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Phenotype Correlation and Intergenerational Dynamics of the Friedreich Ataxia GAA Trinucleotide Repeat

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Summary

The Friedreich ataxia (FA) mutation has recently been identified as an unstable trinucleotide GAA repeat present 7–22 times in the normal population but amplified as many as >1,000 times in FA. Since it is an autosomal recessive disease, FA does not show typical features observed in other dynamic mutation disorders, such as genetic anticipation. We have analyzed the GAA repeat in 104 FA patients and 163 carrier relatives previously defined by linkage analysis. The GAA expansion was detected in all patients, most (94%) of them being homozygous for the mutation. We have demonstrated that clinical variability in FA is related to the size of the expanded alleles: milder forms of the disease—late-onset FA and FA with retained reflexes—are associated with shorter expansions, especially with the smaller of the two expanded alleles. Absence of cardiomyopathy is also associated with shorter alleles. Dynamics of the GAA repeat has been investigated in 212 parent-offspring pairs. Meiotic instability showed a sex bias: paternally transmitted alleles tend to decrease in a linear way that depends on the paternal expansion size, whereas maternal alleles can either increase or decrease. A different pattern of intergenerational variation was also observed, depending on the genetic status of the sib: patients had shorter expansions than were seen in heterozygous carriers. This finding has been interpreted as a postzygotic event. Finally, we have observed that the size of the expansion remains constant in the population through carriers.

Introduction

Friedreich ataxia (FA) is the most common early-onset hereditary ataxia, with an estimated prevalence of 2–4/100,000 in several European populations (Winter et al. 1981; Romeo et al. 1983; Polo et al. 1991; López-Arlandis et al. 1995). FA is an autosomal recessive degenerative disease characterized by onset at <20 years of age, mainly around puberty; relentlessly progressive ataxia of gait and limbs; absence of tendon reflexes; loss of position and vibratory sense; dysarthria; extensor plantar responses; and axonal sensory neuropathy (Geoffroy et al. 1976; Harding 1981). Cardiomyopathy is very frequent but is not detected in all patients (Harding and Hewer 1983), and diabetes mellitus is found in 10% of affected individuals. The main pathological changes occur in the dorsal root ganglia, with loss of large sensory neurones, degeneration of spinocerebellar tracts, and atrophy of large myelinated sensory fibers of peripheral nerves.

The mutated gene in FA (FRDA) was mapped to chromosome 9q13 by linkage to marker loci D9S15 (Chamberlain et al. 1988) and D9S5 (Fujita et al. 1989). Further linkage studies in several populations showed no evidence of genetic heterogeneity (Chamberlain et al. 1989; Pandolfo et al. 1990). In spite of autosomal recessive inheritance and genetic homogeneity, clinical variability is reported in most series, in particular with regard to the age at onset and the progression of the disease. Late-onset FA (LOFA), defined by onset at >20 years of age, was also mapped on the FRDA locus (De Michele et al. 1994), suggesting that this phenotype is an allelic variant of FA. A rarer variant is FA with retained reflexes (FARR). We have also demonstrated that FARR is located within the FRDA region (Palau et al. 1995). Both LOFA and FARR have been thought to represent different allelic mutations. Associated symptoms are variable as well. This clinical heterogeneity can render difficult the disease diagnosis and can hinder genetic counseling, especially in small families with only one affected child.

The isolation of the FRDA gene, called "X25," has recently been reported (Campuzano et al. 1996). It has six coding exons spanning ~80 kb of genomic DNA. X25 encodes a 210-amino-acid protein, frataxin, of unknown function. A second protein, of 171 amino acids, is generated by alternative splicing and, in the 11

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COOH-terminal residues, differs from the main isoform. Most patients show the expansion of a GAA trinucleotide repeat in the first intron of the X25 gene. The GAA motif is present 7–22 times in the normal population, but it is amplified as many as >1,000 times in FA patients. The expanded allele is observed in 98% of FRDA chromosomes. Three point mutations have been identified in the nonexpanded mutant allele of some heterozygous patients: a nonsense mutation, L106X in exon 3; a missense change, I154F in exon 4; and a splicing mutation at the acceptor site at the end of the third intron (Campuzano et al. 1996).

So far, FA is the first autosomal recessive disease identified as being caused by an unstable trinucleotide-expansion mutation. This finding has broken the paradigm involving diseases described earlier as caused by dynamic mutations (Warren 1996). Trinucleotide-repeat disorders commonly have been defined as a group of autosomal dominant or X-linked disorders involving the nervous system and caused by the expansion of a trinucleotide motif in the affected individuals. These diseases usually show classical features of genetic anticipation: early age at onset and more severe phenotype in successive generations. Anticipation has been explained by the meiotic instability of the mutation when transmitted from parents to offspring (Richards and Sutherland 1992; Mandel 1993; La Spada et al. 1994; Ashley and Warren 1995). Because of the disease's autosomal recessive pattern of inheritance, evidence for anticipation has not been reported in FA. Nevertheless, to examine the possibility that clinical variability could be explained by the size of the expansion, we investigated the relationship between the GAA trinucleotide-repeat length and the phenotype in a series of FA patients. We also analyzed the intergenerational dynamics of the mutation and the factors that could influence the genetic instability of the repeat.

