

MOSAICISM IN THE FRAGILE X SYNDROME

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Individuals with the fragile X syndrome generally carry more than 200 copies of the CGG repeat and are described as having the "full" mutation. Methylation of the repeat region usually occurs when the full mutation of greater than 200 repeats is present. (Methylation is the addition of a methyl group to a DNA cytosine base where it precedes a guanine base at a CpG site). This methylation "turns off" the FMR-1 gene so that no protein is made. Among males who have the fragile X mutation, there are a group termed "mosaic" who have different sizes of the repeat expansion in different cells. In other words, some of the cells in mosaic males carry a fully expanded repeat with more than 200 copies while other cells carry a partially expanded repeat described as a "premutation" with 60-200 copies. These individuals, termed "size" mosaics, represent the most common form of mosaic males. In addition, there are occasional males who are "methylation" mosaics carrying the full mutation in both a methylated and an unmethylated state. Mosaic females have also been observed, but for unknown reasons, with much less frequency. As a consequence, the discussion here will focus only on mosaic males.

The origin of Mosaicism in fragile X

Every individual develops from one fertilized egg with a single set of genetic information. A fragile X male inherits a single CGG repeat number from his mother. It is believed that the expansion to the full mutation takes place very early in development after the fertilized egg has divided only a few times. Mosaicism probably occurs because the CGG repeat expands to the full mutation in some cells, whereas in other cells the repeat number fails to increase in size and remains a premutation. Based on the observation that CVS material taken from the placenta at around 10 weeks is frequently unmethylated, the methylation of the expanded repeat appears to occur at around this stage. In the uncommon cases of methylation mosaicism, for some reason, the fully expanded repeat fails to undergo methylation or may be incomplete.

The identification of mosaic status

DNA studies for the fragile X mutation are carried out using both polymerase chain reaction (PCR) and Southern blot analysis. To identify the presence of mosaicism in an individual, both PCR and Southern analysis can be used. Southern analysis is used to determine both the size of repeat expansion and presence of methylation of a site that usually undergoes methylation in the full mutation. PCR gives more accurate size but does not reveal methylation status. Therefore, Southern analysis is the preferred method for the detection of mosaicism. The incidence of mosaicism identified by Southern analysis of affected males in various studies has ranged from about 12% to 41%. The earliest study by Rousseau et al. (1991), observed 19% of affected males to be mosaic. Subsequently, using a very sensitive assay, we found mosaicism was present in 41% of full mutation males (Nolin et al 1994). The variations among these studies are likely due to technical differences and varying sensitivities in the Southern analysis procedures.

Because males with a mosaic fragile X status have a premutation size repeat as well as the full mutation, they make some FMR1 protein. Thus, mosaicism can also be determined by the presence of the FMR1 protein in individual cells. At the present time this procedure is not being used for routine clinical studies. However, an autopsy study of the brain of a male with a full mutation seen in blood showed that some brain cells were making FMR protein and led to the suggestion that all full mutation males may be mosaic in some percentage of their cells (de Graff et al. 1995).

Developmental delay in mosaic males

Despite the fact that mosaic males synthesize some protein, they are nevertheless developmentally delayed. One explanation may be that the amount of FMR1 protein present is insufficient to permit normal development. Alternatively, because DNA studies for determination of fragile X status are usually carried out on blood cells, these results may not accurately reflect the molecular pattern in the brain. Mosaicism in different tissues such as skin and blood has been investigated in a few individuals (Dobkin et al. 1996). For some males, a very similar mosaic pattern has been observed in both the skin and blood, whereas extreme variation has been noted in others. Thus, on an individual basis it may be difficult to predict whether mosaicism in blood correlates with that in brain.

Several studies have attempted to examine the question whether mosaicism is associated with higher mental function by comparing groups of mosaic males with full mutation males. The relationship between IQ and mosaicism has been examined and failed to show any association in the several studies by Rousseau (1991, 1994). Since fragile X males are easily aroused and difficult to test, these results were perhaps not surprising. Subsequently, we assessed the relation of mosaicism to adaptive scores, rather than IQ (Cohen et al., 1996). In this analyses the caregivers, usually parents, were asked a standardized series of questions regarding their sons' behavior and quantitative scores for function were derived using Vineland scores. These studies have suggested that although mosaic males have mental retardation, they function at a higher level than do full mutation males who do not have a mosaic pattern on Southern analysis. More recent studies of FMR1 protein expression using antibody staining by Tassone et al. (1999) have shown a positive correlation between IQ testing and degree of protein expressed in mosaic males and partially methylated full mutation males. Thus, mosaicism appears to be associated with a better prognosis.