

# Experience with molecular and cytogenetic diagnosis of fragile X syndrome in Brazilian families

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## ABSTRACT

We report on the cytogenetic and DNA analysis of 55 families with the fragile X (FMR-1 locus) mutation (318 individuals and 15 chorionic villi samples). A total of 129 males were investigated, 54 mentally normal and 75 presenting mental retardation. Among the 54 normal males, 11 had the premutation, and none expressed the fragile site. The full mutation was detected in 73 retarded males, and 14 (18%) presented a premutation along with the full mutation (mosaics). All of them manifested the fragile site. The frequencies of fragile site expression correlated positively with the sizes of the expansion of the CGG repeats ( $\Delta$ ). Among 153 normal females, 85 were found to be heterozygous for the premutation and 15 had the full mutation. In the premutated females the fragile site was not observed or it occurred at frequencies that did not differ from those observed in 53 noncarriers. Cytogenetic analysis was thus ineffective for the diagnosis of premutated males or females. Among the 51 heterozygotes for the full mutation, 36 (70%) had some degree of mental impairment. As in males, a positive correlation was detected between the frequencies of fragile site manifestation and the size of the expansion. However, the cytogenetic test was less effective for the detection of fully mutated females, than in the case of males, since 14% false negative results were found among females. Segregation analysis confirmed that the risk of mental retardation in the offspring of heterozygotes increases with the length of  $\Delta$ . The average observed frequency of mental retardation in the offspring of all heterozygotes was 30%. There was no indication of meiotic drive occurring in female carriers, since the number of individuals who inherited the mutation did not differ from the number of those inheriting the normal allele. No new mutations were detected in the 55 genealogies studied here.

## INTRODUCTION

Fragile X syndrome (fra(X)) is the most frequent inherited disease which causes mental retardation. This X-linked disorder has an estimated incidence of 4.4/10,000 in males and 4.1/10,000 in females (Sherman *et al.*, 1984). The mutation causing the fragile X syndrome has been characterized as an unstable expansion of a CGG trinucleotide repeat in the 5' untranslated region of the FMR-1 gene (Oberlé *et al.*, 1991; Verkerk *et al.*, 1991; Yu *et al.*, 1991). The CGG repeat is highly

polymorphic in the normal population, ranging from six to 50 repeats, and becomes unstable when the repeat number exceeds 50 copies (Fu *et al.*, 1991). Oberlé *et al.* (1991) proposed the classification of fragile X mutations into premutations and full mutations. When 50-200 copies of the CGG repeats (expansions up to 600 bp) are present, individuals are carriers of the premutation, which is usually unmethylated on the active X and has no clinical or cytogenetic manifestations (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 of

the repeats greatly exceeds 200 (expansions larger than 600 bp up to thousands of base pairs) and abnormal methylation of the nearby CpG island occurs. The full mutation is associated with silencing of transcription (Pieretti *et al.*, 1991; Sutcliffe *et al.*, 1992) and expression of the fragile site at Xq27.3 (FRAXA). Mental retardation in all males and in a fraction of carrier females are manifestations of the full mutation. Some individuals with the full mutation may also have the premutation in some of their cells, being called "mosaics".

The present study on Brazilian fragile X families aimed to address some questions regarding: 1) correlation between cytogenetic, phenotypic and molecular data; 2) transmission patterns of the mutation; 3) segregation mode of the FMR-1 mutation in the offspring of female carriers.

## MATERIAL AND METHODS

### Subjects

A total of 318 individuals from 55 fragile X positive families and 15 chorionic villi samples (including seven prenatal diagnosis cases and eight control samples) were analyzed with probe StB12.3.

### Cytogenetic analysis

Peripheral blood lymphocytes were cultured for fragile X investigation for 72 h in TC 199 with the addition of an inductor of folate-sensitive fragile sites: 26 µg/ml trimethoprin, 300 µg/ml thymidine or  $2 \times 10^{-7}$  M FUdR. Affected males and females usually had 100 cells scored, in one or two culture systems. Normal male and female carriers each had 100-300 cells scored, 100 per culture system.