Subjects and Methods

FA patients were ascertained on the basis of Harding's (1981) essential diagnostic criteria: autosomal recessive inheritance or isolated cases, onset at <20 years of age, progressive unremitting ataxia of gait and limbs, absence of lower-limb deep-tendon reflexes, and evidence of sensory axonal neuropathy. Most patients were evaluated for the presence of cardiomyopathy, by electrocardiogram and/or echocardiogram.

DNA from peripheral leukocytes of each patient, carrier parents, sibs, and other related family members was amplified by PCR, in a PTC-100 MJResearch thermocycler and by use of primers GAA-F (5′-GGGATTGGTTGCCAGTGCTTAAAAGTTAG-3′) and GAA-R (5′-GATCTAAGGACCATCATGGCCACACTTGCC-3′) (Campuzano et al. 1996), in thin-walled tubes in a final volume of 25 μl, including 250 ng of DNA; 5 pmol of each primer; 250 μM each of dATP, dCTP, and dTTP; 166.6 μM dGTP (Pharmacia); 83.3 μM 7-deaza-dGTP (Boerhinger Mannheim); and 1.75 units of the Expanded Long Template PCR System, a mix containing *Taq* and *Pwo* DNA polymerases (Boerhinger Mannheim). Amplification was conducted by use of a long-PCR protocol: initial denaturation at 94°C for 3 min, 20 cycles each at 94°C for 20 s and 68°C for 8 min, followed by a further 17 cycles in which the length of the 68°C step was increased by 15 s/cycle. Seven microliters of the product were run in a 0.8% agarose gel at 100 V for 4 h. After ethidium bromide visualization, each sample was scored for the presence of both normal and expanded alleles (the size of each allele is $457 + 3n$ bp, with n being the number of GAA triplets). Since in some cases the PCR products corresponding to the expanded repeats appeared as a smear, hybridization experiments with a digoxigenin-dUTP–labeled oligonucleotide $(GAA)_{10}$ probe were performed at 52°C overnight, and the product was washed twice in 0.5 × SSC/0.1% SDS at 52°C for 20 min.

Results

Analysis of the GAA Expansion and Clinical Phenotypes

The trinucleotide-GAA expansion within the first intron of the X25 gene was investigated in 104 patients and 163 carrier relatives from 75 unrelated Spanish families, 1 Portuguese family, and 1 Argentinean family. On the basis of clinical data, patients were classified in one of four clinical groups: (1) classic FA, if the patient fulfilled Harding's essential criteria (68 subjects); (2) late-onset FA (onset at >20 years of age) (13 subjects); (3) FA with retained lower-limb tendon reflexes (9 subjects); and (4) unclassified patients (14 FA patients for whom age at onset was not available). The expansion was found in all patients, independent of the clinical phenotype-classic or variant. The familial segregation pattern was in accordance with previous results, obtained by indirect haplotype analysis. No reversion to normal allele size was observed. Ninety-eight patients were homozygous for the expanded GAA motif, whereas six patients were compound heterozygous, showing only one expanded allele. Three of these six had typical FA with cardiomyopathy (group 1); in one the disease started at the age of 26 years, and cardiomyopathy was also evident (group 2); the other two patients belonged to group 4, for which no complete clinical data were available. A point mutation could be defined in the nonexpanded allele from patient LF3, with classical FA. This mutation, previously reported by Campuzano et al. (1996), involved the splicing acceptor site at the end of intron 3 of the X25 gene, 385-2(G→T). Overall, the GAA

Table 1

Statistical Analysis of Clinical Groups

Clinical Characteristic	Allele S	Allele L	Alleles S and L
	Mean ± SD		
Phenotype:			
Classic (n = 59)	700 ± 211	930 ± 186	807 ± 168
LOFA (n = 12)	351 ± 155	656 ± 193	509 ± 132
FARR (n = 9)	512 ± 203	887 ± 191	700 ± 115
Cardiomyopathy:			
Present (n = 54)	667 ± 239	922 ± 207	791 ± 181
Absent (n = 16)	489 ± 247	727 ± 205	608 ± 192
	Student's t-Test		
Classic vs. LOFA	5.39 ($P < .0001$)[a]	4.75 ($P < .0001$)[a]	5.80 ($P = .0001$)[a]
Classic vs. FARR	2.48 ($P = .016$)[a]	.63 ($P = .52$)	1.85 ($P = .068$)
LOFA vs. FARR	−2.06 ($P = .053$)	−2.77 ($P = .012$)[a]	−3.47 ($P = .003$)[a]
Cardiomyopathy vs. no cardiomyopathy	2.57 ($P = .012$)[a]	3.29 ($P = .002$)[a]	3.49 ($P = .001$)[a]

[a] Statistically significant.

expansion was observed in 97% (196/208) of FRDA chromosomes.

