Table 1

Properties of Screening Set 8

Collection	No. of STRPs	Heterozygosity ± SD	Average Spacing ± SD (cM)	Fraction of Dinucleotide-Repeat Polymorphisms
High density	387	.77 ± .06	10.4 ± 3.9	11.4%
Low density	169	.78 ± .05	25.4 ± 8.2	5.9%

is more straightforward for longer-repeat-length markers (Sheffield et al. 1995). Occasionally, STRPs, especially tetranucleotide STRPs, exhibit alleles that have size differences other than integer multiples of the repeat length. STRPs that showed these "noninteger" alleles were largely excluded from Screening Set 8. Only 8.5% of Screening Set 8 markers exhibited common noninteger alleles, and most of these were tetranucleotide STRPs with alleles differing in size by only even numbers of nucleotides.

Screening Set 8 STRPs have been robustly tested in linkage projects involving a total of well over 2 million genotypes. Protocols involving silver staining, as well as radioisotopic and fluorescent labeling, of the amplification products have been applied to analysis of the markers. Combinations ("combos") of two to five screening-set STRPs for simultaneous amplification have been generated and are being improved continually (see the Marshfield web site). CEPH-family allele frequencies for the Screening Set 8 markers also are available from the Marshfield web site. Primers for the Screening Set 8 markers can be purchased from Research Genetics. Future upgrades to Screening Set 8 likely will involve the shifting of PCR primers for selected markers, to optimize marker combinations, and the addition of new STRPs, to reduce average spacing to ~5 cM.

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References

Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nat Genet 380:152–154

Dubovsky J, Sheffield VC, Duyk GM, Weber JL (1995) Sets of short tandem repeat polymorphisms for efficient linkage screening of the human genome. Hum Mol Genet 4:449–452

Ott J (1991) Methods of linkage analysis. In: Analysis of human genetic linkage, rev ed. Johns Hopkins University Press, Baltimore, pp 54–81

Reed PW, Davies JL, Copeman JB, Bennett ST, Palmer SM, Pritchard LE, Gough SCL, et al (1994) Chromosome-specific microsatellite sets for fluorescence-based, semi-automated genome mapping. Nat Genet 7:390–395

Sheffield VC, Weber JL, Buetow KH, Murray JC, Even DA, Wiles K, Gastier JM, et al (1995) A collection of tri- and tetranucleotide repeat markers used to generate high quality, high resolution human genome-wide linkage maps. Hum Mol Genet 4:1837–1844

Utah Marker Development Group, The (1995) A collection of ordered tetranucleotide-repeat markers from the human genome. Am J Hum Genet 57:619–628

Weber JL, Wang Z, Hansen K, Stephenson M, Kappel C, Salzman S, Wilkie PJ, et al (1993) Evidence for human meiotic recombination interference obtained through construction of a short tandem repeat–polymorphism linkage map of chromosome 19. Am J Hum Genet 53:1079–1095

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The Effect of Parental Gender on the GAA Dynamic Mutation in the FRDA Gene

To the Editor:

Within a cooperative study, we recently isolated the defective gene (X25) causing Friedreich ataxia (FRDA), an autosomal recessive neurodegenerative disorder. X25 encodes a 210–amino acid protein, frataxin, whose function is unknown. Frataxin mRNA levels are reduced in FRDA patients. The most-frequent mutation is the expansion of a $(GAA)_n$ trinucleotide repeat in the first X25 intron. Normal chromosomes contain 8–22 copies of the triplet, whereas FRDA chromosomes contain >200 copies. In addition, we described few patients with point mutations (Campuzano et al. 1996). The expansion of trinucleotide repeats has been previously demonstrated to be the mutational mechanism associ-

ated with eight human diseases (Bates and Lehrach 1994). Trinucleotide repeats occur both in coding and noncoding regions of the gene. Although trinucleotide repeats in the normal size range are relatively stable, expanded repeats are highly variable when transmitted from one generation to the next. For the eight previously described diseases, meiotic instability is generally associated with a mutational bias toward an increase in repeat number. Here, we analyze intergenerational variability in FRDA chromosomes in parent-carrier child pairs. In addition, we studied the stability of FRDA expanded alleles in male gametogenesis, directly analyzing male germ cells.

