


Mild Neurological Signs in *FMR1* Premutation Women in an Unselected Community-Based Cohort

Marsha R. Mailick, PhD,^{1*}  Jinkuk Hong, PhD,¹ Arezoo Movaghar, PhD,¹ Leann DaWalt, PhD,¹ Elizabeth M. Berry-Kravis, MD, PhD,² Murray H. Brilliant, PhD,^{1,3} Jaime Boero, MD, PhD,³ Peter K. Todd, MD, PhD,^{4,5} and Deborah Hall, MD, PhD²

¹Waisman Center, University of Wisconsin-Madison, Madison, Wisconsin, USA

²Department of Neurological Sciences, Rush University, Chicago, Illinois, USA

³Marshfield Clinic Research Institute, Marshfield, Wisconsin, USA

⁴Department of Neurology, University of Michigan, Ann Arbor, Michigan, USA

⁵Ann Arbor Veterans Administration Healthcare Center, Ann Arbor, Michigan, USA

ABSTRACT: Background: Premutation-sized (55–200) CGG repeat expansions in the *FMR1* gene cause fragile X-associated tremor/ataxia syndrome (FXTAS). Most studies of premutation carriers utilized reverse ascertainment to identify patients, leading to a selection bias for larger repeats. As shorter CGG premutation repeats are common in the population, understanding their impact on health outcomes has a potentially large public health footprint.

Objective: The study's objective was to compare an unselected group of premutation carriers ($n = 35$, 55–101 CGG repeats) with matched controls ($n = 61$, 29–39 CGG repeats) with respect to FXTAS-type signs using structured neurological assessments.

Methods: Three neurologists independently rated signs, using an adapted version of the FXTAS Rating Scale (Leehey MA, Berry-Kravis E, Goetz CG, et al. *FMR1* CGG repeat length predicts motor dysfunction in premutation carriers. *Neurology*. 2008). This was a double-blind study, as genetic status (premutation vs. control) was known neither by the participants nor by any of the neurologists. Analyses controlled potentially confounding

comorbid conditions in the electronic health record (eg, osteoarthritis and stroke) and probed the association of age with signs.

Results: Although there was no overall difference between carriers and controls, among individuals without any potentially confounding comorbid diagnoses, there was a statistically significant age-associated elevation in FXTAS-type signs in premutation carriers compared to controls.

Conclusions: Among those who do not have other comorbid diagnoses, women who have CGG repeats at the lower end of the premutation range may be at greater risk for ataxia and parkinsonism than their age peers, although their overall risk of developing such clinical features is low. This study should provide reassurance to those who share characteristics with the present cohort. © 2021 International Parkinson and Movement Disorder Society

Key Words: *FMR1* premutation; fragile X-associated tremor/ataxia syndrome; FXTAS Rating Scale

The *fragile X mental retardation 1* (*FMR1*) gene plays a critical role in normal neurological development and functioning, regulating the translation of approximately 30% of all transcripts in the synaptic proteome.¹

Changes in the CGG repeat copy number within the 5' untranslated region of *FMR1* result in three distinct disorders—fragile X syndrome (FXS), fragile X-associated primary ovarian insufficiency (FXPOI),

*Correspondence to: Dr. Marsha R. Mailick, Waisman Center, University of Wisconsin-Madison, Madison, WI 53705, USA; E-mail: marsha.mailick@wisc.edu

Relevant conflicts of interest/financial disclosures: Marsha R. Mailick: chair, Scientific Advisory Board, John Merck Fund Developmental Disabilities Program. PI: R01 R01 HD082110. Co-I, Signature Research Project: U54 HD090256. Jinkuk Hong: none. Arezoo Movaghar: none. Leann DaWalt: MPI: R01 HD084563. Elizabeth M. Berry-Kravis: funding from Asuragen Inc., which is directed to RUMC in support of rare disease programs. She receives no personal funds

and has no relevant financial interest in this commercial entity. Murray H. Brilliant: none. Jaime Boero: none. Peter K. Todd: none. Deborah Hall: none.

Received: 25 January 2021; **Revised:** 12 April 2021; **Accepted:** 7 May 2021

Published online 12 June 2021 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.28683

and fragile X-associated tremor/ataxia syndrome (FXTAS). FXS is a neurodevelopmental disorder that is the most prevalent inherited cause of intellectual disability and autism, resulting from expansions of >200 CGG repeats and transcriptional silencing of *FMR1*.² FXS is inherited from a mother who carries an *FMR1* premutation.³ The premutation is defined by 55–200 CGG repeats and occurs in approximately 1 in 150–200 women and 1 in 450 men.^{4,5} Premutation carriers are at risk for FXTAS and FXPOI, both of which are clinically and mechanistically distinct from FXS.^{6,7} FXPOI is the most prevalent genetic cause of impaired ovarian functioning and infertility.⁶ FXTAS, which is the focus of the present research, is a late-onset and progressive neurodegenerative disorder, manifested in a subset of premutation carriers after age 50.^{6,8,9}