### DNA analysis

DNA isolated from peripheral blood lymphocytes or chorionic villi samples was doubly digested with *EcoRI* and *EagI*, subjected to electrophoresis in 0.7% agarose gels, and transferred onto nylon membranes. Probe StB12.3 was labeled with  $\alpha^{32}\text{P}$ -dCTP by random priming (BRL) and hybridized to the filters at 42°C in a hybridization solution containing 50% formamide. Filters were washed to a stringency of  $2 \times \text{SSC}$  and exposed to KODAK XOMAT films at -70°C, from two to seven days.

Hybridization with probe StB12.3 (kindly provided by F. Rousseau, INSERM, Strasbourg, France) after *EcoRI* and *EagI* digestion reveals normal frag-

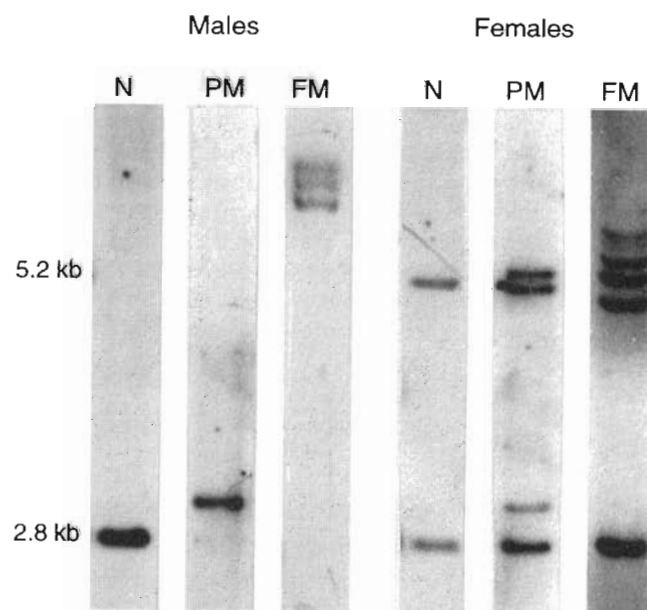
ments of 2.8 kb (in the unmethylated active X chromosome) and 5.2 kb (in the methylated inactive X chromosome). Premutations can be visualized as an increase ( $\Delta$ ) up to 500-600 bp (either in the 2.8 kb normal fragment or in the 5.2 kb methylated fragment). The full mutation is always methylated and seen above the fragment of 5.2 kb, with a  $\Delta$  larger than 500 bp. Figure 1 shows the patterns of hybridization with probe StB12.3.

## RESULTS

Mental status and molecular characterization status of the 318 individuals from the 55 fragile X families were determined (Table I).

### Premutated males (normal transmitting males)

Among the 54 phenotypically normal males, possible carriers of the mutation, 11 premutations were detected with  $\Delta$  ranging from 50 to 300 bp. Six of these normal transmitting males had their chromosomes studied and all of them were fragile X negative. Also, in



**Figure 1** - Patterns of hybridization with probe with StB12.3 after *EcoRI* and *EagI* digestion. **Males:** N, noncarrier of the mutation showing a normal 2.8-kb fragment; PM, normal transmitting male with a premutated fragment larger than 2.8 kb; FM, fully mutated individual with methylated fragments larger than 5.2 kb; **Females:** N, noncarrier of the mutation showing a 2.8-kb unmethylated fragment from the active X and a methylated 5.2-kb fragment from the inactive X; PM, premutated carrier with two unmethylated fragments, one normal and one expanded, from the active X, and two methylated fragments, one normal and one expanded, from the inactive X; FM, carrier of the full mutation showing normal fragments of 2.8 kb (from the active X) and 5.2 kb (from the inactive X), and multiple methylated expanded fragments larger than 5.2 kb.

**Table I** - Distribution of 318 subjects studied with StB12.3 probe, according to mental and molecular status.

Sex	Mental status	Mutation		Normal
		Premutation	Full mutation	
Males (129)	normal	11		43
	retarded		73	2
Females (189)	normal	85	15	53
	slow-learners/retarded		36	

a group of 12 normal control males who were shown to be noncarriers, none expressed the fragile site.

### Affected males

Among 75 mentally retarded males, 73 presented the full mutation. In the remaining two, mental retardation seemed to have another etiology, and both were fragile X negative. Among the 73 carriers of the full mutation, 14 were mosaics. Full mutations had  $\Delta$  ranging from 400 to several thousands base pairs, with a mean of  $1475 \pm 745$  bp. One mosaic male presented an exceptional unmethylated fragment with  $\Delta = 800$  bp. In another mosaic an unmethylated normal-sized fragment (2.8 kb) was present: in a first *EcoRI-EagI* digest this normal fragment was not visualized, but it was detected during the development of a PCR screening routine test for affected males (Haddad *et al.*, 1996). The coexistence of the normal fragment with the full mutation could be clearly confirmed after *BglII* digestion and hybridization with the StB12.3 probe.

