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Maternal *FMR1* Premutation Allele Expansion and Contraction in Fraternal Twins

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Fragile X syndrome results from an expansion of the CGG trinucleotide repeat in the 5′ untranslated region of the Fragile X Mental Retardation 1 (*FMR1*) gene. Expansion of a maternal premutation allele is the mechanism by which a full mutation allele arises; contraction of a maternal premutation allele is rare. Here we report on both an expansion and contraction of a maternal *FMR1* premutation allele in fraternal twins. The propositus was the product of a 29-week gestation twin pregnancy and was referred for *FMR1* testing due to developmental delay. A *FMR1* full mutation with complete methylation was observed on Southern blot analysis. Evaluation of the maternal *FMR1* gene by PCR revealed a normal and premutation allele with CGG repeat numbers of 30 and 93, respectively. Subsequent *FMR1* testing on the twin sister of the propositus detected CGG repeat numbers of 30 and 54. The *FMR1* CGG repeat number of the reproductive partner was 30. The *FMR1* CGG repeat 30 allele in the twin sister was determined to be of paternal origin and the *FMR1* allele with a CGG repeat number of 54 was of maternal origin. This observation is particularly interesting not only because of the concomitant donation of a *FMR1* expanded and contracted premutation allele in a twin pregnancy but also because of the significant degree of contraction (39 repeats) of the maternal premutation allele. © 2013 Wiley Periodicals, Inc.

Key words: Fragile X syndrome; trinucleotide repeats; premutation contraction

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INTRODUCTION

Fragile X syndrome is one of the most commonly inherited forms of intellectual disability with an estimated incidence of 1 in 4,000 males and 1 in 4,000–8,000 females [Maddalena et al., 2001]. The condition results from an expansion of the CGG trinucleotide repeat in the 5′ untranslated region of the Fragile X Mental Retardation 1 (*FMR1*) gene on Xq27.3 which results in hypermethylation of *FMR1* and transcriptional silencing of the gene [Fu et al., 1991; Verkerk et al., 1993]. This 5′ untranslated region is polymorphic with normal alleles ranging from 5–44 repeats, gray zone alleles containing between 45 and 54 repeats, premutation alleles from 55–200 repeats, and full mutation alleles containing 200 repeats or more [Maddalena et al., 2001].

Expansion to more than 200 repeats results in hypermethylation and subsequent silencing of the *FMR1* gene product [Sutcliffe et al., 1992]. The loss of the fragile X mental retardation protein (FMRP) is responsible for Fragile X syndrome [Pieretti et al., 1991]. Fragile X syndrome comprises severe mental disability, developmental delay, autistic-like manifestations and hyperactivity, elongated facies and large testicles, among others [Sherman, 1991; Warren, 1997]. In contrast, high levels of *FMR1* transcript with preserved levels of FMRP are found in premutation allele carriers [Kenneson et al., 2001]. It is thought that transcript toxicity and the sequestration of proteins by FMR1 mRNA binding may be the cause of late onset fragile X-associated tremor ataxia syndrome (FXTAS) and fragile X-associated premature ovarian insufficiency (FXPOI) observed in some premutation carriers [Marozzi et al., 2000; Jacquemont et al., 2003; Handa et al., 2005; Hagerman, 2012].

Alleles with CGG repeats within the normal range are stably transmitted; gray zone or premutation alleles can increase in size from generation to generation [Snow et al., 1993; Nolin et al., 2003]. Repeat expansion is thought to occur through either the incorporation of looped DNA intermediates on the nascent lagging strand, on the template of the lagging strand, or during replication fork stalling and restarting [Mirkin, 2006]. More recently though, evidence suggests that large expansions may result from loop formation arising from repair of single-strand breaks during exci-

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sion of damaged DNA bases; small changes in repeat sizes may occur due to DNA polymerase slippage during replication [McMurray, 2010].

MATERIALS AND METHODS

Patient Samples

Genomic DNA was extracted from peripheral blood specimens using the QIAGEN Puregene DNA extraction reagents or Qiagen BioRobot EZ1 and the corresponding DNA blood kit (QIAGEN, Inc, Germantown, MD).