FXTAS is defined clinically by intention tremor, gait ataxia, and cognitive decline due to memory issues and executive dysfunction. Pathologically, FXTAS presents with ubiquitinated inclusions in neuronal and non-neuronal cells, white matter disease, and global brain atrophy.¹⁰ Considerably more research on FXTAS has been conducted on men than women, due to the more severe manifestation and higher penetrance of the syndrome in men (affecting approximately 40% of carrier men vs. 16% of carrier women over age 50). The lower prevalence and milder severity of FXTAS in women is due to the effect of the nonmutated *FMR1* gene on the second X chromosome.¹¹ Most past studies identified participants from specialized FXS clinics or via family cascade testing,^{12,13} possibly resulting in ascertainment bias. For this reason, the prevalence and severity of FXTAS-type symptoms in premutation carriers in the general population have not been adequately studied and may differ from what is reported in the research literature.

Two major factors affect the diagnosis and manifestation of FXTAS in premutation carriers—age and CGG repeat number. After age 50, the diagnosis of FXTAS becomes increasingly likely¹⁴ and is more frequent in those with more than 100 CGG repeats.^{14,15} Although younger carriers, as well as carriers who have lower numbers of repeats, may manifest mild clinical features similar to FXTAS, this has been studied in only a few investigations.^{12,16-18}

Present Research

These gaps in past research shaped the present investigation, which was designed to advance the understanding of FXTAS-type signs among premutation carriers in the general population. To accomplish this, we accessed a unique biobank that included DNA samples from 20,000 members of a single health-care

system.¹⁹ These DNA samples were screened to identify premutation carriers.²⁰ Unlike past research, none of these individuals were identified based on family history of FXS or via an FXS specialty clinic, nor were they aware of their status as premutation carriers. Thus, a study of this cohort offers a window into how FXTAS-type signs may be manifested in unselected premutation carriers in the general population. Given the more limited research on premutation women, the focus of the present study is on how premutation women differ in FXTAS-type signs from matched controls, based on a structured neurological assessment.

Importantly, this was a double-blind study where neither the participants nor the three rating neurologists were aware of who was a premutation carrier versus a control. When enrolling in the biobank, members consented to give access to their electronic health records (EHRs) for research, and thus, in addition to the neurological assessment, we reviewed the EHRs to identify any potentially confounding comorbid diagnoses (eg, hip replacement and stroke) that might affect both patients and controls and that would need to be controlled in the present analysis.

We examined two research questions. Do premutation carrier women and controls differ in the severity of the neuromotor signs associated with FXTAS? Is there evidence of more severe FXTAS-related neuromotor signs associated with advancing age? We hypothesized that premutation carriers would have more FXTAS-related neuromotor signs than controls, and these would become greater with advancing age.

Patients and Methods

Participants were drawn from the Marshfield Clinic's Personalized Medicine Research Project (PMRP), a population-based cohort (n = 20,353, 40% of the eligible population of the Marshfield Epidemiologic Study Area, a 19-zip code region around Marshfield, Wisconsin, and a 9-zip code area in northern Wisconsin) who consented to share their EHRs and DNA for research. PMRP recruitment began in 2002. Health records were digitized back to 1979, and thus an average of 40 years of EHRs are available for these members. Institutional Review Board (IRB) approval was obtained from the Marshfield Clinic and University of Wisconsin-Madison. According to the initial protocol, participants consented at the time of enrollment in PMRP to contribute their de-identified EHRs and DNA to be used in research without an expectation of return of research results. Additional written consent was obtained before the neurological assessment, which per IRB was not described as a study related to *FMR1* or FXTAS.

Identification of Premutation Carriers and Controls

We screened all PMRP participants for whom DNA samples were available ($n = 19,989$, including 11,526 women) using procedures described previously.²⁰ The aim of screening was to identify premutation carriers (ie, those with 55–200 CGG repeats), resulting in the identification of 72 premutation carrier women. Next, the specific number of CGG repeats within the premutation range was quantified for each carrier. Subsequently, carrier status and repeat number were confirmed in an additional assay using the Asuragen AmpliX Kit,^{21,22} conducted in the Rush University Medical Center Molecular Diagnostics Laboratory (supervised by author E.M. Berry-Kravis). Buccal swab kits were provided to participants who obtained their own samples, returned via courier packages. DNA was isolated using standard methods. The same CGG repeat assays were followed to identify a pool of control women (24–40 CGG repeats) who were matched with premutation carriers at a ratio of approximately 2:1 ($n = 137$ controls) based on age and duration of receiving care in the Marshfield health-care system.

