Clinical and Genetic Abnormalities in Patients with Friedreich's Ataxia

Alexandra Dürre, M.D., Mireille Cosse, M.D., Yves Agid, M.D., Ph.D., Victoria Campuzano, Ph.D., Claude Mignard, M.D., Christiane Penet, Jean-Louis Mandel, M.D., Ph.D., Alexis Brice, M.D., and Michel Koenig, M.D., Ph.D.

Abstract

Background Friedreich's ataxia, the most common inherited ataxia, is associated with a mutation that consists of an unstable expansion of GAA repeats in the first intron of the frataxin gene on chromosome 9, which encodes a protein of unknown function.

Methods We studied 187 patients with autosomal recessive ataxia, determined the size of the GAA expansions, and analyzed the clinical manifestations in relation to the number of GAA repeats and the duration of disease.

Results One hundred forty of the 187 patients, with ages at onset ranging from 2 to 51 years, were homozygous for a GAA expansion that had 120 to 1700 repeats of the trinucleotides. About one quarter of the patients, despite being homozygous, had atypical Friedreich's ataxia; they were older at presentation and had intact tendon reflexes. Larger GAA expansions correlated with earlier age at onset and shorter times to loss of ambulation. The size of the GAA expansions (and particularly that of the smaller of each pair) was associated with the frequency of cardiomyopathy and loss of reflexes in the upper limbs. The GAA repeats were unstable during transmission.

Conclusions The clinical spectrum of Friedreich's ataxia is broader than previously recognized, and the direct molecular test for the GAA expansion on chromosome 9 is useful for diagnosis, determination of prognosis, and genetic counseling. (N Engl J Med 1996;335:1169-75.)

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FRIEDREICH'S ataxia, an autosomal recessive neurodegenerative disorder, is the most common hereditary ataxia. Its estimated prevalence in European populations is 1 in 50,000. In 1988 the locus of the genetic defect was mapped to chromosome 9.¹ The frataxin gene, encoding a protein of unknown function, was recently identified, and, surprisingly, most of the mutations appeared to be unstable expansions of a GAA repeat in the first intron.² Ninety-four percent of patients with the classic form of the disease were found to be homozygous for the GAA expansion. The causal role of mutations in the frataxin gene was proved by the identification of rare compound heterozygotes with an expansion in one allele and a point mutation in the other.³

A cardinal feature of Friedreich's ataxia is ataxia of all four limbs, associated with cerebellar dysarthria, absent reflexes in the lower limbs, sensory loss, and pyramidal signs. The onset of symptoms is usually before 20 years of age, and the progression of the disease is relentless. Skeletal deformities and cardiomyopathy are found in a majority of patients, who also have an increased frequency of impaired glucose tolerance and diabetes. The presence of these signs is strongly suggestive of Friedreich's ataxia.

The diagnostic criteria were revised by Harding⁴ to include patients with onset up to the age of 25 and to take into account the incomplete clinical presentation in patients in whom the duration of disease was less than five years. Families with at least two patients meeting these criteria revealed linkage to chromosome 9q13,⁵ although an autosomal recessive deficiency of vitamin E may produce similar symptoms.⁶ The existence of patients with autosomal recessive cerebellar ataxia who had intact tendon reflexes⁷ or disease of late onset⁸ but with linkage to chromosome 9q13⁹-¹³ suggested that the clinical spectrum is broader than previously thought.

We screened 187 patients with progressive ataxia...
for GAA expansion in the frataxin gene. In 140 patients who were homozygous for the expansion, we measured the size of the repeats and examined phenotype–genotype correlations.

METHODS

Patients and Families
We screened 187 patients from 147 families, selected because they had progressive, unremitting cerebellar ataxia of proved or possible autosomal recessive inheritance, for GAA expansion in the frataxin gene. Consanguinity was documented in 21 families, and 75 patients had no affected relatives. All but 10 patients were initially referred to the Fédération de Neurologie at the Salpêtrière Hospital in Paris or to the Department of Neurology at St. Pierre Hospital on the French island of Réunion in the Indian Ocean and were examined between May 1990 and March 1996. All patients were examined by one of the authors according to a standardized protocol, and age at onset of symptoms, disease progression, and clinical signs associated with cerebellar ataxia were recorded. According to the essential diagnostic criteria, the patients had typical Friedreich's ataxia. Patients in this group had a disease duration of at least five years, with onset of symptoms before the age of 25, progressive ataxia of gait and limbs, absent knee and ankle jerks, and extensor planter responses. Ten other patients with disease duration of less than five years were also classified as having typical Friedreich's ataxia; all these patients presented with no extensor plantar reflex, and two of them had intact reflexes in the lower limbs. Among the other 74 patients, at least one of the essential criteria was still unmet five years after diagnosis, but all had progressive, unremitting cerebellar ataxia compatible with autosomal recessive inheritance. Nine had at least one sibling with typical Friedreich's ataxia.

