Friedreich ataxia: The clinical picture

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■ **Abstract** Friedreich ataxia (FRDA) is a rare autosomal recessive hereditary disorder that affects approximately 1 in 50,000 Caucasians. It is caused by hyperexpansion of GAA repeats in the first intron of the frataxin gene. Initial symptoms of FRDA usually appear around the beginning of the second decade of life. In addition to neuro-pathological disabilities such as ataxia, sensory loss, and muscle weakness, common signs are scoliosis, foot deformity, and hypertrophic cardiomyopathy. Approximately 10 % of patients with FRDA develop diabetes. The neuronopathy in the dorsal root ganglia, accompanied by the loss of peripheral sensory nerve fibres and the degeneration of posterior columns of the spinal cord, is a hallmark of the disease and is responsible for the typical combination of signs and symptoms specific to FRDA. Variation in neurophysiological abnormalities is correlated with the size of the GAA repeat expansion and likely accounts for individual variation in the progression of FRDA. Despite a range of disease severity, most patients will lose their ability to walk, stand, or sit without support within 10 to 15 years of disease onset. In addition to a review of the clinicopathological features of FRDA, a discussion of recent advances in our understanding of the underlying molecular mechanisms is provided.

■ **Key words** inherited ataxia · sensory loss · muscle weakness · gait instability · hypertrophic cardiomyopathy · dysarthria · pyramidal tract degeneration · frataxin gene mutation · gene silencing

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Introduction

Nicholaus Friedreich, Professor of Medicine in Heidelberg, first described in 1863 a disease characterised clinically by ataxia and pathologically by a "degenerative atrophy of the posterior columns of the spinal cord" that could affect several children of unaffected parents [1]. Now known as Friedreich ataxia (FRDA), this autosomal recessive hereditary disease is the most common of the early-onset hereditary ataxias in Caucasians. In addition to progressive neurological disability, FRDA causes a hypertrophic cardiomyopathy and an increased risk of diabetes mellitus. Skeletal abnormalities such as kyphoscoliosis and pes cavus are common. The first symptoms usually are noticed around the time of puberty. On average, after 10 to 15 years of disease, progressive gait and limb ataxia eventually results in the need for a wheelchair and for help with all activities of daily living. Some patients have a very severe cardiomyopathy that can cause premature death due to cardiac insufficiency or arrhythmia. The complications of diabetes may further contribute to disease burden and disability. As is the case for almost all neurodegenerative diseases, no proven treatment that can stop the progression of FRDA is now known. Furthermore, no effective symptomatic treatments (e.g., comparable with levodopa for Parkinson disease) can improve the patient's condition without affecting the progression of the neurodegenerative process.

Friedreich Ataxia: Clinicopathological Features

In Caucasians, FRDA has a prevalence of around 2×10^{-5} and accounts for half of overall cases of hereditary ataxia

and for three quarters of those with onset before the age of 25 years [2]. The disease does not occur in non-Caucasians because it is the result of a unique founder mutation (discussed below). Friedreich described the essential clinical and pathological features of the disease as a "degenerative atrophy of the posterior columns of the spinal cord", leading to progressive ataxia, sensory loss, and muscle weakness, often associated with scoliosis, foot deformity, and heart disease. In the late 1970s and early 1980s, a renewed interest in this disease and the availability of modern biochemical and molecular genetic approaches led to the establishment of rigorous diagnostic criteria for use in the selection of families for research [3]. In 1996, the discovery of the mutated gene in FRDA resulted in a direct molecular diagnostic test [4], whose availability allowed neurologists to characterise the full clinical spectrum of the disease.

The typical age of onset for FRDA is late in the first decade or early in the second decade of life, but this may vary substantially even between siblings [2]. Late-onset cases, showing symptoms only after age 25 years (late-onset Friedreich ataxia [LOFA]), have been reported, although the disease rarely emerges as late as the sixth or seventh decade. Late onset is a phenomenon that is explained in part now by the dynamic nature of the underlying mutation. Gait instability and generalised clumsiness are typical presenting symptoms. Scoliosis, first diagnosed as idiopathic, often is already present when neurological symptoms appear. In rare cases, hypertrophic cardiomyopathy becomes symptomatic before the onset of ataxia. Ataxia is of mixed cerebellar and sensory type. Truncal ataxia results in swaying and gait becomes broad-based, with frequent loss of balance, requiring at first intermittent, then constant support. Limb ataxia causes increasing difficulty in activities requiring dexterity and precision, such as writing, dressing, and handling utensils. Ataxia is progressive and unremitting, although periods of stability are frequent at the beginning of the illness. Limb weakness of central origin appears and then worsens with the progression of FRDA. It initially affects proximal muscles, then becomes generalised and significantly contributes to disability. Weakness confounds the evaluation of ataxia in advanced cases and eliminates some ataxic features, such as intention tremor. Some amyotrophy, particularly in the hands, is very common. On average, 10 to 15 years after onset, patients lose the ability to walk, stand, and eventually sit without support [2, 3]. However, disease progression is variable, and those with mild disease may still be ambulatory decades after onset, while those with severe disease may become wheelchair-bound within a few years. Dysarthria consisting of slow, jerky speech with sudden utterances is almost universal. It usually begins within 2 years of disease onset and progresses until speech becomes almost unintelligible. Dysphagia, particularly for liquids, is a feature of very advanced disease, for which

