Very Late-Onset Friedreich Ataxia Despite Large GAA Triplet Repeat Expansions

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Background: Most patients with Friedreich ataxia (FRDA) have abnormal GAA triplet repeat expansions in both X25 genes. The size of the GAA expansion in the shorter of the 2 expanded alleles correlates significantly with parameters of clinical severity and is inversely related to the age at onset.

Objectives: To describe the clinical and molecular genetic findings in a patient with very late-onset FRDA and to review the literature.

Patient and Methods: A 58-year-old white woman with mild progressive gait disturbance of 15 years' duration whose examination revealed mild incoordination was analyzed for mutations in the X25 gene. A combination of long-range polymerase chain reaction and genomic Southern blot analyses were used to identify GAA expansions in intron 1 of the X25 gene. To uncover evidence of somatic variability in triplet repeat length, DNA isolated from several tissue samples was similarly analyzed. Single-strand conformational polymorphism analysis was used to screen for mutations spanning the entire coding sequence of frataxin and all intron-exon junctions of the X25 gene.

Results: DNA isolated from blood leukocytes revealed GAA triplet repeat expansions in both X25 genes, which were estimated to contain 835 and 1200 repeats. Similar expansions were detected in DNA isolated from lymphoblasts, fibroblasts, buccal cells, and sural nerve, with estimated mean (±SD) lengths of the shorter and longer expansions being 854 (±69) and 1283 (±72) triplets, respectively. A review of reported cases of late-onset Friedreich ataxia (25-39 years) and very late-onset Friedreich ataxia (>40 years) demonstrated that this is the first instance of a patient presenting with very late-onset FRDA despite carrying more than 800 GAA repeats in both expanded X25 alleles.

Conclusions: This unique case of very late-onset FRDA highlights a limitation in our ability to accurately predict the phenotype in FRDA based solely on the size of the GAA expansion. Other genetic or environmental factors may significantly modify disease severity in FRDA.

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Friedreich ataxia (FRDA), an autosomal recessive disease, is the most common inherited ataxia. It typically begins before 25 years of age with progressive gait and limb ataxia. It is usually accompanied by dysarthria, loss of position and vibration senses, absent deep tendon reflexes, and pathologic extensor plantar responses. In addition, most patients have hypertrophic cardiomyopathy, and some have either diabetes mellitus or impaired carbohydrate tolerance. The disease is progressive, and most patients are wheelchair bound 15 years after onset. Death usually occurs in the third or fourth decade of life.

Friedreich ataxia is commonly associated with an abnormal GAA trinucleotide repeat expansion in intron 1 of the X25 gene located at chromosome 9q13. Normal and FRDA chromosomes have 7 to 38 and 66 to more than 1700 repeats, respectively. The GAA expansion accounts for 98% of FRDA chromosomes and rare patients are compound heterozygous for expansions and deleterious point mutations within the X25 gene. The X25 gene encodes frataxin, a mitochondrial protein with a putative function in iron transport. Patients have a marked deficiency of X25 messenger RNA and frataxin, which results in cell death mediated by mitochondrial dysfunction.

For an autosomal recessive disease, patients with FRDA display an unusual degree of clinical variability. It is now recognized that up to 25% of patients may be considered atypical with respect to the established diagnostic criteria. Patients
MATERIALS AND METHODS

Tissue samples were collected following informed consent according to standard procedures. DNA from leukocytes, lymphoblasts, fibroblasts, and biopsied subcutaneous nerve was isolated following proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation. Polymerase chain reaction (PCR)–ready DNA from buccal epithelium was obtained using a buccal swab DNA extraction kit (MasterAmp; Epicenter Technologies, Madison, Wis). Long-range PCR and genomic Southern blot analyses of the GAA expansion in intron 1 of the X25 gene were performed exactly as described elsewhere.6-8 Individual X25 exons (1-5A and 5B) were amplified by PCR using previously reported primer pairs and cycling conditions.3 Single-strand conformational polymorphism analysis was performed using a previously described protocol.26

may show delayed age at onset (arbitrarily subdivided into late-onset FRDA [LOFA; 25-39 years] and very late-onset FRDA [VLOFA; ≥40 years]), retained deep tendon reflexes,17 and unusually gradual disease progression as seen in some Acadians (FRDA-Acad).18 Patients with delayed onset may also exhibit mild clinical impairment, slower progression of disease, and fewer secondary complications or associated manifestations.4,16,19-21 Following the identification of the GAA expansion, a remarkable correlation was found between the age at onset and size of the GAA expansion.4,5,20-23 Patients with LOFA and VLOFA typically have shorter GAA expansions than those with the earlier age of onset FRDA (VLOFA).7,8 As is significant tissue variability in GAA repeat length,24 we now describe a patient with a mild FRDA phenotype whom we diagnosed as having VLOFA. Unexpectedly, she had large GAA expansions within both X25 genes, which were found to be relatively mitotically stable in various tissues.

