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Examination of Factors Associated with Instability of the FMR1 CGG Repeat

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Summary

We examined premutation-female transmissions and premutation-male transmissions of the FMR1 CGG repeat to carrier offspring, to identify factors associated with instability of the repeat. First we investigated associations between parental and offspring repeat size. Premutation-female repeat size was positively correlated with the risk of having full-mutation offspring, confirming previous reports. Similarly, premutation-male repeat size was positively correlated with the daughter's repeat size. However, increasing paternal repeat size was associated also with both increased risk of contraction and decreased magnitude of the repeat-size change passed to the daughter. We hypothesized that the difference between the female and male transmissions was due simply to selection against full-mutation sperm. To test this hypothesis, we simulated selection against full-mutation eggs, by only examining premutation-female transmissions to their premutation offspring. Among this subset of premutation-female transmissions, associations between maternal and offspring repeat size were similar to those observed in premutation-male transmissions. This suggests that the difference between female and male transmissions may be due to selection against full-mutation sperm. Increasing maternal age was associated with increasing risk of expansion to the full mutation, possibly because of selection for smaller alleles within the offspring's soma over time; a similar effect of increasing paternal age may be due to the same selection process. Last, we have evidence that the reported association between offspring sex and risk of expansion may be due to ascertainment bias. Thus, female and male offspring are equally likely to inherit the full mutation.

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Introduction

Fragile X syndrome, a form of X-linked mental retardation, is caused by unstable expansion of a CGG repeat in the 5′ UTR of the FMR1 gene (Fu et al. 1991; Verkerk et al. 1991). Under normal circumstances, this CGG repeat is polymorphic, with individuals possessing 6–54 copies of the repeat. The number of repeats usually is inherited without change, from parent to child (Fu et al. 1991; Heitz et al. 1992; Yu et al. 1992; Brown et al. 1993; Snow et al. 1993). In families with fragile X syndrome, however, the repeat has a propensity to expand in size, in each generation. Once the number of repeats has expanded to $\geqslant$200, the CGG repeat and surrounding region become hypermethylated. This aberrant methylation of FMR1 causes transcriptional inactivation (Pieretti et al. 1991; Sutcliffe et al. 1992; McConkie-Rosell et al. 1993). Thus, individuals who possess >200 copies of the repeat are missing the gene product, an RNA-binding protein called "FMRP," and are at risk to develop symptoms of fragile X syndrome (Ashley et al. 1993). These individuals are said to carry the full-mutation form of the repeat. Other family members, who carry 55–200 CGG repeats, are not at risk to develop symptoms of the disorder, although their repeat exhibits intergenerational instability. These family members are said to carry the premutation form of the repeat.

The mechanism causing expansion of the CGG repeat remains to be defined. It is known that the initial susceptibility to instability is related to the AGG interruption pattern within the repeat sequence. Normally, a single AGG interrupts the repeat sequence every 10 or so CGG repeats (Eichler et al. 1994; Hirst et al. 1994; Kunst and Warren 1994; Snow et al. 1994; Zhong et al. 1995). Thus, most normal alleles have one to three AGG interruptions. In contrast, most premutation alleles have either no AGG or only a single AGG interruption at the 5′ end of the repeat (Eichler et al. 1994; Snow et al. 1994; Zhong et al. 1995). Because of these observations, it has been hypothesized that the length of uninterrupted CGG repeats on the 3′ end of the repeat determines the susceptibility to instability. It appears that this threshold

for stability is ~35 CGG repeats (Eichler et al. 1994). Evidence for cis-acting factors involved in the susceptibility to instability and/or for other characteristics of the repeat sequence comes from association studies of the repeat allele and markers flanking the repeat (for review, see Morris et al. 1995). Results from those studies suggest that there may be different mutational pathways that cause normal alleles to become unstable and that these pathways are associated with different haplotypes (Eichler et al. 1996).

Once an allele is unstable and has a total repeat length >55, expansion of the repeat size is significant. The mechanism(s) related to this massive expansion, or hyperexpansion, is also unknown. However, several factors are known to influence that instability. First, the sex of the carrier parent plays an important role. Expansion from a premutation allele to a full-mutation allele occurs only in female transmissions of the unstable gene, not in male transmissions. Second, the parental repeat size is associated with the magnitude of the expansion. In females, the risk of expansion to the full mutation in the next generation increases with increasing repeat size of the maternal allele (Fu et al. 1991; Heitz et al. 1992; Yu et al. 1992; Snow et al. 1993; Nolin et al. 1996; Sherman et al. 1996). In contrast, in premutation males, increasing paternal repeat size is associated with increasing probability for contraction in repeat size in the next generation (Nolin et al. 1996).

