

Distribution of CGG Repeats and FRAXAC1/DXS548 Alleles in South American Populations

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In order to assess the molecular variability related to fragile X (*FMR1* locus), we investigated the distribution of CGG repeats and DXS548/FRAXAC1 haplotypes in normal South American populations of different ethnic backgrounds. Special attention was given to Amerindian Wai-Wai (Northern Brazil) and Ache (Paraguay), as well as to Brazilian isolated communities of African ancestry, the remnants of *quilombos*. Comparison of samples from quilombos, Amerindians, and the ethnically mixed, but mainly European-derived population of São Paulo revealed that the 30-copy allele of the fragile X gene is the most frequent in all groups. A second peak at 20 repeats was present in the population of São Paulo only, confirming this as a European peculiarity. The distribution of DXS548 and FRAXAC1 alleles led to a high expected heterozygosity in African Brazilians, followed by that observed in the population of São Paulo. Amerindians showed the lowest diversity in CGG repeats and DXS548/FRAXAC1 haplotypes. Some rare alleles, for example, the 148-bp (FRAXAC1) or 200-bp (DXS548) variants, which seem to be almost absent in Europe, occurred in higher frequencies

among African Brazilians. This suggests a general trend for higher genetic diversity among Africans; these rarer alleles could be African in origin and would have been lost or possibly were not present in the groups that gave rise to the Europeans.

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KEY WORDS: fragile X; CGG repeats; microsatellite markers; population genetics

INTRODUCTION

Fragile X syndrome is one of the most frequent inherited forms of mental retardation. It results from expansions in the CGG repeat in the 5' untranslated region of the *FMR1* gene. This CGG region is polymorphic in the normal population and varies in size from 6 to about 50 repeats. A classification of fragile X mutations into premutations and full mutations has been proposed [Oberlé et al., 1991]. When 50–200 copies of CGG repeats are present, individuals are carriers of the premutation and it has apparently no negative effects on mental development [Rousseau et al., 1991]. When transmitted by females, the premutation has a high probability of expanding into a full mutation, and the risk of expansion increases with the size of the premutation [Heitz et al., 1992; Yu et al., 1992]. In carriers of the full mutation, the number of copies greatly exceeds 200 and transcription silencing of the *FMR1* gene occurs [Pieretti et al., 1991].

It has been questioned how CGG repeats in the normal size range (6–50 repeats) may expand into premutations, generating new disease alleles. Linkage disequilibrium with microsatellite markers flanking the *FMR1* gene was studied to determine the origin of fragile X mutations in populations from several countries, and certain predisposing haplotypes have been identified [reviewed in Chiurazzi et al., 1996a]. We also found

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strong linkage disequilibrium of the fragile X mutation with two DXS548/FRAXAC1 haplotypes when we compared fragile X and normal chromosomes in the Brazilian population [Mingroni-Netto et al., 1999].

The risk of expansion of a normal repeat into a pre-mutation allele was found to be associated with the interspersed of the CGG tract with AGGs, which seemed to protect normal CGG repeats from expanding into disease alleles [Hirst et al., 1994; Kunst and Warren, 1994; Snow et al., 1994; Eichler et al., 1995, 1996]. The latter authors proposed two main pathways for the origin of the mutation. In the first, large alleles of the DXS548 and FRAXAC1 markers (haplotype 2-1 or 204/158 bp) are associated with high numbers of CGG repeats, leading to predisposed chromosomes, which tend to gradually increase their number of repeats. Alternatively, there is another main risk haplotype (6-4 or 196/152 bp), which is not necessarily associated with high numbers of CGG copies, but to sequences that appear particularly prone to expand because of the loss of AGG interruptions. These alleles could progress rapidly into larger alleles through the instability of pure CGG tracts, mainly at the 3' end of the repeat. These above-indicated arrangements correspond to the two most frequently observed haplotypes in affected males in Brazil [Mingroni-Netto et al., 1999]. The two pathways may be interpreted as the result of unidentified cis-acting factors acting on or solely dependent on the structure of the repeats themselves.

Recently, new insights about the origin of fragile X mutations have emerged through population studies in African Americans [Crawford et al., 2000a, 2000b]. Several important differences were observed between African and European-derived American populations: two haplotypes, not previously associated with the mutation among Caucasians, were found to be frequent in African American fragile X chromosomes (4-4 or 200/152 bp and 3-4 or 202/152 bp). These data suggest that the haplotype associations identified in Caucasians may not be influenced by cis-acting sequences. On the other hand, they are consistent with the idea that the Caucasian mutational history differs from that of Africans: initial mutational events occurred on different haplotype backgrounds in different ethnic groups. Based on CGG structures associated with the most frequent fragile X haplotypes in African Americans, they proposed that the lack of a proximal (5') AGG interruption is a novel factor involved in CGG repeat instability [Crawford et al., 2000b].

In the present study we determined the distribution of CGG repeats and linked DXS548/FRAXAC1 haplotypes in South American populations of different ethnic backgrounds. Special attention was given to Amerindians and isolated communities of African ancestry. Before 1888, when slavery was abolished in Brazil, many communities (*quilombos*) were founded by African slaves who had escaped or were abandoned by the owners of the land where they worked. Today, these communities are referred to as remnants of quilombos, and they remain at least partially genetically isolated. It is estimated that there are 700 such communities spread over the Brazilian territory. They can be regarded as relics of the

original African contribution to the composition of the present Brazilian population. We studied communities located in the states of Bahia (Rio das Rãs group) and Maranhão (Pontal and Cajual).

Individuals from two Amerindian populations, the Ache and the Wai-Wai, were also studied. Wai-Wai is a generic identification of a group of people who speak dialect variations of the linguistic family Parukoto-Charumã, of the Carib stock. They live in the Mapuera River basin, medium and high Nhamundá, headwaters of the Jatapu and Trombetas Rivers, at the frontier between Brazil and Guiana. The Wai-Wai have been extensively studied from demographic and genetic points of view [Callegari-Jacques et al., 1996]. The Ache are one of the most southerly representatives of the tropical forest Tupi linguistic stock. They reside in the Alto Paraná area of eastern Paraguay and have been extensively studied ecologically, medically, and demographically [Hill and Hurtado, 1996].

