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Mosaic *FMR1* Deletion Causes Fragile X Syndrome and Can Lead to Molecular Misdiagnosis:

A Case Report and Review of the Literature

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Abstract

The most common cause of fragile X syndrome is expansion of a CGG trinucleotide repeat in the 5' UTR of *FMR1*. This expansion leads to transcriptional silencing of the gene. However, other mutational mechanisms, such as deletions of *FMR1*, also cause fragile X syndrome. The result is the same for both the expansion mediated silencing and deletion, absence of the gene product, FMRP. We report here on an 11-year-old boy with a cognitive and behavioral profile with features compatible with, but not specific to, fragile X syndrome. A mosaic deletion of 1,013,395 bp was found using high-density X chromosome microarray analysis followed by sequencing of the deletion breakpoints. We review the literature of *FMR1* deletions and present this case in the context of other *FMR1* deletions having mental retardation that may or may not have the classic fragile X phenotype.

Keywords

deletion; mosaic; mental retardation; social anxiety; fragile X syndrome

INTRODUCTION

Fragile X syndrome is one of the most common causes of inherited mental retardation, representing approximately 2–3% of all cases of mental retardation. Fragile X syndrome is caused by absence or severely reduced expression of the *FMR1* protein, FMRP [Penagarikano et al., 2007]. The most common cause of fragile X syndrome is expansion of a CGG trinucleotide repeat in the 5' UTR of the *FMR1* gene. This expansion leads to DNA methylation, aberrant heterochromatinization and silencing of the *FMR1* gene resulting in the absence of the gene product, FMRP. However, other mutational mechanisms, such as deletions of *FMR1*, can also cause fragile X syndrome. Deletions ranging in size from a single nucleotide to several Mb, involving all or a segment of *FMR1*, have been found in fragile X patients.

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METHODS AND RESULTS

Patient

The patient is an 11-year-old boy with a cognitive and behavioral profile compatible with, but not specific to, fragile X syndrome (Fig. 1). The patient has mild mental retardation with verbal greater than nonverbal skills [Differential Ability Scales (SS): Composite = 56, Verbal = 70, Nonverbal = 62, Spatial = 54] mild language delay [Clinical Evaluation of Language Fundamentals-4 (SS): Composite = 70, Language Content = 88, Working Memory = 60; Receptive One Word Picture Vocabulary Test = 86, Expressive One Word Picture Vocabulary Test = 95], and borderline-mildly impaired adaptive skills [Vineland Adaptive Behavior Scales (SS): Composite = 73, Communication = 66, Socialization = 84, Daily Living Skills = 79]. He also displays moderate to severe social avoidance, compatible with social anxiety, and ADHD-like symptoms by the Conner's Rating Scales (inattention, hyperactivity, impulsivity) and by measures of attention and executive functioning (Wide Range Assessment of Memory and Learning-Second Edition, Delis Kaplan Executive Function System) that have responded well to treatment with selective serotonin reuptake inhibitors (SSRIs) and methylphenidate, respectively. Overall, the patient is physically a well-developed boy with mild hypotonia and no major anomalies. He has some joint laxity and some minor facial characteristics of fragile X (e.g., prominent jaw and thickening of nasal bridge), but not all of the manifestations usually seen in this disorder (e.g., normal size ears with incomplete cartilage). In summary, this patient has a more subtle presentation of fragile X syndrome than is typically seen. The patient had either inconclusive or normal results from standard clinical laboratory testing for fragile X and was referred to our laboratory for further analysis of the *FMR1* gene.

