Friedreich’s ataxia
Revision of the phenotype according to molecular genetics

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Summary
Friedreich’s ataxia is an autosomal recessively inherited neurodegenerative disorder caused by expansions of an unstable GAA trinucleotide repeat in the STM7/X25 gene on chromosome 9q. We studied the (GAA)n polymorphism in 178 healthy controls and 102 patients with idiopathic ataxia. The repeat size ranged from 7 to 29 (GAA)n motifs on normal chromosomes and from 66 to 1360 trinucleotide repetitions in Friedreich’s ataxia patients. Meiotic instability of expanded alleles was observed without significant differences in maternal and paternal transmissions. Thirty-six of 102 patients were typed homozygous for expanded (GAA)n alleles. Twenty-seven of these presented with the typical Friedreich’s ataxia symptoms and nine patients with an atypical Friedreich’s ataxia phenotype. Before molecular genetic diagnosis had been performed seven of these patients had been classified as early onset cerebellar ataxia and two as idiopathic sporadic cerebellar ataxia of late onset. In contrast, in one family with typical Friedreich’s ataxia phenotype we did not find an expanded allele; this suggests that there can be either point mutations in the X25 gene on both chromosomes or locus heterogeneity in Friedreich’s ataxia. The phenotypic spectrum of Friedreich’s ataxia is much broader than considered before. Early onset, areflexia, extensor plantar responses and reduced vibration sense should no longer be considered essential diagnostic criteria of Friedreich’s ataxia. In comparison with the non-Friedreich’s ataxia group hypertrophic cardiomyopathy seems to be the only symptom specific for Friedreich’s ataxia. However, it is not obligatory. The phenotype is significantly influenced by the number of GAA repeats with close genotype–phenotype relationships when the smaller of the two alleles is considered. Repeat length correlated inversely with age at onset, onset of dysarthria and progression rate. In conclusion, molecular genetic analysis appears mandatory for the diagnosis and genetic counselling of Friedreich’s ataxia. The molecular genetic test should be applied not only to patients with typical Friedreich’s ataxia phenotype but also in all cases of idiopathic autosomal recessive or sporadic ataxia.

Keywords: Friedreich’s ataxia; trinucleotide repeat; diagnostic criteria; genotype; phenotype

Abbreviation: PCR = polymerase chain reaction

Introduction
Friedreich’s ataxia is the most common hereditary ataxia in central Europe, with an estimated prevalence of 1 in 50 000. Although Nikolaus Friedreich described this form of ataxia more than a century ago in a series of five papers (Friedreich 1863–1877), the full spectrum of the phenotype and characteristics separating Friedreich’s ataxia from other forms of ataxia are still under debate. Diagnostic criteria for Friedreich’s ataxia have been developed in two large series by Geoffroy et al (1976) and by Harding (1981). Geoffroy et al. (1976) found that onset was before the age of 20 years, progressive ataxia of gait and limbs, dysarthria, decrease in position sense and/or vibration sense in lower limbs, muscle weakness and deep tendon areflexia in lower limbs were obligatory symptoms. Harding (1981) postulated onset of symptoms before 25 years, progressive ataxia of gait and limbs, absent knee and ankle jerks, extensor plantar responses, development of dysarthria within 5 years of disease onset and motor nerve conduction velocity of >40 m/s in upper limbs with small or absent sensory action potentials. Further frequent but not obligatory symptoms are scoliosis, pes cavus, cardiomyopathy of the hypertrophic non-obstructive type, optic atrophy, deafness, and diabetes.

Chamberlain et al. mapped the Friedreich’s ataxia gene to chromosome 9 in 1988. By the identification of recombination...
events, the candidate region was successively reduced to 450, 300 and then 150 kb (Chamberlain et al., 1993; Duclos et al., 1994; Rodius et al., 1994; Montermini et al., 1995). Linkage analysis demonstrated that the Friedreich’s ataxia phenotype is broader than previously described. Klockgether et al. (1993, 1996), De Michele et al. (1994) and Palau et al. (1995) identified families with autosomal recessive ataxia linked to chromosome 9q13 but with a later onset of the disease or retained tendon reflexes.

Recently, mutations have been identified in the X25 gene on chromosome 9q13 (Campuzano et al., 1996), which codes for frataxin, a protein of unknown function. It is a matter of debate whether X25 is part of the STM7 gene which codes for a 2.7-kb transcript with phosphatidylinositol-4-phosphate 5-kinase activity (Carvajal et al., 1996; Chamberlain et al., 1997; Cossé et al., 1997). The most common mutation in Friedreich’s ataxia is an expanded GAA trinucleotide repeat in intron 1 of the X25 gene (Campuzano et al., 1996; Dürr et al., 1996; Filla et al., 1996). Healthy persons are characterized by 7–22 GAA repetitions, while patients carry 200–900 GAA repeats in this locus. In the original report, homozygosity for expanded alleles was demonstrated in 71 of 74 Friedreich’s ataxia patients (Campuzano et al., 1996). In five additional patients, heterozygous for expanded alleles, point mutations in the X25 gene were demonstrated. Together these findings proved the disease-causing character of mutations in this gene.

