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The Neuropathology of Late-Onset Friedreich's Ataxia

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Abstract

Friedreich's ataxia (FRDA) affects very young persons. In a large series, the mean ages of onset and death were 11 and 38 years, respectively. The clinical spectrum of FRDA has expanded after genetic confirmation of the mutation became a routine laboratory test. The main cause of death in juvenile-onset FRDA is cardiomyopathy whereas patients with late-onset are more likely to succumb to neurological disability or an intercurrent illness. Many patients with early onset now survive for 20 years or longer. This study made a systematic comparison of the neuropathology in 14 patients with juvenile onset and long survival, and five patients with late onset and long survival. Mean ages of onset ($\pm$ standard deviation) were 10 ± 5 and 28 ± 13 years, respectively. Disease durations were 33 ± 11 and 47 ± 11 years, respectively. Cross-sectional areas of the thoracic spinal cord were greatly reduced from the normal state but did not differ between the two groups. Similarly, the neurons of dorsal root ganglia were significantly reduced in size in both juvenile- and late-onset cases of FRDA. The dentate nucleus showed severe loss of neurons as well as modification and destruction of corticonuclear terminals in all FRDA patients. Delayed atrophy of the dentate nucleus is the likely cause of the ataxic phenotype of FRDA in late-onset cases, but the reason for the delay is unknown. Frataxin levels in the dentate nucleus of two patients with late onset were similar to those of seven patients with juvenile onset.

Keywords

Age of onset; Dentate nucleus; Dorsal root ganglion; Frataxin; Friedreich's ataxia; Spinal cord

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Introduction

Friedreich's ataxia (FRDA) is an autosomal recessive disease that affects central and peripheral nervous systems; heart; skeleton; and endocrine pancreas. In her large series of patients, Harding [1] reported a mean age of onset of 10.52 years and a mean age of death of 37.54 years. De Michele et al. [2] defined "late onset" as 24.4 years (range 21–29 years) but the identification of the mutation as a homozygous guanine-adenine-adenine (GAA) trinucleotide repeat expansion in intron one of the frataxin gene [3] dramatically broadened the spectrum of the disease including age of onset [4]. First signs of ataxia at the age of 40 years or above are no longer unusual [4], and some patients are not diagnosed during life [5]. Short GAA expansions convey a more benign phenotype including late onset [4] and protracted course [5]. Generally, patients with very late-onset FRDA have no cardiomyopathy or diabetes mellitus but die from disabling ataxia or illnesses that are unrelated to FRDA. Many patients with juvenile-onset of FRDA now also survive for decades despite cardiomyopathy. Heart disease in FRDA evolves independently of the neurological manifestations of FRDA and may precede ataxia [6]. In rare cases, rapidly progressive cardiomyopathy leads to early death before the neurological phenotype becomes evident. Such cases offer insight into the progression of FRDA in the nervous system. Little is known about the neuropathological differences between late-onset and juvenile-onset long-surviving FRDA patients. This report presents a comparison of the main nervous system lesions in these two groups. Dentate nucleus (DN) and dorsal root ganglia (DRG) bear the brunt of the disease [7, 8] though classical teaching emphasizes atrophy of the spinal cord and dorsal spinal roots [9]. The observations point toward progressive disease of the DN as the main reason for neurological disability in patients with late-onset FRDA.

Patients and Methods

The Institutional Review Board of the VA Medical Center, Albany, N.Y., USA, has approved the human-subjects research underlying this report. Patients with FRDA of "juvenile-onset with long survival" (JOFLS) were defined as having the onset of their disease at an age of 20 years or less and a survival of 20 years or more. "Late-onset" FRDA with long survival (LOFLS) was defined as onset after the age of 20 years and survival of 20 years or more. Table 1 lists details of sex, age of onset, age of death, disease duration, GAA trinucleotide repeat expansions, and causes of death. Specimens from 14 patients with JOFLS and five patients with LOFLS were available. All cases included the DN. Adequate tissue samples of mid-thoracic spinal cord were available from 12 patients with JOFLS; four patients with LOFLS; and six normal controls. DRG were available from 11 of 14 JOFLS patients, three of five LOFLS patients, three controls for JOFLS (age range, 36–54 years) and seven controls for LOFLS (age range, 65–71 years).

