liberal, allowing the diagnosis of FRDA in the early stages of the disease

Criteria	Geoffroy et al, 1976	Harding, 1981
Primary (essential for diagnosis)	(1) Onset before the end of puberty (never after the age of 20 years), (2) progressive ataxia of gait, (3) dysarthria, (4) loss of joint position or vibration sense, (5) absent tendon reflexes in the legs, (6) muscle weakness	(1) Age of onset of symptoms before the age of 25 years, (2) progressive unremitting ataxia of limbs and of gait, (3) absence of knee and ankle jerks
Secondary	(1) Extensor plantar responses, (2) pes cavus, (3) scoliosis, (4) cardiomyopathy	(1) Dysarthria, (2) extensor plantar responses
Additional		If secondary criteria are absent, the following have to be present: (1) an affected sib fulfilling primary and secondary criteria, (2) median motor nerve conduction velocities of greater than 40 m/s thus excluding cases of type I hereditary motor and sensory neuropathy (HMSN)

Table 2 Percentage of the study population showing specific signs and complications of FRDA in three series<sup>6, 20, 51</sup>

	Harding <sup>6</sup>	Dürr et al <sup>20</sup>	Delatycki et al <sup>51</sup>
Gait ataxia	—	100	100
Limb ataxia	99	99	100
Lower limb muscle weakness	88	67	—
Diminished vibration sense	73	78	88
Pes cavus	55	55	74
Dysarthria	97	91	95
Extensor plantar responses	89	79	74
Cardiomyopathy	66*	63	65
Diabetes	10	32	8
Scoliosis	79	60	78
Reflexes LL	0.9	12	2
Sphincter disturbance	—	23	41
Decreased visual acuity	18	13	—
Hearing loss	8	13	—

LL = lower limbs. — = not reported. \* = Based on abnormal ECG excluding 9.6% where ECG changes were described as insignificant.

nately had FRDA. Cases of FRDA meeting these criteria are defined as typical FRDA, those that do not meet the essential criteria are labelled atypical.<sup>6</sup>

Mean onset of symptoms in FRDA was  $10.52 \pm 7.4$  years (range 1.5–27) in Harding's series of 115 patients<sup>6</sup> and  $15.5 \pm 8$  years (range 2–51) in a recent series of 140 patients.<sup>20</sup> The difference can at least partly be explained by the fact that Harding's series only included patients fulfilling diagnostic criteria which include onset before 25 years,<sup>6</sup> whereas the later series include patients with late onset FRDA.<sup>20</sup> The natural history of FRDA is slow progression of neurological symptoms. Loss of ambulation occurs on average  $15.5 \pm 7.4$  years after disease onset (range 3–44).<sup>6</sup> Death occurs after a variable time with Harding finding an average age of death of  $37.5 \pm 14.4$  years (range 21–69).<sup>6</sup> The commonest cause of death is cardiomyopathy.

Nerve conduction studies characteristically show absent sensory nerve action potentials as well as absent spinal somatosensory evoked potentials, although these may be reduced or even normal early in the disease course.<sup>10</sup> Motor nerve conduction velocities are reduced to a lesser extent than sensory nerve action potentials.

It is noteworthy that despite the diagnostic confusion over the 133 years between the initial disease description and the finding of its molecular mechanism, the initial observations of Friedreich were very accurate in terms of diagnosis and mechanism. Richter wrote: "Details have been added or changed, and some of the physiologic interpretations proposed by Friedreich are no longer tenable, but

the main clinical and pathological observations and ideas are sound and enduring".<sup>21</sup>

## Pathology

The main sites of pathology in FRDA are the dorsal root ganglia, posterior columns of the spinal cord, corticospinal tracts, and the heart. Macroscopically there is a small spinal cord with the posterior and lateral columns particularly affected.<sup>10</sup> The nervous system changes appear to be a dying back process from the periphery.<sup>22</sup> This process appears to affect the longest and largest myelinated fibres. In the posterior columns, demyelination is seen. In particular, the large fibres arising in the dorsal root ganglia are affected. There is striking involvement of Clarke's columns and the dentate nucleus. The corticospinal tract involvement, which is more severe laterally and distally,<sup>23</sup> explains the common finding of upgoing plantar responses. A distal axonopathy affecting larger myelinated nerve fibres is also present.<sup>24</sup> In the cerebellar cortex, there is only mild neuronal loss.<sup>25</sup> Reduced phospholipid levels have been found in the cerebellar and occipital cortex of brains of those with FRDA.<sup>26</sup> This was interpreted as being the result of a widespread alteration in cellular phospholipid metabolism given that these are areas characterised by minimal neuronal loss.

