Original Article

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Anxiety disorders in fragile X premutation carriers: Preliminary characterization of probands and non-probands

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Summary **A very high proportion of individuals with fragile X syndrome (FXS) (*FMR1* full mutation, > 200 CGG repeats) experience clinically significant anxiety. Recent evidence suggests that adult fragile X premutation carriers (55-200 CGG repeats) also are at risk for anxiety disorders, and they demonstrate limbic system alterations mediated by FMRP and/or elevated *FMR1* mRNA that may explain this heightened risk. However, less is known about psychiatric symptoms including anxiety among children and adolescents with the premutation. We completed structured DSM-IV based diagnostic interviews focused on current anxiety in 35 children, adolescents or young adults with the premutation (ages 5-23 years, M = 11.3 ± 4.3; 27 male; 20 probands and 15 non-probands) and 31 controls (ages 5-18 years, M = 9.9 ± 3.6; 22 males). Among premutation carriers, 70.6% met criteria for at least one anxiety disorder (most frequently generalized anxiety disorder, specific phobia, social phobia, or obsessive compulsive disorder), compared to 22.6% of controls and 9.8% of the general population in this age range. Premutation carriers with intellectual disability, male gender, and proband status were associated with the highest rates of anxiety disorders. However, non-probands did have higher rates of having any anxiety disorder (40.0%) compared to general population norms. Although the results implicate anxiety as a target of screening and intervention among youth with the premutation, larger studies of unselected samples from the population of premutation carriers are needed to confirm and specify the degree and extent of psychiatric disorders in this condition.**

Keywords: Premutation carriers, proband, fragile X syndrome (FXS), anxiety, social phobia, specific phobia, intellectual disability

1. Introduction

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability (ID) and the most common known genetic cause of autism. It is caused by a trinucleotide expansion (CGG) of greater than 200 CGG repeats in the 5' untranslated region of the fragile X mental retardation 1 gene (*FMR1*) located on the X chromosome and occurs in approximately 1 per 5,000 males and 1 in 2,500-8,000 females (*1-6*). The inheritance pattern of fragile X is based on progressive generational expansion of the repeat size passed down from mother to child. Individuals are normally categorized based on the size of the CGG repeat expansion, in which normal alleles have 5-44 CGG repeats, while full mutation alleles have > 200 CGG repeats (*7*). Premutation carriers have a molecular phenotype characterized by abnormally elevated *FMR1* mRNA, which positively tracks with CGG repeat size within the premutation range. The fragile X premutation has an expansion of between 55 and 200 repeats, contributing to risk for expansion to a full mutation on transmission from mother to offspring in a single generation. The prevalence of the fragile X premutation in the general population is approximately 1 in 260-815

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males and 1 in 130-290 females, which in comparison to FXS is relatively high (*8*).

Individuals with the *FMR1* premutation are at risk for the two well-established phenotypes, the fragile X-associated primary ovarian insufficiency (FXPOI) and fragile X-associated tremor/ataxia syndrome (FXTAS). FXPOI occurs in approximately 20% of females with the premutation compared to 1% of the general population and is defined by cessation of menses before age 40 (*9,10*). FXTAS is a neurodegenerative disease seen in a significant proportion of older males and smaller number of older females with the premutation. Symptoms of FXTAS include intention tremor, cerebellar ataxia, neuropathy, autonomic dysfunction, cognitive decline, and brain atrophy with white matter disease (*11-15*).