The mean frequency of cells with the fra(X) was 18.2% after trimethoprin induction and 23.5% after FUdR induction. In a sample of 28 males studied with both systems, a test for paired data showed that FUdR was significantly more efficient as an inductor of the fragile site than trimethoprin ( $t = 2.82$ , d.f. = 27;  $P < 0.01$ ). There was no significant difference in fra(X) expression between mosaics ( $N = 8$ ) and nonmosaics ( $N = 42$ ) studied with trimethoprin ( $t = 1.23$ , d.f. = 48;  $P > 0.20$ ) or between mosaics ( $N = 6$ ) and nonmosaics ( $N = 40$ ) studied after FUdR induction ( $t = 0.02$ , d.f. = 44;  $P > 0.20$ ).

A significant correlation was detected between  $\Delta$  (when the middle part of smears or the mean  $\Delta$  of multiple fragments were taken into account) and the frequency of fragile X expressing cells after trimethoprin ( $r = 0.404$ , d.f. = 35;  $0.01 < P < 0.02$ ) and FUdR induction ( $r = 0.365$ , d.f. = 34;  $0.02 < P < 0.05$ ), in nonmosaic cases. No significant correlation was

detected between age of affected males and mean  $\Delta$  of the full mutation ( $r = -0.225$ , d.f. = 49;  $0.10 < P < 0.20$ ). Twelve pairs of affected brothers were compared in order to investigate a possible effect of maternal age on  $\Delta$  of affected males. The mean  $\Delta$  of the older brothers did not differ significantly from that of the younger brothers ( $t = 1.00$ , d.f. = 22;  $P > 0.20$ ). There was no significant correlation between  $\Delta$  of affected males and maternal ages ( $r = 0.241$ , d.f. = 44;  $0.10 < P < 0.20$ ).

### Female carriers of the premutation

In a group of 153 intellectually normal females, 53 were not carriers of expansions in the FMR-1 locus. In the remaining 100, the premutation was identified in 85 and the full mutation in the remaining 15.  $\Delta$  Ranged from 50 to 500 bp and mean  $\Delta$  was 198 bp among premutated females. In one case, the premutation appeared as different-sized fragments, similarly to what is often observed in full mutations.

The mean frequency of fra(X) expression was 0.41% with trimethoprin induction ( $N = 50$ ), 0.37% with FUdR ( $N = 48$ ) and 0.76% with thymidine ( $N = 50$ ). Fra(X) frequencies in premutation carriers and in noncarriers did not differ in the three culture systems ( $t = 1.66$ , d.f. = 73;  $0.05 < P < 0.10$  after trimethoprin induction;  $t = 0.66$ , d.f. = 65;  $P > 0.20$  after FUdR induction;  $t = 1.70$ , d.f. = 73;  $0.05 < P < 0.10$  after thymidine induction). Thus, chromosome analysis was inefficient for the detection of premutated female carriers.

### Fully mutated females

Fifty-one female carriers of the full mutation were diagnosed: 26 were mentally retarded, 10 were slow-learners and 15 were intellectually normal. Three of them also presented small unmethylated fragments in the premutation range and were thus classified as mosaics. Expansions in the methylated fragments ranged from 400-3,000 bp.

The mean cytogenetic expression of fra(X) was 11.67% ( $N = 30$ ), 16.4% ( $N = 27$ ) and 9.73% ( $N = 26$ ) with trimethoprin, FUdR and thymidine, respectively. FUdR induction increased expression significantly when compared to thymidine ( $t = 2.48$ , d.f. = 19;  $0.02 < P > 0.05$ ), but not to trimethoprin ( $t = -0.62$ , d.f. = 19;  $P > 0.20$ ).

