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Neurological and Endocrine Phenotypes of Fragile X Carrier Women

Deborah Hall[1], **Kristina Todorova-Koteva**[2], **Shrikant Pandya**[3], **Bryan Bernard**[1], **Bichun Ouyang**[1], **Maura Walsh**[3], **Tamar Pounardjian**[3], **Cory Deburghraeve**[4], **Lili Zhou**[5], **Molly Losh**[6], **Maureen Leehey**[7], and **Elizabeth Berry-Kravis**[1,5,8]

[1]Department of Neurological Sciences, Rush University, Chicago, IL

[2]Department of Internal Medicine, Rush University, Chicago, IL

[3]Rush Medical College, Rush University, Chicago, IL

[4]Department of Anesthesia, University of Illinois,, Chicago, IL

[5]Department of Biochemistry, Rush University, Chicago, IL

[6]Roxelyn and Richard Pepper Department of Communication Sciences and Disorders, Northwestern University, Evanston, IL

[7]Department of Neurology, University of Colorado, Aurora, CO

[8]Department of Pediatrics, Rush University, Chicago, IL

Abstract

Introduction—Women who carry *fragile X mental retardation 1 (FMR1)*gene premutation expansions frequently report neurological or endocrine symptoms and prior studies have predominantly focused on questionnaire report of medical issues.

Methods—Premutation carrier women (n=33) and non-carrier controls (n=13) were recruited and evaluated by a neurologist, neuropsychologist, and endocrinologist. Blood and skin biopsies were collected for molecular measures. Scales for movement disorders, neuropathy, cognitive function, psychiatric symptoms, sleep, and quality of life were completed.

Results—The average age of the women was 51 years (n=46) and average CGG repeat size was 91 ± 24.9 in the *FMR1* premutation carrier women. Seventy-percent of the premutation carrier women had an abnormal neurological examination. Premutation carrier women had significantly higher scores on the FXTAS Rating Scale, more neuropathy, and difficulty with tandem gait compared to controls. Central sensitivity syndromes, a neuroticism profile on the NEO Personality Profile, and sleep disorders were also prevalent. Discrepancies between subject report and examination findings were also seen.

Corresponding author: Deborah Hall, MD PhD, Department of Neurological Sciences, Rush University, 1725 West Harrison Street, Suite 755, Chicago, IL 60612, Deborah_A_Hall@rush.edu.

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Conclusions—This pilot study suggests that women with the *FMR1* premutation may have a phenotype that overlaps with that seen in FXTAS. Additional research with larger sample sizes is warranted to better delineate the clinical features.

Keywords

fragile X-associated tremor/ataxia syndrome; fragile X mental retardation 1 gene; fragile X syndrome; premutation

INTRODUCTION

Fragile X-associated disorders result in significant morbidity. These disorders include intellectual disability, infertility, and progressive movement disorders later in life. They are caused by a CGG repeat expansion in the *fragile X mental retardation 1 (FMR1)* gene. Fragile X syndrome (FXS), the most common cause of inherited intellectual disability and autism, occurs in 1/4000 children and results from an expansion of >200 CGG repeats (full mutation), with resultant hyper-methylation, of the *FMR1* gene. All mothers of individuals with FXS are obligate carriers of a gene expansion, either the full mutation or the premutation (55–200 repeats). The prevalence rate for premutation carriers is 1/151–1/209 women in the USA.(1) Although children with FXS have been extensively studied, much less is known about the premutation carrier mothers. Primary ovarian insufficiency is seen in roughly a quarter of these women,(2) but surveys of premutation carrier (PMC) women suggest that other neurological and endocrine disorders may also be present.(3–5)

FMR1 has a long stretch of a variable number of trinucleotide repeats, mostly CGG with a few AGG interspersions at the 5’end.(6) Premutation carrier men with 55–200 repeats have higher than normal levels of *FMR1* mRNA and mildly reduced *FMR1* protein (FMRP) levels.(7) *Antisense FMR1* (*ASFMR1)* is a novel gene that that is transcribed in reverse across the CGG repeat region of *FMR1*. It is upregulated in *FMR1* PMC, with altered alternative splicing.(8) Elevated antisense transcripts may be associated with neurological phenotypes in *FMR1* PMC men with parkinsonism,(9) but have not been evaluated in PMC women with milder phenotypes. These molecular changes in PMC women may account for abnormal clinical phenotypes, similar to the molecular pathophysiologic mechanisms seen in FXTAS and PMC men.(10)

