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Two New Cases of FMR1 Deletion Associated with Mental Impairment

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Summary

Screening of families clinically ascertained for the fragile X syndrome phenotype revealed two mentally impaired males who were cytogenetically negative for the fragile X chromosome. In both cases, screening for the FMR1 trinucleotide expansion mutation revealed a rearrangement within the FMR1 gene. In the first case, a 660-bp deletion is present in 40% of peripheral lymphocytes. PCR and sequence analysis revealed it to include the CpG island and the CGG trinucleotide repeat, thus removing the FMR1 promoter region and putative mRNA start site. In the second case, PCR analysis demonstrated that a deletion extended from a point proximal to FMR1 to 25 kb into the gene, removing all the region 5′ to exon 11. The distal breakpoint was confirmed by Southern blot analysis and localized to a 600-bp region, and FMR1-mRNA analysis in a cell line established from this individual confirmed the lack of a transcript. These deletion patients provide further confirmatory evidence that loss of FMR1 gene expression is indeed responsible for mental retardation. Additionally, these cases highlight the need for the careful examination of the FMR1 gene, even in the absence of cytogenetic expression, particularly when several fragile X-like clinical features are present.

Introduction

The fragile X syndrome is associated with the presence of an expanded trinucleotide array within the first exon of the FMR1 gene (Kremer et al. 1991; Oberle et al. 1991; Yu et al. 1991). Expansion results in extensive hypermethylation of the promoter and the repeat itself (Bell et al. 1991; Vincent et al. 1991; Hansen et al. 1993) and the loss of FMR1 gene expression (Pieretti et al. 1991; Sutcliffe et al. 1992). FMR1 expression occurs throughout fetal development in multiple tissues (Pieretti et al. 1991), including specialist neuronal cell types later in development, which may be of significant phenotypic importance (Abitbol et al. 1993; Devys et al. 1993; Hinds et al. 1993). While the function of the FMR1 protein is not yet fully understood, evidence that it plays a role in RNA metabolism has come from (1) the identification of two motifs found within RNA binding proteins—the RGG box and KH domain (Siomi et al. 1993; Gibson et al. 1993)—and (2) the fact that in vitro the FMR1 protein demonstrates an RNA binding activity (Siomi et al. 1993). A point mutation converting Isoleucine-367 to an asparagine residue has been identified in an individual with some of the features of fragile X syndrome (De Boulle et al. 1993). This mutation lies within the highly conserved amino acids of the KH domain and is suggested to alter the putative RNA binding activity of the protein (Siomi et al. 1993, 1994).

Additional evidence that the loss of FMR1 gene expression causes the fragile X syndrome phenotype has come from studying cytogenetically FRAXA-negative males with the clinical features of the fragile X syndrome–carrying deletions. A de novo microdeletion removing proximal flanking DNA and the first four exons of the FMR1 gene, including the CpG island and the CGG array, has been described in a mentally retarded male (Wöhrle et al. 1992). This deletion extends from >90 kb proximal to FMR1 and removes at least the first five exons of the gene. In a second case, the deletion spans >2,500 kb of DNA and completely removes the gene (Gedeon et al. 1992). In both cases large regions of DNA are deleted, and the loss of other genes, particularly proximal or adjacent to FMR1 could not be excluded. More recently, a small, 1.6-kb deletion that removes the promoter region but leaves a portion of the $(CGG)_n$ array intact (Meijer et al. 1994) has been described. In this case, the deletion had segregated through several generations, including via male transmission, suggesting that the FMR1 gene product is not essential for spermatogenesis.

In this paper, we describe two cases of de novo deletions of portions of the FMR1 gene in mentally impaired males. In the first case, the individual is a mosaic of normal and deletion chromosomes, the deletion extending 660 bp across the FMR1 promoter. This removes the CpG island,

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the putative start site of FMR1 transcription, and the first 128 bp of the mRNA, including the trinucleotide array. In the second case, a deletion was found in all the proband's cells and extended across ⩾35 kb of DNA, ending between exons 10 and 11 of FMR1. These cases highlight the occurrence of deletions within FMR1 and are discussed in the context of their identification in routine diagnosis. Implications about the role of FMR1 in early development are also discussed.

Patients, Material, and Methods

Clinical Presentation

Case 1.—The proband presented at 8 years of age with a history of nocturnal epileptiform seizures. He was born at term, after an uneventful pregnancy; early development was reported to be normal, but at the time of referral, he was being investigated for learning and behavioral difficulties. There was no family history of note. Electroencephalography was performed, and it revealed focal epileptiform seizures; a computed-tomography brain scan was reported as normal. His seizures were eventually controlled by anticonvulsive therapy, but his learning difficulties remained and resulted in him being transferred to a school for students with special educational needs. At age 12 years, he was reinvestigated because of aggressive behavior, and physical examination revealed obesity, large ears, normal sized testes (6 ml), and a large head (59 cm, >97th percentile). Cytogenetic investigation revealed a normal 46,XY karyotype with no fragile X in 100 cells.

Case 2.—The proband is a 25-year-old moderately mentally retarded male referred for further diagnosis because of behavioral problems, with marked hyperactivity, echolalia, hand biting, and flapping. He presented with a typical fragile X phenotype with high and broad forehead, long facies, large ears, and large testes (40 ml). Chromosome analysis revealed a normal 46,XY male karyotype, and fragile X screening was negative in 300 cells in three different M199 cultures.