SUBJECTS AND METHODS

Subjects

African Brazilians

- 54 individuals (22 males and 32 females) from the remnant of quilombo Pontal. This community comprises 312 individuals of African ancestry and is located near the town of Bequimão, state of Maranhão.
- 35 individuals (12 males and 23 females) from the community of Cajual. Cajual is an island with 216 inhabitants, near the town of Alcântara, state of Maranhão.
- 166 individuals (47 males and 119 females) from quilombo communities located in the region of Rio das Rãs, near the town of Bom Jesus da Lapa, state of Bahia. The Rio das Rãs group comprises a set of quilombo communities with about 3,000 inhabitants; most samples were obtained from the largest communities of Rio das Rãs and Brasileira. Other smaller quilombos in the same region are Capão do Cedro, Enchu, and Bom Retiro.

Amerindians

- 26 Ache individuals (14 males and 12 females) from eastern Paraguay, whose samples were obtained at the Arroyo Bandera and Chupa Pou reservations.
- 16 Wai-Wai (6 males and 10 females) living near the frontier between Brazil and Guiana.

Euro-Brazilians

- 64 normal chromosomes from heterozygous mothers of fragile X patients studied in our genetic counseling unit [Mingroni-Netto et al., 1999]. The ethnically mixed population of São Paulo is predominantly European in origin, but as revealed by other molecular studies of Brazilian populations, European-derived people in Brazil have certainly experienced a considerable amount of genetic admixture with native (Amerindians) and African-derived persons.

The population of São Paulo is representative of this ethnic heterogeneity.

The localization of all samples studied is shown in Figure 1.

Methods

PCR amplifications of FRAXAC1 and DXS548 alleles were performed as previously described [Mingroni-Netto et al., 1999]. The allele nomenclature used here and their correspondence to other denominations are presented in Tables I and II.

PCR amplifications of CGG repeats at locus *FMR1* were performed with primers c and f [Fu et al., 1991], according to a protocol previously described [Kenneson et al., 1997], except that "the perfect match" (Stratagene) was not employed.

Electrophoresis conditions and sizing of alleles have also been described [Mingroni-Netto et al., 1999]. The CGG repeat number was determined through electrophoresis in sequencing acrylamide gels and comparison of the strongest radioactive PCR bands with an M13mp18 sequencing ladder. According to the original sequence [Fu et al., 1991], the modal 308-bp PCR product

corresponded to the 29 CGG allele. However, after sequencing some individuals from the total sample, in an ABI 377, it was clear that the modal repeat number corresponded to 30 copies. Therefore, one repeat was added to the estimated copy number after gel electrophoresis.

The methods for obtaining population variability estimates (expected and observed heterozygosity levels) and of fixation indexes (F) are described in standard textbooks such as Nei [1987].

RESULTS AND DISCUSSION

Distribution of CGG Repeats

The distribution of the CGG repeats found in all populations studied is presented in Table III. The most frequent fragile X CGG repeat observed in Euro-Brazilians from São Paulo, Quilombo remnants (African Brazilians), and Amerindians was that of 30 copies.

The modal repeat number in several populations of European ancestry was reported as 30 [Brown et al., 1993; Chiurazzi et al., 1996c; Kunst et al., 1996] or 29 [Fu et al., 1991; Jacobs et al., 1993; Macpherson et al., 1994; Syrrou et al., 1996]. We observed after comparison



Fig. 1. Localization of the populations studied. PO = Pontal, CA = Cajual, RR = Rio das Rãs, AC = Ache, WW = Wai-Wai, SP = São Paulo.

TABLE I. Correspondence Between Allele Denominations for DXS548 Alleles

| Reference | Alleles | | | | | | |
|---|---------|--------|--------|--------|--------|--------|--------|
| | 194 bp | 196 bp | 198 bp | 200 bp | 202 bp | 204 bp | 206 bp |
| Riggins et al. [1992] and present study | | | | | | | |
| Macpherson et al. [1994] | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| Chiurazzi et al. [1999] | T40 | T42 | T44 | T46 | T48 | T50 | T52 |
| Crawford et al. [2000a] | 195 | 197 | 199 | 201 | 203 | 205 | 207 |

of the strongest band with an M13mp18 sequencing ladder that the modal repeat number was 29. The same method was employed by others [Fu et al., 1991; Jacobs et al., 1993]. However, preliminary sequencing results from our own laboratory have shown 30 CGGs for individuals previously classified as 29 after gel electrophoresis. So, we considered that the modal repeat number is in fact 30 and added one triplet to our former classification of alleles. Discrepancy is probably due to an artifact of mobility of the repeat on gel [Warren and Nelson, 1994]. It is likely, as others have previously observed [Chiurazzi et al., 1999], that some differences in the modal repeats in different populations may occur as a consequence of problems in allele standardization: most European populations must have a mode of 30 repeats. This, however, could be classified as 29 in some laboratories, depending on the method of size determination.

The distribution of CGG repeats in the mixed population of São Paulo shows a secondary peak at 20 (or 19 copies after electrophoresis), with a frequency of 19.0%. This peak is absent in quilombos and Amerindians. The population from São Paulo comprises an admixture of genes from several ethnic groups, including an important Portuguese contribution through colonization and Amerindian and African contributions, obtained through their historical interbreeding. Later on, in the last hundred years, other immigrants came from Europe (Italians, Spaniards, and Germans) and Asia (Japanese and Chinese). Despite this admixture, the CGG distribution observed in São Paulo is very similar, including the peak around 20 copies, to the large majority of European samples reported in the literature. On the other hand, in the African samples described to date [Chiurazzi et al., 1996b (Bamileke, Cameroon); Kunst et al., 1996 (Wolof and Mandenka, Senegal); Eichler and Nelson, 1996 (Baka and Mbuti Pygmy)] and among African Americans [Eichler et al., 1995; Crawford et al., 2000a], the 19-or 20-copy allele is rare. In quilombos, the absence of the peak around 19 or 20 copies might explain somewhat the lower expected

heterozygosity of CGG repeats in African Brazilians, when compared to those of São Paulo (Table III). In Japanese [Arinami et al., 1993], Chinese [Pang et al., 1999], and other Asians [Eichler et al., 1995; Chen et al., 1997], this peak does not occur either. Our results corroborate that the high frequency of 19 or 20 copies is a European peculiarity. One could speculate that this allele increased its frequency in Western Europe, probably much later than the early dispersals from ancestral populations coming from Africa. In Asians, a second peak around 36 or 37 copies seems to be characteristic [Arinami et al., 1993; Eichler et al., 1995; Chen et al., 1997; Pang et al., 1999]. The higher frequency of 20-copy alleles among Europeans and 36-copy alleles among Asians seem to be a signature of founder effects in groups that gave rise to the present Asian and Western European populations.