CGG Repeat Quantification of the FMR1 Gene

DNA was analyzed using the AmplideX^{TM} *FMR1* PCR kit by Asuragen Inc. (#46082 Austin, TX) according to the manufacturer's instructions. Briefly, 20 ng of DNA was PCR amplified using a proprietary polymerase to amplify GC rich DNA sequences with 3 primers [Juusola et al., 2012]. A fluorescently tagged reverse primer amplified the CGG repeat containing region of the human *FMR1* when paired with the forward primer. A third primer specific for CGG repeat sequences coupled with the reverse fluorescent primer provided amplification of "stutter" peaks. The assay enables the precise determination of CGG repeats and determines the presence of AGG interruptions due to the inability of the 15-mer CGG repeat primer to anneal to AGG sequences. The PCR products were subjected to capillary electrophoresis using a Pop-7 matrix on an Applied Biosystems 3130*xl* Genetic Analyzer for detection and analyzed using GeneMapper v4.1 software (Life Technologies, Carlsbad, CA).

Southern Blot Analysis

Genomic DNA was digested with restriction endonuclease EcoRI and the methyl-sensitive enzyme EagI. Following electrophoresis on a 0.7% gel, the digested DNA was transferred by vacuum to a nylon membrane, and hybridized with a ^{32}P radiolabeled probe specific for the CGG trinucleotide repeat in exon 1 of *FMR1* [Hirst et al., 1991]. Bands were visualized by autoradiography following 3–7 days at −70°C.

DNA Fingerprint Analysis

The AmpFlSTR® Profiler Plus^{TM} ID PCR Amplification Kit (#4330284 Applied Biosystems, Carlsbad, CA) allows for amplification of 9 polymorphic STR loci and 1 gender specific locus, Amelogenin. The fluorescently tagged PCR products were subjected to capillary electrophoresis using a Pop-7 matrix on an Applied Biosystems 3130*xl* Genetic Analyzer and analyzed using the GeneMapper v4.1 software.

RESULTS

The propositus was the product of a dizygotic twin pregnancy with a pre-term 29-week gestation presumably due to the fact that at least 60% of all twins are born before the 37th week of pregnancy and account for 15% of all preterm births in the United States. [Santolaya and Faro, 2012]. Bronchopulmonary dysplasia resolved by age 5 months. He was diagnosed with hypotonia at 24 months and with developmental delay/intellectual disability by age 32 months. His total language equivalent at this stage was comparable to that of a 21-month-old. A sensory integration disorder with symptoms including photosensitivity, gagging after certain odors, oral fixation, and facial tactile aversion was described. At this age, autism, Asperger, and attention deficit hyperactivity disorder were ruled out and the patient was referred for Fragile X DNA testing. Following confirmation of a fully methylated CGG repeat allele of >200 in the propositus by Southern blot analysis, peripheral blood samples of at-risk family members (including maternal aunt, and first cousin) were submitted for analysis.

The propositus had a full mutation allele with complete methylation due to the exclusive presence of a smear above 5.2 kb and the absence of any distinct band at 2.8 kb (Fig. 1). The mother and maternal aunt were premutation carriers and the twin sister was not affected but had a slightly expanded gray zone allele. The father and cousin's DNA demonstrated CGG repeats within the normal range.

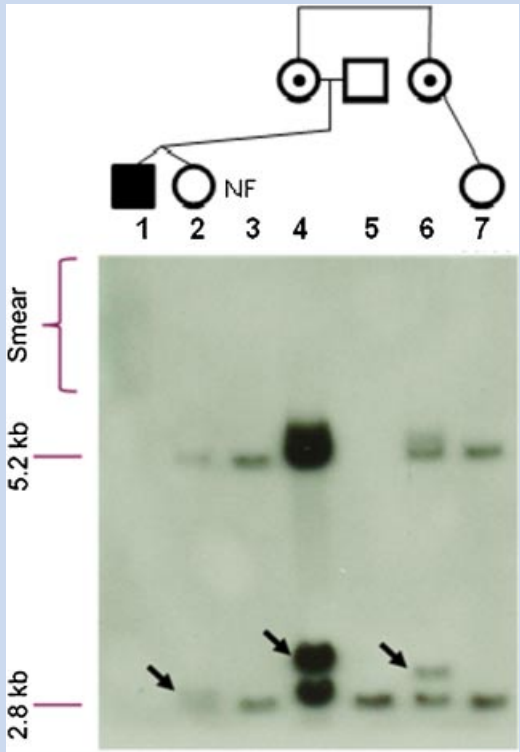

FIG. 1. Nuclear pedigree and Southern blot analysis of propositus and family. Digestion of genomic DNA with EcoRI and methyl-sensitive restriction endonuclease EagI, a full mutation with complete methylation (smear) is observed in the propositus. Gray zone and premutation alleles are seen (arrows) in the twin sister (54 repeats), mother (93 repeats), and maternal aunt (90 repeats). Normal alleles with CGG repeats of 30 are approximately 2.8 kb. Lane #1. Propositus, 2. Sister, 3. Normal female control, 4. Mother, 5. Father, 6. Aunt, 7. Cousin.

