Letters to the Editor

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Reverse Mutation in Fragile X Syndrome

To the Editor:

The fragile X syndrome is the most common cause of familial mental retardation, with an incidence of ~1/1,500 in males and 1/2,500 in females (Sherman 1991). The clinical expression includes moderate to severe mental retardation, macroorchidism, dysmorphic facial features and behavior disturbances (Fryns 1989), In 1991, the FMR-1 gene was isolated from the region of the fragile X site (Verkerk et al. 1991). The fragile X phenotype has been found, in most cases, to be characterized at the molecular level by expansion of a $(CGG)_n$ repeat and hypermethylation of a CpG island identified in the 5′-UTR of the FMR-1 gene (Bell et al. 1991; Heitz et al. 1991; Oberlé et al. 1991; Vincent et al. 1991). It has been proposed, and some evidence has been shown, that germ cells carry only premutation alleles and that expansion occurs at a postzygotic stage. A few cases of reduction of the $(CGG)_n$ repeat in fragile X syndrome have been reported. These reductions were from a larger premutation to a smaller premutation (Rousseau et al. 1991; Wöhrle et al. 1993), in female-to-male transmission (Rousseau et al. 1991), from full mutation to a mosaic pattern (Yu et al. 1992; Väisänen et al. 1994), reduction from mosaic full-mutation/premutation females (Fu et al. 1991) or regression from premutation to normal (Brown et al. 1994). We present here the novel observation of a phenotypically normal female carrying a nonmosaic full-mutation allele in somatic cells who transmits a premutation allele to her daughter. This daughter has three mosaic offspring with the full mutation and the premutation. Two of them are monozygotic (MZ) twins sharing a concordant mutation pattern. They are monoamniotic monochorionic, which indicates a late form of twinning (Philips 1993).

The pedigree of the family is depicted in figure 1. Individuals 3, 4, 8, 9, 10, and 11 have a typical fragile X syndrome phenotype. Individual 6 carries a premutation allele, and no symptoms of the fragile X syndrome were observed. Individuals 2 and 7 do not have fragile X syndrome.

All individuals had a normal karyotype in peripheral lymphocytes under standard conditions. Chromosome analysis under folate-deprived conditions (Sutherland 1977) expressed the Xq27.3 fragile X site in all affected individuals and carrier females tested except individual 6, who did not express the fragile X site.

For molecular analysis of FRAXA, DNA from peripheral blood was digested with *Eco*RI-*Eag*I and hybridized with probe StB12.3 according to conditions described elsewhere (Oberlé et al. 1991) (fig. 1). The normal males (individuals 2 and 7 and a normal male control 13) show an unmethylated 2.8-kb fragment that represents the normal active X chromosome. The normal females (individual 5 and a normal female control 12) present an unmethylated 2.8-kb fragment (normal active) and a methylated 5.2-kb fragment (normal inactive).

Individual 1 is a clinically unaffected female carrier with the normal 2.8- and 5.2-kb fragments and an expanded 6.4-kb fragment (400 repeats) in the methylated full-mutation range. Individual 6 is an unaffected female carrier and presents a 2.8-kb and an more intense 5.2-kb fragment but does not show a fragment or smear in the full-mutation range. This individual presents two fragments in the premutation range, an unmethylated fragment of ~3.06 kb and a less intense 5.46-kb methylated fragment. Mosaicism in lymphocytes was excluded after repeated testing. To determine the exact size of this premutation, we digested the DNA with *Pst*I and hybridized with probe StB12.XX as described by Rous-

