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The Fragile X Premutation in Carriers and Its Effect on Mutation Size in Offspring

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Summary

The pattern of inheritance in the fragile X (fra(X)) mutation follows a multistage intergenerational process in which the premutation evolves into the full mutation and the characteristic phenotype of the fra(X) syndrome after passing through oogenesis or a postzygotic event. Findings from our multicenter study confirm a strong direct relationship between fra(X) premutation size in the mother and probability of a full mutation in offspring with the mutation. Remarkably, the best-fitting equations are nonlinear asymptotic functions. The close approximation to both the logistic model and Gompertz suggests a process of accumulation of errors in DNA synthesis, as has been proposed previously. We also note that a larger-than-expected number of daughters of transmitting males have premutations that are smaller than their fathers', and that proportion is significantly higher than the proportion of daughters whose premutations are smaller than their mothers'. Intergenerational decreases in premutation size have been reported in other trinucleotide-repeat disorders and also appear to be parent-of-origin specific. Thus, while intergenerational expansion to the full mutation in fra(X) may manifest a postzygotic event, decreases in mutation size may occur during or prior to meiosis.

Introduction

The fragile X (fra(X)) mutation is the leading cause of inherited mental retardation (MR). Prevalence estimates range from 1:2,250 males (Sherman et al. 1984) to 1:1,000 males (Webb et al. 1986), although recent evidence suggests that it may be as common as Down syndrome (Rousseau et al. 1994).

The defective gene in fra(X) syndrome has been named the "fra(X) mental retardation-1 (FMR-1) gene" (Verkerk et al. 1991). Normally, it has 6–52 copies of a CGG repeat and is thus polymorphic in the general population (Fu et al. 1991). Unaffected carrier males and females have an increased number of repeats—the "premutation"—to an upper limit of ~230 copies. Affected males and females have much larger expansions—the "full mutation"—ranging in size from 230 to several thousand copies. Full mutations are accompanied by methylation associated with failure of gene transcription and appearance of the characteristic phenotype of fra(X) syndrome (Heitz et al. 1991; Hanzlik et al. 1993; Verheij et al. 1993).

The pattern of inheritance of the fra(X) mutation suggests that the full mutation evolves following a multistep, intergenerational process (Pembrey et al. 1985; Laird 1987). That is, once the premutation is evident, it increases in size and is transformed into the full mutation after passing through oogenesis or during a postzygotic event (Wöhrle et al. 1993; Reyniers et al. 1993). Recent studies (Fu et al. 1991; Snow et al. 1993) have reported that the risk of conversion to the fully mutated state is directly related to the size of the premutation and thereby resolves the issue of penetrance manifested by Sherman's "paradox" (Sherman et al. 1984).

The paradigm of expansion inherent in the fra(X) mutation has also been described in other disorders, notably myotonic dystrophy (DM) (Harley et al. 1992), spinal and bulbar dystrophy or Kennedy disease (La Spada et al. 1991), Huntington disease (Huntington Disease Collaborative Research Group 1993), spinocerebellar ataxia type I (Orr et al. 1993), FRAXE (Knight et al. 1993), and hereditary dentatorubral-pallidoluysian atrophy (Koide et al. 1994). Currently, several quantitative versions have been advanced that elaborate on the mechanism. Morton and McPherson (1992) proposed a model wherein the outcomes associated with Sherman's paradox are produced by an inexorable increase in the size of the premutation, from a stable (S) to unstable state (Z) to the fully mutated condition (L). Sherman and Ashley (1993) also conjecture that expansion to full mutation in the FMR-1 gene occurs in a multistep linear progression, and they employ computer simulations to

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predict population estimates of the full mutation, on the basis of the size of an unstable premutation. Viewing subsequent generations as a transition matrix in a Markov process, Kolehmainen (1994) constructed a population model similar to that of Sherman and Ashley but included probabilities associated with decreases in premutation size in successive generations.

