Mosaicism for the fragile X syndrome full mutation and deletions within the CGG repeat of the FMR1 gene

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Abstract

The main mutation responsible for the fragile X syndrome is the expansion of an untranslated CGG repeat in the first exon of the FMR1 gene, associated with the hypermethylation of the proximal CpG island and the CGG repeat region, and repression of transcription of FMR1. Fragile X syndrome mosaicism has been described as the coexistence of the full mutation and the premutation. We present here two cases of mosaicism for the full mutation in the FMR1 gene and deletions involving the CGG repeat region. In one case the deletion removed 113 bp proximal to the CGG repeat and part of the repeat itself, leaving 30 pure repeats, and representing 17% of lymphocytes of the patient. The 5′ breakpoint of this deletion falls outside the putative hotspot for deletions in the CGG region of FMR1. In the second case the deleted region only involved the CGG sequence (leaving 15 pure repeats), with normal sequences flanking the repeat; this deleted ("normal") FMR1 was estimated to be in about 31% of blood lymphocytes. This second case can be considered a true regression of the CGG FMR1 expansion to a normal sized allele, although in mosaic form.

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Fragile X syndrome mosaicism has been described as the coexistence of the full mutation and the premutation and has been detected with a frequency of about 20%, with a higher prevalence in males.[1-4] Recently, six cases of mosaicism for the full mutation and a deletion in the CGG repeat region of FMR1 have been reported, the deletions being present in 5 to 40% of lymphocytes[5 6] and in 85% of fibroblasts.[7] In five of these cases, the 5′ breakpoint of the deletion was found between 75–53 bp upstream of the CGG repeat, suggesting that this region is a hotspot for deletions.[6 7] We present here two further cases of mosaicism for the full mutation in the FMR1 gene and deletions involving the CGG repeat region.

Patient 1 was a 12 year old boy, who scored 16 on the Hagerman checklist[8] and had an IQ of 54 on the Terman-Merrill scale. Patient 2 belongs to a family with two mentally retarded sons and a normal daughter. He scored 18 in the Hagerman checklist and had an IQ of 45.

Southern blot analysis with probe StB12.3 after double digestion with *Eco*RI and *Eag*I in patient 1 showed a 6·1–6·7 kb smear corresponding to the FMR1 full mutation, and an additional fragment of 2·6 kb (instead of the expected 2·8 kb *Eco*RI/*Eag*I fragment in normal subjects (fig 1A). Densitometric analysis showed that 17% of the patient's blood lymphocytes had the 2·6 kb fragment. Analysis of the family members showed that the mother and the maternal grandfather had premutations (about 156 and 112 CGG repeats, respectively). To determine the molecular basis of the deleted fragment, we performed PCR analysis using primers flanking the CGG repeat. Whereas primers P1[2] and P2 (5′-TTGTA-
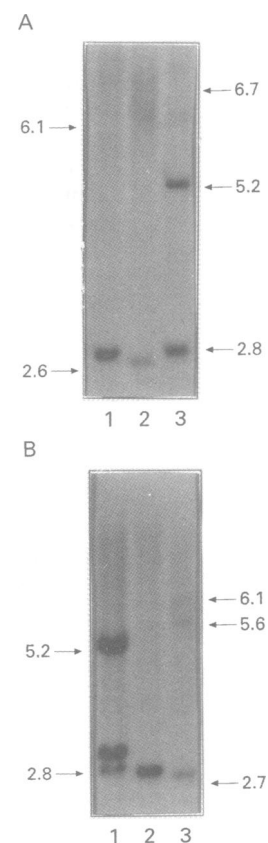

Figure 1 Analysis of two fragile X patients showing mosaicism for the full mutation and a deletion within the CGG repeat of FMR1. Southern blotting was performed with probe StB12.3 hybridised to EcoRI/EagI double digestion. (A) Lane 1 is a normal male showing a 2·8 kb fragment. Lane 2 is patient 1 and shows a 6·1–6·7 kb smear and a 2·6 kb fragment. Lane 3 is a normal female showing two fragments of 2·8 and 5·2 kb. (B) Lane 1 is a carrier female. Lane 2 is a normal male showing a 2·8 kb fragment. Lane 3 is patient 2 and shows a 5·6–6·1 kb smear and a 2·7 kb fragment. Size numbers are in kilobases (kb).
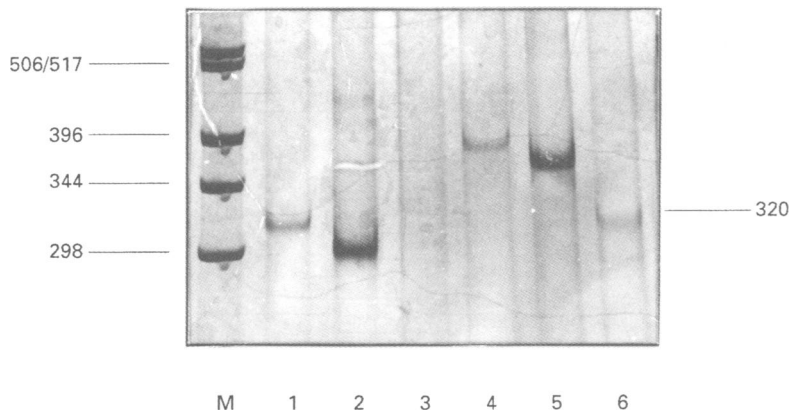
Figure 2 Determination of the sizes of two deletions in the first exon of the FMR1 gene. Lanes 1 and 4 are a male control sample. Lanes 2 and 5 are patient 2. Lanes 3 and 6 are patient 1. PCR for lanes 1 to 3 was with primers P1 and P2 (immediately flanking the CGG repeat), whereas for lanes 4 to 6 it was with primers A and 571R (located 5' and 3' with respect to P1 and P2). Lane M is a molecular weight marker. Size numbers are in bp. Primers P1 and P2 failed to amplify in patient 1 (lane 3).