Individuals with full FXS demonstrate high rates of anxiety disorders, which are regularly observed in clinical practice and through detailed research-based psychiatric interviews with parents (*16*). Though it was previously believed that fragile X premutation carriers develop normally through childhood, and young and middle adulthood, there is evidence to suggest that perhaps at least a subgroup is affected (*17-19*). For example, there is evidence of significant executive function (EF) deficits in carriers, particularly males (*20,21*), although this has not been consistently found in all studies (*22*). Females, on the other hand, are less likely to demonstrate EF deficits (*23,24*), but may be prone to mood and anxiety disorders, notably major depressive disorder, panic disorder without agoraphobia, agoraphobia without panic disorder, and social phobia (*25,26*). Although these symptoms were previously attributed to stresses on mothers with the premutation raising children with FXS, the participants in the study by Roberts and colleagues (*25*) retrospectively reported significant distress before the children were born. Franke and colleagues (*27*) carried out a remarkable study of mothers with the premutation to determine whether psychological problems were related to the premutation itself or to the stress of raising a developmentally impaired child. This study compared 13 mothers with the full mutation, 61 mothers with the premutation, 17 women with the premutation who were siblings of the first two groups but did not have children with FXS, and 18 women siblings without the *FMR1* mutation and without children, and 42 mothers without the *FMR1* mutation who had children with autism. The study used a psychiatric interview to obtain DSM-IV diagnoses and to assess personality disorders. Mothers with a premutation, as well as their siblings with the premutation but without affected children, were more likely to be diagnosed with social phobia than a control group of mothers of children with autism. A recent large family survey of children with the full mutation and premutation, in which parents were simply asked whether their child had been diagnosed with or been treated for a range of conditions, showed that 33.3% of 57 males and 35.6% of 119 females with the premutation were identified as having significant anxiety, compared to 8.8% of males and 15.3% of females with normal *FMR1* alleles matched for age and family income (*28*). However, it should be noted that Hunter and colleagues (*29*) examined mood and anxiety in 119 males and 446 females age 18-50 ascertained from families with a history of FXS and from the general population. Repeat length was not associated with anxiety, but was marginally associated with depression and negative affect in males and negative affect only in females. Thus the authors concluded that phenotypic differences were subtle and had a small effect size. However, elevated mRNA or reduced FMRP play a more important role in clinical outcomes among carriers than CGG size alone. For example, psychological symptoms, such as anxiety and obsessive-compulsive features are associated with abnormal elevation of *FMR1* mRNA in adult male premutation carriers with and without FXTAS (*15*). Also, both reduced FMRP and elevated mRNA contribute to alterations in limbic function and symptom expression in young adult carriers (*30,31*), providing a gene-brain-behavior basis for an understanding of emergence of these difficulties.

Anxiety disorders are among the most common psychiatric disorders in the general population, occurring in 2.4-10.7% of children (*32-34*). In a large epidemiological study funded by the National Institutes of Mental Health (Center for the Study of Emotion and Attention; CSEA-NIMH, 35), 9.8% of children met criteria for an anxiety disorder (n = 1,289, 9-17 years) (*36*). The most common anxiety disorders in the NIMH study were social phobia (4.5%), overanxious disorder (3.1%) and separation anxiety (2.3%).

The goal of the present study was to assess the frequency of anxiety disorders among children, adolescents and young adults who carry the *FMR1* premutation. Further, we sought to determine whether clinical features such as autism, intellectual disability and proband status, might be associated with the presence of anxiety disorders in premutation carriers.

2. Materials and Methods

2.1. *Participants*

Participants included 35 individuals with the *FMR1* premutation [27 males, ages 5.20-20.14, M = 11.04 (3.85); 8 females, ages 4.98-22.96, M = 12.13 (5.79)], and 31 healthy controls with normal *FMR1* alleles [22 males, ages 5.07-17.83, M = 9.73 (3.37); 9 females, ages 5.74-17.40, M = 10.26 (4.33)] (Table 1). Fifty-seven percent of the participants with the *FMR1* premutation were the first in their family pedigree to come to the attention of a clinician (probands), leading to fragile X DNA testing (17 males, 3 females). The remainder of premutation carriers (42.9%) was identified by cascade DNA testing