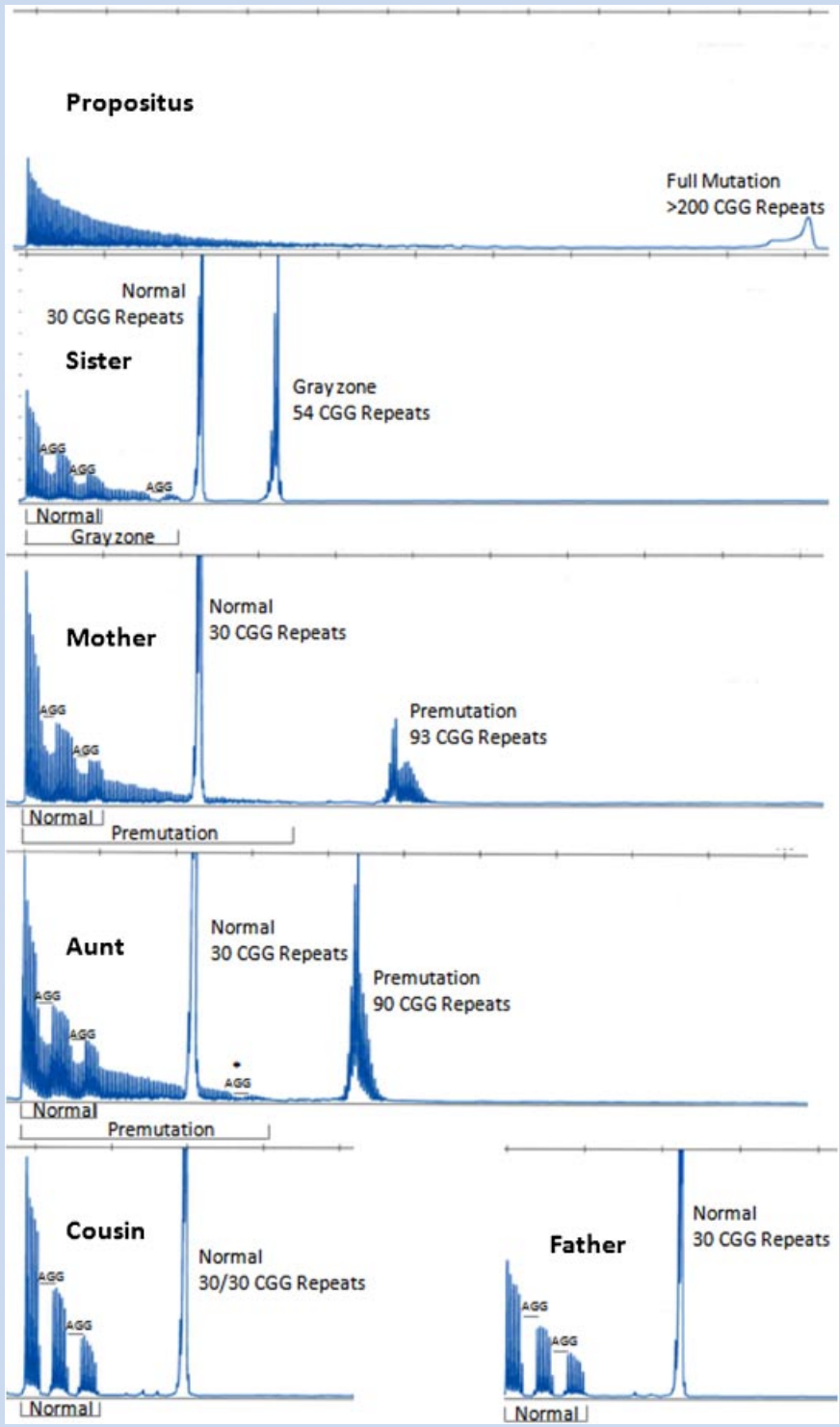

FIG. 2. Amplidex™ *FMR1* PCR Results. Electopherograms from the members of this family. This platform allows for the detection of the >200 CGG repeat allele in the propositus. The presence of two alleles were detected in the sister with CGG repeat lengths of 30 (normal) and of 54 (gray zone). The maternal *FMR1* gene had two alleles with CGG repeat lengths of 30 (normal) and of 93 (premutation). The aunt had two alleles with CGG repeat lengths of 30 (normal) and 90 (premutation). The cousin was homozygous for *FMR1* CGG repeats of 30 (normal). The *FMR1* CGG repeat in the paternal sample is 30 (normal). AGG interruptions are marked; potential AGG interruption in the aunt is noted with an asterisk. No interruptions are detected in the full mutation allele of the propositus.

PCR was used to assess the precise CGG repeat number in each family member using the Amplidex^{TM} *FMR1* PCR kit (Fig. 2). The full mutation of the propositus was approximately 270 CGG repeats. The mother had both a *FMR1* normal and premutation allele corresponding to 30 and 93 CGG repeats, respectively. Interestingly, the twin sister's *FMR1* CGG repeat numbers were 30 and 54, suggesting a contraction of the maternal premutation allele. The maternal aunt had a normal and premutation allele corresponding to 30 and 90 CGG repeats; the paternal allele had 30 CGG repeats and the first cousin was homozygous for 30 *FMR1* CGG repeats.

The Asuragen Amplidex^{TM} technology also queries the quantity and position of the AGG interruptions within the CGG repeats [Yrigollen et al., 2012]. By looking closer at the products from the stutter peaks created, one can infer the composition of the allele. As expected, the full mutation allele of the propositus contains no AGG interruptions as can be appreciated by a full array of continuous stutter peaks. The sister, the mother, and the aunt all have two AGG interruptions in their normal allele. The mother's premutation allele has no appreciable AGG interruptions although the propositus' sister contracted, gray zone allele appears to have one AGG interruption. Further, the aunt's premutation allele appears to contain one AGG interruption detected between the normal and premutation peak. The father and cousin's 30 CGG repeat alleles have AGG interruptions at identical locations.

