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 phosphoimager 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 FMR1 and FMR1NB 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 TGAAAAACTGGAAGAAATCCAA (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 FMR1 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 FMR1 in a fragile X male [Luganbeel 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 FMR1 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 FMR1, 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 FMR1. 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 intact. Both FMR1 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 FMR1 gene. On the other X chromosome, she carries a small deletion at the 5’ end of FMR1 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 FMR1. A third case of a deletion extending into the CGG repeat flanking sequence, but not disrupting expression of FMR1, was reported by Han et al. [2006]. They report a highfunctioning 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 FMR1

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 FMR1 is relatively devoid of genes with the closest being SLITRK2 located 2,100 kb proximal to
The region distal to FMR1 is relatively more gene-rich with AFF2 (the FMR2 gene) being the closest gene of known function. There is ~550 kb of sequence between the 3′ end of FMR1 and the 5′ end of AFF2. Many of the patients carrying large deletions spanning FMR1 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 FMR1 and FMR2. The mother of this patient was found to carry a premutation FMR1 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 FMR1 or FMR2 had no detectable signal, indicating the deletion spanned at least 900 kb. They were able to PCR amplify segments of FMR1, 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, FMR1 is silenced due to expansion of the CGG repeat to a full mutation. They suggest that there is total loss of FMR1 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 FMR2 due to the mosaic deletion, although neither FMR1 nor FMR2 expression studies were done. Consistent with other patients that carry deletions that encompass FMR1 and FMR2, this patient has severe mental retardation.

There are other reports of deletions known to encompass both FMR1 and FMR2 [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 FMR1, FMR2, 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 FMR1 and FMR2 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 FMR1 and FMR2 deletions, two males and one female, were all reported to have seizures, leading to the suggestion that absence of both FMR1 and FMR2 can cause epileptic activity [Moore et al., 1999].

However, there are several other reports describing patients carrying deletions removing both FMR1 and FMR2, who do not have seizures. There are two earlier reports of patients with deletions encompassing both FMR1 and IDS who were affected with Hunter syndrome [Clarke et al., 1992; Birot et al., 1996]. FMR2 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 FMR1 and IDS in a female with Hunter syndrome.
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 \textit{FMR1} and the \textit{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 \textit{FMR1} and \textit{FMR2} de novo deletion was reported by Dahl et al. [1995]. They characterize a ~600 kb deletion that removes the \textit{FMR1}, \textit{FMR2}, \textit{IDS}, and \textit{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 \textit{FMR1}, \textit{FMR2}, and the \textit{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 \textit{FMR1} and \textit{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 \textit{FMR1}. These reports illustrate there is not a clear genotype/phenotype correlation for manifestation of seizures in patients with mutations in \textit{FMR1} and \textit{FMR2}.

In addition to Hunter syndrome and myotubular myopathy, other phenotypic abnormalities noted in patients with contiguous gene deletions that include \textit{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 \textit{FMR1} function.

**Mosaic Deletion of \textit{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 \textit{FMR1} and \textit{FMR1NB}, and no adjacent genes such as \textit{FMR2}, are affected. PCR and quantitative Southern analyses indicates that 90% of his lymphocytes carry the deletion. Thus, in terms of \textit{FMR1} expression, this patient is more similar to mosaic \textit{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 CGG repeat allele. Since we detected faint signal of the 23 CGG 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.

Acknowledgments

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References


Fig. 1.
Photograph of the patient with mosaic deletion of FMR1. 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 \textit{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. \textbf{Lane 1}, normal female; \textbf{lane 2}, the patient being tested; \textbf{lane 3}, normal male; \textbf{lane 4}, male with typical CGG expansion and methylation; \textbf{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**