Clinical Fragile X Testing

Blood from the patient was sent for standard fragile X syndrome testing at two different clinical laboratories. Testing consisted of assessment of CGG repeat length by PCR and *FMR1* DNA methylation by Southern analysis. After inconclusive results at both clinical laboratories, which in one instance reported weak signal for normal range CGG repeats, a sample was submitted to our laboratory for *FMR1* gene sequencing. During sequencing, it was observed several of the exons, notably at the 5' end of the gene and the promoter of *FMR1*, did not amplify efficiently, though enough product was able to be generated for sequence analysis. These and the previous results suggested that a mosaic deletion may be present. CGG repeat analysis by PCR and Southern analysis, specifically looking for evidence of a deletion, was then performed. CGG repeat analysis consists of PCR amplification using fluorescently labeled amplification primers flanking the CGG repeat, followed by separation of the products by capillary electrophoresis. The patient had a CGG repeat length of 23 CGGs, well within the normal range. However, compared to another normal male sample with 23 CGGs, the peak area was approximately 10-fold less in the patient's DNA sample (Fig. 2A). In the Southern blot, measurement of the 2.7 kb band intensity by phosphorimager analysis indicated there was ~10-fold reduction in intensity relative to an unaffected male tested in parallel (Fig. 2B). Together, the PCR and Southern analyses indicate the patient has a mosaic deletion involving *FMR1* in ~90% of his lymphocytes with 10% of his lymphocytes carrying an intact *FMR1* gene. Sequencing of the *FMR1* gene did not identify any mutations. Testing of the patient's mother identified a 23 and a 30 CGG repeat allele with a normal female pattern on the Southern blot, indicating she does not carry a premutation allele. The sample was then submitted to the research laboratory for further evaluation.

High-Density X Chromosome Microarray Analysis

Array comparative genomic hybridization (aCGH) was performed using a high-density microarray (P/ N: B3754001-00-01, Design name: HG18_CHRX_FT) from NimbleGen Systems to further characterize the deletion (Fig. 3A,B). The array consists of 385,000 oligo

probes ranging from 50 to 75 nucleotides applied to a glass slide using photomediated synthesis chemistry. The probes tile along the forward strand of the X chromosome at an average intermarker distance of 340 bp after repeat masking. Sample preparation and hybridization were performed in accordance with the manufacturer's instructions. In brief, 2 µg of genomic DNA from the patient and a male reference sample were sonicated to generate 500–2,000 bp fragments. After fragmentation, the two samples were labeled with Cy3 and Cy5, respectively, during whole genome amplification using random 9 mers labeled with Cy3 or Cy5. Fifteen micrograms each of these labeled amplification products were combined and then hybridized to the microarray for 16 hr at 42°C. After hybridization, the arrays were washed and scanned at a 5 µm resolution using an Axon 4000B scanner.

PCR Amplification and Sequencing of the Breakpoint

NimbleScan and Signal Map analysis software from NimbleGen were used to analyze the signal ratios on the array. A contiguous region spanning over 1 Mb encompassing *FMRI* and *FMRI*NB showed a depressed signal (−0.33) relative to flanking sequence (0.001) on the X chromosome (Fig. 3A,B). To confirm the deleted sequence, primers were designed to flank the potential break points. At the 5' end of the deletion, a primer in the forward direction was designed to sequence just upstream of the first probe (CHRXFS146047722) having depressed signal (−0.231). Likewise, a primer in the reverse orientation was designed to sequence downstream of the last probe (CHRXFS147055822) showing depressed signal (−0.483). Using these primers, a 3.5 kb amplicon was generated with the patient's DNA, but not with control DNA. This amplicon was gel eluted and sequenced in both directions.

PCR was performed using the LA Taq enzyme kit from Takara Bio, Inc. (Otsu, Shiga, Japan). Fifty nanograms of genomic template was used. The primer sequence for the upstream or 5' end was AGGCTAATATCCTGGACGAAC (Hg18, ChrX starts at 146047123) and the downstream or 3' end was TGAAAACTGGAAGAAATCCAA (Hg18, ChrX starts at 147063517). Twenty-five microliters reaction volumes were made such that the final primer concentration was 0.2 µM, final dNTP concentration was 0.25 mM, buffer plus magnesium, and 1.5 U of LA Taq were used. The cycling conditions were 94°C for 4 min, 35 cycles of 94°C for 20 sec and 60°C for 8 min, with a final extension of 72°C for 5 min. The 3.5 kb band was purified from an agarose gel using Qiagen's Gel Elution Kit (Cat. No. 28704) (Valencia, CA). The PCR product was bidirectionally sequenced using standard dideoxy chain termination methods from Applied Biosystems, Inc. (Foster City, CA) (Fig. 4).

