

Autism Spectrum Disorders and Attention-Deficit/ Hyperactivity Disorder in Boys with the Fragile X Premutation

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ABSTRACT. Fragile X syndrome (FXS) is caused by a full mutation expansion (>200 CGG repeats) in the *FMR1* gene that results in a deficiency of the fragile X mental retardation protein. Although most individuals with the premutation (55–200 CGG repeats) are considered unaffected by FXS, recent case studies have documented children with the premutation who have cognitive deficits, behavioral problems, and/or autism spectrum disorders. The objective of this study was to compare the prevalence of autism spectrum disorders (ASD) and attention-deficit hyperactivity disorder (ADHD) symptoms in boys with the premutation who presented as probands, in brothers with the premutation who did not present as probands, and in normal brothers of premutation and/or full mutation carriers. Participants included 43 male children: 14 probands who presented to clinic, 13 nonprobands who were identified through cascade testing (routine genetic testing of family members after identification of a proband) and confirmed to have the premutation, and a control group of 16 male siblings of individuals with the fragile X premutation or full mutation who were negative for the *FMR1* mutation. Participants came from 1 of 2 collaborative sites: University of California, Davis and La Trobe University in Australia. Parents completed the Conners' Global Index-Parent

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Version for assessing symptoms of ADHD and the Social Communication Questionnaire (SCQ) for identifying symptoms of ASD. Children who were in the ASD range on the SCQ ($n = 13$) underwent further evaluation with either the Autism Diagnostic Observation Schedule-Generic ($n = 10$) or the Autism Diagnostic Interview-Revised ($n = 3$). A final diagnosis of ASD included clinical assessment utilizing DSM-IV-TR criteria in addition to the standardized assessments. There was a higher rate of ASD in boys with the premutation presenting as probands ($p < 0.001$) or nonprobands ($p < .04$) compared with sibling controls without the premutation. In addition, probands had a significant increase in ADHD symptoms compared with controls ($p < .0001$). Of the probands, 93% had symptoms of ADHD and 79% had ASD. In the nonproband premutation group, 38% had symptoms of ADHD and 8% had ASD. Thirteen percent of sibling controls had symptoms of ADHD and none had ASD. IQ scores were similar in all 3 groups ($p = .13$), but the use of psychotropic medications was significantly higher in probands with the premutation compared with that in controls ($p < .0001$). Developmental problems have been observed in premutation carriers, particularly those who present clinically with behavioral difficulties. Although this study is based on a small sample size, it suggests that premutation carriers, even those who do not present clinically, may be at increased risk for an ASD and/or symptoms of ADHD. If the premutation is identified through cascade testing, then further assessment should be carried out for symptoms of ADHD, social deficits, or learning disabilities. *J Dev Behav Pediatr 27:137-144, 2006.* Index terms: *FMR1, autism, FXTAS, RNA toxicity, ADHD, FMRP.*

INTRODUCTION

Fragile X syndrome (FXS) is the most common heritable form of mental retardation, affecting approximately 1 in 3600 males and 1 in 6000 females.¹ The syndrome is caused by a trinucleotide (CGG) repeat expansion in the 5' untranslated region of the Fragile X Mental Retardation 1 (*FMR1*) gene on the X chromosome.² Based on the size of the CGG repeat, the *FMR1* allele is classified as normal (5–44 repeats), gray zone (45–54), premutation (55–200), or full mutation (>200).³ Full mutation expansions generally result in hypermethylation of the CGG repeat and adjacent promoter region, transcriptional silencing, and the consequent loss of *FMR1* protein (FMRP).

