

# Increased prevalence of seizures in boys who were probands with the *FMR1* premutation and co-morbid autism spectrum disorder

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**Abstract** Seizures are a common co-occurring condition in those with fragile X syndrome (FXS), and in those with idiopathic autism spectrum disorder (ASD). Seizures are also associated with ASD in those with FXS. However, little is known about the rate of seizures and how commonly these problems co-occur with ASD in boys with the *FMR1* premutation. We, therefore, determined the prevalence of seizures and ASD in boys with the *FMR1* premutation compared with their sibling counterparts and population prevalence estimates. Fifty premutation boys who presented as clinical probands ( $N = 25$ ), or non-probands (identified by cascade testing after the proband was

found) ( $N = 25$ ), and 32 non-carrier controls were enrolled. History of seizures was documented and ASD was diagnosed by standardized measures followed by a team consensus of ASD diagnosis. Seizures (28%) and ASD (68%) were more prevalent in probands compared with non-probands (0 and 28%), controls (0 and 0%), and population estimates (1 and 1.7%). Seizures occurred more frequently in those with the premutation and co-morbid ASD particularly in probands compared with those with the premutation alone (25 vs. 3.85%,  $p = 0.045$ ). Although cognitive and adaptive functioning in non-probands were similar to controls, non-probands were more likely to meet the diagnosis of ASD than controls (28 vs. 0%,  $p < 0.0001$ ). In conclusion, seizures were relatively more common in premutation carriers who presented clinically as probands of the family and seizures were commonly associated with ASD in these boys. Therefore, boys with the premutation, particularly if they are probands should be assessed carefully for both ASD and seizures.

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## Introduction

Fragile X syndrome (FXS) is the most common known single gene cause of autistic disorder and heritable form of intellectual disability (Belmonte and Bourgeron 2006; Sherman 2002). It is caused by an expansion of CGG trinucleotide repeats (>200) in the 5' untranslated region of the fragile X mental retardation 1 (*FMR1*) gene. The premutation of this gene (55–200 CGG repeats) is relatively common in the general population with prevalence estimates ~1 in 130–250 females and 1 in 250–810 males (Dombrowski et al. 2002; Fernandez-Carvajal et al. 2009).

Clinical symptoms of developmental delays, autism spectrum disorders (ASD) and attention deficit/hyperactivity

disorder (ADHD) have been reported to be associated with the fragile X premutation (Aziz et al. 2003; Bailey et al. 2008; Chonchaiya et al. 2009a, b; Clifford et al. 2007; Farzin et al. 2006; Loesch et al. 2007); however, many children and young adults with the premutation may have no clinical symptoms (Hunter et al. 2008). It is not known why some individuals have clinical involvement, while others do not. These differences may be related to variations in the expression differences of the *FMRI* gene at varying CGG repeat lengths, variations in *FMRI* protein (FMRP) levels, background genetic effects and/or environmental effects. All premutation carriers have elevated *FMRI* mRNA leading to a gain-of-function toxicity to the neuron. Studies of the neurons of the premutation knock-in (KI) mouse demonstrated enhanced cell death by 21 days in culture compared with controls (Chen et al. 2010; Qin et al. 2011). Dendritic spines of premutation neurons have decreased length and complexity compared with wild type neurons in culture (Chen et al. 2010). Moreover, less complexity of dendritic arbors and decreased mature synapses were demonstrated in the medial prefrontal cortex, amygdala, and hippocampus of the KI mouse compared to controls (Qin et al. 2011). Furthermore, behavioral phenotypes including hyperactivity, social impairments, progressive spatial processing deficits, and profound impairments on the passive avoidance test were observed in both *Fmr1* KI and knockout mice suggesting that learning and memory were affected in both of these types of mutations (Hunsaker et al. 2009; Qin et al. 2011). Children with a fragile X premutation show features including developmental delay, ASD, ADHD, and behavior problems as mentioned earlier in some boys but less frequently in girls (Aziz et al. 2003; Bailey et al. 2008; Chonchaiya et al. 2009b; Clifford et al. 2007; Farzin et al. 2006; Loesch et al. 2007). There is only one previous report of seizures in boys with the premutation and this demonstrated that 11.3% had seizures of 57 boys from a family survey. (Bailey et al. 2008).