FMR1 PMC women have been shown to have primary ovarian insufficiency and psychiatric issues. In a study of 507 PMC women, ovarian dysfunction increased with *FMR1* repeat size up to 100 repeats.(2) A French-German cooperative study with psychiatric interviews showed that PMC women are more likely to be diagnosed with a social phobia than mothers of autistic children.(11) A more recent study using data from a national fragile X parent survey looked at co-occurring psychiatric conditions in 199 PMC women and showed that these women had frequently been diagnosed or treated for depression, anxiety, or attention problems.(12) In addition, the neurological signs of fragile X-associated tremor ataxia syndrome (FXTAS) occur in a small percentage of PMC women,(13) although these symptoms are much more common in PMC men due to the lack of a second protective X

chromosome. FXTAS is a progressive neurodegenerative disorder manifesting as kinetic tremor, gait ataxia, and executive dysfunction typically after the age of 55.(14)

In recent years, fragile X research groups have tried to better understand the genotype-phenotype relationship in *FMR1* PMC women by doing chart review and questionnaire studies, supplemented by a small amount of examination data. The first study included 334 PMC carrier women who were asked to self-report their medical history using a structured questionnaire covering neurological, psychiatric, and autoimmune disorders.(3) This study showed no difference in reporting of any questionnaire items compared to non-carriers. Specific questions related to FXTAS were not included, but women were asked to report all medical diagnoses. However, PMC women with ovarian insufficiency reported higher rates of thyroid problems, depression, and anxiety. In contrast, a second study investigating 146 PMC women with and without FXTAS compared to 69 age-matched controls showed a striking difference in medical symptoms.(5) This study showed that PMC women with definite or probable FXTAS had an increased prevalence of thyroid disease, hypertension, seizures, peripheral neuropathy, and fibromyalgia. The non-FXTAS premutation carriers had more complaints of muscle pain, persistent paresthesias, and history of tremor. A third smaller questionnaire study conducted in PMC women who were daughters of men with FXTAS showed that these women have a higher prevalence of neurological symptoms including tremor, balance issues, memory problems and dizziness, menopausal symptoms, sleep problems, and anxiety.(4)

It is imperative to determine the phenotype of PMC women given that they are the primary caregivers for children with fragile X syndrome and for their own parents with the neurodegenerative disease FXTAS. The anticipated outcome of this effort was that PMC women would have a distinct neurological and endocrine phenotype that could be described and targeted for treatment by their own health professionals. The overall purpose of this study was to further prior work by determining the neurological and endocrine phenotypes of PMC women using examinations and laboratory testing done by specialists in these fields. In addition, correlation with molecular characteristics was performed. This project was planned as a pilot study. The primary hypotheses were that FXTAS rating scale scores would be higher in PMC women, endocrinopathy more frequent, and that these relationships would be modified by molecular characteristics. Secondary hypotheses were that the Weschler Abbreviated Scale of Intelligence(15) (WASI) score would be lower and the anxiety inventory (Beck Anxiety Inventory, BAI)(16) and depression scale scores (Center for Epidemiologic Studies Depression Scale, CES-D)(17) would be higher in PMC women compared to controls. Other secondary hypotheses were that thyroid disease, adrenal dysfunction and pituitary problems would be more common in PMC women compared to controls. Exploratory hypotheses were that PMC women would have higher/abnormal scores on the neuropathy scale and other neuropsychological tests compared to controls.

MATERIALS AND METHODS

Study Participants

Subjects were recruited through the Fragile X-associated Disorders Program at Rush University. The majority were women who had a family history of fragile X-associated

disorders and had either children or parents who were being treated in the program. Women had to be over the age of 18 and only two women were recruited from each family to reduce bias. Controls were either friends of the subjects, in-laws, or healthy volunteers who were recruited from advertising of the study in the general population. All interested controls who qualified were included. The study was approved by the Rush Institutional Review Board.

Neurological Measurements—Each subject saw a neurologist (DAH) who performed a neurological history and standardized neurological examination. The following scales were chosen and performed based on abnormalities reported in prior PMC and FXTAS studies. Movement disorders were evaluated using the FXTAS Rating Scale. This scale is a tool developed to measure the motor signs of FXTAS(18) and quantifies the phenomenology and severity of tremor and balance problems. The scale was constructed by combining three published rating scales commonly used to assess tremor, ataxia, and parkinsonism.(19–21) Neuropathy was evaluated using two scales: the World Health Organization (WHO) classification scale for neuropathy-related symptoms and the Total Neuropathy Score, which was modified to only include the grading of symptoms and signs (without nerve conduction studies and quantitative sensory tests).(22) Headache classification using the International Classification of Headache Disorders, 2nd Edition was used to determine the presence and type of headache in each woman.(23) Each woman was asked regarding the presence of 'central sensitivity syndromes' based on standardized definitions, to include: chronic fatigue syndrome, irritable bowel syndrome, temporomandibular disorder, myofascial pain syndrome, restless legs syndrome, periodic limb movements of sleep, multiple chemical sensitivity, primary dysmenorrheal, female urethral syndrome, and post-traumatic stress disorder.(24) Diagnostic criteria for fibromyalgia were performed.(25)