To better understand the underlying mechanisms of hyperexpansion, other factors also have been examined. First, increasing maternal age may be associated with increased instability. One report has noted that, among siblings with the full mutation, the younger sibling more often inherited a larger expansion (Mornet et al. 1993). The authors of that have suggested that this may be due to a maternal age effect or to selection against the larger allele within an individual over time. Second, there have been two reports that suggest that the sex of the offspring is associated with the risk of expansion. These reports found that male offspring were more likely to receive larger expansions from their premutation or full-mutation mothers than were female offspring (Rousseau et al. 1994; Loesch et al. 1995). Third, a familial factor(s), independent of parental sex and repeat size, has been identified that causes repeat sizes within sibships to be more similar than those among sibships (Nolin et al. 1996). The nature of this familial factor has not yet been defined. However, preliminary analyses indicate that it does not seem to be due to the internal sequence of the repeat (Nolin et al. 1996), nor to the haplotype background (Murray et al. 1997).

It is clear that a more in-depth analysis of the factors that influence hyperexpansion of the repeat in the FMR1 gene would provide insight into that mechanism. Thus, we have analyzed transmissions of the FMR1 CGG repeat of premutation females and premutation males to their carrier offspring, to identify factors that may be associated with the expansion mechanism.

Subjects and Methods

Information on repeat size, sex, birth date, and pedigree was collected for carrier parent–carrier offspring pairs of 434 premutation mothers and 144 premutation fathers. These data were collected through both prospective and retrospective means. The "prospective" cases (50 premutation mother transmissions) have been described elsewhere and originated from data on prenatal and live-born pregnancy outcomes of known fragile X carrier females (Sherman et al. 1996). Data for each case included a pedigree and DNA results on repeat size in both the mother and the offspring. Because of the prospective collection, these cases were considered to be free of ascertainment bias. These prospective cases were kindly contributed by those investigators noted in the Acknowledgments. The "retrospective" cases originated from families that had been clinically ascertained through a proband with fragile X syndrome. Each transmission was assessed to determine whether it was involved in the ascertainment of the family. If so, the transmission was excluded from analysis. Retrospective cases were contributed from four centers. Hunter Genetics, Newcastle, New South Wales (affiliation of H.R. and G.T.) contributed 244 premutation mother transmissions and 37 premutation father transmissions. The New York State Institute for Basic Research, Staten Island (affiliation of A.E.G., S.L.N., and W.T.B.) contributed 112 premutation mother transmissions and 85 premutation father transmissions. The remaining cases (28 premutation mother transmissions and 22 premutation father transmissions) were contributed by J. C. Self Research Institute, Greenwood Genetics Center, Greenwood, SC (affiliation of C.E.S.) and by the Department of Genetics, Emory University, Atlanta (affiliation of A.E.A.-K. and S.L.S.).

Molecular analyses of repeat size were performed at the contributing center, by either PCR or restriction digestion followed by Southern blotting. In general, small premutation repeat sizes (<90 repeats) were estimated by PCR, for greater accuracy. Larger premutation and full-mutation repeat sizes were estimated by the less accurate method of restriction digestion and Southern blotting, because of the difficulty in amplification of these larger repetitive alleles. Examples of the protocols for the molecular analyses have been described by Brown et al. (1993). Information on the size of a full mutation usually was not available, for several reasons. First and foremost, the size was not determined because of the lack of clinical relevance. Second, an accurate estimate of repeat size was difficult to determine because of both

low resolution on Southern blots and the possible complication of mosaicism. Therefore, for analyses that included full-mutation cases, the repeat-size status was treated as a binary variable (i.e., 0 = premutation, and 1 = full mutation).

The goal of the present study was to identify factors involved in the variation of instability of the CGG repeat. Thus, with respect to premutation-female transmissions, only transmissions from mothers with $\leqslant 90$ repeats were eligible for study, since mothers with >90 repeats have a 94%–100% risk of expansion to the full mutation (Fu et al. 1991; Heitz et al. 1992; Yu et al. 1992; Snow et al. 1993; Nolin et al. 1996; Sherman et al. 1996). All premutation-male transmissions were eligible for the study, since all their daughters carry a premutation allele. The premutation fathers had repeat sizes in the range of 56–130 repeats.

To examine factors affecting offspring repeat size, linear regression was used. The dependent variable was either offspring repeat size or the repeat-size difference between parent and child. The independent variables were parental repeat size, parental age, grandparental origin of the mutation, and offspring sex. Logistic regression was used to examine two factors: (1) the risk, among the premutation-female transmissions, of expansion to the full mutation and (2) the risk, among premutation-female transmissions and premutation-male transmissions, to pass an expansion, versus a contraction or no change in repeat size. Thus, the dependent variable was either the mutational status (i.e., premutation or full mutation) of the offspring or whether the parent had passed an expansion to his or her offspring. The independent variables were the same as those used in the linear-regression analyses. Results of logistic regression are reported as odds ratio (OR) with 95% confidence interval (95% CI) and correspond to the probability of either having a full-mutation child or passing an expansion to the child, depending on the analysis. The reported *P* values for logistic regression correspond to the hypothesis that the coefficient of the independent variable is different from 0. All statistical analyses, including Pearson correlations, *t*-tests, and linear- and logistic-regression analyses, were performed with Statistical Analysis System (SAS) software release 6.07.

