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Typical Friedreich's Ataxia without GAA Expansions and GAA Epansions Wthout Typical Friedreich's Ataxia

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ORIGINAL COMMUNICATION

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Abstract We clinically assessed and performed polymerase chain reaction analysis for the GAA trinucleotide repeat expansion in 103 patients from 73 families in Ireland, with a prior clinical diagnosis of Friedreich's ataxia (FA) or an unclassified progressive ataxic syndrome. The patients were classified as "typical" or "atypical" FA according to Harding's mandatory clinical diagnostic criteria. All patients underwent blood glucose analysis, and electrocardiography and echocardiography was performed in 99 and 101 patients, respectively. Mutation screening for expanded CAG trinucleotide repeats, associated with spinocerebellar ataxia (SCA) 1, 2, 3 and 6 was performed in 86 patients overall, including all GAA negative patients. Forty-nine of 56 typical patients and 13 of 47 atypical patients were either homozygous or heterozygous for the GAA expansion. Seven patients with a typical FA phenotype were negative for the GAA expansion. Although one of these patients had vitamin E deficiency, and two had raised α-fetoprotein levels, three other GAA negative patients with a typical FA phenotype had no other identifiable cause for their ataxia, once again raising the possibility of locus heterogeneity in FA. It is also possible that these patients have two point mutations in the X25 gene, or that they have another ataxic syndrome mimicking the FA phenotype. Two families who were homozygous for the GAA expansion exhibited intrafamilial phenotypic variability. Only one GAA negative patient had the SCA 3 mutation, and this was the only patient in the study with a possible autosomal dominant inheritance pattern. In the homozygous GAA population typical patients had significantly more repeats on the smaller allele than atypical patients, and there was an inverse relationship between the number of repeats on the smaller allele and the age at presentation. There was also an inverse relationship between the repeat size on both the larger and the smaller of the two alleles and the age at becoming wheelchair bound. There was no significant relationship between repeat size and the other indices of disease severity, including the presence or absence of diabetes or cardiomyopathy. This is the first large study of an Irish population with progressive ataxia that has shown a similar phenotype/genotype relationship to studies of FA in other European and non-European populations. The relatively low sensitivity and specificity of Harding's clinical diagnostic criteria must be appreciated when clinically assessing patients with a progressive ataxic syndrome. Although molecular genetic analysis now plays an essential role in diagnosis and classification, patients with a typical FA phenotype without any identifiable cause for their ataxia exist.

Key words Friedreich's ataxia · GAA trinucleotide repeat · Mandatory diagnostic criteria · Intrafamilial phenotypic variability · Sensitivity and specificity

Introduction

In 1981, Harding [1] studied 115 patients with Friedreich's ataxia (FA) and proposed what are commonly known as "Harding's" criteria for the clinical diagnosis of this autosomal recessive syndrome [1]. The mandatory clinical diagnostic criteria which emerged from this landmark study include: (a) an age at onset below 25 and definitely below 27 years, (b) ataxia of all four limbs, (c) ataxia of gait, (d) lower limb areflexia and (e) presumed or proven autosomal recessive inheritance. The frequency of other findings such as dysarthria, extensor plantar responses, abnormal lower limb vibration sensation and joint position sensation varied depending on the duration of the disease. Although considered important "secondary diagnostic criteria" for entry into Harding's study, dysarthria was noted in only 60 % of patients 5 years after disease onset, and extensor plantar responses in only 88.7 % of patients overall.

Subsequent to the identification of a GAA trinucleotide repeat expansion in the first intron of the X25 gene on chromosome 9q13–21.1 [2], interest in the relationship between the genotype and the severity of the phenotype of FA has been kindled. Several studies have established that normal chromosomes contain 7–29 GAA repeats, whereas FA chromosomes carry between 66–1700 [2–7]. Campuzano et al. [2] studied 79 patients with "typical" FA (criteria not specified in the report) and found that 71 were homozygous and 8 patients were heterozygous for the GAA expansion.

In light of this genetic information, the sensitivity and specificity of the clinical diagnostic criteria of Harding [1] and Geoffrey et al. [8] have come under scrutiny. To assess the relationship between the phenotype and genotype of FA, we performed an observational analytical study on 103 patients from 73 families in Ireland, with a prior clinical diagnosis of FA or an unclassified progressive ataxic syndrome. We also assessed the sensitivity and specificity of Harding's diagnostic criteria for identifying patients who were heterozygous or homozygous for the GAA expansion.