modified foods and eventually gastrostomy feedings are required. Cognitive functions generally are well preserved, although subtle abnormalities, particularly of executive functions, have been detected [5]. Because it involves substantial physical disability, FRDA may have a substantial impact on academic, professional, and personal development.

The neuropathology of FRDA is specific to this disorder [6] and results in a typical combination of signs and symptoms. Sensory neuronopathy in the dorsal root ganglia (DRG), accompanied by loss of sensory fibres in peripheral nerves and degeneration of posterior columns in the spinal cord, is the hallmark of this disease. Larger neurons that carry proprioceptive information are most often affected, resulting in loss of position and vibration sense and abolished reflexes. Perception of light touch, pain, and temperature decreases with advancing disease. Progressive degeneration of pyramidal tracts leads to extensor plantar responses and muscular weakness. Some patients with more limited sensory neuronopathy retain reflexes; in these cases, pyramidal involvement may lead to hyperreflexia and spasticity, to the point of mimicking a spastic paraplegia. The relative impact of sensory neuropathy and of pyramidal tract degeneration clearly varies from patient to patient, resulting most often in a typical clinical picture of areflexia associated with extensor plantar responses. In some cases, one component prevails. Such partial presentations usually are observed in milder cases of the disease.

Fixation instability with square-wave jerks (SWJs) is the typical oculomotor abnormality in FRDA. This disorder is evident early in the disease course and tends to worsen over time. Gaze-evoked nystagmus has been described in a variable proportion of cases, but in this author's experience, nystagmus is rare in FRDA. In some reported cases, SWJ may have been interpreted as nystagmus. Ophthalmoparesis does not occur. However, about 30% of patients develop optic atrophy, with or without visual impairment, and about 20% develop sensorineural hearing loss [2, 3].

Neurophysiological studies of the peripheral nervous system (PNS) reveal a severe reduction or complete loss of sensory nerve action potentials (SNAPs), with motor and sensory (when measurable) nerve conduction velocities (NCVs) within or just below the normal range. These neurophysiological abnormalities do not worsen over time but correlate with the size of the causal GAA triplet repeat expansion in the FXN gene, indicating that the underlying pathology is established early and does not significantly progress [7]. In the central nervous system (CNS), degeneration of both peripheral and central sensory fibres causes dispersion and delay of somatosensory evoked potentials (SEPs) and brainstem auditory evoked potentials (BAEPs). Visual evoked potentials (VEPs) are commonly reduced in amplitude but not

delayed. Central motor conduction velocity, reflecting pyramidal tract function, is lower than normal. Contrary to sensory involvement, this slowing becomes more severe with increasing disease duration, indicating a progressive degeneration of pyramidal tracts during the disease course [8].

Structural neuroimaging by computed tomography (CT) or magnetic resonance imaging (MRI) typically shows an atrophic cervical spinal cord. Cerebellar cortical atrophy is mild and is commonly seen only in advanced cases. The dentate nucleus is instead severely affected in FRDA – a feature that can be detected with MRI [9]. Newer MRI approaches, including tractography, may have the potential to reveal degeneration of specific fibre systems, but have been systematically studied in this disease only recently [10]. Preliminary studies with phosphorus magnetic resonance spectroscopy (P-MRS) in the CNS showed this technique to be useful in evaluating mitochondrial dysfunction. Results revealed decreases in parameters related to adenosine triphosphate (ATP) synthesis efficiency, which had been found previously in the heart and skeletal muscle of these patients [11]. However, such studies are currently limited to specialised centers. It is noteworthy that older metabolic imaging studies with fluorodeoxyglucose (FDG) positron emission tomography (PET), which showed a diffusely increased FDG uptake in the brain in early, still ambulatory FRDA, also suggested inadequate utilisation of energy sources [12].