Definition of the disease-causing mutations offers the opportunity to examine every patient with ataxia for a possible diagnosis of Friedreich’s ataxia. In this report we provide data on the frequency of expanded GAA repeats in 102 German patients with idiopathic sporadic or autosomal recessive ataxia, and determine meiotic instability of expanded alleles. Furthermore, we provide evidence for locus heterogeneity in Friedreich’s ataxia. Finally, we redefine the phenotypic spectrum of Friedreich’s ataxia in patients with genetically confirmed diagnosis, and study the influence of the repeat length on the phenotype.

Patients and methods

Patients
We examined a series of 102 consecutive patients from 92 unrelated families attending our ataxia clinic because of idiopathic, progressive ataxia and inheritance compatible with autosomal recessive or sporadic disease. All patients were of German origin. Families suggesting autosomal dominant inherited ataxia were excluded from the study.

All patients were examined clinically by the same neurologist (L.S.). According to the diagnostic criteria of Harding (1981), 32 patients presented with typical Friedreich’s ataxia. These patients had early onset at <25 years of age, progressive ataxia of gait and limbs, dysarthria, absent knee and ankle jerks, and extensor plantar responses. In 70 patients the phenotype was atypical for Friedreich’s ataxia. This group included 21 patients with early onset cerebellar ataxia (onset at <25 years of age), 34 patients with idiopathic sporadic cerebellar ataxia of late onset (at >25 years of age) and 15 patients with multiple system atrophy of predominantly cerebellar type. The diagnosis of multiple system atrophy was made in cases with ataxia as an initial or predominant symptom in combination with Parkinsonism and autonomic failure (Wenning et al., 1994).

Furthermore, parents from six families with genetically confirmed Friedreich’s ataxia patients have been studied for the transmission of expanded GAA repeats. The cohort of anonymous healthy blood donors who provided control data for this study has been described in Epplen et al. (1997b).

(GAA)n repeat analysis
Two separate blood samples were taken from each of the patients and were analysed independently to prevent interchange. DNA was extracted from EDTA-treated blood samples following the protocol of Miller et al. (1988). The (GAA)n repeat length in the first intron of the X25 gene was determined by polymerase chain reaction (PCR), utilizing the primers GAA-104F and GAA-629R (Filla et al., 1996). The Perkin Elmer XL long PCR reagents were used under standard conditions suggested by the manufacturer. Amplifications consisted of the following steps: an initial denaturation for 5 min at 94°C and 30 cycles for 1 min at 94°C, 30 s at 60°C and 3 min at 70°C. Fragments were separated on 1.5% agarose gels. For visualization of poorly amplified large PCR fragments, gels were dried and hybridized with a radioactive labelled (TCT)6 probe (Epplen, 1991). The size of expanded alleles was determined by the least squares method by comparing the relative mobilities of the PCR products with those of known DNA size markers according to Schaffer and Sedoroff (1981). The number of (GAA)n repeats was estimated by subtracting the size of the adjacent single copy sequence. The single (GAA)n repetitive sequence may contain mutations in the perfect stretch of GAA trinucleotides. Two independent analyses were performed for each sample. For size determination of non-expanded (GAA)n repeats, an aliquot of the PCR product was digested with the restriction enzyme Sau3AI to yield fragments of 200 + 3n bp (n = number of GAA motifs), separated on 5% polyacrylamide gels, blotted onto nylon membranes (Amersham) and hybridized with a radioactive labelled (GAA)6 oligonucleotide. Comparison with a sequencing ladder allowed estimation of the number of trinucleotides.

Statistics
Allele frequencies, genotype frequencies and heterozygosity rate were obtained as described (Epplen et al., 1995). Comparison of means were calculated using the unpaired t test. Frequencies were compared with the χ2 test using Yate’s correction when appropriate. Regression analysis was
Friedreich's ataxia: genotype and phenotype

Fig. 1 Distribution of \((\text{GAA})_n\) repeat lengths in 36 patients with Friedreich's ataxia.

performed with a linear model for the correlations mentioned in the text.