The commonest cardiac lesion is hypertrophic cardiomyopathy, but less commonly a hypokinetic cardiomyopathy may be seen, which usually occurs following cardiac hypertrophy.<sup>27</sup> Iron deposits in the myocardium have been reported.<sup>28, 29</sup>

## Molecular genetics

The gene mutated in FRDA was mapped to chromosome 9 in 1988.<sup>30</sup> The location of the gene was narrowed down to 9q13–21.1 by subsequent linkage studies.<sup>31, 32</sup> This was followed by six years of intensive work by a number of research groups culminating in the cloning of the gene in 1996.<sup>33</sup>

The gene was initially called *X25* and later changed to *FRDA*. It contains seven exons (1–5a, 5b, 6). The commonest transcript arises from exons 1–5a. This is a 210 amino acid protein, frataxin. By alternate splicing, exon 5b can be transcribed and here a 171 amino acid protein arises. Exon 6 is non-coding.<sup>33</sup> Species conservation was found particularly for amino acids 141–167 that are encoded by exons 4 and 5a in *Caenorhabditis elegans* and *Saccharomyces*

*cerevisiae*. Campuzano *et al*<sup>33</sup> were unable to find sequence homology between this protein and others of known function in published databases.

Mutation analysis was undertaken on 184 FRDA patients. It was shown that 71/79 patients with typical FRDA were homozygous for a GAA expansion in intron 1 of *FRDA* and that the other 8/79 were compound heterozygotes for a point mutation and an expansion. No expansions of the GAA repeat were found in 77 people who did not suffer from FRDA.<sup>33</sup> Normal alleles contain between six and 34<sup>15 34</sup> uninterrupted GAA repeats whereas expanded alleles contain 67-1700 repeats.<sup>14 20</sup> Thus FRDA is so far unique among trinucleotide repeat disorders in that it is autosomal recessive, the repeat is intronic, and it is the only disease known to be the result of expansion of a GAA trinucleotide repeat.

Campuzano *et al*<sup>33</sup> showed by northern blotting that the highest level of expression of *FRDA* is in heart with intermediate levels in liver, skeletal muscle, and pancreas. Similar studies of the central nervous system showed high levels of expression in the spinal cord, less in the cerebellum, and very little in the cerebral cortex. Messenger RNA levels are very low or undetectable in patients compared to controls.<sup>33</sup> Furthermore, protein levels are low, but not absent, in various tissues in FRDA patients including cerebral and cerebellar cortex, skeletal muscle, and lymphoblasts.<sup>35</sup> There is an inverse correlation between the size of the smaller GAA repeat and the amount of residual frataxin in lymphoblasts.<sup>35</sup>

Mouse embryo studies show that frataxin expression increases from day 10 to 14.<sup>36 37</sup> Expression is seen in the developing forebrain, thoracolumbar region of the spinal cord, and large neuronal cells of the dorsal root ganglia. Outside the central nervous system, high levels of frataxin expression are seen in the developing heart, liver, skeletal muscle, thymus, skin, teeth, and brown fat.

It has been shown that the reduced mRNA levels are the result of inhibition of transcription and not at the level of post-transcriptional RNA processing. The level of mRNA is inversely related to the size of the GAA repeat.<sup>38 39</sup> Replication of plasmids with long GAA tracts of about 250 repeats, in COS-7 cells, were significantly inhibited compared to plasmids carrying shorter GAA repeats.<sup>38</sup> The GAA expansion forms an unusual DNA structure which is stable and it is believed that this interferes with transcription.<sup>39</sup> There is evidence that the uninterrupted GAA expansion adopts conformations (probably triplexes) which results in self-association of the DNA.<sup>40</sup> This phenomenon has been called "sticky DNA" and is unprecedented in DNA biochemistry.