Heart disease is most commonly asymptomatic, but it may contribute to disability and may cause premature death in a significant minority of patients, particularly in those with earlier age of onset. Shortness of breath and palpitations are the usual initial symptoms. The electrocardiogram shows inverted T-waves in essentially all patients with FRDA, ventricular hypertrophy in most, conduction disturbances in about 10%, and supraventricular ectopic beats and atrial fibrillation occasionally. Echocardiography demonstrates concentric hypertrophy of the ventricles or asymmetrical septal hypertrophy. In severe cases, the cardiomyopathy progresses to a dilatative stage with severe heart failure [13].

About 10% of patients with Friedreich ataxia develop diabetes mellitus, and about 20% have carbohydrate intolerance. The mechanisms are complex, with both β-cell dysfunction and peripheral insulin resistance, possibly resembling other mitochondrial disorders [14].

Kyphoscoliosis may cause pain and cardiorespiratory problems. Pes cavus and pes equinovarus may further affect ambulation. Autonomic disturbances, most commonly cold and cyanotic legs and feet, become increasingly frequent as the disease progresses. Sphincteric problems are rare.

The Friedreich Ataxia Gene

The mutated gene in FRDA (FXN) is localized in the proximal long arm of chromosome 9 [15]. The major mRNA transcript has a size of 1.3 Kb and corresponds to five exons, numbered 1 to 5a; minor transcripts that include an alternative exon 5b have unknown functional significance [4]. The encoded protein, frataxin, is predicted to contain 210 amino acids. Frataxin has an N-terminal leader peptide that directs its subcellular localisation to mitochondria.

The FXN gene is expressed in all cells but at variable levels, which can be accounted for only in part by differences in mitochondrial content. In adult humans, FXN mRNA is most abundant in the heart and spinal cord, followed by liver, skeletal muscle, and pancreas [4]. In mouse embryos, FXN mRNA expression starts in the neuroepithelium at embryonic day 10.5 (E10.5), and it reaches its highest level at E14.5 and into the postnatal period. At E16.5, the highest levels of FXN mRNA are found in the spinal cord, particularly at the thoracolumbar level, in the dorsal root ganglia, in proliferating neural cells in the periventricular zone, in the cortical plates, and in the ganglionic eminence. FXN mRNA expression is also high in heart, liver, and brown fat [16, 17].

Current knowledge of frataxin function and of the consequences of frataxin deficiency is summarised in the review in this supplement by Pastore and Pandolfo.

Mutations Causing Friedreich Ataxia

FRDA is caused by a unique mutation, the hyperexpansion of a GAA triplet repeat in the first intron of the FXN gene [4]. The expression of frataxin is substantially reduced by this mutation. FRDA is a genetic recessive disease; therefore, affected individuals have GAA expansions in both homologues of chromosome 9. Although GAA repeats in normal chromosomes consist of up to ~40 triplets, disease-associated repeats contain from ~70 to more than 1000 triplets, most commonly 600–900. Heterozygous carriers are clinically normal. This is the most common disease-causing triplet repeat expansion identified to date. A study in France revealed a prevalence of carriers of 1 in 90 [18]. To date, no other disease has been recognised as being caused by the expansion of a GAA triplet repeat.

Other mutations that either change the amino acid sequence of frataxin or result in complete loss of expression are very rare. To date, all patients with FRDA found to carry such mutations were compound heterozygotes for the GAA expansion [4, 19]. As was demonstrated in knockout animal models of FRDA, a minimal residual level of functional frataxin is necessary to survive through embryonic development (see review by Pastore and Pandolfo in this supplement).

The GAA expansion allows the synthesis of some frataxin that is structurally and functionally normal because the coding sequence is not involved in the mutation. Residual levels of frataxin vary according to the length of the expansion and the cell type. In peripheral blood leukocytes, frataxin levels range from about 5% to 30% of normal. Because the expansion size determines the level of residual frataxin expression, it has an influence on the severity of the phenotype. A direct correlation has been firmly established between the size of the GAA repeat and an earlier age of onset, an earlier age of confinement to a wheelchair, more rapid rate of disease progression, cardiomyopathy, and the presence of non-universal disease manifestations, such as diabetes, optic atrophy, and hearing loss, which are indicative of more widespread degeneration [20–22]. Differences in GAA expansion, however, account for only about 50% of the variability in age of onset, indicating that other factors also influence the phenotype. These may include somatic mosaicism for expansion sizes, variation and interruptions in the repeat sequence, modifier genes, and environmental factors. Variation in the mitochondrial DNA sequence also may play a role. Mitochondrial haplogroup analysis has suggested that haplogroup U may be protective, thereby delaying disease onset and lowering the rate of cardiomyopathy [23]. The mechanism of this protection is still unknown.