Results

Premutation-Female Transmissions

Maternal Repeat Size.—As previous studies have shown, examination of all the premutation-female transmissions revealed that the mother's repeat size is an important predictor of the mutational status of her offspring (OR 1.09 [95% CI = 1.06–1.12]). The mean repeat size in mothers of full-mutation offspring was 74.09, compared with 68.10 in mothers of premutation offspring. Therefore, the risk of having a full-mutation child increased as the mother's repeat size increased, as shown in figure 1. To identify additional risk factors, we controlled for the effect of the mother's repeat size in the subsequent analyses, by including it as an independent variable in the regression analyses.

Maternal Age.—Next, we examined whether maternal age was associated with the mutational status of the offspring. We found a significant age effect after adjusting for the mother's repeat size (OR 1.07 [95% CI = 1.03–1.12]). This observation could be either a true maternal-age effect or due to selection against large alleles in the soma of the offspring over time; that is, since most siblings are tested simultaneously for their carrier status, older siblings could have smaller repeat sizes compared with those in younger siblings, because of somatic selection against larger alleles. Unfortunately, age-at-testing data were not available for the retrospective cases. However, we were able to compare retrospective cases, in which the offspring were tested at various developmental time points, and prospective cases, in which the offspring were tested at the same developmental time point. We hypothesized that, if there is a true maternal-age effect, it would be present in both the retrospective and the prospective cases. In contrast, if selection is occurring, the apparent maternal-age effect should be observed only among the retrospective cases. Since the retrospective cases were tested at various developmental

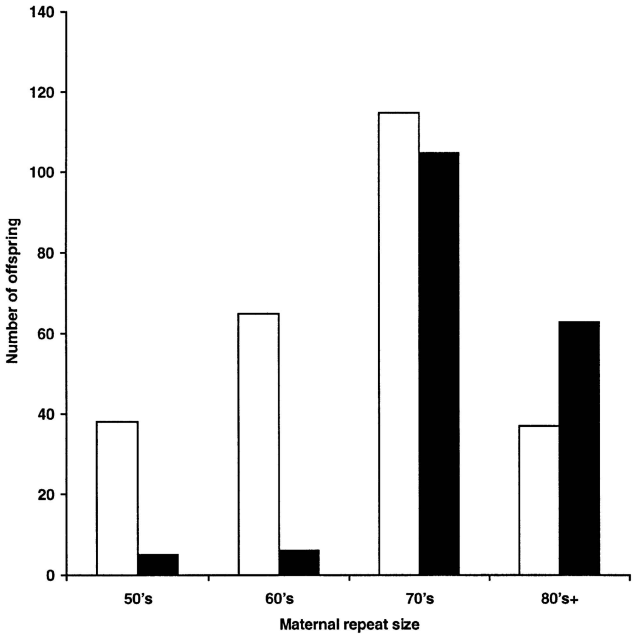

Figure 1 No. of premutation offspring (*unblackened bars*) and full-mutation offspring (*blackened bars*) of premutation females, based on maternal repeat size.

time points, this would allow for the effects of selection to mimic a maternal-age effect. In contrast, the prenatal cases were tested at the same developmental time; thus, all cases would have had an equal amount of time for selection to occur, and no maternal-age effect should be observed. Comparison of the mean maternal age of retrospective cases versus that of prenatal cases shows that the maternal-age effect was restricted to the retrospective cases (table 1). This was confirmed by logistic regression, with adjustment for maternal repeat size (table 1). These results suggest that the observed maternal-age association is due to selection against larger alleles over time in the offspring.

Sex of Offspring.—Elsewhere it has been reported that the male offspring of premutation or full-mutation mothers receive a larger repeat size than do female offspring (Rousseau et al. 1994; Loesch et al. 1995). This may be a true effect, or it may result from ascertainment biases that spuriously increase the number of transmissions from premutation mothers to premutation daughters. At least two such biases are obvious. The first relates to who is tested within a sibship. Nonsymptomatic females are tested more often for carrier status than are their male counterparts, since only females are at risk of transmitting the full mutation to their offspring. Thus, this bias may increase the number of transmissions to premutation-female offspring, relative to the number of transmissions to premutation-male offspring. The second bias relates to the observed decreased fitness of symptomatic full-mutation carrier females (Sherman et al. 1984). Specifically, mothers of probands (i.e., females who have reproduced) are more likely to be premutation carriers than full-mutation carriers. Thus, inclusion of transmissions from the carrier grandmother to the mother of the proband may spuriously increase the number of premutation-female offspring, relative to the number of full-mutation female offspring. Given these potential biases, we examined the association between the sex of the offspring and his or her repeat size, under three correction schemes. In the first scheme, we performed the general correction as described in Subjects and Methods: we excluded all transmissions involved in the ascertainment of the family. Under the second scenario, we excluded sibships in which not all offspring had been tested. Last, we excluded all transmissions from carrier grandmothers to mothers of probands. Table 2 summarizes the number of cases and the results of each analysis. The results indicate that the association of sex of the offspring with the offspring's mutational status diminishes with correction of potential biases.