The instability of GAA repeat.—Since it is not possible to discriminate between maternally and paternally inherited alleles in patients, we selected 26 parent-carrier child pairs (16 mother-carrier child and 10 father-carrier child) from 21 FRDA pedigrees. Haplotype analysis performed previously allowed us to identify the transmitted allele unambiguously. We analyzed the GAA repeat size in the first intron of the X25 gene according to previously described methods (Filla et al. 1996). Ten percent glycerol was added to PCR mixture, to better amplify the largest expanded alleles. We did not observe intergenerational variation of the repeat length in the normal alleles, whereas expanded alleles appeared unstable. Parent and child gender and GAA repeat size and delta (delta = child GAA repeat size minus parent GAA repeat size) are shown for each pair (table 1). We detected a contraction of the mutated allele in 14 pairs (54%), an expansion in 8 (31%), and no variation in the remaining 4 (15%). The contraction/expansion ratio was 1.75:1. Delta means were −166 (SD = 83) for contractions and 197 (SD = 130) for expansions. The number of triplets in offspring (mean = 863; SD = 179) did not differ significantly from that in parents (mean = 892; SD = 137). When the percentage of variation on the parent GAA repeat size (absolute[delta]/parent GAA size × 100) was calculated, no statistically significant difference between contractions (mean = 18.2%; SD = 9.4%) and expansions (mean = 26.7%; SD = 22.1%) was found.

Parental gender effect on GAA repeat size variation.—Figure 1 shows the relationship between the variation in the repeat length and the parental gender. Among the 13 maternally inherited alleles whose size varied, 8 increased and 5 decreased. Among the paternally derived alleles, all nine changes were contractions. Contraction/expansion ratio differed according parental gender (Fisher's exact test $P = .004$). No relationship was observed between variation in repeat length and child gender. We found an inverse correlation between maternal GAA triplet number and delta ($r_p = -.60$; df = 14; $P < .01$) (fig. 2).

GAA repeat length variation in male gametogenesis.—To study GAA repeat length variation in male gametogenesis, we amplified the repeat from sperm DNA and from the corresponding nucleated blood cells from three FRDA gene carriers. DNA was extracted from semen samples according to previously reported procedures (Telenius et al. 1995). To evaluate GAA repeat length, blood and sperm PCR products were loaded on 1% denaturing agarose gel and transferred on a nylon membrane (Hybond N+) by alkaline blot. Filter was hybridized using a 5′-DIG–labeled GAA primer and detected by colorimetric system (Boehringer). In all three cases, the PCR amplification of sperm DNA and blood DNA yielded products of the same size from the normal allele (data not shown). Amplification of the sperm-expanded allele yielded a diffuse array of products shorter than the corresponding allele from blood (fig. 3).

GAA repeat length variation and FRDA haplotypes.—We previously demonstrated (Pianese et al. 1994) the absence of recombination events between six closely linked polymorphisms (GS4, MS, GS2, 26p, FD1, and MLS1) and the FRDA gene in these pedigrees. We found no common extended haplotype associated with alleles prone to contract or to expand. In addition, the FD1-MLS1 haplotype, which we demonstrated to be in linkage disequilibrium with the FRDA gene (Pianese et al. 1994), was not associated with contracting or ex-

Table 1

Analysis of the GAA Repeat in 26 Parent-Carrier Child Pairs from 21 FRDA Pedigrees

Pedigree	Parent/Child Gender[a]	Parent/Child GAA Repeat No.	Delta
3	F/F	1031/919	−112
4	F/F	943/867	−76
6	F/M	952/1160	208
7	F/M	823/973	150
9	M/F	869/667	−202
10	M/M	950/851	−99
11	M/F	867/641	−226
15	M/F	824/690	−134
16	F/F	722/1110	388
16	M/M	952/631	−321
18	F/F	1017/1135	118
18	F/F	1017/1017	0
20	F/M	1066/1005	−61
21	F/M	974/1154	180
21	M/M	1005/765	−240
22	M/M	1074/847	−227
23	F/M	678/618	−60
24	F/F	1074/873	−201
25	F/F	618/745	127
27	M/F	816/707	−109
27	M/F	816/816	0
37	F/M	900/919	19
37	F/M	900/900	0
38	F/F	577/965	388
39	F/F	997/997	0
43	M/F	739/478	−261

[a] M =male; F = female.