FMRP is important for the development and maturation of dendritic spines and synaptic connections,^{4,5} and the lack of FMRP is responsible for the cognitive, physical, and behavioral impairments that are characteristics of FXS.^{6,7} The degree of cognitive impairment ranges from normal intelligence with learning disabilities to mental retardation.⁸ FXS is also associated with increased risk for deficits in other domains, including executive function, sustained attention, working memory, and social function even in those without mental retardation.^{8,9}

While the cognitive and behavioral phenotype of the full mutation has been described in numerous reports,^{8–11} much remains to be understood about the phenotypic effects of the premutation allele. Most individuals with the premutation are considered unaffected cognitively,^{12,13} but more recent studies have documented cognitive or behavioral deficits in a subgroup of males and rarely females with the premutation.^{14–18} Many cognitive and behavioral problems affecting individuals with a premutation allele, including social deficits, autism spectrum disorders (ASD) and attention problems, seem to be mild variants of those seen in full mutation carriers. This clinical picture may be related to mild FMRP deficits commonly reported in affected individuals with the premutation.^{17,19}

Premutation carriers also develop symptoms that are not associated with full mutation alleles; that is, the symptoms

are not due to lowered/absent FMRP. Approximately 21% of female carriers develop premature ovarian failure, defined as cessation of menses, before the age of 40 years.^{20–22} A second condition, fragile X-associated tremor-ataxia syndrome (FXTAS) has recently been identified in older male carriers^{23–30} and, less commonly, in female carriers.^{31–34} Just before the recognition of the FXTAS clinical phenotype, Tassone et al³⁵ discovered that *FMR1* mRNA levels are increased from 2- to 8-fold above normal in peripheral blood leukocytes of premutation carriers, and that this increase correlates with an increased number of CGG repeats within the premutation range. The presence of increased levels of *FMR1* mRNA in premutation carriers is thought to result in a “toxic” gain-of-function of the mRNA per se, whereby one or more proteins become sequestered by the increased levels of the expanded CGG repeat; this process is accompanied by the formation of mRNA-containing intranuclear inclusions.^{36,37}

The prevalence of the premutation in the general population (1 per 813 males; 1 per 259 females)^{38,39} is much greater than the prevalence of the full mutation alleles. Therefore, clinical involvement in premutation carriers may have a greater impact in the general population. Moreover, clinical involvement in premutation carriers may occur from both/ either lowered FMRP levels and/or increased *FMR1* mRNA, raising the prospect of mechanistic diversity of clinical involvement in the premutation range. To begin to address the broader issue of clinical involvement from a neurodevelopmental perspective, the aim of the current study was to assess the frequency of behavioral problems in boys with the premutation, in those identified clinically as the proband, and in a nonproband group of carriers identified through cascade testing in fragile X families.

METHODS

Subjects

Forty-three white male subjects, ages 4 to 22 years (10.3 ± 5.0 years), were enrolled in the study. Most participants (24/43) were recruited and assessed at the University of California, Davis; the remaining cases (19/43) were recruited

and evaluated at La Trobe University, Victoria, Australia. All known premutation carriers between 4 and 22 years of age who presented to clinic at both collaborative sites were invited to participate in the study. For the premutation subjects, inclusion in the study was based on documentation of a premutation allele (CGG repeat size between 55 and 200 repeats) using polymerase chain reaction (PCR) and Southern blot analysis; control siblings were confirmed to be negative for the premutation allele (CGG repeat size <55). Fourteen (9.3 ± 4.8 years) were probands ascertained through clinical presentation and confirmed as carriers of the premutation allele; 13 (11.5 ± 5.1 years) were nonprobands with the premutation allele who were identified through pedigree analysis and cascade testing in fragile X families after a proband was found with either a full mutation or a premutation allele; and 16 (10.4 ± 5.5 years) were male sibling controls without the premutation. In a survey of 670 fragile X family pedigrees of probands with either the premutation or full mutation analyzed in the United States, 65 had gone through cascade testing in the past. We identified 29 males who were nonproband *FMR1* premutation carriers in the age range of interest for this study. We attempted to contact all of these families, but 16 had incorrect telephone information and could not be located. Of the remaining 13 families, 8 participated, 1 refused participation, and 4 were unable to participate because of time constraints. All sibling controls from the 8 families who participated were included, in addition to 2 sibling controls from local families who had a full mutation proband. The Australian subjects were recruited from the La Trobe University register of 59 families participating in the other ongoing National Institutes of Health/National Health and Medical Research Council (NIH/NHMRC)-supported fragile X studies. These families had initially been ascertained through clinical admissions of probands identified as full mutation or premutation at the Victorian Genetic Health Services at the Royal Children's Hospital in Melbourne. In these families, 3 were premutation probands in the appropriate age for the study. The nonproband premutation carriers ($n = 5$) were subsequently identified through cascade testing of the initially ascertained families conducted by the La Trobe investigator (D.Z.L.). All the proband ($n = 3$) and nonproband premutation carriers ($n = 5$) identified in Australia consented to participate in this study. In addition, 11 male sibling controls were recruited from the 8 families with a proband or nonproband and 5 families with a full mutation proband. The sibling controls were similar in age to the premutation group (probands and nonprobands combined). All aspects of this study were approved by the Institutional Review Board of the University of California, Davis and by the ethics committees of La Trobe University and the Royal Children's Hospital in Melbourne. All participants signed written informed consent.