GAAAGCGCCATTGGAGCCC-3'), which are close to the CGG repeat core, failed to amplify a product, a fragment of about 320 bp was obtained with primers A and 571R[9] (fig 2). Sequence analysis with these primers showed a deletion of 113 bp of the non-variable sequence, upstream of the CGG repeat region and part of the CGG region itself, leaving 30 pure repeats at the 3' end of this region (fig 3). Since the expanded region in this patient was between 300 and 500 CGG repeats, the total estimated length of the deletion (if it derived from the full mutation) was between 900 and 1600 bp. PCR in the mother only amplified the normal allele of 23 CGG repeats, different from the 30 CGG repeats detected in her son.

In patient 2, Southern blot analysis showed a 5·6–6·1 kb smear and an additional fragment at 2·7 kb (fig 1B). Family studies showed a carrier mother with a premutation inherited from her father, who is a normal transmitting male (about 133 and 91 CGG repeats, P1–P2 and A-571R, respectively), and the brother of the proband had the full mutation, but not the 2·8 kb fragment. His sister does not have any clinical manifestation of fragile X syndrome, but is also a carrier of the full mutation. PCR analysis using primers flanking the CGG repeat detected fragments of 298 and 385, respectively (fig 2). Sequence analysis of the PCR product containing the CGG repeat showed an allele of 15 pure repeats, without loss of additional material. Densitometric analysis showed that 31% of the cells contained the deleted allele. PCR in the mother amplified only one allele of 29 CGG repeats, different from the 15 CGG repeats detected in her son.

Both patients with the classical Martin-Bell syndrome phenotype show the coexistence of the full mutated FMR1 gene and a deleted or a "normal" FMR1 gene. Several cases of mosaicism for the full mutation and deletions within the CGG repeat region of FMR1 have been described recently.[5-7 10] Although our two patients apparently show similarities to those previously described, the molecular characterisation indicated several differences.

The first case (patient 1) differs from the five cases described previously[6 7] in that the deletion includes the Chi-like sequence, located 5' to the deletions described by de Graaff et al,[6] and the deletion conserved part of the CGG repeats (30 pure repeats). A further patient mosaic for the full mutation and a 660 bp deletion, including the CGG repeat, has recently been described.[5] In this case and in our patient 1, the 5' endpoint was located outside the Chi-like sequence, making its involvement in the mechanism causing the deletions questionable (fig 3). Other mutations in this region include a 1·6 kb germline deletion involving the promoter of FMR1 and part of the CGG repeat[11] and a YAC clone derived from a fragile X patient with about 350 CGG repeats that showed a deletion of 43 bp 5' to the CGG repeat and 43 pure repeats. Thus, the 5' endpoint of several deletions in the CGG repeat region also extend proximally to the putative hotspot and the 3' endpoint of some deletions falls within the CGG repeat.

A similar case to our case 2 was reported by van den Ouweland et al[10] in a patient who showed mosaicism for the full mutation and a "normal" FMR1 gene with 21 and 33 repeats, because of the deletion of the allele expanded in the mother. In both cases the "normal" alleles were unmethylated. These two cases can be considered as true regressions of the CGG FMR1 expansion to a normal size allele, although in mosaic form.

In the case described by van den Ouweland et al[10] and in patient 2 the estimated percentage of FMR1 cells without the full mutation that should be considered as being "normal" is about 50% (an estimation from the figure provided by the authors) and 31% (with densitometric analysis), respectively. A recent study has shown that the FMRP levels depend on both the methylation status and the length of the CGG repeat.[12] Thus, since the methylation status and CCG length is normal in these two patients, we expect them to have detectable levels of FMR1 mRNA and FMRP. However, the postulated levels of between 31% and 50% of FMRP in these patients might not be enough to prevent the clinical abnormalities of the fragile X syndrome that they have. This is in agreement with a recent report that shows a wide range of FMRP levels (between 10% and 60%) in fragile X syndrome males with mosaicism for the full mutation and the premutation.[13] A major difference between the mosaic males and these two cases is that the repeat length is normal. Finally, we do not know the proportions or the existence of this type of mosaicism in other tissues from these cases. The presence of the "normal" allele at relatively high levels in these mosaic fragile X patients shows that the full mutation and its methylation is the determinant of the phenotype, regardless of the presence of a normal copy of the gene.