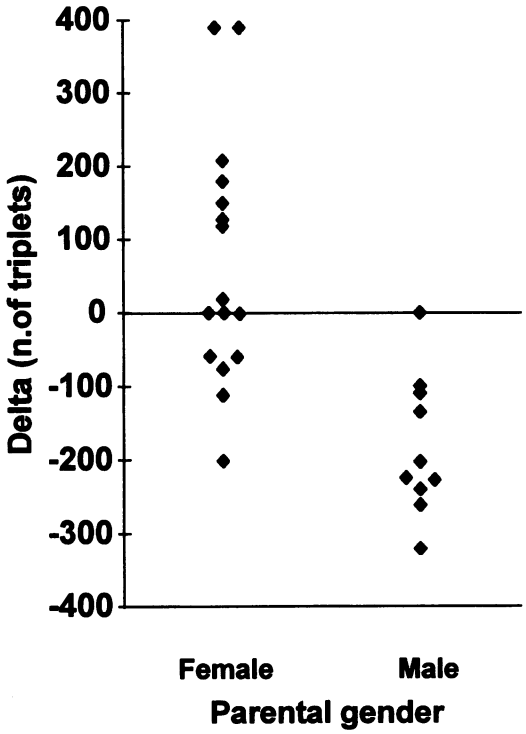

Figure 1 Relationship between intergenerational repeat length variation and parental gender. Contraction/expansion ratio differed according parental gender (Fisher's exact test $P = .004$).

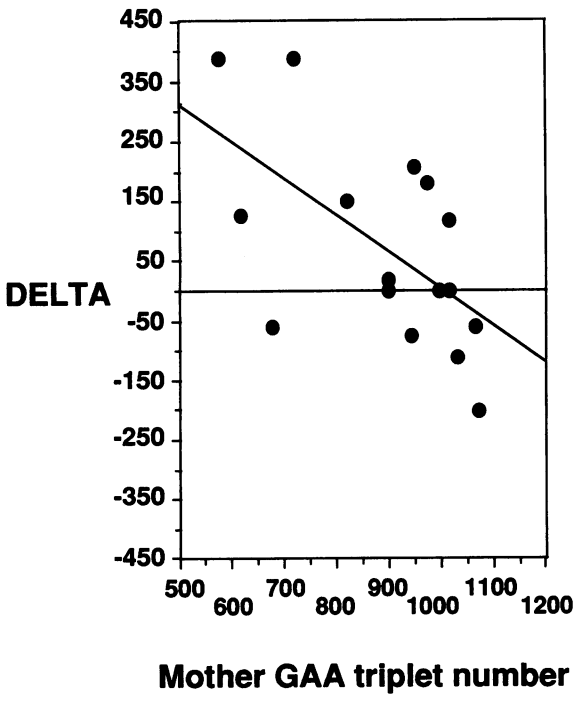

Figure 2 Correlation between mother GAA triplet number and delta. $r_p = -.060$; df = 14; $P < .01$.

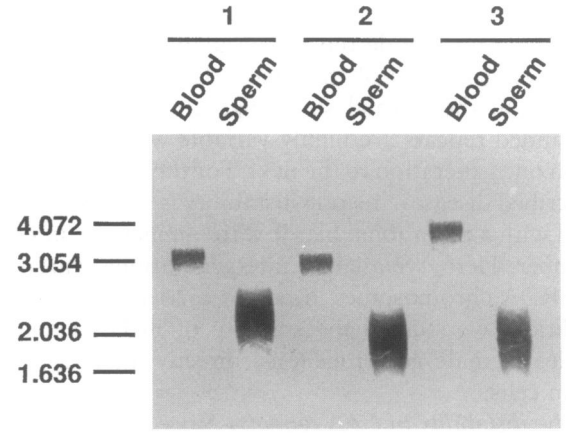

Figure 3 PCR amplification of sperm DNA and blood DNA from three FRDA gene carriers. Normal alleles are not shown. To better define expanded alleles in this experiment, normal alleles flowed out of the gel.

panding alleles. In pedigree 21, both parents carried the same extended haplotype (table 1). Although family data failed to reveal consanguinity, grandparents were born in the same restricted area. Therefore, it seems likely that they could carry the same FRDA gene. In this family, one child inherited an expanded allele from the mother and another child inherited a contracted allele from the father. In pedigrees 18 and 37, the same allele was inherited by two children from the mother, expanded in one case and unchanged in the other one. In pedigree 27, the same allele was inherited by two children from the father, contracted in one case and unchanged in the other.

The factors affecting trinucleotide repeat stability, normal allelic variation, and appearance of new disease alleles are not fully understood. FRDA is the first autosomal recessive disorder caused by a trinucleotide expansion (Campuzano et al. 1996). Expanded alleles are transmitted by asymptomatic carriers whose prevalence is rather high (1/70) in the general population (Filla et al. 1992). FRDA expanded alleles show a frequency of 1/140 chromosomes, at least one order of magnitude higher than any other characterized trinucleotide expansion. We demonstrated instability of the GAA expanded repeat during parent-offspring transmission in 85% of the cases. This variability is comparable with that observed in other trinucleotide expansions (Bates and Lehrach 1994). We also found a tendency to contract during parent-child transmission, whereas the other dynamic mutations show a tendency for gaining repeat units (Bates and Lehrach 1994). Few exceptions exist. Sperm typing in X-linked spinal and bulbar muscular atrophy suggests that large normal alleles have a mutation frequency somewhat higher than small alleles, with contractions outnumbering expansions, but that expansion is favored once a threshold size is reached (Zhang et

al. 1994). Furthermore, cases of reverse mutation with contraction to normal size have been rarely observed in myotonic dystrophy (Brunner et al. 1993) and in fragile X syndrome (Antiñolo et al. 1996).