In one patient with FRDA, onset was at 15 years but severe cardiomyopathy led to his death 4 years later. Spinal cord and DN of this case were available for comparison with JOFLS and LOFLS.

Immunocytochemistry of DN and Spinal Cord

Six- μ m-thick sections of the cerebellar hemisphere were stained by immunocytochemistry with mouse monoclonal antibodies to neuron-specific enolase (NSE; Chemicon, Temecula, CA, USA) and glutamic acid decarboxylase (GAD; MBL International, Woburn, MA USA). Sections of spinal cord were stained for myelin basic protein (MBP) with a monoclonal antibody from Covance (San Diego, CA, USA). The antibodies were diluted to 2 μ g protein/ml (NSE) or 1 μ g protein/ml (GAD; MBP). Sections were dewaxed by standard techniques, and endogenous peroxidase activity was suppressed by immersion in 3% hydrogen peroxide in methanol (30 min). Antigen retrieval for NSE and GAD consisted of a 30-min-long incubation at 95°C in a diluted proprietary “decloaking” solution named DIVA by the supplier (Biocare Medical, Concord, CA, USA). Immunoreactivity of MBP was enhanced by an overnight exposure of the sections to 80% ethanol at 4°C. The sequence of incubations was similar to a previously described procedure [7] and is summarized here as follows (washing steps omitted): primary antibody→biotinylated anti-mouse IgG (Sigma, St. Louis, MO, USA; 0.6 μ g protein/ml)→horseradish peroxidase-labeled streptavidin (Sigma)→diaminobenzidine/hydrogen peroxide. Pale reaction product was enhanced by brief exposure to a 1% solution of osmium tetroxide. Sections were dehydrated and covered by standard methods.

Measurements of Thoracic Spinal Cord Cross-Sectional Area

Paraffin sections of 6 μ m thickness were stained with hematoxylin and eosin or Cresyl Violet and photographed at a magnification of 1.25X. A single photograph generally sufficed for FRDA whereas multiple exposures were needed for sections of normal controls. Photos of the control specimens were pieced together to obtain a cohesive cross-sectional area. Final enlargement was 40X. The total cross-sectional area in mm² was obtained by tracing the outline of the spinal cord on a Zeiss MOP-3 particle analyzer.

Quantitative Determinations of Neuronal Sizes in DRG

All DRG came from lumbar or sacral roots. Six- μ m-thick sections were stained by hematoxylin and eosin, or Cresyl Violet. Ten separate neurons of each ganglion with distinct nucleoli were photographed at a magnification of 63X under oil immersion and stored as digital images. After printing, final enlargement was 1,950X. The neuronal cell body was outlined by a pencil mark and traced with the cross-hair device of the Zeiss MOP-3 particle analyzer. Results were expressed as μ m². A common artifact in DRG obtained by autopsy was separation of the neuron from its capsule of satellite cells. In sections with this phenomenon, the pencil mark was placed just inside the row of satellite cells. This method differs from the practice of Ohta et al. [10] who placed the measuring limit to a point midway between the retracted neuron and its satellite cells.

Enzyme-Linked Immunosorbent Assay of Frataxin in the Dentate Nucleus

Frozen samples of DN were available from 7 FRDA patients in the JOFLS group, two patients in the LOFLS group, and controls (aged 49 years and 52 years, respectively). Tissue

samples weighing 50–100 mg were obtained by a 4-mm wide cork borer, weighed, and immediately homogenized in two volumes of an ice-cold extraction buffer by three bursts of ultrasonication (5 s). The buffer contained 50 mM tris (pH 7.5), 150 mM NaCl, 1% octylphenoxypolyethoxyethanol (Triton X-100), 1% nonylphenylpolyethyleneglycol (Nonidet P-40), 5 mM ethylenediaminetetraacetic acid (EDTA), 5 mM ethyleneglycol bis(β -aminoethylether) tetraacetic acid (EGTA), and 10% protease inhibitor cocktail (Sigma, St. Louis, MO, USA) [11]. After incubation on ice for 1 h, the homogenate was centrifuged for 2 h at $14,000 \times g$. The supernatant was collected, and a 50 μ l-aliquot was diluted 1:10 with phosphate-buffered saline (pH 7.4) to reduce the concentrations of EDTA and EGTA to below 0.1%. The diluted extracts were centrifuged at $14,000 \times g$ for 45 min through centrifugal filter devices with a molecular weight cut-off of 30 kDa (Millipore, Billerica, MA, USA). The filtrate was used for enzyme-linked immunosorbent assay (ELISA). The method was similar to double-sandwich ELISA of ferritin that was previously used in this laboratory for DN ferritin [7]. The plates were coated with monoclonal anti-frataxin (0.1 μ g protein/ml; Mitosciences, Eugene, OR, USA). The second antibody was rabbit polyclonal anti-frataxin that was generously supplied by Drs. Grazia Isaya and Oleksandr Gakh (Rochester, MN, USA). The same investigators also provided human recombinant frataxin that was used to standardize ELISA. Aliquots of tissue extract corresponding to 2, 5, and 6.67 mg original wet tissue yielded colored reaction product that absorbed light at 492 nm in the range of 75 pg to 4 ng human recombinant frataxin. Optimal curve fitting of standards was based on absorbances at 492 nm vs. the logarithm of the amounts of frataxin.