Of these potential sample members, 10 carriers and 21 controls were unable to provide informed consent at the time of data collection for the present study due to dementia, residence in long-term-care settings, or death. An additional 10 carriers and 17 controls declined to participate in the larger study. The neurological assessment required an in-clinic visit and agreement to being video-recorded. Those who no longer lived in the area or who refused video recording were not included in the current report. The single exclusion criterion was having a child with a neurodevelopmental disorder (3 premutation carriers and 4 controls).

Rates of participation of eligible carriers and controls were comparable (72.2% and 72.3%, respectively), and the two groups did not differ in rates of refusal, inability to participate due to dementia or residence in a long-term-care setting, or death before this study. There was no association between participation and diagnoses of depression and anxiety, as noted in the EHR ($F = 0.21$, $P = 0.644$). Notably, the recruiter for the study was blind to premutation versus control group status.

Neurological Assessment

A video-recorded structured neurological assessment (FXTAS Rating Scale)¹⁴ was administered to 35 premutation carriers and 61 controls by a clinical neurologist at the Marshfield Clinic (J. Boero). In addition, the video recordings were independently rated by two movement disorder neurologists with expertise in performing FXTAS-related evaluations (D. Hall and P.K. Todd).

The FXTAS Rating Scale was the dependent variable in the present analysis. It was developed to assess the three major motor features of FXTAS—tremor, ataxia, and parkinsonism.^{14,23} We used an adapted version of the measure (adapted by D. Hall), which comprises 56 items from the Clinical Rating Scale for Tremor, the International Cooperative Ataxia Rating Scale, the Unified Parkinson's Disease Rating Scale, and the Unified Huntington's Disease Rating Scale. Most items were rated from 0 (normal) to 4 (severe impairments), with 0 signifying normal in all items. For each item, the ratings from the three neurologists were averaged, and the items were summed up to create scores for the three subdomains as well as the total score. The percentage of agreement across raters for the total score averaged 93.2%. Because the current data set had many 0s (thus high rates of agreement among raters), an alternative to the Kappa statistic was employed.²⁴ Gwet's AC,²⁵ which is a more appropriate metric of agreement in such instances, averaged 0.91. See Supplementary Table S1 for the interrater reliability of the three subscale scores.

Comorbidities in EHRs

The EHRs of participants were screened to identify potentially confounding comorbid diagnoses that might result in motor dysfunction not due to FXTAS. A list of such diagnoses was developed by one of the authors (P.K. Todd). The EHRs of study participants were independently mined by another author (A. Movaghar), and the specific codes that were found in the EHRs of study participants are provided in Table 1. Premutation carriers and controls were classified into two groups—those having any of the diagnoses and those with no such diagnoses.

Statistical Analysis

The distribution of scores on the FXTAS Rating Scale and its subscales was first checked to determine if any would be considered statistical outliers (using Cook's D). No outliers were detected. Subsequently, the statistical analysis included four steps. First, independent sample t tests were used to compare premutation carriers and controls on the FXTAS Rating Scale. Second, to evaluate the effect of potentially confounding comorbid diagnoses in the EHR, two-way ANCOVAs (analysis of covariances) were used to examine mean differences in FXTAS Rating Scale scores by premutation status and by any of the comorbid diagnoses. Third, for the primary question addressed by this study, multiple regression was used to examine three-way interactions to determine if the association between premutation status and FXTAS Rating Scale scores varies based on age and any of the potentially confounding comorbid diagnoses. Fourth, we conducted two sensitivity analyses. We removed the eight

TABLE 1 Potentially confounding comorbidity diagnoses recorded in participants' electronic health records

Category	Description	ICD10
Cerebrovascular disease	Brainstem stroke syndrome	G46.3
	Cerebrovascular disease, unspecified	I67.9
Gait problems	Abnormalities of gait and mobility	R26
	Ataxic gait	R26.0
	Difficulty in walking, not elsewhere classified	R26.2
	Other abnormalities of gait and mobility	R26.89
	Unspecified abnormalities of gait and mobility	R26.9
Hip osteoarthritis	Ataxia, unspecified	R27.0
	Unilateral primary osteoarthritis, right hip	M16.11
	Unilateral primary osteoarthritis, left hip	M16.12
	Unilateral osteoarthritis resulting from hip dysplasia, unspecified hip	M16.3
Hip replacement	Osteoarthritis of hip, unspecified	M16.9
	Presence of right artificial hip joint	Z96.641
	Presence of left artificial hip joint	Z96.642
Knee osteoarthritis	Presence of unspecified artificial hip joint	Z96.649
	Bilateral primary osteoarthritis of knee	M17.0
	Unilateral primary osteoarthritis, unspecified knee	M17.10
	Unilateral primary osteoarthritis, right knee	M17.11
	Unilateral primary osteoarthritis, left knee	M17.12
Knee replacement	Osteoarthritis of knee, unspecified	M17.9
	Presence of right artificial knee joint	Z96.651
	Presence of left artificial knee joint	Z96.652
	Presence of artificial knee joint, bilateral	Z96.653