Results

The frequency of \((\text{GAA})_n\) repeats on normal and Friedreich's ataxia chromosomes

\((\text{GAA})_n\) repeats determined for 353 normal chromosomes ranged from 7 to 29 GAA motifs (heterozygosity rate 0.72). Expanded alleles were also identified resulting in a carrier rate of 1/60 to 1/90 for the German population. Allele frequencies and population genetic aspects are described in Epplen et al. (1997a).

Thirty-six out of 102 ataxia patients (21 females and 15 males) included in this study were homozygous for the \((\text{GAA})_n\) repeat expansion. Two patients with a typical Friedreich's ataxia phenotype carried only one expanded \((\text{GAA})_n\) allele and they are expected to be compound heterozygotes. Compound heterozygotes with an expanded allele on one chromosome and a point mutation on the other chromosome have been described previously (Campuzano et al., 1996). The size of expanded \((\text{GAA})_n\) repeats ranged from 66 to 1360 with ~50% of the expanded alleles between 700 and 900 GAA motifs (Fig. 1).

Transmission instability

Six families with 25 parent/offspring pairs were analysed to evaluate meiotic instability of expanded \((\text{GAA})_n\) repeats. We calculated changes in repeat length between parents and offspring presuming the minimal alterations possible in every mother/father/child trio. Since we did not assign the parental origin of alleles in the offspring transmission instability may be underestimated. The expanded Friedreich's ataxia alleles transmitted from the parents displayed size differences from expansions up to 448 to contractions up to 292 GAA motifs. The \((\text{GAA})_n\) repeat expanded further in 14 of 25 transmissions, contracted in 10 and was stable in one. Expansion of the \((\text{GAA})_n\) repeat was observed more often in female offspring (11 female versus three male), whereas contractions of the \((\text{GAA})_n\) tract were more frequent in males (three female versus seven male). The significance of the differences between maternal and paternal transmissions was only borderline \((P = 0.05, \chi^2\) test). In maternal transmissions, changes in repeat length ranged from –217 to +448 GAA units (mean 59 ± 172, \(n = 15\)) and in male transmissions from –292 to +119 GAA units (mean –67 ± 121, \(n = 10\)) without significant differences \((P = 0.0564,\) unpaired \(t\) test).

Similar size differences were observed when smaller and larger expansions were transmitted and when smaller and larger alleles of individual sibling pairs were compared.

Expansion by >400 repeat units was the exception and was found in one family where the mother carried an intermediate sized allele of 38 GAA repetitions. The \((\text{GAA})_{38}\) allele expanded to \((\text{GAA})_{446}\) in her first son, a carrier of the Friedreich's ataxia mutation, and to \((\text{GAA})_{346}\) in her affected daughter. In the second son, the maternal allele expanded from \((\text{GAA})_{38}\) to only \((\text{GAA})_{66}\) (Fig. 2). Since he displayed ataxia, the \((\text{GAA})_{66}\) motif is the shortest expanded repeat ever observed in a case of Friedreich's ataxia.

Genotype versus phenotype in Friedreich's ataxia

Thirty-two patients presented with the typical Friedreich's ataxia phenotype according to the criteria of Geoffroy et al. (1976) and Harding (1981). Of these, 27 patients were homozygous and two were heterozygous for an expanded \((\text{GAA})_n\) repeat in the X25 gene. Three patients from one family (two siblings and their uncle) had the typical Friedreich's ataxia phenotype but had two repeats in the normal range. Vitamin E levels were normal in all affected family members. Since family history did not indicate consanguinity, point mutations in the X25 gene on both chromosomes are unlikely. These facts point to locus heterogeneity in Friedreich's ataxia.

In 70 patients with a phenotype atypical for Friedreich's ataxia according to the essential diagnostic criteria (Geoffroy...
Clinical features in 36 patients homozygous for an expanded (GAA)$_n$ repeat in the X25 gene were analysed to revise spectrum and characteristics of the Friedreich’s ataxia phenotype (Table 2). Thirty-four genetically confirmed Friedreich’s ataxia patients had disease onset before the age of 25 years. Two of thirty-six (6%) patients had a late onset form, with first symptoms at the age of 26 and 36 years. All Friedreich’s ataxia patients of our series had progressive ataxia of gait and limbs as well as dystarxia. However, onset of dystarxia varied from 0 to 19 years after disease onset. In contrast to the criteria of Harding, the majority of our patients (28/32) experienced dystarxia >5 years after gait disturbance. Knee jerks were preserved in six of 36 (17%) patients and ankle jerks in three, despite the disease duration being 13–26 years. One of these patients even had patella and ankle clonus.

Plantar responses were normal in two of 36 (6%) patients during the whole course of the disease (duration 15 and 38 years). In two further patients we observed loss of extensor plantar responses later in the disease, associated with progressive lower limb weakness and atrophy. Sensory deficits were frequent, affecting predominantly vibration and joint position sense with accentuation in distal lower limbs.