Statistical Analysis

Thoracic spinal cord areas of JOFLS and LOFLS were compared to each other and normal controls by analysis of variance (ANOVA) with significance accepted at $p < 0.05$. The comparison of neuronal size in DRG was based on repeated-measures ANOVA with a fixed effect of group (JOFLS, JOFLS controls, LOFLS, LOFLS controls) and a random repeat measure of the neuronal size nested within subjects. The logarithms of the neuronal sizes were used for analysis to correct for upwardly skewed distributions. Multiple comparisons were by Fisher's protected least significant difference test and a significance level at $p < 0.05$.

Results

Figure 1 shows the dramatic effect of FRDA on DN neurons and their afferent terminals. Neuronal loss in JOFLS and LOFLS patients is approximately the same, as assessed by NSE reaction product. Glutamic acid decarboxylase reaction product around unstained neurons ("N" in Fig. 1d) may be interpreted as GABA-ergic terminals arising from Purkinje cells. Small neurons with very dense NSE reaction product persist and likely represent GABA-ergic nerve cells because pale cytoplasmic GAD reaction product is also present in similar small neurons (arrows in Fig. 1 d–f). The sections of FRDA patients show an overall decline of the density of GAD-positive terminals, but clusters of grumose degeneration are widespread (Fig. 1e–f). Figure 1f, representing a patient with very late onset (50 years) and protracted duration (33 years), suggests a slightly better retention of GAD-reactive terminals. A systematic comparison, however, of GAD immunoreactivity in DN of four

additional patients with JOFLS (Fig. 2a–d) and four LOFLS (Fig. 2e–h) does not allow the conclusion that late onset and long survival invariably present better retention of corticonuclear terminals.

Figure 3 compares the DN in two young patients with very different disease durations with a normal control. The DN in a 19-year-old man with rapidly fatal cardiomyopathy but little ataxia over a 4-year-long course (Fig. 3a) contains many more neurons than the DN of a 24-year-old man with a more protracted illness (14 years) (Fig. 3c). The DN from the patient with short survival, however, may not be entirely normal because neurons are generally smaller (Fig. 3a) than normal (Fig. 3e). Artifact due to delayed fixation cannot be excluded. GAD reaction product in the DN of the patient with short survival remains abundant, and GAD-reactive grumose degeneration is absent. These clusters of abnormal GABA-ergic terminals are very prominent in the DN of a patient with a more protracted neurological disease (Fig. 3d).

Figure 4 displays a comparison of the thoracic spinal cord profiles of a normal control, JOFLS, and LOFLS after immunocytochemical visualization of MBP. Thoracic spinal cord profiles are significantly different (ANOVA; $p=0.003$). In 12 patients with JOFLS, the cross-sectional area is $20.5\pm5.3\text{ mm}^2$ (mean $\pm$ standard deviation), which is not significantly different from the mean of 4 cases with LOFLS ($21.9\pm3.1\text{ mm}^2$) (Fisher's test; $p=0.65$). Both JOFLS and LOFLS areas are significantly different from the mean of six normal thoracic spinal cords ($30.4\pm5.6\text{ mm}^2$): JOFLS vs. normal, $p=0.006$; LOFLS vs. normal, $p=0.015$. The thoracic spinal cord area of the 19-year-old man who died from cardiomyopathy within 4 years of onset is 34 mm^2 , which is in the normal range.