(Continues)

TABLE 1 Continued

Category	Description	ICD10
	Presence of unspecified artificial knee joint	Z96.659
Other movement disorders	Parkinson's disease	G20
	Myoclonus	G25.3
	Abnormal involuntary movements	R25
	Essential tremor	G25
Neuropathy	Tremor, unspecified	R25.1
	Idiopathic progressive neuropathy	G60.3
	Hereditary and idiopathic neuropathy, unspecified	G60.9
	Inflammatory polyneuropathy, unspecified	G61.9
	Polyneuropathy, unspecified	G62.9
	Idiopathic peripheral autonomic neuropathy	G90.0
Radiculopathy	Radiculopathy, lumbar region	M54.16
Rheumatoid arthritis	Rheumatoid arthritis with rheumatoid factor of unspecified hip without organ or system involvement	M0/5.79
	Rheumatoid arthritis with rheumatoid factor, unspecified	M05.9
	Rheumatoid arthritis, unspecified	M06.9
Spondylolisthesis	Spondylolisthesis, site unspecified	M43.10
	Spondylolisthesis, lumbar region	M43.16
	Fusion of spine, site unspecified	M43.2
Spinal stenosis	Spinal stenosis, site unspecified	M48.00
	Spinal stenosis, occipito-atlanto-axial region	M48.01
	Spinal stenosis, thoracic region	M48.04
	Spinal stenosis, lumbar region	M48.06
	Spinal stenosis, lumbar region without neurogenic claudication	M48.061
	Spinal stenosis, lumbar region with neurogenic claudication	M48.062

Note: Codes are organized into 12 categories using the ICD10 hierarchies or similarity of diagnoses.

participants (2 premutation and 6 controls) who had comorbidities in their EHRs involving movement disorders that were not signs of FXTAS (eg, abnormal involuntary movements, drug-induced akathisia; see Table 1, “Other movement disorders” category) and then repeated the analysis. Subsequently, following the approach of Leehey et al,¹⁴ we restricted the analysis to participants aged 50 or older to specifically focus on those at the highest risk of FXTAS-type signs. Due to the small size of the sample, an alpha level of $P = 0.05$ was used throughout.

Results

Descriptive Data

Participants averaged 57.2 years of age (SD [standard deviation] = 13.7), with a range from 26 to 86. Most ($n = 69$, 71.9%) were aged 50 or older. There was no significant age difference between premutation carriers and controls, due to matching. However, those with potentially confounding comorbid diagnoses were significantly older than those without such diagnoses (age 64.2 [SD = 12.9] vs. 52.2 [SD = 12.0], $P < 0.001$). The CGG repeat length of the controls ranged from 29 to 39 repeats (mean = 31.6). The CGGs of premutation carriers ranged from 55 to 101 (mean = 68.5), spanning the lower range of the premutation (Fig. 1). Among the premutation carriers, there was no association between CGG repeat number and FXTAS Rating Scale scores (total or any subscale scores, all $P > 0.24$).

Table 2 presents comparisons between premutation and control participants regarding potentially confounding comorbid diagnoses in the EHR. Many participants had at least one such diagnosis, but there was no significant difference between premutation and controls in the percentage with diagnoses (45.7% vs. 39.3%, respectively), nor were there group differences in individual diagnostic categories. The most common comorbid diagnosis was osteoarthritis of the knee.

Research Question 1: Comparison of Motor Signs in Premutation Carriers and Controls

Next, we compared premutation carriers and controls with respect to the FXTAS Rating Scale and the subscales. The two groups did not differ significantly on either the total scale score ($P = 0.458$) or any of the subscales ($P > 0.412$; see Supplementary Table S2). Next, to separate the effects of premutation status and potentially confounding comorbid diagnoses in the EHR, two-way ANCOVAs were conducted. There were significant interaction effects between premutation status and comorbid diagnoses with respect to the total score of the FXTAS Rating Scale and the subdomains of parkinsonism and ataxia ($P < 0.05$). The control

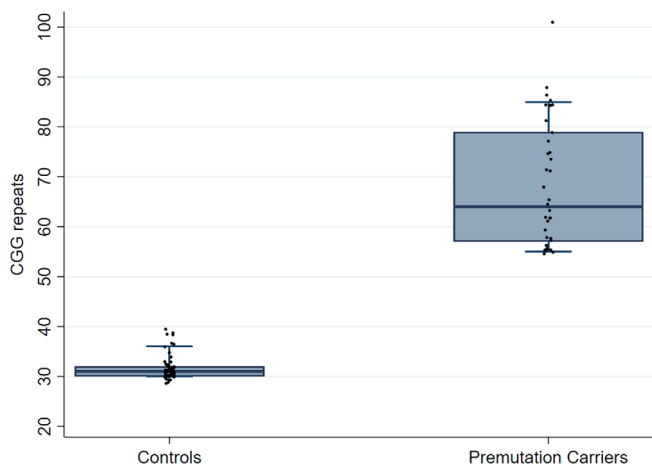


FIG. 1. Distributions of CGG repeats by premutation carriers versus controls.

participants with comorbid diagnoses had substantially more severe impairments than the other three groups (see Supplementary Table S3).