DISCUSSION AND REVIEW OF LITERATURE

In 1997, Hammond et al. provided a comprehensive review of 23 deletions involving *FMRI* in patients with mental retardation [Hammond et al., 1997]. Including those cited by Hammond et al., we have identified 37 reports in the literature (a total of 71 deletions, listed in Tables I and II). There are two reported cases of deletions less than 10 bp in size, a 1 bp deletion in exon 5 of *FMRI* in a fragile X male [Lugenbeel et al., 1995] and 5 bp deletion near the start codon in a male with developmental delay, but with normal levels of FMRP, indicating that this deletion is unrelated to the patient's phenotype [Hegde et al., 2001]. The remaining 56 deletions fall into two distinct classes: small deletions (<10 kb) restricted to the 5' end of the gene that are due to instability of the CGG repeat and large deletions, up to 13 Mb in size, which may be cytogenetically visible and often include adjacent genes. The large deletions generally are not associated with CGG repeat instability. It is important to note there may be deletions in other regions of *FMRI* that have not been identified, since these regions of the gene are not routinely interrogated during fragile X testing.

Small Deletions Due to CGG Repeat Instability

Most small deletion cases are concomitant with full (>200 CGG repeats) and premutation (55–200 CGG repeats) size alleles and occur during the transmission of premutation alleles from mother to child [Meijer et al., 1994; van den Ouweland et al., 1994; Hirst et al., 1995; de Graaff et al., 1995; Quan et al., 1995a; Mannermaa et al., 1996; Mila et al., 1996; Schmucker et al., 1996; de Graaff et al., 1996; Gronskov et al., 1997; Hammond et al., 1997; Loesch et al., 1997; Orrico et al., 1998; Grasso et al., 1999; Petek et al., 1999; Schmucker and Seidel, 1999; Garcia Arocena et al., 2000; Gasteiger et al., 2003; Fan et al., 2005; Han et al., 2006]. The deletion may be located entirely within the CGG repeat, sometimes referred to as a contraction or a “reverse mutation”, or the deletion may extend into sequences on the 5′ and/or 3′ side of the CGG repeat. A chi-like element, located between the transcription initiation site and the CGG repeat tract, has been reported to be a hotspot for these deletions [de Graaff et al., 1995]. There have been several other reports of the 5′ break of the deletion occurring near this chi-like element, verifying that this is indeed a hotspot for deletion [Quan et al., 1995a; Gronskov et al., 1997; Petek et al., 1999; Fan et al., 2005]. However, there are numerous other proximal breakpoints identified 5′ to the CGG repeat (see Table I). The 5′ breakpoints have been reported to range from within the CGG repeat [de Graaff et al., 1996] up to >2,700 bp upstream of the CGG repeat [Grasso et al., 1999]. Similarly, the 3′ breakpoints can also vary widely and range from within the CGG repeat to 8,700 bp downstream of the CGG repeat. These deletions often disrupt the promoter and/or the start codon in exon 1, located immediately upstream and downstream of the CGG tract, respectively, resulting in the absence of gene expression from the deleted allele.

There are three reports, however, of deletions limited to a region between the transcription start site, located 129 bp upstream of the CGG repeat, and the translation initiation codon in exon 1 of *FMRI*, located 69 bp downstream of the CGG repeat [de Graaff et al., 1996; Gronskov et al., 1997; Han et al., 2006]. These cases illustrate the CGG repeat and the immediate flanking sequences are not required for expression of *FMRI*. The first case was reported by de Graaff et al. [1996] in a male fragile X patient who was mosaic for a full mutation and a small deletion in 28% of his lymphocytes. The 5′ boundary of the deletion was within the CGG repeat and the 3′ boundary was located 30 bp downstream of the repeat, leaving the ATG start codon in exon 1 intact. Both *FMRI* transcript and FMRP were detected indicating the deletion did not impair expression of the gene. The second case is an unaffected female sister of a male with the full mutation reported by Gronskov et al. [1997]. This girl carries a large terminal deletion on the long arm of one X chromosome, removing the entire *FMRI* gene. On the other X chromosome, she carries a small deletion at the 5′ end of *FMRI* that extends from 63 to 67 bp upstream of the CGG repeat to 30–34 bp downstream of the repeat. This deletion was probably produced as a regression of a full mutation from her affected mother. Normal amounts of FMRP were detected in the daughter’s lymphocytes. This demonstrated she had favorable skewing of X inactivation with silencing of the X chromosome carrying the large terminal deletion with the active X chromosome carrying the small deletion within *FMRI*. A third case of a deletion extending into the CGG repeat flanking sequence, but not disrupting expression of *FMRI*, was reported by Han et al. [2006]. They report a high functioning male who carries premutation and full mutation alleles, along with a deletion that extends from 42 bp upstream of the CGG repeat to 1 bp downstream of the repeat. Twenty-two percent of the normal level of FMRP was detected in his lymphocytes, consistent with the deletion not disrupting expression.

