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Clinical phenotypes of a juvenile sibling pair carrying the fragile X premutation

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Abstract

Individuals with alleles containing 55–200 CGG repeats in the fragile X mental retardation (*FMR1*) gene are premutation carriers. The premutation allele has been shown to lead to a number of types of clinical involvement, including shyness, anxiety, social deficits, attention deficit hyperactivity disorder (ADHD) and executive function deficits. Some of these problems could be due to mild deficits of the fragile X protein (FMRP) and a possible developmental effect of the elevated *FMR1* mRNA observed in carriers. In addition, two abnormal phenotypes specific to the premutation have been described. Primary ovarian insufficiency (FXPOI), defined by cessation of menses prior to age 40, occurs in 20% of females with the premutation. The other phenotype, fragile X-associated tremor/ataxia syndrome (FXTAS), affects some older adult premutation carriers.

Premutation females typically have one expanded allele (> 55 CGG repeats) and one normal allele (< 54 CGG repeats). This study describes the cognitive, behavioral and molecular profile of a female with two alleles in the premutation range (60 and 67 CGG repeats) in comparison to her brother with a similar premutation size (65 CGG repeats). Both exhibited high IQ scores, anxiety, and some physical features associated with fragile X syndrome. This comparison allows us to examine the effect of the premutation in this male-female pair while controlling for environmental and background genetic factors.

Keywords

Compound premutation; FMR1; Fragile X

INTRODUCTION

Fragile X syndrome is characterized by an expansion of > 200 CGG repeats in the 5' untranslated region of the *FMR1* gene while the fragile X premutation is characterized by a CGG repeat expansion ranging from 55 to 200 [Maddalena, 2001]. Frequencies of the premutation allele in Canadian studies have been reported as approximately 1 in 250 for females and 1 in 800 for males [Rousseau et al., 1995; Dombrowski et al., 2002]. However,

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This work is dedicated to the memory of Matteo.

in other ethnic populations, this rate is higher; for instance, the prevalence of the premutation in females in Israel is closer to 1 in 113 and in Spain the premutation in males is 1 in 250 [Hagerman and Hagerman, 2002; Toledano-Alhadeff et al., 2001; Fernandez-Carvajal, 2009]. Although the premutation status was once believed to be free of clinical symptoms, findings over the past few years indicate that CGG-expansion in the premutation range is associated with a spectrum of clinical involvement including a neurodegenerative disorder known as fragile X-associated tremor ataxia syndrome (FXTAS) [Hagerman et al., 2001; Berry-Kravis et al., 2007] and fragile x-associated primary ovarian insufficiency (FXPOI) [Sherman, 2000; Wittenberger et al., 2007]. Although those with a premutation usually have normal intellectual abilities, reports have recorded intellectual disability (ID), attention deficit hyperactivity disorder (ADHD), anxiety and autism spectrum disorders in some children with premutations [Aziz et al., 2003; Cornish et al., 2005; Farzin et al., 2006; Goodlin-Jones et al., 2004; Moore et al., 2004] as well as anxiety and depression in adults [Roberts et al., 2009; Hagerman, 2006; Bourgeois, 2009].

It is uncommon for a female to present with a fragile X mutation on both of her X-chromosomes. This phenomenon occurs when both of her parents carry a premutation allele, which is more likely to occur in consanguineous unions. Only two other studies have reported on females with two premutation alleles. The most recent by Esch et al. [2009] reported FXPOI in two of three sisters derived from a consanguineous union. Another study of three sisters without consanguinity described the neuropsychological profiles of three women who all carried two premutation alleles. They reported no deficit in any of the sisters' measures of verbal performance or deficits in executive functions or in the visual spatial domain [Mazzocco and Holden, 1996]. Here, we describe a sibship of another biologically unrelated union: a female carrier of two premutation alleles and her brother who carries a single premutation allele and examine the differences between the two individuals. While both individuals have comparable CGG-repeat sizes in the premutation range and are similar in age, they present with different cognitive and behavioral profiles.