The expansion size could be measured in all carrier individuals (163 FRDA chromosomes), in 89/98 homozygous patients, and in the 6 heterozygous patients (184 FRDA chromosomes). In the other nine patients the expanded alleles were visualized as a smear and were not included in the study. In the overall population, the size of the expansion was 210–1,350 trinucleotides, with a mean length of 800 repeats. In patients, the mean ± SD length mutation was 753 ± 217 repeat units, with a range of 210–1,210 repeats. In homozygous affected individuals we also analyzed the mean sizes of the smaller allele (allele S) and the larger allele (allele L). Mean ± SD sizes were 625 ± 232 repeats (range 210–1,180) for allele S and 880 ± 201 (range 310–1,210) for allele L.

Correlation between Clinical Phenotype and GAA-Repeat Length

We investigated the sizes of alleles S and L in 89 homozygous patients, both for well-defined expansions and for their correlation with the clinical phenotype. Mean values for each group are shown in table 1. Statistical differences (Student's t-test) between groups were observed for both alleles. The highest mean length differences were obtained between classic and LOFA groups, for both alleles. Significant differences were also obtained when classic phenotypes were compared with FARR phenotypes, for allele S, and when LOFA variants were compared with FARR variants, for allele L. Differences between LOFA and FARR, for allele S, did not reach statistical significance. Comparison of alleles' mean values, between the three groups, evidenced significant differences between LOFA and both the classic and the FARR phenotypes.

We further analyzed the distribution of allele S sizes, with regard to the clinical phenotype (fig. 1). Eighty-five percent (11/13) of LOFA patients had the allele S <500 repeats, whereas 80% (48/62) of classic cases had the allele S >500 repeats. The size of alleles S from the nine FARR patients were distributed around the 500-repeat value.

Since evident differences were observed between classic and LOFA patients, relating late onset with shorter alleles S, we investigated correlation between age at onset and the length of the repeat, for both allele S and allele L. The mean ± SD age at onset in the whole series was 12.2 ± 7.2 years (range 1–30 years). Highly

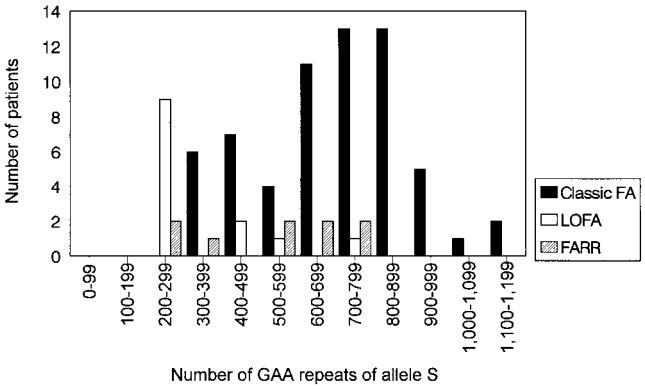

Figure 1 Distribution of allele S sizes for each clinical group. The x-axis represents the GAA expansion, in number of repeat units.

significant correlation was obtained for allele S ($r = -.58$; one tailed $P < .0001$ by Spearman's correlation), and regression analysis gave an R^2 value of .29, indicating that approximately one-third of the variation in the age at onset can be explained by the number of repeat units of allele S.

Electrocardiographic and/or echocardiographic data were obtained from 67 homozygous patients. Hypertrophic cardiomyopathy was diagnosed in 54/67 subjects. Analysis of the GAA alleles showed that patients with no cardiomyopathy had expansion sizes smaller than was seen in patients with heart disease, with the differences being more significant for allele L (table 1). We further investigated whether, between clinical groups, there was a different distribution of presence of cardiomyopathy. We could determine that only 2/13 (15.4%) LOFA patients had heart disease, whereas 5/59 (8.5%) classic patients had no evidence of cardiomyopathy ($\chi^2 = 33.6$; $P < .0001$). Most FARR patients showed signs of heart disease.