Light touch and temperature appreciation was less affected and pain was well recognized in all patients. One patient with a long-standing disease complained of teasing dysesthesiae. Difficulties in keeping the feet warm were observed in all patients, often causing severe problems in later stages of the disease.

Macro square-wave jerks were the most frequent oculomotor finding (69%) in our series, followed by impaired visual control of the vestibulo-ocular reflex (43%), gaze evoked nystagmus (39%), reduced optokinetic nystagmus (38%), impaired smooth pursuit (32%) and saccadic dysmetria (21%). Loss of rapid eye movements as described in autosomal dominant forms of hereditary ataxias has not been observed. External ophthalmoplegia has rarely been described in Friedreich’s ataxia. We observed partial ptosis in four patients of our series (11%), developing later in the disease when patients were already confined to wheelchairs. Horizontal or vertical gaze palsy, as it is seen in autosomal dominant cerebellar ataxias (Schöls et al., 1996), was not observed. Eleven of 28 Friedreich’s ataxia patients (39%) presented with hearing problems manifesting mostly as problems in speech discrimination. However, three patients had major sensorineural deafness requiring a hearing aid. Loss of visual acuity (two patients, 6%), mild optic atrophy (one patient) and diabetes (two patients, 6%) are well-known symptoms of Friedreich’s ataxia which were rare in the patients of our series. One patient developed torticollis after 13 years of the disease. Torticollis aggravated gait disturbance by hindering visual control of feet movements. However, a relationship between dystonia and the Friedreich’s ataxia mutation remains doubtful. Scoliosis was present in 27 of 32 Friedreich’s ataxia patients (84%). It was present in all patients with onset before 10 years of age and was more severe in early onset cases. Foot deformity was associated with scoliosis in most instances. Pes cavus was present in 29 of 38 Friedreich’s ataxia patients (76%) and two additional patients had flat feet. Intelligence was assessed clinically and in three patients using the Hamburg–Wechsler adult intelligence scale was used. No intellectual deficits were established.

### The Friedreich’s ataxia phenotype: spectrum and characteristics

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Table 2 Frequencies of clinical signs in genetically proven Friedreich’s ataxia patients

<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>Typical FA/FA (n = 29)</th>
<th>Atypical FA/FA (n = 9)</th>
<th>Typical FA/Atypical FA/FA not FA (n = 3)</th>
<th>Atypical FA/FA not FA (n = 61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset before age 25 years*</td>
<td>29/29</td>
<td>7/9</td>
<td>3/3</td>
<td>14/61</td>
</tr>
<tr>
<td>Gait ataxia*</td>
<td>29/29</td>
<td>9/9</td>
<td>3/3</td>
<td>61/61</td>
</tr>
<tr>
<td>Limb ataxia*</td>
<td>29/29</td>
<td>9/9</td>
<td>3/3</td>
<td>61/61</td>
</tr>
<tr>
<td>Dysarthria*</td>
<td>29/29</td>
<td>9/9</td>
<td>3/3</td>
<td>53/61</td>
</tr>
<tr>
<td>Lower limb areflexia*</td>
<td>29/29</td>
<td>3/9</td>
<td>3/3</td>
<td>10/61</td>
</tr>
<tr>
<td>Extensor plantar responses*</td>
<td>29/29</td>
<td>7/9</td>
<td>3/3</td>
<td>25/61</td>
</tr>
<tr>
<td>Decreased vibration sense (&lt;6/8)</td>
<td>25/27</td>
<td>5/9</td>
<td>3/3</td>
<td>33/50</td>
</tr>
<tr>
<td>Optic atrophy</td>
<td>0/12</td>
<td>1/9</td>
<td>0/3</td>
<td>2/9</td>
</tr>
<tr>
<td>Reduced visual acuity</td>
<td>2/23</td>
<td>0/9</td>
<td>0/3</td>
<td>1/15</td>
</tr>
<tr>
<td>Nystagmus</td>
<td>10/27</td>
<td>4/9</td>
<td>3/3</td>
<td>26/42</td>
</tr>
<tr>
<td>Square wave jerks</td>
<td>21/26</td>
<td>3/9</td>
<td>0/3</td>
<td>0/33</td>
</tr>
<tr>
<td>VOR</td>
<td>11/26</td>
<td>4/9</td>
<td>1/2</td>
<td>19/28</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>9/19</td>
<td>2/9</td>
<td>0/3</td>
<td>7/41</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>20/26</td>
<td>5/7</td>
<td>0/3</td>
<td>24/43</td>
</tr>
<tr>
<td>Weakness (foot dorsiflexion)</td>
<td>21/26</td>
<td>3/9</td>
<td>3/3</td>
<td>9/42</td>
</tr>
<tr>
<td>Amyotrophy (lower leg muscles)</td>
<td>13/21</td>
<td>2/9</td>
<td>3/3</td>
<td>10/41</td>
</tr>
<tr>
<td>Incontinence</td>
<td>4/26</td>
<td>0/8</td>
<td>0/3</td>
<td>19/41</td>
</tr>
<tr>
<td>Scoliosis</td>
<td>23/24</td>
<td>4/8</td>
<td>1/3</td>
<td>3/38</td>
</tr>
<tr>
<td>Foot deformity</td>
<td>25/29</td>
<td>6/9</td>
<td>2/3</td>
<td>12/40</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0/26</td>
<td>2/9</td>
<td>0/3</td>
<td>1/21</td>
</tr>
<tr>
<td>T-wave inversion in ECG</td>
<td>23/26</td>
<td>6/9</td>
<td>0/3</td>
<td>0/23</td>
</tr>
<tr>
<td>HCM in echocardiography</td>
<td>19/24</td>
<td>5/8</td>
<td>0/3</td>
<td>0/10</td>
</tr>
<tr>
<td>Abnormalities in ECG or Echo</td>
<td>26/27</td>
<td>6/9</td>
<td>0/3</td>
<td>0/25</td>
</tr>
<tr>
<td>Axonal sensory neuropathy*</td>
<td>19/19</td>
<td>8/8</td>
<td>2/2</td>
<td>15/20</td>
</tr>
<tr>
<td>CMCT prolonged in MEP</td>
<td>7/7</td>
<td>5/5</td>
<td>0/2</td>
<td>1/14</td>
</tr>
<tr>
<td>Cervical cord atrophy on MRI</td>
<td>10/10</td>
<td>6/9</td>
<td>1/2</td>
<td>1/15</td>
</tr>
<tr>
<td>Cerebellar atrophy on MRI</td>
<td>5/10</td>
<td>3/9</td>
<td>2/2</td>
<td>11/15</td>
</tr>
</tbody>
</table>

