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. 1,2 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. 3 Normal and FRDA chromosomes have 7 to 38 and 66 to more than 1700 repeats, respectively. 3-5 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. 3,6-8 The X25 gene encodes frataxin, a mitochondrial protein with a putative function in iron transport. 3,9-12 Patients have a marked deficiency of X25 messenger RNA 13 and frataxin, 14 which results in cell death mediated by mitochondrial dysfunction.

For an autosomal recessive disease, patients with FRDA display an unusual degree of clinical variability. 1 It is now recognized that up to 25% of patients may be considered atypical with respect to the established diagnostic criteria. 1,4,15 Patients...
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, and unusually gradual disease progression as seen in some Acadians (FRDA-Acad). Patients with delayed onset may also exhibit mild clinical impairment, slower progression of disease, and fewer secondary complications or associated manifestations. Individual X25 exons (1-5A and 5B) were amplified by PCR using previously reported primer pairs and cycling conditions. Single-strand conformational polymorphism analysis was performed using a previously described protocol.

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 she occasionally needed to hold on to a support to avoid falling. 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
analysis for the CAG triplet repeat expansions seen in spinocerebellar ataxia types 1 and 3 showed normal results.

Both LOFA and VLOFA account for up to 25% of patients with FRDA. Following the discovery of the GAA trinucleotide repeat expansion in FRDA, various authors 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 shorter GAA expansion (GAA-1) accounts for less than 20%.4,19,24,25

Transcription caused by the expanded GAA X25 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). 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.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 unusual, a rare case of dysarthria was reported 19 years after onset of disease.30

In our patient, the presence of cardiomyopathy clearly correlated with her genotype but was incompatible with her VLOFA status. Homozygous 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.5,19,24,25 Our patient, whom we studied, is the first exception to that observation. In the 8 patients with VLOFA other than our patient, the num-
Summary of Reported Cases of Very Late-Onset Friedreich Ataxia (VLOFA)\textsuperscript{*}

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<th>9</th>
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<tr>
<td>Age at onset, y</td>
<td>40</td>
<td>45</td>
<td>51</td>
<td>40</td>
<td>48</td>
<td>40</td>
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<td>Disease duration, y</td>
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<td>5</td>
<td>27</td>
<td>8</td>
<td>17</td>
<td>11</td>
<td>8</td>
<td>15</td>
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<tr>
<td>Ambulant (Amb) or wheelchair bound (Wc)</td>
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<td>Amb</td>
<td>Wc</td>
<td>Amb</td>
<td>Wc</td>
<td>Amb</td>
<td>Amb</td>
<td>Wc</td>
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<tr>
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<td>–</td>
<td>–</td>
<td>Mild</td>
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<td>260</td>
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<td>260</td>
<td>360</td>
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</table>

* The sources of the patients with VLOFA are as follows: numbers 1-3 are described in reference 4; 4-7, references 20 and 24; 8, reference 21; and 9 is patient 105. Proven or suspected compound heterozygotes were not used in this survey. Plus sign indicates present; minus sign, absent; plus or minus sign, equivocal; and question mark, unknown. GAA-1 and GAA-2 refer to the sizes of the shorter and longer expansions seen in homozygous expansion cases, respectively.

Somatic mosaicism in triplet repeat length as a result of mitotic instability is well-known. Triplet repeat lengths estimated from peripheral blood samples may, therefore, not necessarily be indicative of the situation in other pathologically relevant tissues, such as the dorsal columns, dorsal root ganglia, and spinocerebellar tracts. In fact, the GAA repeat has previously been shown to have significantly differing sizes within different parts of the brain, and among various tissues, including lymphoblasts, fibroblasts, peripheral nerve, and sperm. We have previously shown a shorter repeat length in a pe-
ripheral nerve sample compared with blood in a patient mildly affected with FRDA.20 Interestingly, this patient was also shown to have a proportion of blood lymphocytes carrying a heterogeneous mix of fully contracted normal GAA repeat alleles.33 In our patient (patient 105), however, only limited somatic variability was demonstrable among blood leukocytes, skin fibroblasts, buccal cells, and sural nerve. The patient’s mild ataxic phenotype may, however, suggest the possibility of a contracted GAA repeat specific to her spinocerebellar tissue.

Barring somatic variability in triplet repeat length, other mechanisms warrant formal consideration. We and others13,29 have previously shown that the transcriptional blockade associated with expanded GAA tracts may be mediated by an unusual, higher-order structure assumed by this sequence. It is possible that sequence alterations within or immediately flanking the GAA expansion may have a destabilizing effect on such a structure,35 resulting in a lesser degree of transcriptional inhibition and consequently a milder phenotype. Limited sequence analysis of intron 1 immediately flanking the GAA expansions and within the 3’ end of both expansions revealed no alterations (data not shown).

Analyses of FRDA sibling pairs and FRDA-Acad indicate that there are likely to be genetic or environmental modifiers of the phenotype in FRDA. De Michele et al25 found that age at onset in siblings was a significant independent determinant of age at onset in patients. Excluding the obvious effect of the correlation between GAA expansion sizes among siblings by multiple regression analyses, they concluded that shared genetic or environmental factors, or both, might account for this correlation. Schölz et al33 analyzed sibling pairs whose repeat lengths differed by less than 100 (n = 5) and 120 to 420 (n = 4) triplet repeats. Surprisingly, their ages at onset showed remarkable correlation despite the lack of strict correlation with repeat sizes. On the other hand, the brother of our patient apparently had a typical FRDA phenotype based on the history of disease progression. Others have noted a similar intrafamilial disparity in phenotypic severity, despite the proven31,36 or assumed37 (including our case) similarity of mutant alleles. The latter examples also indicate the presence of modifiers, discordance for which seem to exert variant phenotypic effects.

The patients with FRDA-Acad show a slightly later age at onset and a more gradual progression than typical FRDA, being wheelchair bound on average 7 years later than the typical patient with FRDA.20 The frequency of all other manifestations in FRDA-Acad (excluding cardiomyopathy) are similar to typical FRDA. Moreover, FRDA-Acad in 44 patients was reported to be associated with similar GAA expansion sizes seen in typical FRDA.20 The molecular basis for this phenotypic variation is unknown but is speculated to be determined by an element(s) acting in cis given the shared core haplotype specific to this population. Our patient denied knowledge of any Acadian ancestry. However, similar genetic and possibly environmental factors may be responsible for her milder phenotype. We were unable to detect any X25 mutations that involved the entire frataxin coding sequence and all X25 splice junctions, similar to what was shown in patients with FRDA-Acad.20

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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