Patients and methods

Patients were included in the study if they had a prior clinical diagnosis of FA or an unclassified progressive ataxic syndrome established by their neurologist or consultant physician. The age at disease onset was not specified as an inclusion or exclusion criterion. The vast majority of patients were referred for assessment by the Friedreich's Ataxia Society of Ireland, with a smaller proportion referred by consultant neurologists in the Republic of Ireland. A detailed history, including family history, was taken, and each patient was assessed clinically by one of the authors [D. J. H. M. (n=82), R. P. M. (n=16) or M.K (n=5)] using a standardised protocol based on the Kurtzke Functional Systems Neurological Examination [9]. One patient was Chinese, the genealogy of one patient was unknown, and one patient was the offspring of a Romanian mother and an Irish father. The parents of the remaining 100 patients were Irish. "Typical" patients had to fulfil all of Harding's mandatory diagnostic criteria, including a pattern of inheritance consistent with an autosomal recessive disorder. Due to the absence of dysarthria and extensor plantar responses in a significant proportion of patients in Harding's original series, these criteria were not considered to be "mandatory" for the diagnosis. The remaining patients were classified as having "atypical" FA, as were those with ophthalmoparesis or ophthalmoplegia, because horizontal or vertical gaze palsy and external ophthalmoplegia were not included in classical descriptions of the disease at the outset of the study.

The indices of severity used to assess the relationship between the phenotype and genotype of the disease included: age at onset of the disease; duration to assistance to walk; duration to becoming wheelchair-bound; age at becoming wheelchair-bound; and presence or absence of diabetes, cardiomyopathy or retained deep tendon reflexes. Age at onset was defined as the age at which the first neurological symptom was noted, and disease duration refers to the duration between the age at onset and the age at examination. Duration to assistance to walk refers to the duration in years from the onset of the disorder until requiring permanent assistance to walk (permanently requiring either a stick or walking aid or the assistance of another person to walk). Age at becoming wheelchair-bound was defined as the age when the patient was permanently dependent on a wheelchair, and duration to becoming wheelchair-bound refers to the difference in years between the age at onset and the age at becoming wheelchair-bound.

Random blood glucose was measured in all patients and, if elevated, a fasting and 2-h post-prandial assay was performed. Patients were considered to be diabetic if they had a definite prior diagnosis or a fasting blood glucose greater than 6.7 mmol/l. Electrocardiography (ECG) and two-dimensional transthoracic echocardiography (ECHO) was performed in 99 and 101 patients, respectively. Current ECGs were unavailable on one sibling pair, and the abnormalities noted on their recordings in 1982 were therefore used, but otherwise all ECGs were performed at the time of assessment. Each ECG was classified as normal or abnormal by a consultant cardiologist (D. P. M.), who was blinded to the clinical categorisation and molecular genetic status of the patients. The ECHOs were evaluated by the same cardiologist for changes of concentric left ventricular hypertrophy, non-concentric left ventricular hypertrophy, asymmetrical septal hypertrophy, dilated cardiomyopathy, valvular defects and other detailed indices of cardiac function. One patient with a possible autosomal dominant inheritance pattern subsequently had a transoesophageal echocardiogram to confirm the results of the transthoracic ECHO.

Polymerase chain reaction (PCR) analysis for the GAA trinucleotide repeat expansion was performed in all patients and screening for spinocerebellar ataxia (SCA) 1, 2, 3 and 6 mutations was performed in 86 patients, including all those who were GAA negative. Genomic DNA was extracted from peripheral blood lymphocytes using a standard salting out procedure [10]. To estimate the size of the GAA repeat in intron 1 of the frataxin (X25) gene, PCR was performed on DNA samples using the following primers: GAA–104F: 5′-GGCTTAAACTTCCCACACGTGTT and GAA–629R: AGGACCATCATGGCCAC ACTT [4] with the Boehringer-Mannheim Long Template PCR system. PCR conditions were 94°C for 2 min, 17 cycles at 94°C for 10 s, 68°C for 2 min 30 s followed by 20 cycles of 94°C for 10 s, 68°C for 2 min 30 s with the addition of 20 s to the extension time per cycle, and a final extension at 68° for 10 min. This generates a PCR product of (500+3n) base pairs, where n is the number of GAA repeats. The size of the PCR product was estimated using appropriate size standards (Gibco BRL) and the Phoretix one-dimensional programme (Phoretix International). Screening for SCA 1, 2, 3 and 6 mutations was performed by published methods [11–14].

In typical patients who were negative for the GAA expansion,

levels of vitamin E [15, 16] and hexosaminidase A [17] were measured to exclude deficiency states that can produce an ataxic syndrome resembling FA. We also measured α-fetoprotein levels and performed preliminary cytogenetic analysis to look for consistent numerical or structural abnormalities in G-banded metaphases identified in ataxia telangiectasia. In addition, we performed lipoprotein electrophoresis to exclude abetalipoproteinaemia or hypobetalipoproteinaemia, mutation screening for hereditary motor and sensory neuropathy (HMSN) I, and measured the amplitude of the sural sensory nerve action potential (SNAP) and the median nerve motor conduction velocity (MCV) [1].

Simple linear regression was used to explore the relationship between selected continuous variables, and the resulting regression models are illustrated in the text where appropriate. The *t* test was used for comparison of group means, and cross-tabulation methods were used for screening of patient characteristics.