VOR = vestibulo-ocular reflex; ECG = electrocardiogram; HCM = hypertrophic non-obstructive cardiomyopathy; CMCT = central motor conduction time; MEP = motor evoked potentials; MRI = magnetic resonance imaging; FA = Friedreich’s ataxia. *Essential diagnostic criteria according to Harding (1981).

**Electrophysiological, echocardiographic and MRI findings**

Axonal neuropathy of predominantly sensory type was confirmed in all 27 Friedreich’s ataxia patients who underwent nerve conduction studies. This type of neuropathy was typical but not specific for Friedreich’s ataxia. At least 17 patients, in which Friedreich’s ataxia was not confirmed genetically (two with typical and 15 with atypical Friedreich’s ataxia phenotype), had indistinguishable forms of neuropathy. Furthermore, several patients with autosomal dominant cerebellar ataxias presented with similar forms of polyneuropathy (our unpublished results).

Motor evoked potentials in Friedreich’s ataxia patients demonstrated severely prolonged central motor conduction time (>mean + 4 SDs) in 19 of 24 arms (first dorsal interosseous muscle) and in five of seven legs (tibialis anterior muscle). Additionally, no potential could be recorded after cortical stimulation from 11 tibialis anterior muscles. Peripheral motor conduction time was normal in all but two of 34 extremities. Prolongation of central motor conduction time tended to be less pronounced in Friedreich’s ataxia patients with an atypical phenotype.

In somatosensory evoked potentials following stimulation of tibial as well as median nerves, peripheral potentials were often missing or their latencies were prolonged (Erb’s point 8/12, lumbar spine 6/12). Central sensory conduction was impaired (missing cortical potentials despite preserved peripheral potentials or prolonged inter-peak intervals) in five out of seven median nerve evoked potentials and in eight out of eight tibial nerve evoked potentials.

Visual evoked potentials were abnormal in 17 of 29 Friedreich’s ataxia patients with bilateral changes in all patients. Six patients had prolonged P100 latencies (>123 ms) up to 136 ms with normal amplitudes, seven patients had reduced amplitudes (<4 µV) with normal latencies and one patient had a delayed P100 with reduced amplitudes. Additionally, in three patients no visual evoked potential could be recorded.

Brainstem auditory evoked potentials were normal in six out of 17 recordings, absent in seven cases and with signs of diffuse brainstem alteration in four patients (deformed waves III, IV and V or a prolonged inter-peak interval I–V).

Sympathetic skin responses were variable within the Friedreich’s ataxia group (10 recordings). Five patients had...
high amplitudes corresponding to increased sweating of feet and hands. Five patients had very small or missing potentials at least to the feet, in three cases despite obvious hyperhidrosis.