Table 1. Participant descriptive data

Items	Premutation Carriers			Controls			*t*-test
	Total	Males	Females	Total	Males	Females	
Age N	35	27	8	31	22	9	
M (SD)	11.29 (4.30)	11.04 (3.85)	12.13 (5.79)	9.88 (3.61)	9.73 (3.37)	10.26 (4.33)	t (64) = 1.43,
Range	4.98-22.96	5.20-20.14	4.98-22.96	5.07-17.83	5.07-17.83	5.74-17.4	p = 0.158
FSIQ* N	35	27	8	31	22	9	
M (SD)	93.49 (25.68)	90.30 (23.72)	104.25 (30.66)	114.16 (14.15)	113.59 (13.21)	115.56 (17.00)	t (54) = -4.11,
Range	36-141	36-141	40-126	85-143	88-136	85-143	p < 0.000
Proband Status N (%)							
Proband	20 (57.1)	17 (63.0)	3 (37.5)	0 (0%)	0 (0)	0 (0)	
Non-proband	15 (42.9)	10 (37.0)	5 (62.5)	31 (100%)	22 (100)	9 (100)	
Intellectual Disability N (%)							
IQ Below 80	11 (31.4)	9 (33.3)	2 (25.0)	0 (0)	0 (0)	0 (0)	
IQ Above 80	24 (68.6)	18 (66.7)	6 (75.0)	31 (100)	22 (100)	9 (100)	
ADOS Category N (%)							
No ASD	25 (73.5)	19 (70.4)	6 (85.7)	31 (100)	22 (100)	9 (100)	
ASD	5 (14.7)	5 (18.5)	0 (0.0)	0 (0)	0 (0)	0 (0)	
Autism	4 (11.8)	3 (11.1)	1 (14.3)	0 (0)	0 (0)	0 (0)	

*Full Scale IQ (IQ tests included WASI, WISC-IV and DAS-II). ADOS, Autism Diagnostic Observation Schedule; ASD, Autism Spectrum Disorder.

(10 males, 5 females). Controls were recruited through announcements and flyers in the community and local school districts. Race and ethnicity data were collected in accordance with NIMH funded project requirements. The majority of the sample was Caucasian (86.4%) and not Hispanic or Latino (62.1%). Twelve (34.3%) premutation carriers and 2 (6.5%) controls were taking psychoactive medications at the time of assessment. For carriers, medications included: SSRI/antidepressant (n = 3), antianxiety/sedative (n = 1), antipsychotic (n = 3), stimulant (n = 3), and anticonvulsant (n = 3). For controls, medications included: SSRI/antidepressant (n = 1), antipsychotic (n = 1), and stimulant (n = 1). Ten of the 35 premutation carriers had a sibling with the *FMR1* full mutation.

2.2. *Measures*

The Anxiety Disorders Interview Schedule for DSM-IV: Parent Report Version (ADIS-IV; *37*) is a structured interview designed to assess and diagnose the presence of anxiety disorders according to DSM-IV criteria. ADIS-IV was used specifically to measure the severity and occurrence of anxiety disorders through parent ratings of disorder features and symptomology. The parent ratings indicate either the severity of distress or the amount of interference the item has on the person's overall functioning (0 = none to 8 = very severe). ADIS-IV has demonstrated good test-retest reliability (k = 0.73) and excellent inter-rater reliability (k = 0.80-1.0) between the parent- and child- report version of the ADIS for both principal diagnosis and individual anxiety disorders (*38,39*). Administration of the ADIS takes approximately two hours and was completed with the primary caregiver, usually the mother. ADIS has been used extensively in published studies of anxiety across many settings and populations (*40-44*). It has also been used in validation studies (*45-48*) and a federally-funded pediatric anxiety treatment trial (*49*). Finally, our group has validated the use of the ADIS in a population of children, adolescents and young adults with FXS, with and without ID (*16*).

Intelligence testing was conducted by a trained clinician. Due to the wide age range of participants, several measures were used, including the Wechsler Intelligence Scales: WASI (43.9%), WPPSI-III (6.1%), WISC-IV (30.3%), WAIS-III (6.1%). In addition, the Stanford-Binet Intelligence Scale, Fifth Edition (12.1%), and Leiter International Performance Scale-Revised (1.5%) were also used. Those with an IQ score below 80 were classified as having an ID (borderline range inclusive).

The presence of a possible autism spectrum disorder (ASD) was screened using the Social Communication Questionnaire (SCQ; *50*) and confirmed as necessary with the Autism Diagnostic-Observation Scale (ADOS-G; *51*). All diagnostic assessments used to determine ASD status were administered by a trained clinician. The control group did not include any participants with a previously-diagnosed disorder and all had an SCQ score within the normal range.

CGG repeat size and methylation status were determined for all participants on genomic DNA isolated from peripheral blood mononucleated cells (*PBMC*) using PCR and Southern Blot analysis as previously described (*52,53*). qRT-PCR by Taqman assay was used to measure *FMR1* mRNA expression levels as reported in Tassone and colleagues (*54*).