Results

Within our study population of 103 patients, 67 had a positive family history of an autosomal recessive ataxic syndrome, 35 had a sporadic ataxic syndrome with no other family members affected, and one patient had an ataxic syndrome of presumed autosomal dominant inheritance. The parents of three affected siblings were possibly fifth or sixth cousins, and one sib pair had parents who were third cousins; otherwise, a history of consanguinity was not elicited.

There were 56 men and 47 women, with a mean age at onset of 11.2 years (range: 1–54 years) and a mean age at examination of 31.2 years (range: 11.5–71 years). The oldest patient (age at onset 54 years and age at examination 71 years) was found to have a history of excessive alcohol consumption on assessment at our clinic and was considered to have an alcohol-related ataxic syndrome.

Categorisation of the study population according to phenotype revealed 56 typical and 47 atypical patients. The results of the molecular genetic analysis on these two groups are summarised in Table 1.

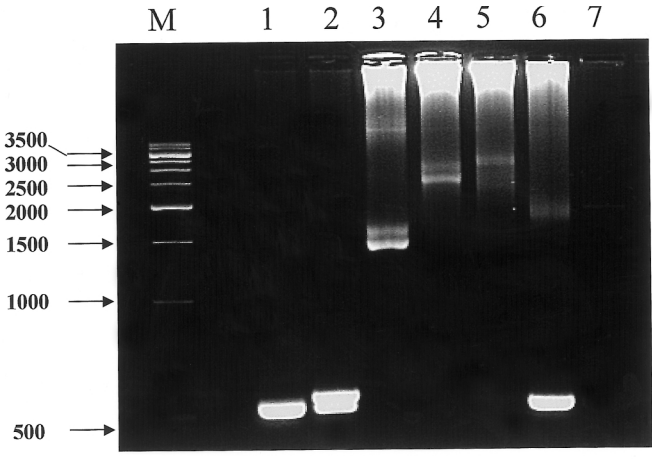
Within the homozygous population (those with expanded repeats on each allele; *n*=58) the mean GAA repeat length for the larger of the two alleles (GAA 1) was 885 (range 534–1200) and for the smaller of the two alleles (GAA 2) was 762 (range 333–1053). A sample agarose gel obtained from the patients in the study is shown in Fig. 1.

The frequency of the predominant clinical characteristics of the phenotype/genotype subgroups are summarised in Table 2.

Table 1 Phenotype vs. molecular genetic status: absolute number of patients in each group (*GAA HOMO* GAA expansions identified on both alleles, GAA *hetero* GAA expansion identified on one allele only, GAA *Neg* no GAA expansion identified)

Phenotype	Total	GAA Homo	GAA Hetero	GAA Neg
Typical FA	56	47	2	7
Atypical FA	47	11	2	34

Fig. 1 1.5 % agarose gel showing PCR amplification products from X25 intron 1 GAA repeat expansions. *M* Size marker (sizes shown in base pairs).



Typical homozygotes

Within the typical homozygous subgroup (*n*=47), ataxia of gait was the presenting symptom in 80.9 % of patients and was present in all patients on examination. The incidence of dysarthria, extensor plantar responses and abnormal lower limb joint position sensation was similar to that observed in Harding's series, whereas scoliosis (91.5 %), abnormal lower limb vibration sensation (89.4 %), pes cavus (80.9 %) and hearing abnormalities (22.4 %) were more frequent. Nystagmus was noted in 80.9 % of patients in this subgroup compared to only 20 % of patients in Harding's series. However, the nystagmus was only mild, often not sustained, and gaze-evoked on horizontal or vertical gaze in 45 %, moderate in 30 % and severe in 6 % of patients on examination. If one excludes patients with only mild nystagmus which may not have been pathological, the frequency of this finding falls to 36 % which is similar to the incidence of nystagmus reported in other recent studies in the literature [3, 7]. Only moderate or severe nystagmus was considered when calculating the frequency of this finding in the other phenotype/genotype subgroups. An unexpectedly large percentage of patients had titubation (45.7 %) and abnormal lower limb superficial sensation (50 %). The latter was defined as reduced or increased appreciation of light touch, pin-prick or temperature sensation. None of these patients were diabetic, and peripheral cyanosis was identified in only four of the 23. Diabetes was less common (6.5 %) and optic atrophy or suspicious optic disc pallor was noted in only 6.4 % of typical homozygotes in comparison to 30.4 % of Harding's patients.

