

## Short reports

# Prenatal diagnosis of the fragile X syndrome: loss of mutation owing to a double recombinant or gene conversion event at the FMR1 locus

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### Abstract

The fragile X syndrome, an X linked mental retardation syndrome, is caused by an expanded CGG repeat in the first exon of the FMR1 gene. In patients with an expanded repeat the FMR1 promoter is methylated and, consequently, the gene is silenced and no FMR1 protein (FMRP) is produced, thus leading to the clinical phenotype. Here we describe a prenatal diagnosis performed in a female from a fragile X family carrying a large premutation. In chorionic villus DNA of the male fetus the normal maternal CGG allele and a normal pattern on Southern blot analysis were found in combination with the FRAXAC2 and DXS297 allele of the maternal at risk haplotype. A second chorionic villus sampling was performed giving identical results on DNA analysis and, in addition, expression of FMRP was shown by immunohistochemistry. We concluded that the male fetus was not affected with the fragile X syndrome. Subsequent detailed haplotype analysis showed a complex recombination pattern resembling either gene conversion or a double crossover within a 20 kb genomic region.

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Keywords: loss of mutation; FMR1 gene; FMR1 protein

The fragile X syndrome is the most frequent form of inherited mental retardation with a frequency of 1:4000 in males and 1:6000 in females.<sup>1</sup> The CGG repeat in the FMR1 gene, which is expanded to >200 repeats in patients, is polymorphic but stably inherited in the normal population, ranging from 6-52 repeat units. Alleles of up to 200 CGGs, designated premutations, are unstable upon transmission to offspring.<sup>2-4</sup> Both females and males, the so-called normal transmitting males, can carry premutations, but these can only expand to a full mutation after female meiotic transmission.<sup>5</sup> The vast majority of patients have a methylated full mutation, while in about 20% of the cases a mosaic pattern of premutation and full mutation, and sometimes normal, alleles is found. A decrease in the number of

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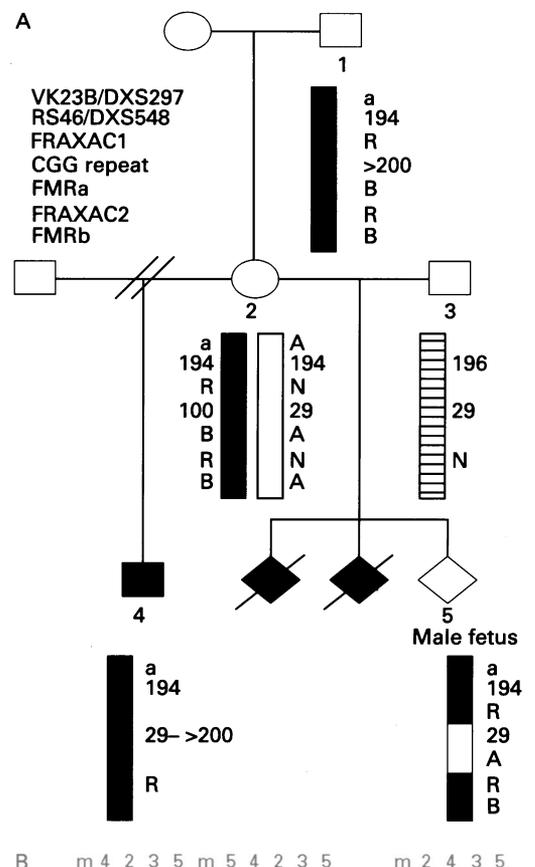


Figure 1 (A) Pedigree of the fragile X family with the haplotype data. (B) Southern blot analysis of genomic DNA. The numbers above the lanes correspond to the numbers in the pedigree. Fragment sizes are indicated in kb at the side of the figure. The HindIII and EcoRI/EagI digests were hybridised with probe pP2 and the PstI digests with probe pPX6.<sup>14</sup> Note that the index patient is a mosaic for alleles in the normal, premutation, and affected range.