Thirty-two patients had one or more children. Eighty-one of the 114 families with GAA expansions were French. In 74 of these families, both parents came from France. In the remaining seven, the other parent came from Italy (three), Belgium (two), Germany (one), or Morocco (one). There were also families from Réunion (17), Algeria (five), Spain (three), Italy (three), Portugal (two), Morocco (one), Greece (one), and Argentina and Uruguay (one).

GAA-Repeat Analysis
GAA repeats were analyzed by Southern blotting. In the initial report, the expansion mutation was detected by Southern blot analysis of DNA digested with the EcoRI restriction enzyme. In the present study, we used the BstI/HKAI enzyme, which yields a smaller target DNA fragment (2.4 kb instead of 8.2 kb), allowing a more accurate estimation of the number of GAA repeats and a better resolution between the two alleles. Ten micrograms of genomic DNA was digested by BstI/HKAI (New England Biolabs, Beverly, Mass.), electrophoresed in 0.7% agarose gel, blotted on Hybond-N+ membranes (Amersham, Little Chalfont, United Kingdom), and hybridized with a 32P-radiolabeled 663-bp genomic fragment containing exon 1. The fragment was obtained by the polymerase chain reaction with the forward primer GAA-GTTCCTCGTTT and the reverse primer CGCGCGGT-GTTCGCCG and cloned in pGEM-T. A 1-kb–ladder marker (GIBCO BRL, Gaithersburg, Md.) was run and hybridized in parallel for size measurement. Blots were washed in 0.2% saline sodium citrate (1× saline sodium citrate) at 0.15 M sodium chloride and 0.015 M sodium citrate and 0.1 percent sodium dodecyl sulfate twice at room temperature and twice at 60°C and exposed for autoradiography at −80°C with an intensifying screen.

Statistical Analysis
Means were compared with use of nonparametric tests and Student’s t-test, and frequencies were compared with use of the chi-square and Yates' corrected chi-square test, when appropriate. Means are given with standard deviations. Regression coefficients with the quadratic model were calculated for the correlations between the number of GAA repeats and both age at onset and duration of disease until the patient became confined to a wheelchair.

RESULTS
Frequency and Size of GAA Expansions
Expanded GAA repeats were found on both alleles of the frataxin gene in 140 patients from 114 families (Table 1). Nine patients from six families were heterozygous for the expansion mutation and were known or expected carriers of a point mutation on the other allele, as previously shown in five families. Among the patients who fulfilled Harding’s criteria for Friedreich’s ataxia, 94 percent were homozygous for the GAA expansion and the remaining 6 percent were heterozygous. Surprisingly, 46 percent of the patients who did not meet all the diagnostic criteria for Friedreich’s ataxia were also homozygous for the GAA expansion. Thirty-eight patients had two alleles with GAA repeats in the normal size range, which excludes the diagnosis of Friedreich’s ataxia.

In the 140 patients who were homozygous for the GAA expansion, the number of repeats ranged from 120 to 1700 (Fig. 1); the normal frataxin gene has between 8 and 22 GAA repeats. Most of these patients had two expanded alleles of different sizes, and no detectable somatic instability. In 28 patients the two expanded alleles appeared as a single band on Southern blotting. The mean numbers of GAA repeats on the smaller and the larger alleles were 630±230 and 890±230, respectively. In a few instances two major expanded alleles were detected, along with less intense bands in the expanded range, probably reflecting somatic mosaicism. Only the bands corresponding to the stronger signals were taken into account. The expansion was unstable during transmission, accounting for the variable number of GAA repeats among siblings (Fig. 2).