Sample Analysis

DNA was isolated from peripheral blood lymphocyte samples by standard techniques, and direct visualization of the FMR1 region was carried out by hybridization to the DNA probe Ox1.9 after restriction digestion with *Hin*dIII or *Eco*RI, and Ox0.55 after restriction with *Pst*I (Hirst et al. 1991*a*; Nakahori et al. 1991). For intron analysis, probe K (PCR XI-XII; see below and as originally described by Eichler et al. 1993) was prepared by PCR and was purified by preparative gel electrophoresis. Hybridization probes were prepared by random-primed labeling, and filters were hybridized overnight, washed to a stringency of 1–0.5 × SSC and exposed to Kodak XAR5 film at −70°C.

Promoter-Region PCR

PCR amplification across the FMR1 promoter was carried out using several sets of primer pairs (see fig. 1). Primers C130–721 amplify across the CG-rich promoter region of FMR1. The conditions used were as follows: PCR was cycled through 35 cycles of 98°C for 15 s and 70°C for 10 min in a Hybaid Omnigene thermal cycler. Each 10-μl reaction contained 100 ng of genomic DNA, 0.5 μM of each oligonucleotide, 200 μM dATP, 200 μM dTTP, 200 μM dGTP, 200 μM dCTP, 5% dimethylsulfoxide, and 0.5 units of wild type Pfu polymerase (Stratagene). This protocol allows the amplification of the CGG array and promoter products to be visualized with ethidium bromide (Hirst et al. 1994): C131, 5′-CTGAGTGCACCTCTGCAGAAATGGGCGGTT; C129, 5′-ACCGGAAGTGAAACCGAAACGGAGCTGAGC (the inverse of 722); C130, 5′-TGGAGTTGCAGCTAATGCTCTGCTCCCATTCAG; 721, 5′-AGCCCCG CACTTCCACCACCAGCTCCTCCA; and 722, 5′-GCTCAGCTCCGTTTCGGTTTCACTTCCGGT.

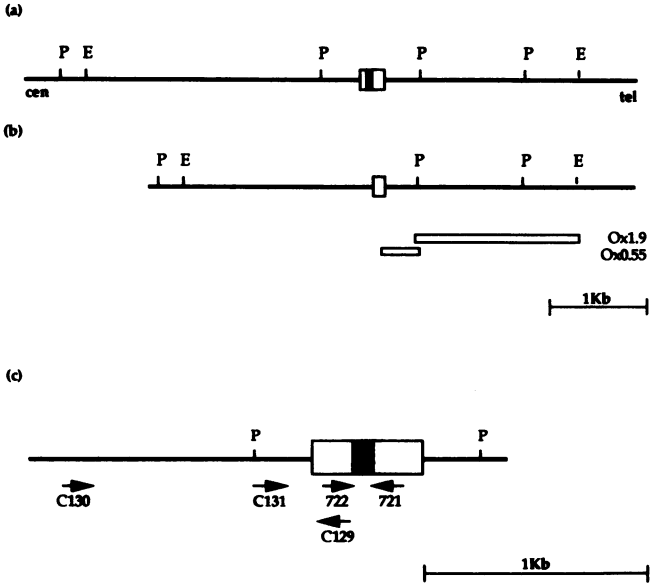

Figure 1 *a*, Restriction map of the normal 5′ region of FMR1, showing the first exon (unblackened box) and trinucleotide array (blackened box). *b*, Restriction map of the deletion chromosomes found in case 1. *c*, Positions of the 721, 722, C131, C129, and C130 primers, shown relative to the trinucleotide repeat. Restriction sites are shown: P = *Pst*I; E = *Eco*RI.

Deletion PCR Analysis

PCR was performed with primers for the markers M759, DXS548, G9L, FRAXAC1, FMR1-CGG, intron IV-V, intron VIII-IX, intron IX-X, intron X-XI, intron XI-XII, intron XVII-3′end, and 141R in standard buffer containing 2O0 μM each dNTP for 30 cycles of 95°C for 15 s; annealing temperature (T_m) for 1 min; and 72°C for 1.5–3 min in a Hybaid Omnigene thermal cycler. Intron PCR primers were as described by Eichler et al. (1993), and all primer pairs were used at 55°C. PCR primer pairs for the

markers FRAXA-AC1 and DXS548 were as described by Richards et al. (1991) and Verkerk et al. (1991). For markers M759, G9L, and 141R (described in Hirst et al. 1991*b*), each primer, product size, concentration of $MgCl_2$, and T_m is listed below. PCR products were resolved by electrophoresis through a 2% agarose gel and scored as present or absent: M759F, 5′-GAAGGGCCAGCAAATTGC; R, 5′-CTGTAGACTACTTGATGTGA (109 bp, 1.5 mM, 53°C); G9LF, 5′-AGGTTCAGTGTTGACATTGGGA; R, 5′-GCATATCAGACTCAACATGTGCA (500 bp, 1.5 mM, 62°C); 141R F, 5′-AATTCATCAAGAGGATGTAA-TAAC; and R, 5′-CCATTCAGGTTCATATTGCTTAA (500 bp, 3 mM, 58°C).

