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Expansion of the CGG Repeat in Fragile X in the FMR1 Gene Depends on the Sex of the Offspring

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Summary

Analysis of 139 mother-to-offspring transmissions of fragile X CGG triplet repeats revealed that the repeat expansion is enhanced in mother-to-son transmissions compared with mother-to-daughter transmissions. Evidence has been based on analysis of mother-offspring differences in the size of repeat (in kb), as well as on comparisons between proportions of male and female offspring with premutations, and full mutations, inherited from mothers carrying a premutation. Mean difference in the repeat size from mother-son transmissions was 1.45 kb, compared with mother-daughter transmissions of 0.76 kb. The difference is due primarily to a greater proportion of male than female offspring with full mutation from the premutation mothers and also to a higher frequency of reduction in repeat size from mothers to daughters than from mothers to sons. Our findings suggest the possibility of an interaction of the normal X homologue in a female zygote with the FMR1 sequence on the fragile X during replication to account for the lower level of expansion in mother-to-daughter transmissions relative to mother-to-son transmissions.

Introduction

Fragile X syndrome, the most common inherited cause of intellectual disability, is associated with amplification of a CGG triplet repeat in the 5′ UTR of the first exon of the FMR1 gene (Verkerk et al. 1991; Yu et al. 1991). The syndrome, which consists of cognitive deficits and behavioral and physical anomalies, is caused by failure to produce the protein FMRP (Pieretti et al. 1991). The transcription of the FMR1 gene is turned off by hypermethylation of the CpG island in the 5′ region of this gene, occurring in a majority of subjects with the size of expansion >0.6 kb (equivalent to >200 repeats) (Rousseau et al. 1991). The CGG repeat number is polymorphic in the normal population and varies in size from 6 to ~52 repeats, which are usually stable during transmission (Fu et al. 1991; Arinami et al. 1993; Brown et al. 1993; Snow et al. 1993; Zhong et al. 1994). Small expansions of 52–200 repeats, defined as "premutations," can expand further, upon female transmission, and the risk of expansion is generally related to the size of the premutation (Fu et al. 1991; Yu et al. 1992). The expansion of the CGG repeat within the fragile X size range accounts for the anticipation phenomenon characterized by increased severity or penetrance of the clinical phenotype in the descendants of female carriers, but some proportion of female carriers transmit a reduced fragment to their offspring (Rousseau et al. 1991; Fu et al. 1992; Heitz et al. 1992; Chiurazzi et al. 1994; Mulley et al. 1995; D. Z. Loesch, V. Petrovic, D. I. Francis, and H. Slater, unpublished data).

The mechanism responsible for the observed repeat expansion is still not fully clarified, but some predisposing factors have been identified. Apart from the size of CGG repeat and the sex of a parent being the major determinants of instability, the loss of AGG triplets, which are normally interspersed throughout the CGG repeat, was postulated as a factor predisposing to mispairing and replication slippage within the repeat region (Eichler et al. 1994; Snow et al. 1994). It has also been shown that certain haplotypes associated with CGG repeats are more prevalent in fragile X than in normal populations (Richards et al. 1992; Macpherson et al. 1994; Snow et al. 1994).

Mispairing or slippage (Schlotterer and Tautz 1992), which are believed to generate expansion or reduction in the size of CGG repeat, may take place in mitosis and in meiosis. The observed discrepancy between the presence of the full mutation in somatic cells and a premutation in germ-line cells of fragile X males, who always transmit the premutation to their offspring, gave rise to a hypothesis that the full mutation regresses to premutation in their gametes, with selective advantage for gametes with premutation (Reyniers et al. 1993). On the other hand, the results from a fragile X tissue culture (Wöhrle et al. 1993), chorionic villus biopsies from early fetuses, and blood samples from monozygotic twin pairs (Devys et al. 1992) indicated that the expansion of the