Measures

The same measures were utilized at the University of California, Davis and La Trobe University, Australia.

Molecular Testing. Genomic DNA was isolated from peripheral blood lymphocytes (5 mL of whole blood) using

standard procedures (Puregene Kit, Gentra Inc, Minneapolis, MN), as previously described by Tassone et al.³⁵ Molecular testing including Southern blot and PCR were as previously described by Saluto et al.⁴⁰ and Tassone et al.⁴¹

Cognitive and Behavior Assessments. Cognitive testing included the Wechsler Preschool and Primary Scale of Intelligence, Third Edition⁴² for children between 3 and 7 years of age. Children 7 and 16 years of age were given the Wechsler Intelligence Scale for Children, Third Edition.⁴³ We administered cognitive testing on all probands, 10 of 13 nonprobands, and all controls.

The Conners' Parent Rating Scales-Revised: Short (CPRS-R:S)⁴⁴ was used to assess symptoms of attention-deficit hyperactivity disorder (ADHD) and evaluate other problem behaviors in children. The scale has a large normative database to support the instrument's reliability and validity. The CPRS-R:S contains 10 items and covers a subset of the subscales and items on the long parent form, including hyperactivity, inattention, and oppositional behavior. Each item is scored on a scale from 0 (not true at all, never, seldom) to 3 (very much true, very often, very frequent) for a maximum total score of 30 points. A total raw score of 15 or higher and a standardized *T* score of 65 or higher suggest symptoms consistent with a diagnosis of ADHD. The coefficient alphas for internal reliability range from a low of 0.86 to a high of 0.94 for the short form of the CPRS-R. The identification of ADHD symptoms was confirmed by authors in the United States (R.J.H.) and in Australia (J.C. and D.L.) who utilized the DSM-IV-TR diagnostic criteria for ADHD.⁴⁵ In addition, a structured medical history interview was also conducted, which included teacher comments and clinical examination incorporating activities that require concentration.

The Social Communication Questionnaire (SCQ)⁴⁶ was used to evaluate autism spectrum symptoms in all subjects. Based on the original Autism Diagnostic Interview-Revised (ADI),⁴⁷ the SCQ is a 40-item questionnaire composed of yes-or-no questions completed by a parent or primary caregiver. The Lifetime Form of the SCQ, which focuses on the child's entire developmental history, is used to provide a total score that is interpreted in relation to the specific cutoff point of 15. A total score of 15 or higher indicates a significantly increased likelihood of an autism spectrum disorder (ASD). Subjects with SCQ scores at or above 15, or who raised clinical concerns for ASD, were referred for a more complete evaluation using the ADI or the Autism Diagnostic Observation Schedule (ADOS). Scores on the SCQ and ADI are typically highly concordant and are substantially unaffected by age, gender, language level, and performance IQ.⁴⁸ DSM-IV-TR⁴⁵ criteria were also used by authors/clinicians in the United States (R.J.H.) and Australia (J.C. and D.L.) to obtain a diagnosis of either Autistic Disorder or Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS).