All patients in this category had abnormalities on their ECG, and ECHO was abnormal in 32 of 46 typical homozygotes (69.6 %). Concentric left ventricular hypertro-

Table 2 Frequencies of clinical characteristics in the different phenotype/genotype subgroups (*GAA HOMO* GAA expansions identified on both alleles, GAA *hetero* GAA expansion identified on one allele only, GAA *Neg* no GAA expansion identified, *SS* soft touch and/or pin prick and/or temperature sensation)

Sign	Typical			Atypical		
	GAA Homo (*n*=47)	GAA Hetero (*n*=2)	GAA Neg (*n*=7)	GAA Homo (*n*=11)	GAA Hetero (*n*=2)	GAA Neg (*n*=34)
Optic atrophy	3/47	0/2	0/7	2/11	0/2	0/34
Suspicious optic disc pallor	0/47	0/2	3/7	3/11	1/2	7/34
Nystagmus	17/47	1/2	3/7	6/11	0/2	12/34
Broken-up pursuit eye movements	27/46	1/2	3/7	3/11	0/2	8/34
Broken-up saccades	18/45	1/2	3/7	5/11	0/2	4/33
Dysarthria	43/47	2/2	5/7	11/11	1/2	24/34
Head tremor	21/46	0/2	2/7	2/11	0/2	7/34
Gait Ataxia (or not testable due to weakness)	47/47	2/2	7/7	11/11	2/2	30/33
Pyramidal weakness						
Upper limbs	23/47	1/2	3/7	6/11	0/2	10/34
Lower limbs	40/47	2/2	6/7	2/11	1/2	15/34
Distal weakness						
Upper limbs	3/47	1/2	0/7	1/11	1/2	3/34
Lower limbs	8/46	0/2	4/7	2/11	1/2	7/34
Areflexia						
Upper limbs	42/47	2/2	6/7	5/11	0/2	8/34
Lower limbs	47/47	2/2	7/7	3/11	0/2	8/34
Extensor plantar response	43/47	1/2	3/7	11/11	1/2	14/34
Abnormal vibration sensation						
Upper limbs	21/47	2/2	5/7	2/10	2/2	8/32
Lower limbs	42/47	2/2	6/7	9/10	2/2	13/32
Abnormal joint position sensation						
Upper limbs	25/45	1/2	4/7	3/10	0/2	2/33
Lower limbs	38/45	1/2	6/7	9/10	1/2	10/32
Abnormal SS						
Upper limbs	13/47	0/2	0/7	1/10	1/2	3/33
Lower limbs	23/46	1/2	2/7	8/10	2/2	8/33
Distal amyotrophy						
Upper limbs	15/47	0/2	2/7	2/11	1/2	5/34
Lower limbs	31/47	0/2	4/7	8/11	1/2	9/34
Scoliosis	43/47	1/2	1/7	6/11	1/2	10/34
Pes Cavus	38/47	1/2	5/7	8/11	0/2	11/34
Diabetes	4/47	0/2	0/7	0/11	0/2	2/34
Abnormal ECG	45/45	0/1	3/6	11/11	2/2	13/34
Abnormal ECHO	32/46	0/2	0/6	6/11	1/2	5/34

phy was the commonest abnormality on ECHO (37%), followed by asymmetrical septal hypertrophy (19.6%) and non-concentric left ventricular hypertrophy (13%). Dilated cardiomyopathy was present in one patient (2.2%) who also had features of concentric left ventricular hypertrophy.

Typical heterozygotes

The two typical heterozygous patients are presumed compound heterozygotes, but to date we have identified a GAA expansion on only one allele in each patient, estimated at 222 and 852 repeats, respectively. They are presumed to carry a point mutation on the other allele, but mutation screening for the I154F and G130V mutations and single-strand conformation analysis of all five exons have so far been negative (data not shown).

Typical negative patients

The clinical details of the seven typical patients who had GAA repeats within the normal range are outlined in Table 3. By definition these patients fulfilled all of Harding's mandatory diagnostic criteria [1], but it is noteworthy that three of these patients were still not wheelchair-bound 40, 25 and 22 years after disease onset. One patient presented with titubation, and another with "leg spasms", which are uncommon presenting symptoms of typical FA [1]. Patient 1 had choreiform movements of the hands and face, which has been previously described in FA [18], and is still ambulatory with assistance at 48 years of age. Nerve conduction studies revealed a severe sensory axonal neuropathy with a median nerve MCV of 42 m/s. Patient 6 also had choreiform movements of the hands and face with an absent sural SNAP and a markedly reduced median nerve MCV of 32.3 m/s. He has a 25-year-old atypical GAA negative sister, who was classified as atypical purely due to a left lateral rectus palsy. Her disease course was more rapid, and she became wheelchair-bound at age 19; her sural SNAP was absent also with a median nerve MCV of 45.4 m/s. These three patients had elevated α-fetoprotein levels, but vitamin E, hexosaminidase A levels and lipoprotein electrophoresis were normal, and preliminary cytogenetics for ataxia telangiectasia, and mutation screening for HMSN I was negative. None of these patients had any apparent telangiectasia, but it is possible that they have ataxia telangiectasia mimicking the typical FA phenotype in two; further screening for the genetic defect associated with ataxia telangiectasia is in progress. It is of interest that two of these patients had peripheral nerve conduction studies compatible with typical FA [1]. Patient 3 had a talipes equinus deformity of both feet and was noted to be ataxic while walking at 2 years of age. His nerve conduction studies were compatible with the predominantly sensory axonal neuropathy described in FA. His sister also has an early-onset ataxic syndrome, but in addition she has a history of developmental delay, learning disability, short stature, pigmentary retinopathy and a short PR interval on ECG and is negative for the GAA trinucleotide repeat expansion. Patient 5 had normal cardiac findings but had nerve conduction studies consistent with typical FA also. Patient 7 has a 59-year-old brother in the atypical GAA negative category who has a more slowly progressive disease course with retained deep tendon reflexes, and he did not become wheelchair-bound until 38 years after disease onset. Patient 7 had normal nerve conduction studies and both siblings had reduced vitamin E levels with normal biochemical and genetic screening tests otherwise. Screening for a mutation in the α-tocopherol transfer protein on chromosome 8q13, which may cause autosomal recessive ataxia with vitamin E deficiency [19], is underway in this sib pair. Patient 2 refused to have further tests performed to look for an alternative cause for his ataxia. Four patients in this subgroup had abnormalities on their ECG compatible with changes described in FA; one of these patients had raised α-fetoprotein levels and another had reduced vitamin E levels. None had evidence of cardiomyopathy on ECHO, as described in FA to date [20, 21].