Large Deletions Involving *FMRI*

Large deletions (>10 kb; Table II) are not generally associated with premutation alleles, but are caused by meiotic or mitotic ectopic recombination. Other genes located proximally and/or distally may also be lost resulting in additional phenotypes. The region proximal to *FMRI* is relatively devoid of genes with the closest being *SLITRK2* located 2,100 kb proximal to

FMRI. Conversely, the region distal to *FMRI* is relatively more gene-rich with *AFF2* (the *FMRI2* gene) being the closest gene of known function. There is ~550 kb of sequence between the 3' end of *FMRI* and the 5' end of *AFF2*. Many of the patients carrying large deletions spanning *FMRI* have typical findings of fragile X syndrome [Schmidt et al., 1990; Gedeon et al., 1992; Wohrle et al., 1992; Tarleton et al., 1993; Gu et al., 1994; Trotter et al., 1994; Hirst et al., 1995], with some patients having additional manifestations depending upon the other genes being deleted [Clarke et al., 1992; Dahl et al., 1995; Quan et al., 1995b; Birot et al., 1996; Wolff et al., 1997; Moore et al., 1999; Parvari et al., 1999; Fengler et al., 2002].

There is only one reported example of a large deletion associated with transmission of a premutation. Fengler et al. [2002] describe a male with severe mental retardation and classic fragile X features who is mosaic for a deletion that removes both *FMRI* and *FMRI2*. The mother of this patient was found to carry a premutation *FMRI* allele, leading the authors to suggest the deletion occurred on the X chromosome carrying the expanded CGG repeat, though this was not formally tested by marker analysis. Southern blot analyses probing for *FMRI* or *FMRI2* had no detectable signal, indicating the deletion spanned at least 900 kb. They were able to PCR amplify segments of *FMRI*, although not the CGG repeat tract, suggesting nondeleted alleles were present. The inability to amplify the CGG tract was interpreted to be due to CGG repeat expansion in the minority of cells carrying the nondeleted X chromosome. The authors postulate that in these cells, *FMRI* is silenced due to expansion of the CGG repeat to a full mutation. They suggest that there is total loss of *FMRI* expression in this patient, mainly due to the deletion present in 90% of cells, with the remaining 10% of cells carrying a CGG expansion. In addition they suggest that this patient has significantly reduced expression of *FMRI2* due to the mosaic deletion, although neither *FMRI* nor *FMRI2* expression studies were done. Consistent with other patients that carry deletions that encompass *FMRI* and *FMRI2*, this patient has severe mental retardation.

There are other reports of deletions known to encompass both *FMRI* and *FMRI2* [Clarke et al., 1992; Dahl et al., 1995; Birot et al., 1996; Wolff et al., 1997; Moore et al., 1999; Probst et al., 2007]. In addition to severe mental retardation, seizures are reported in some of these patients. A male patient reported by Wolff et al. [1997] carries a 13 Mb deletion, removing *FMRI*, *FMRI2*, *SOX3* as well as other genes. This patient has severe growth and mental retardation and epilepsy. His phenotypically normal mother and his slightly delayed sister carry the same deletion. Neither the mother nor the sister is reported to have seizures. X inactivation analysis indicated the mother had more favorable skewing, with the deleted X chromosome inactive in >95% of her lymphocytes. The slightly affected daughter carried the deleted X chromosome on the inactive X in 80–85% of her lymphocytes. In the same report, Wolff et al. also describe an unrelated female patient who carries a similar sized 12 Mb deletion. This patient has an overgrowth phenotype, severe mental retardation, seizures, and hypothyroidism. X inactivation analysis indicated the deleted X chromosome is on the inactive X in 50% of her lymphocytes. In a report by Moore et al. [1999] a patient with a *FMRI* and *FMRI2* deletion was also described. This male patient carries a de novo 6.5 Mb deletion. He has severe mental retardation, autistic features, a large head (>97th centile), seizures and facial characteristics of fragile X syndrome. These three patients with *FMRI* and *FMRI2* deletions, two males and one female, were all reported to have seizures, leading to the suggestion that absence of both *FMRI* and *FMRI2* can cause epileptic activity [Moore et al., 1999].