Research Question 2: Age-Related Differences in Motor Severity

To examine whether the FXTAS Rating Scale scores of carriers and controls differed with advancing age, multiple regression analyses were carried out, including a three-way interaction effect term (age \times premutation status \times presence vs. absence of comorbid diagnoses), predicting symptom severity. The three-way interaction term was the primary test of the study's hypothesis. There were significant three-way interaction effects on the FXTAS Rating Scale total score ($P < 0.001$) and the two subdomains of ataxia ($P < 0.001$) and parkinsonism ($P = 0.005$), with a trend-level interaction for the tremor subdomain ($P = 0.052$) (see Table 3, Figure 2, and Supplementary Figures S1–S3). Among those women who had no potentially confounding comorbid diagnoses in their EHR, it was only among the premutation group where FXTAS signs were more severe with advancing age, a pattern not evident among the controls. In contrast, among those who had at least one such diagnosis, it was the control group that showed more severe signs associated with advancing age, likely due to the comorbidity, a pattern not evident among the carriers.

Sensitivity Analyses

We removed 8 participants with potentially confounding other movement disorder diagnoses from the analysis (see Tables 1 and 2). The significant three-way interactions reported earlier (for total score, parkinsonism, and ataxia) remained significant when these 8 participants were removed from the analysis. In addition, the interaction term predicting the tremor subscale

TABLE 2 Comparison of premutation carriers and controls based on potentially confounding comorbidity code categories in the electronic health records

	Controls (n = 61)	Premutation carriers (n = 35)	F-values	P-values
Percentage having at least one potentially confounding comorbidity code category	39.3% (24)	45.7% (16)	0.30	0.582
Total number of unique categories of confounding comorbidity codes among individuals with at least one comorbidity code	2.12 (1.5) [1, 7]	1.69 (1.2) [1, 5]	0.64	0.430
Percentage (n) with each category of potentially confounding comorbidity codes				
Cerebrovascular disease	1.6% (1)	0% (0)	0.61	0.437
Gait problems	11.5% (7)	5.7% (2)	0.94	0.334
Hip osteoarthritis	8.2% (5)	2.9% (1)	1.34	0.250
Hip replacement	4.9% (3)	0% (0)	2.06	0.155
Knee osteoarthritis	26.2% (16)	25.7% (9)	0.05	0.831
Knee replacement	1.6% (1)	8.6% (3)	2.61	0.110
Other movement disorders	9.8% (6)	5.7% (2)	0.47	0.657
Neuropathy	3.3% (2)	8.6% (3)	1.18	0.280
Radiculopathy	3.3% (2)	5.7% (2)	0.29	0.589
Rheumatoid arthritis	1.6% (1)	2.9% (1)	0.14	0.713
Spondylolisthesis	1.6% (1)	2.9% (1)	0.15	0.696
Spinal stenosis	6.6% (4)	5.7% (2)	0.05	0.822

TABLE 3 Multiple regression models predicting FXTAS Rating Scale total score and subscale scores (n = 96)

	Total score			Tremor			Ataxia			Parkinsonism		
	b	SE	P-value	b	SE	P-value	b	SE	P-value	b	SE	P-value
Age ^a	0.15	0.12	0.200	0.08	0.05	0.072	0.03	0.04	0.481	0.02	0.05	0.653
PM status (premutation = 1)	3.89	2.46	0.118	0.14	0.96	0.887	1.91	0.84	0.025	1.61	0.99	0.106
Any comorbidity codes (any code = 1)	5.73	2.47	0.023	1.23	0.96	0.203	2.06	0.84	0.016	2.08	0.99	0.039
Age × PM status	0.29	0.19	0.123	0.04	0.07	0.596	0.13	0.06	0.049	0.11	0.08	0.156
Any comorbidity codes × age	0.69	0.18	<0.001	0.10	0.07	0.158	0.28	0.06	<0.001	0.27	0.07	<0.001
Any comorbidity codes × PM status	-7.01	3.86	0.073	-0.71	1.50	0.640	-2.85	1.31	0.033	-2.79	1.55	0.075
Age × PM status × any comorbidity codes	-1.05	0.28	<0.001	-0.21	0.11	0.052	-0.44	0.10	<0.001	-0.32	0.11	0.005
Constant	9.19	1.49	<0.001	4.06	0.58	<0.001	3.23	0.51	<0.001	1.46	0.60	0.017
R ²	0.511			0.294			0.538			0.424		

^aAge is mean centered.