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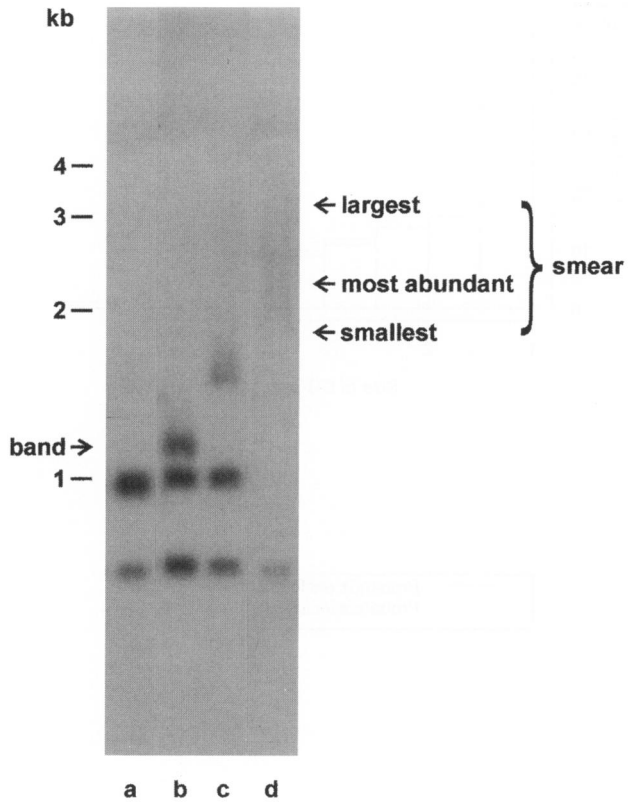

Figure 1 An autoradiograph of *Pst*I digests of DNA probed with Pfax3 and Ps8. Ps8 is a control probe that gives an invariate 0.8-kb band (below the 1-kb fragment) in all subjects. Lane a, Normal female subject who has the normal 1-kb band. Lane b, Female fragile X carrier who has a premutation detected as a discrete band slightly larger than 1 kb. Lane c, Female carrier with a small, full mutation detected as a small smear. Lane d, Male with a full mutation detected as an extensive smear where the range of mutation sizes (arrows) were measured.

CGG repeat on the maternally derived fragile X chromosome takes place in an early postzygotic stage. This also implies there is some distinction between paternally and maternally derived premutations, when one considers the fact that only the latter one expands to the full mutation.

Several surveys of fragile X families provided estimates of the rate of expansion or reduction in the number of repeats in parent to offspring transmissions, depending on the size of expansion and the sex of a parent (Heitz et al. 1992; Yu et al. 1992; Arinami et al. 1993; Snow et al. 1993; Väisänen et al. 1994). However, the estimates give the overall risk for the offspring, regardless of their sex. The purpose of this study was to establish whether the risk of expansion is the same or different in daughters and sons of fragile X female heterozygotes. In order to minimize ascertainment bias due to preferential screening of sons or daughters in the sibships, all available offspring have been tested for the size of CGG expansion, and probands of either sex were omitted from analysis. Our findings have shown that the rate of the CGG repeat expansion inherited from the mother depends on sex of the offspring.

Material and Methods

Family Sample

This study is based on a sample of 52 extended Caucasian families attending outpatient clinics in Victoria and South Australia. As described in studies published earlier (Loesch et al. 1993*a*, 1993*b*), all available branches of these families led to by a proband were followed up by one of us (D.Z.L.), and all available individuals were tested for the size of CGG repeat, regardless of sex, and the presence or absence of clinical features of fragile X. All the probands ascertained by us (including five females) were omitted from analysis, as a standard correction for ascertainment bias. In ~1/3 of our families, a proband(s) was ascertained in another state or country so that the data from a proband's family branch were often not available to us. Resulting from these procedures, our sample considered in the present study includes 30 two-generation and 22 three-generation families, with the average sibship size of 2.5, ranging from 1 to 4. In the three-generation families, females in the second generation were considered twice in calculations of expansion rate: as the mothers of the youngest generation children and as the daughters of the oldest generation mothers, respectively. In addition, six mother-male fetus and eight mother-female fetus pairs have been considered in analyses presented, because no obvious differences in distributions or transmission patterns with and without inclusion of these pairs have been encountered.

DNA Analysis

The method used for detection of FRAXA mutations has been described in detail elsewhere (Yu et al. 1991, 1992). DNA was prepared from peripheral blood and chorionic villus samples by using a commercial kit (Progen). Each sample (3.5 μg) was digested with the restriction enzyme *Pst*I. After agarose gel electrophoresis, restriction fragments containing the FRAXA (CGG)n repeat were detected by autoradiography of Southern blots in which the radiolabeled probe pfxa3 was used. Another X-linked anonymous probe pS8 was used along with pfxa3 in cohybridization to confirm the presence of mutations, i.e., reduced relative 1-kb (normal) signals in females. pS8 gave an invariate 0.8-kb signal with all samples. Male and female samples were run in mixed batches as they were collected. Gels included control normal and abnormal DNA samples and a ladder of molecular-weight markers. A calibration curve was constructed by using the molecular-weight markers relating DNA fragment size to migration distance through the