Fra(X) expression was significantly higher in fully mutated females than in premutated females in all

three induction systems (trimethoprin:  $t = 7.51$ , d.f. = 31;  $P < 0.01$ ; FUdR:  $t = 6.89$ ; d.f. = 27;  $P < 0.01$ ; thymidine:  $t = 5.52$ ; d.f. = 25;  $P < 0.01$ ). The correlation between fra(X) expression and  $\Delta$  was investigated in females with only one methylated fragment (without smears). A significant positive correlation was found only with FUdR induction ( $r = 0.582$ , d.f. = 11;  $0.02 < P < 0.05$ ). The mean size of expansions ( $\Delta$ ) did not differ between affected ( $1,146 \pm 463$  bp) and normal ( $1,013 \pm 354$  bp) fully mutated females ( $t = 0.978$ , d.f. = 43,  $P > 0.20$ ).

Table II summarizes the data on fragile site expression in premutated, fully mutated and noncarriers of the FMR-1 mutation, after FUdR induction.

### Isolated cases and origin of mutation

In nine of the 55 fra(X), positive families studied, the proband was an isolated case of mental retardation. In all 55 families, the full mutation was always inherited from female carriers, either in the premutated or in the fully mutated state. No new mutation was ever detected in the genealogies.

### Chorionic villi samples

DNA analysis was performed in 15 chorionic villi samples: seven were studied aiming at the diagnosis of fragile X syndrome and eight were analyzed for control studies of methylation in chorionic villi. Among the seven cases carried for diagnosis purposes, six were normal and in one male fetus a smear of large fragments ( $> 6.0$  kb), characteristic of the full mutation, was detected. The study of methylation in the eight control samples (six 46,XX; one 46,XY and one

**Table II** - Fragile X frequency in 152 individuals, carriers and noncarriers of fragile X mutation, studied after FUdR induction.

	0	$\leq 3\%$	$> 3\%$	Total	Mean
<b>Females</b>					
Noncarriers	14	5	0	19	0.29%
Carriers of the premutation ( $\Delta < 500$ pb)	35	12	0	47*	0.37%
Carriers of the full mutation ( $\Delta \geq 500$ pb)	4	3	21	28	16.14%
<b>Males</b>					
Noncarriers	8	0	0	8	0
Carriers of the premutation ( $\Delta < 300$ pb)	4	0	0	4	0
Carriers of the full mutation ( $\Delta > 400$ pb)	1	1	44	46	23.50%

\*Two individuals in this group had  $\Delta \geq 500$  pb and were included in the premutation group because expanded fragments were unmethylated.

45,X) revealed only 2.8 kb normal unmethylated fragments, indicating that the CpG island of the FMR-1 gene was not methylated.

### Segregation analysis of FMR-1 mutation

In the group of eleven normal male carriers of the premutation, six had a total of 13 female offspring, whose DNA was studied. In six females,  $\Delta$  increased in about 50 bp and its length remained unchanged in the other seven.

An additional sample of 24 females belonging to 10 fragile X genealogies was included for segregation analysis of the offspring of heterozygotes. Only those females with at least 50% of their offspring tested for the presence of the mutation were considered for this analysis (Table III).

The number of individuals who inherited mutated alleles did not differ from the number of those who inherited normal alleles ( $\chi^2 = 0.77$ ;  $0.3 < P < 0.50$ ). Therefore, there is no indication of meiotic drive occurring in fragile X syndrome.

**Table III** - Segregation analysis in the offspring of premutated and fully mutated heterozygotes.

Female carriers	Male offspring				Female offspring					
	FMR	PM	N	P	FMR	FMN	PM	N	P	R
<b>Premutated</b>										
$\Delta \leq 100$ bp (N = 22)	14 (7)	2	15	77.8%	8 (7)	2	15	13	29.2%	26.7%
$100 < \Delta \leq 200$ bp (N = 44)	49 (27)	6	23	81.8%	14 (9)	8	7	22	37.5%	29.8%
$200 < \Delta \leq 500$ bp (N = 12)	12 (6)	0	5	100.0%	3 (2)	1	0	9	66.7%	41.7%
Total (N = 78)	75 (40)	8	43	83.3%	25 (18)	11	22	44	35.3%	31.6%
<b>Fully mutated (N = 17)</b>										
	20 (11)	0	4	100.0%	7 (5)	0	0	10	100.0%	50.0%

FMR = Carriers of full mutation and mentally retarded; PM = carriers of premutation; N = noncarriers of the mutation; P = penetrance of mental retardation; FMN = carriers of full mutation and mentally normal; R = risk of mental retardation in the offspring. In parentheses the numbers after the exclusion of probands.

