Friedreich Ataxia

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Friedreich ataxia is an autosomal recessive degenerative disease that primarily affects the nervous system and the heart. It is named after its original description as a "degenerative atrophy of the posterior columns of the spinal cord" by Nicholaus Friedreich, who was a professor of medicine in Heidelberg in the second half of the 19th century. The full extent of the Friedreich ataxia phenotype and its genetic epidemiology could only be appreciated after a direct genetic test became available in 1996. At the same time, the complex pathogenesis of Friedreich ataxia started to be unraveled. Herein, I review our current knowledge of the disease and how it is contributing to the development of therapeutic approaches.

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Symptoms of Friedreich ataxia (FRDA) typically become evident around puberty,[1] but age at onset may vary substantially, even within a sibship. The earliest onset may be around the age of 2 years, while late-onset FRDA is defined as having onset after the age of 25 years.[2,3] Occasionally, patients become clearly symptomatic in their sixth or seventh decade. Variability of age at onset is now in part explained by the dynamic nature of the underlying mutation.[1,2]

The first symptom is usually gait instability, though scoliosis may already be present when neurologic symptoms appear, and, in rare cases, hypertrophic cardiomyopathy is diagnosed before the onset of ataxia. Ataxia of mixed cerebellar and sensory type is the cardinal symptom. It affects the trunk, with swaying, imbalance, and falls, and the limbs, with increasing difficulty in activities of daily living, such as dressing, handling utensils, and writing. Ataxia is progressive and unremitting, though periods of stability are frequent at the beginning of the illness. With progression, gait becomes broad-based, with frequent losses of balance requiring intermittent and then constant support. Fine motor skills deteriorate and dysmetria and intention tremor become evident. Limb weakness of central origin is a constant feature of advanced FRDA, in which it may become severe. Some amyotrophy, particularly in the hands, is also very common. On average, 10 to 15 years after onset, patients lose the ability to walk, stand, and sit without support.[1] However, evolution is variable. There are patients with mild cases who are still ambulatory decades after onset and those with severe cases who must use a wheelchair in a few years.[1,4] Dysarthria consisting of slow, jerky speech with sudden utterances is a relatively early feature and progresses until speech becomes almost unintelligible. Dysphagia, particularly with liquids, appears with advancing disease, requiring modified foods and eventually a nasogastric tube or gastrostomy feedings. Nystagmus is not prominent in FRDA, the most common oculomotor abnormality being instability of fixation with frequent square-wave jerks.[3] Cognitive functions are generally well preserved, but subtle executive dysfunction may be common. In any case, because of substantial physical disability, FRDA often has a substantial effect on academic, professional, and personal development.

Pathologically, specific vulnerability of different neuronal types is evident.[5] The somatosensory system conveying proprioceptive information to the brain and cerebellum is affected early and severely. Large primary sensory neurons in the dorsal root ganglia are atrophied and disappear early in the course of the disease. Their loss leads to an axonal sensory peripheral neuropathy and to the atrophy of the posterior columns of the spinal cord (as indicated in the title of the original Friedreich article[6]), which is the basis of the loss of vibration and position sense that affects these patients and of the sensory component of their ataxia. Loss of neu-

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rons in the nucleus thoracicus posterior results in atrophy of the spinocerebellar tracts and loss of proprioceptive input to the cerebellum, which cause, with the severe atrophy of the dentate nucleus, the main source of cerebellar output pathways, the cerebellar component of ataxia. Corticocerebellar atrophy and Purkinje cell loss are mild or moderate and generally late, so these patients have no obvious cerebellar atrophy on brain imaging studies. Involvement of the auditory sensory neurons and pathways may also be found, as in optic atrophy.[1] On the motor side, the main finding is the progressive degeneration of the corticospinal tracts, which is more evident distally, suggesting a dying-back process.[5]

A hypertrophic cardiomyopathy with thickening of the left ventricular wall and septum is detected by heart ultrasonography in most patients, but it is often asymptomatic. On the other hand, the electrocardiogram is almost always abnormal in the repolarization phase, indicating that subclinical heart disease is almost universal in FRDA. When present, cardiac symptoms initially consist of palpitations and shortness of breath. In a few cases, cardiomyopathy progresses to the point of causing heart failure or fatal arrhythmias, sometimes at a young age.[1] Pathologically, there is a real hypertrophy of cardiomyocytes at the beginning, followed by their loss and replacement by connective tissue when cardiomyopathy progresses toward heart failure and a final dilation stage.[7] Foci of necrosis as well as iron deposits in surviving myocardial cells can be seen.[8]

Kyphoscoliosis and talipes cavus are common skeletal abnormalities found in patients with FRDA.[1] Because these abnormalities are present in other neuromuscular disorders as well, they are thought to be secondary to nervous system involvement. Scoliosis may aggravate cardiorespiratory problems and progress to the point of requiring surgery. Talipes cavus may render gait, already compromised by ataxia, even more difficult. Its surgical correction may be indicated in some still ambulatory patients to delay loss of ambulation.