REPORT OF A CASE

A 58-year-old white woman (patient 105) presented with mild gait disturbance that was slowly progressive during the past 15 years. Although she walked unassisted, she occasionally needed to hold on to a support to avoid falling. Her gait disturbance was more noticeable when at work on an offshore oil rig. She denied excessive alcohol consumption. She denied having any weakness, tingling, or back pain. Her vision, hearing, and swallowing were intact. Her speech was nasal and had remained unchanged. Bladder and bowel function was normal. Abnormal routine electrocardiogram had prompted a cardiology consultation. A 2-dimensional echocardiogram revealed concentric left ventricular hypertrophy and moderate diffuse hypokinesia. Her parents had died of unrelated causes. She is 1 of 6 children, and a brother who was ataxic since the age of 7 years became wheelchair bound and subsequently died at 31 years of age with a clinical diagnosis of FRDA.

Her blood pressure was normal. On neurologic examination, she was alert and scored 28/30 on the Mini-Mental State Examination. Cranial nerves were intact. Muscle strength and tone were normal. She had mild foot and leg muscle wasting. Sensation to pinprick was diminished distal to her knees and wrists. Vibration and position senses were severely impaired at the toes, and vibration sense was moderately reduced in her fingers. Deep tendon reflexes were absent in her lower limbs and diminished in her upper limbs. She had bilateral Babinski sign. Her gait was slightly wide based, with an inability to tandem walk, and she would lose her balance when turning. She was able to walk on her toes or heels without assistance, although with marked unsteadiness. She could not balance herself when attempting to stand on either foot. The results of a Romberg test were positive. The results of a finger-to-nose test were normal, and a heel-to-shin test revealed minimal dysmetria.

Complete blood cell counts, routine chemistry test results, vitamin E levels, and thyroid function test results were normal. Rheumatoid factor was slightly elevated. The level of vitamin B12 was in the low normal range, and methylmalonic acid and homocysteine levels were normal. Brain magnetic resonance imaging scans revealed mild diffuse cortical atrophy. Cervical magnetic resonance imaging showed minimal spondylosis with normal spinal cord size and signal. On nerve conduction studies, right sural, median, ulnar, and radial sensory responses were absent. Peroneal, tibial, and ulnar compound motor action potential amplitudes were at the lower limit of normal. Mild active denervation was limited to the abductor hallucis muscle.

RESULTS

Long-range PCR analysis of intron 1 of the X25 gene, using DNA obtained from the patient's peripheral blood sample, showed 2 large expanded alleles diagnostic of FRDA (Figure 1, top). The expanded repeats were estimated to contain 835 and 1200 GAA triplets. Homozygous expansions were confirmed by Southern blot analysis of leukocyte genomic DNA (Figure 1, bottom). To explore the extent of somatic variability in triplet repeat length, we performed DNA analysis on all available tissues, which included a transformed lymphoblastoid cell line, primary skin fibroblasts established from a skin biopsy specimen, buccal epithelial cells from a mouthwash, and a sural nerve biopsy specimen. As seen in Figure 2, long-range PCR analysis revealed that the GAA expansion sizes within the various assayed tissues were similar to that seen in peripheral blood. Overall, slight variation was seen in the mean (±SD) lengths of the individual GAA expansions, which ranged from 795 to 960 triplets for the shorter allele (854 ± 69) and from 1200 to 1360 triplets for the longer allele (1283 ± 72).

Single-strand conformational polymorphism analysis of 6 X25 exons (1-5A and 5B) showed no evidence of mutations involving the entire frataxin coding sequence and all splice junctions (data not shown). DNA
larger allele (GAA-2) accounts for less than 20%.4,19,24,25 To 73% of the variation in the age at onset, the size of the expansions (GAA-1). Although the latter accounts for 33% ease severity and the length of the shorter of the 2 GAA expansions were detectable in all tissue samples tested, and their respective triplet repeat lengths were estimated to be the following: 105B = 835/1200, 105L = 795/1250, 105F = 795/1360, 105N = 875/1360, and 105MW = 960/1250. L and W indicate lanes containing a DNA size marker (with sizes indicated in kilobases) and a water blank, respectively. The unshaded and filled squares indicate samples from a normal control and a patient with Friedreich ataxia, respectively. EA and NA depict the positions of the expanded and normal alleles, respectively.

