

Prenatal diagnosis of a hypermethylated full fragile X mutation in chorionic villi of a male fetus

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Abstract

Fragile X syndrome, one of the most common human genetic diseases, is characterised by a unique genetic mechanism which involves dynamic mutation because of a heritable unstable DNA sequence and abnormal DNA methylation. Direct detection of the dynamic mutation and its methylation status at the DNA level would facilitate reliable tests for prenatal and postnatal diagnosis of the disease and for carrier detection. However, it has been suggested that DNA methylation can not be used as the basis for prenatal diagnosis as the CpG island is not always methylated in chorionic villus DNA. We report here a male fetus exhibiting both extensive somatic heterogeneity and abnormal hypermethylation of the full fragile X mutation in chorionic villus DNA as well as in fetal tissue DNA. Our results indicate that both somatic heterogeneity and hypermethylation of the full fragile X mutation are events that are clearly detectable in the 11th to 12th week of pregnancy.

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The fragile X syndrome is the most common cause of familial mental retardation, but has a very unusual mode of inheritance. It has very recently been characterised as involving a dynamic mutation because of a heritable unstable DNA sequence, p(CCG)n/p(CGG)n, at the FRAXA locus.^{1,2} A candidate gene, FMR-1, located by the unstable DNA sequence, was identified and a CpG island adjacent to the FMR-1 gene was shown to be methylated and FMR-1 transcription repressed in affected subjects. The findings of a new genetic mechanism and abnormal DNA methylation have shed light of the unusual genetics of the syndrome, providing the basis for highly reliable diagnostic tests both for carrier detection¹⁻⁴ and for prenatal diagnosis.⁵⁻⁹ For prenatal diagnosis, DNA analysis can routinely be performed on chorionic villi samples. However, while the DNA methylation correlates completely with fragile X phenotype in affected subjects, abnormal methylation of the full mutation has not always been detected in chorionic villus DNA.^{5,8,9}

We report here a case of a male fetus in which both extensive somatic heterogeneity of the full fragile X mutation and abnormal hypermethylation at a CpG island was found in chorionic villus DNA at 11 weeks of gestation. Similar patterns were detected in the

brain and other tissues of the same fetus after termination of the pregnancy in the 12th week.

Materials and methods

FRAGILE X FAMILY

The family was referred for prenatal studies to evaluate an 11 week pregnancy for fragile X syndrome. The pregnant mother was a non-penetrant obligate carrier, having had a previous son who is fully penetrant for fragile X syndrome. In our previous study,⁴ Southern blot analysis of her DNA with the probe pPCRfx1,³ which detects the dynamic mutation associated with the fragile X syndrome, confirmed that she is a carrier of the premutation. Her father was found to be a normal transmitting male (NTM) and her first son carried the full fragile X mutation with extensive somatic heterogeneity. Transcervical chorionic villus sampling was performed at 11 weeks of gestation. The polymerase chain reaction (PCR) using primers specific for the Y chromosome showed the fetus to be male (data not shown).

DNA SAMPLES AND SOUTHERN BLOT ANALYSIS

DNA was extracted from blood leucocytes obtained from family members, from the chorionic villus sample (CVS), and from fetal tissue obtained after termination of the pregnancy at 12 weeks. Samples of DNA (7 µg) were digested to completion with the restriction enzyme *Pst*I or *Eco*RI plus *Eag*I, and the DNA fragments were separated through a 0.8% agarose gel electrophoresis and subjected to Southern blot hybridisation using a DNA probe, pPCRfx1, as described previously.^{3,4}

Results

The results of Southern blot analysis using pPCRfx1 are shown in fig 1. The fragile X genotypes of the family members and their methylation patterns were determined by *Pst*I and *Eco*RI + *Eag*I assay, respectively. In all non-carriers, the DNA probe pPCRfx1 detects a 1.0 kb band in the *Pst*I assay, and a 2.8 kb band derived from an active X chromosome and a 5.2 kb band from an inactive one in the *Eco*RI + *Eag*I assay. As shown in fig 1, the pregnant mother (lane 3) had the pattern typical of a premutation carrier, with her normal X chromosome giving a 1.0 kb band and her fragile X chromosome giving a 1.3 kb band, which corresponds to the range of premutation. The *Eco*RI + *Eag*I assay showed the

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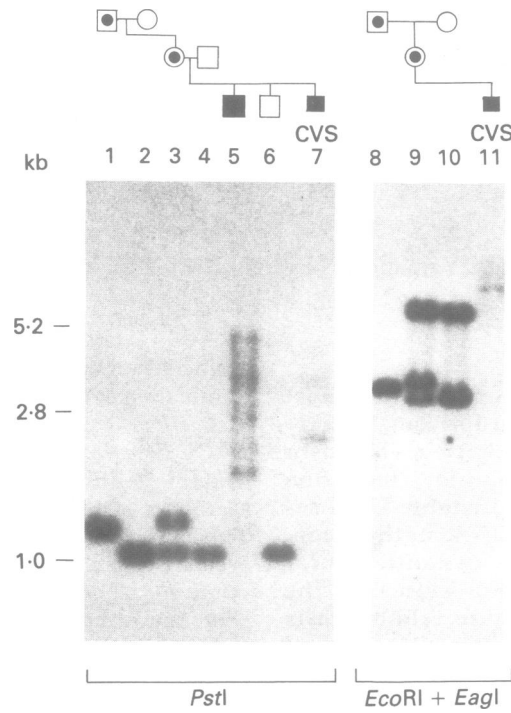