#### **Intergenerational instability, premutations, and origin of mutations**

As in other trinucleotide repeat disorders, the GAA repeat that underlies FRDA is unstable in its transmission from parent to offspring.<sup>41</sup> Maternal transmission may result in a larger or

smaller allele in offspring. By contrast, the GAA repeat size almost always decreases when transmitted by a male.<sup>42-45</sup> The size of the triplet repeat influences the direction of instability with smaller alleles more prone to increase in size and larger ones to decrease.<sup>42 45</sup>

A small percentage of alleles are beyond the size found in the normal range but are smaller than alleles generally found in those affected with FRDA. These have been called premutation alleles.<sup>14 15 34 44</sup> Such alleles are prone to very large expansions in one generation. Most expansions of this type do not cause FRDA because the allele inherited from the other parent is likely to be normal. Therefore, the incidence of such events is unknown.

Sperm studies have shed some light on these findings. Males heterozygous for a full length GAA repeat have GAA repeat sizes in sperm which are smaller than those seen in lymphocyte DNA.<sup>43</sup> A premutation carrier was found to have GAA repeat number in sperm intermediate between that in his leucocytes and his son's smaller allele.<sup>44</sup> This is suggestive of a two stage expansion, meiotic and mitotic. Sperm studies on FRDA patients also led to the conclusion that instability occurs pre- and postzygotically.<sup>45</sup> Size contraction is greater in transmission to homozygotes than to their carrier sibs.<sup>42 45</sup> This possibly indicates a postzygotic interaction of the two FRDA alleles or postzygotic negative selection against cells containing larger expansions and thus lower levels of frataxin.<sup>42 45</sup>

Normal sized alleles have a bimodal distribution; most (about 83%) have between six and 12 repeats, while the second smaller group (about 17%) have between 14 and 34 repeats.<sup>15 34</sup> These groups have been called small normal and large normal alleles respectively. The haplotype background of expanded alleles and large normal alleles is very similar. By contrast, the haplotype background of small normal alleles is different.<sup>15</sup> This strongly suggests that expanded alleles arise from large normal alleles as has been described in other trinucleotide repeat disorders, such as Huntington's disease.<sup>46</sup> There appears to be an equilibration of alleles expanding from the large normal range to pre- and full mutations to compensate for mutations lost through reduced reproductive fitness of those affected with FRDA, and alleles which decrease from expansions to normal alleles. This last mentioned phenomenon has been observed in sperm of a person with FRDA.<sup>45</sup> The presence of a single or few initial founder events leading to a large normal allele is suggested by the common haplotype background found in FRDA patients.<sup>15 47</sup> The fact that very similar haplotypes were found in North African and Yemenite subjects with large normal and expanded alleles suggests the founder event is very old, predating the split of these populations.<sup>15</sup>

#### **Genotype-phenotype correlation**

With the identification of the gene mutated in FRDA, a number of studies examined the question of whether there is a correlation

between the size of the GAA repeats and the presence and timing of various features of the disease.<sup>20 42 48-51</sup> About 50% of the variation in the age of onset is accounted for by the size of the smaller allele. All these studies apart from one<sup>50</sup> show a far greater contribution from the smaller than larger allele to disease parameters and complications.

Two of the major complications of FRDA are cardiomyopathy and diabetes mellitus. Different groups had different conclusions with regard to the association between these complications and allele size (table 3).<sup>20 42 48 50 51</sup>

Overall, it can be concluded that the size of the GAA repeat length in each allele is important in predicting the age of onset and some features of FRDA. The smaller of the two alleles is more important in this regard. The smaller allele size inversely correlates with the amount of residual frataxin present in lymphoblasts, providing a biological explanation for the observed genotype-phenotype correlation with this allele.<sup>35</sup> As for other trinucleotide repeat disorders, repeat sizes cannot be used accurately to predict prognosis in a person.<sup>41</sup>

Of interest is the finding from the USA and Germany that subjects unaffected by FRDA but with non-insulin dependent diabetes mellitus are significantly more likely to have large normal alleles (but not expansions) than those without this disease.<sup>52</sup> This finding was not replicated by French<sup>53</sup> or Danish studies.<sup>54</sup>

Once the chromosomal localisation of the FRDA gene was known, a number of studies were done to assess whether this is the only gene FRDA is linked to and whether some atypical phenotypes are linked to the same gene.