Atypical negative patients

Within the atypical subgroup the majority (34/47) did not have expanded GAA repeats. One of these patients, who originated from Hong Kong, had an expanded CAG repeat in the SCA 3 gene on chromosome 14q32.1. He first developed left lower limb ataxia and weakness at 39 years of age, with mild dysarthria a year later, and was the only patient in the study with a history consistent with an autosomal dominant ataxic syndrome. His mother developed ataxia and dysarthria at 40 years of age, and his sister had a history of "unsteadiness" beginning at 35 years of age. He did not have linguofacial fasciculations, dystonia or extrapyramidal features at the time of examination. None of the remaining GAA negative patients exhibited the SCA 1, 2, 3 or 6 mutations. One atypical negative female patient had a low vitamin E level (0.7 mg/100 ml; normal range 0.9–1.6), in addition to cerebellar vermian atrophy and mild atrophy of the mid-brain and pons on magnetic resonance imaging; she had subjectively stabilised on 400 mg oral vitamin E daily. Vitamin E levels are not available on any other atypical negative patients, as this was not part of our study protocol.

Ataxia of gait was also the commonest presenting symptom (61.8 %) in this subgroup. These patients were classified as atypical for the following reasons: 76.5 % had retained deep tendon reflexes in the legs, 14.7 % had an age at onset greater than 25 years, 14.7 % did not have ataxia of all four limbs, and gait ataxia was absent in 8.8 % of patients on examination. Dysarthria (70.6 %), extensor plantar responses (41.2 %), pes cavus (32.4 %) and scoliosis (29.4 %) occurred less frequently than in Harding's original series, whereas nystagmus was more commonly observed (35.3 %). Abnormalities compatible with those reported in FA were seen on the ECG in 38.2 % and concentric left ventricular hypertrophy on ECHO was reported in 14.7 %.

Atypical homozygotes

11 homozygous patients were excluded from the typical category for failing to fulfil all of Harding's mandatory diagnostic criteria. Seven had retained deep tendon reflexes alone; one had a late age at onset (28 years) and exhibited 534 and 333 repeats on GAA 1 and GAA 2 respectively; one patient had both retained reflexes and an age at onset at 29 years, and exhibited 662 repeats on GAA 1 and GAA 2. Two patients in this group had abnormalities of eye movement (one had moderate abduction weakness bilater-

Table 3 Typical/GAA negative (*SS* soft touch and/or pin prick and/or temperature sensation, *AOG* = ataxia of gait z, *ULA* = upper limb ataxia, *NIA* = not applicable)