Diabetes mellitus is much more frequent in patients with FRDA than in the general population. A combination of insulin resistance and inadequate insulin response contributes to diabetes in FRDA; both forms are likely to be a direct consequence of the mitochondrial dysfunction that occurs in this disease.

Friedreich ataxia is the most common inherited ataxia in individuals of Western European origin and is also found in those of North African and Middle Eastern origin, but it has not been reported in other ethnic groups. The reason for this restricted distribution is the unique occurrence of the causative mutation. The differential diagnosis, once tumoral, vascular, toxic, and inflammatory causes of ataxia have been ruled out, involves other recessive degenerative and metabolic ataxias. The **Table** summarizes the distinctive clinical and laboratory findings in FRDA vs the most common recessive ataxias that are in the differential diagnosis.

THE FRIEDREICH ATAXIA GENE

The mutated gene in individuals with FRDA (*FXN*; OMIM 606829) is localized in the proximal long arm of chromosome 9.[9] The main messenger RNA has a size of 1.3 kilobases and corresponds to 5 exons (numbered 1-5a). Minor splice variants containing an alternative exon 5b and a sixth noncoding exon exist, but their functional significance is unknown.[10] The encoded protein, predicted to contain 210 amino acids, is called frataxin. Frataxin has been shown to be a mitochondrial matrix protein.[11] It is highly conserved in evolution, with homologues in all eukaryotes and in many prokaryotes. Simple unicellular organisms as yeast can survive without frataxin, but frataxin is lethal for higher organisms, including mice,[12] which die early in embryonic development. In conditional frataxin knockout mice, total lack of frataxin results in the eventual demise of the targeted cells. The reason frataxin is essential for survival, particularly during embryonic development, and its mechanism(s) of cell death are still unknown.

The *FXN* gene is expressed in all cells, but at variable levels in different tissues and during development.[10,13,14] Differences in mitochondrial mass cannot fully account for differences in *FXN* expression. Overall, *FXN* expression is high in cells that are affected by the disease, but variation in frataxin abundance, which is similar throughout the central nervous system, does not obviously explain the selective vulnerability of specific neurons as primary sensory neurons. In adult humans, frataxin messenger RNA is most abundant in the heart and spinal cord, followed by the liver, skeletal muscle, and pancreas. In mouse embryos, expression starts in the neuroepithelium at embryonic day 10.5 and reaches its highest level at embryonic day 14.5 and into the postnatal period. In developing mice, the highest levels of frataxin messenger RNA are found in the spinal cord, particularly at the thoracolumbar level, and in the dorsal root ganglia. The developing brain is also very rich in frataxin messenger RNA, which is abundant in the proliferating neural cells in the periventricular zone, cortical plates, and ganglionic eminence. Expression is also high in the heart, liver, and brown fat.

FRATAXIN FUNCTION AND FRDA PATHOGENESIS

Frataxin targets the mitochondrial matrix, where an N-terminal targeting sequence is removed by the mitochondrial processing peptidase. Structurally, mature frataxin is a compact, globular protein that contains an N-terminal α helix, a middle β-sheet region composed of 7 β strands, a second α helix, and a C-terminus in an extended conformation.[15] The α helices are folded upon the β sheet, with the C-terminus filling a groove between the 2 α helices. On the outside, a ridge of negatively charged residues and a patch of hydrophobic residues are highly conserved. The acidic ridge is essential for function, as shown by structural and functional in vitro studies and by complementation experiments in frataxin-deficient yeast using specific missense mutants, and is likely to be involved in iron-mediated protein-protein interactions.[16]

Though the function of frataxin is still partly debatable, there is general agreement that it is involved in cellular iron homeostasis and that its deficiency results in multiple enzyme deficits, mitochondrial dysfunction, and