The bolded row indicates the exact probability for the significance level rather than <.001 (the three-way interaction). For the total scale, the exact probability is P = .00034; For the ataxia sub-scale, the exact probability is P = .00001.

Abbreviations: FXTAS, fragile X-associated tremor/ataxia syndrome; b, regression coefficient; SE, standard error; PM, premutation.

became statistically significant in the regression models that did not include these participants (see Supplementary Table S4).

Subsequently, we restricted the analysis to participants who were aged 50 years or older (n = 69) to focus on those at or above the age at which clinical

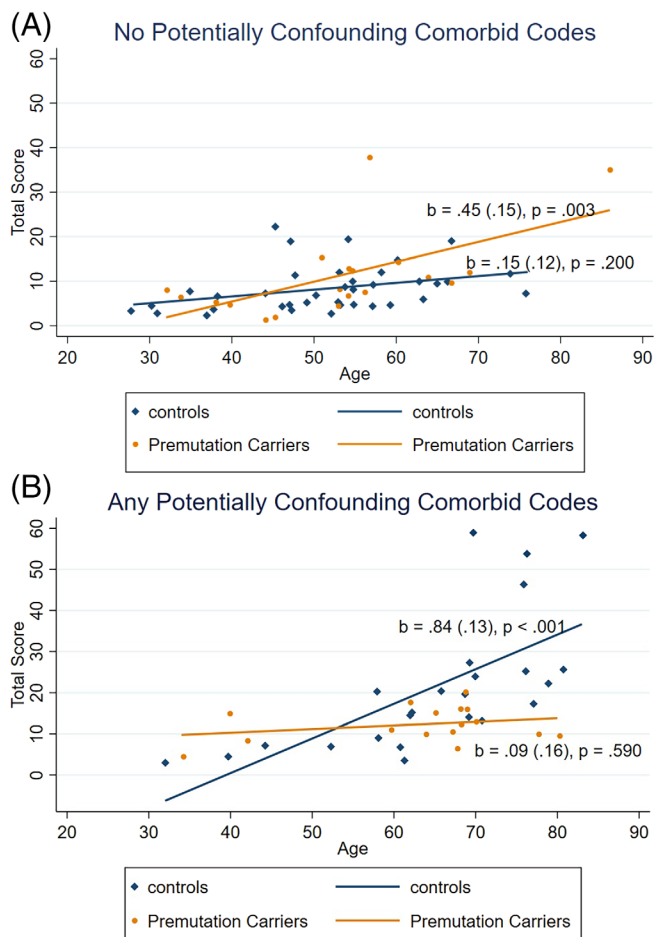


FIG. 2. Scatter plots and fitted lines showing three-way interactions: age, premutation status, and potentially confounding comorbid diagnoses codes on fragile X syndrome (FXS) Rating Scale total score. The fitted lines are drawn from data points, illustrating the bivariate associations between age and the total FXTAS (fragile X-associated tremor/ataxia syndrome) Rating Scale. For each fitted line, the unstandardized regression coefficient (simple slope), the standard error of the coefficient (in parentheses), and the *P*-value are presented. (A) Participants who did not have potentially confounding comorbid codes. (B) Participants who had any potentially confounding comorbid codes.

signs of FXTAS tend to be observed among premutation carriers. The same pattern of results was found as reported in Table 3, namely that among those without potentially confounding comorbidities, the premutation carriers had significantly greater impairments with advancing age, a pattern not evident among controls (see Supplementary Table S5).

Discussion

Based on a population-based cohort, the present study yielded information about FXTAS-type neurological signs in a group of premutation carriers not often evaluated in previous research—premutation women with lower numbers of CGG repeats, some of whom were younger than age 50 years, who were ascertained

in an unbiased fashion. Overall, these women had FXTAS Rating Scale scores that were no different from their peers who had CGG repeats in the normal range. As premutation carriers with shorter repeat lengths are much more common in the population than those with repeats in the upper premutation range, the present results are significant from a public health perspective.

Although none of the participants were diagnosed with FXTAS, and although initial comparisons did not reveal greater evidence of mild clinical FXTAS-type neurological signs in premutation carriers than controls, after comorbid diagnoses were controlled, an age-associated vulnerability of premutation carriers was observed with respect to the total FXTAS Rating Scale and the ataxia and parkinsonism subscales. In other words, it was only among otherwise-healthy carriers that we begin to see any impact of the CGG repeat. The same pattern was observed when we restricted the data to only those aged 50 years and older and to those who had no other movement disorder-type comorbid codes.