## DISCUSSION

Our findings about normal male carriers of the premutation having small expansions not associated with fragile X expression are in accordance with many other reports. Rousseau *et al.* (1994) reported an increased frequency of low fra(X) expression among premutated males when compared to controls, but in our sample this difference was not detected. The conclusion is the same regarding cytogenetic detection of premutated females, which did not differ in fra(X) expression from noncarriers. It demonstrates that cytogenetic analysis is ineffective in detecting premutation carriers.

All 85 premutated females and 11 premutated males had normal intelligence. In one premutated normal female, more than one premutation fragment was present, an indication of the instability that is often present among fully mutated individuals. This has already been described and seems to be a rare event (Fu *et al.*, 1991; Devys *et al.*, 1992; Rousseau *et al.*, 1994).

In the sample of affected males, some exceptional patterns of hybridization were detected. One mosaic male had a normal-sized fragment (Mingroni-Netto *et al.*, 1996). Since a full mutation has never been observed to arise *de novo*, it is not conceivable that the inherited fragment was the normal-sized allele, which later expanded mitotically into a full mutation. It is reasonable to assume that this exceptional pattern resulted from mitotic instability of the full mutation.

In another mosaic male an unmethylated fragment with  $\Delta = 800$  bp was detected. Similar patterns have already been reported (Tarleton *et al.*, 1992; McConkie-Rosell *et al.*, 1993; Loesch *et al.*, 1993). In the large multicenter study of Rousseau *et al.* (1994) this pattern was detected in only one case. According to Loesch *et al.* (1993), expansions with  $\Delta$  ranging from 600-1,200 bp may present variable degrees of methylation and this may influence the severity of mental retardation.

We confirmed the general trend of high expression of fra(X) in male carriers of the full mutation. As shown in Table II, only two out of 46 males studied with FUdR had low or no expression of fra(X), and it never occurred simultaneously in two induction systems. This supports previous recommendations that cytogenetic investigation of mentally retarded males should be performed with at least two induction systems (Jacky *et al.*, 1991). It was only recently that in our laboratory one affected fully mutated male failed to express the fragile X in two induction systems, but this would account for less than 1% of all affected males studied. In the multicenter study of Rousseau *et al.*

(1994) an overall frequency of 1.5% fra(X) negatives among fully mutated males was reported. Thus, we believe that cytogenetic analysis is reliable in detecting male carriers of full mutation, but care should be taken with low-expressing and fra(X) negative males whose clinical picture strongly suggests the syndrome. They should be checked by DNA analysis. The situation is clearly different when we consider cytogenetic detection of fully-mutated females. In spite of showing higher expression of fra(X) than the premutated females (Table II), some of them did not express the fragile site at all. For instance, 4/27 did not express fra(X) after FUdR induction, and 3/27 expressed it at frequencies lower than 3%. Among 43 fully mutated females whose chromosomes were studied, six failed to express the fragile site in one or more systems of induction. In the multicenter study of Rousseau *et al.* (1994), 5% did not express fra(X) at all and 4% expressed it at low frequencies. These data indicate that detection of fully mutated females is less efficient than that of fully mutated males. It has been argued that X-inactivation might affect cytogenetic expression of fra(X), but our previous data do not support this idea (Mingroni-Netto *et al.*, 1994). On the other hand, our present data indicate a correlation between  $\Delta$  and fra(X) expression in fully mutated females. We also detected a significant correlation between fra(X) expression and  $\Delta$  when we considered the mean size of multiple fragments or smears in fully mutated males, as observed by other authors (Yu *et al.*, 1992; Staley *et al.*, 1993; De Vries *et al.*, 1993). Thus, the CGG repeats are probably the site of fragility themselves, and larger expansions increase the probability of visualization of the fragile site.

The proportion of mosaics among affected males was 18%, which is in the range of frequencies from 10 to 25% reported in the literature (Rousseau *et al.*, 1991; Snow *et al.*, 1993; Steinbach *et al.*, 1993; Rousseau *et al.*, 1994). Cytogenetic expression of fragile X did not differ significantly between mosaics and nonmosaics and this was also observed before (Snow *et al.*, 1993; Staley *et al.*, 1993; Rousseau *et al.*, 1994). De Vries *et al.* (1993) found more mosaics among males aged more than 20 years, suggesting an age-dependent process in which the premutation would tend to expand into a full mutation. This trend was not confirmed in our study, since the mean age of mosaics did not differ from the mean age of nonmosaics (Mann-Whitney confidence interval and test,  $W = 560.0$ ;  $P > 0.05$ ). The proportion of fully mutated mosaic females was 6%, exactly the same reported in the multicenter study of Rousseau *et al.* (1994).