Figure 1 Direct detection of the fragile X mutation (*PstI* assay) and its methylation status (*EcoRI*+*EagI* assay) by Southern blot analysis with the DNA probe pPCRfx1. Lanes 3 and 9 are samples from the pregnant mother who is an obligate carrier having had a previous son (lane 5) with fragile X syndrome. Lanes 7 and 11 are DNA obtained from chorionic villus sampling at 11 weeks of gestation. Lanes 1 and 8 are samples from a normal transmitting male and the remainder are from normal relatives.

majority of DNA fragments with the premutation to be unmethylated. Her father (lane 1) was a normal transmitting male with a premutation and his fragile X chromosome was also not methylated (lane 8). Her first son (lane 5) had the pattern typical of a full mutation showing extensive somatic heterogeneity ranging from 1.6 to 4.0 kb.

The fetal DNA from the chorionic villus sample (CVS, lane 7) showed that the fetus lacked the normal 1.0 kb band, which was replaced by multiple bands ranging from 2.0 to 4.0 kb, this being a pattern typically observed in males with fragile X syndrome. They appeared to be hypermethylated (lane 10). After the termination of the pregnancy at 12 weeks of gestation, DNA samples were obtained from placental villi, brain, and liver tissues. The results of Southern blot analysis on these materials with pPCRfx1 are shown in fig 2. In the *PstI* assay, the DNA samples from all three fetal tissues (lanes 3, 4, and 5) examined showed a very similar banding pattern of the DNA fragments. The majority of the DNA fragments were in the full mutation range, giving 2.0 to 5.0 kb bands, with a small fraction in the premutation range at 1.2 kb. The *EcoRI*+*EagI* assay indicated that the DNA fragments with the full mutation (lanes 8, 9, and 10) were totally hypermethylated, but the ones with the premutation were not methylated. Although the patterns of dynamic mutations were slightly different from the results

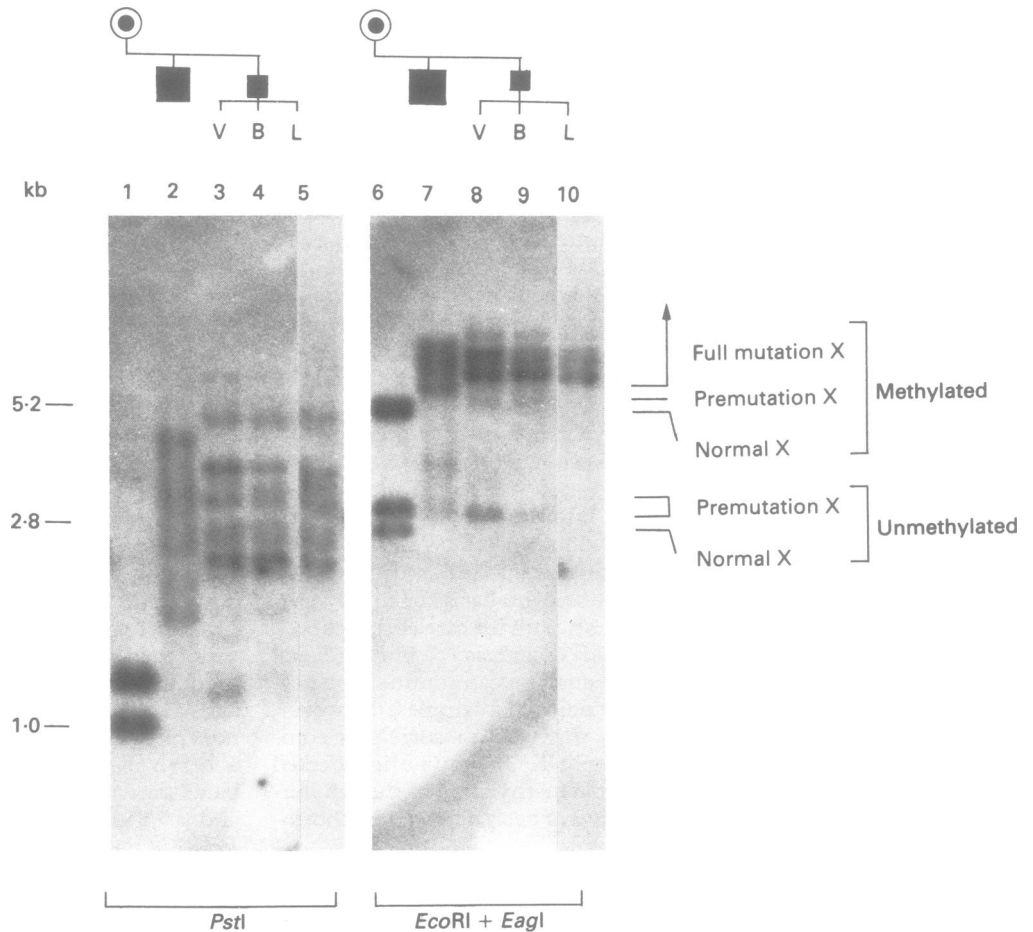


Figure 2 Southern blot analysis of DNA samples isolated from fetal tissues obtained after termination of the pregnancy at 12 weeks of gestation (V=placental villi, B=brain, L=liver). Lanes 1 (and 6) and 2 (and 7) are DNA samples obtained from leucocytes of the pregnant mother and her first affected son, respectively. The fragile X mutations and their methylation status in the *EcoRI*+*EagI* assay are shown on the right.