What is the clinical significance of these findings? It is valuable to note that the CGG repeat is not the dominant driver of related signs and symptoms in this cohort of women. The participants in the present study differ from many participants in past studies who generally had larger numbers of CGG repeats than those evaluated here.^{14,15,26} Nevertheless, there was evidence of a statistically significant pattern of ataxia and parkinsonism signs in premutation carriers who did not have potentially confounding diagnoses. This may suggest that these women were manifesting early signs of FXTAS, they have neurological signs that are not related to underlying FXTAS pathology, or they have other yet-to-be diagnosed movement disorders. Additional studies are warranted to determine which of these explanations accounts for the results. Future research should also probe the possible effect of reproductive aging on such symptoms, as premutation carriers experience menopause earlier than controls.

The study design addressed the risk of ascertainment bias by recruiting participants from a general health-care system rather than from families of individuals with FXS or from FXS specialty clinics. Thus, the study of this cohort can offer insights into how mild clinical FXTAS-type signs may be manifested in unselected premutation carrier women in the general population. A notable feature of the study is that it brought together sources of data—DNA, neurological assessments, and EHRs—that are not often integrated into a single research endeavor. Were it not for each of these diverse data sources and the integrative analytic approach, the underlying pattern of symptoms would not have been detected. Another strength of the present study was the double-blind approach and the inclusion of independent ratings from three neurologists with high interrater reliability.

The study was limited by a number of factors, particularly the small sample size, which limited statistical power. Due to this limitation, the present study warrants replication in larger cohorts. In addition, we did not have neuroimaging data needed for a formal FXTAS diagnosis. We also did not have access to activation ratios on participants, which have been found to be important in past studies using the FXTAS Rating Scale.¹⁴ Why the control group participants with confounding diagnoses were substantially more impaired than the other three groups is not clear. This pattern may be a chance finding of the present study, or it might point to an area for investigation in future research. Further, the scale may be picking up neurological signs related to other comorbidities rather than FXTAS itself. Finally, among women who did not have confounding diagnoses, an association between FXTAS Rating Scale scores and age was observed among premutation carriers but was not observed among controls; however, this observation was based on cross-sectional data and warrants confirmation in longitudinal research.

Conclusion

Women who have CGG repeats at the lower end of the premutation range may be at greater risk for ataxia and parkinsonism signs than their age peers, although their overall risk of developing such conditions is low. Importantly, the findings of the present study cannot be attributed to ascertainment bias. Rather, these signs were detected among an unselected group of premutation carrier women in the general population. The severity of such signs was more subtle than that which was observed in past studies of family members of children with FXS and only evident among otherwise-healthy carriers. This should provide reassurance to premutation carriers who share characteristics with the present cohort. ●

Acknowledgments: Grant support for this research was provided by R01 HD082110. Support was also provided by the Waisman Center Core Grant (U54 HD090256) and the Marshfield Clinic Research Institute. We specially thank the participants of the Personalized Medicine Research Project and Renee Makuch and Terrie Kitchener for their coordination of data collection across sites.