Premutation-Male Transmissions

Two factors that may influence the change in repeat size in male transmissions were examined among the 144 father-daughter pairs: paternal repeat size and age.

Paternal Repeat Size.—The father's repeat size was positively correlated with the daughter's repeat size ($r = .65$, $P = .0001$) and explained ~43% of the variance. To further understand this association, we examined both the magnitude of the intergenerational change from father to daughter and the direction of the change (i.e., expansion vs. either contraction or no change). We found that the magnitude of the change decreased as the father's repeat size increased ($r = -.19$, $P = .025$). To examine the direction of the change, logistic regression was performed with the dependent variable indicating whether there was an expansion. This analysis showed that the risk of expansion decreased with increasing repeat size in the father (OR 0.96 [95% CI = 0.94–0.99]). This association is depicted in figure 2.

This negative association between risk of expansion and paternal repeat size could represent different mutational mechanisms in males versus females. Alternatively, the same mechanism may be occurring but there may be strong selection against full-mutation alleles only in sperm, not in eggs. This limited selection would explain why paternal transmissions of the full mutation are not observed. To test this, we analyzed a subset of female transmissions that would be observed if there were a comparable selection against full-mutation transmissions in females; that is, we omitted those female transmissions that expanded to the full mutation. As in male transmissions, the risk of expansion decreased with increasing maternal repeat size (OR 0.96 [95% CI = 0.93–0.99]). Mothers who passed an expansion to their premutation offspring had an average repeat size of 67.23, compared with 70.32, the average repeat size in mothers who did not pass an expansion to their pre-

Table 1

Association between Maternal Age and Risk for Expansion

	Mean Age of Mother of (years)			
Type of Case	Premutation Offspring	Full-Mutation Offspring	*t*-Test *P*	Odds Ratio (CI)
Retrospective	25.82 ($n = 203$)	27.17 ($n = 132$)	.02	1.06 (1.02–1.11)
Prenatal	30.71 ($n = 17$)	31.3 ($n = 20$)	.82	1.01 (.86–1.18)

mutation offspring (fig. 3). However, the association between the magnitude of the change and the parental repeat size was not observed among female transmissions ($r = -.05$, $P = .45$). This failure to observe an association may be due to the smaller range of repeat sizes among the mothers (52–90), compared with that among the fathers (56–130).

Paternal Age.—Next we examined paternal age as a risk factor after controlling for the effect of the father's repeat size. No association was observed between the father's age and the daughter's repeat size ($r = .07$, $P = .49$). However, there was a trend in that the magnitude of change increased with the father's age ($r = .15$, $P = .12$). Also, the direction of the change was associated with paternal age: Older fathers were more likely to pass on expansions, rather than contractions or no change in repeat size, than were younger fathers (OR 1.10 [95% CI = 1.03–1.18]). The average age among fathers who passed on an expansion was 31.84 years, compared with 27.98 years, the average age among fathers who passed on a contraction or no change in repeat size (*t*-test $P = .0034$).

Again we examined the subset of premutation-female-to-premutation-offspring transmissions, to test the hypothesis that the basis of the paternal-age effect was the same as that of the maternal-age effect. As in the case of the male transmissions, we did not detect an association between the mother's age and the offspring's repeat size ($r = -.06$, $P = .38$). In contrast to male transmissions, there was also no association between maternal age and either the magnitude of the change ($r = -.004$, $P = .95$) or the direction of the change (OR 1.02 [95% CI = 0.97–1.07]). However, these analyses should be interpreted with caution, since the different ranges of parental repeat sizes—that is, 52–90 among the mothers, versus 56–130 among the fathers—may have affected the results.

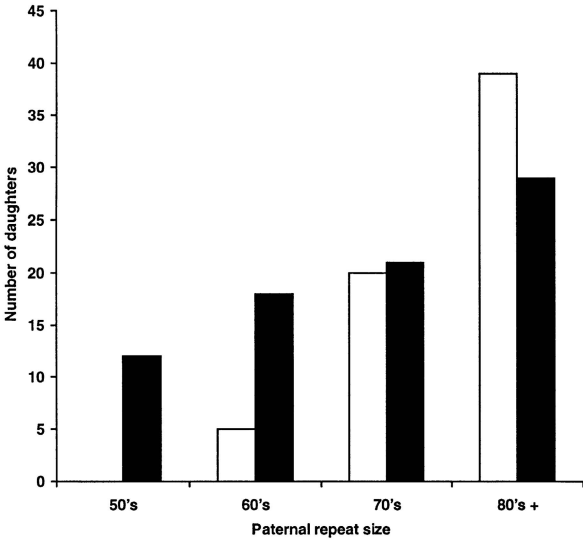

Figure 2 No. of premutation daughters of premutation males, based on paternal repeat size and whether the daughter received an expansion. Unblackened bars represent either a contraction or no repeat-size change, from father to daughter, and blackened bars represent repeat-size expansions, from father to daughter.