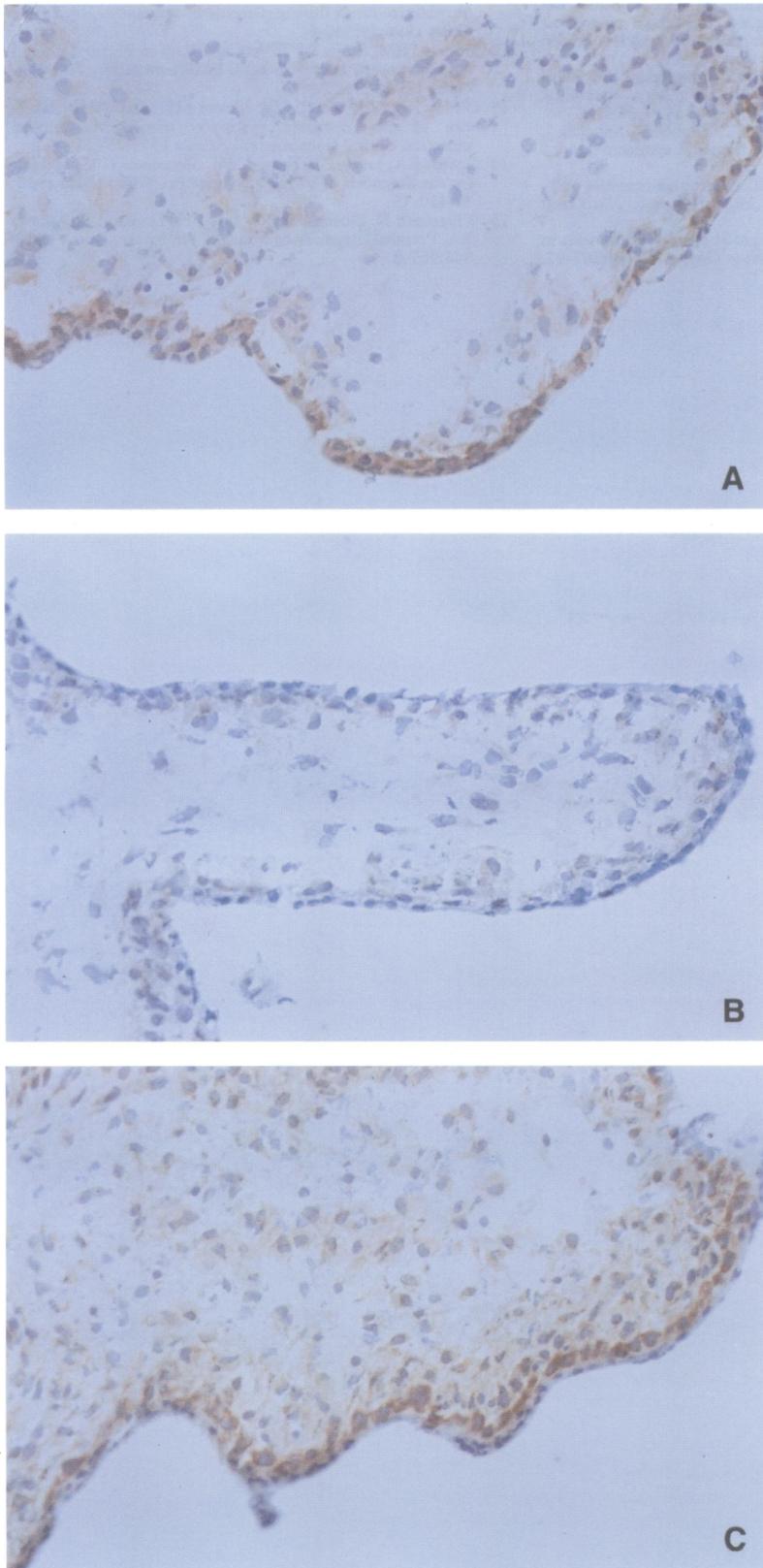
CGGs is a rare phenomenon. Regression from a full mutation allele to a premutation is rare, and regression from a full mutation to a normal sized allele has never been described.<sup>6</sup> Regression within the premutation range is not

an uncommon phenomenon but regression from a premutation to a repeat of normal size is extremely rare.<sup>5 7-11</sup>

A female carrier of a large premutation (about 100 CGGs) from a well studied fragile X family<sup>12</sup> requested her third prenatal diagnosis. Her son, the index case, is a mosaic for normal, premutation, and methylated expanded alleles. With her second partner she had two previous pregnancies monitored by DNA analysis and an affected female and male fetus were diagnosed. Both pregnancies were terminated. PCR analysis of the chorionic villus DNA from the male fetus, obtained at 10 weeks 5 days of gestation, showed a normal CGG allele of 29 repeats, identical to the maternal normal allele. Southern blot analysis showed a normal, unmethylated hybridisation pattern (fig 1). However, analysis of the intragenic FRAXAC2 marker showed the maternal at risk allele R. Subsequent analysis of the proximal marker DXS297 showed the at risk allele as well. A number of STRs on different chromosomes were tested to confirm that the chorionic villus sample was from this couple. Since there was no time to perform extensive haplotype analysis, these results were given to the parents. In order to have a fast result, they decided to have a second chorionic villus sampling which took place at 15 weeks of gestation. In addition to repeated DNA analysis, an immunohistochemical test was performed to determine whether FMRP was expressed in the cytotrophoblasts of the chorionic villi. Repeated PCR and Southern blotting analysis confirmed the initial results and the presence of the FMR1 protein was shown (fig 2). It was concluded that the male fetus was not affected with the fragile X syndrome. This was presented to the parents who decided to continue the pregnancy. Recent follow up at the age of 6 months showed a healthy boy with normal psychomotor development.

Detailed haplotype analysis was indicative of a complex recombination pattern in the fetal DNA in a region of about 20 kb encompassing the FMR1 gene (fig 1). The male fetus has received the non-risk haplotype from his mother as far as the regions immediately flanking and spanning the CGG repeat in the FMR1 gene are concerned, while the remainder is derived from the at risk haplotype. One transition between the haplotypes is located between FRAXAC1 and the CGG repeat and the other between FMRA and FRAXAC2 which is about 12.5 kb downstream of the CGG repeat region.

The occurrence of a double recombination in such a small genomic region is highly unlikely. Two similar loss of mutation cases showing a "patchwork" of normal and at risk alleles, probably owing to gene conversion events, have been described in a fragile X and a myotonic dystrophy family.<sup>9 13</sup> The patchwork pattern in both the FMR1 and DM cases is analogous to discontinuous gene conversion tracts, as seen in members of multigene families.



**Figure 2** FMRP expression in chorionic villi from a control fetus (A, 12.5 weeks), a fetus with a full mutation (B, 12.5 weeks), and the case described here (C, 15 weeks). FMRP is present in cytotrophoblast cells (brown precipitate) in villi from the control sample and the case described here, whereas in the fetus with a full mutation no FMRP can be detected. Chorionic villi were embedded in Tissue-Tek (Miles Inc USA) within four hours of sampling and immediately frozen in liquid nitrogen. Thick cryostat sections (7  $\mu$ m) were cut and incubated to show FMRP with an indirect immunoperoxidase technique.<sup>15</sup>

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