ECG or echocardiographic equivalents of cardiomyopathy were found in all but four of the 36 patients of our series who underwent cardiac investigations. In the ECG, T-wave inversion was present in 29 out of 35 patients. Hypertrophy of the inter-ventricular septum and the left ventricular wall were found in 24 out of 32 patients. Two patients had normal ECGs despite abnormalities in echocardiography, exertional dyspnoea and palpitations. Four patients had abnormal ECGs but normal echocardiographic findings.

MRI demonstrated atrophy of the upper cervical spinal cord in 16 out of 19 (84%) patients investigated. In contrast, only eight out of 19 (42%) patients had mild cerebellar atrophy.

**Influence of the (GAA)\textsubscript{n} repeat length on the phenotype**
The 36 patients homozygous for expanded (GAA)\textsubscript{n} repeats were analysed for effects of the repeat size on the phenotype (Fig. 3 and Table 3). We named the smaller allele GAA1 and the larger repeat expansion GAA2 in each patient.

Age at onset was significantly influenced by (GAA)\textsubscript{n} repeat size with longer repeats being associated with earlier onset of the disease. The GAA1 group had a strong effect ($r = -0.61$, $P < 0.0001$, $n = 36$) being responsible for ~37% of variability in age at onset (Fig. 3A). The GAA2 group did not significantly influence onset of symptoms ($r = -0.22$, $P = 0.19$, $n = 36$). An inverse relationship with GAA1 repeat length was found for onset of dysarthria ($r = -0.43$, $P < 0.05$), dysphagia ($r = -0.42$, $P = 0.05$) and motor skill impairment ($r = -0.32$, $P < 0.09$) as well as age when chair-bound ($r = -0.53$, $P < 0.01$). Furthermore, repeat length is, in part, responsible for variability of progression, determined as duration of the disease prior to the patients becoming confined to a wheelchair (Fig. 3B). There was a strong correlation between age at onset and the age when patients were chair-bound (Fig. 3C).

To study the influence of repeat length on frequency of Friedreich’s ataxia symptoms, we compared patients with smaller expansions with patients with larger expansions. Analysis was performed with thresholds of (GAA)\textsubscript{n} at <400, <500, <600 and <700 for the smaller allele (GAA1) and for the size of both alleles (GAA1 + GAA2) <1000, <1200, <1400 and <1500. Differences were most pronounced with a threshold of 500 repeats for the GAA1 allele (Table 3). In patients with smaller expansions (GAA1 < 500), onset of the disease, onset of dysarthria and loss of ambulation was significantly delayed compared with those in patients with larger repeats. In the group with smaller alleles, there were more patients with an atypical Friedreich’s ataxia phenotype and fewer patients confined to wheelchairs. Furthermore, extensor plantar responses, areflexia, weakness, amyotrophy and scoliosis were observed more often in association with larger repeats (Table 3). Only four Friedreich’s ataxia patients in our series showed no signs of cardiomyopathy in the ECG.
Table 3 Influence of the size of the smaller expanded repeat (GAA1) on the phenotype in Friedreich’s ataxia

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Number of repeats</th>
<th>GAA1 &lt; 500 (n = 11)</th>
<th>GAA1 &gt; 500 (n = 25)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset (years)</td>
<td>19.5 ± 6.7 13–36</td>
<td>11.8 ± 4.3 5–19</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>19.2 ± 8.4 7–38</td>
<td>19.9 ± 8.9 5–42</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Age when chair-bound (years)</td>
<td>33.8 ± 8.8 20–44</td>
<td>21.3 ± 4.8 15–30</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Progression to wheelchair (years)*</td>
<td>16.0 ± 6.6 5–26</td>
<td>10.0 ± 3.4 5–19</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Age at onset of dysthria (years)</td>
<td>23.7 ± 5.4 14–30</td>
<td>19.5 ± 6.4 10–29</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Age at onset of upper limb ataxia (years)</td>
<td>23.4 ± 3.3 18–28</td>
<td>18.2 ± 7.2 10–39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Typical Friedreich’s ataxia phenotype
- GAA1 and GAA2 represent the numbers of GAA repeats on the smaller and on the larger expanded allele of each patient.
- ‘–’ = absent; ‘+’ = present/mild; ‘+++’ = moderate; ‘++++’ = severe; F = female; M = male.