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TYPICAL FRIEDREICH’S ATAXIA (N = 113)</th>
<th>ATYPICAL FRIEDREICH’S ATAXIA (N = 74)</th>
<th>TOTAL (N = 187)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous for GAA-repeat expansion</td>
<td>106 (89)</td>
<td>34 (25)</td>
<td>140 (114)</td>
</tr>
<tr>
<td>Heterozygous for GAA-repeat expansion</td>
<td>7 (5)</td>
<td>2 (1)</td>
<td>9 (6)</td>
</tr>
<tr>
<td>Without GAA-repeat expansion</td>
<td>0</td>
<td>38 (27)</td>
<td>38 (27)</td>
</tr>
</tbody>
</table>

*In one family, the point mutation in the other allele of the frataxin gene has been identified.
Features of Patients Homozygous for the GAA Expansion

Clinical Characteristics

Of the 140 patients homozygous for the GAA expansion, 72 were women and 68 were men. The mean age at onset of the disease was 15.5±8 years (range, 2 to 51), and the mean age at examination was 31±13 years (range, 7 to 77). The mean time until the patient became confined to a wheelchair was 10.8±6 years (range, 1 to 25). The presenting symptom was gait ataxia in all patients except for seven in whom scoliosis was diagnosed before the ataxia. The overall clinical picture is detailed in Table 2. In addition, the following signs and symptoms were observed: facial dysmorphia (five patients in three families), seizures (two patients), dystonic postures (two patients), myoclonus (five patients), postural tremor (two patients), limited eye gaze with preserved vestibulo-ocular movements (five patients), and mental retardation (five patients). These features might be associated with, but not caused by, the mutation.

Thirty-four of the 140 homozygous patients had an atypical clinical presentation for one or more of the following reasons: an age of more than 25 years at onset (19 patients); retained tendon reflexes in lower limbs, including 4 patients with brisk reflexes (13 patients); and absence of extensor plantar response (21 patients). In addition, 10 patients who had had the disease for less than five years had an incomplete clinical picture but were homozygous for the expansion, confirming the diagnosis of Friedreich’s ataxia.

Correlation of Age at Onset and Rate of Disease Progression with GAA-Expansion Size

We determined the correlations between the size of the smaller and the larger expansions for each pair of alleles and both age at onset and duration of disease before the patient became confined to a wheelchair. An inverse relation was found between the size of the smaller GAA expansion and both age at onset \((r = -0.75, P < 0.001)\) (Fig. 3) and time until confinement to a wheelchair \((r = -0.49, P < 0.005)\). The correlation was less strong between the size of the larger allele and age at onset \((r = -0.5, P < 0.001)\). However, the sizes of the two alleles were not independent \((r = 0.5, P < 0.001)\), possibly because of consanguinity or a founder effect in a substantial proportion of the families. This observation may explain in part the correlation between the size of the larger allele and age at onset. Only the number of GAA repeats in the smaller expansion was used in subsequent calculations.

Correlation of Phenotype with GAA-Expansion Size and Duration of Disease

The frequency of some clinical signs increased with the size of the GAA expansion (Table 3), where-
A total of 63 patients were tested in our study. Diabetes or impaired glucose tolerance was present in 32 patients. Abnormal visual evoked potentials were present in 34 patients. Abnormal brain-stem evoked potentials were present in 61 patients. Cardiomyopathy on echocardiography was present in 63 patients. Axonal neuropathy was defined as abnormal motor-nerve conduction velocity in the upper limbs with small or absent sensory action potentials. A total of 63 patients were tested in our study.

**TABLE 2. FREQUENCY OF CLINICAL SIGNS IN 140 PATIENTS HOMOZYGOUS FOR THE GAA EXPANSION AND IN PATIENTS STUDIED BY HARDING.**