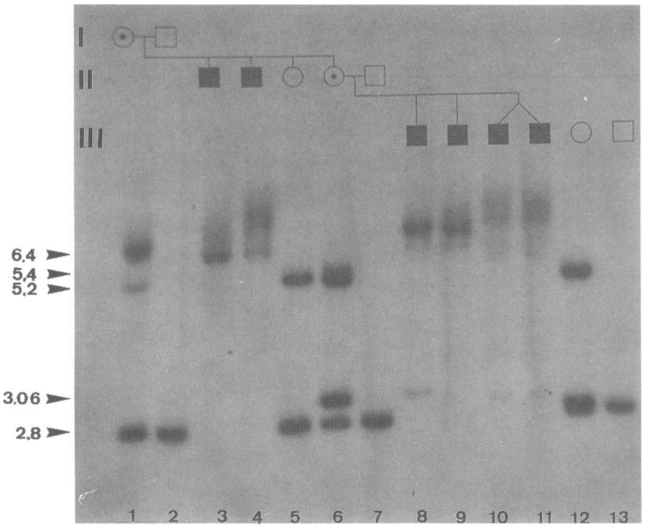

Figure 1 Analysis with probe StB12.3 of *Eco*RI-*Eag*I-digested DNA. Lane 1, Carrier grandmother, showing a 2.8-kb fragment (normal active), a faint 5.2-kb fragment (normal inactive), and a 6.4-kb fragment in the full-mutation range. Lane 2, Grandfather, showing a 2.8-kb fragment. Lane 3, Affected son, showing a 6.2-kb fragment (~333 repeats) and a diffuse smear. Lane 4, Affected son, with a very diffuse smear. Lane 5, Noncarrier daughter, with normal 2.8-kb and 5.2-kb fragments. Lane 6, Carrier daughter, presenting the normal 2.8-and 5.2-kb fragments and two fragments in the premutation range, an unmethylated 3.06-kb fragment and a methylated 5.46-kb fragment. Lane 7, Normal male, with a 2.8-kb fragment. Lane 8, Affected mosaic grandson, showing an intense 7.5-kb fragment (770 repeats), a diffuse smear, and a very faint fragment in the unmethylated premutation range. Lane 9, Affected grandson, with a 7.25-kb fragment (680 repeats) and a smear in the full-mutation range. Lanes 10 and 11, MZ twin, sharing a similar mutation pattern. Lane 12, Control female. Lane 13, Control male.

seau et al. (1992). Only one intense fragment, with an increase in size of 0.26 kb (111 repeats), appeared, which indicates that only one premutation exists with an unmethylated and a methylated fraction (data not shown). All the affected males (individuals 3, 4, 8, 9, 10, and 11) showed discrete fragments or diffuse smears in the full-mutation range. Individuals 8, 10, and 11 presented unmethylated premutations and methylated full mutations. The MZ twins share a concordant smear in the full-mutation range and an identical premutation allele. The *Eco*RI-*Eag*I filter was hybridized with probe F33 to confirm complete digestion (data not shown) as described by Heitz et al. (1991).

The expansion from premutation to full mutation in *FMR-1* takes place only on the maternal X chromosome (Oberlé et al. 1991). The premutation never expands to full mutation when transmitted by a normal transmitting male (NTM), and daughters of NTMs never show cytogenetic or clinical expression of fragile X syndrome (Oberlé et al. 1991). The specific mutational mechanism of the $(CGG)_n$ expansion and also the exact moment at which expansion to full mutation occurs, whether during early embryogenesis or during maternal meiosis, remain to be elucidated. That expansion to full mutation occurs at a postzygotic stage is supported by the observation in the described family that the premutated female has three mosaic offspring with the full mutation and the premutation and one with only the full mutation. The observation of mosaic offspring in this family would be consistent with the hypothesis that premutation alleles frequently found in affected individuals represent the inherited maternal premutation and that expansion to full mutation occurs in a window of early development on maternally inherited X chromosomes (Wöhrle et al. 1993).

If expansion to full mutation occurs at a postzygotic stage, it probably takes place before the twinning event. The monoamniotic monochorionic type of twinning, such as the sibs presented in this report, is indicative of a late form of twinning, probably between days 10 and 20 after fertilization. If the full mutation is not present in germinal cells, which separate from somatic cells around day 5, expansion would take place between days 5 and 20. It is likely that the similar (Devys et al. 1992; Malgrem et al. 1992; present report) or discordant (Kruyer et al. 1994) mutation pattern that has been observed in MZ twins would be related to the moment of separation of the embryo. In such a case, the later the twinning event, the higher the probability of having an identical genotype, since the mutation pattern would have been already established.

The data presented here and those of Reyniers et al. (1993), showing that fragile X syndrome males with the full mutation in their leukocytes have only the premutation in their sperm, would be consistent with the full mutation never being present in gametes. It would support expansion as a postzygotic event. Central to this discussion would be the knowledge of whether female gametes carry the full mutation. The finding of the reverse mutation presented here would be consistent with the full mutation being absent in female gametes, as in the case of the mother transmitted a premutation allele, although, ultimately, to prove that gametes of female fragile X carriers have only premutation alleles, it would be necessary to test oocytes directly.

Since the discovery of the molecular defect underlying the fragile X syndrome, the use of probes StB12.3 and StB12.XX has shown to be a reliable diagnostic tool. Accurate information on the risk of premutation-to-full-mutation transition is very important for adequate genetic counseling. The findings reported here are consistent with expansion occurring in a particular period of early development. Further studies on the moment and

sequence of expansion and their relationship to the methylation event are likely to provide new insight into the molecular mechanism leading to the manifestations of fragile X syndrome.

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Exclusion of Linkage between Cleft Lip With or Without Cleft Palate and Markers on Chromosomes 4 and 6

To the Editor:

Nonsyndromic cleft lip with or without associated cleft palate (CLP) is a common craniofacial defect, occurring in ~1/1,000 live births. While the defect generally oc-