Discussion

We have confirmed that increasing maternal repeat size is significantly associated with the risk of having full-mutation offspring, an association that has been well documented by previous reports (Fu et al. 1991; Heitz et al. 1992; Yu et al. 1992; Snow et al. 1993; Nolin et al. 1996; Sherman et al. 1996). We also have shown that the father's repeat size is positively correlated with the daughter's repeat size. Moreover, examination of the magnitude and direction of the intergenerational change revealed a negative association with the father's repeat size, confirming previous results that the frequency of contractions in male transmissions increases with increasing paternal repeat size (Nolin et al. 1996). To determine whether this observation represented a mechanistic difference between premutation-male transmissions and premutation-female transmissions or was simply a function of selection against the full mutation in sperm but not against full mutation in eggs, we examined a subset of female transmissions that would be observed

Table 2

Association between Sex of Offspring and Risk of Expansion

	Proportion of Offspring with Full Mutation (%)		*P*	
Ascertainment Scheme (No. of Cases)	Males	Females	t-Test	Logistic Regression
Removal of cases associated with ascertainment (434)	.46	.38	.06	.07
Removal of incompletely ascertained sibships (338)	.48	.43	.38	.34
Removal of transmissions to proband's mother (298)	.48	.50	.63	.71

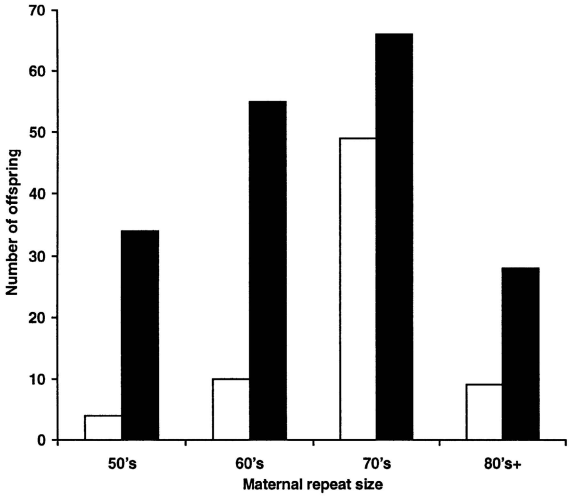

Figure 3 No. of premutation offspring of premutation females, based on maternal repeat size and whether offspring received an expansion. Unblackened bars represent either a contraction or no repeat-size change, from mother to offspring, and blackened bars represent repeat-size expansions, from mother to offspring.

if a similar selection had occurred in eggs; that is, we examined premutation-female-to-premutation-offspring transmissions. The results indicated a negative association between the mother's repeat size and the direction of the intergenerational change, similar to that observed in the paternal transmissions. This suggests that the difference between premutation-male transmissions and premutation-female transmissions may be simply selection against the full mutation in sperm.

Thus far, the only definitive evidence supporting a selective force in fragile X gametes is found in full-mutation male carriers. Elsewhere it has been demonstrated that full-mutation males have premutation sperm (Reyniers et al. 1993). However, at the time of that study, it was not known whether this observation was due to the gametes being spared from somatic expansion or to selection against full-mutation sperm. Recently, Malter et al. (1997) examined full-mutation male fetuses of various developmental stages and found evidence to support the selection hypothesis. They observed that a full-mutation male fetus of 13 wk gestation had full-mutation pro-spermatogonia and no detectable premutation pro-spermatogonia. In contrast, a full-mutation male fetus of 17 wk gestation exhibited both full-mutation pro-spermatogonia and premutation pro-spermatogonia. These observations, taken with the observations by Reyniers et al. (1993), suggest that a selective process removes full-mutation alleles in full-mutation males until most, if not all, of the sperm carry a premutation allele. Furthermore, Malter et al.'s (1997) examination of full-mutation female fetuses at 16 and 17 wk gestation did not detect the presence of premutation alleles in the ovaries, providing no evidence for selection against full-mutation eggs. These data support the hypothesis that the lack of expansion to the full mutation in male transmissions is due simply to differences in the stringency of selection against full-mutation gametes, in males versus females. Interestingly, examination of the ratio of X-bearing sperm to Y-bearing sperm in a premutation male revealed no deviation from the expected ratio of 1.0 (H. E. Malter, A. E. Ashley-Koch, S. L. Sherman, and S. T. Warren, unpublished data). This may not be an unexpected result, since, because of incomplete cytokinesis during mitosis and meiosis, the progeny of a spermatogonial cell mature within a common syncytium and, thus, the progeny can share gene products via cytoplasmic bridges (Braun et al. 1989). Therefore, if a spermatogonial cell is carrying a full mutation that is deficient for the FMR1 gene product, FMRP, then all the progeny, including both X-bearing sperm and Y-bearing sperm, would be selected against. Moreover, since the X chromosome normally is inactivated around the onset of meiosis during sperm development (Ayoub et al. 1997), FMRP probably is expressed only prior to this. Alternatively, the selection against full-mutation sperm may not result from a lack of FMRP but, rather, may occur during mitotic divisions of the spermatogonia, because of the inability to faithfully replicate or retain the large number of CGG repeats in the full-mutation allele. Both of these hypotheses suggest that selection against full-mutation sperm occurs premeiotically. It should be noted that, as well, other observations are consistent with a selection process in females. Several investigators have observed that premutation—but not full mutation—carrier women have an increased risk of premature ovarian failure (POF) (Conway et al. 1995; Partington et al. 1996; Vianna-Morgante et al. 1996), although these observations have not always been replicated by other investigators (Kenneson et al. 1997). The underlying mechanism of this observed POF in these premutation females is not clear. The POF may be due to loss of eggs carrying mutated alleles. However, if this is the case, then one would expect, as well, to observe an increased risk of POF in full-mutation carrier women. Alternatively, the POF may be due to an overall loss of eggs, both those carrying mutated alleles and those carrying nonmutated alleles. The later hypothesis would be consistent with a lack of selection against full-mutation eggs.