Most cases of typical FRDA are linked to chromosome 9 but not all.<sup>31 55-57</sup> Rarely, families with typical FRDA are not linked to the chromosome 9 locus suggesting that a second locus for FRDA may exist.<sup>58 59</sup> Ataxia with vitamin E deficiency (AVED) can cause a very similar phenotype to FRDA<sup>60</sup> and is the result of mutations in the  $\alpha$ -tocopherol transfer protein gene on chromosome 8.<sup>61</sup> If a patient has a phenotype that could be the result of FRDA but molecular testing is negative, then vitamin E levels should be tested.

Late onset Friedreich ataxia (LOFA), which is defined as onset of FRDA after 25 years of age, was shown to be linked to the same locus as typical FRDA.<sup>62 63</sup> FRDA with retained reflexes (FARR) was similarly shown to be linked to the same chromosome 9 locus.<sup>64 65</sup> The Acadian form of FRDA (FRDA-Acad) is

characterised by slower progression than classical FRDA and is not associated with cardiomyopathy and diabetes mellitus. Spastic ataxia is also seen in the Acadian population (SPA-Acad). Both FRDA-Acad and SPA-Acad are linked to the same chromosome 9 locus as classical FRDA.<sup>55 66</sup> These linkage findings were confirmed by mutation analysis of FRDA.<sup>20 48 50</sup> Additionally, with the hindsight afforded by molecular diagnosis, it has been found that spastic paraparesis can be the presenting feature of FRDA.<sup>67 68</sup> Occasional unexpected presentations of FRDA have also been reported, including pure sensory ataxia<sup>69</sup> and chorea.<sup>70</sup> The diagnostic criteria of Geoffrey *et al*<sup>71</sup> and Harding<sup>6</sup> have proven to be highly specific although they will miss a small number of cases of FRDA including LOFA and FARR.<sup>20 49 50 72</sup>

### Point mutations

Seventeen point mutations have so far been described in FRDA.<sup>73 74</sup> About 4% of those with FRDA are compound heterozygotes for a point mutation on one allele and an expansion on the other allele. This means that approximately 2% of all FRDA mutations among the patient population that have GAA expansions are point mutations.<sup>33 74</sup> No patients homozygous for point mutations have been described. The chances of a person with FRDA having homozygous point mutations is about 1:2500<sup>16</sup> and therefore the population incidence of a person being homozygous for a point mutation is expected to be approximately one in 100 million!

Three point mutations appear to be relatively common. I154F is found among southern Italians with a carrier frequency of about 1:3300.<sup>33</sup> This mutation, when present with a GAA expansion on the other allele, is associated with a phenotype indistinguishable from typical FRDA.<sup>48</sup> The ATG→ATT (M1I) mutation of the start codon has been identified in three independent families in whom haplotype analysis suggests a common founder.<sup>75</sup> G130V has been found in patients from a number of places around the world and is associated with an atypical phenotype in all four families described with this mutation.<sup>73 74 76</sup> In particular these patients have slower disease progression despite age at onset being the same as typical FRDA, they all have brisk knee reflexes, and dysarthria is minimal or absent.<sup>73 74 76</sup>

Comparing the clinical parameters of those with point mutations to those homozygous for a GAA expansion, Cossee *et al*<sup>74</sup> found a significantly earlier age of onset, less dysarthria, and more frequent optic disc pallor in those with point mutations. No other parameters were significantly different. This study pooled data from all their point mutation patients to reach these conclusions. The size of the GAA repeat will presumably be important in phenotype determination and again the small numbers make this difficult to assess. Looking at all point mutations described, it can be concluded that point mutations associated with absent functional frataxin result in a severe phenotype,

Table 3 Analysis of the correlation of small allele size with the presence of cardiomyopathy and diabetes mellitus in various series<sup>20 42 48 50 51</sup>

Feature	Reference				
	20	42	48	50	51
Cardiomyopathy	<0.05	<0.05	<0.05*	NS	NS
Diabetes	NS	<0.001	—	NS	NS

NS = statistically significant correlation not found.