The derivation of the twin sister's *FMR1* alleles of 30 and 54 was investigated. In one scenario, the mother may have donated the 54 allele from a contracted *FMR1* premutation allele with the 30 allele of paternal origin. Alternatively, the 30 allele could have been of maternal origin with the 54 allele derived from alternative paternity. Lastly, a laboratory error could have occurred. To verify the origin of each *FMR1* allele and ensure no laboratory error, DNA fingerprint studies were performed, and paternity was confirmed as stated (data not shown). These results confirmed a maternal contraction of the 93 *FMR1* CGG repeat premutation allele as the origin of the 54 *FMR1* CGG repeat seen in the twin sister.

DISCUSSION

Extensive follow-up on the family showed that the twin sister was diagnosed with cerebral palsy, a common complication in a twin pregnancy, and is the likely cause of her developmental delay [Santolaya and Faro, 2012]. Further investigation uncovered four relatives with intellectual disability, three males and one female. Six female family members were termed "slow," two "autistic" and two others with "anger issues." Although the diagnoses of these extended family members were not confirmed by us, the possibility of Fragile X syndrome or symptoms associated with expanded alleles are possible. This case depicts the utility of genetic testing on not just the nuclear family but also the extended family.

This case is an interesting one in that a maternal *FMR1* germline premutation allele of 93 CGG repeats expanded to a full mutation in the gamete she donated to one offspring yet significantly contracted (by 39 repeats) to a gray zone allele to a second offspring in a dizygotic twin pregnancy. To our knowledge there has only been one more similar case documented in the literature as identified in siblings from a twin pregnancy. Nolin et al. [2011] describe a cohort of Fragile X families one of which contained a maternal allele of 150 repeats which transmitted a contracted CGG 82 repeat allele to one offspring and a full mutation allele to a second offspring in a twin pregnancy.