2.3. *Procedures*

All participants (and parents, if applicable) signed either a consent or assent to participate in the study under the

approval of the institutional review board (IRB). As part of a larger study, participants were seen for 1 to 3 days examining physiological correlates of anxiety in individuals with neurodevelopmental disorders. A list of current medications was reported by the parents. The ADIS was administered by an experienced licensed clinical psychologist (D.H.) or graduate level student (L.C., L.A., A.C.) who had passed reliability training on the instrument, as described previously (*16*). Any discrepancies or disagreements of diagnosis were handled by case discussion and reviewed by a licensed clinical psychologist for final diagnosis (D.H.). Administration was standardized to collect specific information to aid in the differential diagnoses of intellectual disability (ID) and autism (AUT), as described previously (*16*). DSM-IV adaptations for children were used for children with or without ID, as well as for adults with ID. As for adults without ID, standard DSM-IV criteria were used.

During the ADIS, interviewees were asked to provide specific examples regarding symptom description in order to ensure comprehension and proper symptom endorsement. For example, if fear of spiders was endorsed in the specific phobia section, the interviewee was asked to describe the reaction, the last time it occurred, the consistency of the fear, and the severity and type of interference in daily functioning. The interviewee was also asked whether the participant "reported" fear or whether they had "observed" a reaction indicating distress or a fearful response. The information collected provided the interviewer with enough data to make any diagnostic adjustments, if necessary.

2.4. *Statistical analysis*

Proportion tests (*z*-tests) (*55,56*) were carried out using the SPSS Custom Tables module to determine if the prevalence of anxiety disorders in the current study groups were significantly different from the prevalence in previous studies of the general population. General population prevalence rates were taken from the largest published NIMH epidemiological study of psychiatric disorders in children and adolescents (n = 1,285) using DSM-III-R criteria (*36*). Alpha values were adjusted using a Bonferroni correction. Given the potential impact of having a sibling with FXS on expression of anxiety, we conducted Chi-Square analyses to examine the association between having an affected sibling and the presence or absence of each anxiety disorder type.

Correlations were used to assess the association between the number of anxiety disorders and both mRNA and number of CGG repeats.

3. Results

3.1. *Rates of anxiety disorders*

Among all premutation carriers, 70.6% (n = 25) met criteria for at least one anxiety disorder, while 22.6% (n = 8) of the control group met criteria for at least one anxiety disorder (Table 2). The most common anxiety disorders in the premutation carrier group were generalized anxiety disorder (37.1%; n = 13), specific phobia (31.4%; n = 11), social phobia (28.6%; n = 10) and obsessive-compulsive disorder (22.9%; n = 8). The most common anxiety disorders in the control group were social and specific phobia (each 12.9%; n = 4). To further characterize anxiety among key clinical aspects of the premutation, rates were examined by gender, among those with and without an intellectual disability (ID), and by proband status (Table 3). Overall, more males (76.9%) than females (50.0%), those with ID (81.8%) than without ID (62.5%), and probands (94.7%) than non-probands (40.0%) met criteria for at least one disorder. Both males and females had a similar pattern of anxiety although twice as many females met criteria for separation anxiety. The pattern among those with an ID was somewhat different compared to those without an ID. Separation anxiety and selective mutism were more common among those with ID, while those without an ID had higher rates of GAD and OCD. Probands and non-probands had similar patterns of anxiety disorders, although many more probands met criteria for social phobia (40.0%) and specific phobia (50.0%) compared to non-probands (13.3% and 6.7%, respectively). Chi-Square analyses showed that there was no association between having a sibling with FXS and presence of any anxiety disorder (all $p > 0.25$).

3.2. *Comparison of anxiety disorder rates with the general population*

Both the premutation, and to a lesser extent the control group participants with average IQ had higher rates of anxiety compared to the general population (Table 4). Premutation carriers had significantly higher rates of social, specific and GAD compared to the general population, as well as a rate of having any anxiety disorder (all $p < 0.0083$ after controlling for multiple

Table 2. Percentage of premutation carriers and control group meeting criteria for DSM-IV anxiety disorders

Anxiety Type	Premutation carriers (%) (n = 35)	Controls (%) (n = 31)
Any disorder	70.6	22.6
Separation anxiety	8.6	6.5
Social phobia	28.6	12.9
Specific phobia	31.4	12.9
Panic disorder	0	0
Agoraphobia	0	0
GAD	37.1	3.2
OCD	22.9	3.2
PTSD	8.6	6.5
Selective mutism	8.6	0

GAD, generalized anxiety disorder; OCD, obsessive compulsive disorder; PTSD, post-traumatic stress disorder.