Table. Clinical and Laboratory Findings in FRDA and Common Recessive Ataxias

Characteristic	Disease				
	FRDA	AVED	AOA Type 1	AOA Type 2	ARSACS
Age at onset, range, y	2-60	2-50	2-18	2-28	1-Adulthood
Clinical feature					
Ataxia	+	+	+	+	+
Dysarthria	+	+	+	+	+
Proprioceptive loss	+	+	+	+	+
Areflexia	+	+	+	+	±[a]
Babinski sign	+	+	–	+	+
Muscle weakness	+	+	+	+	+
Distal amyotrophy	+	±	+	+	+
Spasticity	±	±	–	–	+
Oculomotor apraxia	–	–	+	±	–
Cognitive impairment	–	±	±	±	±
Sensorineural hearing loss	±	–	–	–	–
Head titubation	–	±	–	–	–
Dystonia	±	±	±	±	–
Chorea	±	–	+	±	–
Scoliosis	+	±	±	±	±
Pes cavus	+	±	±	±	±
Cardiomyopathy	+	±	–	–	–
Diabetes mellitus	±	–	–	–	–
Laboratory investigation					
Biochemistry	–	Vitamin E <3 mg/L	Hypoalbuminemia, hypercholesterolemia	α-Fetoprotein >20 ng/mL, increased IgG and IgA levels, hypercholesterolemia, increased serum CK concentrations	–
MRI finding	Cervical spinal cord atrophy	Mild cerebellar atrophy	Cerebellar atrophy	Cerebellar atrophy	Superior vermis atrophy
Electrophysiology	Axonal sensory neuropathy	Axonal sensory neuropathy	Axonal sensorimotor neuropathy	Axonal sensorimotor neuropathy	Axonal and demyelinating sensorimotor neuropathy
Ophthalmologic finding[b]	Optic atrophy	Retinopathy			Retinal striation
Gene	*FXN*	*TTPA*	*APTX*	*SETX*	*SACS*

Abbreviations: AOA, ataxia with oculomotor apraxia; ARSACS, autosomal recessive spastic ataxia of Charlevoix-Saguenay; AVED, ataxia with vitamin E deficiency; CK, creatine kinase; FRDA, Friedreich ataxia; MRI, magnetic resonance imaging; +, typical features; ±, uncommon features; –, absent.

SI conversion factor: To convert α-fetoprotein to micrograms per liter, multiply by 1.0.

[a] Ankle reflexes are gradually lost, but other reflexes remain quick.

[b] Ophthalmologic abnormalities not present in all patients.

oxidative damage. Frataxin can bind ferrous iron through the negatively charged amino acids in its surface; it promotes the mitochondrial synthesis of iron-containing molecules, iron-sulfur clusters (ISCs),[17] and heme[18]; and it controls the ability of iron to perform redox chemistry.

Iron-sulfur clusters are prosthetic groups for a diverse group of proteins. Frataxin directly interacts with components of the mitochondrial ISC synthesis "machinery," in particular the "scaffold" protein IscU, where the first assembly of ISCs takes place, and with the sulfur donor IscS.[17] In mitochondria, ISC-containing proteins are involved in processes, such as electron transport (complexes I, II, and III of the respiratory chain), energy metabolism (aconitase), DNA repair (NTH1/MIH), and iron handling (ferrochelatase). All these activities are reduced to a variable extent when frataxin level is low, as in patients with FRDA, or absent, resulting in mitochondrial dysfunction.[19] Iron-sulfur cluster–containing proteins are also found in the cytosol and nucleus, where they are involved in regulating iron metabolism (cytosolic aconitase/iron regulatory protein), purine synthesis (phosphoribosylpyrophosphate amidotransferase), purine catabolism (xanthine oxidase), protein synthesis and DNA repair (RpS3). At least some of these extramitochondrial ISC-containing enzymes also have reduced activity when frataxin is deficient, possibly contributing to the disruption of cellular homeostasis.[20] The involved mechanism is still controversial: either cytosolic and nuclear ISCs are synthesized in the mitochondria and subsequently exported or there is extramitochondrial ISC synthesis and an extramitochondrial pool of frataxin takes part in it and possibly other functions.[21]

Heme synthesis defects are not evident in patients with FRDA, though they occur after *FXN* disruption in simple model organisms as yeast. Frataxin is possibly not as critical for heme synthesis as it is for ISCs, or it has a higher affinity for the heme-synthesizing protein ferrochelatase than for IscU, as determined in vitro with purified components,[18,22] which allows this process to proceed normally even when frataxin is too low to support normal ISC synthesis.