Seizures are a common co-occurring condition in those with the full mutation and in those with idiopathic ASD (Berry-Kravis et al. 2010; Brooks-Kayal 2010; Garcia-Nonell et al. 2008). There is evidence that seizures are associated with the diagnosis of autistic disorder in those with the full mutation (Berry-Kravis et al. 2010; Garcia-Nonell et al. 2008). However, little is known about the rate of seizures and how commonly these problems co-occur with ASD in boys carrying the *FMRI* premutation. We, therefore, studied this association in two distinct groups of boys with the premutation. The first group included boys initially seen at our clinic who were the first to be identified to have a mutation in the *FMRI* gene in their families (probands). All clinical probands were referred for behavior problems, but none of them were referred due to

seizures. The initial diagnosis of the premutation in the probands was made at the Fragile X Research and Treatment Center, University of California, Davis M.I.N.D. Institute due to the genetic testing for developmental delays and/or ASD for most participants (80%). The rest of the probands were referred to the M.I.N.D. Institute after the premutation diagnosis was made by another clinician who wanted further input related to behavioral/neurodevelopmental problems associated with the premutation.

The second group of boys (non-probands) was found to have a premutation through cascade testing in families once a proband was diagnosed with either a full mutation or a premutation. Our results were compared to siblings who did not have the premutation (controls). In addition, we compared our findings to population prevalence studies of seizures and ASD in boys and/or young men from recent large national surveys in the US (Centers for Disease Control and Prevention 2009; Cowan et al. 1989; Haerer et al. 1986; Hauser et al. 1991; Kobau et al. 2008; Kogan et al. 2009; Murphy et al. 1995; Pallin et al. 2008; Theodore et al. 2006).

## Materials and methods

### Participants

Fifty boys with the premutation with a mean age of 9 years 5 months (SD 4 years 8 months) and 32 controls with a mean age of 9 years 2 months (SD 5 years 3 months) were recruited in studies at the Fragile X Research and Treatment Center, University of California, Davis M.I.N.D. Institute between the years of 2003 and 2009. Of 42 boys with the premutation, 39 (92.9%) were Caucasian non-Hispanic, 2 (4.8%) were Hispanic, and 1 (2.4%) was Asian. Of 28 sibling controls, 23 (82.1%), 3 (10.7%), and 2 (7.1%) were Caucasian, Hispanic, and Asian, respectively.

A description of young male individuals who presented as probands, non-probands, and controls is illustrated in Table 1. The premutation allele and *FMRI* mRNA quantification was obtained using Southern Blot and polymerase chain reaction (PCR)-based genotyping analyses as previously described (Tassone et al. 2000; Tassone et al. 2008). All controls were documented to be negative for mutations in the *FMRI* gene (CGG repeats <45). This study was approved by the Institutional Review Board of the University of California, Davis Health System.

### Study protocol

After the written informed consent was obtained, all participants underwent a full medical history and physical examination by one author (RJH), who has expertise in fragile X-associated disorders, or clinicians who were

**Table 1** Characteristics of study participants

Variable	A = control			B = non-proband			C = proband			p value		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	A versus C	A versus B	B versus C
Age	32	9.2	5.27	25	10	5.08	25	8.86	4.23	0.7929	0.5455	0.4138
CGG repeats	29	28.55	4.97	25	94.36	29.78	25	86.08	42.86	<0.0001*	<0.0001*	0.3239
<i>FMR1</i> mRNA levels	18	1.45	0.32	20	3.13	1.3	23	2.88	1.6	0.0006*	0.0001*	0.5087
Full scale IQ	22	105.5	17.08	22	98.18	16.64	19	78.84	21.69	<0.0001*	0.1932	0.0014*
Vineland adaptive behavioral composite	12	91.17	12.33	13	92.23	13.97	18	68.83	15.63	0.0001*	0.8534	<0.0001*

N number of participants, SD standard deviation

\* Significant level at 0.05, adjusted for multiple testing

trained and supervised by her. The medical history covered a review of developmental delays/regression or behavioral problems. Medical problems were documented including clinical seizures (onset, duration, characteristics, frequency, any loss of consciousness or neurological deficit, associated symptoms, cortical electrical activity recorded by an electroencephalography (EEG), and anticonvulsant medications), in addition to a review of systems, and medication use. To be documented as having clinical seizures, the participants had to have sought pediatric professional help for these problems and to have been previously diagnosed and/or treated by a physician for seizures (physician-documented seizures).