Meiotic Instability of the GAA-Trinucleotide Repeat

The meiotic instability of the GAA expansion could be observed in several ways: length-mutation variation was present in almost all parent-offspring transmissions, and affected siblings from multiplex families showed different expanded-allele sizes. In addition, we observed allelic size variation in patients expected to be homozygous by descent. We found different-size alleles in children of 5 consanguineous families (in two families, parents were first cousins; in three marriages, parents were second cousins) and in 16/19 individuals of 11 nonconsanguineous families for which we had previously demonstrated, for a genomic region spanning ⩾450 kb around the FRDA locus, homozygosity of FRDA haplotypes (data not shown). All these FRDA haplotypes were very rare (<2%) in the general Spanish population. Thus, it could be argued that carrier patients are homozygous by descent (Monrós et al. 1994). The range of allelic differences in these patients was 100–730 repeat units (fig. 2).

Expansion-Size Distribution in Patients and Carriers

We performed size-distribution analysis of the GAA expansion by taking into account the parental sex and the genetic status of the offspring. We first analyzed whether there were differences between paternal and maternal allele sizes: no significant differences were found between means ± SDs (820 ± 280 and 890 ± 200, respectively; $t = -1.49$, not significant by Student's t-test), but allelic distributions had different variances ($F = 6.26$; $P = .014$ by Levene test of variance) because 77.7% (28/36) of smaller alleles (<500 repeats) belonged to the fathers' group (fig. 3). Also, no differences were observed between the mean ± SD length in carrier parents (855 ± 250) and the mean ± SD length in carrier children (815 ± 250) (t-test not significant), but significant differences were obtained when means for progenitors were compared with those for affected children ($t = -2.71$; $P = .007$). Overall, the mean ± SD number of GAA-repeat units in carriers was 840 ± 250, whereas in patients it was 753 ± 217. An influence of the status of the individual on the length of the mutation was suspected on the basis of these results.

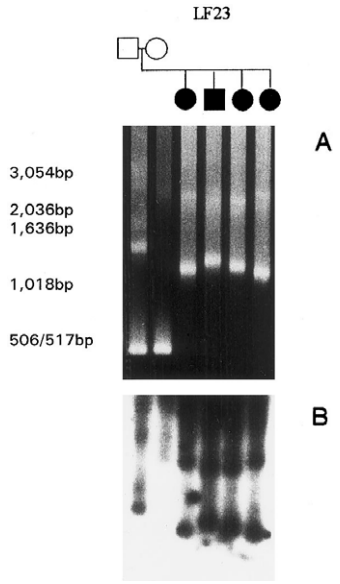

Figure 2 PCR analysis of the GAA repeat in family LF23. Patients had onset of the disease at >20 years of age, and two sibs (II-1 and II-3) showed retained lower-limb reflexes. *A*, DNA amplification showing both parents, heterozygous for the expansion, and the four children, homozygous for two expanded alleles. The largest allele size is ~680 repeats, and the smallest is ~250 repeats. *B*, Hybridization of the GAA-amplified alleles with oligonucleotide probe $(GAA)_{10}$ (the signal of nonexpanded alleles in parents was very weak and is not observed). Patients were homozygous for a very rare FR1-FR2-FAD1-FR8-FR7-FR5 extended haplotype, 5-8-B-1-6-9 (Monrós et al. 1996). The existence of significant size differences between allele S and allele L in each patient that are coincident on the same associated haplotype confirms the great meiotic instability observed in the GAA-trinucleotide repeat.

Intergenerational Variation: Size and Sex Parental Effects

For analysis of the instability of the GAA expansion, 212 parent-offspring transmissions were available. First, carrier sibs were identified by linkage analysis with FRDA-locus flanking markers (49 transmissions). With regard to parent–affected child pairs, the intergenerational variation was measured only when the parental origin of the mutation (father vs. mother) could be established. In this way, 100 transmissions from 39 families could be studied. In 25 families, the paternal and maternal expansion sizes were different enough to allow us