Haplotype PCR Analysis

PCR amplification for the polymorphic markers DXS548, FRAXAC1, and DXS1113 (IDS) was carried out in the presence of [^{35}S]-dATP, under conditions as described by Hirst et al. (1993), with the primers described by Weber et al. (1993) (DXS113), Verkerk et al. (1991) (DXS548), and Richards et al. (1991) (FRAXAC1). Direct detection of the FMR1 trinucleotide array was carried out using primers 721 and 722 (as above), as described by Fu et al. (1991), incorporating [^{32}P]-dCTP directly, except that 30 cycles of amplification were performed. Alleles were resolved on 5% denaturing polyacrylamide gels and were visualized by autoradiography with Kodak XAR5 film.

Sequence Analysis

The deletion PCR product of 1,100 bp (see fig. 1) from the PCR amplification with primers C130–721 was isolated from low-melt agarose and sequenced directly using the exo$^-$*Pfu* cycle sequencing reagents (Stratagene) supplemented with [^{32}P]-dCTP. The reactions were cycled through 30 cycles of 98°C for 15 s and 70°C for 1 min, in the presence of either the 721 or the C130 primer. Products were seperated on 5% polyacrylamide gels and were visualized by autoradiography on Kodak XAR5 film.

FMR1 Transcript Analysis

Total mRNA was isolated from a cell line established from individual 2 and was used as template for random-primed synthesis of cDNA. Two primer pairs were used to amplify regions of FMR1 from this cDNA. Primer pair 1 amplifies FMR1 exons 2 and 3, and primer pair 2 amplifies FMR1 exons 13–18. As a positive control, a primer pair for actin was also used: primer pair 1—F5′-AA-CAACTGGCAGCCTGATAGGCAGATTCCA and R5′-TTTAGCTAACCACCAACAGCAAGG; primer pair 2—F5′-GAGCAGTTGCGACAGATTGGA and R5′-GAA-TTATGCAGTTTAGGGTAC; and actin—F5′-GCCAAC-ACAGTGCTGTCTGG and R5′-TGAGCAGTATGA-GGACGAACGAC.

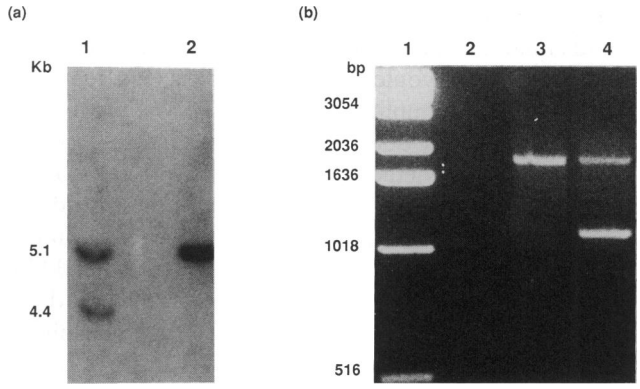

Figure 2 *a*, Southern blot analysis of case 1. Genomic digestion with *Eco*RI reveals the presence of a smaller hybridizing fragment of 4.4 kb (track 1) compared with the normal 5.1 kb (track 2). *b*, Ethidium bromide–stained 2% agarose gel, showing the PCR-amplification products obtained from PCR with primers 721–C130. Lane 1, Size marker ΦX174/*Hae*III. Lane 2, Negative control. Lane 3, Control female DNA. Lane 4, Case 1. The normal amplification product is ~1,800 bp, and the deletion fragment is estimated to be ~1,100 bp in size (see Results).

Results

Screening for the expanded trinucleotide within FMR1 was carried out using DNA probe Ox1.9 (see fig. 2). In two cases, this Southern blot analysis revealed no amplification of the FMR1 trinucleotide repeat, but other alterations were present.

Case 1: An FMR1 Promoter-Deletion Mosaic

On digestion with *Eco*RI and detection with the DNA probe Ox1.9, a fragment of 4.4 kb was present in addition to the wild-type 5.1-kb fragment (see fig. 1). With the enzyme *Pst*I, in combination with the probe Ox0.55, an additional fragment, of 2.7 kb, was present (data not shown). This smaller fragment was visible in ~40% of the patient's peripheral blood lymphocytes, and was confirmed on a second, independent lymphocyte sample. Neither parent was found to carry this deletion. On studying the position of restriction sites around these DNA probes, this series of altered restriction fragments is best interpreted as a deletion of DNA encompassing the 5′ region of FMR1 and removing the proximal *Pst*I site detected by Ox0.55 (see fig. 2). Deletion in this region would result in both a smaller *Eco*RI fragment and an increase in size of the *Pst*I fragment, as the next *Pst*I site is immediately proximal to the *Eco*RI site.