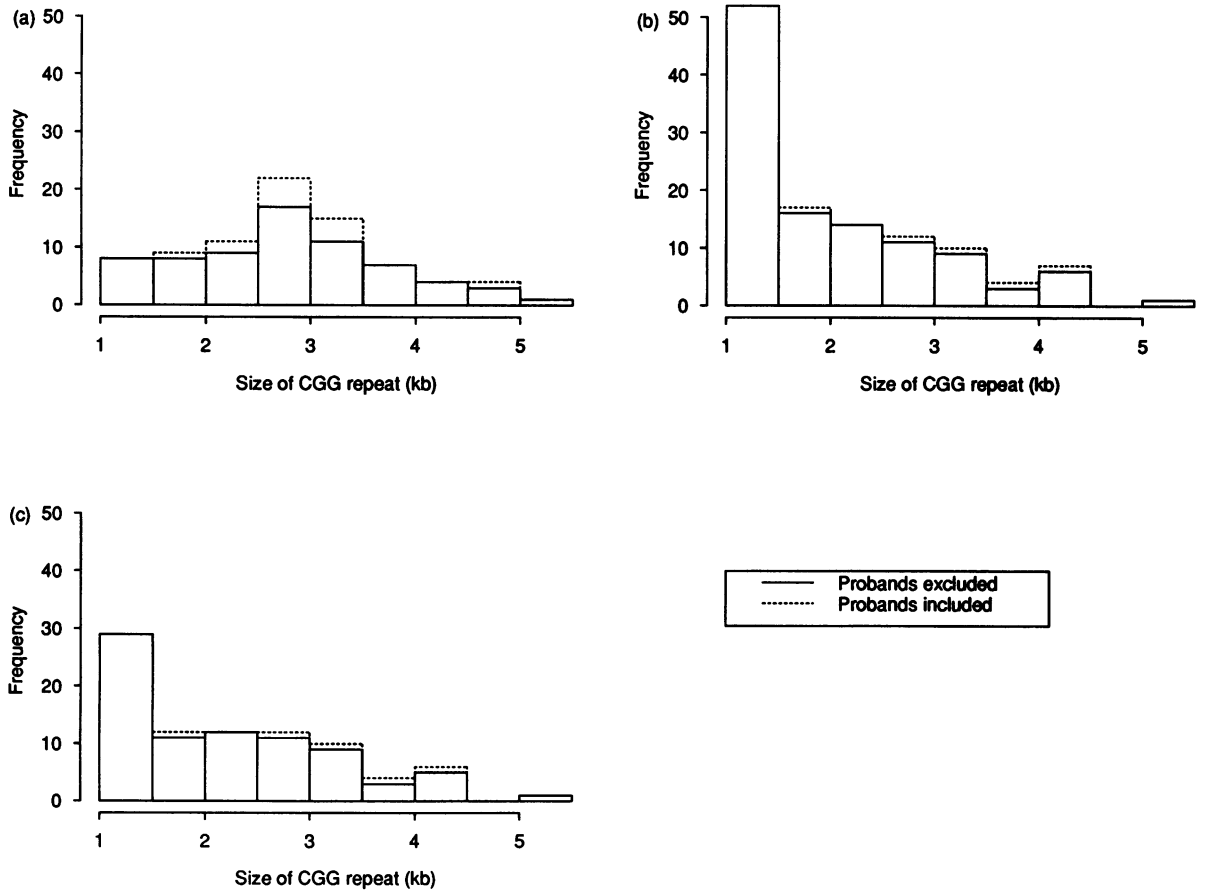

Figure 2 Frequency distributions of different expansions of CGG repeat (in kb) in fragile X samples of sons (*a*) and all daughters (*b*) of female carriers and in a subsample of their daughters from the youngest generation (*c*). Vertical axis represents percentage frequencies, and horizontal axis, the size of CGG repeat (in kb).

gel. The migration distance for discrete bands was measured according to the generally adopted procedure using the band midpoint (see fig. 1). The normal size of the *Pst*I fragment is 1.0 kb, and the size of the mutant fragile X fragments lies between 1.0 and 5.0 kb. The precision with which band sizes were calculated was ±0.05 kb for a band size of 1 kb, decreasing to ±0.2 kb for a band size of 5 kb. These errors in no way alter the interpretation of the data. For mutations revealed as smears, migration distances were measured for the smallest, largest, and most dense regions. The results presented in this paper are based on the average for all three regions. However, to rule out possible bias caused by an average being taken in subjects with multiple bands, we performed the same analyses using respectively, the smallest and the largest band, with the same result (data not shown). This is the predictable outcome, when one considers that in our data we have not encountered true size mosaics, so that multiple bands were all within the full-mutation range.