On the basis of data collected from our multicenter study, we have obtained a sufficiently large sample from which we can report several important findings. First, although there is a strong and significant linear relationship between premutation size in the mother and probability of full mutation in her offspring, asymptotic growth curves provide a more coherent fit. Second, premutation size is but one of two components needed to determine the aggregate risk estimate of a full mutation to offspring from a population of females with premutations. The second factor must be the frequency distribution of premutation alleles. Weighting premutation size by its relative frequency of occurrence produces the best estimate of aggregate risk for the full mutation. Finally, we demonstrate that there is a significantly higher proportion of daughters of transmitting males with smaller premutations than their fathers, compared with the proportion of daughters of transmitting females, and that decreased sizes in offspring occur more frequently in the fra(X) population than has been thought previously. Our findings have implications for identifying the mechanisms that influence the relationship between size of the mutation transmitted from parent to offspring.

Subjects, Material, and Method

Subjects

One hundred pedigrees from whom DNA was tested were obtained from four sites in North America: (1) 44 from the Mayo Clinic in Rochester, MN; (2) 25 from the Ongwanada Resource Centre in Kingston, Ontario; (3) 18 from Baylor College of Medicine, Houston; and (4) 13 from the Genetics and IVF Institute in Fairfax, VA. DNA from 18 of the 25 families screened by the Ongwanada Resource Centre was also tested by the molecular genetics lab at the Baylor College of Medicine. Eighty-seven pedigrees obtained from the Mayo Clinic, Ongwanada Resource Centre, and Baylor College of Medicine had been evaluated in an earlier investigation of the premutation population (Fisch et al. 1994). Many of these pedigrees have been reported previously in the literature (Fu et al. 1991; Snow et al. 1993; Levinson et al. 1994).

Forty-five pedigrees contained at least three generations of individuals from whom DNA was obtained: (1) 18 from the Mayo Clinic in Rochester, MN; (2) 13 from the Genetics and IVF Institute in Fairfax, VA; (3) 11 from the Ongwanada Resource Centre in Kingston, Ontario; and (4) 3 from Baylor College of Medicine, Houston. Although also used in the general analysis, the 13 families from the Genetics and IVF Institute were obtained specifically to examine three-generation pedigrees.

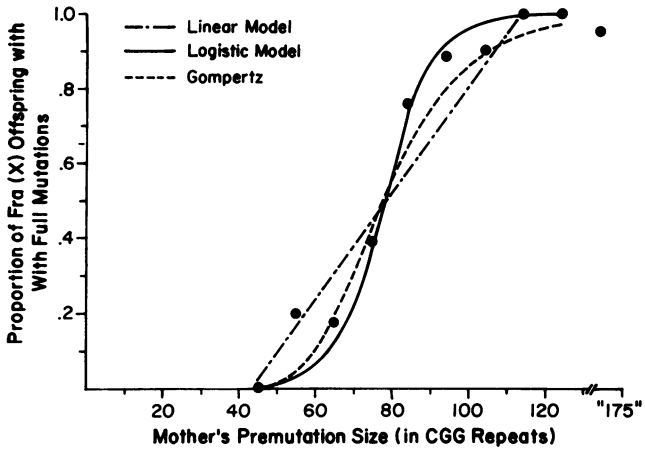

Figure 1 Probability of risk of a full mutation in offspring given the fra(X) mutation in offspring, as a function of the mother's premutation size, in number of CGG repeats. Closed circles represent midpoints of premutation-size categories (see table 1A).

Material

Assays based on PCR product were used to determine the length of CGG repeats in individuals who were carriers of the premutation. Individuals with either large premutations (>130 repeats) or full mutations were identified from Southern blots. Procedural aspects of DNA analysis by PCR and Southern blot have been described elsewhere (Fu et al. 1991; Snow et al. 1993; Levinson et al. 1994).