Although sample sizes were limited, in Amerindians only 29 and 30 copies of the repeat were present. South American Indians were previously studied by Eichler and Nelson [1996]: 10 Karitiana, 10 Mayans, and 12 Surui. Only two repeat lengths (29 and 30) were found among Mayan and Surui, and four alleles (29, 30, 40, and 41) were reported among the Karitiana. These findings suggest that genetic variability of CGG repeats is much lower among Amerindians than Africans.

FRAXAC1 and DXS548 Alleles Distribution

The distribution of FRAXAC1 alleles presented in Table IV shows some peculiarities of the quilombo communities. A higher expected heterozygosity was observed for all the African ancestry communities. Similar values were obtained in the Bamileke of Cameroon [Chiurazzi et al., 1996b]. This can be partially explained by the occurrence of a 156-bp allele, which is absent in the São Paulo sample and in Amerindians, and the presence of other rare alleles among Africans. It is remarkable that the rare 148-bp allele is present in the mixed population of São Paulo and in quilombo communities in frequencies of 1.4% to 3.8%, respectively. This allele has been reported only once in Europe, in a sample from the United Kingdom [Jacobs et al., 1993]. On the other hand, it was reported in the Bamilekes [Chiurazzi et al., 1996b] and in Mandenka of Senegal and African Americans [Kunst et al., 1996]. This suggests that this allele is probably African in origin and was not present in the African populations that migrated to Europe, or was subsequently lost.

The same trend toward higher genetic diversity in the African-derived communities was observed in the DXS548 locus (Table V). Rare alleles in São Paulo, like

TABLE II. Correspondence Between Allele Denominations for FRAXAC1 Alleles

| Reference | Alleles | | | |
|---------------------------------------|---------|--------|--------|--------|
| | D | C | B | A |
| Richards et al. [1991] | | | | |
| Zhong et al. [1993] and present study | 152 bp | 154 bp | 156 bp | 158 bp |
| Macpherson et al. [1994] | 4 | 3 | 2 | 1 |
| Zhong et al. [1995] | 18 | 19 | 20 | 21 |
| Chiurazzi et al. [1999] | T36 | T38 | T40 | T42 |
| Crawford et al. [2000a] | 109 | 111 | 113 | 115 |

TABLE III. Distribution of CGG Repeats in Normal X Chromosomes From Quilombo Communities (African Brazilians), Amerindians, and Euro-Brazilians From São Paulo

| No. of CGG repeats | Quilombos | | | | | | Rio das Rás' group | | | | | | Amerindians | | | Euro-Brazilians | | |
|-------------------------|-------------------|------|-------------------|------|-------------------|------|--------------------|------|--------|------|-------|------|-------------|------|---------|-----------------|-----------|------|
| | Pontal | | Cajual | | Rio das Rás | | Brasileira | | Others | | Total | | Ache | | Wai-Wai | | São Paulo | |
| | N | % | N | % | N | % | N | % | N | % | N | % | N | % | N | % | N | % |
| 15 | 0 | | 0 | | 1 | 1.2 | 0 | | 0 | | 1 | 0.4 | 0 | | 0 | | 0 | |
| 17 | 0 | | 0 | | 0 | | 0 | | 1 | 0.8 | 1 | 0.4 | 0 | | 0 | | 0 | |
| 20 | 0 | | 1 | 1.7 | 6 | 7.2 | 2 | 3.9 | 2 | 1.6 | 10 | 3.8 | 0 | | 0 | | 11 | 18.6 |
| 21 | 0 | | 1 | 1.7 | 2 | 2.4 | 0 | | 2 | 1.6 | 4 | 1.5 | 0 | | 0 | | 2 | 3.4 |
| 22 | 1 | 1.2 | 2 | 3.4 | 5 | 6.0 | 1 | 2.0 | 7 | 5.5 | 13 | 5.0 | 0 | | 0 | | 0 | |
| 23 | 5 | 5.8 | 0 | | 2 | 2.4 | 2 | 3.9 | 5 | 3.9 | 9 | 3.4 | 0 | | 0 | | 3 | 5.1 |
| 24 | 1 | 1.2 | 4 | 6.9 | 1 | 1.2 | 0 | | 1 | 0.8 | 2 | 0.8 | 0 | | 0 | | 0 | |
| 25 | 1 | 1.2 | 0 | | 4 | 4.8 | 0 | | 1 | 0.8 | 5 | 1.9 | 0 | | 0 | | 0 | |
| 26 | 2 | 2.3 | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 2 | 3.4 |
| 27 | 1 | 1.2 | 0 | | 1 | 1.2 | 1 | 2.0 | 0 | | 2 | 0.8 | 0 | | 0 | | 0 | |
| 28 | 3 | 3.5 | 0 | | 2 | 2.4 | 0 | | 1 | 0.8 | 3 | 1.1 | 0 | | 0 | | 0 | |
| 29 | 25 | 29.1 | 9 | 15.5 | 22 | 26.5 | 6 | 11.8 | 33 | 26.0 | 61 | 23.4 | 1 | 6.7 | 1 | 20 | 10 | 17.0 |
| 30 | 27 | 31.4 | 27 | 46.5 | 19 | 22.9 | 22 | 43.1 | 49 | 38.6 | 90 | 34.5 | 14 | 93.3 | 4 | 80 | 16 | 27.1 |
| 31 | 11 | 12.8 | 7 | 12.1 | 15 | 18.1 | 7 | 13.7 | 12 | 9.4 | 34 | 13.0 | 0 | | 0 | | 6 | 10.2 |
| 32 | 3 | 3.5 | 0 | | 1 | 1.2 | 1 | 2.0 | 2 | 1.6 | 4 | 1.5 | 0 | | 0 | | 2 | 3.4 |
| 33 | 1 | 1.2 | 1 | 1.7 | 0 | | 1 | 2.0 | 0 | | 1 | 0.4 | 0 | | 0 | | 0 | |
| 34 | 1 | 1.2 | 0 | | 0 | | 1 | 2.0 | 1 | 0.8 | 2 | 0.8 | 0 | | 0 | | 0 | |
| 35 | 0 | | 2 | 3.4 | 0 | | 2 | 3.9 | 5 | 3.9 | 7 | 2.7 | 0 | | 0 | | 0 | |
| 36 | 0 | | 1 | 1.7 | 0 | | 3 | 5.9 | 1 | 0.8 | 4 | 1.5 | 0 | | 0 | | 0 | |
| 37 | 0 | | 1 | 1.7 | 0 | | 1 | 2.0 | 1 | 0.8 | 2 | 0.8 | 0 | | 0 | | 0 | |
| 38 | 1 | 1.2 | 0 | | 1 | 1.2 | 1 | 2.0 | 1 | 0.8 | 3 | 1.1 | 0 | | 0 | | 0 | |
| 39 | 2 | 2.3 | 1 | 1.7 | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | |
| 40 | 0 | | 0 | | 0 | | 0 | | 1 | 0.8 | 1 | 0.4 | 0 | | 0 | | 1 | 1.7 |
| 41 | 0 | | 1 | 1.7 | 1 | 1.2 | 0 | | 0 | | 1 | 0.4 | 0 | | 0 | | 3 | 5.1 |
| 42 | 1 | 1.2 | 0 | | 0 | | 0 | | 1 | 0.8 | 1 | 0.4 | 0 | | 0 | | 2 | 3.4 |
| 43 | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 1 | 1.7 |
| Total | 86 | | 58 | | 83 | | 51 | | 127 | | 261 | | 15 | | 5 | | 59 | |
| Expected heterozygosity | 0.802 ± 0.020 | | 0.702 ± 0.038 | | 0.831 ± 0.015 | | 0.770 ± 0.036 | | — | | — | | — | | — | | 0.842 | |
| Observed heterozygosity | 0.469 ± 0.109 | | 0.478 ± 0.084 | | 0.424 ± 0.108 | | 0.500 ± 0.063 | | — | | — | | — | | — | | — | |
| F | 0.416 ± 0.073 | | 0.319 ± 0.084 | | 0.489 ± 0.067 | | 0.351 ± 0.115 | | — | | — | | — | | — | | — | |