Intrafamilial variability
Clinical and genetic data were available for eight sibling-pairs (Table 4). Repeat sizes differed between siblings by 0–420 GAA repeats for the smaller alleles (GAA1). In five sibling-pairs with <100 GAA motifs difference, the global clinical impression was that of a similar course of the disease. In contrast, in three sibling-pairs with size differences of 120–420 GAA units, the sibling with the smaller repeat had a less severe course of the disease. The only exception was the unexpected early loss of ambulation in Patient 8–1 (Table 4).
4). Differences in the development of weakness, amyotrophy, scoliosis and age when chair-bound were especially obvious. Surprisingly, differences in age at onset between siblings did not correlate strictly with repeat sizes.

**Discussion**

In this study we determined the distribution of (GAA)$_n$ repeats in normal and expanded alleles of the X25 gene. Precise definition of the normal repeat range is essential for genetic counselling. The range of the (GAA)$_n$ motif in healthy Germans includes up to 29 GAA repetitions. The heterozygosity rate in healthy controls exceeds 70%. Such a high heterozygosity rate allows demonstration of two distinct alleles within the normal range in most individuals not having Friedreich’s ataxia. Consequently, repeated analyses in search for expanded alleles can be avoided where insufficient PCR amplification is suspected, as in allele carriers or compound heterozygotes.

We detected an intermediate (GAA)$_{38}$ allele with high transmission instability expanding to 66, 446 and 486 GAA motifs in the offspring. Furthermore, our data suggest that the intermediate interval is much smaller than has been reported previously (22–200 GAA motifs; Campuzano et al., 1996; Filla et al., 1996). We identified a (GAA)$_{66}$ and a (GAA)$_{>300}$ allele in an ataxia patient whose affected sister carried two alleles with >200 GAA repeats. These circumstances strongly suggest that (GAA)$_{66}$ represents a disease-related expansion. Therefore, the intermediate range is limited from 30 to 66 GAA units or less.

Our data confirm that expanded GAA repeats are the most common mutation causing Friedreich’s ataxia in Germany. The range of expanded repeat sizes in our series is substantially larger than in the original report (Campuzano et al., 1996) including 66–1360 GAA motifs. In our series of German Friedreich’s ataxia patients we found 5% (two of 38) presumed to be compound heterozygotes. Equal frequencies were observed in patients of different ethnic origin (Campuzano et al., 1996; Dürre et al., 1996; Filla et al., 1996).

As demonstrated before in other trinucleotide diseases (Huntington’s disease, dentatorubro-pallidoluysian atrophy and the spinocerebellar ataxias type 1, type 2 and type 3), the expanded repeat causing Friedreich’s ataxia is unstable during transmission. In all of these (Huntington’s disease, dentatorubro-pallidoluysian atrophy and the spinocerebellar ataxias type 1, type 2 and type 3), expanded alleles show a tendency to expand further especially in paternal transmissions causing anticipation (earlier onset and more severe cause of the disease in later generations; for review, see Hammans, 1996). In contrast, in Friedreich’s ataxia we found similar frequencies of expansions and contractions, without significant influence of the parental sex. However, our data refer to a small sample and should be controlled in larger cohorts.

Expanded alleles were lacking in a family with the typical Friedreich’s ataxia phenotype, including two siblings and their uncle. Hereditary vitamin E deficiency which had been described to imitate the Friedreich’s ataxia phenotype (Ben Hamida et al., 1993; Ouahchi et al., 1995) was excluded. The constellation with affected individuals in two generations makes it extremely unlikely that affected individuals in this family are homozygous for point mutations in the X25 gene. Therefore, our data suggest locus heterogeneity in Friedreich’s ataxia. Recently, a Spanish kindred with similar clinical features has been reported (Smeyers et al., 1996). The Friedreich’s ataxia locus on chromosome 9 was excluded by linkage analysis. Linkage analysis was performed to exclude the X25 gene formally in the family described here. Remarkably, neither family has signs of cardiomyopathy; this may be a common feature in this second type of Friedreich’s ataxia.

Diagnostic criteria and the phenotypic spectrum of Friedreich’s ataxia have been a matter of debate since the original description more than a century ago. The finding of the molecular cause of Friedreich’s ataxia promises to settle this issue. Here we investigated a large series of idiopathic degenerative ataxias with a family history compatible with autosomal recessive or sporadic disease in order to revise the phenotype according to genetic data. Our data confirm that the phenotypic spectrum of Friedreich’s ataxia is much broader than considered before. Nine out of 36 patients homozygous for the (GAA)$_n$ repeat expansion (25%) presented with a phenotype atypical for Friedreich’s ataxia according to the criteria of Geoffroy et al. (1976) and Harding (1981). A similar percentage of atypical cases has been encountered by Dürre et al. (1996). According to our data the only obligatory symptom of Friedreich’s ataxia is progressive ataxia of gait and limbs (Table 2). It is regularly accompanied by dysarthria, axonal (predominantly sensory) neuropathy and prolonged central motor conduction time in motor evoked potentials. At least dysarthria may develop as late as 15 years after disease onset. Early onset (before 25 years of age), extensor plantar responses, areflexia and reduced vibration or position sense of the lower limbs are frequent but by no means obligatory symptoms for diagnosis of the disease. On the other hand, severe spasticity cannot exclude Friedreich’s ataxia on clinical grounds.