While the maternal *FMR1* CGG repeat size is an important risk factor of expansion to a full mutation with the greater the number the greater the risk [Fu et al., 1991; Heitz et al., 1992; Vaisanen et al., 1994]; both *cis*- and *trans*-acting factors may contribute [McMurray et al., 2010]. *Cis*-acting factors including AGG repeats, which are normally separated by every 9–10 or so CGG repeats in normal sized alleles [Zhong et al., 1995], are diminished in number or absent from larger repeat tracts in the premutation and full mutation ranges [Snow et al., 1994; Ludwig et al., 2009]. These interruptions are known to reduce the risk of premutation instability and thus the risk of expansion to a full mutation [Eichler et al., 1994; Yrigollen et al., 2012]. The length of the uninterrupted 3′ end of the repeat is a risk factor such that the expansion rate increases with greater than 24 uninterrupted CGG repeats [Sullivan et al., 2002]. *Trans*-acting factors, whose effects on allele stability are not fully understood, include those involved in the repair of single-strand DNA breaks [Liu et al., 2009; McMurray, 2010]. Remarkably, some alleles are more prone to expand than others as is the rare case of expansion of a gray zone allele to a full mutation allele over two generations [Fernandez-Carvajal et al., 2009], while distinct haplotypes may explain premutation expansion in some populations [Eichler et al., 1996; Levesque et al., 2009].

The maternal premutation allele in our case does not have any detectable AGG interruptions. This fact increased the odds for this allele to expand to a full mutation as has been previously shown by Yrigollen et al. [2012]. However, the propositus' sister gray zone allele contains an AGG interruption. Since the maternal aunt has an AGG interruption in her premutation allele the possibility exists that (1) the mother also has an AGG repeat that is not detected and is thus retained in the contracted 54 allele inherited by her daughter; or (2), loop formation from repair of single-strand breaks during excision of damaged DNA bases occurred [McMurray, 2010], as did a contraction and an AGG sequence were acquired.

To put our results in perspective, significant contractions or reversions of a *FMR1* premutation or gray zone allele occur rarely and few have been documented. A literature search revealed only 37 reported cases of maternal allele contractions ranging from a decrease of 1–147 repeats [Fu et al., 1991; Zhong et al., 1993; Van Den Ouweland et al., 1994; Vits et al., 1994; Brown et al., 1996; Gasteiger et al., 2003; Nolin et al., 2003; Rife et al., 2004; Tabolacci et al., 2008; Nolin et al., 2011; Sorensen et al., 2013]. In approximately 28% of these cases a reversion to a normal allele was achieved. As is shown in Figure 3, the degree of maternal allele contraction appears to increase with increasing CGG repeat length. It is unclear how many contractions occur and remain unknown due to lack of ascertainment within the family. Few cases of paternal contractions are reported; three of these with a decrease from 42 to 40 (−2), from 65 to 54 (−11), and 130 to 34 (−96) CGG repeats [Vaisanen et al., 1994, 1996; Nolin et al., 2011]. Four additional cases of paternal contractions were documented in the cohort described by Rife et al. [2004].Interestingly, this group determined a statistical increase in the odds of contraction in paternal alleles rather than maternal alleles.

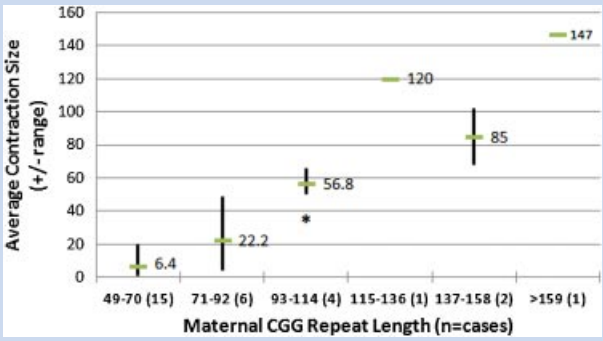

FIG. 3. Correlation between contraction size and maternal CGG repeat length. The average contraction size is listed next to the horizontal line for each maternal allele length group. The range of the contractions documented for each group is shown as vertical bars. The number of cases documented for each group appears in parenthesis. Although contraction of a premutation allele is rare, the documented cases suggest that the greater the CGG repeat length of the maternal allele, the greater the degree of contraction. The contraction of the case described in this document is depicted as an asterisk. Not included in this figure are the six contractions reported by Rife et al. [2004] because the authors did not disclose the CGG repeat numbers.

This report describes both the expansion and contraction of a maternal *FMR1* premutation allele with 93 CGG repeats to offspring in a dizygotic pregnancy. This case serves as a reminder that the factors associated with the likelihood of expansion or contraction of a premutation *FMR1* allele are not fully understood.

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