However, there are several other reports describing patients carrying deletions removing both *FMRI* and *FMRI2*, who do not have seizures. There are two earlier reports of patients with deletions encompassing both *FMRI* and *IDS* who were affected with Hunter syndrome [Clarke et al., 1992; Birot et al., 1996]. *FMRI2* would also be deleted in these patients since it is located between these two genes, though that was not known at the time. Clarke et al. characterize a de novo large deletion at Xq27-q28 that removes *FMRI* and *IDS* in a female with Hunter

syndrome, but no seizures were reported [Clarke et al., 1991; Clarke et al., 1992]. Birot et al. [1996] report a male patient who carries a 5 Mb deletion that results in the loss of *FMR1* and the *IDS* gene. This mutation is also carried by his slightly affected mother and his severely affected sister. The brother, who is affected with Hunter syndrome, was not reported to have seizures, whereas his sister, who was not affected with Hunter syndrome, was reported to have significant mental retardation and had seizures since the age of four. The mother was reported to have highly skewed X inactivation in her lymphocytes, whereas the mentally retarded sister with seizures had random X inactivation in her lymphocytes. A female with moderate myotubular myopathy and mental retardation with a *FMR1* and *FMR2* de novo deletion was reported by Dahl et al. [1995]. They characterize a ~600 kb deletion that removes the *FMR1*, *FMR2*, *IDS*, and *MTM1* genes. X inactivation studies indicate the deleted X chromosome is inactive in only 20% of her lymphocytes (i.e., 80% of her active X chromosomes carry the deletion). Seizures were not noted for this patient. Recently, a female with a ~2.7 Mb deletion was described by Probst et al. [2007]. The deletion identified in this patient by chromosomal microarray analysis was found to span *FMR1*, *FMR2*, and the *IDS* genes. This patient had overgrowth, macrocephaly, and marked developmental delay. Interestingly, the 12-year-old sister of the patient, who did not carry the deletion and had normal intelligence, also had an overgrowth phenotype, suggesting in this family the overgrowth is unrelated to the X chromosome deletion.

Altogether, 12 individuals were reported to carry deletions encompassing both *FMR1* and *FMR2*, 4 males and 8 females. Of the four males, two patients had seizures and two patients, one with Hunter syndrome and one with mosaicism for the deletion, did not have seizures. Of the eight females, two had seizures, and six did not have seizures. Interestingly, a patient with seizures described by Hirst et al. [1995] has a small deletion restricted to the 5' end of *FMR1*. These reports illustrate there is not a clear genotype/phenotype correlation for manifestation of seizures in patients with mutations in *FMR1* and *FMR2*.

In addition to Hunter syndrome and myotubular myopathy, other phenotypic abnormalities noted in patients with contiguous gene deletions that include *FMR1* are obesity [Hirst et al., 1995; Quan et al., 1995b], cherubism [Quan et al., 1995a], overgrowth [Wolff et al., 1997; Parvari et al., 1999], growth retardation [Wolff et al., 1997], and macrocephaly [Meijer et al., 1994; Moore et al., 1999]. With the possible exception of obesity and macrocephaly, which both have been seen in both small deletion and large deletion patients, these features are isolated and may be unrelated to the deletion. Obesity and a Prader-Willi like phenotype has been described in a subset of fragile X patients with the CGG expansion mutation [de Vries et al., 1993], indicating the obesity noted for these deletion patients is probably due to loss of *FMR1* function.