References

- Darnell JC, Van Driesche SJ, Zhang C, et al. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell* 2011;146(2):247–261. <https://doi.org/10.1016/j.cell.2011.06.013>.
- Brown W, Fragile X. In: Hagerman R, Hagerman P, eds. *Syndrome Diagnosis, Treatment and Research*. Baltimore, MD: The Johns Hopkins University Press; 2002.
- Nolin SL, Lewis FA 3rd, Ye LL, et al. Familial transmission of the FMR1 CGG repeat. *Am J Hum Genet* 1996;59(6):1252–1261. <https://pubmed.ncbi.nlm.nih.gov/8940270>.
- Seltzer MM, Baker MW, Hong J, Maenner M, Greenberg J, Mandel D. Prevalence of CGG expansions of the FMR1 gene in a US population-based sample. *Am J Med Genet B Neuropsychiatr Genet* 2012;159B(5):589–597. <https://doi.org/10.1002/ajmg.b.32065>.
- Tassone F, Iong KP, Tong T-H, et al. FMR1 CGG allele size and prevalence ascertained through newborn screening in the United States. *Genome Med* 2012;4(12):100. <https://doi.org/10.1186/gm401>.
- Hagerman R, Hagerman P. Advances in clinical and molecular understanding of the FMR1 premutation and fragile X-associated tremor/ataxia syndrome. *Lancet Neurol* 2013;12(8):786–798. [https://doi.org/10.1016/S1474-4422\(13\)70125-X](https://doi.org/10.1016/S1474-4422(13)70125-X).
- Roberts JE, Tonnsen BL, McCary LM, Ford AL, Golden RN, Bailey DB. Trajectory and predictors of depression and anxiety disorders in mothers with the FMR1 premutation. *Biol Psychiatry* 2016;79(10):850–857. <https://doi.org/10.1016/j.biopsych.2015.07.015>.
- Hagerman RJ, Hagerman P. Fragile X-associated tremor/ataxia syndrome — features, mechanisms and management. *Nat Rev Neurol* 2016;12(7):403–412. <https://doi.org/10.1038/nrneurol.2016.82>.
- Hall DA, Robertson E, Shelton AL, et al. Update on the clinical, radiographic, and neurobehavioral manifestations in FXTAS and FMR1 premutation carriers. *Cerebellum* 2016;15(5):578–586. <https://doi.org/10.1007/s12311-016-0799-4>.
- Berry-Kravis E, Abrams L, Coffey SM, et al. Fragile X-associated tremor/ataxia syndrome: clinical features, genetics, and testing guidelines. *Mov Disord* 2007;22(14):2018–2030. <https://doi.org/10.1002/mds.21493>.
- Jacquemont S, Hagerman RJ, Leehey MA, et al. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. *JAMA* 2004;291(4):460–469. <https://doi.org/10.1001/jama.291.4.460>.
- Allen EG, Charen K, Hipp HS, et al. Clustering of comorbid conditions among women who carry an FMR1 premutation. *Genet Med* 2020;22(4):758–766. <https://doi.org/10.1038/s41436-019-0733-5>.
- Hagerman RJ, Leavitt BR, Farzin F, et al. Fragile-X-associated tremor/ataxia syndrome (FXTAS) in females with the FMR1 premutation. *Am J Hum Genet* 2004;74(5):1051–1056. <https://doi.org/10.1086/420700>.
- Leehey MA, Berry-Kravis E, Goetz CG, et al. FMR1 CGG repeat length predicts motor dysfunction in premutation carriers. *Neurology* 2008;70(16 Part 2):1397. <https://doi.org/10.1212/01.wnl.0000281692.98200.f5>.
- Tassone F, Adams J, Berry-Kravis EM, et al. CGG repeat length correlates with age of onset of motor signs of the fragile X-associated tremor/ataxia syndrome (FXTAS). *Am J Med Genet B Neuropsychiatr Genet* 2007;144(4):566–569. <https://doi.org/10.1002/ajmg.b.30482>.
- Hall D, Tassone F, Klepitskaya O, Leehey M. Fragile X-associated tremor ataxia syndrome in FMR1 gray zone allele carriers. *Mov Disord* 2012;27(2):297–301. <https://doi.org/10.1002/mds.24021>.
- Liu Y, Winarni TI, Zhang L, Tassone F, Hagerman RJ. Fragile X-associated tremor/ataxia syndrome (FXTAS) in grey zone carriers. *Clin Genet* 2013;84(1):74–77. <https://doi.org/10.1111/cge.12026>.
- Shelton AL, Cornish KM, Kraan CM, Lozano R, Bui MI, Fielding J. Executive dysfunction in female FMR1 premutation carriers. *Cerebellum* 2016;15(5):565–569. <https://doi.org/10.1007/s12311-016-0782-0>.
- McCarty CA, Wilke RA, Giampietro PF, Wesbrook SD, Caldwell MD. Marshfield clinic personalized medicine research project (PMRP): design, methods and recruitment for a large population-based biobank. *Per Med* 2005;2(1):49–79. <https://doi.org/10.1517/17410541.2.1.49>.
- Maenner MJ, Baker MW, Broman KW, et al. FMR1 CGG expansions: prevalence and sex ratios. *Am J Med Genet B Neuropsychiatr Genet*. 2013;162(5):466–473. <https://doi.org/10.1002/ajmg.b.32176>.

21. Chen L, Hadd A, Sah S, et al. An information-rich CGG repeat primed PCR that detects the full range of fragile X expanded alleles and minimizes the need for southern blot analysis. *J Mol Diagn* 2010;12(5):589–600. <https://doi.org/10.2353/jmoldx.2010.090227>.
22. Grasso M, Boon EMJ, Filipovic-Sadic S, et al. A novel methylation PCR that offers standardized determination of FMR1 methylation and CGG repeat length without southern blot analysis. *J Mol Diagn*. 2014;16(1):23–31. <https://doi.org/10.1016/j.jmoldx.2013.09.004>.
23. Hall DA, Stebbins GT, Jacquemont S, et al. Clinimetric properties of the fragile X-associated tremor ataxia syndrome rating scale. *Mov Disord Clin Pract* 2019;6(2):120–124. <https://doi.org/10.1002/mdc3.12708>.
24. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. *Fam Med* 2005;37(5):360–363.
25. Gwet KL. Computing inter-rater reliability and its variance in the presence of high agreement. *Br J Math Stat Psychol* 2008;61(1):29–48. <https://doi.org/10.1348/000711006X126600>.
26. Jacquemont S, Leehey MA, Hagerman RJ, Beckett LA, Hagerman PJ. Size bias of fragile X premutation alleles in late-onset movement disorders. *J Med Genet* 2006;43:804–809.

Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.