The frequency of deletions involving the CGG repeat in our series of 134 fragile X affected males with the CGG expansion in the FMR1 gene is 1·5%, being the frequency of premutation/full mutation mosaicism of 13·4%[4] (unpublished data).
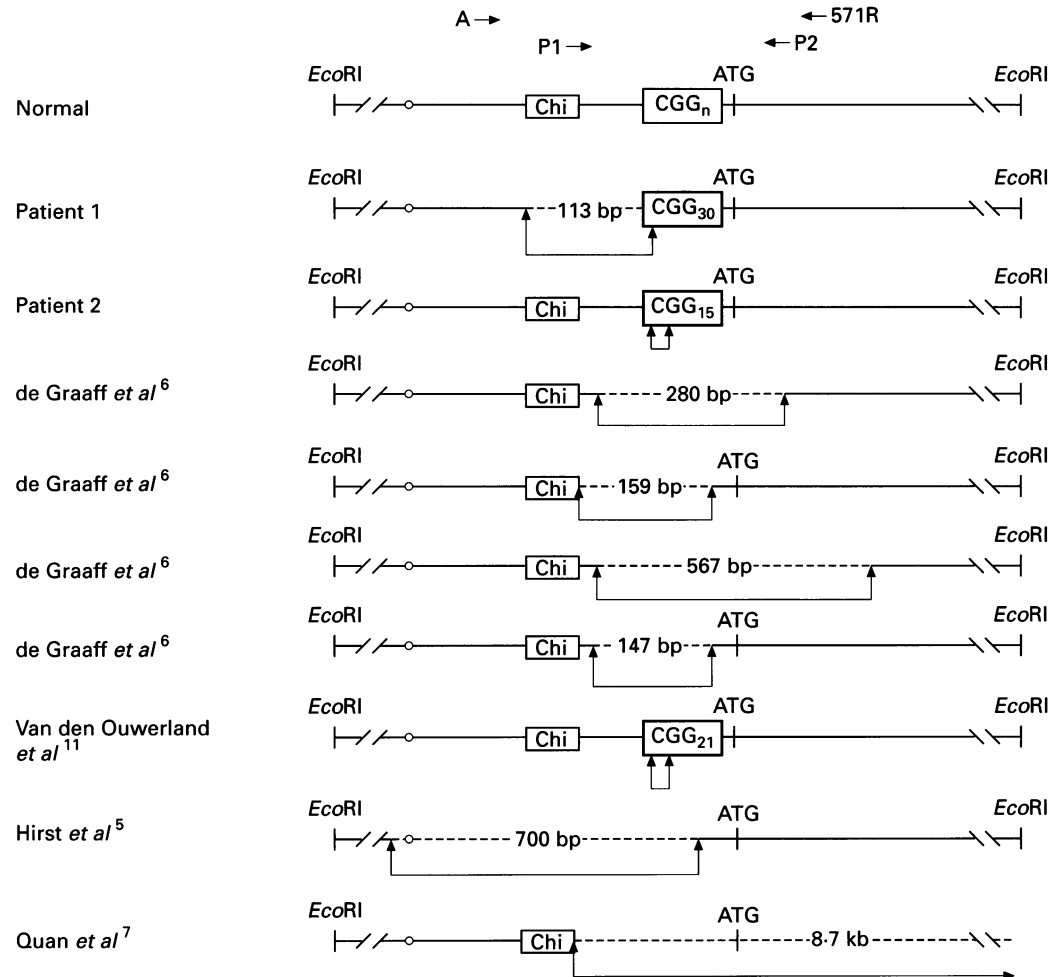
Figure 3   Schematic representation of deletions in the CGG region of the FMR1 gene in patients showing mosaicism for the full mutation. The reported cases (patients 1 and 2) are shown first. Other cases of mosaicism for the full mutation and deletions within the CGG repeat region of FMR1 are shown only for comparison purposes. The region corresponding to the normal FMR1 gene is shown at the top of the figure. The EcoRI sites are indicated. The vertical arrows and dashed lines indicate the extent of the deletions, which is also indicated in base pairs (bp). The open circle shows the initiation of transcription. The boxes indicate the CGG repeats that show partial deletion (from premutation or full mutation to a normal sized allele) and that consist of pure CGG repeats. The ATG site and the Chi-like sequence are indicated.

PCR amplification of the CGG repeat using primers close to the repeat core might lead to failure in the amplification of alleles containing deletions in this region. Thus, it is advisable to use primer pairs that are located outside the commonly deleted region, such as those described by Chong *et al*.[9]

It is also important to emphasise that this type of mosaicism could not be detected in a PCR based screening for fragile X syndrome, owing to the fact that a normal sized allele is amplified with serious consequences in the context of pre- and postnatal diagnosis. These types of mutations and the methylation status related to FMR1 stress the importance of combining Southern blot analysis and PCR for the diagnosis of fragile X syndrome.

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