We found homozygotes for (GAA)$_n$ repeat expansions in patients previously classified as early onset cerebellar ataxia as well as in patients considered to suffer from idiopathic sporadic cerebellar ataxia. Our data suggest the usefulness of the Friedreich’s ataxia test for all patients with idiopathic ataxia compatible with autosomal recessive or sporadic disease. Currently, the diagnosis of Friedreich’s ataxia can be excluded clinically only in patients with multiple system atrophy (criteria of Wenning et al., 1994) and in patients with severe olivopontocerebellar atrophy on CT or MRI.

Cardiomyopathy of the hypertrophic non-obstructive type was the only sign that was exclusively found in genetically confirmed Friedreich’s ataxia patients of our series. Cardiomyopathy was not present in a family with a typical
Friedreich’s ataxia phenotype but their (GAA)n repeats were in the normal range. Therefore, cardiomyopathy appears to be specific for Friedreich’s ataxia. However, cardiomyopathy is not obligatory for diagnosis, since four of our 36 patients homozygous for expanded (GAA)n repeats showed no cardiac symptoms, ECG changes or echocardiographic abnormalities. Concerning ECG in Friedreich’s ataxia, it is noteworthy that ECG changes, but not echocardiographic abnormalities, were inconsistent in repeated recordings in a limited number of patients. The pathogenesis of hypertrophic non-obstructive cardiomyopathy in Friedreich’s ataxia is unclear, but seems to be directly related to repeat expansions since the X25 gene is highly expressed in heart tissue (Campuzano et al., 1996). Recent studies suggest that at least some forms of cardiomyopathy may be caused by apoptosis in the heart (Colucci et al., 1996).

The phenotype of Friedreich’s ataxia is partially determined by the size of (GAA)n expansions. The repeat length influences age at onset, onset of dysarthria, onset of motor skill impairment and progression rate. The (GAA)n block in the smaller repeat range appears to be more decisive for the phenotype than the larger allele. Since the Friedreich’s ataxia mutation is supposed to cause a loss of function (Campuzano et al., 1996; Dürr et al., 1996), the correlation between repeat length and severity suggests that smaller expansions do not totally abolish processing of the frataxin RNA. Clinical features were significantly different in Friedreich’s ataxia patients with <500 GAA units compared with those with longer GAA blocks. Since similar differences were not observed at thresholds of 400, 600 or 700 GAA, our data suggest, that, beyond a threshold of ~500 GAA repeats, the residual expression of frataxin is too low to influence the clinical presentation. One may hypothesize that very long purine stretches (>500 GAA units) in intron 1 of the X25 gene prevents the processing of hnRNA (heterogenous nuclear RNA) into mature mRNA (messenger RNA).

The extensive meiotic instability of expanded (GAA)n blocks suggests that the correlation between repeat sizes in peripheral blood and disease phenotype have to be interpreted with caution. Repeat length may differ in leukocytes from tissues affected by the disease due to somatic mosaics. This may explain, in part, phenotypic variability observed independently of the repeat length. Comparison of repeat lengths in different tissues of Friedreich’s ataxia patients is required to approach this question. The finding of disease-causing mutations is of great help in the diagnostic and genetic counselling of patients and families with Friedreich’s ataxia. Despite clear influences of the repeat size on the phenotype, there are substantial variations even in patients with similar repeat lengths, so that the size of the expansion cannot be used to predict the individual course of the disease in clinical or genetic counselling. Molecular genetics sheds light on the large phenotypic variability of the disease, which explains the severe diagnostic problems existing before the disease-causing mutations had been identified. It becomes obvious that the criteria elaborated by Geoffroy et al. (1976) and Harding (1981) can no longer be regarded as essential for the diagnosis of Friedreich’s ataxia but they are still the best diagnostic tool from the clinical point of view. Finding the Friedreich’s ataxia genotype in patients previously classified as having early onset cerebellar ataxia and idiopathic sporadic cerebellar ataxia makes genetic analysis indispensable in excluding the diagnosis of Friedreich’s ataxia. On the other hand, the increasing evidence of locus heterogeneity in Friedreich’s ataxia requires a genetic confirmation of the diagnosis. Furthermore, molecular genetics provides a potential tool to elucidate the pathogenesis of Friedreich’s ataxia, and it raises hopes for new therapeutic strategies in the future.

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