In addition to selection in the gametes, our observations suggest that selection also occurs in somatic cells. Elsewhere, a positive correlation between maternal age and full-mutation–offspring repeat size has been observed (Mornet et al. 1993). The investigators in that study hypothesized that this observation could be due to either a true maternal-age effect or selection against

larger alleles in the soma of the offspring over time. To test this hypothesis, we compared retrospectively ascertained live-birth cases versus prenatally diagnosed cases. The live-birth cases would have been tested at different ages, whereas the prenatally diagnosed cases were tested at the same developmental time point. A significant association with maternal age was found among the live-birth cases but not among the prenatally diagnosed cases. This suggests that the association is due to selection against larger alleles within the soma of the offspring over time. Similarly, examination of paternal transmissions identified an association between paternal age and intergenerational changes in repeat size. As the father ages, the risk that he will pass on an intergenerational expansion increases. This association, as well, most likely is due to somatic selection; however, no prenatal paternal transmissions were available to allow us to test this hypothesis.

Although these results suggest the presence of somatic selection within the individual over time, there were limitations to our analysis. First, the tissue sampled among the retrospective cases was blood and was embryonic in origin, whereas the tissue sampled among the prenatal cases was either amniocyte or chorionic villus and was extraembryonic in origin. If there are differing selection mechanisms occurring among the different tissues, our interpretation of the results may be incorrect. Furthermore, in most cases, repeat analysis was performed on transformed cell lines of the samples. Selection mechanisms affecting transformed cell lines may be quite different than those that affect tissues in vivo. Second, there were only a small number of prenatal cases ($n = 37$) available for analysis. For these reasons, further analysis of the association between parental age and offspring repeat size is warranted, to confirm that the association is due to somatic selection.

If somatic selection does occur, then somatic mosaicism of repeat size may be expected as a result. Mosaicism is not uncommon among individuals with the full mutation (Rousseau et al. 1991*a*, Nolin et al. 1994), and it also has been observed among premutation individuals (Moutou et al. 1997). Moreover, Rousseau et al. (1991*b*) observed that somatic mosaicism appeared to decrease with increasing age in full-mutation females. These investigators observed that the patterns of full-mutation alleles in older women (age >40 years) were more homogeneous than the patterns observed in younger women. Although this trend was not statistically significant, it is consistent with the prediction of somatic selection.

Data obtained from transgenic mouse models of trinucleotide-repeat disorders conflict with the theory of somatic selection. A mouse model of Huntington disease (HD) has displayed both increasing somatic and intergenerational instability with increasing age, in paternal transmissions (Mangiarini et al. 1997). In addition, a mouse model of spinocerebellar ataxia type 1 has displayed increasing intergenerational instability with increasing age, in maternal transmissions (Kaytor et al. 1997). Both of these transgenic mouse models appear to display true parental-age effects. Moreover, in the HD mouse, somatic instability increased with age, which directly contradicts the observations by Rousseau et al. (1991*b*). It is difficult to correlate the results from the transgenic mice with those from humans, because the effects of cis-acting factors in the transgenes, as well as the integration positions of the transgenes within the mouse genome, are not well understood. More data on the transgenic mouse models need to be obtained before we can extrapolate those results to humans.

We also have identified factors, specific to premutation-female transmissions, that are associated with a woman's risk of having a full-mutation child. First, in myotonic dystrophy, another disorder caused by expansion of a trinucleotide repeat, it has been well documented that there is a statistically significant excess of transmitting grandfathers of individuals with the congenital form of the disorder (Harley et al. 1993; Lavedan et al. 1993; Lopez de Munain et al. 1995). To determine whether this association also is present in fragile X syndrome, the parental origin of the mother's premutation was examined. The results indicated that, similar to situation for myotonic dystrophy, women who inherited the fragile X premutation from their fathers were more likely to have a full-mutation child than were women who inherited the mutation from their mothers. However, this observation was not statistically significant and, thus, was only a trend (OR 1.44 [95% CI = 0.87–2.38]). This observation is consistent with the predictions from a previous model, which examined the effects of expansion during meiosis and mitosis (Ashley and Sherman 1995). This meiotic/mitotic model assumed that maternally inherited somatic—but not gametic—alleles were subjected to additional expansion, whereas paternally inherited somatic alleles were not. Thus, the model predicted that, when women with similar somatic repeat sizes are compared, women who inherited the mutation from their father would be at greater risk of having full-mutation offspring than would women who inherited the mutation from their mother; this is because women who inherited the mutation from their father would have similar repeat sizes in their somatic and gametic cells, whereas women who inherited the mutation from their mother would have smaller repeat sizes in their gametic cells, because the former group of women had been spared the maternal somatic expansion process. However, the meiotic/mitotic model cannot explain the observed association, because the assumptions of the model, specifically those concerning selection against full-mutation sperm, are incorrect.