Sign	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Gender	Male	Male	Male	Male	Male	Male	Male
Age at presentation	8 years	1 year	2 years	17 years	2 years	6 years	8 years
Presenting symptom	Titubation	AOG	AOG	Leg spasms	AOG	AOG	ULA
Disease duration	40 years	63 years	22 years	25 years	11 years	30 years	40 years
Duration to assistance to walk	32 years	15 years	16 years	24 years	N/A	21 years	12 years
Duration to being wheelchair-bound	N/A	26 years	N/A	N/A	N/A	23 years	12 years
Scoliosis	No	No	Yes	No	No	No	Yes
Pes cavus	Yes R, L	Yes R	No	Yes R, L	No	Yes R, L	Yes R, L
High medial arches	N/A	N/A	Yes R, L	N/A	Yes R, L	N/A	N/A
Suspicious optic disc pallor	Yes	Yes	No	No	Yes	No	No
Choreo-athetosis	Hands, face	No	No	No	No	Hands, face	No
Titubation	Yes	No	No	No	No	No	Yes
Nystagmus	Yes	Yes	No	Yes	No	Yes	Yes
Dysarthria	Moderate	Severe	No	No	Mild	Mild	Severe
Limb tone	Hypotonic	Hypotonic	Spastic	Hypotonic	Normal	Normal	Hypotonic
Distal amyotrophy	Legs	Arms, legs	Legs	No	No	Arms, legs	No
Pyramidal weakness	Moderate legs	Severe arms, moderate legs	No	Mild right arm, moderate legs	Moderate legs	Moderate legs	Mild arms, moderate legs
Distal weakness	No	Severe legs	Severe legs	No	No	Severe legs	Severe legs
Areflexia arms	Yes	Yes	Yes	Yes	Yes	Yes	Normal biceps, triceps
Areflexia legs	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Plantar response	Equivocal	Equivocal	Equivocal/up	Up	Absent	Equivocal	Up
Ataxia arms	Moderate	Severe	Mild	Mild	Mild	Moderate	Moderate
Ataxia legs	Moderate	Wheelchair bound	Moderate	Moderate	Moderate	Severe	Wheelchair bound
Ataxia of gait	Severe	Severe	Severe	Moderate	Moderate	Severe	Severe
Abnormal vibration sensation							
Upper limbs	No	Yes	Yes	Yes	Yes	Yes	No
Lower limbs	Yes	No	Yes	Yes	Yes	Yes	Yes
Abnormal joint position sensation							
Upper limbs	No	Yes	Yes	Yes	Yes	No	No
Lower limbs	No	Yes	Yes	Yes	Yes	Yes	Yes
Abnormal SS. lower limbs	No	No	No	Yes	No	Yes	No
ECG	Normal	N/A	Right axis deviation	Borderline left atrial hypertrophy	Normal	Right axis deviation	Short PR interval
ECHO	Normal	N/A	Normal	Mildly impaired LV diastolic function	Normal	Normal	Normal

ally, and one had mild limitation of upgaze bilaterally), but were otherwise typical in all respects. This latter patient, with restricted upgaze, exhibited 1144 and 869 repeats on GAA 1 and GAA 2 respectively, and had a typical brother with 924 and 731 GAA repeats. Two brothers in this subgroup, aged 25 and 27 years, had retained upper and lower limb reflexes, and PCR testing revealed that one had 571 and the other 626 repeats on GAA 2. They had a typical homozygous areflexic brother (aged 34 years) who had 879 repeats on GAA 2, confirming previous reports of intrafamilial phenotypic variability in FA [22].

Atypical heterozygotes

The two patients in this category are interesting. One patient has had a very slowly progressive disease course beginning at 10 years of age, with minimal ataxia and profound lower limb spasticity. He had 954 expanded GAA repeats on one allele and a G130V mutation on the other. The second patient is the father of two typical homozygous affected sons, and some of the details of this pedigree with possible pseudo-dominant inheritance have been outlined previously [23].

We were able to use microsatellite markers in and around the FA locus to investigate inheritance in five pairs of clinically affected siblings (including the three typical negative patients with atypical siblings), and one set of four atypical GAA negative female siblings. In two sib pairs with atypical FA, involvement of the FA locus was excluded. In the three sib pairs with one typical and one atypical patient in each, involvement of the FA locus was excluded in one pair (patient 3). However, alleles were shared in the two sib pairs with raised α-fetoprotein levels and vitamin E deficiency respectively. In the sibship of four affected sisters, two sibs shared alleles at all loci tested, whereas involvement of the FA locus was excluded in the other two (data not shown).

Phenotype/genotype relationship in the homozygous population

Within the homozygous population, there was an inverse relationship between the repeat size on GAA 2 and the age at onset of the disorder (P=0.03). There was also a significant inverse relationship between repeat size on both GAA 1 (P=0.0009) and GAA 2 (P=0.01) and the age at becoming wheelchair-bound. Otherwise, there was no significant relationship between the GAA repeat size on either allele and the other indices of disease severity (Table 4).

Typical homozygous patients as a group had significantly more repeats on GAA 2 than atypical patients (793±19.7 vs. 642±38.6, P=0.001). Because all homozygotes had abnormalities on their ECG, it was not possible to compare the mean repeat size between patients who did and those who did not have features of cardiomyopathy on the ECG. There was no significant difference in the mean repeat length on GAA 1 or GAA 2 between patients who did and those who did not have cardiomyopathy overall on ECHO. However, subgroup analysis revealed that 6 patients with non-concentric left ventricular hypertrophy on ECHO had significantly more repeats on GAA 1 than the 51 patients without non-concentric left ventricular hypertrophy (985 vs. 874, P=0.04; two-sample t test assuming unequal variance). Due to the unbalanced nature of these samples this statistically significant result may not be truly representative of the FA population as a whole, and its clinical significance is debatable. There was no significant difference in repeat size between patients who did and those who did not have concentric left ventricular hypertrophy, asymmetrical septal hypertrophy or dilated cardiomyopathy.

Comparison of mean GAA repeat lengths on the two alleles between patients with and without diabetes did not reveal any significant differences between the subgroups (GAA 1, P=0.6; GAA 2, P=0.26: two-sample t test, assuming unequal variance). However, the low incidence of diabetes in this study (4 diabetic vs. 54 non-diabetic homozygotes) did not facilitate a clinically representative result either. Within the homozygous group there was no significant difference in the number of expanded repeats between patients with and without areflexia (GAA 1, P=0.55; GAA 2, P=0.21).