— = result not reported.

\*Monros *et al*<sup>48</sup> found that cardiomyopathy had a significant correlation with both alleles, but was more significant with the larger of the two.

whereas with missense mutations it cannot be predicted whether they will result in a mild or severe disease course.<sup>73 74</sup>

### Implications of the advances in the molecular genetics of FRDA for clinical practice

The ability to diagnose FRDA by molecular means with a high sensitivity and specificity is a great advance in clinical practice for the following reasons.

For patients with clinically typical FRDA, a specific and rapid diagnosis can be made in most patients and in the 4% compound heterozygous for a point mutation, the presence of one expanded allele indicates that the diagnosis is likely.

Atypical patients can usually be definitively diagnosed as having or not having FRDA. This is very helpful in counselling such families. Those heterozygous for an expansion remain problematical. Are they compound heterozygotes with a point mutation or are they carriers and the finding is a red herring? This can only be answered by mutation analysis for point mutations.

For a subject with FRDA who has a partner who is a carrier there is a 1:2 risk of having an affected child. The a priori risk for a person with FRDA having a child with the condition is approximately 1:200 unless there is consanguinity.

Carrier testing is available for relatives of those affected and their partners. Thus very accurate advice regarding risks for their offspring can now be given. The small possibility that a point mutation is present needs to be incorporated into counselling. Thus if the partner of a carrier does not have an expanded allele, the chance that they carry a point mutation is about 1:5000 (taking a FRDA carrier rate of 1:100 and that 2% of mutations are point mutations). Therefore the risk of this couple having a child with FRDA is about 1:20 000 which is only about twice the background risk of the general population.

Prenatal diagnosis is available by direct mutation detection. This aspect of FRDA was recently reviewed.<sup>16</sup>

### Pathogenesis

Before the cloning of *FRDA*, a number of pathogenic mechanisms had been explored to try to explain the underlying cause of FRDA. Lipoamine dehydrogenase deficiency had been found although it was unclear whether this was primary or secondary.<sup>77</sup> Another group found low pyruvate carboxylase in liver and fibroblasts,<sup>78</sup> but later withdrew this finding for fibroblasts.<sup>79</sup> The finding of reduced mitochondrial malic acid in cultured fibroblasts from FRDA patients aroused much interest,<sup>80</sup> but subsequent studies failed to replicate these results.<sup>81 82</sup>

With the discovery of the gene that is mutated in FRDA, rapid advances have been made in deciphering the underlying pathogenesis. There is much evidence to suggest that FRDA results from mitochondrial iron accumulation leading to cellular damage and death

by the production of toxic free radicals by Fenton chemistry (fig 1). The Fenton reaction is:  $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}^\bullet$ .

$\text{OH}^\bullet$  is the hydroxyl radical and is known to be highly toxic to cells by reacting with many intracellular elements including DNA, membrane lipids, and carbohydrates.<sup>83</sup> Evidence to support this as the underlying cause of FRDA includes the following.

#### MITOCHONDRIAL LOCALISATION OF FRATAXIN

The N-terminal region of frataxin was predicted by detailed sequence comparison with known proteins to contain a mitochondrial targeting sequence.<sup>36 84</sup> Using frataxin fused to a reporter protein, human frataxin has been shown to be a nuclear encoded mitochondrial protein.<sup>36 85</sup> Colocalisation of specific anti-frataxin antibodies and anti-mitochondrial antibodies provided precise independent evidence that frataxin is a nuclear encoded mitochondrial protein.<sup>35</sup>

#### EVIDENCE THAT MITOCHONDRIAL IRON OVERLOAD AND RESPIRATORY DEFICIENCY UNDERLIES FRDA

The yeast frataxin homologue (*YFH1*) was discovered not in the pursuit of the pathogenesis of FRDA, but in the study of genes involved in cellular iron metabolism.<sup>85</sup> The product of *YFH1*, Yfh1p, was found to be a high copy number suppressor of a mutant that could not grow on iron limited media.<sup>85</sup> The homology with *FRDA* was subsequently noted. When *YFH1* was deleted (*Δyfh1*), the following was observed.