Mornet *et al.* (1993), studying pairs of fully mutated brothers, reported that the mean  $\Delta$  of older

brothers was smaller than that of younger brothers. This could be explained either as a maternal age effect on the size of the mutation or an age-dependent decrease in size in affected individuals. Since the authors had detected an inverse correlation between  $\Delta$  and maternal age, they inferred a maternal age effect on the size of the transmitted mutation. We did not detect any difference between  $\Delta$  of older and younger brothers, nor a correlation of  $\Delta$  with maternal age. Furthermore, the age of affected males did not correlate significantly with the size of expansions. Therefore, the possibility of an effect of maternal or patients' age on  $\Delta$  was not confirmed by our data.

Seventy percent of carriers of the full mutation were females with mental disabilities. The frequencies of mental impairment in different studies ranged from 52 to 82% (De Vries *et al.*, 1996). These authors observed a significant correlation between the proportion of normal active FMR-1 and IQ in fully mutated females, but no correlation between  $\Delta$  and IQ, indicating that X-inactivation might explain why 30% of fully mutated females are not affected by mental retardation. We compared  $\Delta$  of mentally impaired and normal fully mutated females and did not detect any significant difference. It seems therefore that size of expansions is much less important than X-inactivation in determining the mental status of fully mutated females. It is assumed that the clinical phenotype in fragile X syndrome depends on the lack of expression of the FMR-1 gene. Among fully mutated females, the size of the full mutation would not be of great importance, since the FMR-1 gene is silenced by methylation; the proportion of cells with the active X bearing the full mutation in crucial tissues would determine the phenotype.

The absence of new mutations in our genealogies is in line with the fact that, although the incidence of the syndrome is fairly high, no new mutation has ever been detected, generating either a premutated or a fully mutated individual. This has practical implications in genetic counseling, since every time a fully mutated male is diagnosed, it means that his mother is certainly a carrier of the mutation, either in the premutated or in the fully mutated state.

Our study of methylation in chorionic villi showed that the CpG island of the FMR-1 gene is unmethylated in the normal fetus by the 10th week of embryonic development. Methylation of the CpG island has been reported in a few normal female fetuses (Devys *et al.*, 1992; Luo *et al.*, 1993), but the available data allow the conclusion that the methylation pattern of the inactive X chromosome is rarely present in chorionic villi of normal females at about the 10th week of gestation, while the abnormal methylation pattern of the full

mutation may be present or absent (Rousseau *et al.*, 1991; Sutherland *et al.*, 1991; Devys *et al.*, 1992; Sutcliffe *et al.*, 1992).

In the female offspring of 11 normal premutated males, only the premutation was present, in accordance with other studies. Expansions increased by 50 bp or did not increase in these carrier females, in contrast to the trend of large increases in the offspring of premutated and fully mutated females. Recently, daughters of transmitting males have been reported with smaller expansions than those present in their fathers and the frequency of these reductions is significantly higher than that occurring in the offspring of female carriers, which is a rare event (Fisch *et al.*, 1995). We did not observe reduction of expansion in the offspring of premutated male or female carriers, resulting in a smaller premutation than that present in parents, but the number of cases reported in the literature is increasing (Antinolo *et al.*, 1996; Väisänen *et al.*, 1996). In our sample, the few cases of apparent reduction of mutation occurred in the offspring of premutated and fully mutated females, and originated smaller fragments coexisting with larger fragments, as mosaics. In some cases, fully mutated mothers had fully mutated offspring with part of the fragments in smears being smaller than the maternal ones (Mingroni-Netto *et al.*, 1996).