Neuropsychological Measurements

A neuropsychologist (BB) evaluated each subject, blinded to gene status, with the following measures chosen due to prior studies published in PMC and FXTAS. The WASI provided verbal IQ, performance IQ, and scores on tests of specific ability.(15) The Behavioral Dyscontrol Scale is a brief scale of executive function and was used to test the ability to regulate purposeful, goal-directed activity and perform activities of daily living.(26) The Controlled Oral Word Association Test measures verbal fluency and correlates with measures of executive function.(27) The Symbol Digit Modalities Test measures processing speed.(28) Digit Span Forward, Backward, and Sequencing measures attention and working memory by having the subject repeat a sequence of numbers forward, backward, and in sequential order.(29) The Logical Memory Test is a brief memory measure which has the subject remember details of a paragraph.(29)

The following surveys were administered, scored, and interpreted by the neuropsychologist and neurologist. The BAI is a questionnaire which queries symptoms of anxiety during the past week, to include nervousness, inability to relax, and heart pounding or racing.(16) The CES-D Scale was used to measure depressive symptoms.(17) The Sickness Impact Profile was used for comparing health status and assessing quality-of-life.(30) The NEO personality inventory was used to assess quantitative dimensions of normal personality traits.(31) Sleep questionnaires were completed and included the Epworth sleepiness scale to measure

daytime sleepiness in adults(32) and the Pittsburgh Sleep Quality Index (PSQI), which assesses sleep quality and disturbances over a one month interval.(33)

Endocrine Measurements

An endocrinologist evaluated each patient, blinded to gene status, with an examination and review of the following endocrine laboratory studies drawn at the study visit. The presence or absence of an endocrinopathy was determined by the evaluating endocrinologist. The endocrine labs were chosen by the endocrinologist as appropriate tests to diagnose normal function of each endocrine organ. Thyroid disease was defined by abnormal level of thyroid blood test (TSH, T4, and/or thyroid antibodies: thyroid peroxidase and thyroglobulin) or in patients who had known thyroid disease and were on thyroid medication. Ovarian dysfunction was defined by a high level of FSH in premenopausal women who have not had oophorectomy. In most cases, pre-menopausal women had a day 3 FSH. Pituitary dysfunction was screened by prolactin level, and patients who had high prolactin level (above upper end of normal) were suspected to possibly have pituitary dysfunction. Adrenal dysfunction was screened by 8 am cortisol and ACTH, and patients who had 8 am cortisol <10 mcg/dl underwent 250 mcg cosyntropin stimulation test to rule out adrenal insufficiency. A cortisol level above 18 mcg/dl at 30 min or 60 min after 250 mcg Cosyntropin ruled out adrenal insufficiency. Hemoglobin A1c was added partway through the study. Pre-Diabetes was defined as HbA1c 5.7–6.4% and diabetes as >6.5%.

Molecular Measurements—Serum samples and full thickness skin biopsies were performed with a 3mm punch under local anesthesia with lidocaine. Blood samples were sent to the Rush University Molecular Diagnostic Laboratory (Berry-Kravis lab) for molecular testing. DNA was isolated from blood samples. *FMR1* PCR with quantification of allele-specific CGG repeat length and identification of AGG interspersions(34, 35) was performed by utilizing commercially available kits (Asuragen, Inc., Austin TX). Analysis and calculation of the CGG repeat size and the activation ratio (the percent of cells with the normal X on the active X chromosome) were carried out via densitometric image analysis as described in previous studies.(36) RNA was purified using Qiagen's RNeasy kit, treated with DNase, screened for contamination, then cDNA synthesis was performed and cDNA amplified using primers for *FMR1* mRNA anti-sense *FMR1* (*ASFMR1)* mRNA and splice variants. Relative expression of *FMR1* mRNA and *ASFMR1* splice variants was quantified using real time PCR as described using a standard curve assay and compared to a control assay of *GUS* expression.(8) *ASFMR1* splice variant levels were determined in fibroblasts. For FMRP, lymphocytes were isolated from 10 ml human whole blood and cells were lysed in cell lysis buffer and total protein was quantified using the BCA protein assay (Thermo Scientific Cat # 23235). FMRP was quantified by Luminex sandwich capture immunoassay adapted from LaFauci et al.(37)