Results

Evaluation of Risk for the Full Mutation

We assessed offspring from 140 females with premutations. Not all normal offspring were tested. To evaluate the risk of producing an offspring with a full mutation, we proceeded as follows: To eliminate ascertainment bias, we removed all index cases from analysis. Consequently, there remained 110 female carriers who bore 174 offspring with FMR-1 gene mutations. Carriers were allocated to one of nine premutation allele–size categories. Premutation sizes were grouped into classes of 10 CGG repeats (50–59, 60–69, etc.) according to the convention adopted by Fu et al. (1991). Then we calculated the number of offspring to females within each premutation-size group. From each premutation-size category, we computed the proportion of offspring with a full mutation. These data are presented in figure 1 and table 1A. Premutation-size categories are

Table 1

Frequencies for Mothers' Premutation-Size Categories

A. s_i and No. of Mothers for Mothers's Premutation-Size Categories

	Data for Mothers' Premutation-Size Category								
	50–59	60–69	70–79	80–89	90–99	100–109	110–119	120–129	⩾130
S_i[a]	.20 (5)	.17 (23)	.39 (38)	.76 (39)	.89 (35)	.91 (11)	1.0 (9)	1.0 (4)	.95 (20)
No. of mothers	3	12	21	20	25	8	8	2	11

B. Relative Frequency of f_i for Mothers' Premutation-Size Categories

	Frequency of f_i for Mothers' Premutation-Size Category								
	50–59	60–69	70–79	80–89	90–99	100–109	110–119	120–129	⩾130
Males	.04	.15	.12	.19	.23	.04	.08	.04	.12
Females	.02	.12	.19	.18	.19	.10	.06	.03	.12

[a] Numbers in parentheses are number of offspring.

represented by their midpoint values. Results show an increase in risk to offspring for the full mutation as the size of the mother's premutation increases. To assess the quantitative relationship between size of the mother's premutation and the probability that an offspring with the mutation acquired the full mutation, we computed a simple linear regression using relative frequency of full mutation along with midpoint values from their respective premutation-size category. Since it contained widely dissimilar sizes, the largest premutation class (⩾130) was omitted from analysis. Output from the analysis indicates that the Pearson correlation between the size of the mother's premutation allele and the offspring's probability of acquiring a full mutation is $r = .96$ (adjusted $R^2 = .91$; $P < .0001$). That is, mothers' premutation size accounted for 91% of the variance in the relative frequency with which the offspring bore the full mutation. The linear regression relating probability of a full mutation, P(FM), as a function of premutation size (P_{size}) is

$$P(\text{FM}) = -.60\ (\pm.14) + .014\ (\pm.002)\ P_{size}. \quad (1)$$

However, visual inspection of the data suggests that a linear relationship is not the best-fitting function. Examination of the standardized residuals from the regression equation reveals a systematic trend in the error term, but a test for autocorrelation in the residuals (Durbin-Watson statistic = 1.23) is inconclusive. The sigmoid shape of the points plotted implies that a nonlinear asymptotic function is more suitable. Consequently, the data were reanalyzed and fitted using both logistic regression and a Gompertz curve (Croxton and Cowden 1949). Outcome from logistic regression produces a correlation of $r = .95$ (adjusted $R^2 = .90$; $P < .0001$). Correlation to the Gompertz function was $r = .96$ (adjusted $R^2 = .92$; $P < .01$). The Durbin-Watson statistic was not significant for either nonlinear function, indicating no autocorrelation in the error term.

We examined whether there was a difference in premutation size between males and females. To compare the relative frequency distributions of premutation size in females and males, we proceeded in two stages. First, all females with premutations were separated into two groups: (1) females who either were offspring of transmitting males or (as yet) had no offspring of their own ($n = 46$) and (2) females who were transmitting carriers ($n = 110$). The two groups were compared with one another. Median number of CGG repeats for the first group was 90, with a semi-interquartile range (SIQR) of 75–107. Median number of repeats for the second group was also 90, with SIQR of 73–100. Premutation sizes in the two groups were not significantly different from another. Consequently, premutation sizes for females were pooled ($n = 156$).