ASD was assessed by parent report for participants >4 years utilizing the Social Communication Questionnaire (SCQ), an efficient screening tool for ASD which provides a strong agreement with the Autism Diagnostic Interview, Revised (ADI-R) scores (Rutter et al. 2003). The SCQ consisted of 40 yes-or-no questions completed by a parent, focusing on children's overall developmental history particularly in social and communication domains. A total score of 15 or above indicates a significantly increased likelihood of ASD. The diagnosis of ASD was confirmed by standardized measures including the Autism Diagnostic Observation Schedule (ADOS), the Autism Diagnostic Interview, Revised (ADI-R), and the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (American Psychiatric Association 2000; Le Couteur et al. 1989; Lord et al. 2000) followed by a multidisciplinary team consensus agreement as previously described (Harris et al. 2008). ASD was categorized into autistic disorder, pervasive developmental disorders not otherwise specified (PDD-NOS) and Asperger's syndrome. Severity of social impairment associated with ASD was completed by parents of children who were 4–18 years of age using social responsiveness scale (SRS) (Constantino and Gruber 2005). The SRS is a 65-item rating scale that covered five subscales of children's social deficits including social awareness, social cognition, social communication, social

motivation, and autistic mannerisms. *T* score for each subscale and total *T* score were then computed. A *T* score of 60–75 indicates clinically significant impairments in reciprocal social behaviors or mild to moderate difficulties in daily social interactions. A *T* score above 75 indicates severe interference in daily social interactions and is strongly associated with a clinical diagnosis of ASD.

The cognitive ability was assessed with age appropriate measures, including the Mullen scales of early learning (Mullen 1995), the Wechsler scale of intelligence third or fourth edition (Wechsler 1991, 1997, 1999, 2002, 2003), and the Stanford-Binet intelligence scale, fifth edition (Roid 2003). Children's adaptive functioning was also assessed using the Vineland adaptive behavioral scales, second edition (Vineland-II) (Sparrow et al. 2005). The Mullen scales of early learning is not an IQ test, but it is a standardized developmental and cognitive test frequently used in young children with an age ranging from birth to 68 months (Mullen 1995). It provides an early learning composite which was utilized as a proxy for the full scale intelligence quotient (FSIQ) in this study. The Mullen scales of early learning was used to assess cognitive ability in only 3 probands, 3 non-probands, and 3 sibling controls.

#### Statistical analysis

Continuous variables were compared using one-way analysis of variance (ANOVA) (Tables 1, 2). Dichotomous categorical variables including the diagnosis of seizures and ASD (Table 3) were compared between probands and non-probands using Fisher's exact test. Prevalence of such problems was compared to general population estimates using one-sample binomial test. We utilized this latter approach because large studies provide stable estimates of these problems in boys and/or young men as described above and summarized in Tables 3 and 4. All *p* values reported are two-sided and the significance level is 0.05. Moreover, *p* value adjustment for multiple testing was based on the false discovery rate (FDR) criterion.

**Table 2** ASD characteristics documented by the SCQ, the ADOS, and the SRS scores in each group of study participants

Variable	A = control			B = non-proband			C = proband			p value		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	A versus C	A versus B	B versus C
SCQ	12	2.33	2.39	12	3	2.73	17	16.59	8.23	<0.0001*	0.7755	<0.0001*
ADOS score	9	1.33	1.87	13	6.54	5.8	19	8.95	5.41	0.0006*	0.0219*	0.1905
SRS total score	7	43.57	8.83	12	51.58	9.56	16	74.56	15.3	<0.0001*	0.1865	<0.0001*

N number of participants, SD standard deviation

\* Significant level at 0.05, adjusted for multiple testing

Raw *p* values are reported and a significant *p* value after FDR adjustment is indicated by an asterisk (Tables 1, 2, 3).

## Results

### Characteristics of study participants

There was no significant difference in a mean age among each group of participants (Table 1). With regard to molecular variables including CGG repeats and *FMRI* mRNA levels, those with the premutation (both probands and non-probands) had higher CGG repeats and elevated *FMRI* mRNA levels compared with sibling controls without the premutation (Table 1) as expected. There were no differences in the FSIQ nor the Vineland adaptive behavioral composite between non-probands and sibling controls. Although there were no differences in molecular variables between probands and non-probands, probands were more likely to have lower cognitive ability and adaptive functioning than non-probands and sibling controls (Table 1).