*Δyfh1* yeast grew poorly on fermentable carbon sources and formed abnormally small colonies.<sup>36 85 86</sup> This is typical of so called "petite" strains of yeast which display respiratory deficiency.<sup>36 86</sup>

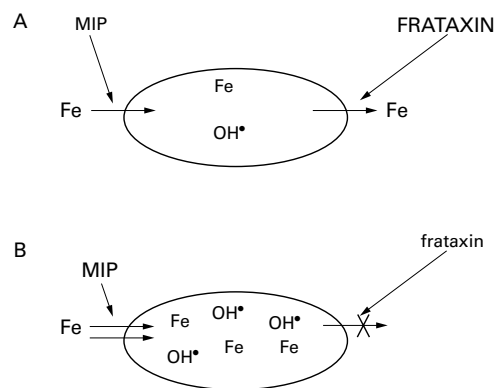


Figure 1 Diagrammatic representation of the likely pathogenesis of FRDA. (A) The normal situation in the mitochondrion is shown with iron influx and efflux maintaining low mitochondrial iron (Fe) and free radical ( $\text{OH}^\bullet$ ) levels. Frataxin is likely to be acting directly at the level of iron efflux. Yeast data suggest that frataxin may also be indirectly limiting the influx of iron into mitochondria by reducing mitochondrial intermediate peptidase (MIP) activity. (B) The situation believed to exist in FRDA. Reduced frataxin results in inhibition of the efflux of mitochondrial iron. This leads to reduced cytosolic iron, which results in induction of iron uptake systems and this in turn results in further iron uptake into mitochondria. The increased iron uptake may be in part the result of overactivity of MIP owing to the absence of frataxin. The excess mitochondrial iron leads to excess production of toxic free radicals leading to cell damage and death.<sup>92 93 101</sup>

*Ayfh1* whole yeast contained twice as much iron as wild type yeast and 10 times the amount of mitochondrial iron.<sup>85 87</sup>

*Ayfh1* yeast were hypersensitive to oxidants including H<sub>2</sub>O<sub>2</sub>,<sup>85 87</sup> iron, and copper.<sup>87</sup> These substances all inhibited the growth of *Ayfh1* to a greater extent than wild type yeast. *Ayfh1* yeast lost mitochondrial DNA becoming so called *rho*-.<sup>36 85 86</sup>

Respiration rates of *Ayfh1* yeast were less than 10% of wild type.<sup>36</sup>

Further evidence that FRDA is the result of mitochondrial dysfunction came from mitochondrial enzyme studies. These showed that there was deficient activity of iron-sulphur containing enzymes in endomyocardial biopsies from two unrelated FRDA patients, but the enzyme activities were normal in skeletal muscle, lymphocytes, and fibroblasts.<sup>88</sup> Iron sulphur proteins are very sensitive to oxidative stress.<sup>89</sup> Additionally, fibroblasts from FRDA patients were more sensitive to oxidant stress than control fibroblasts.<sup>90</sup>

The finding of iron deposits in myocardium from people with FRDA is further evidence that the yeast findings are relevant to human FRDA.<sup>28 29</sup> It has been shown that fibroblasts from patients with FRDA have higher mitochondrial iron levels than is seen in control fibroblasts.<sup>91</sup> This is the first evidence of mitochondrial iron overload in cells from FRDA patients.

While the exact mechanism by which frataxin is involved in mitochondrial iron homeostasis is unknown, investigation of the yeast frataxin homologue indicates that the protein is involved in iron efflux from mitochondria.<sup>92</sup> This study of Radisky *et al*<sup>92</sup> provides strong evidence that the iron overload is primarily responsible for the pathology of FRDA and is not the result of mitochondrial damage from another cause, with secondary iron accumulation. Recent evidence has shown that Yfh1p interacts with mitochondrial intermediate peptidase (YMIP), a metalloprotease required for maturation of ferrochelatase and other iron using proteins.<sup>93</sup> When there is diminished Yfh1p, there is activation of YMIP leading to mitochondrial iron uptake. Thus it appears that Yfh1p regulates mitochondrial iron directly at the level of iron efflux and indirectly through regulation of YMIP activity.