<table>
<thead>
<tr>
<th>Refs.</th>
<th>Description</th>
<th>5’ Break (bp 5’ to CGG)</th>
<th>3’ Break (bp 3’ to CGG)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Small deletions in FMR1 (&lt;10 bp) that do not involve CGG repeat instability</strong></td>
<td></td>
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</tr>
<tr>
<td>Lugenbeel et al. [1995]</td>
<td>Male with typical features of fragile X syndrome with a single de novo nucleotide deletion in exon 5 of FMR1</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Hegde et al. [2001]</td>
<td>Male with developmental delay with a 5 bp deletion near the translation initiation codon of FMR1. Normal amount of FMRP produced in lymphocytes, excluding fragile X syndrome</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Small deletions (10–10,000 bp) due to CGG repeat instability</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meijer et al. [1994]</td>
<td>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</td>
<td>~1,600 bp</td>
<td>Within the CGG repeat</td>
</tr>
<tr>
<td>van den Ouweland et al. [1994]</td>
<td>Male with fragile X syndrome with deletion (contraction) entirely within the CGG repeat</td>
<td>Within the CGG repeat</td>
<td>Within the CGG repeat</td>
</tr>
<tr>
<td>Hirst et al. [1995]</td>
<td>Patient 1: Male with learning disabilities, aggressive behavior, obesity, and seizures mosaic for normal allele and a 660 bp deletion</td>
<td>594 bp</td>
<td>25 bp</td>
</tr>
<tr>
<td>Quan et al. [1995a]</td>
<td>Male with MR and cherubism mosaic for an 8.7 kb deletion</td>
<td>85 bp</td>
<td>8,700 bp (intron 1)</td>
</tr>
<tr>
<td>de Graaff et al. [1995]</td>
<td>Four unrelated patients with fragile X syndrome who are mosaic for full mutations and small deletions</td>
<td>Pt. 1.53 bp</td>
<td>178 bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pt. 2.85 bp</td>
<td>25 bp</td>
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<td></td>
<td>Pt. 3.74 bp</td>
<td>444 bp</td>
</tr>
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<td></td>
<td>Pt. 4.74 bp</td>
<td>24 bp</td>
</tr>
<tr>
<td>Mannermaa et al. [1996]</td>
<td>Male with fragile X syndrome and obesity mosaic for full mutation and a deletion. FMRP was not analyzed</td>
<td>31 bp</td>
<td>61 bp</td>
</tr>
<tr>
<td>de Graaff et al. [1996]</td>
<td>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 FMR1 transcript and protein indicating regulatory elements are intact</td>
<td>Within the CGG repeat</td>
<td>30 bp</td>
</tr>
<tr>
<td>Schmucker et al. [1996]</td>
<td>Male with fragile X syndrome mosaic for full mutation and deletion</td>
<td>168 bp</td>
<td>256 bp; (intron 1)</td>
</tr>
<tr>
<td>Mila et al. [1996]</td>
<td>Patient 1: Male with fragile X syndrome mosaic for a full mutation and a deletion</td>
<td>113 bp</td>
<td>Within the CGG repeat</td>
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<tr>
<td></td>
<td>Patient 2: Mosaic male with a full mutation and a deletion entirely within the CGG repeat</td>
<td>Within the CGG repeat</td>
<td>Within the CGG repeat</td>
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<tr>
<td>Hammond et al. [1997]</td>
<td>Male with fragile X syndrome with a ~300 bp deletion</td>
<td>~300 bp</td>
<td>Within the CGG repeat</td>
</tr>
<tr>
<td>Gronskov et al. [1997]</td>
<td>Phenotypically normal female carrying a large deletion (Xq24-qter) on one X chromosome and a 144 bp deletion. Normal levels of FMRP were detected</td>
<td>63–67 bp</td>
<td>30–34 bp</td>
</tr>
<tr>
<td>Loesch et al. [1997]</td>
<td>Contraction of premutation to normal size allele in 9 different premutation mother-child transmissions</td>
<td>Within the CGG repeat</td>
<td>Within the CGG repeat</td>
</tr>
<tr>
<td>Orrico et al. [1998]</td>
<td>Male with severe MR who is mosaic for full mutation and contracted 7 CGG repeat allele</td>
<td>Within the CGG repeat</td>
<td>Within the CGG repeat</td>
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<tr>
<td>Petek et al. [1999]</td>
<td>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</td>
<td>76 bp</td>
<td>91 bp</td>
</tr>
<tr>
<td>Grasso et al. [1999]</td>
<td>8 unrelated patients with fragile X syndrome mosaic for full mutations and deletions</td>
<td>Within the CGG repeat</td>
<td>Within the CGG repeat</td>
</tr>
<tr>
<td></td>
<td>Patients 1–4: all deletions entirely within CGG repeat</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patient 5: 157 bp deletion with a 7 bp insertion</td>
<td>84 bp</td>
<td>24 bp</td>
</tr>
</tbody>
</table>

*Am J Med Genet A. Author manuscript; available in PMC 2009 June 17.*
<table>
<thead>
<tr>
<th>Refs.</th>
<th>Description</th>
<th>5’ Break (bp 5’ to CGG)</th>
<th>3’ Break (bp 3’ to CGG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schmucker and Seidel [1999]</td>
<td>Two males with fragile X syndrome who are mosaic for normal, premutation, and full mutation alleles</td>
<td>Within the CGG repeat</td>
<td>Within the CGG repeat</td>
</tr>
<tr>
<td>Garcia Arocena et al. [2000]</td>
<td>Male with fragile X syndrome mosaic for a full mutation and a deletion</td>
<td>438 bp</td>
<td>420 bp</td>
</tr>
<tr>
<td>Gasteiger et al. [2003]</td>
<td>Normal female with contraction of permutation to 10 CGG repeats</td>
<td>Within the CGG repeat</td>
<td>Within the CGG repeat</td>
</tr>
<tr>
<td>Fan et al. [2005]</td>
<td>Female with fragile X syndrome mosaic for a full mutation and 210 bp deletion</td>
<td>63–65 bp</td>
<td>86–88 bp</td>
</tr>
<tr>
<td>Han et al. [2006]</td>
<td>Mildly affected male with full, premutation, and deletion</td>
<td>42 bp</td>
<td>1 bp</td>
</tr>
</tbody>
</table>
### TABLE II

**Large deletions of FMR1**

<table>
<thead>
<tr>
<th>Refs.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schmidt et al. [1990]</td>
<td>Female with mental retardation with a ~10 Mb deletion at Xq27.1q27.3</td>
</tr>
<tr>
<td>Clarke et al. [1992]</td>
<td>Female with Hunter syndrome with a 3–5 cM deletion encompassing FMR1 and IDS genes (as well as FMR2)</td>
</tr>
<tr>
<td>Gedeon et al. [1992]</td>
<td>Male with typical features of fragile X syndrome carrying a deletion removing FMR1 and ~2.5 Mb of flanking sequences. Deletion probably does not include FMR2</td>
</tr>
<tr>
<td>Wohrle et al. [1992]</td>
<td>Male with fragile X syndrome with a deletion of less than 250 kb extending from sequence proximal to FMR1 to within the gene, removing at least 5 exons of FMR1</td>
</tr>
</tbody>
</table>