FMR3 is a novel gene associated with FRAXE CpG island and transcriptionally silent in FRAXE full mutations

Jozef Gécz

Abstract

We have identified a novel gene, FMR3, originating from the FRAXE CpG island. The FMR3 gene is transcribed from the opposite strand to the FMR2 gene. Analogous to the silencing of the FMR1 and FMR2 genes, FMR3 transcription is extinguished by FRAXE full mutation. Although the role of FMR3 in FRAXE associated mild to borderline mental retardation is not yet clear, lack of expression of FMR3 in FRAXE full mutation males means that the FMR3 gene is potentially involved.

Keywords: FMR2; FMR3; FRAXE; mental retardation

FRAXE associated mental retardation (FRAXE MR) is the most prevalent form of non-specific X linked mental retardation (MRX) affecting at least 1 in 23423 males.1–4 The molecular basis for this disorder has been identified as the absence5–6 or truncation5 of the FMR2 protein. This is a consequence of either methylation of the FRAXE CpG island in subjects with the full mutation (>200 CCG copies) and consequent transcription silencing of the FMR2 gene,7 or deletion within the FMR2 gene.58 No FMR2 specific point mutations have been reported so far. The 5' end of the FMR2 gene lies within the FRAXE CpG island. Although the transcription of the FRAXE CCG repeat as part of the FMR2 gene has been suggested,9 there are currently no experimental data supporting this.

In silico search for FMR3 was prompted by identification of a mildly mentally retarded patient with a submicroscopic deletion of FMR2 intron 3 sequences,8 which has no apparent effect on expression of the FMR2 gene (unpublished data). Database searches (EST division of GenBank) with genomic sequence around FRAXE identified a single EST, AI470948. The sequence of this EST starts from the FRAXE NotI site, only 3 bp from the putative FMR2 transcription start site10 and only 23 bp proximal to the FRAXE CCG repeat (fig 1A). Comparison of EST AI470948 and FRAXE genomic sequence (AC002368) identified two exons separated by a 2.1 kb intron (fig 1B). RT-PCR analysis across this intron showed that this EST originated from a new gene, FMR3, transcribed in the opposite orientation to FMR2. Transcription of FMR3 from the opposite strand to that of the FMR2 gene was also confirmed using gene specific primer synthesis of cDNA followed by PCR (results not shown).
Northern blot analysis with the 1.2 kb insert of the EST AI470948 identified ∼3.8 kb low abundance transcript expressed in adult brain (medulla and spinal cord) (fig 2, left panel). In the search for the 3' end of FMR3, two ESTs derived from the same cDNA clone (N66183 and N99175) were identified more proximal (∼6 kb) to the FRAXE CpG island. A 541 bp PCR probe just distal to the polyadenylation signal observed in the EST N66183 was designed and generated by RT-PCR on human adult brain mRNA using standard techniques. The following oligonucleotide primers were used: F10 5'-CTA TGC TGC TAT GCA ACG ACG-3' and R5 5'-ACA CTT AGC ACT GCT GAT GTC ACC-3'. Northern blot hybridisation showed the same size transcript (∼3.8 kb) as detected with the EST AI470948 clone insert (fig 2, right panel). However, in addition to the ∼3.8 kb transcript one larger and two smaller sized transcripts were detected (arrows, fig 2, right panel). It is not yet clear whether these transcripts are the result of an alternative splicing of the FMR3 gene or alternative transcription start sites.

In searching for additional introns and exons within the FMR3 gene, EST AI470948 was fully sequenced and the region between ESTs AI470948 and N66183 was subdivided into 500-900 bp regions and amplified from oligo dT primed human adult brain cDNA. No additional exons were detected, which would indicate that the FMR3 gene is composed of only two exons. Careful analysis of the region of the FMR3 transcript did not show any protein coding open reading frame (ORF) within the FMR3 transcript, although smaller ORFs (up to 51 amino acids) were present. Thus, based on currently available information, we can only speculate that the FMR3 is a protein coding gene.

To investigate whether this gene is subject to transcriptional repression in subjects with FRAXE CCG full mutations, fibroblast RNA from seven FRAXE full mutations, one FRAXE premutation, two known FMR2 deletion patients, and controls was analysed by RT-PCR. The result of this experiment is shown in fig 3. As in the FMR2 gene (fig 3, middle panel) the newly identified gene FMR3 is transcriptionally silenced in FRAXE full mutations (fig 3, upper panel). FMR3 transcripts were detected in a FRAXE premutation and in controls. FMR3 transcription was also detected in two FMR2 deletion patients. These results show that absence of FMR3 expression might also contribute to the FRAXE MR phenotype.

The mental retardation in FRAXE is mild to borderline (50<IQ<85) without any dysmorphic features. There are at present six documented cases of FRAXE full mutations with a clinically normal phenotype, although the level of FMR2 expression was tested in only two of them. Identification of yet another gene, FMR3, associated with the FRAXE fragile site is intriguing, especially the extent of any contribution this gene might have to FRAXE MR. Currently, there is only one FMR2 mutation (deletion of exons 2 and 3) which affects only the FMR2 gene (where a truncated protein would be produced) and not FMR3. All other FRAXE full mutations so far tested have had both FMR2 and FMR3 transcripts

Figure 2  Northern blot analysis of FMR3. FMR3 probes (5' and 3') were hybridised to human brain II Northern blot (Clontech). FMR3 transcript of ∼3.8 kb was detected in medulla and spinal cord. Additionally, two smaller and one larger transcript (of slightly lower abundance) were detected with the 3' end FMR3 probe (right panel, indicated by arrows). A 5' end probe 1.2 kb insert of EST AI470948 was used. The 3' end probe was derived by RT-PCR based on the sequence of EST N66183 (see text).
absent. Thus, it is difficult to estimate the extent of the contribution of FMR3 to the FRAXE MR clinical phenotype and to establish whether the affected phenotype is the result of more than one gene.

Several deletions of the FMR3/FMR2 5' end region have been described. These were identified either from a FRAXA full mutation male or females with premature ovarian failure (POF, fig 1A). While the contribution of the FMR2/FMR3 genes to the FRAXA phenotype of the deletion patient of Brown et al. is difficult to assess (because of the severity of the FRAXA phenotype), the four deletions reported by Murray et al. may help to determine the relative contributions of the FMR2/FMR3 genes to POF, as these affect transcription starts of either the FMR3 or the FMR2 gene.

The identification of a second gene associated with the FRAXE (CCG) repeat is novel and may help to resolve unanswered questions about FRAXE MR and the aetiology of POF.

I thank G Sutherland and J Mulley for critical reading of the manuscript, C Derwas, M Hillman, and M Mangelsdorf for laboratory assistance, and colleagues on a previous paper who provided patient samples. This work was supported by the National Health and Medical Research Council of Australia.