Thus, further data are needed, both to confirm an association between parental origin of the mutation and risk of having a full-mutation offspring and to determine the cause of this association.

Second, examination of our data revealed that the observed association of offspring sex and risk of expansion probably was due to ascertainment problems. These ascertainment problems stem from two sources. First, nonsymptomatic females are more likely to be tested than are nonsymptomatic males. Second, full-mutation females are less likely to reproduce—and, therefore, are less likely to have a proband offspring—than are premutation females. These sources of bias may be present in the previous analyses, reporting that male offspring receive larger expansions (Rousseau et al. 1994; Loesch et al. 1995).

This study represents the collaboration of many centers conducting fragile X research. The advantage of this collaborative approach is that large numbers of premutation-female transmissions and premutation-male transmissions can be analyzed, providing greater statistical power. The disadvantage of this approach is that there is variation, in repeat-size estimation, among laboratories—and that, therefore, the pooling of data can introduce error. However, 82% of the premutation-female transmissions and 85% of the premutation-male transmissions in this analysis were contributed by only two centers, one in Australia and one in the United States, which should greatly reduce the error that is due to data pooling.

In conclusion, analyses of transmissions from premutation females and premutation males to their carrier offspring suggest that the mechanism(s) of expansion in premutation females and premutation males may be similar and that selection against full-mutation alleles in sperm is the basis for the lack of expansion to the full mutation in male transmissions. More molecular data are needed for definition of the mechanism of expansion, but results from this study support analysis of familial data as a valuable approach toward a better understanding of the nature of the instability of the expansion of FMR1 CGG repeats.

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References

Ashley AE, Sherman SL (1995) Population dynamics of a meiotic/mitotic expansion model for the fragile X syndrome. Am J Hum Genet 57:1414–1425

Ashley CT, Wilkinson KD, Reines D, Warren ST (1993) FMR1 protein: conserved RNP family domains and selective RNA binding. Science 262:563–566

Ayoub N, Richler C, Wahrman J (1997) Xist RNA is associated

with the transcriptionally inactive XY body in mammalian male meiosis. Chromosoma 106:1–10

Braun RE, Behringer RR, Peschon JJ, Brinster RL, Palmiter RD (1989) Genetically haploid spermatids are phenotypically diploid. Nature 337:373–376

Brown WT, Houck GE, Jeziorowska A, Levinson FN, Ding X, Dobkin C, Zhong J, et al (1993) Rapid fragile X carrier screening and prenatal diagnosis using a nonradioactive PCR test. JAMA 270:1569–1575

Conway GS, Hettiarachchi S, Murray A, Jacobs PA (1995) Fragile X premutations in familial premature ovarian failure. Lancet 346:309–310

Eichler EE, Holden JA, Popovich BW, Reiss AL, Snow K, Thibodeau SN, Richards CS, et al (1994) Length of uninterrupted CGG repeats determines instability in the FMR1 gene. Nat Genet 8:88–94

Eichler EE, Macpherson JN, Murray A, Jacobs PA, Chakravarti A, Nelson DL (1996) Haplotype and interspersion analysis of the FMR1 CGG repeat identifies two different mutational pathways for the origin of the fragile X syndrome. Hum Mol Genet 5:319–330

Fu YH, Kuhl DPA, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJMH, et al (1991) Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 67:1047–1058

Harley HG, Rundle SA, MacMillan JC, Myring J, Brook JD, Crow S, Reardon W, et al (1993) Size of the unstable CTG repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. Am J Hum Genet 52: 1164–1174

Heitz D, Devys D, Imbert G, Kretz C, Mandel JL (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

Hirst MC, Grewal PK, Davies KE (1994) Precursor arrays for triplet repeat expansion at the fragile X locus. Hum Mol Genet 3:1553–1560

Kaytor MD, Burright EN, Duvick LA, Zoghbi HY, Orr HT (1997) Increased trinucleotide repeat instability with advanced maternal age. Hum Mol Genet 6:2135–2139

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

Lavedan C, Hofmann-Radvanyi H, Shelbourne P, Rabes J-P, Duros C, Savoy D, Dehaupas I, et al (1993) Myotonic dystrophy: size- and sex-dependent dynamics of CTG meiotic instability, and somatic mosaicism. Am J Hum Genet 52: 875–883

Loesch DZ, Huggins R, Petrovic V, Slater H (1995) Expansion of the CGG repeat in fragile X in the FMR1 gene depends on the sex of the offspring. Am J Hum Genet 57:1408–1413