Further controversies about the function of frataxin include the physiological relevance of its ability to form in vitro high molecular weight complexes with iron when the metal is present at a high concentration.[23] Such complexes would be important for iron detoxification and protection from oxidative damage,[24] but data suggest that this function is dispensable.[25] Additional findings also suggest possible roles of frataxin in directly stimulating the respiratory chain[26] and in mobilizing antioxidant defenses,[27,28] but the involved mechanisms have not been elucidated. **Figure 1** illustrates the currently postulated functional roles of frataxin.

Frataxin deficiency clearly leads to oxidative damage, as indicated by much evidence in a model system and in patients with FRDA. Several mechanisms may be involved and act synergistically: iron in mitochondria is not efficiently used and gets engaged in redox chemistry with oxygen radicals generated as a by-product of the respiratory chain; and ISC deficiency in complexes I, II, and III causes impaired electron transport and enhanced oxygen radical production by the respiratory chain. In patients with FRDA, oxidative stress is revealed by increased plasma levels of malondialdehyde (a lipid peroxidation product),[29] increased levels of urinary 8-hydroxy-2′-deoxyguanosine (a marker of oxidative DNA damage),[30] decreased plasma free glutathione,[31] and increased plasma glutathione *S*-transferase activity.[32] Increased free radical production was directly demonstrated in cultured cells that were engineered to produce reduced levels of frataxin.[33] Intriguingly, no evidence of oxidative stress was obtained in studies on conditional knockout mouse models.[34] However, interfering factors may have affected these results, including the admixture of cells with normal frataxin with progressively disappearing cells with no frataxin and the almost complete shutdown of the respiratory chain in the latter.

Mitochondrial dysfunction is also a consequence of frataxin deficiency. It has been directly and noninvasively demonstrated in skeletal muscle and hearts of patients with FRDA by phosphorus magnetic resonance spectroscopy that showed a reduced rate of adenosine triphosphate synthesis compared with healthy controls and individuals who have other neurological diseases.[35] Activation of stress pathways in frataxin-deficient cells supports the pathogenic role of mitochondrial dysfunction and oxidative stress in causing cell atrophy and death. Studies on cultured PC12 cells, rat pheochromocytoma cells that can be differentiated into neurons by adding nerve growth factor, showed an increased expression and activity of the mitogen-activated kinase kinase 4/c-Jun N-terminal kinase pathway, which may, at first, be a protective response but may also eventually trigger apoptosis.[36]

MUTATIONS CAUSING FRDA

The hyperexpansion of a GAA-triplet repeat in the first intron of *FXN* is the mutation found, so far, in all individuals with FRDA.[10] Most patients are homozygous for this mutation. A few, estimated between 2% and 5% in different countries, are compound heterozygous for the GAA expansion and a different mutation that leads to *FXN*

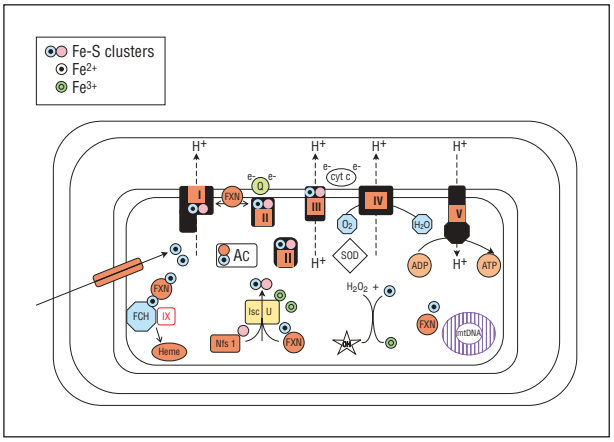

Figure 1. Postulated functions of frataxin (FXN): (1) Frataxin is a general iron chaperone, providing Fe^{2+} to ferrochelatase (FCH) for heme biosynthesis, mitochondrial iron-sulfur (Fe-S) clusters biogenesis, and maintenance of the mitochondrial aconitase (Ac) Fe-S cluster. (2) Frataxin may have a direct interaction with respiratory chain complexes (I-V). (3) Frataxin prevents oxidative stress and protects mitochondrial proteins and mitochondrial DNA (mtDNA) from free Fe^{2+}. It prevents Fenton reaction by converting Fe^{2+} to Fe^{3+} and thus prevents hydroxyl radical formation. ADP indicates adenosine diphosphate; ATP, adenosine triphosphate; cyt c, cytochrome c; e^-, electron; SOD, superoxide dismutase.