We evaluated males with premutations in a comparable fashion. Of the 26 males with premutations, 16 were transmitting males and 10 had no offspring of their own. Median numbers of CGG repeats in these two groups were 88 and 91, respectively. A Mann-Whitney test of the two groups indicated no significant differences ($P > .10$). Therefore, the data for males were also pooled. The relative frequencies of male and female premutations are presented in figure 2 and table 1B. Median premutation size for females was 90; median premutation size for males was 89. A χ^2 test of binomial samples revealed that there were no significant differences in the

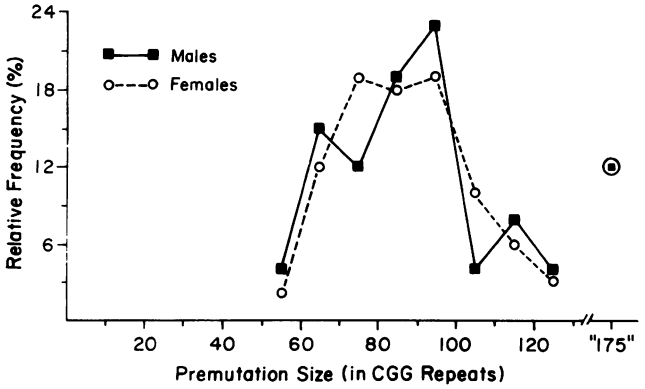

Figure 2 Relative frequency of premutation size in males with premutations (n = 26) and females with premutations (n = 156). Filled squares represent males; open circles represent females. Squares and circles represent midpoints for premutation-size categories (see table 1B).

proportion of females for any premutation allele–category size ($\chi^2_{(8)} = 3.31$; $P > .50$). Therefore, the frequency distributions of premutation size in males and females are not significantly different from one another (see table 2).

We also examined the probability of risk for a full mutation for the population with a fra(X) mutation. Given that the risk that an offspring with the fra(X) mutation will acquire the full mutation is directly related to the size of the premutation in the carrier mother, and given the relative frequency of occurrence of premutation sizes, we computed the probability that offspring with a mutation would present with a full mutation, as follows: Let R_i = mean CGG repeat number for the ith premutation category; f_i = relative frequency of occurrence of a premutation in the ith premutation category; and s_i = probability that an offspring of a female carrier with mean repeat number R_i will have a full mutation. Then the point estimate of the probability of a full mutation, P(FM), is the sum of the joint probabilities of relative frequency of premutation size, f_i, and the probability that any offspring will have a full mutation, given R_i, as in equation (2).

Table 3

Relative Size of Daughter's Premutation Compared with That of the Transmitting Parent

Size of Daughter's Premutation vs. Transmitting Parent's	Sex of Transmitting Parent		Total
	Male	Female	
Smaller	10	1	11
Equal	3	1	4
Larger	13	44	57
Total	26	46	72

$$P(\text{FM}) = f_1 s_1 + \ldots + f_n s_n = \sum f_i s_i \qquad (2)$$

for $i = 1, \ldots, n$, where n = number of size categories. Using the relative frequencies of f_i for females (see table 1B) and s_i from table 1A, and assuming that the mutation is inherited in offspring, we calculated P(FM), the aggregate risk that an offspring of a female with a premutation would manifest the full mutation, and found it to be .70 (±.035).

Differences in Premutation Size, as a Function of the Sex of the Parent

We also compared the premutation-allele size in daughters of carriers with a premutation, according to whether daughters acquired the premutation from their father or mother. Of 60 male and female carriers with premutations who transmitted the premutation to their offspring, 54 had daughters with premutations. Eighteen parents were male, and 36 were female. Of the 72 daughters of transmitting parents, 26 were offspring of transmitting males, and 46 were from females. Ten (38%) of the 26 daughters of transmitting males had smaller premutations than their fathers, whereas only 1 (2%) of 46 daughters of transmitting females exhibited a smaller premutation than her mother. The data are presented in table 3.