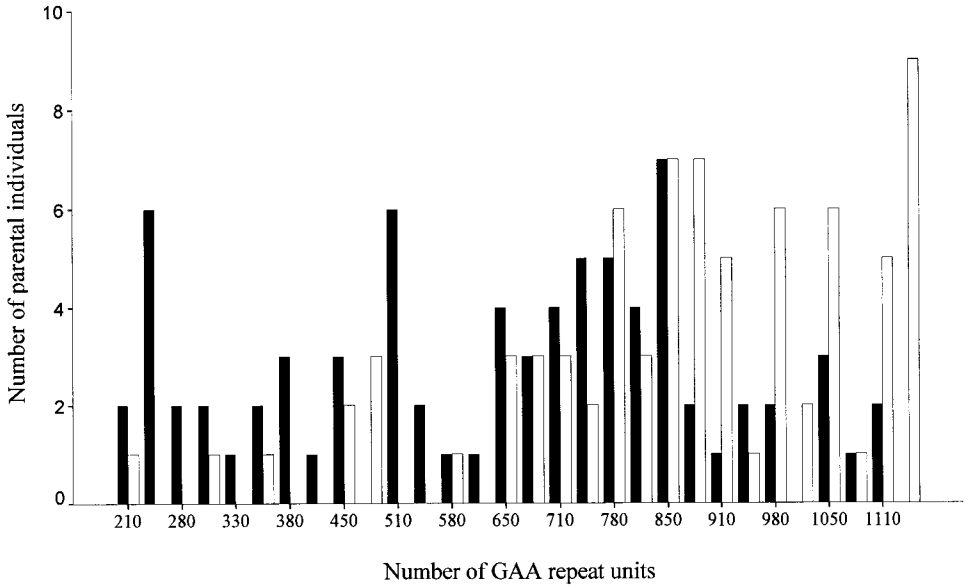

Figure 3 Distribution of both paternal (*black bars*) and maternal (*unblackened bars*) expanded alleles. The x-axis represents the GAA expansion, in number of repeat units.

to distinguish between them (e.g., see fig. 2), allowing us to ascertain the origin of 75 patients' expanded alleles. Eleven couples with similar expansion length had one affected sib who showed a single expanded band, so the parental origin of the intergenerational variation could be determined also in these 22 transmissions. Three additional transmissions were established in three compound heterozygous patients from three families in which a well-defined or putative (abnormal SSCP band) point mutation was segregating. The total number of parent-child pairs that we could analyze was 149. The size of the mutation in the offspring correlated with the size of the parental mutation ($r = .81$; $P < .0001$). We then analyzed whether there was an effect of the progenitor sex in the expansion transmission: when transmissions were classified with the parental sex (70 paternal and 79 maternal) being taken into account, the correlation remained significant (father-child, $r = .83$, $P < .0001$; mother-child, $r = .78$, $P < .0001$), but paternal and maternal alleles behaved differently in transmission. The mean ± SD size of the analyzed paternal alleles was 750 ± 290 repeats, whereas the mean ± SD size of the paternally inherited alleles in the offspring was 640 ± 250 repeats ($t = -5.63$; $P < .0001$). Conversely, maternal transmission did not affect the mean ± SD length of the expansion (890 ± 215 repeats in both generations). Paternally inherited FRDA mutations were shorter than maternally inherited mutations ($t = -5.77$; $P < .0001$), and this effect of the sex-transmitting parent was not due to the fact that fathers carry smaller expansions. The correlation between the length of the GAA repeat in the offspring and the sex of the transmitting parent is shown in figure 4.

We investigated the pattern of intergenerational variation after male and female meioses and found that the dynamics for the GAA expansion were significantly different between sexes (table 2). Paternal expanded alleles decreased in 70% of transmissions (mean decrement −110 repeat units), whereas maternal alleles were more

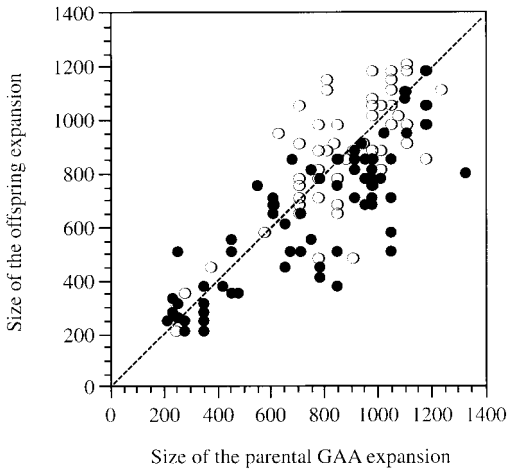

Figure 4 Correlation of the GAA expansion (expressed in repeat units), between parents and offspring. The x-axis represents the GAA-repeat length of the carrier parents, and the y-axis represents the GAA-repeat length of the mutant gene of the affected and carrier children. Parent–child pair correlation is indicated by black dots (paternal) and unblackened circles (maternal).

Table 2

Parental Sex Influence on Transmission of GAA Expansion to Offspring

GAA-Expansion Variation	Parental (n = 70)	Maternal (n = 79)
Decrease	49 (70%)	29 (37%)
No variation	5 (7%)	16 (20%)
Increase	16 (23%)	34 (43%)

NOTE.—χ^2 = 16.89, 2 df, P = .0002.

stable and showed an equilibrium between increase and decrease (no mean variation).

Effect of the Genetic Status on the Mutation Length

We previously have shown that the mean length of carriers' mutations remains invariable through generations but that, with regard to their parents, a significantly shorter size is observed in patients' expansions. To explain these findings, we compared the mean length and intergenerational variation between affected and carrier sibs by analyzing 100 parent-patient transmissions and 49 parent-carrier transmissions. Significant differences were obtained when we examined the pattern of variation in both groups by the χ^2 test (table 3): patients' alleles decreased in 60% of transmissions (mean decrease 75 repeat units) and carriers' alleles tended to expand (53% of transmissions), although the mean length increase was very low (+20 repeats) (means difference between groups, $t = -3.56$; $P = .001$).