MATERIALS AND METHODS

Subjects

This family initially contacted the Fragile X Research and Treatment Center at the UC Davis MIND Institute in Sacramento (CA) with questions regarding assessment and treatment of the maternal grandfather's FXTAS symptoms. At the time of initial contact, none of the members within the immediate family unit, except for the mother, had been tested for the *FMR1* gene. Further diagnostic testing identified the male sibling as a premutation carrier and the female sibling as a carrier of two premutation alleles (thus, no normal allele was present). The female sibling's results indicated further testing of the paternal side of the family. After obtaining informed consent from all four family members, this family underwent cognitive and behavioral evaluations and detailed medical history. A protocol was approved by the Institutional Review Board at the University of California, Davis. The maternal grandfather, with a known diagnosis of FXTAS, was unable to be assessed due to the severity of his FXTAS symptoms and difficulties associated with traveling to our facility. While both parents were also assessed, we will focus mainly on describing the children's characteristics, since this unique male-female sibling pair offers an opportunity to compare the possible effect of two premutation alleles across gender under comparable age, repeat-size, environmental and background genetic conditions.

Molecular analysis

DNA was extracted from whole blood (3–5ml) using the Gentra Puregene Blood Kit by Qiagen (Valencia, Ca), following the standard protocol. Repeat sizes were determined using

PCR amplification and Southern blot analysis of the *FMR1* gene according to the conditions described in Tassone et al. [2008].

FMRP expression levels were measured using Western blot analysis according to conditions described in [Iwahashi, 2009], with some modifications. Proteins were extracted using MPER buffer (Pierce, Rockford, IL) with Complete (Roche, Nutley, NJ) protease inhibitors. FMRP expression level values were measured using Western Blot analysis. A mean was obtained for each sample from 4 independent runs. GAPDH was used for the normalization. Anti-FMRP antibodies (IC3) and anti-GAPDH antibodies were obtained from Millipore (Billerica, MA).

Quantification of *FMR1* mRNA expression levels was performed using 7900 sequence detector (Applied Biosystems). β -glucuronidase (GUS) was used as reference. Details are as in Tassone et al. [2000].

Cognitive and Behavioral Measures

To assess overall cognitive ability, the children were administered either the Wechsler Abbreviated Scale of Intelligence (WASI) [Wechsler, 1999] or Wechsler Intelligence Scale for Children-fourth edition (WISC-IV) [Wechsler, 2003]. Both measures were able to provide standardized Full Scale IQs (FSIQ). The WASI provided Verbal IQ (VIQ) and Performance IQ (PIQ) measures while the WISC-IV provided measures of verbal comprehension, perceptual reasoning, working memory and perceptual speed, respectively. Adaptive Behavior was assessed using the Vineland Adaptive Behavior Scale – Second Edition: Parent Survey Interview Form (VABS-II) [Sparrow et al., 2005]. VABS-II reported an overall Vineland Adaptive Behavior Composite (VABC) and measures of Communication, Daily Living Skills, and Socialization. Characteristics of autism were evaluated using the Autism Diagnostic Observation Schedule (ADOS) [Lord et al., 1999], the Social Responsiveness Scale (SRS) [Constantino and Gruber, 2005], and the Social Communication Questionnaire (SCQ) [Rutter et al., 2003]. Additional behavioral questionnaires included the Aberrant Behavior Scale (ABC) [Aman et al., 1985] and the Behavior Assessment System for Children—Second Edition: Parent Rating Scale (BASC-II: PRS) [Reynolds and Kamphaus, 2004]. The Short Sensory Profile (SSP) [Dunn, 1999] was also completed to explore sensory deficit. The Anxiety Disorder Interview Schedule for DSM-IV: Parent Report Version (ADIS-IV) [Silverman and Albano, 2004] was also administered for both children. This semi-structured parent interview based on the DSM-IV criteria assessed the presence, severity, and interference level of a variety of anxiety-related disorders. A diagnosis on the ADIS-IV meets criteria for a clinical diagnosis by DSM-IV criteria. The ADIS-IV was utilized in a recent study presenting evidence of a high prevalence of anxiety disorders in fragile X syndrome [Cordeiro et al., in press]. Finally, to address his ADHD, the male sibling was administered the Integrated Visual and Auditory Continuous Performance Task (IVA-CPT) [Sandford et al., 1995].