Table 2

Relative Frequencies of Premutation Sizes

	Frequencies For Premutation-Size Category								
	50–59	60–69	70–79	80–89	90–99	100–109	110–119	120–129	≤130
Males	1	4	3	5	6	1	2	1	3
Females	3	18	29	28	30	16	10	4	18
Total	4	22	32	33	36	17	12	5	21
P_i[a]	.75	.82	.91	.88	.84	.95	.86	.80	.88

[a] Ratio of the number of females in premutation class i to the total number of males and females in class i.

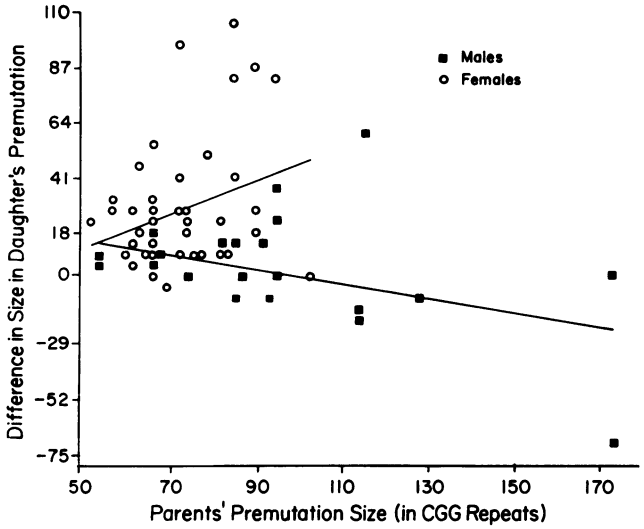

Figure 3 Relationship of parents' premutation size (number of CGG repeats) to difference in premutation size in daughters. Uppermost regression line shows relationship between mother's premutation size and the difference in premutation size in their daughters. Lower regression line shows the relationship between father's premutation size and the difference in premutation size in their daughters.

If we regard the models developed by Morton and MacPherson (1992) and Sherman and Ashley (1993) as applicable, then offspring whose premutations are as large as or larger than their parents' form a single, natural class. Consequently, we collapsed table 3 into a 2×2 table in which daughters' premutations as large as or larger than their parents' form one class, while daughters' premutations that are smaller than their parents' form a second class; then we ascertained whether the difference in proportions between male and female transmitting carriers was particularly unusual. We computed the probability of its occurrence by using Fisher's exact test. The result indicated that the difference was significant, $P(10,1) = .00008$.

Premutation-allele sizes in daughters were compared with those of their parents by calculating the premutation-size difference between parent and daughter (P_{diff}) and comparing it with the parent's allele size. Given the previously obtained differences with regard to the sex of the parent, P_{diff} was evaluated according to whether the daughter was a descendant of a transmitting male or transmitting female. Results are presented in figure 3. Separate regression analyses were conducted for transmitting fathers and transmitting mothers.

The relationship between the difference in premutation size, P_{diff}, in daughters paired with their mothers is represented by the linear regression in equation (3):

$$P_{diff} = -22(\pm 25) + .67\ (\pm .33)\ P_{mother}\ . \qquad (3)$$

The correlation coefficient between P_{diff} and P_{mother} was $r = .29$ ($P < .05$).

The relationship between P_{diff} in daughters and P_{father} is represented by the linear regression in equation (4):

$$P_{diff} = 28\ (\pm 12) - .26\ (\pm .12)\ P_{father}\ . \qquad (4)$$

The correlation coefficient between P_{diff} and P_{father} was $r = -.40$ ($P < .05$). That is, there is a modest and significant positive correlation between the increase in premutation size in daughters of transmitting females and the size of their mother's allele. Contrariwise, there is a modest and significant negative correlation between the difference in premutation size in daughters of transmitting males and the size of the father's allele.