loss of function.[10,37] Repeats in normal chromosomes contain up to approximately 38 triplets, and disease-associated repeats contain from approximately 70 to more than 1000 triplets, most commonly 600 to 900. Heterozygous carriers are clinically healthy. This is the most common disease-causing triplet-repeat expansion identified so far, about 1 in 100 Europeans being a carrier. No other disease has been recognized to date to be caused by an expansion of GAA triplets.

The GAA-expansion mutation results in partial silencing of *FXN* and therefore in low levels of frataxin. The other rare mutations are either missense changes that cause the encoded protein to be nonfunctional or only partially functional or are null alleles. As demonstrated in knockout animals, complete lack of frataxin leads to early embryonic death. Homozygosity for expanded GAA repeats allows the synthesis of some structurally and functionally normal frataxin, at least around 5% of normal levels. Had this expansion never appeared, FRDA would very likely not exist or would be extremely rare, because all other known frataxin mutations in homozygosity may cause a loss of function that is too severe to allow survival through development. Even when a frataxin point mutation that was known to cause incomplete loss of function (G130V) (as judged by complementation frataxin–deleted yeast) was inserted into the homologous mouse gene by homologous recombination, death of homozygous embryos resulted (Panos Ioannou, oral communication, 2003). In fact, FRDA has never been diagnosed in individuals from ethnic groups in which the GAA expansion is not found.

The FRDA-associated expansion shows instability when transmitted from parent to child.[10] Expansions and contractions can both be observed and are equally likely after maternal transmission, while contractions are most common after paternal transmission.[38] In this regard, FRDA resembles the other diseases associated with very large expansions in

noncoding regions, including fragile X syndrome and myotonic dystrophy, and differs from the diseases that are caused by CAG repeats in coding regions, such as dominant ataxias and Huntington disease, in which size increases typically occur after paternal transmission.

The GAA expansion is also unstable mitotically and even in postmitotic cells. It is open to discussion whether one of the factors contributing to the specific vulnerability of some cell types in FRDA, such as dorsal root ganglia sensory neurons, is the inherent tendency of the GAA repeat in these cells to undergo further expansion.[39]

The FRDA-associated GAA repeat lies in the middle of a repetitive Alu sequence of the Alu-Sx subfamily. It is preceded by an average stretch of 16 A nucleotides, apparently derived from an expansion of the canonical $(A)_5(TACA)_6$ sequence linking the 2 halves of Alu sequences. Alleles at the GAA-repeat site can be subdivided into 3 classes depending on their length: short normal alleles of fewer than 12 GAA triplets (SN, approximately 82% in Western Europeans), long normal alleles of 12 or more GAA triplets (LN, approximately 17% in Western Europeans), and pathologic expanded alleles (E, approximately 1% in Western Europeans). The length polymorphism of the GAA repeat in normal alleles suggests that it was generated by 2 types of events. Small changes, plus or minus 1 trinucleotide, were likely the consequence of occasional events of polymerase slippage during DNA replication followed by misrealignment of the newly synthesized strand by 1 or a few repeat units (stuttering). Conversely, the passage from the SN to the LN group was probably an exceptional event. Linkage disequilibrium studies indicate that E and LN alleles appear genetically homogeneous, while SN represents a heterogeneous class of alleles.[40] It is possible that the event that created LN alleles was the sudden duplication of an SN allele containing 8 or 9 GAA triplets, creating an LN allele with 16 or 18 GAA triplets. The passage from LN to E alleles probably involved a second genetic event of the same kind, which generated very long LN alleles containing 30 to 34 GAA triplets still on the same haplotype background as the shorter LN alleles from which they were derived. By reaching the instability threshold, estimated as 32 GAA triplets, they form a reservoir for expansions. This length is close to the instability threshold for other triplet repeat–associated disorders, such as those involving CGG and CAG repeats.

One of the mechanisms underlying the instability of E alleles may be strand displacement during DNA replication. For this phenomenon to occur, the displaced strand has to form some kind of secondary structure. A single DNA strand containing a GAA repeat is able to form different types of secondary structures, which may be involved in instability. The triplex-forming ability of long GAA repeats may be involved in repeat instability by causing DNA-polymerase stalling and by forming a target for protein binding.