In order to confirm these observations, a PCR strategy to amplify various 5′ regions of FMR1 was adopted (see fig. 1). This included an assay that can amplify across the CGG trinucleotide repeat and CpG island region and give products visible by ethidium bromide staining, rather than the usual radioactive detection (Hirst et al. 1994). As this individual is a mosaic of normal and deletion chromo-

somes, only the direct amplification across the deletion would confirm its presence, as the fragment from the normal chromosome would always coamplify. With primers C130–721, which amplify across the CGG repeat to a region proximal to the deleted *Pst*I site, a deletion fragment of ~1,100 bp was detected, coamplified with the normal fragment of 1,800 bp (see fig. 1). The size of these products confirmed that the deletion was limited to the region immediately proximal to the FMR1 gene and was ~700 bp in size, in good agreement with that estimated by Southern blot analysis. No other combination of primers amplified the deletion region, all showing only the presence of the normal sized fragment. This confirms the extent of the deletion, as for each of these combinations of primers, one or both of each primer pair are within the deleted region.

In order to localize the endpoints of the deletion precisely, the C130-721 deletion PCR fragment was purified from agarose, directly sequenced, and compared with the sequence of the 5.1-kb FMR1 *Eco*RI fragment present in the GenBank database (see fig. 3). This shows that the deletion extends from position 2125, proximal to the CpG island, to position 2792, distal to the CpG island. No additional DNA appears to be present at this breakpoint. A more detailed analysis of this region, and a comparison with the known start of transcription (Hwu et al. 1993), shows that this deletion has removed the transcription initiation site (+1) and, based on this start site, extends to position +128 of the FMR1 mRNA. The truncation of the 5′ coding region thus ends prior to the initiation site for peptide synthesis (ATG), which lies at position +181 (Ashley et al. 1993).

Case 2: A Large Deletion Extending to the Exon 1 1 Region

The affected proband in this family appeared to have no hybridizing fragments with the DNA probe Ox1.9 (data not shown), suggesting that a deletion of this region was present. To confirm this and to screen other family members, it was necessary to screen with polymorphic markers, as only heterozygous markers would be informative for deletions in female family members. In PCR assays, the markers FMR1-CGG, FRAXAC1, DXS548, and IDS (DXS1113) confirmed that the deletion was a de novo event (see fig. 4) and that it extended to cover FRAXAC1 and the FMR1-CGG array. The deletion chromosome carries a flanking haplotype derived from the grand-paternal X chromosome and has thus most likely arisen from a chromosome carrying 19 copies of the CGG trinucleotide (see fig. 4). Additional typing of the grandmother showed that she also carried the FMR1-$(CGG)_{51}$ allele, indicating that despite its length, this allele is stable through three meioses (data not shown).

The extent of this de novo deletion was investigated further by scoring the presence or absence of other flanking markers across the region in the proband. These PCR markers are shown in figure 5, and their presence or absence is scored accordingly. This demonstrated that the deletion started proximal to FMR1, somewhere between G9L and FRAXAC1, and extended distal to the region between exons 10 and 11. Because of the lack of proximal markers, we have been unable to narrow down the proximal extent of the breakpoint further. Confirmation of this

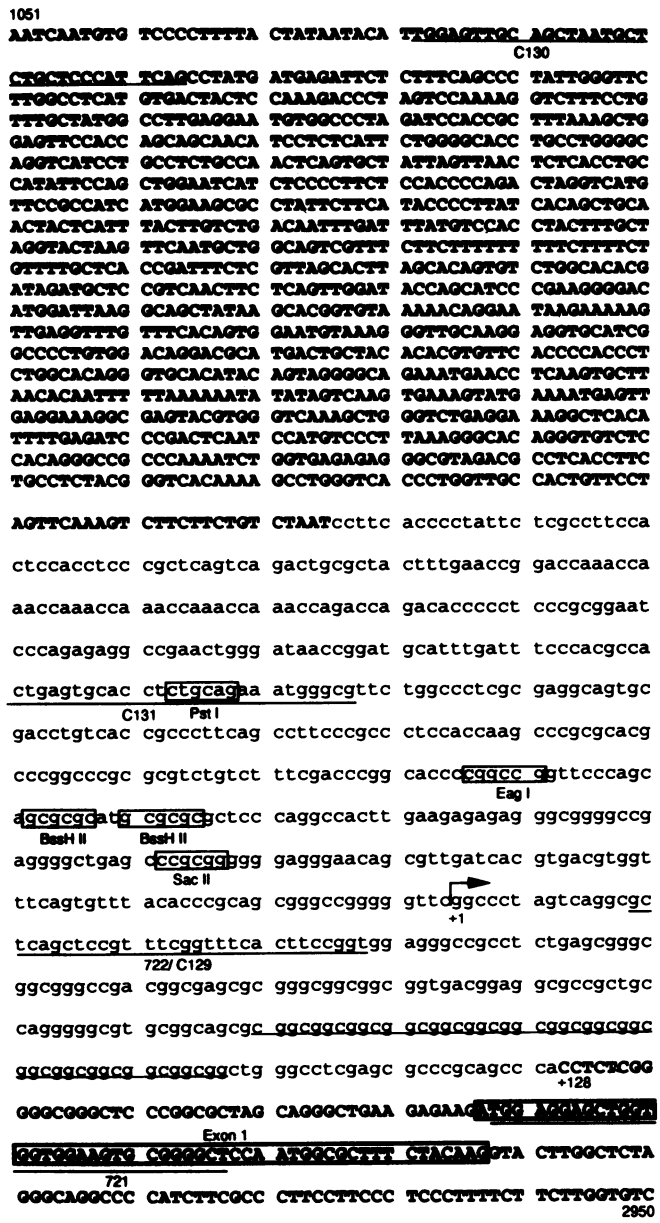

Figure 3 Sequence of the FMR promoter regions (coding strand), encompassing nucleotides 1051–2950. The positions of all the PCR primers used in the deletion study are underlined, and critical restriction sites are highlighted in boxes. Also shown are the transcription initiation site (+1) (as mapped by Hwu et al. (1993), the position of the deletion endpoint (+128), and the position of the CGG array (underlined). The portion of the 5′ coding region encoding protein is highlighted as exon 1. Lowercase letters represent the deletion found in case 1, and the numbering is based on the GenBank database FMR1 sequence HSFXDNA.