The sensitivity of Harding's clinical diagnostic criteria for identifying patients who were GAA positive was 79 % (49/62). The specificity of these criteria for identifying patients who were GAA negative was also relatively low at 83 % (34/41).

Table 4 Regression relationship between the repeat size on GAA 1 and GAA 2 and the indices of severity of FA (*AP* age at onset of the disease, *AWB* age at becoming wheelchair-bound, *DTA* duration to assistance to walk, *DTW* duration to becoming wheelchair-bound)

	Slope of line	t	P	R^2
GAA 1 vs. AP (n=56)	–4.920	–1.38	0.17	0.034
GAA 2 vs. AP (n=56)	–9.460	–2.18	0.03	0.081
GAA 1 vs. AWB (n=42)	–7.767	–3.58	0.0009	0.243
GAA 2 vs. AWB (n=42)	–7.604	–2.66	0.01	0.151
GAA 1 vs. DTA (n=50)	–2.043	–0.36	0.72	0.003
GAA 2 vs. DTA (n=50)	–1.556	–0.23	0.82	0.001
GAA 1 vs. DTW (n=42)	–4.984	–1.33	0.19	0.043
GAA 2 vs. DTW (n=42)	–6.196	–1.33	0.19	0.043

Discussion

In this study we assessed 103 patients with a progressive ataxic syndrome, and with the exception of only one, all had a history consistent with an autosomal recessive disorder. One hundred patients were of definite Irish descent, and this is the first large phenotype/genotype study of progressive ataxia in this genetically homogeneous European population. We strictly applied Harding's mandatory diagnostic criteria, as outlined above, in classifying patients as typical or atypical. This contrasts with other papers on this topic that included extensor plantar responses [3, 7, 24], dysarthria [7], and sensory axonal neuropathy [5] as essential diagnostic criteria for typical FA. Despite specifying these criteria, Dürr et al. [3] included ten patients in the typical group who did not have extensor plantar responses, including two who had intact lower limb reflexes. Campuzano et al. [2] did not specify the diagnostic criteria for typical patients at all, and Filla et al. [4] and Monrós et al. [5] classified patients as typical if their age at onset was below 20 years of age. Lamont et al. [23] studied 56 patients who fulfilled Harding's diagnostic criteria for FA (with the exception that no upper age limit was set and areflexia was not essential), and all were homozygous for the GAA trinucleotide repeat expansion.

These differences in the criteria used to classify patients may in part be responsible for the reported variability in the percentage of typical patients harboring the GAA expansion in different series. If the presence of at least one GAA expansion is taken as a requirement for the diagnosis of FA, Harding's criteria identified only 47 out of a total of 62 GAA positive patients as having typical FA. This yields a relatively low diagnostic sensitivity of 79 % for the clinical diagnostic criteria in this study. This contrasts with the 100 % clinical diagnostic sensitivity in several series [2, 5, 6, 23], but compares favourably with a sensitivity of 76.3 % and 75.8 % in the studies by Schöls et al. [7] and Dürr et al. [3] (despite variable application of the criteria by the latter group). We identified two affected homozygous siblings, one of whom was typical and the other was classified as atypical due to mild reduction in upgaze bilaterally. Dürr et al. [3] recorded "limited eye gaze with preserved vestibulo-ocular movements" in five homozygous patients also. Therefore, in the absence of other significant variables, eye movement abnormalities should no longer exclude patients from the typical category.