Lopez de Munain A, Cobo AM, Poza JJ, Navarrete D, Martorell L, Palau F, Emparanza JI, et al (1995) Influence of the sex of the transmitting grandparent in congenital myotonic dystrophy. J Med Genet 32:689–691

Malter HE, Iber JC, Willemsen R, de Graaff E, Tarleton JC, Leisti J, Warren ST, et al (1997) Characterization of the full fragile X syndrome mutation in fetal gametes. Nat Genet 15:165–169

Mangiarini L, Sathasivam K, Mahal A, Mott R, Seller M, Bates GP (1997) Instability of highly expanded CAG repeats in mice transgenic for the Huntington's disease mutation. Nat Genet 15:197–200

McConkie-Rosell A, Lachiewicz AM, Spiridigliozzi GA, Tarleton J, Schoenwald S, Phelan MC, Goonewardena P, et al (1993) Evidence that methylation of the FMR-1 locus is responsible for variable phenotypic expression of the fragile X syndrome. Am J Hum Genet 53:800–809

Mornet E, Jokic M, Bogyo A, Tejada I, Deluchat C, Boue J, Boue A (1993) Affected sibs with fragile X syndrome exhibit an age-dependent decrease in the size of the fragile X full mutation. Clin Genet 43:157–159

Morris A, Morton NE, Collins A, Lawrence S, Macpherson JN (1995) Evolutionary dynamics of the FMR1 locus. Ann Hum Genet 59:283–289

Moutou C, Vincent M-C, Biancalana V, Mandel J-L (1997) Transition from premutation to full mutation in fragile X syndrome is likely to be prezygotic. Hum Mol Genet 6: 971–979

Murray A, Macpherson JN, Pound MC, Sharrock A, Youings SA, Dennis NR, McKechnie N, et al (1997) The role of size, sequence and haplotype in the stability of FRAXA and FRAXE alleles during transmission. Hum Mol Genet 6: 173–184

Nolin SL, Glicksman A, Houck GE Jr, Brown WT, Dobkin CS (1994) Mosaicism in fragile X affected males. Am J Med Genet 51:509–551

Nolin SL, Lewis FA III, Ye LL, Houck GE Jr, Glicksman AE, Limprasert P, Li SY, et al (1996) Familial transmission of the FMR1 CGG repeat. Am J Hum Genet 59:1252–1261

Partington MW, Moore DY, Turner GM (1996) Confirmation of early menopause in fragile X carriers. Am J Med Genet 64:370–372

Pieretti M, Zhang F, Fu YH, 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

Reyniers E, Vits L, De Boulle K, Van Roy B, Van Velzen D, de Graaff E, Verkerk AJMH, et al (1993) The full mutation in the FMR-1 gene of male fragile X patients is absent in their sperm. Nat Genet 4:143–146

Rousseau F, Heitz D, Biancalana V, Blumenfeld S, Kretz C, Boue J, Tommerup N, et al (1991*a*) Direct diagnosis by DNA analysis of the fragile X syndrome of mental retardation. N Engl J Med 325:1673–1681

Rousseau F, Heitz D, Oberle I, Mandel J (1991*b*) Selection in blood cells from females carriers of the fragile X syndrome: inverse correlation between age and proportion of active X chromosmes carrying the full mutation. J Med Genet 28: 830–836

Rousseau F, Heitz D, Tarleton J, MacPherson J, Malmgren H, Dahl N, Barnicoat A, et al (1994) Higher rate of transition from fragile X premutations into full mutation in males than in females suggest post-conceptional expansion of the CGG repeats. Am J Hum Genet Suppl 55:A240

Sherman SL, Meadows KL, Ashley AE (1996) Examination of factors that influence the expansion of the fragile X mutation in a sample of conceptuses from known carrier females. Am J Med Genet 64:256–260

Sherman SL, Morton NE, Jacobs PA, Turner G (1984) The marker (X) syndrome: a cytogenetic and genetic analysis. Ann Hum Genet 48:21–37

Snow K, Doud LK, Hagerman R, Pergolizzi RG, Erster SH, Thibodeau SN (1993) Analysis of a CGG sequence at the FMR-1 locus in fragile X families and in the general population. Am J Hum Genet 53:1217–1228

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 3:1543–1551

Sutcliffe JS, Nelson DL, Zhang F, Pieretti M, Caskey CT, Saxe D, Warren ST (1992) DNA methylation represses FMR-1 transcription in fragile X syndrome. Hum Mol Genet 1: 397–400

Verkerk AJMH, Pieretti M, Sutcliffe JS, Fu Y, Kuhl DPA, Pizzuti A, Reiner O, et al (1991) Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 65:905–914

Vianna-Morgante AM, Costa SS, Pares AS, Verreschi IT (1996) FRAXA premutation associated with premature ovarian failure. Am J Med Genet 64:373–375

Yu S, Mulley J, Loesch D, Turner G, Donnelly A, Gedeon A, Hillen D, et al (1992) Fragile-X syndrome: unique genetics of the heritable unstable element. Am J Hum Genet 50: 968–980

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