Additionally, both parents were administered the Wechsler Adult Scale of Intelligence – Fourth Edition (WAIS-IV) [Wechsler, 2008] which also reported FSIQ, VIQ and PIQ. Self-assessments including the Beck Anxiety Inventory (BAI) [Beck and Steer 1993] and Symptom Checklist-90-R (SCL-90-R) [Derogatis, 1992] assessed various symptoms, including anxiety. Both adults were also administered the Structured Clinical Interview – I (for DSM-IV) Disorders – Non-Patient Edition (SCID-I/NP) [First et al., 2002] to explore various clinical diagnoses and the IVA-CPT to assess ADHD.

Individual medical and neurological histories as well as family pedigree and brief family clinical history were also collected via parent report.

RESULTS

Female Proband

The female sibling was seven years, two months of age at the time of assessment. Her mother's pregnancy was normal with no complications at birth. Her developmental history was normal. Aside from 3 months of speech and language therapy to help with a lisp, she was not currently receiving speech and language or occupational therapies.

However, behaviorally she had a long history of shyness, poor eye contact and social anxiety. Though she spoke consistently in the home, she had selectively mute tendencies and would not speak in certain situations. She had a history of temper tantrums and excessive chewing on her hair. She exhibited some obsessive-compulsive tendencies including refusal to throw trash away from her room (plastic wrap, yogurt containers, lollipop sticks, and scraps of paper). Her mother reported that these behavioral problems had increased within the three months prior to her assessment. Aside from these behavioral characteristics, she did very well academically, was a creative artist and was interested in socializing with peers despite anxiety and shyness.

On physical examination she was selectively mute with poor eye contact. She had mild fragile X-associated physical characteristics including hyperextensible finger joints (metacarpophalangeal [MP] extension to 90 degrees), easily dislocated thumbs, a mildly broad forehead, and flat feet. In cognitive testing, the WASI reported a full scale IQ of 123, which is considered superior intellectual ability. There was notable split in her verbal and performance IQs: verbal IQ of 107, a performance IQ of 135. On the VABS-II, she scored 120 on communication, 105 on Daily Living Skills, and 106 on Socialization with an Adaptive Behavior Composite of 109. She scored within typical ranges on the ADOS, SRS and SCQ. While she also scored in the typical ranges on the behavioral questionnaires (ABC and BASC-II), items relating to irritability were slightly elevated. She also did not exhibit any sensory deficits on the SSP. On the ADIS, she met criteria for the diagnosis of social phobia and generalized anxiety disorder. This was consistent with our clinical evaluation and medical history, and it was verified by meeting DSM-IV criteria for these diagnoses.

On molecular studies, her Southern blot and PCR analysis confirmed presence of two premutation alleles of 60 and 67 CGG repeats in size. QRT-PCR analysis revealed her *FMR1* mRNA expression level was elevated (2.39 ± 0.14) compared to typical controls (mean = 1.26 ± 0.26), in agreement with previous reports [Allen et al., 2004; Kenneson et al., 2001; Tassone et al., 2000]. *FMRP* expression levels measured by Western blot analysis were within the normal range (Table I, Fig 1 and Fig 2).

Male Propositus

Her brother was eight years, 11 months of age at the time of assessment. His mother's pregnancy and his birth history were normal. While he met gross and fine motor developmental milestones appropriately, his language was delayed, saying words at 2.5 years and phrases at three years of age. As a result, he received speech and language therapy from two to four years of age. Additionally, due to sensory integration difficulties, he was diagnosed with Sensory Integration Dysfunction at 6 years of age and was treated with occupational therapy from grades 1 – 3.

Compared to his sister, he exhibited considerable behavioral deficits, especially with respect to attention and hyperactivity. Past psychoeducational reports provided by the family indicated that he was diagnosed with ADHD utilizing DSM-IV criteria. Like his sister, he chewed excessively on his sleeves, had occasional poor eye contact, intermittent aggression, tantrums, and anxiety on a regular basis. He also picked at scabs repetitively, had poor self-

modulation, and presented with controlling, competitive, and rigid behaviors. He too, did well in school, but teachers corroborated ADHD tendencies by reporting that he had difficulty focusing and sitting still in class. He complained daily about somatosensory symptoms, such as stomach aches and gastrointestinal problems.