MECHANISMS OF GENE SILENCING BY THE EXPANDED GAA REPEAT

The mechanism of *FXN* silencing by the expanded GAA repeat has been the object of intense investigation, mostly because its understanding may offer a target for treatment. Because of the presence of an intact frataxin coding sequence in affected individuals on at least 1 chromosome, by partially overcoming the silencing imposed by the expanded GAA repeat, we can expect that sufficient levels of *FXN* expression may be restored to block or slow down disease progression or even, if the intervention is made before symptoms appear, to prevent disease development. Considering that carriers are clinically normal, pushing *FXN* expression to about half of normal in affected individuals may be enough to avoid disease. Clues to the mechanism of *FXN* silencing came from studies on the DNA structure and properties of the GAA repeat and, more recently, studies on the structure of chromatin in the region of the repeat. Studies on DNA structure moved from the fact that the GAA-repeat DNA contains only purines (R, G, and A) in 1 strand and pyrimidines (Y, C, and T) in the complementary strand. Such R·Y sequences have been known for some time to adopt a peculiar structure called a triplex, at least in the test tube. Triplexes are 3-stranded nucleic acid structures in which a third strand occupies the major groove of the DNA double helix and is kept in place by a special type of base pair, called Hoogsteen pairs, with the bases in the double helix. In intramolecular triplexes, the R·Y DNA folds back onto itself to form the triple helix structure. Four different isomers may form: 2 based on R·R·Y structures, in which the R strand from 1 part of the sequence pairs with the double R·Y helix from another part of the sequence, leaving a single-stranded Y region; and 2 based on Y·R·Y structures, in which it is the Y strand that pairs with the double helix. Intermolecular triplexes are formed between oligonucleotides or polynucleotides (DNA or RNA) and target R·Y sequences on duplex DNA. R·R·Y triplexes are formed at a neutral pH by a GAA repeat as found in FRDA. Further investigations showed that an even more complicated structure, called *sticky DNA*, may be formed by such repeats.[41] Sticky DNA consists of complexes formed by joining 2 DNA segments at the triplex region through the exchange of the third GAA-containing (R) strand. There is a clear correlation between the length of a GAA repeat, which confers the ability to form sticky DNA, and its pathogenicity in FRDA. The length threshold to form sticky DNA is about 59 repeats and the shortest reported pathologic allele in FRDA is 66 repeats. Furthermore, interruptions in the GAA repeat sequence prevent sticky DNA formation and are present in some rare long, nonpathogenic alleles of the *FXN* repeat. Sticky DNA is a strong inhibitor of transcription in vitro, as it impedes the progression of RNA polymerase, providing a possible direct mechanism for *FXN* silencing. However, in the eukaryotic nucleus, DNA is not free in solution, but it is associated with proteins, histones, and other factors to form chromatin. Chromatin may adopt a tightly condensed conformation that impedes access to transcription factors and is associated with gene silencing, or it may adopt an open, transcription-permissive structure. Closed, condensed chromatin corresponds to the heterochromatic regions, which are very poor in active genes, described by cytologists and cytogeneticists. Open, decondensed chromatin instead characterizes the transcriptionally active euchromatin. Chromatin condensation is linked by posttranslational modification

of histones, including acetylation, methylation, and ubiquitination. Evidence that expanded *FXN* GAA repeats are associated with heterochromatin first came from studies in transgenic mice. When a reporter gene was associated with a long GAA repeat in transgenic mice, it turned out to be silenced in a proportion of cells, resembling a phenomenon known to *Drosophila* geneticists as *position effect variegation*. Position effect variegation occurs when a gene is placed in the vicinity of a heterochromatic region and is caused by a spreading of heterochromatin to involve the gene itself. On the basis of the transgenic mice data, it was proposed that the GAA repeat acts as a seed for chromatin condensation, resulting in the silencing of any associated gene.[42] According to this view, *FXN*, or at least part of it, would be included into a heterochromatic region and silenced when it contains an expanded GAA repeat. Recent data indeed show that characteristic heterochromatin-associated posttranslational modifications of histones occur in the vicinity of the expanded GAA repeat in the lymphocyte DNA of patients with FRDA. Changes include an increase in histone H3 lysine 9 (H3K9) trimethylation and a decrease in acetylation at H3K14, H4K5, H4K8, and H4K12, all markers of a heterochromatic state.[43] In particular, H3K9 trimethylation is known to trigger binding of heterochromatin protein 1, which then recruits other factors that participate in chromatin condensation and gene silencing.