TABLE IV. Distribution of FRAXAC1 Alleles in Normal X Chromosomes From Quilombo Communities (African Brazilians), Amerindians, and Euro-Brazilians From São Paulo

| FRAXAC1 alleles | Quilombos | | | | | | Amerindians | | | | | | Euro-Brazilians | | | | | | | |
|-------------------------|--------------------|------|---------------|------|---------------|------|---------------|------|--------|------|-------|------|-----------------|------|---------------|------|------------------------|------|---|--|
| | Rio das Rás' group | | | | | | | | | | | | | | | | | | | |
| | Pontal | | Cajual | | Rio das Rás | | Brasileira | | Others | | Total | | Ache | | Wai-Wai | | São Paulo ^a | | | |
| N | % | N | % | N | % | N | % | N | % | N | % | N | % | N | % | N | % | N | % | |
| 158 bp | 1 | 1.2 | 0 | | 4 | 4.4 | 2 | 3.5 | 5 | 3.6 | 11 | 3.9 | 0 | 0 | 0 | 0 | 7 | 10.9 | | |
| 156 bp | 5 | 5.9 | 2 | 3.8 | 13 | 14.4 | 4 | 7.0 | 22 | 15.9 | 39 | 13.7 | 0 | 0 | 0 | 0 | 0 | | | |
| 154 bp | 43 | 50.6 | 31 | 58.5 | 41 | 45.6 | 38 | 66.7 | 62 | 44.9 | 141 | 49.5 | 29 | 80.6 | 14 | 77.8 | 43 | 67.2 | | |
| 152 bp | 33 | 38.8 | 18 | 34.0 | 26 | 28.9 | 11 | 19.3 | 44 | 31.9 | 81 | 28.4 | 7 | 19.4 | 4 | 22.2 | 12 | 18.8 | | |
| 150 bp | 0 | | 0 | | 4 | 4.4 | 0 | | 3 | 2.2 | 7 | 2.5 | 0 | 0 | 0 | 0 | 1 | 1.6 | | |
| 148 bp | 3 | 3.5 | 2 | 3.8 | 2 | 2.2 | 2 | 3.5 | 2 | 1.4 | 6 | 2.1 | 0 | 0 | 0 | 0 | 1 | 1.6 | | |
| Total | 85 | | 53 | | 90 | | 57 | | 138 | | 285 | | 36 | | 18 | | 64 | | | |
| Expected heterozygosity | 0.589 ± 0.021 | | 0.540 ± 0.033 | | 0.684 ± 0.022 | | 0.511 ± 0.048 | | — | | — | | 0.313 ± 0.057 | | 0.346 ± 0.078 | | 0.501 | | — | |
| Observed heterozygosity | 0.484 ± 0.090 | | 0.429 ± 0.108 | | 0.486 ± 0.084 | | 0.429 ± 0.108 | | — | | — | | 0.583 ± 0.142 | | 0.429 ± 0.187 | | — | | — | |
| F | 0.178 ± 0.106 | | 0.206 ± 0.131 | | 0.290 ± 0.085 | | 0.161 ± 0.106 | | — | | — | | — | | — | | — | | — | |

^aMingroni-Netto et al. [1999].