Figure 5 compares a DRG of a normal control with JOFLS and LOFLS. The microphotographs were taken from comparable subcapsular regions where neurons are normally most abundant. The paucity of large neurons in the FRDA cases is apparent. The large number of nodules of Nageotte in JOFLS (Fig. 5b) may indicate a more aggressive disease process. Neuronal size in LOFLS (Fig. 5c), however, are not visibly larger than those in JOFLS (Fig. 5b).

The box plot in Fig. 6 displays the results of systematic measurements of neuronal areas in DRG of JOFLS, LOFLS, and matching controls. The unweighted means and standard deviations for JOFLS, JOFLS-controls, LOFLS, and LOFLS-controls are, in μm^2 , 1721 ± 776 , 2667 ± 1193 , 2142 ± 1255 , and 2793 ± 1559 , respectively. Neuronal areas are significantly different among the groups (ANOVA; $p=0.001$). The difference between the control groups is not significant (Fisher's test; $p=0.79$). Both JOFLS and LOFLS are significantly different from their control groups ($p=0.003$ and $p=0.033$, respectively). The mean neuronal area in DRG of LOFLS is somewhat larger than that of JOFLS, but this difference does not achieve statistical significance ($p=0.17$).

Frataxin levels in LOFLS patients 17F and 18F (Table 1) were 26 and 25.8 ng/g wet weight, respectively. They were similar to those in JOFLS patients 5F (18.9), 6M (17.5), 7F (40.5), 10F (18.9), 11M (14.9), 13M (17.7), and 14F (21.6). Levels in two control DN were 137 and 263 ng/g wet weight, respectively.

Discussion

Long and Short Survival in FRDA and Correlation with GAA Repeats

The most common cause of death in FRDA is cardiomyopathy (Table 1) but patients with LOFLS generally have only the ataxic phenotype. In 13 of 14 patients with JOFLS (92.9%), heart disease was an immediate or contributing cause of death. In contrast, cardiomyopathy was the cause of death in only 1 of 5 patients with LOFLS (20%). Patients without cardiomyopathy generally have short GAA expansions on the smaller of the two alleles, and the data in Table 1 confirm the observations by others [4, 12]. The longer allele may not be irrelevant for disease duration and the neuropathological phenotype (Table 1). Patients with LOFLS have significantly shorter GAA expansions on *both* alleles when compared to JOFLS. Two-tailed t-tests of mean GAA trinucleotide repeat lengths yield $p=0.026$ for the longer alleles, and $p=0.014$ for the shorter alleles. Emphasis on a tight correlation between the shorter GAA trinucleotide repeat expansion and the clinical and pathological phenotypes may no longer be justified. Epigenetic phenomena in impaired frataxin gene transcription must also be taken into consideration (review in [13]). Frataxin levels in the dentate nucleus of LOFLS patients were not higher than in JOFLS, but measurement of this protein in autopsy tissues may not be an appropriate measure of disease severity. Loss of mitochondria-rich neurons and afferent terminals in the DN readily explain reduced levels in both groups though frataxin may have been higher at one time in patients with LOFLS.

The Natural History of the Lesion in DN, DRG, and Spinal Cord

The better condition of the DN in rapidly fatal FRDA-related cardiomyopathy (Fig. 3a–b) suggests that loss of neurons and corticonuclear terminals in more typical FRDA cases (Fig. 1b–c and e–f) is due to *atrophy* rather than hypoplasia. A combination of hypoplasia *and* atrophy, however, may exist in DRG and dorsal roots (DR). The absence of large myelinated fibers in DR may be attributed to lack of large DRG neurons [8, 14], but the smaller than normal neuronal size is not necessarily due to atrophy. DR actually display a normal abundance of axons and, peculiarly, a greater degree of myelination in comparison with the normal state [8]. It is unknown whether lack of neurons with a diameter in excess of 54 μm [14] represents postnatal shrinkage or developmental failure. Koeppen et al. [8] recently summarized the evidence for a developmental deficit of DRG in FRDA by attention to the neural crest.

The examination of DRG of older patients with FRDA must consider changes that are due to advancing age [15]. Nodules of Nageotte are more common in DRG of normal older persons, and Gardner [16] estimated that the number of neurons in the DRG of the eighth and ninth thoracic roots decreased by 30% between the ages of 50 and 80 years. In contrast, Ohta et al. [10] could not confirm a numerical reduction in DRG of the first sacral root of older persons. Also, neuronal diameters remained the same after acquiring a stable status after the first decade of life. This study is especially important for the interpretation of the reduced cytoplasmic areas of nerve cells reported here.