General Intergenerational Dynamics of the GAA Expansion

We investigated the final characterization of the dynamic mutation associated with FA by subclassifying transmissions according to the four possible different pairs: father–affected child (n = 52), father–carrier child (n = 18), mother–affected child (n = 48), and mother–carrier child (n = 31). Results with regard to the mean variation that each possible intergenerational change had on the length of the GAA expansion are summarized in table 4. Contractions were predominant among patients, the more important and frequent length reductions having a paternal origin. Among carriers, paternal decrements were compensated by maternal increments. The general behavior of the GAA-expansion transmission is shown in table 5.

Table 3

Children's Genetic-Status Influence on Intergenerational Variation of GAA Expansion

GAA-Expansion Variation	Patients (n = 100)	Carrier Sibs (n = 49)
Decrease	60 (60%)	18 (37%)
No variation	16 (16%)	5 (10%)
Increase	24 (24%)	26 (53%)

NOTE.—χ^2 = 12.46, 2 df, P = .002.

Table 4

Intergenerational Variation of GAA Expansion, with Regard to Both Parental Origin and Child's Clinical Status

Variation	Mean ± SD Repeat Units
Parental origin:	
Group 1: FA patients (n = 52)	−130 ± 160
Group 2: FA carrier sibs (n = 18)	−30 ± 150
Maternal origin:	
Group 3: FA patients (n = 45)	−20 ± 115
Group 4: FA carrier sibs (n = 31)	60 ± 60

NOTE.—Results of Student's t-test are as follows: group 1 vs. group 2—$t = -2.25$, $P = .028$; group 1 vs. group 3—$t = -3.72$, $P < .001$; group 3 vs. group 4—$t = -2.63$, $P = .01$; and group 2 vs. group 4—$t = -2.0$, $P = .05$.

Figure 5 plots the intergenerational variation of the GAA expansion after female (*top*) and male (*bottom*) meioses, in relation to the size of the parental mutation, distinguishing between affected and carrier sibs. The behavior difference between sexes is clearly reflected. Maternal transmissions behave randomly: no linear regression can be traced, and size variation (expansion and contraction) seems to be independent of the original maternal allele size. Intergenerational increases are observed for the whole range of maternal mutation, despite the fact that longer increases (as much as 350 bp) are observed only in carrier sibs, whereas those in patients do not expand >100 bp. Conversely, in paternal transmissions a pattern exists that confirms their tendency to decrease. Increments are observed only in the lower-number range (<800) of paternal repeats that are larger for carriers than patients, as observed in mothers' transmissions. Decrements are larger as the father allele size increases, but linear regression is different for carrier and affected sibs.

Discussion

FA is the 10th inherited neurological disease to be associated with a dynamic mutation, but, to date, it is the only one described in an autosomal recessive disorder. Dominant trinucleotide-repeat disorders show instability of the expanded repeat in parent-offspring transmissions (La Spada et al. 1994; Ashley and Warren 1995). Typically, the repeat allele tends to expand, although contractions have been reported also. Pheno-

Table 5

General Behavior of GAA-Expansion Transmission

	FA Patients		FA Carrier Sibs	
Parental Expansion	Variation Mean	No. of Cases	Variation Mean	No. of Cases
Paternal:				
Decrease	−190	39 (75%)	−140	10 (56%)
No variation	0	4 (8%)	0	1 (5%)
Increase	+70	9 (17%)	+110	7 (39%)
Maternal:				
Decrease	−130	21 (44%)	−140	8 (26%)
No variation	0	12 (25%)	0	4 (13%)
Increase	+80	15 (31%)	+150	19 (61%)

typic variability in these disorders has been correlated with the length of the expanded alleles. The larger the repeat, the more severe is the clinical phenotype. Dynamic mutations help to explain the molecular pathogenesis of the phenomenon of anticipation, in which increased repeat length correlates with more seriously affected individuals in succesive generations within a family.

In FA there is no evidence for genetic anticipation. However, FA shows clinical variation, with milder forms that differ from the classic phenotype. Several questions arise regarding to the clinical variability of FA: Does size variation of the expanded alleles explain the clinical spectrum of the disorder? Does the FA-associated GAA expansion show intergenerational instability? How do the parental alleles behave during transmission to offspring?

To answer these questions, we have investigated the GAA-trinucleotide repeat in 104 FA patients from 77 unrelated families. All patients had at least one expanded allele: 98 individuals were homozygous for the expansion, whereas 6 of them were compound heterozygous with one expanded allele and one nonexpanded allele. Overall, the GAA expansion was found in 97% of FRDA chromosomes.