Two mitochondrial proteins have been identified which play a role in the maturation of frataxin. Ssc2p is a homologue of mt-Hsp70, a heat shock protein, and is involved in Yfh1p maturation in yeast,<sup>94</sup> while mitochondrial processing peptidase beta (MPPbeta) is involved in human frataxin processing.<sup>95</sup>

The question arises as to why some tissues are affected in FRDA and not others. Part of the explanation relates to frataxin expression. As already noted, frataxin expression is highest in the heart, liver, skeletal muscle, and pancreas and is minimal in the cerebral cortex.<sup>33</sup> This expression pattern mirrors the affected organs to an extent, although the high liver expression, for example, does not correlate with pathology. This may be because affected tissues are non-dividing (neurones, heart, pancreas), but

liver and skeletal muscle are composed of dividing cells.<sup>96</sup> Different tissues have different sensitivity to oxidative stress and may use different ways of dealing with that stress.<sup>89</sup> Thus the organ specificity of pathology in FRDA may be explained by the combination of different expression patterns of frataxin and different requirements for frataxin in dealing with mitochondrial iron and, as a consequence, oxidative stress.

### The future

The three years since the cloning of the *FRDA* gene have seen great advances in the understanding of the basis of FRDA. It has opened the way for possible therapeutic advances based on the discoveries of what frataxin does and what happens when it is reduced in quantity.

Initial enthusiasm for trialing iron chelators waned with the realisation that the main available chelator, desferrioxamine, reduces intracellular iron but its ability to remove mitochondrial iron is unknown. It has a significant side effect profile and its effect on people who do not have generalised iron overload is not well studied. FRDA patients have normal serum iron and ferritin levels.<sup>97</sup> In addition, it has been shown in *in vitro* studies that desferrioxamine in the presence of reduced iron causes a marked decrease in aconitase activity.<sup>98</sup> Aconitase is one of the iron-sulphur enzymes whose activity was found to be reduced in myocardium in FRDA patients.<sup>88</sup>

Antioxidant therapy has, in theory, a role to play in treating FRDA. It has been proposed that reducing the load of free radicals will slow the progression of the disease. Early reports of treating small patient numbers are promising but larger trials are required.<sup>98</sup>

There have been many recent reports of advances in gene therapy approaches for neurological and cardiac disease. FRDA has a number of features that suggest that this therapeutic modality may one day play a role in its management. Cardiomyopathy is the commonest cause of death in FRDA, and the heart may be relatively easy to deliver genes to. Another reason for optimism with the prospect of gene therapy for FRDA is that there is a low level of frataxin present in all patients, and therefore immunological considerations will be less of a concern than in conditions with complete absence of a particular protein.

If a treatment is shown to benefit people with FRDA, then as patients can be identified presymptomatically with full neurological function, the intervention may be able to prevent the appearance of symptoms.

One of the problems with assessing treatment regimens for FRDA is the difficulty in observing benefit owing to the slow progression of the disease and the lack of surrogate end points. For example, if a treatment slowed the progression but did not reverse any clinical manifestations, this benefit could take many years to detect. One possible solution is to produce animal models. A number of groups are in the process of making animal models but none has yet been published. Another important path in monitoring therapeutic efficacy is to

develop laboratory based assays that reflect the benefit of a particular treatment. So far no such assay exists, although some show promise. These include protoporphyrin IX<sup>99</sup> and <sup>31</sup>P magnetic resonance spectroscopy which show that FRDA patients have lower rates of mitochondrial ATP production in skeletal muscle than controls.<sup>100</sup>

Our knowledge of FRDA has advanced more during the last three years than in the 133 years from the initial description to gene discovery. There is appropriate optimism that, based on this knowledge, therapies will be found which will alleviate the inevitable and tragic fate that is faced by those affected by Friedreich ataxia.

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