Table 3. Percentage of premutation carriers meeting criteria for clinical anxiety disorders

Anxiety type	Gender		ID status		Proband status	
	Male	Female	ID	Non-ID	Proband	Non-proband
Any disorder	76.9%	50.0%	81.8%	62.5%	94.7%	40.0%
Separation anxiety	7.4%	12.5%	18.2%	4.2%	10.0%	6.7%
Social phobia	29.6%	25.0%	27.3%	29.2%	40.0%	13.3%
Specific phobia	29.6%	37.5%	54.5%	20.8%	50.0%	6.7%
Panic disorder	0	0	0	0	0	0
Agoraphobia	0	0	0	0	0	0
GAD	37.0%	37.5%	27.3%	41.7%	45.0%	26.7%
OCD	22.2%	25.0%	0	33.3%	25.0%	20.0%
PTSD	11.1%	0	9.1%	8.3%	15.0%	0
Selective Mutism	11.1%	0	18.2%	4.2%	10.0%	6.7%

GAD, generalized anxiety disorder; OCD, obsessive compulsive disorder; PTSD, post-traumatic stress disorder.

Table 4. *Z*-test of proportions comparing non-proband premutation carriers to control group and general population rates of clinical anxiety disorders

Anxiety Type	Non-proband $n = 15$	Controls[a] $n = 31$	General population rates[b]
Any disorder	40.0%	24.1%	9.8%*
Separation anxiety	6.7%	6.5%	2.3%
Social phobia	13.3%	12.9%	4.5%
Specific phobia	6.7%	12.9%	1.3%
Panic disorder	0	0	N/A
Agoraphobia	0.	0	1.4%
GAD	26.7%	3.2%^	3.1%**
OCD	20.0%	3.2%	N/A
PTSD	0	6.9%	N/A
Selective mutism	6.7%	0	N/A

[a] Control for multiple comparisons used, significant differences are $p < 0.01$. [b] Control for multiple comparisons used, significant differences are $p < 0.007$. * Difference is significant at the corrected *p*-value. ** Difference was not significant after control for multiple comparisons. GAD, generalized anxiety disorder; OCD, obsessive compulsive disorder; PTSD, post-traumatic stress disorder.

Table 5. *Z*-test of proportions comparing rates of anxiety disorders in control and premutation groups to general population rates

Anxiety Type	General population	Premutation[a] $n = 35$	Controls[a] $n = 31$
Any disorder	9.8%	70.6%*	24.1%
Separation anxiety	2.3%	8.6%	6.5%
Social phobia	4.5%	28.6%*	12.9%
Specific phobia	1.3%	31.4%*	12.9%**
Panic disorder	N/A	0	0
Agoraphobia	1.4%	0*	0*
GAD	3.1%	37.1%*	3.2%
OCD	N/A	22.9%	3.2%
PTSD	N/A	8.6%	6.9%
Selective Mutism	N/A	8.6%	0

[a] Control for multiple comparisons used, significant differences are $p < 0.0083$. * Difference is significant at the corrected *p*-value. ** Difference was not significant after control for multiple comparisons. GAD, generalized anxiety disorder; OCD, obsessive compulsive disorder; PTSD, post-traumatic stress disorder.

comparisons) (Table 4). The control group had significantly higher rates of specific phobia compared to the general population, but this was no longer significant after controlling for multiple comparisons. Both premutation carriers and controls had significantly lower rates of agoraphobia compared to the general population.

The rates of anxiety disorders among non-probands were not significantly different from the rates among the control group or the general population, with the exception of the rate of *any anxiety disorder* compared to the general population (non-probands = 40.0%; general population = 9.8%; $p = 0.0172$, after control for multiple comparisons) (Table 5). Both the premutation and control group participants with average IQ had higher rates compared to the general population rates.