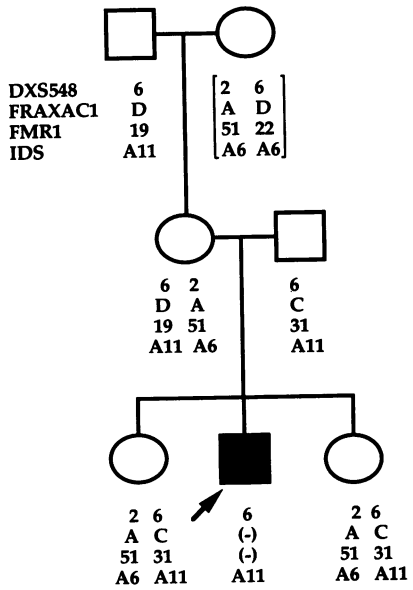

Figure 4 Haplotype analysis of the family of case 2. Typings are reported for the markers DXS548, FRAXAC1, FMR1-CGG, and IDS (DXS1113) (see Patients, Material, and Methods for details of amplification). Typings within brackets indicate that the phase of these chromosomes could not be determined.

PCR data was obtained by directly analyzing genomic DNA from the proband with a DNA hybridization probe that detects the region immediately distal to exon 11 (probe K in fig. 5). This probe detects altered restriction fragments with every enzyme tested except *Eco*RI, confirming this as the distal breakpoint region (data not shown). Further restriction analysis was performed to create a map around this breakpoint region (see fig. 5). Comparison with the normal restriction map (fig. 5) shows that the breakpoint must occur within the 600 bp between the retained *Eco*RI site and the missing *Hin*dIII site (marked with an asterisk [*] in fig. 5), some 25 kb from the FMR1 promoter region. Thus, the proband has lost the first 10 exons of the FMR1 gene.

In order to confirm that these deletion chromosomes did not express either FMR1 or any truncated products from an internal promoter region, cells from this individual were analyzed for FMR1 transcription using reverse transcription–PCR. Total mRNA was isolated from a lymphoblastoid cell line established from individual 2 and used for cDNA synthesis. Primer pairs that amplify varying regions of FMR1 were then assayed. Primer pair 1 detected exons 2 and 3, which are deleted in this individual, while primer pair 2 detected exons 13–18, which were not deleted. As can be seen from figure 6, neither pair gave rise to a product in individual 2; thus, it appears that no region of FMR1 was transcribed.

Discussion

The fragile X syndrome is most often associated with the presence of an expanded CGG trinucleotide array within the first exon of the FMR1 gene, which, on hypermethylation, results in the loss of gene expression. While this mechanism of mutation is predominant, several other mutations have been described, including several deletions and one case of a point mutation. Here we describe two new cases of de novo deletions within the FMR1 gene that have given rise to mental retardation and some degree of the fragile X phenotype.

The deletion in case 1 extends across the CpG island and removes the putative transcription initiation site and the first 128 bp of the mRNA, including the CGG trinucleotide. Within this promoter region are several recognition sites for the SP1 activator and the putative TATA box (Hwu et al. 1993). Thus, we would expect that the proportion of cells within this individual carrying this deletion do not express the FMR1 gene. The establishment of a clonal cell line carrying the deletion chromosome should confirm this. While several of the usual features of the Martin-Bell phenotype are absent, it is difficult to determine whether this is because the patient is prepubescent or because of the mosaicism. We do feel, however, that the nature of the mental impairment and the presence of the large head and ears in this patient, when associated with a deletion in the FMR1 promoter, are causally linked. Thus we believe that his milder clinical phenotype is due to the distribution of cells carrying the deletion chromosome within his body. In case 2, the deletion is present in 100% of the peripheral blood lymphocytes of the proband, as PCR assays for deleted markers failed to show any amplified products. The extent of this deletion in the rest of the proband's tissues has not yet been investigated. In order to contribute to all cell types within the proband, the deletion must have arisen either in the formation of the mother's germ-line cells or very early in embryogenesis. This is in contrast to case 1, which is clearly a postzygotic event.