TABLE V. Distribution of DXS548 Alleles in Normal X Chromosomes From São Paulo, From Quilombo Communities (African Brazilians), Amerindians, and Euro-Brazilians From São Paulo

| DXS548 alleles | Quilombos | | | | | | Amerindians | | | | | | Euro-Brazilians | | | | | | | |
|-------------------------|--------------------|------|---------------|------|---------------|------|---------------|------|--------|------|-------|------|-----------------|-------|---------|-------|------------------------|------|---|--|
| | Rio das Rás' group | | | | | | | | | | | | | | | | | | | |
| | Pontal | | Cajual | | Rio das Rás | | Brasileira | | Others | | Total | | Ache | | Wai-Wai | | São Paulo ^a | | | |
| N | % | N | % | N | % | N | % | N | % | N | % | N | % | N | % | N | % | N | % | |
| 206 bp | 1 | 1.2 | 0 | | 1 | 1.1 | 0 | | 0 | | 1 | 0.4 | 0 | 0 | 0 | 0 | 2 | 3.3 | | |
| 204 bp | 6 | 7.0 | 2 | 3.7 | 6 | 6.9 | 0 | | 5 | 3.9 | 11 | 4.1 | 0 | 0 | 0 | 0 | 6 | 10 | | |
| 202 bp | 7 | 8.1 | 3 | 5.6 | 10 | 11.5 | 5 | 9.6 | 8 | 6.2 | 23 | 8.6 | 0 | 0 | 0 | 0 | 0 | | | |
| 200 bp | 3 | 3.5 | 1 | 1.9 | 12 | 13.8 | 4 | 7.7 | 13 | 10.1 | 29 | 10.8 | 0 | 0 | 0 | 0 | 1 | 1.7 | | |
| 198 bp | 8 | 9.3 | 4 | 7.4 | 5 | 5.7 | 4 | 7.7 | 21 | 16.3 | 30 | 11.2 | 0 | 0 | 0 | 0 | 2 | 3.3 | | |
| 196 bp | 19 | 22.1 | 11 | 20.4 | 12 | 13.8 | 10 | 19.2 | 23 | 17.8 | 45 | 16.8 | 0 | 0 | 0 | 0 | 9 | 15.0 | | |
| 194 bp | 42 | 48.8 | 33 | 61.1 | 38 | 43.7 | 29 | 55.8 | 59 | 45.7 | 126 | 47.0 | 38 | 100.0 | 26 | 100.0 | 39 | 65.0 | | |
| 192 bp | 0 | | 0 | | 3 | 3.4 | 0 | | 0 | | 3 | 1.1 | 0 | 0 | 0 | 0 | 0 | | | |
| 190 bp | 0 | | 0 | | 87 | | 0 | | 0 | | 0 | | 0 | 0 | 0 | 0 | 1 | 1.7 | | |
| Total | 86 | | 54 | | 87 | | 52 | | 129 | | 268 | | 38 | | 26 | | 60 | | | |
| Expected heterozygosity | 0.691 ± 0.028 | | 0.575 ± 0.046 | | 0.749 ± 0.025 | | 0.631 ± 0.042 | | — | | — | | — | | — | | 0.542 | | — | |
| Observed heterozygosity | 0.500 ± 0.088 | | 0.429 ± 0.108 | | 0.471 ± 0.086 | | 0.368 ± 0.111 | | — | | — | | — | | — | | — | | — | |
| F | 0.277 ± 0.081 | | 0.254 ± 0.104 | | 0.371 ± 0.072 | | 0.416 ± 0.108 | | — | | — | | — | | — | | — | | — | |

^aMingroni-Netto et al. [1999].

202 and 200 bp, were observed in higher frequencies in the quilombos. There is a remarkable lack of genetic variation in this locus and FRAXAC1 among Amerindians (Tables IV and V). It would be interesting to see if the same results are verified in larger samples.

The general trend for higher heterozygosity in our African-derived communities is in agreement with the general finding of higher levels of African diversity in mtDNA, a number of autosomal microsatellites, and several Y chromosome loci [Jorde et al., 2000; Ingman et al., 2000]. This gives support to an African origin of modern *Homo sapiens* or, alternatively, to suggestions of larger effective population sizes in Africa or earlier population expansions in Africa than in Europe or Asia [Bowcock et al., 1994; Jorde et al., 1995, 1997; Shriver et al., 1997].

It is interesting that Brazilian quilombos still show similarity with the original African diversity, detected in their high expected heterozygosity indices. This diversity still persists despite the fact that populations are small and were at least partially isolated for centuries. This might be due to: 1) genetic admixture in the foundation of quilombos, since Brazilian slaves belonged to different African ethnic groups; and 2) genetic admixture with Europeans and Amerindians; there are records, for at least some of the most studied

quilombos, that Amerindians and even Europeans joined Africans in their resistance to the colonial slavery society. Also, it is well known that slave women often had children fathered by their Portuguese masters. The extent of this admixture is being addressed by a number of Brazilian geneticists who are estimating Amerindian and European genetic contribution toward quilombos and Brazilian Caucasian populations using molecular markers [Alves-Silva et al., 2000].

A low genetic diversity in Amerindians is not unexpected, since they usually live in small groups, with limited gene flow between tribes. These populations are much older than the quilombos and seem to have experienced more geographic isolation, leading to extensive founder effects and bottlenecks.

In all three systems tested, there appears to be a deficit in observed, when compared to expected, heterozygosity. This led to very high estimates of F in all groups. In São Paulo, F could not be calculated because the sample was obtained from normal X chromosomes of fragile X mothers; thus, all were heterozygotes for the fragile X mutation. For combining the F values obtained for the three loci of each population, we averaged the individual F values by the reciprocals of their variances. We obtained F values of 0.3173, 0.2757, 0.3982, and 0.3151 for Pontal, Cajual, Rio das Rãs, and Brasileira,

TABLE VI. DXS548/FRAXAC1 Haplotypes in Males, and Females Whose Haplotypes Could Be Determined (Homozygotes in One of the Microsatellites)