The specificity of Harding's clinical diagnostic criteria in this study was also relatively low at 83 %, due to the seven GAA negative patients who fulfilled all of the mandatory criteria for typical FA. Within this subgroup, patient 1 was still not wheelchair-bound at 48 years of age. Although outside the mean age range of 25.14±15.5 years, he is still within the overall age range for becoming wheelchair-bound of 11–58 years [1]. In the original series of patients dysarthria was recorded in 96.5 %, scoliosis in 79.1 %, abnormal lower limb joint position and vibration sensation in 78.3 % and 73 % respectively, and pes cavus in 54.9 % of patients [1]. The plantar responses were extensor in 88.7 %, absent in 10.4 % and flexor in 0.9 % of Harding's patients. Therefore none of these findings were universal, and their absence should not warrant the exclusion of these patients from the typical category. However, it is unusual that two of the typical negative patients did not have dysarthria at 22 and 25 years after disease onset. Three patients in the typical negative subgroup had siblings who were classified as atypical. In this study and in another recent report [22], families have been identified containing some homozygous members exhibiting the typical phenotype and others with preserved or exaggerated reflexes. This confirms that intrafamilial phenotypic variability exists in FA, and a family history of an atypical sibling should not rule out the possibility of typical FA in another sibling. Conversely, the identification of intrafamilial phenotypic variability should alert one to the possibility of another inherited ataxic syndrome, and further tests including vitamin E, hexosaminidase A and α-fetoprotein levels etc. should be measured if mutation screening for the GAA expansion is negative. One typical negative patient had an atypical GAA negative sister whose phenotype was inconsistent with classic FA, and although unlikely, it is possible that these siblings are affected by two distinct ataxic syndromes. This theory is supported by the exclusion of involvement of the FA locus on microsatellite analysis in this sib pair. However, it is more probable that they have phenotypic variations of a recessive ataxic syndrome distinct from but mimicking FA in the brother. Four typical negative patients had abnormalities on their ECG that have been described in FA, and one had mildly impaired left ventricular diastolic function on ECHO. Although features of cardiomyopathy on ECG or ECHO support a clinical diagnosis of FA even with negative PCR results, these findings are not specific for FA because cardiomyopathy has been described with vitamin E deficiency also [25]. At least three patients in the typical negative category could have two point mutations in the X25 gene, although this has never been described to date. It is possible, as suggested by others [7, 26], that there is locus heterogeneity in FA, and that there is another, as yet unidentified, mutation causing clinical FA. Locus heterogeneity was investigated using microsatellite markers closely linked to the FA locus. This analysis can exclude the involvement of the identified FA locus (if alleles are not shared), but cannot prove involvement, because one in four siblings will share alleles by chance. This is illustrated by the finding of shared alleles in two sib pairs, one with vitamin E deficiency and one with possible ataxia telangiectasia. We were able to exclude FA in three of six families tested and to provide strong evidence for exclusion in a fourth sibship. This approach is useful when families suspected of having FA are found to be GAA expansion negative, before extensive screening for double point mutations is undertaken.

We identified 11 atypical patients who were homozygous for the GAA expansion. Our results confirm the suggestions of previous authors [27, 28] that the clinical spectrum of FA extends to include patients with retained deep tendon reflexes and late-onset disease. In addition, the incidence of abnormal lower limb superficial sensation has been underestimated in FA to date.

In concordance with previous reports [3–7, 23], we found a significant inverse relationship between the GAA repeat size on the smaller of the two alleles and the age at presentation of the disorder. There was also a significant inverse relationship between repeat size on both the smaller and larger of the two alleles and the age at becoming wheelchair-bound. In contrast with other studies [3, 6, 7], our results and those of Filla et al. [4] did not demonstrate a significant relationship between repeat size and the duration to becoming wheelchair-bound. In this series GAA repeat size did not significantly affect the duration to assistance to walk, a relationship not assessed in other studies. Dürr et al. [3] reported that patients with cardiomyopathy on ECHO had significantly more repeats on the smaller of the two alleles than those without, and Filla et al. [4] reported similar findings for the smaller, larger and mean number of repeats overall. Monrós et al. [5] concluded that patients with cardiomyopathy had larger expansions than those without, with the difference being more significant for the larger allele. We did not find any significant difference in the mean repeat size on the smaller or larger allele between patients with and without cardiomyopathy on ECHO. In this regard we confirm the findings of Montermini et al. [6], although they did not specify the exact number of patients in their study who had ECHOs performed, and their definition of cardiomyopathy differed from ours. None of typical negative patients had diabetes mellitus, and the overall incidence of diabetes in the homozygous population (6.9 %) was lower than previously reported by several authors [1, 3, 4, 24], and slightly higher than in the study by Schöls et al. [7]. The I154F mutation was not found in any of the presumed compound heterozygotes of Irish descent. Because all I154F cases to date are from a restricted area in southern Italy [2, 4], this mutation may not be an important cause of FA in other European or non-European populations.

Thirty-four of a total of 102 patients with a presumed autosomal recessive ataxic syndrome were GAA negative, did not have the SCA 1, 2, 3 or 6 mutations, and to date have no identifiable cause for their ataxia. These patients warrant further biochemical, molecular genetic, neurophysiological and neuroradiological investigations to determine the cause of their ataxia. If negative, linkage analysis and subsequent screening of the X25 gene to identify previously described and novel point mutations may facilitate a molecular genetic diagnosis in these patients, because it is possible that two point mutations could produce a *forme fruste* of FA. It is more likely that these patients have another, as yet unidentified, ataxic syndrome genetically distinct from FA.

Therefore, when assessing patients with a progressive ataxic syndrome in a clinical setting, strict application of Harding's mandatory diagnostic criteria may fail to identify GAA positive patients in up to 21 % of cases and may yield a false positive clinical result in up to 17 % of cases. These results alert us to the limitations of the clinical diagnostic criteria, and stress the importance of molecular genetic analysis for the GAA trinucleotide repeat expansion in all patients with a presumed autosomal recessive or sporadic ataxic syndrome. If this is negative, it is mandatory to search for disorders that mimic FA and to perform detailed neurophysiological investigations to exclude HMSN type I [1]. When counselling patients, especially those who are GAA negative, who have been informed for years that they have FA, it is useful to clarify their phenotypic and genotypic status as outlined in this study.

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