The GAA repeat size on FRDA alleles showed a negatively skewed distribution, with the majority of chromosomes having shorter allele length than the mode (Filla et al. 1996). The prevalence of contractions on expansions is consistent with this kind of distribution. The major factor determining contraction of FRDA alleles is paternal transmission. In our sample, all fathers transmit shortened or unvaried alleles to their children. These data were also confirmed by sperm DNA analysis. In all three cases, the amplification of the FRDA gene from sperm yielded a diffuse array of shorter products in comparison with blood. To test preferential amplification of the shortest alleles, we added increasing amounts of blood DNA to sperm DNA during amplification. We could detect the longest alleles at 20% of blood DNA concentration. We suggest that the majority of the gametes carry alleles shorter than blood, but we are unable to define their size distribution. Parental gender effect on repeat length variation has been described in other triplet diseases, occurring on male transmission in CAG repeat diseases and female transmission in fragile X syndrome and myotonic dystrophy. Parental gender effect is more evident in FRDA, apparently conditioning the direction of the variation. Also, GAA repeat size affected instability, since the tendency to expand was more pronounced in the maternal shortest alleles. Further analyses on larger samples are needed to clarify the role of allele size on its variability.

In this study, we found no association between extended haplotypes of the region and FRDA allele tendency to expand or to contract. In addition, the analysis of several instances of intergenerational transmission of the same allele confirms that the parental gender effect is more important than the putative effect of *cis*-acting elements.

In summary, our data suggest that (i) the FRDA GAA repeat is highly unstable during meioses, (ii) contractions outnumber expansions, (iii) both parental source and sequence length are important factors in variability of FRDA expanded alleles, and (iv) the tendency to contract or expand does not seem associated with particular haplotypes. The emerging picture of FRDA gene variability seems to be different from that proposed for other triplet diseases.

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References

Antiñolo G, Borrego S, Cabeza J C, Sánchez R, Sánchez J, Sánchez B (1996) Reverse mutation in fragile X syndrome. Am J Hum Genet 58:237–239

Bates G, Lehrach H (1994) Trinucleotide repeat expansions and human genetic disease. Bioessays 16:277–284

Brunner HG, Jansen G, Nillesen W, Nelen MR, de Die CE, Howeler CJ, van Oost BA, et al (1993) Brief report: reverse mutation in myotonic dystrophy. N Engl J Med 328:476–479

Campuzano V, Montermini L, Moltò MD, Pianese L, Cossèe M, Cavalcanti F, Monros E, et al (1996) Friedreich ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science 271:1423–1427

Filla A, De Michele G, Cavalcanti F, Pianese L, Monticelli A, Campanella G, Cocozza S (1996) The relationship between trinucleotide (GAA) repeat length and clinical features in Friedreich ataxia. Am J Hum Genet 59:554–560

Filla A, De Michele G, Marconi R, Bucci L, Carillo C, Castellano AE, Iorio L, et al (1992) Prevalence of hereditary ataxias and spastic paraplegias in Molise, a region of Italy. J Neurol 239:351–353

Pianese L, Cocozza S, Campanella G, Castaldo I, Cavalcanti F, De Michele G, Filla A, et al (1994) Linkage disequilibrium between FD1-D9S202 haplotypes and Friedreich's ataxia locus in a central-southern Italian population. J Med Genet 31:133–135

Telenius H, Almqvist E, Kremer B, Spence N, Squitieri F, Nichol K, Grandell U, et al (1995) Somatic mosaicism in sperm is associated with intergenerational $(CAG)_n$ changes in Huntington disease. Hum Mol Genet 4:189–195

Zhang L, Leeflang EP, Yu J, Arnheim N (1994) Studying human mutations by sperm typing: instability of CAG trinucleotide repeats in the human androgen receptor gene. Nat Genet 7:531–535

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Contamination of Sequence Databases with Adaptor Sequences

To the Editor:

Because of the exponential increase in the amount of DNA sequences being added to the public databases on