Mosaic Deletion of *FMR1*

The patient we present has mild mental retardation, moderate to severe social anxiety and attention deficit/hyperactivity disorder-like symptoms. Importantly, this patient does not have all the facial findings distinctive of fragile X syndrome. This is, to our knowledge, the first report of a large deletion resulting in a milder phenotype. His mild phenotype is probably due to the mosaicism and that only *FMR1* and *FMR1NB*, and no adjacent genes such as *FMR2*, are affected. PCR and quantitative Southern analyses indicates that 90% of his lymphocytes carry the deletion. Thus, in terms of *FMR1* expression, this patient is more similar to mosaic *FMR1* expansion mutations found in higher-functioning fragile X males. Since the deletion is mosaic it must have occurred post-zygotically, suggesting that other tissues may carry higher or lower levels of the deletion. Testing of the patient's mother demonstrated that she carries a 23 and a 30 CCG repeat allele. Since we detected faint signal of the 23 CCG repeat allele by PCR, these results suggest that this patient inherited this allele from his mother. Therefore,

these data indicate that the deletion occurred on a chromosome with a normal size CGG repeat and is not associated with instability of premutation size CGG repeat.

The deletion removes 1,013,395 bp of sequence and deletes the entire *FMR1* gene, as well as the neighboring *FMR1NB* gene (Fig. 3A,B). Other genes distal to *FMR1NB*, such as *FMR2*, remain intact. Interestingly, both the 5' and 3' breakpoints of the deletion are located within LINE1 elements, suggesting that this deletion may be due to ectopic recombination between these elements. The break occurs within a 4 bp region (AATG) of microhomology between the proximal and distal LINE1 elements (Fig. 4). Analysis of the entire euchromatic sequence of the X chromosome indicates that LINE1 elements are enriched on this chromosome, covering 29% of the chromosome as compared to 17% in autosomes [Ross et al., 2005]. The enrichment of LINE1 elements on the X chromosome suggests that ectopic recombination between these elements may occur more frequently on the X chromosome.

Importantly, this case illustrates large mosaic deletions involving *FMR1* could potentially be missed by routine clinical testing. This patient has a more subtle presentation of fragile X syndrome and individuals like this patient, who have mosaicism for deletion of *FMR1*, may go undetected by routine fragile X clinical testing. In fact, in the case of this patient, the association of mild mental retardation with marked social anxiety in a young male with mild facial dysmorphism led to the persistent search for a *FMR1* mutation. Therefore, this case also demonstrates deletion/duplication analysis (as well as DNA sequencing) of *FMR1* is warranted in patients with a fragile X phenotype and also in patients with mild mental retardation and behavioral abnormalities characteristic of fragile X syndrome but not the classic fragile X appearance.

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Fig. 1. Photograph of the patient with mosaic deletion of *FMRI*. He shows some (e.g., prominent jaw, thickening of nasal bridge) but not all (e.g., normal size ears) of the distinctive facial features of fragile X syndrome.

Fig. 2. Clinical laboratory findings by standard fragile X testing. **A:** PCR amplification of the *FMR1* CGG repeat of DNA isolated from the patient and a normal male with 23 CGG repeats. **B:** Southern analysis of DNA isolated from this patient with atypical fragile X syndrome. **Lane 1**, normal female; **lane 2**, the patient being tested; **lane 3**, normal male; **lane 4**, male with typical CGG expansion and methylation; **lane 5**, molecular weight marker.

Fig. 3. High-density X chromosome array analysis of the patient and sequencing of the junction fragment. **A:** View of entire X chromosome. Arrow indicates deleted region. **B:** View of X chromosome from coordinates 145,400,000 to 147,800,000. The top track graphs individual probes while the bottom track depicts 15,000 bp windows of average signal. Deleted region can be seen as reduced signal extending from 146,047,696 to 147,061,090.

Fig. 4. Sequence of the junction fragment (bottom portion of panel) and corresponding breakpoints in the Ensembl genome browser (top portion of the panel). The proximal break is 146,047,696 and the distal breakpoint is 147,061,090 resulting in the deletion of 1,013,395 nucleotides. The 4 bp AATG homologous sequence between the two LINE1 elements is indicated by the yellow boxed sequence.