The Autism Diagnostic Observation Schedule-Generic (ADOS-G)⁴⁹ is a semistructured grouping of activities designed to evaluate social and communicative behaviors related to the diagnosis of ASDs. The ADOS-G has been psychometrically validated across a wide range of ages and severity levels in autism and provides cutoff scores for

autistic disorder and ASDs based on scores in the domains of communication and social reciprocity. All subjects in this study were assessed with Module 3 based on their language levels.

The (ADI-R)⁵⁰ is a standardized, semistructured clinical interview for caregivers of children and adults. There are 111 items that focus on behavior in 3 domains: reciprocal social interaction, communication and language, and restricted and repetitive, stereotyped interests and behaviors. Questions are organized by content area, and definitions of all behavioral items are provided. Questions refer to current behavior and behaviors occurring between the ages of 4 and 5 years, the developmental period when autistic-type behaviors are most likely to be present. Several questions probe whether a behavior is current or was ever present. For each item, the clinician gives a score ranging from 0 to 3. A score of 0 is given if no atypical behaviors were present between 4 and 5 years of age or at present; a score of 2 indicates "definite abnormal behavior"; and a score of 3 is reserved for "extreme severity" of the specified behavior. An algorithm is generated based on those questions most sensitive to an autism diagnosis. To meet autism criteria on the ADI, scores must be above threshold in each of the 3 domains; the scores are based on the most abnormal behavior during any time period. The ADI does not discriminate between children with autism and other pervasive developmental disorders.

The diagnosis of autism was therefore based on clinical assessment using DSM-IV-TR and the following measures: SCQ, ADOS, or ADI, to generate an overall diagnosis that was agreed upon by our clinical/research team.

Parent Interview and Review of School Records. All parents underwent a structured medical history interview previously utilized in patients with the *FMR1* mutation⁵¹ to collect information about the developmental and social history, medical problems, including attention problems at home and school, learning disabilities, treatment needs and responses, special education, speech-language and occupational therapies, and medications used in treatment. School records were obtained with permission of the parent and reviewed for additional information regarding attentional difficulties and special education services.

RESULTS

Statistical analyses were conducted using SPSS for Windows (SPSS Inc, Chicago, IL). Descriptive statistics and results of comparisons between groups with the premutation and the control group are shown in Table 1. Nonparametric tests were used to assess group differences on all variables of interest due to lack of normality.

Potential confounding effects of age and IQ were first examined by comparing the 3 groups of boys using the Kruskal-Wallis test. This analysis revealed no significant differences in either age ($\chi^2 = 2.36, p = .31$) or IQ ($\chi^2 = 4.14, p = .13$); however, it should be noted that 4 of the probands and 4 of the nonprobands had below average Full Scale IQ (FSIQ) (<85). Next, analogous Kruskal-Wallis

tests with the total Social Communication Questionnaire (SCQ) score and the Conners' standardized *T* score as the dependent variables and group (control vs nonproband premutation vs proband premutation) as the independent variable revealed significant overall group differences on both the SCQ ($\chi^2 = 27.4, p < .0001$) and Conners' ($\chi^2 = 15.00, p = .001$) standardized *T* scores. To determine group specificity of the overall differences, follow-up comparisons of each pair of groups (control vs nonproband, nonproband vs proband, and control vs proband) using Mann-Whitney tests were conducted. Results revealed that nonproband boys with the premutation had higher SCQ scores than controls (Mann-Whitney *U*, $Z = 2.10, p = .04$). There was no significant difference in Conners' scores between these 2 groups ($Z = 1.34, p = .20$). Examination of differences between nonproband and proband boys with the premutation showed that probands had higher SCQ scores ($Z = 3.84, p < .001$) than nonprobands, and the difference on the Conners' standardized *T* scores approached significance ($Z = 1.84, p = .06$). Finally, comparison of control and proband groups showed highly significant differences on the SCQ and Conners', $Z = 4.69$ and 4.00 , respectively, $p < .0001$.