We do not currently understand if the structural properties of long GAA repeats are related to their ability to trigger chromatin condensation. It is possible that specific proteins recognize the triplex structure and recruit heterochromatin-promoting factors, but it is also possible that the GAA sequence is recognized independently of its ability to form triplexes in vitro. The identification of the involved factors is likely to shed light on this point. The current views on *FXN* silencing by the GAA repeat are shown in **Figure 2**.

Whatever mechanism triggers chromatin condensation at the expanded GAA repeat, specific inhibitors of histone deacetylases (HDACs), the enzymes responsible for removing acetyl residues from histones, can revert these modifications and substantially increase frataxin expression in patients' cells, including nonreplicating ones like primary lymphocytes.[43] Though HDAC inhibitors do not reverse H3K9 trimethylation, they probably prevent heterochromatin protein 1 from binding by increasing H3K14 acetylation, which may sterically impede the contact between heterochromatin protein 1 and trimethylated H3K9. These findings provide a very promising perspective on FRDA treatment and are currently the object of intense research.

OTHER *FXN* MUTATIONS

A few FRDA chromosomes carry GAA repeats of normal length, but have missense, nonsense, or splice-site mutations that ultimately affect the frataxin coding sequence.[10,37] In other cases, portions of *FXN* are missing due to large deletions. As discussed, affected individuals with these mutations are always compound heterozygous for a GAA expansion. In most cases, their phenotypes do not differ from those of patients with typical FRDA, though some

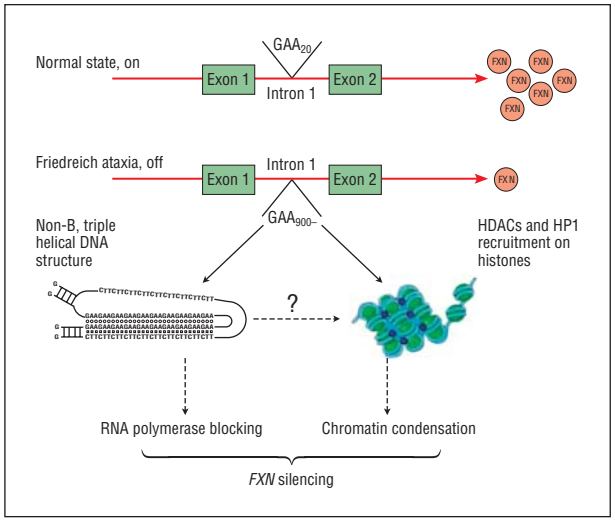

Figure 2. Mechanisms of *FXN* silencing by the expanded GAA repeat. FXN indicates frataxin; HDAC, histone deacetylases; HP1, heterochromatin protein 1.

missense mutations, such as D122Y, R165P, and G130V, cause an atypical and milder clinical presentation with early-onset spastic gait, slow disease progression, absence of dysarthria, retained or brisk tendon reflexes, and mild or no cerebellar ataxia.[37] Some residual activity of these mutated frataxins has been postulated to explain the milder phenotype, and, in fact, the G130V mutation shows some ability to rescue yeast cells that carry a deleted frataxin homologue gene (*yfh1*). Other missense mutations that are associated with a typical, more severe phenotype, such as W173G, are instead unable to complement *yfh1*-deficient yeast, suggesting that they essentially lack any residual activity.[44] Structural studies confirmed that certain missense mutations change critical residues involved in frataxin folding or affect conserved regions thought to be involved in iron binding or protein-protein interactions, so they are likely to result in complete loss of function. For example, the I154F and W155R mutant proteins have a strongly destabilized structure.[45]

Some mutations are predicted to be null alleles. These include insertions and deletions of a single or a few nucleotides, leading to frameshifts, splice-site mutations, and larger deletions. Patients with these mutations have typical FRDA that is sometimes associated with additional symptoms, like abnormal movements, which may indicate more severe disease. For unclear reasons, optic atrophy is more frequent in patients with point mutations of any kind (47%).[37]