Narrow cervical spinal canals on plain X-ray films of patients with FRDA are consistent with hypoplasia of the spinal cord [17], and magnetic resonance imaging (MRI) confirms

thinning of the spinal cord at that level [18, 19]. Normal measurements in the 19-year-old patient with FRDA who died within 4 years of onset, however, implies that thinning of spinal cord in more typical cases also progresses. Neurons in DRG may never reach normal size, and DRG hypoplasia may contribute to thinning of the dorsal columns and a reduction of the anteroposterior and transverse diameters of the spinal cord (Fig. 4; ref [5]).

Clinicoanatomic Correlation

An unexpected outcome of the described study is the principal identity of FRDA lesions in JOFLS and LOFLS. Ultimately, all patients with FRDA, except those succumbing rapidly to cardiomyopathy, appear to undergo the same destruction of their central and peripheral nervous systems. The key question about patients with LOFLS is: What causes the late onset of their neurological manifestations? The likely answer is delayed atrophy of the DN. Morphological observations of autopsy tissues represent end-stage FRDA and do not provide sufficient insight into the course of DN atrophy. Progressive destruction of the DN can now be confirmed by serial MRI in living FRDA patients. Due to its high iron content, the DN can be visualized on T2-weighted images at high field strengths, and changing size and signal hypointensity may be used to monitor the lesion and assess possible therapeutic benefit [20, 21].

A common feature in LFOS patients is the significantly shorter GAA trinucleotide repeat expansion in *both* alleles (Table 1). Since triplet repeats enhance heterochromatin formation and promote gene silencing [13], shorter GAA trinucleotide repeats in LOFLS patients may provide early protection against more serious frataxin deficiency. In theory, frataxin transcription in LOFLS is more efficient because epigenetic effects are less prominent.

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Fig. 1.

GAD in the dentate nucleus of a normal control and patients with JOFLS and LOFLS. The top panel (a–c) shows reaction product of NSE; the bottom panel (d–f) represents GAD. In the GAD-stained section of a normal control (d), neurons are shown as voids (N). (a) and (d), normal control; (b) and (e), JOFLS; (c) and (f), LOFLS. In JOFLS, only a few small NSE-reactive neurons remain (b), and GAD-positive terminals are greatly reduced (e) in comparison with the normal state (d). Neuronal loss in the patient with LOFLS (c) is comparable to JOFLS (b). GAD reaction product is reduced and disorganized (f). Arrows in (d), (e), and (f) point to pale cytoplasmic GAD reaction product in small GABA-ergic neurons of the DN. The clusters of GAD reaction product (*asterisks* in [e] and [f]) represent grumose degeneration. Figures (b) and (e) represent FRDA patient 7F in Table 1; (c) and (f) represent patient 17F in Table 1. Bars, 50 μ m

Fig. 2. GAD in the dentate nucleus of 4 patients with JOFLS and 4 patients with LOFLS. (a)–(d), JOFLS; (e)–(h), LOFLS. Disruption of GAD-reactive terminals affects all DN irrespective of age of onset. The microphotographs correspond to patients listed in Table 1 as follows: (a), 6M; (b), 8F; (c), 11M; (d), 10F; (e) 15F; (f), 16F; (g), 17F; (h), 19M. The length of GAA trinucleotide repeat expansion does not appear to influence the severity of synaptic depletion as assessed by GAD immunocytochemistry. Bars, 50 μ m

Fig. 3.

Progressive atrophy of the DN in FRDA. (a, b), DN in a 19-year-old man with fatal cardiomyopathy but minimal ataxia, and death after a disease duration of 4 years; (c, d) DN of a 24-year-old man with cardiomyopathy and advanced neurological disability after a disease duration of 14 years. (e, f) normal control. (a), (c), (e), immunocytochemistry of NSE; (b), (d), (f) immunocytochemistry of GAD. In the case with rapidly fatal cardiomyopathy ([a]–[b]), numerous neurons are present in the DN though they appear smaller than normal (e). GABA-ergic terminals (b) are preserved, and grumose degeneration is absent. The voids labeled by “N” in (b) and (f) indicate the location of unstained nerve cells. In the patient with comparable age (c, d) but a much longer course, only a few small NSE-reactive neurons remain (c). GAD-immunocytochemistry shows much less reaction product in the neuropil, but many clusters of grumose degeneration are present (arrows in [d]). The DN of the patient with the more serious neurological phenotype (c, d) differs greatly from the normal control (e, f) and the short-term survivor (a, b). GAA trinucleotide repeats: (a, b), 1153/841; (c, d), 1050/700. Bars, 50 μ m

Fig. 4.