Deletions removing regions of FMR1 range in size from 660 bp, in case 1 presented here, to >2,500 kb, in the case described by Gedeon et al. (1992). A breakpoint of a ring chromosome X in a fragile X carrier female has also been described proximal to *DXS548* (Mornet et al. 1993). With this limited number of cases, there is no evidence to date for a clustering of breakpoints. In all cases, it is assumed that the deletion leads to the loss of FMR1 expression. While this is certainly the case for the individual described by Gedeon et al. (1992), short deletions of the 5′ end of FMR1 may serve as truncation events, allowing for transcription to occur if an alternative internal promoter is used. In the case of the 1.6-kb deletion described by Meijer et al. (1994), the FMR1 gene is not expressed as assayed by PCR. A small transcript, of 1.4 kb, has been described elsewhere (Hinds et al. 1993), but, as yet, its relationship to the FMR1 coding region is uncharacterized. In case 2, no transcription over the length of the FMR1 coding region was found by PCR, suggesting that this smaller transcript either arises within the deletion region,

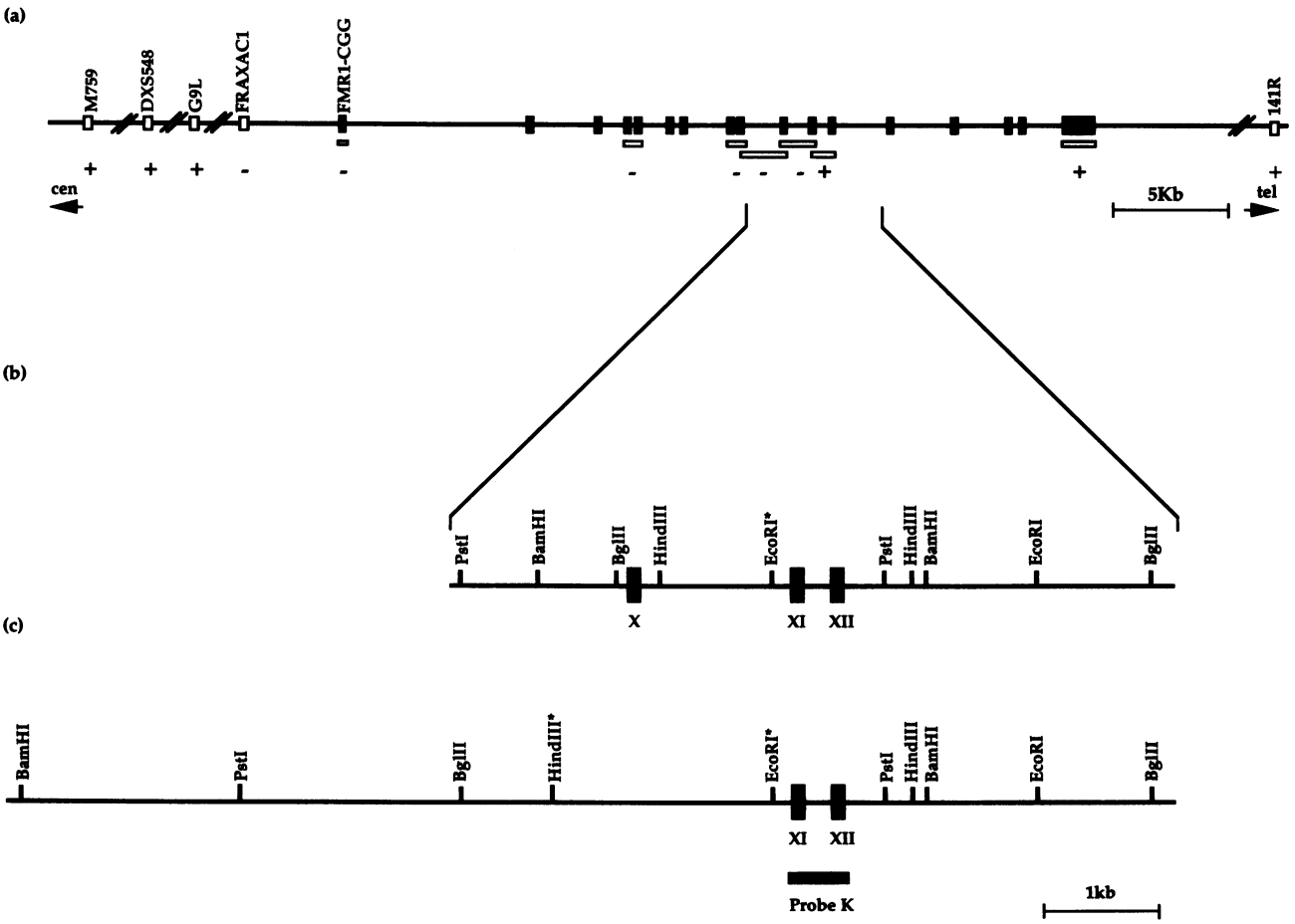

Figure 5 *a*, Map of the FMR1 region, extending from 141R to M759. Only the central portion is shown to scale. Unblackened boxes and bars represent PCR markers tested in case 2 (see Patients, Material, and Methods), and their presence or absence is indicated by + or −, respectively. Solid vertical bars represent the exons of FMR1, as described by Eichler et al. (1993). Restriction maps around exons X, XI, and XII in a normal individual (*b*) and case 2 (*c*) are represented. The position of the probe used for the detection of the breakpoint is shown. Restriction sites highlighted by an asterisk (*) are discussed in the text (see Results).