<table>
<thead>
<tr>
<th>CLINICAL SIGN</th>
<th>THIS STUDY % of patients</th>
<th>HARDING STUDY % of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gait ataxia</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Limb ataxia</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Dysarthria</td>
<td>91</td>
<td>97</td>
</tr>
<tr>
<td>Lower-limb areflexia</td>
<td>87</td>
<td>99</td>
</tr>
<tr>
<td>Knee jerks present</td>
<td>12</td>
<td>0.9</td>
</tr>
<tr>
<td>Loss of vibration sense</td>
<td>78</td>
<td>73</td>
</tr>
<tr>
<td>Extensor plantar reflexes</td>
<td>79</td>
<td>89</td>
</tr>
<tr>
<td>Muscle weakness in lower limbs</td>
<td>67</td>
<td>88</td>
</tr>
<tr>
<td>Scoliosis</td>
<td>60</td>
<td>79</td>
</tr>
<tr>
<td>Pes cavus</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>Horizontal nystagmus</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Wasting of lower limbs</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>Saccadic-pursuit eye movements</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>Swallowing difficulties</td>
<td>27</td>
<td>—</td>
</tr>
<tr>
<td>Wasting of upper limbs</td>
<td>25</td>
<td>49</td>
</tr>
<tr>
<td>Sphincter disturbances</td>
<td>23</td>
<td>—</td>
</tr>
<tr>
<td>Reduced visual acuity</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>Axonal neuropathy*</td>
<td>98</td>
<td>96</td>
</tr>
<tr>
<td>Cardiomyopathy on echocardiography†</td>
<td>63</td>
<td>—</td>
</tr>
<tr>
<td>Abnormal brain-stem evoked potentials‡</td>
<td>61</td>
<td>—</td>
</tr>
<tr>
<td>Abnormal visual evoked potentials§</td>
<td>34</td>
<td>—</td>
</tr>
<tr>
<td>Diabetes or impaired glucose tolerance§</td>
<td>32</td>
<td>10</td>
</tr>
</tbody>
</table>

*Axonal neuropathy was defined as abnormal motor-nerve conduction velocity in the upper limbs with small or absent sensory action potentials.
†A total of 75 patients were tested in our study.
‡A total of 29 patients were tested in our study.
§A total of 34 patients were tested in our study.

Figure 3. Correlation between Age at Onset and the Number of GAA Repeats in the Smaller Allele in the 140 Study Patients. The best fit was obtained with the quadratic model for the regression coefficient \( r = -0.75, P < 0.001 \). The patient with 1000 GAA repeats and onset at 23 years of age had mosaicism for a third expanded fragment of 670 repeats, which might in part explain his late age at the onset of disease.

**DISCUSSION**

The first report on the frataxin gene in Friedreich’s ataxia showed that all patients with the classic form of the disease, as defined by Harding’s diagnostic criteria, carried the GAA expansion, and 94 percent were homozygotes. In the present study, we analyzed a broader sample of patients with progressive ataxia, 40 percent of whom did not meet all of Harding’s criteria, to determine the range of phenotypes associated with mutations in the frataxin gene. Furthermore, we measured the number of GAA repeats by a more accurate technique than that used in the initial report, to see whether the size of the GAA expansion correlated with disease severity or clinical presentation, as in other trinucleotide-expansion diseases such as myotonic dystrophy and Huntington’s disease, or whether instead there was an all-or-none effect on clinical manifestations once a threshold in the size of the mutation was reached, as in the fragile X mental retardation syndrome.

Eighty percent of the patients studied had at least one GAA expansion in the frataxin gene; this result was expected because Friedreich’s ataxia is the chief cause of progressive autosomal recessive ataxia. The diagnostic criteria for Friedreich’s ataxia had been defined with precision, and it was thought that Friedreich’s ataxia was a homogeneous clinical entity. The criteria included autosomal recessive inheritance, onset before the age of 25, the absence of lower-limb reflexes, and the presence of pyramidal signs. Indeed, all the patients who met these criteria had a GAA expansion. The expansion was present on both alleles in 94 percent of these patients and on one allele in 6 percent. The latter patients are presumed...
to carry a point mutation on their nonexpanded allele, as previously demonstrated in several families.2

Although very specific, these diagnostic criteria do not appear to be sensitive enough, since about one quarter of our patients who were homozygous for the expansion lacked at least one of them. There is a wider clinical spectrum in Friedreich’s ataxia than has been previously appreciated. Late onset is not uncommon among patients with homozygous expansions; 19 of our patients (14 percent) had onset between 26 and 51 years of age. Reflexes may be retained in the lower limbs, as observed in 13 patients, and in 4 they were brisk. Linkage analyses in a few families10-13 had suggested that such variant clinical presentations might be allelic with Friedreich’s ataxia.

A clinical analysis of patients with early-onset cerebellar ataxia and retained reflexes suggested that they differed from patients with Friedreich’s ataxia by the absence of cardiomyopathy and optic atrophy,6 the presence of cerebellar atrophy on magnetic resonance imaging (11 of 14 patients),6 and longer times before becoming confined to a wheelchair (33 ± 19 years6 or 21 ± 11 years,6 as compared with 6 ± 0.4 years in Friedreich’s ataxia6). Among our patients, the 10 who presented with clinical and electrophysiologic features of early-onset cerebellar ataxia and with retained reflexes had, in fact, Friedreich’s ataxia with small GAA expansions. Unusual signs (such as mental retardation) were rarely observed and are probably not part of the phenotype of Friedreich’s ataxia.