GENOTYPE-PHENOTYPE CORRELATION AND MODIFYING ALLELES

As expected by the experimental finding that smaller expansions allow a higher residual gene expression, expansion sizes influence the severity of the phenotype. A direct correlation has been firmly established between the size of GAA repeats and earlier age at onset, earlier age when a wheelchair is needed, more rapid rate of disease progression, cardiomyopathy, and presence of nonuniversal disease manifestations, such as diabetes, optic at-

rophy, and hearing loss, which are indicative of a more widespread degeneration.[46]

However, differences in GAA expansions account for only about 47% of the variability in age of onset, indicating that other factors influence the phenotype. These may include somatic mosaicism for expansion sizes, possibly variation and interruptions in the repeat sequence, modifier genes, and environmental factors. None of these has so far been identified, except for variation in mitochondrial DNA, as revealed by haplogroup analysis. In particular, haplogroup U–carrying patients seem to have a delay in disease onset and a lower rate of cardiomyopathy.[47] The involved mechanism of protection is still unknown.

PERSPECTIVES

Progress in understanding the molecular pathogenesis of FRDA has been remarkable. Even more remarkable is that the development of therapeutics is now taking center stage in FRDA research, which unfortunately is still an unusual situation for most other rare genetic disorders, even years after the discovery of the involved genes.

We have learned what the causative mutation for this disease is, the GAA repeat expansion, and we have a rather detailed and coherent picture of the processes involved in its silencing effect on frataxin expression, though many details are still missing. Nevertheless, we have likely identified the main mechanism involved, and we even have preliminary but very encouraging data that this may be partially reversible, which provides an exciting therapeutic approach for the disease.[43] The concern that HDAC inhibitors are molecules that may modify the expression of many genes and therefore have unwanted effects and can become toxic, particularly after a prolonged treatment, is of course justified. Most HDAC inhibitors are being tested for cancer therapy, but some molecules with HDAC inhibitory activity have been used for long-term treatments with adverse effects that are tolerable for a large proportion of patients. The most obvious example is valproic acid, which has HDAC inhibitory activity. Restoration of frataxin expression in FRDA patients' cells is a property of a limited number of HDAC inhibitors that is not related to their overall potency in inhibiting histone deacetylation. This is not unexpected, considering the complexity of the processes that govern the chromatin structure, its effect on gene expression, and the large number of HDACs that have been identified. The current gene-expression profiling technology will undoubtedly allow us to evaluate how drugs that increase frataxin in cellular and animal models of FRDA also affect unrelated genes. Interestingly, the same technologies will also allow us to determine to what extent the changes in gene expression that are the consequence of frataxin deficiency (which are now starting to be characterized[48]) may be corrected by these treatments.

Other therapeutic approaches are also emerging. Erythropoietin appears to increase frataxin in cellular models through a posttranscriptional mechanism[49] and has neuroprotective properties. Erythropoietin is currently being tested in pilot clinical trials, though the induction of erythropoiesis by this drug is clearly a concern for long-term treatment. However, there are some derivatives of erythropoietin, such as carbamylated erythropoietin, that do not induce erythropoiesis and are neuroprotective in experimental models of stroke, neuroinflammation, and trauma.

Different treatment strategies are aimed at the pathogenic cascade that results from frataxin deficiency. Idebenone, a lipid-soluble antioxidant related to coenzyme Q, has shown promising results in preclinical and phase 2 clinical studies[50] and is now being tested in phase 3 trials. The oral iron chelator deferiprone has provided encouraging results in cellular models and in a pilot, open-label study; a phase 2 clinical trial is due shortly. Pioglitazone, an oral antidiabetic drug that stimulates mitochondrial function by acting on the peroxisome proliferator–activated receptor gamma pathway, will soon be tested in a phase 2 trial.

Until recently, properly designed phase 2 and 3 trials have been for rare genetic disorders. In fact, clinical testing of potential treatments for such disorders have too often been hampered by the poor design of many trials, often uncontrolled, underpowered, and with no clearly defined end point. In addition to progress in basic research, the research community has become aware that the advancement of clinical knowledge that allows appropriately designed, controlled, and adequately powered studies is important. This involves the development and validation of rating scales and other tools, including biomarkers, to assess disease progression, functional capacity, and quality of life; a better characterization of the clinical features and natural history of the disease being studied; and the establishment of international consortia of investigators to collect the necessary number of patients for clinical trials. In FRDA, basic research discoveries show the promise for clinically relevant applications and, with proper clinical investigation, give hope that a treatment will be found for this so far incurable, progressive neurodegenerative disease.

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