Results

Distributions of Size of CGG Repeat in Male and Female Subjects

Frequency distributions of the repeat size in male and female individuals in our sample carrying the wide range of CGG expansions are shown in figure 2. Broken lines indicate frequencies with probands that were included in this diagram but were omitted in further analyses. In the males (fig. 2*a*), the distribution of repeat lengths is nearly normal, with some deficit of subjects with expansions >4.0 kb. This deficit may be caused by nonviability of larger expansions, but there are no relevant data published to verify this assumption. In the females, the distribution is obviously skewed toward the lower repeat-size values (fig. 2*b*), and the position of female probands is also indicated by broken lines. One might suspect that the females from parental generations, who are more likely to have lower repeats than their offspring, contribute to the excess of smaller repeats (1.0 < kb

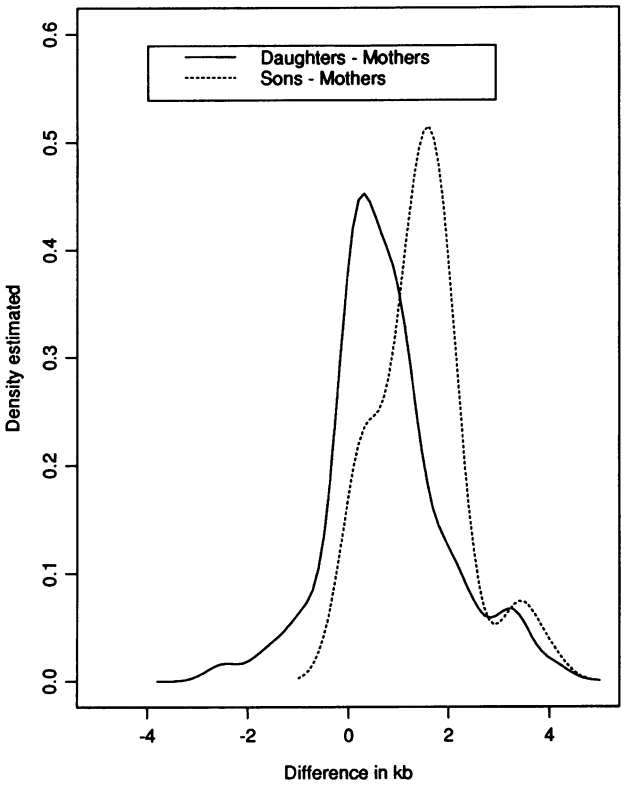

Figure 3 Density function (see Silverman 1986) of individual differences in the size of CGG expansion (in kb) between fragile X mothers and their offspring according to sex.

< 2.5), and thus determine the skewness of the distribution. However, the distribution in figure 2*c*, which is based on a subsample of females only from the youngest generation, shows the same trend, which indicates that there must be some other cause of the observed sex differences.

Mother-Offspring Differences in the Size of Expansion According to Sex of Offspring

In order to be able to explain the reason for differences in the shape of the distribution of expansion sizes between males and females in our sample, we compared the intrapair differences in the size of repeat within pairs of mothers-sons and mothers-daughters. Both premutation and full-mutation mothers were included in the analysis. The density function (smoothed histogram) of the differences in average size (fig. 3) is obviously shifted toward lower values in the daughters compared with the relatively larger differences in the sons. The results of comparison by the *t*-test (in table 1) show that this shift is highly statistically significant. Comparison by the Mann-Whitney nonparametric test yielded similar results, showing a highly significant difference ($P = .0002$) between medians for mother-daughter and mother-son expansion rates.