Linkage studies have shown that both LOFA and FARR variants map to the FRDA locus, suggesting that both phenotypes are allelic variants of FA (De Michele et al. 1994; Palau et al. 1995). In the present study, we have demonstrated that LOFA patients are homozygous for the GAA expansion in all individuals except one, who is heterozygous for the expansion and another unknown mutation. By segregation analysis of FRDA-linked markers, we established that the nonexpanded mutation was associated with a rare (<1%) FRDA haplotype in the Spanish population (Monrós et al. 1996). The mean repeat lengths of expansions were different from those in individuals with the classic phenotype, especially for allele S. In fact, most alleles S in LOFA subjects were <500 repeat units, whereas 80% of classic alleles S had a repeat size larger than the conventional value. These results suggest that phenotype differences between early-onset FA and late-onset FA may be partially explained by differences in the length of the expansion. The inverse correlation between the age at onset and the expansion size of allele S supports this finding as well.

All FARR patients except one exhibited an axonal sensory neuropathy. However, preservation of their tendon reflexes suggests that the physiological pathways of the reflex arch remain functional. The analysis of the GAA repeat confirmed that this less severe new pathology is associated with smaller allele S and smaller allele L, although their respective sizes were larger than those in LOFA.

Hypertrophic cardiomyopathy is diagnosed in 70%–90% of FA patients (Harding and Hewer 1983; Alborilas et al. 1986; Child et al. 1986), despite histological studies suggesting that pathological lesions are present in all individuals (Hewer 1969). By means of electroencephalogram and echocardiogram techniques, cardiomyopathy was recognized in 80% of our patients. We found smaller expansions in patients without cardiomyopathy. An interesting point was the close relationship between late-onset disease and absence of heart-muscle disease. Our findings suggest that absence of cardiac abnormalities depends on both the expansion size and the age at onset.

In conclusion, we have demonstrated that the phenotype is, at least in part, related to GAA-repeat size, especially with regard to allele S length. More benign phenotypes are associated with smaller sizes of the expanded alleles. Two papers recently have reported similar results (Dürr et al. 1996; Filla et al. 1996). In both reports the authors found that the lengths of expanded alleles, especially the smaller alleles, are inversely correlated with both the age at onset of the disorder and shorter times until loss of ambulation. Dürr et al. (1996) and

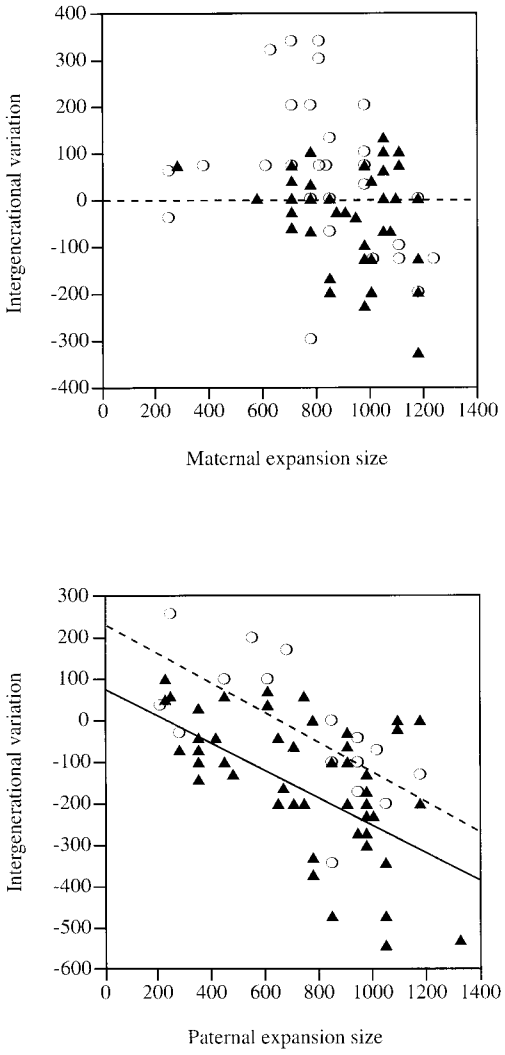

Figure 5 Intergenerational variation in maternal (*top*) and paternal (*bottom*) expanded GAA alleles versus offspring expanded GAA alleles, expressed in repeat units. The x-axis represents the GAA-repeat length of the mutant gene of the carrier parents, and the y-axis represents the intergenerational variation of the parental GAA expansion, calculated on the basis of the GAA-repeat length in the child, either affected or carrier. Patients' alleles are indicated by black triangles, and carriers' alleles are indicated by unblackened circles. Regression curves are indicated in the two panels. *Top,* Common curve for both patients and carrier sibs, indicating that there is no correlation between the intergenerational variation and the mother's allele size. *Bottom,* Correlation between paternal expansion size and affected (*unbroken line*) and carrier (*broken line*) sibs.