Both LOFA and VLOFA account for up to 25% of patients with FRDA.4,10 Following the discovery of the GAA trinucleotide repeat expansion in FRDA, various authors4,5,10,22,24 have reported a striking relation between disease severity and the length of the shorter of the 2 GAA expansions (GAA-1). Although the latter accounts for 33% to 73% of the variation in the age at onset, the size of the larger allele (GAA-2) accounts for less than 20%.4,19,24,25 This is thought to stem from the proportional interference with X25 transcription caused by the expanded GAA triplet repeat sequence, where residual frataxin levels are ultimately determined as a function of the size of the shorter GAA expansion.13,20

Our patient had VLOFA with slow disease progression and mild ataxia despite being homozygous for GAA expansions in excess of 800 repeats. The Table summarizes the current literature on 8 other cases of VLOFA (age at onset, ≥40 years; range, 40-51 years).4,20-22 Patients were followed up for 5 to 37 years after onset of ataxia. Only 2 patients had become wheelchair bound, both of whom were older than 20 years after onset of disease. All the previously described patients with VLOFA had dysarthria, dysmetria, extensor plantars, and absence of cardiomyopathy as constant features. Our patient shared many features but was unique for not having dysarthria and for having cardiomyopathy. Classically, dysarthria starts within 5 years of onset and is seen in almost every patient with FRDA.1,4 Even though the absence of dysarthria in our patient after 15 years is highly unusual, a rare case of dysarthria was reported 19 years after disease onset.5 Cardiomyopathy in FRDA is clearly associated with larger expansions4,10,30 and more severe manifestation of disease20,24 as measured by earlier onset and rapid progression of disease. Only 15.4% of patients with LOFA5,21 and 48% of those with FRDA-Acad20 had cardiomyopathy compared with almost two thirds of all patients homozygous for the GAA expansion4 or 82% to 91.5% with classic FRDA.20-24 Isnard et al30 found echocardiographic evidence of left ventricular hypertrophy in 81% of their patients with more than 770 GAA-1 repeat lengths and in only 14% of those with less than 770 triplets. In our patient, the presence of cardiomyopathy clearly correlated with her genotype but was incompatible with her VLOFA status.

Homoygous GAA expansions, with more than 800 repeats in the shorter of the 2 expanded alleles, have been uniformly associated with the classic FRDA phenotype, with an onset by 25 years of age.4,5,19,20,24 Our patient, whom we studied, is the first exception to that observation. In the 8 patients with VLOFA other than our patient, the num-

**Table:**

<table>
<thead>
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<th>Name</th>
<th>Age at Onset (years)</th>
<th>Sex</th>
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</table>

**References:**

ber of GAA repeats in the shorter and longer expanded alleles ranged from 117 to 260 (mean ± SD, 204 ± 62; n = 8) and 250 to 1033 (mean ± SD, 532 ± 320; n = 8), respectively. On the other hand, the number of GAA repeats within the shorter and longer expanded alleles in 30 LOFA cases4,21-23,31 (S.I.B., C.A.G., P.I.P., and M.M.D., unpublished data, 1999) ranged from 90 to 633 (mean ± SD, 310 ± 155; n = 30) and 230 to 1200 (mean ± SD, 715 ± 297; n = 30), respectively. A 2-tailed, unpaired t test revealed that the difference in the mean repeat lengths of the shorter expansion alleles between patients with LOFA and VLOFA was statistically significant (P = .006). The length of the longer expansion and, to a lesser extent, the mean length of the 2 expansions showed overlap between patients with VLOFA and LOFA (P = .18 and .04, respectively). In contrast, our patient's shorter allele contained 835 repeats, clearly exceeding the known range for the shorter alleles in both VLOFA (99% confidence interval, 128-280) and LOFA (99% confidence interval, 233-388) (P < .001) (Figure 3). The uniqueness of this molecular finding was further highlighted in a recent investigation31 that involved 361 families with adult-onset, idiopathic spinocerebellar ataxia, after excluding those with a diagnosis of FRDA. Thirteen patients, of which 7 had an age at onset of 25 or more years, had molecular evidence of FRDA based on homozygous GAA expansions. All had 600 or fewer repeats in the shorter expanded allele.

The striking discordance between the genotype and phenotype in our patient compared with other LOFA and VLOFA cases and the degree of intrafamilial variability seen in this family make this case unique. Several possibilities are considered in attempting to decipher the etiologic basis of the apparent lack of genotype-phenotype correlation. These include tissue-specific variability in triplet repeat length due to mitotic instability, cis-acting sequence alterations, and other genetic or environmental modifiers.
The diagnosis of VLOFA needs to be considered in patients who present with gait ataxia beginning in the fifth or sixth decade of life. Our case underscores a potential pitfall in phenotype prediction when based solely on the length of the shorter GAA expansion in FRDA. Genetic and prognostic counseling in FRDA needs to be tempered with this information.

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REFERENCES