We confirmed that the risk of affected offspring increases with increasing maternal  $\Delta$  (Table III), as already pointed out by Fu *et al.* (1991), Rousseau *et al.* (1991), Heitz *et al.* (1992), Yu *et al.* (1992), Snow *et al.* (1993) and Fisch *et al.* (1995). In all these studies it was shown that female carriers of premutations with  $\Delta$  larger than 200 bp have a 100% probability of the premutation converting into a full mutation in the offspring. In our sample, the overall rate of conversion of premutation into full mutation was 70% in the offspring of females. Other authors reported rates that ranged from 70 to 77% (Fu *et al.*, 1991; Heitz *et al.*, 1992; Väisänen *et al.*, 1994; Fisch *et al.*, 1995). This leads to an overall risk of 30% of mental retardation in the offspring of premutated females, regardless of the size of the premutation when the penetrance of mental impairment is considered to be 0.70 and 1 among female and male carriers of the full mutation, respectively:  $0.50$  (chance of being male)  $\times 0.50 \times 0.70$  (chance of inheriting the full mutation)  $\times 1$  (penetrance in males)  $+ 0.50$  (chance of being female)  $\times 0.50 \times 0.70$  (chance of inheriting the full mutation)  $\times 0.70$  (penetrance in females). Accordingly, carriers of the full mutation have a probability of affected offspring of 42.5%. In our sample, the proportion of individuals with mental retardation in the offspring of fully mutated females

was 50%, which most probably represents an overestimate (Table III). In our small sample of five fully mutated females born to fully mutated females, all were mentally retarded, when 70% of them would be expected to be affected, considering the proportion of mental retardation among fully mutated females.

The study of syndromes caused by expansions of trinucleotides has raised interesting questions about the origin and maintenance of new mutated alleles. Meiotic drive (or segregation distortion) favoring mutated alleles seems to occur in myotonic dystrophy. Genarelli *et al.* (1994) observed that affected fathers more frequently transmitted the mutant alleles to their offspring. Also, Carey *et al.* (1994) found that males, in the case of the upper-range normal alleles, more frequently transmitted the larger alleles to their offspring. Chakraborty *et al.* (1996) detected preferential transmission of larger alleles, in females. Preferential transmission of mutant paternal alleles was also reported in DRPLA and Machado-Joseph disease (Ikeuchi *et al.*, 1996). These findings raise the possibility that meiotic drive is a common phenomenon in diseases caused by trinucleotide repeat expansion, which may influence their incidence. Our data do not provide any evidence of meiotic drive in fragile X syndrome, at least in the offspring of premutated and fully mutated females. Whether this occurs in the upper-range normal alleles of FMR-1 repeats or is a phenomenon limited to autosomal diseases remains to be clarified.

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## RESUMO

Realizamos análises citogenéticas e moleculares em 55 famílias com a mutação da síndrome do cromossomo X frágil, loco FMR-1 (318 indivíduos e 15 amostras de vilosidade coriônica). Foram estudados 129 indivíduos do sexo masculino, 75 com retardo mental e 54 normais. Entre os 54 normais, 11 eram portadores da pré-mutação e nenhum apresentou o sítio frágil. Foram detectados 73 portadores da mutação completa e 18% eram mosaicos, ou seja, apresentavam também a pré-mutação. Todos expressaram o sítio frágil em pelo menos um dos sistemas de indução utilizados. O tamanho da expansão de trinucleotídeos CGG ( $\Delta$ ) e a frequência de manifestação do sítio frágil apresentaram correlação positiva. Entre as 153 mulheres normais, 85 eram portadoras da pré-mutação e 15 da mutação completa. A frequência de expressão do fra(X) foi

zero ou extremamente baixa entre as pré-mutadas e essa frequência não diferiu da expressão das não portadoras da mutação. Portanto, a análise citogenética é ineficaz na detecção de indivíduos pré-mutados, homens ou mulheres. Entre as 51 mulheres com a mutação completa, 70% manifestaram algum grau de comprometimento mental. Encontramos também correlação entre o  $\Delta$  e a frequência de expressão do fra(X) nessas mulheres. Contudo, a detecção citogenética das mulheres com mutação completa foi menos eficiente do que no caso dos homens, pois 14% de falsos negativos foram observados. A análise de segregação confirmou que o risco de prole afetada aumenta com o  $\Delta$ , e o risco médio de prole afetada para todas as heterozigotas foi de 30%. Não houve indicação de desvio de segregação nas famílias estudadas, pois o número de indivíduos que herdaram a mutação não diferiu do número daqueles que herdaram os alelos normais. Não foi detectada nenhuma mutação nova nas 55 genealogias investigadas.

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