| DXS548/FRAXAC1 haplotypes (bp) | Quilombos | | | | | | Amerindians | | Euro-Brazilians | |
|-----------------------------------|-----------|------|--------|------|--------------------|------|-------------|------|------------------------|------|
| | Pontal | | Cajual | | Rio das Rãs' group | | N | % | São Paulo ^a | |
| | N | % | N | % | N | % | | | N | % |
| 194/154 | 23 | 32.9 | 20 | 46.5 | 61 | 26.4 | 43 | 81.1 | 35 | 58.3 |
| 196/152 ^b | 9 | 12.9 | 4 | 9.3 | 14 | 6.1 | | | 5 | 8.3 |
| 204/152 | 0 | | 0 | | 2 | 0.9 | | | 4 | 6.7 |
| 204/158 ^b | 1 | 1.4 | 0 | | 1 | 0.4 | | | 2 | 3.3 |
| 194/152 | 13 | 18.6 | 6 | 14.0 | 27 | 11.7 | 10 | 18.9 | 2 | 3.3 |
| 196/154 | 5 | 7.1 | 5 | 11.6 | 16 | 6.9 | | | 2 | 3.3 |
| 206/158 | 0 | | 0 | | 0 | | | | 2 | 3.3 |
| 194/158 | 0 | | 0 | | 2 | 0.9 | | | 1 | 1.7 |
| 198/154 | 3 | 4.3 | 1 | 2.3 | 16 | 6.9 | | | 1 | 1.7 |
| 194/148 | 1 | 1.4 | 0 | | 3 | 1.3 | | | 1 | 1.7 |
| 196/148 | 1 | 1.4 | 0 | | 0 | | | | 1 | 1.7 |
| 200/152 ^c | 2 | 2.9 | 0 | | 8 | 3.5 | | | 1 | 1.7 |
| 198/148 | 0 | | 1 | 2.3 | 1 | 0.4 | | | 0 | |
| 200/154 | 0 | | 1 | 2.3 | 10 | 4.3 | | | 0 | |
| 202/152 ^c | 0 | | 1 | 2.3 | 5 | 2.2 | | | 0 | |
| 204/154 | 3 | 4.3 | 0 | | 3 | 1.3 | | | 0 | |
| 202/154 | 3 | 4.3 | 0 | | 13 | 5.6 | | | 0 | |
| 192/154 | 0 | | 0 | | 2 | 0.9 | | | 0 | |
| 194/156 | 2 | 2.9 | 2 | 4.6 | 20 | 8.7 | | | 0 | |
| 200/156 | 0 | | 0 | | 3 | 1.3 | | | 0 | |
| 198/152 | 1 | 1.4 | 2 | 4.6 | 8 | 3.5 | | | 0 | |
| 204/156 | 0 | | 0 | | 2 | 0.9 | | | 0 | |
| 196/156 | 0 | | 0 | | 2 | 0.9 | | | 0 | |
| 196/158 | 0 | | 0 | | 2 | 0.9 | | | 0 | |
| 198/156 | 0 | | 0 | | 2 | 0.9 | | | 0 | |
| Others | 3 | 4.3 | 0 | | 8 | 3.5 | | | 3 | 5.0 |
| Total | 70 | | 43 | | 231 | | 53 | | 60 | |

^aMingroni-Netto et al. [1999].

^bMost frequent haplotypes in fragile X chromosomes among European-derived populations.

^cMost frequent haplotypes in fragile X chromosomes among African Americans [Crawford et al., 2000a].

TABLE VII. DXS548/FRAXAC1/CGG Haplotypes in Males and Females Whose Haplotypes could Be Determined (Homozygotes in Two of the Microsatellites)

| DXS548/FRAXAC1 haplotypes (bp) | Number of CGG repeats | Number of individuals | | | | |
|-----------------------------------|--------------------------|-----------------------|--------|-----------------------|-------------|-----------|
| | | Pontal | Cajual | Rio das Rás' group | Amerindians | São Paulo |
| 194/154 | 20 | | 1 | 3 | | 10 |
| | 21 | | | | | 1 |
| | 23 | 3 | | | | 2 |
| | 26 | | | 1 | | 1 |
| | 28 | 1 | | | | 1 |
| | 29 | 3 | 3 | 6 | | 3 |
| | 30 | 12 | 12 | 30 | 13 | 8 |
| | 31 | 1 | | 5 | | 2 |
| | 32 | | | | | 1 |
| | 34 | | | 1 | | |
| | 36 | | 1 | | | |
| | 37 | | | 1 | | |
| | 41 | | | | | 1 |
| Total | | 20 | 17 | 47 | 13 | 30 |
| 194/152 | 17 | | | 1 | | |
| | 22 | | | 5 | | |
| | 24 | | 2 | | | |
| | 29 | 5 | 2 | 6 | 1 | |
| | 30 | 4 | | 6 | | |
| | 31 | 4 | | 3 | | |
| | 36 | | | 1 | | |
| | 41 | | | | | 1 |
| Total | | 13 | 4 | 22 | 1 | 1 |
| 194/156 | 22 | | | 1 | | |
| | 23 | | | 2 | | |
| | 29 | | | 1 | | |
| | 30 | | | 10 | | |
| | 31 | | 1 | 2 | | |
| | 34 | | | 1 | | |
| Total | | 0 | 1 | 17 | 0 | 0 |
| 196/152 ^a | 22 | 1 | | | | |
| | 23 | 1 | | | | |
| | 25 | | | 2 | | |
| | 28 | 2 | | | | |
| | 29 | 2 | 1 | 5 | | 1 |
| | 30 | 1 | 3 | | | 1 |
| | 31 | | | 1 | | |
| | 32 | | | | | 1 |
| | 34 | | | | | |
| | 35 | | | 3 | | |
| | 42 | | | | | 1 |
| Total | | 7 | 4 | 11 | | 4 |
| 204/158 ^a | 29 | 1 | | | | |
| | 41 | | | | | 1 |
| | 43 | | | | | 1 |
| Total | | 1 | 0 | 0 | 0 | 2 |
| 196/154 | 21 | | | | | |
| | 23 | | | | | 1 |
| | 28 | | | | | 1 |
| | 29 | 2 | | 1 | | |
| | 30 | 1 | 2 | 9 | | |
| | 31 | | 1 | 1 | | |
| | 35 | | 1 | | | |
| | 38 | | | 1 | | |
| Total | | 3 | 4 | 12 | | 2 |
| 200/152 ^b | 29 | | | 4 | | |
| | 30 | | | 1 | | |
| | 31 | 1 | | 1 | | |
| | 32 | | | 1 | | |
| Total | | 1 | 0 | 7 | | |

TABLE VII. (Continued)

| DXS548/FRAXAC1 haplotypes (bp) | Number of CGG repeats | Number of individuals | | | | |
|-----------------------------------|--------------------------|-----------------------|--------|-----------------------|-------------|-----------|
| | | Pontal | Cajual | Rio das Rás' Group | Amerindians | São Paulo |
| 202/152 ^b | 29 | 1 | | 4 | | |
| | 30 | | | 1 | | |
| Total | | 1 | 0 | 5 | 0 | 0 |
| Others | | 7 | 3 | 64 | 0 | 9 |
| Total | | 53 | 33 | 185 | 14 | 48 |

^aMost frequent haplotypes in fragile X chromosomes among European-derived populations.