### Comparison of seizures and ASD between boys with the premutation and controls

In the comparison of ASD between probands and controls, probands were more likely to score higher than controls in all ASD characteristics documented by the SCQ, ADOS, and SRS scores (Table 2). None of the controls had seizures nor ASD. Therefore, probands were more likely to have seizures and ASD than controls (Tables 2, 3). Likewise, these findings were also found between probands and population estimates (Table 3).

Among seven probands who had seizures, 4 had only one seizure type (absence seizures or complex partial seizures); 3 developed more than one seizure type including absence seizures, complex partial seizures, generalized tonic-clonic seizures, and Landau-Kleffner variant. Seizures onset ranged from 14 months to 5 years with a median age of onset at 3 years which generally occurred before the probands were referred to our clinic, such that

the premutation status has been presumably confirmed after the onset of seizures. All probands with seizures had to take anticonvulsant medication to control the seizures. Five of seven children with seizures had abnormal EEG testing documented by their neurologists, but 2 had normal EEGs which were done after the seizures were controlled by anticonvulsant medications.

Regarding the ASD diagnosis in probands with seizures, 6 (85.7%) had ASD (4 had autistic disorder, and 2 had PDD-NOS). Interestingly, those with seizures had a mean IQ significantly lower than that of probands without seizures [60 (SD 12.0) vs. 89.93 (SD18.27), *p* = 0.0013]. Moreover, SCQ was significantly higher in probands with seizures than in those without [24.40 (SD 3.78) vs. 13.33 (SD 7.35), *p* = 0.0066]. However, we caution against over-interpretation due to the small sample size for those with seizures. There were no significant differences in mean age, molecular data (CGG repeats and *FMRI* mRNA levels), Vineland adaptive behavioral composite, ADOS, and SRS scores between probands with and without seizures.

Although SCQ, and SRS scores in non-probands were not significantly different from those of controls, the non-probands had significantly higher scores on the ADOS and a higher rate for ASD diagnosis compared with controls (Table 2) and population estimates (Table 3).

### Comparison of seizures and ASD between probands and non-probands

Probands exhibited more ASD characteristics than non-probands, as indicated by higher SCQ and SRS scores (Table 2) in addition to standardized measures and a team consensus agreement for ASD diagnosis mentioned above. With regard to the ASD diagnosis in 25 probands, 8 had autistic disorder, 7 had PDD-NOS, and 2 had Asperger's syndrome. Whereas out of 25 non-probands, 1 had autistic disorder, 4 had PDD-NOS, and 2 had Asperger's syndrome. Seizures were not seen in any non-probands. Thus, seizures, autistic disorder, and ASD were significantly more frequently diagnosed in probands than in non-probands (Table 3). After excluding probands with seizures from the analyses, there was no significant difference in IQ

**Table 3** Prevalence of seizures, autism, and ASD in those with the premutation compared with conservative population prevalence estimates from recent national surveys in the US

Variable	Value <sup>a</sup> P = population estimate <sup>b</sup>		B = non-proband		C = proband		BC = non-proband and proband		p value <sup>c</sup>			
	Percent	Frequency	Percent	Frequency	Percent	Frequency	Percent	Frequency	B versus C	B versus P	C versus P	BC versus P
Seizures	1	1	0	0	7	28	7	14	0.0096*	<0.0001*	<0.0001*	<0.0001*
	0	25	100	18	72	43	86					
Autistic disorder <sup>d</sup>	1	1	4	8	32	9	18	0.0232*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
	0	24	96	17	68	41	82					
ASD	1	1.7	7	17	68	24	48	0.0101*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
	0	18	72	8	32	26	52					

\* Significant level at 0.05, adjusted for multiple testing

<sup>a</sup> Value: 1 presence, 0 absence<sup>b</sup> Conservative population prevalence estimates—refer to Table 4 of prevalence estimates<sup>c</sup> Comparisons of observed frequency in proband, non-proband and combined group of the *FMR1* premutation to conservative population prevalence estimates, except for B versus C which compares proportions between probands and non-probands<sup>d</sup> Autistic disorder did not include those with pervasive developmental disorders not otherwise specified (PDD-NOS) and Asperger's syndrome

between probands (without seizures), and non-probands. However, the SCQ [13.33 (SD 7.35) vs. 3.00 (SD 2.73),  $p < 0.0001$ ], and SRS total scores [69.64 (SD 14.94) vs. 51.58 (SD 9.56),  $p = 0.001$ ] were still significantly higher in probands without seizures compared with non-probands.