The size of the expansion varied greatly among patients and within and among families. The wide range of GAA repeats, from 120 to 1700, reflects the instability of the expansion during transmission, which is characteristic of all mutations with large trinucleotide-repeat expansions.14 The size of the expansion in Friedreich’s ataxia is similar to those found in fragile X syndrome and myotonic dystrophy, in which the expansion lies in the untranslated region of the corresponding transcript.14 However, it is larger than those found in neurodegenerative disorders such as autosomal dominant cerebellar ataxias resulting from translocated CAG expansions15,16 that lead to a toxic abnormal protein.17

Correlation between the size of the GAA expansion and age at onset of the disease is evidence that the expansion is the cause of the disease rather than being associated by linkage disequilibrium with another, unidentified mutation located elsewhere in the noncoding part of the frataxin gene. The size of the smaller GAA expansion is strongly correlated with age at onset and with the rate of disease progression. The number of repeats on the allele with the smaller expansion accounts for approximately 50 percent of the variability in age at onset, as compared with less than 20 percent for the allele with the larger expansion. Individual variations are large, however, and more than 30 percent of the variance results from unknown factors, so that the predictive value of expansion size is limited.

Autosomal recessive inheritance suggests that the
mutation results in a loss of function. This view is supported by the observation of greatly decreased levels of the frataxin transcript in lymphoblasts from patients and by the fact that in some patients who are heterozygous for the expansion, the other allele carries mutations that result in clear loss of function by interfering with the maturation or translation of frataxin messenger RNA (mRNA). The consequence of the expansion in Friedreich's ataxia is similar to that in fragile X syndrome, where absence of the FMR1 mRNA, protein, or both has been demonstrated. In myotonic dystrophy the expansion is unlikely to create a simple loss of function. If there was a loss of function, one would expect to find point mutations with similar effects in some patients. In Friedreich's ataxia, the correlation observed between clinical severity and the size of the expansion in the smaller allele suggests that small expansions do not totally inhibit transcription or maturation of frataxin mRNA and allow substantial residual expression of the frataxin protein. Above a certain threshold, probably above 700 GAA repeats, the residual expression is too low to influence the clinical presentation. Consequently, the amount of frataxin is driven mostly by the smaller allele.

Both the number of GAA repeats and the duration of disease affect, in varying proportions, the signs associated with cerebellar ataxia. Hypertrophic, most commonly concentric, cardiomyopathy has been recognized as specific for the diagnosis of Friedreich's ataxia and was present in almost all patients with classic Friedreich's ataxia studied by echocardiography. We showed that its frequency increases with the size of the GAA expansion, as does the frequency of the extensor plantar response and skeletal deformities. The correlation of pes cavus and scoliosis with GAA-expansion size might reflect the early onset of peripheral neuropathy. The association of cardiomyopathy with large expansions is important for prognosis, since complications of cardiomyopathy are a frequent cause of death in Friedreich's ataxia. It also suggests that although frataxin mRNA is highly expressed in the heart, there is probably a threshold of residual active protein below which cardiomyopathy appears.

The presence of other signs, such as the extensor plantar response and weakness and wasting in the lower limbs, was determined in part by both the number of GAA repeats and the duration of the disease, whereas the frequency of dysarthria, decreased vibration sense, sphincter disturbances or swallowing difficulties, and hearing or visual loss was associated with increased disease duration alone. Some of these signs were thought to be crucial for differential diagnosis, but our results suggest otherwise. Yet unidentified factors may also contribute to the complex phenotype observed in patients with Friedreich's ataxia.

In conclusion, we demonstrated that the spectrum of Friedreich's ataxia is broader than previously thought, with onset between 26 and 51 years of age in 14 percent of the patients studied and retained lower limb reflexes in 12 percent. Friedreich's ataxia is a progressive disease, so a full clinical presentation is observed only several years after onset, a fact that makes it difficult to diagnose the disease early. In Europe and North America, most cases are sporadic and occur in nonconsanguineous families. Autosomal recessive inheritance cannot be demonstrated in such cases. Delayed diagnosis, however, impairs genetic counseling for future pregnancies. Direct molecular diagnosis through determination of the size of the GAA expansion should become an essential tool in clinical practice, differential diagnosis, and genetic counseling for patients with recessive or sporadic cerebellar ataxias.

REFERENCES