^bMost frequent haplotypes in fragile X chromosomes among African Americans [Crawford et al., 2000a].

respectively. We also calculated the combined F for another sample of 96 females, collected in the same laboratory in São Paulo, for menopause studies [Costa, personal communication]; in this group, $F = -0.020$. Thus, it is likely that high values of F obtained in quilombos may be the consequence of frequent endogamous marriages in small populations or population structures not detected in the collection of samples.

DXS548/FRAXAC1/CGG Haplotypes

Table VI shows the most frequent DXS548/FRAXAC1 haplotypes in all samples, indicating those previously associated with fragile X mutations. Table VII shows DXS548/FRAXAC1/CGG haplotypes. The most frequent DXS548/FRAXAC1 haplotype is 194/154 (7-3), which is frequently associated with the 30-copy allele. The 20-copy allele observed in São Paulo always appeared in a 194/154 background. The main risk haplotype for fragile X in Brazil, 204/158, appeared twice in São Paulo and only once in Pontal and Rio das Rás. In São Paulo, it was associated with the high repeat numbers of 41 and 43. The other risk haplotype for Europeans, the second more frequent in fragile X syndrome in Brazil (196/152 or 7-4) occurred among African Brazilians and in São Paulo associated with different repeat numbers, and did not occur among Amerindians. The risk haplotypes in African Americans, 200/152 or 4-4 and 202/152 or 3-4 [Crawford et al., 2000a], are rare in quilombos and occurred only once in São Paulo. The DNA of a subset of individuals, especially those presenting with risk alleles or haplotypes, will be submitted to sequencing studies. Our aim is to test the hypothesis that lack of 5' AGG interruptions in the CGG tract is also a predisposing factor in CGG expansions [Crawford et al., 2000b].

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REFERENCES

- Alves-Silva J, Santos MS, Guimarães PEM, Ferreira ACS, Bandelt H-J, Pena SDJ, Prado VF. 2000. The ancestry of Brazilian mtDNA lineages. *Am J Hum Genet* 67:444–461.
- Arinami T, Asano M, Kobayashi K, Yanagi H, Hamagushi H. 1993. Data on the CGG repeat at the fragile X site in the non-retarded Japanese population and families suggest the presence of a subgroup of normal alleles predisposing to mutate. *Hum Genet* 93:431–436.
- Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza LL. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* 368:455–457.
- Brown WT, Houck GE, Jeziorowska A, Levinson FN, Ding X, Dobkin C, Zhong N, Henderson J, Brooks SS, Jenkins EC. 1993. Rapid fragile X carrier screening and prenatal diagnosis using a nonradioactive PCR test. *JAMA* 270:1569–1575.
- Callegari-Jacques SM, Salzano FM, Weimer TA, Franco MHL, Mestriner MA, Hutz MH, Schuler L. 1996. The Wai-Wai Indians of South America: history and genetics. *Ann Hum Biol* 23:189–201.
- Chen S-H, Schoof JM, Buroker NE, Scott CR. 1997. The identification of a (CGG)₆ AGG insertion within the CGG repeat of the *FMR1* gene in Asians. *Hum Genet* 99:793–795.
- Chiurazzi P, Macpherson J, Sherman S, Neri G. 1996a. Significance of linkage disequilibrium between the fragile X locus and its flanking markers. *Am J Med Genet* 64:203–208.
- Chiurazzi P, Destro-Bisol G, Genuardi M, Oostra BA, Spedini G, Neri G. 1996b. Extended gene diversity at the *FMR1* locus and neighbouring CA repeats in a Sub-Saharan population. *Am J Med Genet* 64:216–219.
- Chiurazzi P, Genuardi M, Kozak ML, Giovannucci-Uzielli C, Bussani C, Dagna-Bricarelli F, Grasso M, Perroni L, Sebastio G, Sperandio MP, Oostra BA, Neri G. 1996c. Fragile X founder chromosomes in Italy: a few initial events and possible explanation for their heterogeneity. *Am J Med Genet* 64:209–215.
- Chiurazzi P, Pomponi MG, Sharrok A, Macpherson J, Lormeau S, Morel ML, Rousseau F. 1999. DNA panel for interlaboratory standardization of haplotype studies on the fragile X syndrome and proposal for a new allele nomenclature. *Am J Med Genet* 83:347–349.
- Crawford DC, Schwartz CE, Meadows KL, Newman JL, Taft LF, Gunter C, Brown WT, Carpenter NJ, Howard-Peebles PN, Monaghan KG, Nolin SL, Reiss AL, Feldman GL, Rohlf EM, Warren ST, Sherman SL. 2000a. Survey of the fragile X syndrome CGG repeat and the short-tandem-repeat and single-nucleotide-polymorphism haplotypes in an African American population. *Am J Hum Genet* 66:480–493.
- Crawford DC, Zhang F, Wilson B, Warren ST, Sherman SL. 2000b. Fragile X CGG repeat structures among African-Americans: identification of a novel factor responsible for repeat instability. *Hum Mol Genet* 9:1759–1769.
- Eichler EE, Nelson DL. 1996. Genetic variation and evolutionary stability of the *FMR-1* CGG repeat in six closed human populations. *Am J Med Genet* 64:220–225.
- Eichler EE, Hammond HA, Macpherson JN, Ward PA, Nelson DL. 1995. Population survey of the human *FMR1* CGG repeat substructure suggests biased polarity for the loss of AGG interruptions. *Hum Mol Genet* 4:2199–2208.
- Eichler EE, Macpherson JN, Murray A, Jacobs PA, Chakravarti A, Nelson DL. 1996. Haplotype and interspersed analysis of the *FMR1* CGG repeat identifies two different mutational pathways for the origin on the fragile X syndrome. *Hum Mol Genet* 5:319–330.
- Fu Y-K, Kuhl DPA, Pizzuti A, Pieretti M, Sutcliffe JS, Richard S, Verkerk AJMH, Holden JJA, Fenwick RG Jr, Warren ST, Oostra BA, Nelson DL, Caskey CT. 1991. Variation of the CGG repeat at the fragile X site results