Utilizing a χ^2 analysis, there was a significant difference ($p < .0001$) between the percentage of probands who took medication (87%) and controls (0%), but no difference between nonprobands (17%) and controls.

To examine the association between each of the fragile X molecular measures and clinical data, we conducted pairwise Spearman's rho correlations between *FMR1* measures [CGG repeat size, *FMR1* mRNA, and *FMR1* protein (FMRP)] and full-scale IQ, SCQ total scores, and Conners' standardized *T* scores in the premutation group. Results yielded no significant correlations between CGG, FMRP, or mRNA and any of the 3 clinical measures (all correlations > -0.20 and $< +0.20$).

Table 1. Mean Statistics Of Age, *FMR1* Genetic Data, SCQ, And CPRS-R:S Scores

Variable	Siblings	Nonprobands	Probands
	Without premutation (N = 16)	With premutation (N = 13)	With premutation (N = 14)
Age	10.38 ± 5.49	11.54 ± 5.08	9.29 ± 4.78
CGG	26.00 ± 2.70	85.00 ± 14.20	87.00 ± 51.50
<i>FMR1</i> mRNA	1.69 ± 0.25	2.79 ± 1.05	3.13 ± 2.09
FMRP	91.67 ± 3.78	85.75 ± 8.38	75.91 ± 8.42
Full-Scale IQ	104.00 ± 7.57	97.00 ± 12.36	95.00 ± 23.91
SCQ			
(ASD ≥ 15)	1.69 ± 2.02***	4.75 ± 2.63**	18.82 ± 6.84
CPRS-R:S			
(ADHD ≥ 65)	52.62 ± 10.90***	62.36 ± 16.39	75.21 ± 8.88

FMRP indicates fragile X mental retardation protein; SCQ, Social Communication Questionnaire; CPRS-R:S, Conners' Global Index-Parent Version; ASD, autism spectrum disorder; ADHD, attention-deficit hyperactivity disorder.

*Compared to nonprobands, $p < .05$.

**Compared to probands, $p < .001$.

***Compared to probands, $p < .0001$.

Clinical Diagnosis

Thirteen of the 14 probands (93%) and 6 of the 13 nonprobands (38%) met or exceeded cutoffs consistent with attention-deficit hyperactivity disorder (ADHD) as demonstrated by a raw score of 15 or greater on the Conners' Parent Rating Scales-Revised: Short (CPRS-R:S). This was confirmed by examination and the DSM-IV-TR criteria for ADHD symptoms in each case. Two of 16 participants without the premutation (13%) scored above 15 on this measure and had symptoms of ADHD. All 13 probands with symptoms of ADHD had been or were currently being treated with medication; 7 were taking a stimulant, 3 were taking an atypical antipsychotic, 2 were taking a selective serotonin reuptake inhibitor (SSRI), and 1 was taking folate. Of the 6 nonprobands with symptoms of ADHD, 2 were on a stimulant medication. None of the participants without the premutation were receiving medical treatment of symptoms of ADHD.

Symptoms of autism spectrum disorder (ASD) in individuals with the premutation are shown in Table 2. Ten of the 14 probands (71%) scored 15 or greater on the SCQ. Of the remaining 4, 2 scored 14 on this measure. One of the 13 nonprobands (8%) scored above 15 on this measure. None of the individuals without the premutation were found to have symptoms of ASD based on the SCQ measure. The 10 probands who demonstrated symptoms of ASD based on the SCQ were evaluated using the Autism Diagnostic Observation Schedule (ADOS) or Autism Diagnostic Interview (ADI). Four of these 10 were diagnosed with Autistic Disorder, and 4 of the 10 were diagnosed with Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS), utilizing both the standardized measures and the DSM-IV-TR criteria. In addition, 3 individuals who scored 14, 14, and 10 on the SCQ and who all met clinical criteria for PDD-NOS on the DSM-IV-TR were administered either the ADOS (n = 2) or the ADI (n = 1) (see Table 2). The 2 subjects given the ADOS met criteria for PDD-NOS. Case 14, who was given the ADI, scored at the cutoff in the social interaction domain,