#### Association between ASD and seizures in premutation carriers

Among premutation participants, 1 out of 26 without ASD had seizures (3.85%), whereas 6 out of 24 with ASD had seizures (25%). So, there is a significant association between ASD and seizures ( $p = 0.0451$ ) in premutation carriers. However, this association was only seen in probands since seizures were not diagnosed in non-probands in this cohort. There were no significant differences in the molecular data including CGG repeats and *FMR1* mRNA levels among probands/non-probands with and without ASD and also among probands/non-probands with and without seizures.

#### Discussion

This study demonstrates significantly higher rates of ASD in premutation boys (both probands and non-probands), and also a significantly increased prevalence of seizures in probands (28%) compared to non-probands (0%) and to their sibling controls (0%) and population estimates (1%). These findings regarding ASD are in agreement with previous studies (Aziz et al. 2003; Bailey et al. 2008; Clifford et al. 2007; Farzin et al. 2006). However, our cohort included a larger group of participants and a more detailed assessment of ASD and seizures compared with previous studies of premutation carriers. Why some boys with the premutation have ASD is thought to relate to several molecular factors. An increased *FMR1* mRNA level, a molecular hallmark in those with the premutation, has been shown to affect the expression of candidate genes related to autistic disorder, including ubiquitin protein ligase E3A (UBE3A) and cytoplasmic *FMR1* interacting protein (CYFIP) (Handa et al. 2005). Loesch et al. (2009) have demonstrated epigenetic factors that might be involved in mechanisms of neurodevelopmental problems including autistic disorder in those with small CGG expansions. The premutation neuron forms a less complex dendritic tree in neuronal cell cultures and also enhanced cell death compared with control neurons (Chen et al. 2010). These characteristics may lead to reduced brain connectivity and perhaps enhanced vulnerability to environmental toxicity (Hagerman et al. 2010; Paul et al. 2010). The premutation brain also demonstrates areas of reduced FMRP (Handa et al. 2005; Qin et al. 2011) and reduced FMRP levels in



**Table 4** Conservative population prevalence estimates of seizures, autism, and ASD in boys and/or adult men from recent national surveys in the US

Disorders	Sources	Age (years)	Prevalence	95% CI
ASD	ADDM 2006 (CDC 2009)	8	14.5/1,000	13.9–15.1
	NSCH 2007 (Kogan et al. 2009)	3–17	17.3/1,000	14.4–20.7
Seizures	NHAMCS from 1993 to 2003 (Pallin et al. 2008)	All ages	4.3/1,000	3.9–4.7
	CDC 2005 (Kobau et al. 2008)	18–24	7.7/1,000	6.2–9.5
	Atlanta (Murphy et al. 1995)	10	6.7/1,000	–
	Oklahoma study (Cowan et al. 1989)	0–19	5.02/1,000	–
	Rochester (Hauser et al. 1991) <sup>a</sup>	0–24	4.45/1,000	–
	Mississippi (Haerer et al. 1986) <sup>a</sup>	0–24	9.87/1,000	–
	U.S. overall <sup>a</sup>	All ages	5–10/1,000	–

CI confidence interval, ADDM Autism and Developmental Disabilities Monitoring, CDC Centers for Disease Control and Prevention, NSCH National Survey of Children's Health, NHAMCS National Hospital Ambulatory Medical Care Survey

<sup>a</sup> Population prevalence estimates in both genders

the adult brain have also been reported in idiopathic autistic disorder (Fatemi and Folsom 2011).

The imbalance of the excitatory (the mGluR5 pathway), and inhibitory neurotransmitter systems [the gamma-aminobutyric acid A (GABA<sub>A</sub>) pathway] with the absence of FMRP is considered to be the pathophysiology of seizures in those with FXS (Berry-Kravis 2002; Hagerman and Stafstrom 2009). Recently, Chonchaiya et al. (2010) observed increased rates of seizures in children with FXS born to premutation mothers with autoimmune disease suggesting a potential mechanism of seizures in FXS associated with an intergenerational influence from autoimmunity in the premutation mothers. However, little is known about the pathophysiology of seizures in those with the premutation. An increase in neuronal spikes is seen in cell culture of premutation neurons (Chen et al. 2010; Pessah I. 2010, personal communication), so spikes may be intrinsic to premutation neurons resulting in increased vulnerability for seizures in carriers. In the premutation mouse, the level of FMRP decreases with increasing CGG repeat numbers (Brouwer et al. 2008) and preliminary data suggest that the quantitative FMRP level as measured by the new enzyme-linked immunosorbent assay (ELISA) test is significantly lower than normal in some premutation carriers (Iwahashi et al. 2009). Although knowledge of FMRP levels will further our understanding of developmental problems including seizures and ASD in those with the premutation, it will likely be a combination of molecular, environmental, and epigenetic factors, in addition to perhaps intergenerational influences, that have led to the problems presented here in some premutation boys.