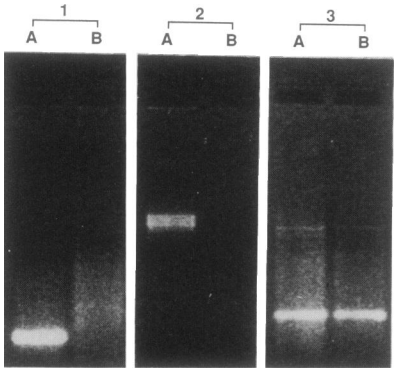

Figure 6 Ethidium bromide–stained 3% agarose gel showing RT-PCR amplification products obtained from lymphoblastoid cell lines from a normal male (lanes A) and individual 2 (lanes B). Panel 1 shows products obtained with primers to FMR1 exons II and III (product size 150 bp). Panel 2 shows products obtained with primers to FMR1 exons XIII-XVII (product size 545 bp). Panel 3 shows products obtained using actin-specific primers as a positive control (product size 210 bp).

splices out the regions tested in our PCR assays, or comes from a related locus that cross-hybridizes in hybridization assays (northern blots) but that is nonhomologous for PCR detection.

Loss of FMR1 gene expression occurs on methylation of the full mutation array in development (Sutcliffe et al. 1992), suggesting that it is present until that event. Indeed, the variable timing of the methylation and expansion event in the developing fetus may well be the cause of the great degree of variation seen in the clinical phenotype of the fragile X syndrome. The occurrence of a similar clinical phenotype in the cases of FMR1 deletions, which do not express FMR1 at any stage of development, as they have a nonfunctional promoter, implies that the major phenotypic effects of loss of FMR1 expression occur because of its absence later in development. Indeed, FMR1 mRNA is found at high levels in nearly all differentiated structures of the brain in a 25-wk-old normal fetus (Abitbol et al. 1993), suggesting that this and similar late expressions contribute to the pathogenesis of the fragile X syndrome.

The deletion in case 1 has arisen from a chromosome carrying 29 triplet repeats, made up of a compound repeat of 5′-$(CGG)_{10}(AGG)(CGG)_9(AGG)(CGG)_8$, while the deletion in case 1 most likely arose from the grandpaternal chromosome carrying 19 repeats, which is a compound repeat $(CGG)_8(AGG)(CGG)_{10}$, both of which are common arrays found in most of the normal population (Hirst et al. 1994). This is in contrast to the deletion described by Meijer et al. (1994), where the deletion has occurred on a chromosome carrying a perfect $(CGG)_n$ array of ~45 triplets. We have also observed a deletion immediately proximal to the FMR1 gene in a male carrying the fully expanded and hypermethylated mutation (Y. Nakahori, M. Hirst, and K. Davies, unpublished data). As most molecular diagnostics for FMR1 mutations are based on the detection of expansion and methylation across the 5.1-kb *Eco*RI fragment, other deletion events associated with expanded arrays may be occurring but are not detected. From the diagnostic aspect, these rare deletions need to be borne in mind, particularly when apparently typical fragile X males are negative for expansions at FMR1.

Deletion events across the FMR1 locus appear to be infrequent, but they will be found in routine molecular investigation into causes of unknown mental impairment or developmental delay. PCR assays to detect the CGG array in the mosaic deletion individual would have detected only a normal allele. Similarly, lack of a CGG-PCR product in case 2 could easily be misinterpreted as a full expansion mutation. Thus, the only reliable test that would detect such cases is a Southern blot assay. To date, all deletion cases have been detected as a consequence of screening with DNA probes for the detection of the expanded trinucleotide, and, thus, all have involvement with the 5′ of FMR1. In other cases, the detection of small deletions or point mutations within FMR1 will depend on the development of screening assays for other portions of the gene. To this end, the PCR assays described by Eichler et al. (1993), which amplify various internal regions of FMR1, could be used as an initial screening tool.

Acknowledgments

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References

Abitbol M, Menini C, Delezoide AL, Rhyner T, Vekemans M, Mallet J (1993) Nucleus basalis magnocellularis and hippocampus are the major sites of FMR-1 expression in the human fetal brain. Nature Genet 4:147–153

Ashley CT, Sutcliffe JS, Kunst CB, Leiner HA, Eichler EE, Nelson DL, Warren ST (1993) Human and murine FMR-1 alternative splicing and initiation downstream of the CGG-repeat. Nature Genet 4:244–251

Bell MV, Hirst MC, Nakahori Y, MacKinnon RN, Roche A, Flint TJ, Jacobs PA, et al (1991) Physical mapping across the fragile X: hypermethylation and clinical expression of the fragile X syndrome. Cell 64:861–866

De Boulle K, Verkerk AJMH, Reyniers E, Vits L, Hendrickx J, Van Roy B, Van Den Bos F, et al (1993) A point mutation in the FMR-1 gene associated with fragile X mental retardation. Nature Genet 3:31–35

Devys D, Lutz Y, Rouyer N, Bellocq JP, Mandel JL (1993) The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X permutation. Nature Genet 4:335–340

Eichler EE, Richards S, Gibbs RA, Nelson DL (1993) Fine structure of the human FMR-1 gene. Hum Mol Genet 2:1147–1153

Fu Y-H, Kuhl DPA, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJMH, et al (1991) Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 67:1–20

Gedeon AK, Baker E, Robinson H, Partington MW, Gross B, Manca A, Korn B, et al (1992) Fragile X syndrome without CGG amplification has an FMR-1 deletion. Nature Genet 1: 341–344