Like his sister, his physical examination also revealed mild fragile X-associated physical characteristics including hyperextensible finger joints (MP extension to 90 degrees) and flat feet. His thumbs showed no hypermobility. He also had a palpably mildly enlarged thyroid gland; follow-up thyroid function studies were recommended, but results are not available at this time. Cognitive testing on the WISC-IV measured a full scale IQ of 113, which is considered above average intellectual ability. He also had a verbal comprehension of 104, perceptual reasoning of 112, working memory of 110, and perceptual speed of 112. On the VABS-II, he scored 100 on communication, 103 on Daily Living Skills, and 92 on Socialization, with an Adaptive Behavior Composite of 96. Like his sister, he was within typical range on the ADOS and SCQ; however, the SRS indicated mild to moderate problems with reciprocal social behavior. On the ADIS, he met criteria for the diagnosis of separation anxiety, social phobia, specific phobia, and generalized anxiety disorder. This was consistent with our clinical assessment and his medical history; he met DSM-IV criteria for these diagnoses. While he did not meet criteria for obsessive-compulsive disorder, he had perseverative thoughts. Unlike his sister, he had elevated sensory scores on the SSP suggesting sensory integration problems. On the IVA-CPT, he scored 62 for the full scale response control and 72 on the full scale attention quotient, indicating significant auditory processing and attention problems.

His CGG repeat size was determined as 65 using both PCR and Southern Blot analysis (fig 1, fig 2). His *FMR1* mRNA level was 2.45 ± 0.16 with no statistical difference in FMRP levels detected compared to the normal control (Table I).

Family History

Both parents were administered the WAIS-IV. The mother was reported to have a FSIQ of 145, VIQ of 142, and PIQ of 140, all within the superior range. The father also scored within the superior range with the following scores: FSIQ of 143, VIQ of 137 and PIQ of 142. While both adults self-reported some anxiety on medical interview, the Beck Anxiety Interview and SCL-90, neither met full diagnostic criteria for anxiety or any other psychiatric diagnosis on the SCID. The mother however, was sub-threshold for diagnoses of social phobia, obsessive thinking and generalized anxiety on SCID, and the father was in the low average range on the IVA-CPT.

The presence of the premutation allele in both parents, mother with 30 and 77 CGG repeats (activation ratio 0.63) and the father with 55 CGG repeats was demonstrated by Southern blot and PCR analysis (Fig. 1 and Fig. 2). Consanguinity was denied (Fig. 3).

Report of family history also indicates the presence of several characteristics, including FXTAS, anxiety and ADD in the extended family. Although anxiety and ADD are common in the general population, they are significantly more common in carriers compared to controls [Bourgeois, in press; Bourgeois, 2009; Farzin et al., 2006]. Maternal grandfather carried a premutation allele of 86 CGG repeats and has been diagnosed with FXTAS with a history of tremor, ataxia and white matter hyperintensities on T2 imaging in the middle cerebellar peduncles (MCP sign). The maternal great grandmother died of Alzheimer disease in her 70's and presented with gait problems prior to death. Three diagnosed premutation carriers on the paternal side (grandmother, aunt and her son) all showed clinical involvement. An undiagnosed uncle with reported ADD and anxiety characteristics and his untested daughter presented with shyness and anxiety. This may present a genetic

predisposition for anxiety, although these relatives should also be tested for premutation involvement. Finally, the paternal grandmother is reported to have had primary ovarian insufficiency (POI) and possible fibromyalgia.

DISCUSSION

The sibling pair reported here are uniquely appropriate for comparing the effect of the premutation allele across gender due to the fact that all copies of the *FMR1* gene between the two subjects have comparable repeat sizes and they share the same living environment. Despite the female sibling's double premutation allele, her brother presented with more significant cognitive and behavioral deficiencies. Contrary to his sister, the male sibling presented with ADHD, early language delay, aggression, somatosensory problems, and poor self-modulation, echoing characteristics similar to those associated with the full mutation. On the other hand, his sister presented with superior intellectual ability, normal language development, and no reported problems with attention or ADHD symptoms. Her high IQ may be related also to background gene effects in an intelligent family.