just below cutoff in the communication area, and significantly above cutoff in repetitive behaviors. The overall diagnosis of PDD-NOS was agreed upon by our clinical/research team based on clinical assessment using DSM-IV-TR and the standardized measures. The nonproband individual with an SCQ score of 23 was diagnosed with autism by both the ADI assessment and the DSM-IV-TR. Eleven out of 14 probands (79%) and 2 out of 13 nonprobands (15%) were receiving special education services that included speech and language and occupational therapies. None of the typically developing participants were receiving these services.

DISCUSSION

This study demonstrated a significantly higher rate of autism spectrum disorder (ASD) and symptoms of attention-deficit hyperactivity disorder (ADHD) in boys with premutation alleles of the *FMRI* gene who presented as clinical probands compared with controls. In addition, nonproband boys with the premutation had a higher rate of ASD symptoms than controls but did not have a higher rate of ADHD symptoms compared with controls. We found that 79% of probands met the criteria for an ASD; 29% for Autistic Disorder and 50% for the milder condition, Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS). In boys with the premutation who were identified by cascade testing (nonprobands), 1 (8%) was diagnosed with Autistic Disorder.

The findings of social deficits and attention problems in young premutation carriers are also supported by the work of others. Cornish et al¹⁸ have recently reported a high rate of social deficits in young adult male carriers. They found that the male premutation carriers had greater difficulty on a task requiring recognition of complex emotions from photographs of eyes compared with controls. They also found difficulties in attention switching in carriers compared with controls. Carrier males prefer fixed routines, display emotional distress at changes in routines, and have a tendency to focus on details, all of which can be seen in

Table 2. Autism Spectrum Disorder Diagnoses in Premutation Carriers

Subject	Consensus Autism Diagnosis	SCQ	ADOS-G			ADI-R		
			Social	Communication	Social-communication Total	Social	Communication	Repetitive Behaviors
2 ^a	Autism	23				10	19	6
7	Autism	25	10	5	15			
8	Autism	28	9	2	11			
10	Autism	29	9	4	13			
12	Autism	21				22	12	5
11	PDD-NOS	14	7	2	9			
13	PDD-NOS	16	5	1	6			
14	PDD-NOS	14				10	7	8
17	PDD-NOS	10	6	2	8			
22	PDD-NOS	20	7	3	10			
26	PDD-NOS	19	6	2	8			
33	PDD-NOS	20	6	3	9			

^aNonproband subject; all other subjects are probands.

SCQ indicates Social Communication Questionnaire; ADOS-G, Autism Diagnostic Observational Schedule-Generic; ADI-R, Autism Diagnostic Interview-Revised; PDD-NOS, Pervasive Developmental Disorder-Not Otherwise Specified.

ASD. Hessler et al⁵² found that the level of *FMRI* mRNA correlated with obsessive-compulsive symptoms and several other types of psychological symptoms in adult male carriers and female carriers who had an X activation ratio less than 0.5 (majority of cells with active, premutation alleles). In the current study, no correlation was found between molecular measures and ADHD or ASD symptoms; however, the current sample size was smaller than that of the study by Hessler et al.⁵²

Neuropsychological deficits, particularly executive function deficits, impulsivity, and attention problems, have also been reported in male carriers.⁵³ Moreover, neuroimaging studies in male carriers, aged 20 to 72 years, demonstrated bilaterally decreased gray matter voxel density in the cerebellum, brainstem, amygdalo-hippocampal complex, caudate, and insula. There was also decreased white matter voxel density in tracts within the frontal and temporal lobes, cerebellum, brainstem, pons, cingulate, and genu of the corpus callosum, as compared with those of aged matched controls.⁵⁴ These patients had not been identified clinically, nor did they have fragile X-associated tremor-ataxia syndrome (FXTAS). More obvious white matter disease and brain atrophy appear as the symptoms of FXTAS become apparent.^{27,55}