Discussion

From pedigrees examined previously, Sherman et al. (1984, 1985) estimated that, for the fra(X) mutation, overall impairment would be 80% in males and 33% in females. The explanation provided by Fu et al. (1991) is that the risk of expansion to the full mutation is directly related to the size of the mother's premutation. However, aggregate risk to offspring must be weighted as a function of the relative frequency with which premutation-allele sizes occur in a population of females with premutations. We found that the probability that offspring with a mutation would exhibit the full mutation is the sum of the joint probabilities of (*a*) relative frequency of mother's premutation size and (*b*) probability of manifesting a full mutation, given that premutation size, a probability that is shown in equation (2).

To establish validity of the risk estimate in equation (2), we compared our results to earlier findings. Having corrected for ascertainment bias, Heitz et al. (1992) noted that 77% of offspring with mutations from mothers with premutations bore full mutations. Väisänen et al. (1994) found that, in 122 transmissions from mothers with a premutation, 92 (75%) were full mutations. The 95% confidence interval that contains our estimate of aggregate risk is .63–.77, which also contains the proportions obtained by Heitz et al. (1992) and Väisänen et al. (1994).

The close fit to both the logistic curve and the Gompertz equation of relative frequency of occurrence of the full mutation in offspring, given mother's premutation size, is itself noteworthy. Historically, logistic and Gompertz curves have described cumulative processes and have been applied to biological patterns involved in growth and development (e.g., see Wosilait et al. 1992) and aging (e.g., see Strehler and Freeman 1980; Riggs 1993). A nonlinear asymptotic increase in the probability that a full mutation will occur as a function of increase in mother's premutation size may reflect an accu-

mulation of errors in DNA synthesis, a paradigm of which has been proposed by Richards and Sutherland (1994). Genetic instability that produces progressive loss of repetitive DNA sequences has been postulated as a mechanism in error theories of human aging (Strehler 1986; Riggs 1993). Recently, Eichler et al. (1994) have shown that absence of interspersed AGG trinucleotides in the FMR-1 gene correlates with instability in normal size alleles and that instability and expansion may involve Okazaki fragment slippage.

Insofar as decreases in mutation size are concerned, other investigators have found reductions in the fra(X) premutation size in successive generations (Oberlé et al. 1991; Heitz et al. 1992; Väisänen et al. 1994), but these events were thought to occur rarely. Our analysis of fra(X) families indicates that intergenerational decreases in premutation size occurred in more than one-third of the daughters of transmitting males. The difference in frequency may be due to the smaller number of families studied previously. Nonetheless, while disabilities are associated with expansion in the mutation in successive generations in fra(X), DM, or Huntington disease, intergenerational decreases in premutation size need to be accounted for as well.

Several studies have reported contraction in families in which disorders are produced by an augmented number of trinucleotide repeats, e.g., in DM (Ashizawa et al. 1992; Abeliovich et al. 1993; Hunter et al. 1993). Moreover, reversals in the DM mutation to normal size alleles have been observed in descendants of fathers (Shelbourne et al. 1992; Brunner et al. 1993*b*). We note that, in one family in which fra(X) has been segregating, a small premutation allele (43 repeats) was stably transmitted through three generations (D. L. Nelson, unpublished data). Several instances of intergenerational decreases in mutation size have been noted in Huntington disease (Andrew et al. 1993). Reductions in mutation size in successive generations have also been reported in families with FRAXE (Knight et al. 1993, 1994). On the other hand, neither systematic increases nor decreases in the number of CAG repeats have been described in successive generations of families with Kennedy disease (La Spada et al. 1991; Amato et al. 1993).