Filla et al. (1996) also observed that patients with cardiomyopathy had larger expansions, for both small and large alleles, whereas only Dürr et al. (1996) found a correlation between the allele sizes and the preservation of tendon reflexes.

Both the length (>200 repeats) of the expansion in FRDA and its location within an untranslated region (intron 1) are similar to those of the expanded trinucleotide repeats associated with fragile X syndrome (FRAXA) (Kremer et al. 1991; Verkerk et al. 1991) and myotonic dystrophy (DM) (Brook et al. 1992). In FRAXA and DM, a size-mutation threshold allows one to distinguish two mutation ranges: the premutation (<200 repeats), which correlates with minimal to nondetectable disease, and the full mutation (>200 repeats), in individuals expressing the complete phenotype. Generally, the step from the premutation to the full mutation is influenced by the length and sex of the parental mutation (Mulley et al. 1992; Tsilfidis et al. 1992; Brunner et al. 1993; Harley et al. 1993; Lavedan et al. 1993).

In FA it is difficult to know whether a premutational range exists, since sample ascertainment bias can occur: asymptomatic carriers are analyzed only when they have an affected child and, as we have demonstrated, already carry the mutation expansion with >200 GAA repeats. Three-generation studies in our series (not shown) have shown that grandparents of affected children also carry long expansions, and we have seen that the mean number of repeats is constant among heterozygous individuals and, thus, that the mean mutation size is maintained in the population. Since carriers do not exhibit a decreased fitness and since some patients do reproduce, loss of FRDA alleles in the population is low when compared with that in DM or FRAXA, and a very low rate of de novo mutations can be expected. Linkage-disequilibrium studies with the FAD1 single-substitution nucleotide polymorphism in normal and FRDA chromosomes from different European origins (Monrós et al. 1996; F. Palau, E. Monrós, M. De Castro, A. Löfgren, C. Van Broeckhoven, and P. Coutinho, unpublished data) suggest that a small number of ancestral mutations are responsible for FA in the Caucasian population. Extended haplotype linkage-disequilibrium analyses in the Spanish population also argue in favor of a small number of founder effects (Monrós et al. 1996). This is a situation similar to that observed in both FRAXA and DM, for which an almost unique origin has been demonstrated (Richards et al. 1992; Imbert et al. 1993; Oudet et al. 1993; Lavedan et al. 1994).

In spite of the lack of information about the existence of a premutation range in FRDA, comparisons with the full mutations in DM and FRAXA still can be made. We have shown that in FRDA there is a sex-dependent compensation mechanism that keeps the GAA mutation size in the populations constant over generations. The tendency for expansion to be observed generally in dominantly inherited dynamic mutations cannot be the rule for FRDA, since it is transmitted by unaffected carriers whose repeats do not expand indefinitely. The full mutations of DM and FRAXA also follow sex-dependent transmission patterns that explain the specific features of each disease. FRAXA expansions, once they have reached the full mutation range, are almost always trans-

mitted by females. As in FRDA, they fail to show the general tendency to increase. Male-patient transmissions are very uncommon, but in a few documented cases it has been demonstrated that fathers pass a premutation to their daughters (Mulley et al. 1992). By contrast, DM maternal expansions always increase, leading to the congenital form in offspring, whereas paternal mutations >500 CTG repeats tend to decrease (Lavedan et al. 1993). In FRDA, we find that female transmissions can either expand or compress the repeat, whatever the size of the maternal repeat. The similarity between the two diseases is that the degree of variation is independent of the maternal mutation length (Lavedan et al. 1993). On the other hand, male FRDA transmissions behave similarly to male DM transmissions (fig. 5, *bottom*). Intergenerational expansions are observed exclusively for the lower-range alleles, whereas longer mutations tend to contract in a linear way that depends on the parental allele size. These results agree with the general hypothesis that in male gametogenesis there might be a selection process, against hyperexpanded alleles or specific repair mechanisms, that would lead to their compression, a process maybe related to the high mitotic rate to which spermatogonia are subjected (Brunner et al. 1993; Harley et al. 1993; Lavedan et al. 1993). Nevertheless, our experiments are based on lymphocyte DNA, and sperm analyses would be necessary to better understand the FRDA mutation dynamics.

The recessive pattern of inheritance in FA has allowed us to define a variation pattern in carriers that is different from that in affected sibs. We have seen in figure 5 that carriers' repeats can expand more than those in patients, with no mean size variation with regard to their parents. By contrast, patients have a mean mutation length significantly shorter than that in their parents, since mainly paternal alleles (75%) but also maternal alleles (44%) lose repeats after one generation. We postulate that these differences may be due to postzygotic events, perhaps through selection against larger alleles in patients.

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