Comparisons between Repeat-Size Categories in Parents and Offspring

Significant differences in the size of expansion between sons and daughters of carrier females have also been observed by using the categorical approach. Table 2 compares frequencies of premutation and full-mutation male and female offspring of mothers carrying a premutation. The results of comparison using the χ^2 test show that the mothers carrying a premutation produced significantly more daughters (35%) than sons (13.5%) within the premutation range, whereas a great majority of their sons (86.5%) and over the half of their daughters (65%) were within the full-mutation range. Among the offspring of a smaller sample of 19 full-mutation mothers, we encountered five daughters and one son with expansion reduced to premutations. The families with triplet-repeat reductions in mother-offspring transmissions have been described in more detail elsewhere (D. Z. Loesch, V. Petrovic, D. I. Francis, and H. Slater, unpublished data). For comparison, 12 fathers carrying premutations and 1 father with a full mutation in our family sample produced 20 daughters, all in the premutation category.

Discussion

We demonstrated significant differences in the expansion rate between fragile X mother-daughter and fragile X mother-son transmissions. We minimized a possibility of an ascertainment bias favoring the male offspring with large expansions, first, by screening all available individuals within sibships irrespective of sex and clinical status and, second, by omitting the probands leading to those sibships as a standard correction for the bias. A strategy of comprehensive screening enabled us to cover a wide range of smaller, as well as larger, repeat expansions in individual families. This may be the reason why the overall expansion rate from premutation to full mutation in our data is lower compared with

Table 1

Means and SDs of the Absolute Differences in the CGG Repeat Size from Mother-Daughter and Mother-Son Transmissions and Results of Comparison between These Transmissions, by *t*-test

Transmission	No.[a]	Difference (kb) Mean	SD	P[b]
Mother-Daughter	78	.76	1.14	.0001
Mother-Son	61	1.45	.91	(df = 136)

[a] Transmissions involving probands are omitted.
[b] *P*-value for the two-tailed test.

Table 2

Number of Male and Female Offspring in Two Categories of CGG Repeat Size, Corresponding to Premutation and Full Mutation from Mothers Carrying a Premutation

	Mothers (P)	Daughters			Sons		
		P	F	Total	P	F	Total
P	74	22	41	63	7	45	52
Percentage	...	34.9	65.1	...	13.5	86.5	...

NOTE.—$\chi^2 = 6.9561$; $P = .00835$ (two-sided); P = premutation ($1.0 < kb \leq 1.6$); F = full mutation ($kb > 1.6$). Average size of repeat considered in subjects with multiple bands.

some reports based on different family samples (i.e., Fu et al. 1991; Heitz et al. 1992), where ascertainment might have favored family branches with obviously affected individuals. In our sample, the segregation ratios (with probands excluded) were 1.09:1.00 for males and 1.19:1.00 for females, and the small deviation from segregation ratio of 1 is not statistically significant ($P = .642$, for male ratio, and $P = .304$, for female ratio).

Since it is very unlikely that the observed differences have been related to ascertainment bias, they are clearly consistent with the suggestions based on the in vitro data that triplet expansion is at least primarily, if not exclusively, a postzygotic phenomenon. Moreover, Wöhrle et al. (1993) and Devys et al. (1992) have shown that the triplet expansion may take place very early in development and during a restricted embryological phase. Our findings suggest that the rate of this expansion depends on the sex of offspring, in that it is enhanced in the male, compared with the female, subjects, but the stage of development when this difference is realized remains speculative.

The known genetic differences between male and female zygotes are the compliments of X and Y chromosomes and imprinting of a small number of known genes. However, there is no known homologue of or gene associated with FMR1 on the Y chromosome, nor has any known imprinted gene any association with FMR1. Little is known of the control of FMR1 expression other than the fact that the gene is subject to Lyonization. It should therefore be considered that the second X chromosome in females has some involvement in restricting the size of expansion of fragile X alleles in female embryos relative to that observed in males.

At this time we have little understanding of the cause of triplet expansion. Replication slippage has been suggested as one possible mechanism (Schlotterer and Tautz 1992; Väisänen et al. 1994) and failure of DNA repair as another (Eichler et al. 1994). Normal replication has been shown to be delayed at the fragile X locus (Hansen et al. 1993), and this may be related to transcriptional inhibition (Pieretti et al. 1991; Verheij et al. 1993). A trans-acting influence of the normal X chromosome in females, which apparently limits triplet expansion, may operate by counteracting one of the above mechanisms. Comprehensive embryological studies of triplet expansion in male and female cells are needed to assess our clinical findings further.

Addendum.—After the manuscript was completed, our attention was drawn to an abstract (Rousseau et al. 1994) reporting the findings that suggested that males are at higher risk of transforming their mother's premutation into full mutation than are females. These data, which are based on different population sample, are in complete agreement with present results.

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