for her first son with fragile X syndrome (lanes 2 and 7), the analysis of the fetal tissues confirmed the result of prenatal diagnosis with chorionic villus sampling.

Discussion

The recent molecular cloning of the FRAXA region has provided a reliable direct method for diagnosing the genotypes of the fragile X syndrome. Thus DNA probes next to the unstable DNA sequence of the p(CCG)n repeat can be used as diagnostic tools for the fragile X genotypes, that is, premutation and full mutation.¹⁻⁴ Methylation of the CpG island adjacent to the repeat has been shown to be completely correlated with the fragile X phenotype in affected subjects.^{1,2,4} Both the fragile X genotype and its methylation status can be determined simultaneously by the DNA probe in DNA samples digested with methylation insensitive (*EcoRI*) and methylation sensitive (*EagI*,^{4,7,8} *BssHII*,⁹ or *SacII*⁵) restriction enzymes.

In prenatal diagnosis, direct detection of the fragile X mutation can be performed on DNA samples obtained from chorionic villus samples (CVS)^{5,6,9} and amniotic fluid (AF).⁷ In the present prenatal study on CVS from an at risk male fetus, we could show that the fetus had indeed inherited the full fragile X mutation, exhibiting somatic heterogeneity. As with the first affected son, who showed hypermethylation of the CpG island in leucocyte DNA, the CVS from the fetus showed clear evidence of DNA methylation at a methylation sensitive *EagI* site (fig 1). After diagnosis of the full mutation in the fetus, the pregnancy was terminated and DNA extracted from fetal tissues, such as placental villi, brain, and liver, showed a very similar pattern of hypermethylation of the full mutation, with extensive somatic heterogeneity in the expansion of the unstable

p(CCG)n repeat (fig 2). These results indicate that both somatic heterogeneity and hypermethylation of the full mutation may be established during a very early stage of embryogenesis, although the time of occurrence can not be determined. As such they are consistent with those reported by Devys *et al*,⁸ who showed that the abnormal methylation characteristic of the full mutation was present in most chorionic villi analysed (eight of nine male or female) by *EcoRI* + *EagI* digestion. A similar finding of hypermethylation in CVS has been described by Sutcliffe *et al*⁹ in their prenatal study of *EcoRI* + *BssHII* digested DNA samples from a male fetus. Furthermore, they showed that FMR-1 gene transcription is repressed in hypermethylated fetal tissues, while the undermethylated chorionic villi expressed the FMR-1 gene. To establish whether the fragile X genotype can be determined by DNA analysis of chorionic villus biopsies, further analysis of the relationship between genotype, methylation, and FMR-1 transcription in fetal tissues is needed.

- 1 Richards RI, Sutherland GR. Fragile X syndrome: the molecular picture comes into focus. *Trends Genet* 1992;8:249-55.
- 2 Mandel JL, Heitz D. Molecular genetics of the fragile-X syndrome: a novel type of unstable mutation. *Curr Opin Genet Dev* 1992;2:422-30.
- 3 Yamauchi M, Seki N, Hori T. Rapid preparation of diagnostic probes for the fragile X syndrome by direct PCR amplification of human chromosomal DNA. *Jpn J Hum Genet* 1992;37:195-203.
- 4 Hori T, Yamauchi M, Seki N, *et al*. Heritable unstable DNA sequences and hypermethylation associated with fragile X syndrome in Japanese families. *Clin Genet* 1993;43:34-8.
- 5 Sutherland GR, Gedeon A, Kornman L, *et al*. Prenatal diagnosis of fragile X syndrome by direct detection of the unstable DNA sequence. *N Engl J Med* 1991;325:1720-2.
- 6 Hirst M, Knight S, Davies K, *et al*. Prenatal diagnosis of fragile X syndrome. *Lancet* 1991;338:956-7.
- 7 Dobkin CS, Ding XH, Jenkins EC, *et al*. Prenatal diagnosis of fragile X syndrome. *Lancet* 1991;338:957-8.
- 8 Devys D, Biancalana V, Rousseau F, *et al*. Analysis of full fragile X mutations in fetal tissues and monozygotic twins indicate that abnormal methylation and somatic heterogeneity are established early in development. *Am J Med Genet* 1992;43:208-16.
- 9 Sutcliffe JS, Nelson DL, Zang F, *et al*. DNA methylation represses FMR-1 transcription in fragile X syndrome. *Hum Mol Genet* 1992;1:397-400.