Gibson T, Rice P, Thompson J, Heringa J (1993) KH domains within the FMR1 sequnce suggest that fragile X syndrome stems from a defect in RNA metabolism. Trends Biochem Sci 18:331–333

Hansen RS, Gartler SM, Scott CR, Chen SH, Laird CD (1992) Methylation analysis of CGG sites in the CpG island of the FMR1 gene. Hum Mol Genet 1:571–578

Hinds HL, Ashley CT, Sutcliffe JS, Nelson DL, Warren ST, Housman DE, Schalling M (1993) Tissue specific expression of FMR1 provides evidence for s functional role in fragile X syndrome. Nature Genet 3:36–43

Hirst MC, Grewal PK, Davies KE (1994) Precursor arrays for triplet repeat expansion at the fragile X locus. Hum Mol Genet 3: 1553–1560

Hirst MC, Knight SJL, Christodoulou Z, Grewal PK, Fryns JP, Davies KE (1993) Origins of the fragile X syndrome. J Med Genet 30:647–650

Hirst MC, Nakahori Y, Knight SJL, Schwartz C, Thibodeau SN, Roche A, Flint TJ, et al (1991*a*) Genotype prediction in the fragile X syndrome. J Med Genet 28:824–829

Hirst M, Rack K, Nakahori Y, Roche A, Bell M, Flynn G, Christodoulou Z, et al (1991*b*) A YAC contig across the fragile X site defines the region of fragility. Nucleic Acids Res 19:3283–3288

Hwu WL, Lee YM, Lee SC, Wang TR (1993) In vitro methylation inhibits FMR-1 promoter. Biochem Biophys Res Commun 193:324–329

Kremer EJ, Pritchard M, Lynch M, Yu S, Holman K, Baker E, Warren ST, et al (1991) Mapping of DNA instability at the fragile X to a trinucleotide repeat sequence p(CCG)n. Science 252:1711–1718

Meijer H, de Graff E, Merckx D, Jongbloed J, de Die-Smulders C, Engelen J, Fryns J, et al (1994) A deletion of 1.6 kb proximal to the CGG repeat of the FMR1 gene causes the clinical phenotype of the fragile X syndrome. Hum Mol Genet 3:615–620

Mornet E, Bogyo A, Deluchat C, Simon-Bouy B, Mathieu M, Thepot F, Grisard M-C, et al (1993) Molecular analysis of a ring chromosome X in a family with fragile X syndrome. Hum Genet 92:373–378

Nakahori Y, Knight SJL, Holland J, Schwartz C, Roche A, Tarleton J, Wong S, et al (1991) Molecular heterogeneity of the fragile X syndrome. Nucleic Acids Res 19:4355–4359

Oberle I, Rousseau F, Heitz D, Kretz C, Devys D, Hanauer A, Boue J, et al (1991) Instability of a 550 bp DNA segment and abnormal methylation in fragile X syndrome. Science 252: 1097–1102

Pieretti M, Zhang F, Fu Y-H, Warren ST, Oostra BA, Caskey CT, Nelson DL (1991) Absence of expression of the FMR-1 gene in fragile X syndrome. Cell 66:817–822

Richards RI, Holman K, Kozman H, Kremer E, Lynch M, Pritchard M, Yu S, et al (1991) Fragile X syndrome: genetic localization by linkage mapping of two microsatellite repeats FRAXAC1 and FRAXAC2 which immediately flank the fragile site. J Med Genet 28:818–823

Siomi H, Choi M, Siomi M, Nussbaum R, Dreyfuss G (1994) Essential role for KH domains in RNA binding: impaired RNA binding by a mutation in the KN domain of FMR1 that causes fragile X syndrome. Cell 77:33–39

Siomi H, Siomi MC, Nussbaum RL, Dreyfuss G (1993) The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein. Cell 74:291–298

Sutcliffe JS, Nelson DL, Zhang F, Pieretti M, Caskey CT, Saxe D, Warren ST (1992) DNA methylation represses FMR-1 transcription in fragile X syndrome. Hum Mol Genet 1:397–400

Verkerk AJMH, Pieretti M, Sutcliffe JS, Fu Y-H, Kuhl DPA, Pizzuti A, Reiner O, et al (1991) Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 65:905–914

Vincent A, Heitz D, Petit C, Kretz C, Oberle I, Mandel J-L (1991) Abnormal pattern detected in fragile X patients by pulsed field gel electrophoresis. Nature 329:624–626

Weber C, Oudet C, Johnson S, Pilia G, Sclessinger D, Hanauer A (1993) Dinucleotide repeat polymorphism close to IDS gene in Xq27.3-q28 (DXS1113). Hum Mol Genet 2:612

Wöhrle D, Kotzot D, Hirst MC, Manca A, Korn B, Schmidt A, Barbi G, et al (1992) A microdeletion of less than 250 kb, including the proximal part of the FMR-1 gene and the fragile site, in a male with the clinical phenotype of fragile-X syndrome. Am J Hum Genet 51:299–306

Yu S, Pritchard M, Kremer E, Lynch M, Nancarrow J, Baker E, Holman K, et al (1991) Fragile X genotype characterized by an unstable region of DNA. Science 252:1179–1181