Had this GAA triplet expansion never occurred, FRDA very likely would not exist, because all other known frataxin mutations that result in homozygosity cause a loss of function that is too severe to allow survival through the development phase. In fact, the disease has never been diagnosed in individuals from ethnic groups in which the GAA expansion is not found, including native populations of the Far East, sub-Saharan Africa, Australia, and North America [24].

The GAA expansion is unstable when transmitted from parent to child [4]. Expansions and contractions are equally likely after maternal transmission, and contractions are most common after paternal transmission [25]. In this regard, FRDA resembles the other diseases associated with large expansions in non-coding regions of the respective genes, such as fragile X and myotonic dystrophy, and differs from diseases that result from CAG repeats in coding regions, such as the dominant ataxias and Huntington disease, in which further expansion typically occurs after paternal transmission. The GAA expansion is also unstable in somatic cells, including post-mitotic cells [26]. It is open to discussion whether the particular tendency of the GAA repeats to expand in some cell types, such as DRG sensory neurons, contributes to the selective vulnerability of these cells in FRDA [27].

In about 2% of FRDA chromosomes, the GAA repeat is of normal length, but other loss-of-function mutations may affect the FXN gene, including missense, non-

sense, or splice site mutations, and, rarely, deletions involving a large portion of the gene [4, 19, 28]. Individuals affected by these mutations are always compound heterozygotes for a GAA expansion. In most cases, they have a typical FRDA phenotype, although some atypical phenotypes have been linked to specific FXN point mutations. D122Y, R165P, and G130V cause a 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 [19]. Complementation studies in yeast showed that these mutations have some residual activity, and missense mutations associated with a typical, more severe phenotype, such as W173G in the Yfh1 yeast, lack any residual activity [29]. Structural studies have confirmed that certain missense mutations change amino acid residues critically involved in frataxin folding or affect conserved regions of frataxin thought to be involved in iron binding or protein–protein interactions (see review by Pastore and Pandolfo in this supplement).

Mechanisms of Frataxin Gene Silencing by the Expanded GAA Repeat

The GAA repeat is a tract of DNA that contains only purines (R) in one strand and pyrimidines (Y) in the other strand. R·Y sequences can adopt a special, three-stranded non-B structure [30–36]. In the test tube, pathological lengths of the FRDA GAA repeat at neutral pH can fold back on themselves and form an intramolecular R·R·Y triplex, in which two R strands and a Y strand associate and leave an unpaired segment of Y strand [37]. Two regions of R·R·Y triplex, in the same or in different DNA molecules, can form a new type of DNA structure by joining through the exchange of the third R strand. This structure, called "sticky DNA", strongly inhibits transcription in vitro, providing an explanation for FXN gene silencing. However, whether the triplex/sticky DNA conformation really occurs in the nucleus, where DNA is tightly packaged in chromatin, in association with proteins that affect its structural properties, remains to be proved. Current data indicate that expanded GAA repeats in the eukaryotic nucleus lead to chromatin remodelling and condensation. When a reporter gene is associated with a GAA repeat in a construct used to create transgenic mice, the reporter gene will be silenced in a proportion of cells. This phenomenon, which is called position effect variegation (PEV), was first observed in genes localised close to heterochromatic regions in *Drosophila melanogaster* [38]. Chromatin condensation is linked to post-translational modification of histones, including changes in acetylation, methylation, phosphorylation, and ubiquitination. Decreased histone acetylation and increased methylation of specific residues do indeed occur in the vicinity of the expanded GAA repeats

in lymphocyte DNA from patients with FRDA [39]. In particular, an increase in trimethylation of histone H3 lysine 9 (H3K9) and a decrease in acetylation at H3K14 and H4K5, H4K8, and H4K12 – all markers of a heterochromatic state [39] – have been observed. Whether a possibly transient formation of triplex or sticky DNA structure is involved in inducing these changes is currently unknown.

It is very important to note that specific histone deacetylase inhibitors (HDACi) can revert these modifications and substantially increase frataxin expression in cells, including non-replicating cells such as primary lymphocytes [40], from patients with FRDA. Increased frataxin expression with HDACi also has been demonstrated in a mouse model that carries expanded GAA repeats in the endogenous frataxin gene [40]. These findings provide a very promising prospect for FRDA treatment and are currently the subject of intense research.

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