TABLE I
Small Deletions of *FMR1*

Refs.	Description	5' Break (bp 5' to CGG)	3' Break (bp 3' to CGG)
Small deletions in <i>FMR1</i> (<10 bp) that do not involve CGG repeat instability			
Lugenbeel et al. [1995]	Male with typical features of fragile X syndrome with a single de novo nucleotide deletion in exon 5 of <i>FMR1</i>	N/A	N/A
Hegde et al. [2001]	Male with developmental delay with a 5 bp deletion near the translation initiation codon of <i>FMR1</i> . Normal amount of FMRP produced in lymphocytes, excluding fragile X syndrome	N/A	N/A
Small deletions (10–10,000 bp) due to CGG repeat instability			
Meijer et al. [1994]	Family with 11 individuals with a deletion extending 1.6 kb proximal of the CGG repeat to the repeat tract removing the promoter. Four males and two carrier females show characteristics of fragile X syndrome	~1,600 bp	Within the CGG repeat
van den Ouweland et al. [1994]	Male with fragile X syndrome with deletion (contraction) entirely within the CGG repeat	Within the CGG repeat	Within the CGG repeat
Hirst et al. [1995]	Patient 1: Male with learning disabilities, aggressive behavior, obesity, and seizures mosaic for normal allele and a 660 bp deletion	594 bp	25 bp
Quan et al. [1995a]	Male with MR and cherubism mosaic for an 8.7 kb deletion	85 bp	8,700 bp (intron 1)
de Graaff et al. [1995]	Four unrelated patients with fragile X syndrome who are mosaic for full mutations and small deletions	Pt.1 53 bp Pt.2 85 bp Pt.3 74 bp Pt.4 74 bp	178 bp 25 bp 444 bp 24 bp
Mannermaa et al. [1996]	Male with fragile X syndrome and obesity mosaic for full mutation and a deletion. FMRP was not analyzed	31 bp	61 bp
de Graaff et al. [1996]	Male with fragile X syndrome with moderate mental retardation mosaic for full mutation and a deletion. Twenty-eight percent of his lymphocytes carry the deletion and produce <i>FMR1</i> transcript and protein indicating regulatory elements are intact	Within the CGG repeat	30 bp
Schmucker et al. [1996]	Male with fragile X syndrome mosaic for full mutation and deletion	168 bp	256 bp; (intron 1)
Mila et al. [1996]	Patient 1: Male with fragile X syndrome mosaic for a full mutation and a deletion	113 bp	Within the CGG repeat
	Patient 2: Mosaic male with a full mutation and a deletion entirely within the CGG repeat	Within the CGG repeat	Within the CGG repeat
Hammond et al. [1997]	Male with fragile X syndrome with a ~300 bp deletion	~300 bp	Within the CGG repeat
Gronskov et al. [1997]	Phenotypically normal female carrying a large deletion (Xq24-qter) on one X chromosome and a 144 bp deletion. Normal levels of FMRP were detected	63–67 bp	30–34 bp
Loesch et al. [1997]	Contraction of premutation to normal size allele in 9 different premutation mother-child transmissions	Within the CGG repeat	Within the CGG repeat
Orrico et al. [1998]	Male with severe MR who is mosaic for full mutation and contracted 7 CGG repeat allele	Within the CGG repeat	Within the CGG repeat
Petek et al. [1999]	Male with mental retardation and typical fragile X facial appearance, without macroorchidism or large ears, mosaic for full mutation, premutation, and a 215 bp deletion	76 bp	91 bp
Grasso et al. [1999]	8 unrelated patients with fragile X syndrome mosaic for full mutations and deletions		
	Patients 1–4: all deletions entirely within CGG repeat	Within the CGG repeat	Within the CGG repeat
	Patient 5: 157 bp deletion with a 7 bp insertion	84 bp	24 bp

Refs.	Description	5' Break (bp 5' to CGG)	3' Break (bp 3' to CGG)
	Patient 6: 352 bp deletion	106–110 bp	166–170 bp
	Patient 7: ~400 bp deletion within 5.2 kb EcoR1 fragment	Not mapped	Not mapped
	Patient 8: Deletion of the 5' end of 5.2 kb EcoR1 fragment	Not mapped	Not mapped
Schmucker and Seidel [1999]	Two males with fragile X syndrome who are mosaic for normal, premutation, and full mutation alleles	Within the CGG repeat	Within the CGG repeat
Garcia Arocena et al. [2000]	Male with fragile X syndrome mosaic for a full mutation and a deletion	438 bp	420 bp
Gasteiger et al. [2003]	Normal female with contraction of permutation to 10 CGG repeats	Within the CGG repeat	Within the CGG repeat
Fan et al. [2005]	Female with fragile X syndrome mosaic for a full mutation and 210 bp deletion	63–65 bp	86–88 bp
Han et al. [2006]	Mildly affected male with full, premutation, and deletion	42 bp	1 bp