It is not known why there is a high rate of ASD or symptoms of ADHD in individuals with the premutation. These problems may be related to subtle abnormalities in white matter in development or growth dysregulation in neurons with the premutation.⁵⁶ Significant increases in white matter, particularly in early childhood, related to an enhanced growth trajectory in autism, have been reported by Courchesne and others.^{57–59} The premutation may be another mechanism of early white matter disease or even enhanced brain growth rates leading to ASD. Animal models are beginning to emerge that may prove helpful in understanding the neuronal toxicity of the premutation.^{60,61}

At the cellular level, there is evidence that the premutation (as RNA) causes toxicity to the neuron, leading to greater vulnerability to apoptosis under stress.⁶² In a study using human cell lines, the premutation caused up-regulation of several genes, including cytoplasmic *FMRI* interacting protein, which controls neuronal connectivity in *Drosophila* by binding to *FMRI* protein (FMRP) and the rho-GTPase, Rac1;⁶³ caspase-8, a protein involved with the death receptor-induced apoptotic pathway; UBE3A, a protein associated with Angelman syndrome, and also linked to autism;⁶⁴ and neurotensin, a component of the hypothalamic-pituitary-gonadal axis, which is important for regulating female fertility.⁶⁵ Our work with both transformed and primary human neural cell lines has demonstrated that the expanded CGG repeat, as RNA, is capable of inducing inclusion formation, increased cell death, and distortions in nuclear morphology in transfected cells in culture.⁵⁶ Overall, there is clear evidence that the premutation allele is toxic to the cell, and this toxicity is likely to be present even early in development.

Subtle deficits in FMRP levels may also occur in the premutation as reported previously;^{17,19,35} however,

the immunocytochemical method is unable to quantify mild deficits in protein level within untransformed cells. Better methodology is needed before this effect can be thoroughly evaluated in premutation carriers.

Our current study is limited by the small numbers of participants in each group and by the lack of more extensive neuropsychological testing. In addition, a diagnosis of ADHD cannot be made clinically according to DSM-IV-TR criteria in an individual with autism or PDD-NOS. Clinically, over one third of patients with ASD meet criteria for symptoms of ADHD, which is true of several of our probands.^{66,67} However, to preserve consistency with the DSM-IV-TR, we have labeled these individuals as having symptoms of ADHD instead of a specifying diagnosis of ADHD.

The finding of significant behavioral involvement in young males with the premutation, including those with a normal IQ suggests the need for *FMRI* DNA testing in the siblings of children who are identified with fragile X syndrome (FXS) or as a proband with the premutation, and highlights the need for medical and behavioral monitoring of these symptoms. Often the parent does not focus on the relatively less severe problems of symptoms of ADHD or social deficits in the sibling when the proband with FXS has more severe problems, including mental retardation or aggression. In fact, the parent may not be aware of the school or social struggles of the child with the premutation, especially if the child has normal intellectual abilities.

The identification of symptoms of ADHD or social deficits in children with the premutation is important from a treatment perspective because most of these children respond well to intervention. Stimulants for symptoms of ADHD have an excellent response rate in the general population with ADHD and in children with FXS and ADHD.^{68,69} If anxiety or ASD is present, medications such as serotonin reuptake inhibitors (SSRIs) or atypical antipsychotics may be helpful.^{69–71} In addition, psychological counseling and behavior interventions can be supportive to both the child and the family.^{72,73} Early intervention for these problems may help the later difficulties of anxiety and depression that are common in older male carriers.⁵²

The current work supports the need for further studies in premutation carriers to better understand the cause of brain dysfunction leading to social deficits and attention problems. In the interim, clinicians should routinely test for the *FMRI* mutation, either pre or full, in children presenting with autism or PDD-NOS, even without obvious physical or cognitive features of FXS. Since there are many causes of ADHD in the general population, we do not recommend routine testing for the *FMRI* mutation with ADHD alone. However, *FMRI* testing should be done in the presence of ADHD symptoms combined with ASD or mental retardation.

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