The overall prevalence of seizures in premutation boys in our study (14%) was in agreement with the study by Bailey et al. (2008) (11.3%), but seizures were only present in probands, with a frequency of 28%. This prevalence of

seizures is very similar to that of FXS, which is ~20% (Berry-Kravis 2002; Musumeci et al. 1999). An important finding in this current study is that those with ASD, particularly probands, had a higher rate of seizures compared with carriers without ASD. Although an increased prevalence of seizures in those with the *FMRI* premutation and co-morbid ASD in our study (25%) is comparable to the prevalence of seizures reported in those with idiopathic ASD (30%) (Tuchman et al. 2010), the prevalence of ASD (85.7%) in those with the premutation and co-morbid seizures is very much higher than the prevalence of ASD in children with epilepsy (30%) who did not have the *FMRI* premutation. Therefore, seizures are an important co-morbid condition which may strongly predict the diagnosis of ASD in those with the premutation. Moreover, similar to the association between seizures and ASD in probands with the premutation, such association has been previously seen in those with the full mutation (Berry-Kravis et al. 2010; Garcia-Nonell et al. 2008). We hypothesize that the presence of seizures in probands may precipitate or exacerbate cellular and molecular changes that can further interfere with brain connectivity leading to ASD as previously documented in those without the *FMRI* premutation who also had seizures. Brooks-Kayal (2010) has previously reported on the deleterious neurotransmitter changes in the CNS with seizures and spike wave discharges. Both ASD and epilepsy in the premutation may share common abnormalities in synaptic structure and function resulting in the imbalances of neurotransmitter systems mentioned earlier (Belmonte and Bourgeron 2006; Tuchman et al. 2010). Although the findings of whether antiepileptic medications have positive psychotropic effects on children with ASD with or without epilepsy are equivocal, improvement in core symptoms of autistic disorder and affective instability, impulsivity, and aggression in addition

to the seizures in those with idiopathic ASD was noted in a few studies (Hollander et al. 2001; Tuchman et al. 2010). We have also seen the same phenomena in those with the premutation or the full mutation in our clinical experiences. However, we do not know for certain whether the seizures are simply a marker for more severe brain dysfunction that would be potentially associated with autistic disorder anyway or whether the seizures are truly additive to the CNS dysfunction leading to ASD. Randomized, placebo controlled trials are needed to explore if early treatment with anticonvulsants may have a long-term benefit in decreasing the prevalence (or severity) of ASD. We recommend a careful medical history to detect the possibility of seizures and an EEG to detect abnormal epileptiform discharges in premutation carriers with ASD particularly those who presented as clinical probands of the family (Chonchaiya et al. 2009a; Hagerman et al. 2009; Matsuo et al. 2010). Likewise, a comprehensive neuropsychological evaluation should be warranted for early detection of ASD in premutation carriers with seizures. Moreover, the premutation status has been generally identified after the onset of seizures. Thus, the fragile X DNA testing should be considered in children with clinical seizures in combination with the diagnosis of ASD or developmental delays.

An important weakness in our study includes a referral bias towards more clinical involvement in the probands, but none of probands were referred due to seizures. Thus, seizures in probands presented here were important clinical involvement which should not be disregarded by clinicians. Although we tried to compensate for this weakness by including the non-proband brothers, overall there may be still a clinical bias for more involvement in these families. However, the molecular details were unknown to the psychologists who carried out the cognitive and behavioral testing, so they were blinded to the proband versus non-proband status and also to the premutation versus non-premutation status. A completely unbiased assessment of premutation development will require the longitudinal study from birth of those identified with the premutation from newborn screening.

In conclusion, seizures and ASD are more prevalent in boys with the premutation, particularly in those who were probands of the family compared with their siblings without a premutation. Therefore, a detailed medical/neurodevelopmental history and assessment is recommended in premutation carriers, especially those who are clinical probands, so that seizures and ASD can be detected early and receive treatment. This study also supports the concept that molecular and neurobiological changes associated with the *FMR1* premutation may increase the risks for ASD and seizure disorders in these children.

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