Size comparison of thoracic spinal cord in a normal control and patients with JOFLS and LOFLS. (a) normal thoracic spinal cord; (b) JOFLS; (c) LOFLS. The spinal cord areas are greatly reduced in FRDA, irrespective of age of FRDA onset. Fiber loss in dorsal columns, spinocerebellar, and corticospinal tracts is also comparable. The sections illustrated in (b) and (c) correspond to FRDA cases 14F and 16F, respectively. Immunostain for MBP. The illustration of the normal spinal cord in (a) was made from three overlapping microphotographs. Bars, 1 mm

Fig. 5. Dorsal root ganglion in a normal control and patients with JOFLS and LOFLS. **(a)** Normal; **(b)** JOFLS; **(c)** LOFLS. In JOFLS **(b)** and LOFLS **(c)**, larger ganglion cells are absent. They are readily

Fig. 6.

Box plot of neuronal areas in DRG of JOFLS, LOFLS, and their respective controls. Individual neuronal sizes are shown on the right; boxes represent the median (*center horizontal line*); the mean (*square symbol*); the 25th and 75th percentile (the *outer box*); and the 5th and 95th percentile (the *whiskers*). Repeated measure ANOVA with Fisher's least significant difference test demonstrates a significant effect of the experimental group ($p=0.001$) with both JOFLS and LOFLS being different from their respective controls ($p=0.003$ and $p=0.033$, respectively) but no difference between either control groups ($p=0.79$) or the JOFLS and LOFLS groups ($p=0.17$) seen in the normal DRG (*asterisks* in [a]). The DRG of JOFLS (**b**) shows an abundance of nodules of Nageotte that are absent in the DRG of LOFLS (**c**). Hematoxylin and eosin. Bars, 50 μm

Table 1
Autopsy specimens of patients with Friedreich's ataxia grouped according to age of onset and disease duration

No. and sex	Age of onset (years)	Age of death (years)	Disease duration (years)	GAA trinucleotide repeats	Cause of death
JOFLS^a					
1F	2	33	31	604/455	Cardiomyopathy; renal failure
2F	7	38	31	750/594	Cardiomyopathy; embolic stroke
3M	16	39	23	491/370	Cardiomyopathy
4F	8	28	20	709/559	Cardiomyopathy; embolic stroke
5F	17	58	41	566/566	Cardiomyopathy; brain hemorrhage
6M	10	38	28	934/429	Cardiomyopathy
7F	11	42	31	990/761	Cardiomyopathy
8F	7	55	48	793/644	Cachexia
9F	6	36	30	840/715	Cardiomyopathy
10F	17	77	60	841/466	Cachexia; cardiomyopathy
11M	3	23	20	1200/1200	Cardiomyopathy
12M	14	50	36	750/400	Cardiomyopathy; myocardial infarction
13M	7	34	27	1114/1114	Cardiomyopathy
14F	17	50	34	723/495	Cardiomyopathy
Mean±SD ^b	10±5	43±14	33±11	808±201/626±253	
LOFLS^a					
15F	22	65	43	631/242	Cachexia
16F	21	79	58	549/356	Cachexia; stroke
17F	20	61	41	694/593	Cardiomyopathy
18F	50	83	33	236/106	Cachexia
19M	29	87	58	170/120	Bronchopneumonia; stroke
Mean±SD ^b	28±13	75±11	47±11	456±238 ^c /283±201 ^c	

^aJOFLS, juvenile-onset FRDA with long survival; LOFLS, late-onset FRDA with long survival

^bSD, standard deviation. FRDA patients 15F and 16F were sisters. In LOFLS patients 18F and 19M, the diagnosis of FRDA was not established during life

^cGAA repeats are significantly longer in JOFLS than LOFLS for both the long (t-test; $p=0.005$) and the short alleles (t-test; $p=0.014$)