The other cases of double premutation females did not report a superior IQ; however, the three sisters reported by Mazzocco and colleagues [1996] showed higher repeat sizes ranging from 77 to 108 CGG repeats, with IQs ranging from 79 to 94. The female presented here is also the only reported double premutation carrier showing such a significant verbal-performance split in intellectual ability. While the female sibling exhibited fewer behavioral deficits and stronger cognitive abilities than her brother, it is important to note some of the similarities in their behavioral profile. They both performed well on the ADOS, but showed similar problems with social anxiety, intermittent poor eye contact, and social phobia. These symptoms are very common among both male and female carriers [Aziz et al., 2003; Farzin et al., 2006; Goodlin-Jones et al., 2004], although they are not uncommon in the general population [Bourgeois, in press; Bourgeois, 2009; Roberts et al., 2009]. They also both showed similar mild obsessive-compulsive behaviors, temper tantrums on a daily basis, and daily anxiety, problems commonly seen in carriers and in those with the full mutation [Bourgeois, 2009; Hagerman, 2006]. Both of these children are being treated with a selective serotonin reuptake inhibitor, sertraline, with a positive response.

Molecular analysis showed a CGG repeat number within the lower premutation range. In both the subjects a slight size reduction of the premutation allele occurred during maternal transmission. In both the female and male cases, the *FMR1*-mRNA levels were slightly elevated as described in previous studies [Tassone et al., 2000; Kenneson et al., 2001; Allen et al., 2004]. FMRP expression determined by Western blot analysis was not significantly different compared to normal controls in any of the carriers in the family likely due to the low CGG repeat number size of their *FMR1* alleles. It is also possible that a small FMRP deficit is present but not detectable by Western Blot analysis.

The behavioral differences between the brother and the sister may be related to many factors including: background genetic effects, sex differences, and environmental effects. Given these significant similarities as well as differences in the cognitive, behavioral, and molecular data, it is of interest to follow both brother and sister longitudinally to see the trajectory of their developmental differences over time. Both of these patients are at risk for FXTAS but it is unclear if a female carrier of 2 premutation alleles (compound heterozygote) will have a higher risk than what is reported in studies of female carriers (one premutation allele and one normal allele) (8–16%) [Coffey et al., 2008; Rodriguez-Revenga et al., 2008].

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Figure 1.

PCR size analysis using QiaXcel capillary system of PCR products derived from: female control (30, 54 CGG repeats) in lane 1, probands' father (55 CGG repeats) in lane 2, probands' mother (30, 77 CGG repeats) in lane 3, female proband (60, 67 CGG repeats) in lane 4, and male propositus (65 CGG repeats) in lane 5.

Figure 2.

Southern blot analysis of genomic DNA isolated from normal control female (lane 2), probands' father (lane 3), probands' mother (lane 4), female proband (lane 5), and male proband (lane 6). The DNA marker, 1 kb ladder, is shown in lane 1. Normal unmethylated band (2.8kb) and normal methylated band (5.2kb) in a female are shown on the left.

Table 1

Cognitive, behavioral, and molecular investigations in 2 premutation siblings

	Female Sibling	Male Sibling
CGG repeat size	59, 65 CGG	63 CGG
FSIQ	123 (94%ile) ¹	113 (81%ile) ²
Verbal IQ ¹	107 (68%ile)	-
Performance IQ ¹	135 (99%ile)	-
Verbal Comprehension ²	-	104 (61%ile)
Perceptual Reasoning ²	-	112 (79%ile)
Working Memory ²	-	110 (75%ile)
Perceptual Speed ²	-	112 (79%ile)
Adaptive Behavior Composite (VABS)	109	96
Communication (VABS)	120	100
Daily Living Skills (VABS)	105	103
Socialization (VABS)	106	92
ADOS	Normal	Normal
Separation Anxiety (ADI-S)	No	Yes
Social Phobia (ADI-S)	Yes	Yes
Specific Phobia (ADI-S)	No	Yes
Generalized Anxiety Disorder (ADI-S)	Yes	Yes

¹ WASI² WISC-IV