TABLE II

Large deletions of *FMR1*

Refs.	Description
Schmidt et al. [1990]	Female with mental retardation with a ~10 Mb deletion at Xq27.1q27.3
Clarke et al. [1992]	Female with Hunter syndrome with a 3–5 cM deletion encompassing <i>FMR1</i> and <i>IDS</i> genes (as well as <i>FMR2</i>)
Gedeon et al. [1992]	Male with typical features of fragile X syndrome carrying a deletion removing <i>FMR1</i> and ~2.5 Mb of flanking sequences. Deletion probably does not include <i>FMR2</i>
Wohrle et al. [1992]	Male with fragile X syndrome with a deletion of less than 250 kb extending from sequence proximal to <i>FMR1</i> to within the gene, removing at least 5 exons of <i>FMR1</i>
Tarleton et al. [1993]	Male with fragile X syndrome with a 3 Mb deletion at Xq27-q28
Trottier et al. [1994]	Male with fragile X syndrome with ~130 kb deletion extending from 70 to 100 kb upstream of the promoter to within <i>FMR1</i>
Gu et al. [1994]	Male with fragile X syndrome with a <100 kb deletion extending from 80 kb upstream of the promoter to intron 7 of <i>FMR1</i>
Hirst et al. [1995]	Patient 2: Male with moderate mental retardation and behavior difficulties carrying a deletion extending from a region proximal to the promoter of <i>FMR1</i> to 25 kb within <i>FMR1</i> (intron 10)
Quan et al. [1995b]	Male with obesity, anal atresia and mental retardation with 9–11 Mb deletion extending 9 Mb proximal to 160–500 kb distal of <i>FMR1</i>
Dahl et al. [1995]	Female with myotubular myopathy and mental retardation carrying a 600 kb deletion encompassing <i>FMR1</i> , <i>FMR2</i> , <i>IDS</i> , and <i>MTM1</i> . Eighty percent of the patient's active X chromosomes in lymphocytes carry the deletion
Biro et al. [1996]	Male with Hunter syndrome carrying a deletion that encompasses both <i>FMR1</i> and <i>IDS</i> (as well as <i>FMR2</i>). Two female relatives carry the same deletion with mild to moderate developmental delay correlating with skewing of X inactivation in lymphocytes
Wolff et al. [1997]	Two unrelated patients with 13 Mb and 12 Mb deletions: Patient 1: Male with severe mental retardation and seizures carrying a 13 Mb deletion encompassing <i>FMR1</i> , <i>FMR2</i> , and <i>Sox3</i> . The same deletion is found in the phenotypically normal carrier mother and in sister with learning difficulties. Skewing of X inactivation in lymphocytes correlates with mental retardation in the mother and sister. Patient 2: Dysmorphic female with moderate to severe mental retardation, seizures, and hypothyroidism carrying a 12 Mb deletion of a similar region of the X chromosome. Deletion is on the inactive X in 50% of lymphocytes
Moore et al. [1999]	Male with severe mental retardation and seizures carrying a 6.5 Mb deletion encompassing <i>FMR1</i> and <i>FMR2</i>
Parvari et al. [1999]	Male with severe mental retardation and overgrowth carrying an 8.5 Mb deletion of <i>FMR1</i> and proximal genes (<i>FMR2</i> is not deleted). The deletion also found in his mother who is mildly delayed but of normal size
Fengler et al. [2002]	Male with severe mental retardation carrying a mosaic deletion of at least 900 kb encompassing <i>FMR1</i> and <i>FMR2</i> . Mother is a premutation carrier
Probst et al. [2007]	Female with severe developmental delay, obesity, and happy demeanor with a ~2.7 Mb deletion encompassing <i>FMR1</i> , <i>FMR2</i> , and <i>IDS</i>