In all but one instance in which we observed decreases in premutation size in offspring, transmission was through the male carrier. Previously, Heitz et al. (1992) noted decreases in premutation size in two daughters of a fra(X) male. Willems et al. (1992) reported on a fra(X) family in which the grandfather exhibited a full mutation but in which his daughter carried the premutation. Väisänen et al. (1994) reported one case in which the premutation transmitted by a male decreased in size in the next generation. Unlike these investigators, Oberlé et al. (1991) found decreases in two daughters of a female with the premutation. In all but one instance of reduction in mutation size reported to have occurred in DM, the transmitting parent has also been the father (Ashizawa et al. 1992; Abeliovich et al. 1993; Brunner et al. 1993*a*; Hunter et al. 1993). In addition, Jansen et al. (1994) found that, in four DM males with long CTG repeats, CTG lengths in sperm were smaller than their lengths in blood. Reyniers et al. (1993) observed only the premutation in sperm from fra(X) males with full mutations present in peripheral lymphocytes. Decreases in mutation size in male-to-female transmissions have been noted in FRAXE families (Knight et al. 1993, 1994). These findings indicate a pattern of parent-specific transmission.

If, as appears to be evident, parent-of-origin–specific transmission is manifest in the fra(X) mutation, a paradigm that could prove useful may emerge from studies of Angelman syndrome (AS) and Prader-Willi syndrome (PWS). Both of the latter result from deletions within 15q11-q13. However, clinical manifestations of AS and PWS are markedly different from one another (Reik 1989; Hall 1990). The absence of paternal DNA in the region results in PWS, whereas maternal deficits in the area result in AS. Differences in methylation patterns (Clayton-Smith et al. 1993) and asynchronous patterns of replication (Kitsberg et al. 1993; Knoll et al. 1994) have been observed in this region in individuals with AS and PWS. Reis et al. (1994) proposed a model in which a *cis*-acting mutation interferes with the expression of imprinted genes in this region.

Despite its variability, early onset of Huntington disease in offspring occurs more frequently when the mutation is inherited from the father (Krawczak et al. 1991; Snell et al. 1993). The opposite is more likely when the mutation is inherited from the mother (Reik 1988; Ridley et al. 1988). Brunner et al. (1993*a*) suggested that the differential influence in maternal and paternal transmission in DM may be due to parent-specific genetic imprinting. Both earlier age at onset and increased severity of DM in offspring of transmitting males ("anticipation") have been reported (Ashizawa et al. 1992; Hunter et al. 1992; Harley et al. 1993). Congenital DM has been associated with maternal transmissions (Lavedan et al. 1993; Mulley et al. 1993). Other investigators (Ashizawa et al. 1993, 1994; Cobo et al. 1993) found a significant negative correlation between the size of the CTG expansion in offspring and the size of the expansion in paternal transmission but noted a near-zero correlation in maternal transmission. As regards daughters of male and female fra(X) transmitters, results obtained by Ashizawa et al. (1994) nearly parallel our own. While intergenerational expansion to the full mutation is suggestive of a postzygotic event, and while *cis*-acting elements are involved in the mutational mechanism (Richards and Sutherland 1994), decreases in mutation size suggest that such events occur prior to embryogenesis.

In conclusion, while anticipation may be a necessary phenomenon of intergenerational expansion in mutations, its occurrence may not be sufficiently inclusive for understanding of the mechanism of heritable instability of DNA, insofar as this mechanism pertains to reductions in mutation size that occur in fra(X) and DM. Therefore, hypotheses that fail to incorporate the possibility of decreases in mutation size may be incomplete. In addition, population estimates obtained from models developed by Morton and MacPherson (1992) or Sherman and Ashley (1993) may not produce exact outcomes. On the other hand, the paradigm provided by Kolehmainen (1994), as well as the revised model by Ashley and Sherman (1994), accommodates both increases and decreases in premutation size and may prove to be a more accurate predictor of population estimates of the fra(X) mutation.

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