3.3. *Relationship to molecular variables*

No significant correlations and no trends were found between molecular variables (highest CGG repeat, mRNA) and the number of anxiety disorders among premutation carriers.

4. Discussion

The results of the study showed that an overall rate of anxiety disorders among a sample of children, adolescents and young adults with the premutation was significantly higher than controls and the general population. After examining the premutation carrier group by gender, proband status and presence of intellectual disability, the rates of having any anxiety disorder were highest among probands (94.7%), and remarkably high among those with an intellectual disability (81.8%) and males (70.6%). With regards to the premutation group, further analysis revealed that the significantly higher rates of many of the anxiety disorders compared to the general population were driven by the rates among the probands. The control group had the same rate of GAD as has been reported in the general population (3.2%), suggesting that the significantly higher rate among the premutation group (37.1%) may not be an artifact of the measure used or study design. The higher rate of any anxiety disorder

among non-probands compared to controls and the general population may be the most important result of the study, and provides perhaps better evidence of increased risk for anxiety among carriers. Clinic referral bias among probands is likely to inflate and overestimate the true rate of anxiety disorders among premutation carriers. For example, a child with developmental problems and anxiety may be referred for *FMR1* testing for FXS, and he/she may be found to carry a premutation allele that is not causally related to the symptoms.

There was no relationship between molecular measures (CGG repeat number or mRNA) and anxiety in our study. The lack of such correlations can be interpreted in a number of ways. First, as has been shown in prior work (*30*), correlations between CGG expansion size or mRNA and behavior may not be evident unless measurement of brain function underlying such behavior is accounted for in providing a key link between genetics and behavior. Second, molecular measures in blood may not be reflected similarly in brain tissue, making it more difficult to establish links between genetic variables and behavior. Third, reduced FMRP that occurs in some carriers, often with higher CGG alleles, could underlie anxiety in the premutation; unfortunately we did not have these measures available for this study. And finally, it is possible that the lack of association with the molecular measures is an indication that the *FMR1* premutation does not actually contribute risk for anxiety disorder. Psychosocial factors may also play a role. For example, an individual's knowledge that he/she has the premutation may contribute to anxiety, as this condition clearly confers risk for ovarian insufficiency and FXTAS, and the simple awareness of having a genetic mutation could be anxiogenic. The anxiety disorders observed in this younger sample of carriers may be consistent with previously published studies of older adults with the premutation who reported an increased rate of some types of lifetime anxiety and mood disorders (*25,26*).

There are several notable limitations of this study. First, interviewers were usually but not always blind to the *FMR1* status of the participants. Second, it is possible that the higher rates of anxiety among probands is at least partially a result of self-selection bias in that parents of probands enrolled in the study often sought assistance for developmental concerns, including related behavioral or emotional symptoms. However, enrollment in this study was continuous enrollment of premutation carriers coming to the center – some being clinic referred, typically probands, and others were siblings of probands or selected from pedigrees for research only. Third, this study assessed the presence of current anxiety disorders and we therefore cannot report the lifetime incidence in this sample. Fourth, the small sample size and the higher proportion of males *vs*. females with the premutation in the study limits the generalizability of findings to the larger population of premutation carriers, and to females, who are more likely to inherent the mutation. Fifth, the premutation group included more individuals with ID and had lower IQs overall than the control group. Ideally we would have chosen to more precisely match IQs. We included those in the borderline range (*70-79*) in the premutation group mainly to improve the sample size, which would have otherwise been too small for analysis. Finally, our results are based on parental report and may not reflect the exact internal states that participants are experiencing. However, the measure used in this study (ADIS-R) has been validated for parental report of symptomology.

A larger study that balances sample characteristics (*i.e*. proband status, autism diagnosis, *etc*.) and recruitment methods to reduce bias, would help validate the findings of this preliminary report. Given our finding of significant anxiety, treatment of these problems needs to be considered by the clinician who cares for these individuals as we have recommended previously (*57,58*).

In conclusion, this study provides evidence that anxiety disorders may be relatively common among children and adolescents with the premutation, especially probands. These findings warrant a thorough clinical assessment and potentially treatment of anxiety symptoms in these individuals.

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