- in genetic instability: resolution of the Sherman Paradox. *Cell* 67:1047–1058.
- Heitz D, Devys D, Imbert G, Kretz C, Mandel J-L. 1992. Inheritance of the fragile X syndrome: size of the fragile X premutation is a major determinant of the transition to full mutation. *J Med Genet* 29:794–801.
- Hill K, Hurtado AM. 1996. *Ache life history: the ecology and demography of a foraging people*. New York: Aldine de Gruyter.
- Hirst MC, Grewal PK, Davies KE. 1994. Precursor arrays for triplet repeat expansion at the fragile X locus. *Hum Mol Genet* 3:1553–1560.
- Ingman M, Kaessmann H, Pääbo S, Gyllensten U. 2000. Mitochondrial genome variation and the origin of modern humans. *Nature* 408:708–713.
- Jacobs PA, Bullman H, Macpherson J, Youings S, Rooney V, Watson A, Dennis NR. 1993. Population studies of the fragile X: a molecular approach. *J Med Genet* 30:454–459.
- Jorde LB, Bamshad MJ, Watkins WS, Zenger R, Fraley AE, Krakowiak PA, Carpenter KD, Soodyall H, Jenkins T, Rogers AR. 1995. Origins and affinities of modern humans: a comparison of mitochondrial and nuclear genetic data. *Am J Hum Genet* 57:523–538.
- Jorde LB, Rogers AR, Bamshad M, Watkins WS, Krakowiak P, Sung S, Keres J, Harpending HC. 1997. Microsatellite diversity and the demographic history of modern humans. *Proc Natl Acad Sci USA* 94:3100–3103.
- Jorde LB, Watkins WS, Bamshad MJ, Dixon ME, Ricker CE, Seielstad MT, Batzer MA. 2000. The distribution of human genetic diversity: a comparison of mitochondrial, autosomal, and Y-chromosome data. *Am J Hum Genet* 66:979–988.
- Kenneson A, Cramer DW, Warren ST. 1997. Fragile X premutations are not a major cause of early menopause. *Am J Hum Genet* 61:1362–1369.
- Kunst CB, Warren ST. 1994. Cryptic and polar variation of the fragile X repeat could result in predisposing normal alleles. *Cell* 77:853–861.
- Kunst CB, Zerylnick C, Karickhoff L, Eichler E, Bullard J, Chalifoux M, Holden JJA, Torroni A, Nelson DL, Warren ST. 1996. *FMR1* in global populations. *Am J Hum Genet* 58:513–522.
- Macpherson JN, Bullman H, Youings AS, Jacobs PA. 1994. Insert size and flanking haplotypes in fragile X and normal populations: possible multiple origins for the fragile X mutation. *Hum Mol Genet* 3:399–405.
- Mingroni-Netto RC, Costa SS, Angeli CB, Vianna-Morgante AM. 1999. DXS548/FRAXAC1 haplotypes in fragile X chromosomes in the Brazilian population. *Am J Med Genet* 84:204–207.
- Nei M. 1987. *Molecular evolutionary genetics*. New York: Columbia University Press.
- Oberlé I, Rousseau F, Heitz D, Kretz C, Devys D, Hanauer A, Boué J, Bertheas MF, Mandel J-L. 1991. Instability of a 550 base-pair DNA segment and abnormal methylation in fragile X syndrome. *Science* 252:1097–1102.
- Pang CP, Poon PMK, Chen QL, Lai KTC, Yin CH, Zhao Z, Zhong N, Lau CH, Lam STS, Wong CK, Brown WT. 1999. Trinucleotide CGG repeat in the FMR-1 gene in Chinese mentally retarded patients. *Am J Med Genet* 84:179–183.
- Pieretti M, Zhang F, Fu Y-H, Warren ST, Oostra BA, Caskey CT, Nelson DL. 1991. Absence of expression of the FMR-1 gene in fragile X syndrome. *Cell* 66:817–822.
- Richards RI, Holman K, Kozman H, Kremer E, Lynch M, Pritchard M, Yu S, Mulley J, Sutherland GR. 1991. Fragile X syndrome: genetic localization by linkage mapping of two microsatellite repeats FRAXAC1 and FRAXAC2 which immediately flank the fragile site. *J Med Genet* 28:818–823.
- Riggins GJ, Sherman SL, Oostra BA, Sutcliffe JS, Feitell D, Nelson DL, van Oost BA, Smits APT, Ramos FJ, Pfendner E, Kuhl DPA, Caskey T, Warren ST. 1992. Characterization of a highly polymorphic dinucleotide repeat 150Kb proximal to the fragile X site. *Am J Med Genet* 43:237–243.
- Rousseau F, Heitz D, Biancalana V, Blumenfeld S, Kretz C, Boué J, Tommerup N, Van der Hagen C, DeLozier-Blanchet C, Croquette M-F, Gilgenkrantz S, Jalbert P, Voelckel MA, Oberlé I, Mandel J-L. 1991. Direct diagnosis by DNA analysis of the fragile X syndrome of mental retardation. *N Engl J Med* 325:1673–1681.
- Shriver MD, Jin L, Ferrell RE, Deka R. 1997. Microsatellite data support an early population expansion in Africa. *Genome Res* 7:586–591.
- Snow K, Tester DJ, Kruckeberg KE, Schaid DJ, Thibodeau SN. 1994. Sequence analysis of the fragile X trinucleotide repeat: implications for the origin of the fragile X mutation. *Hum Mol Genet* 3:1453–1551.
- Syrrou M, Patsalis PC, Georgiou I, Hadjimarcou MI, Costantinou-Deltas CD, Pagoulatos G. 1996. Evidence for high risk haplotypes and (CGG)_n expansion in fragile X syndrome in the Hellenic population of Greece and Cyprus. *Am J Med Genet* 64:234–238.
- Warren SR, Nelson DL. 1994. Advances in molecular analysis of fragile X syndrome. *JAMA* 271:536–542.
- Yu S, Mulley J, Loesch D, Turner G, Donnelly A, Gedeon A, Hillen D, Kremer E, Lynch M, Pritchard M, Sutherland GR, Richards RI. 1992. Fragile-X syndrome: unique genetics of the heritable unstable element. *Am J Hum Genet* 50:968–980.
- Zhong N, Dobkin C, Brown WT. 1993. A complex mutable polymorphism located within the fragile X gene. *Nat Genet* 5:248–253.
- Zhong N, Yang W, Dobkin C, Brown WT. 1995. Fragile X gene instability: anchoring AGGs and linked microsatellites. *Am J Hum Genet* 57:351–361.