Statistical Analyses

Statistical software (SAS 9.2) was used to analyze the data. To evaluate the primary hypothesis, specifically the association of neurological signs and the presence of a premutation as the primary outcome measure, Wilcoxon rank-sum test was used to compare the FXTAS motor rating scale score between the PMC and controls. To evaluate the primary

hypothesis of an association of endocrinopathies and the presence of a premutation, chi-square test was used to compare the presence of an endocrinopathy between PMC and controls. The primary hypotheses were controlled for age using regression models. Correlation analysis and Wilcoxon rank-sum test was performed to evaluate the association between gene effects (activation ratios, AGG interspersions, level of FMRP, *FMR*1 mRNA, and *ASFMR1* transcript levels) and the neurological or endocrine phenotype in PMC as part of the primary hypotheses. Post-hoc analysis was performed with adjustment of age in any significant association between gene effects and the neurological or endocrine phenotype in PMC. Analysis for the secondary hypotheses utilized the presence or absence of thyroid disease, ovarian dysfunction, or adrenal/pituitary problems as determined by the endocrinologist comparing between carriers and controls. Additional secondary hypotheses included analysis of the neuropsychological tests (WASI, BAI, CES-D) in relation to the molecular measures. Finally, exploratory analysis was performed using the neuropathy scales, presence of movement disorders, additional neuropsychological tests, endocrine laboratories, central sensitivity syndromes, Sickness Impact Scale, and NEO personality domains. Secondary and exploratory analyses were measured with either unpaired *t* test, Wilcoxon rank-sum, or Chi-square used as appropriate.

RESULTS

The following women were recruited during the two year duration of the study: *FMR1* PMC (n=33) with average age of 54.2 ± 16.8 years and control women (n=13) with an average age of 47 ± 10.1 years (p=0.18). Average CGG repeat size was 91 ± 24.9 in the *FMR1* PMC women and 31 ± 6.4 in controls (p<0.0001). Ninety-one percent had a family history of fragile X-associated disorders, while only 15% of the controls had a family history (p<0.0001), as expected. Other demographics are located in Table 1.

Seventy-three percent (24/33) of the *FMR1* PMC women and 23% (3/13) of the controls had an abnormal neurological examination (p=0.002) (Table 2). *FMR1* PMC women had significantly higher scores on the FXTAS Rating Scale: with a median of 7 (Interquartile Range, IQR=8) compared to controls at 3 (IQR=5,p=0.005). *FMR1* PMC women had more difficulty with tandem gait (30% vs. 0%, p=0.02) and the Total Neuropathy Score was higher in the *FMR1* PMC women with a median of 2 (IQR=3) compared to controls (median= 0, IQR=1; p=0.02). There were two women who had tremor, ataxia, and prior MRI scans who met criteria for FXTAS. Prevalence of dystonia was higher in PMC women compared to controls, however it did not reach statistical significance [8/33 (24.2%) vs. 0/13, p=0.08]. The number of diagnoses of central sensitivity syndromes was higher in the PMC women compared to controls (p=0.005). PMC women had higher scores on the Sickness Impact Scale than controls [median(IQR): 34.6(85.1) vs. 0(14.6), p=0.005]. There was a significant difference in the NEO personality inventory between groups, with the NEO neuroticism domain at 92.2 ± 38 in PMC women compared to 72.4 ± 18.3 in controls (p=0.02). On the neuropsychological measures, there was no difference between PMC and controls on the WASI (p=0.35) and the CES-D (p=0.15), but PMC women had higher BAI score than controls (7.8 ± 7.5 vs, 3.6 ± 3.5, p=0.01).

There was no significant difference between the presence of an endocrinopathy and specifically thyroid disease and ovarian dysfunction between carriers and controls (all p's > 0.2), although 5/33 PMC women had FXPOI. For the molecular aims, we did not find any significant correlation between the FXTAS motor rating scale score and CGG repeat size (p=0.31), *ASFMR1* spice variant levels (p=0.48), activation ratio (p=0.33), *FMR1* mRNA (p= 0.75), and FMRP (p=0.68) using Spearman correlation. There was no significant difference in these molecular measures between carriers with and without endocrinopathy (all p's > 0.05). There was a negative correlation between FXTAS motor rating scale and AGG number (p=0.005), but this relationship was no longer significant after controlling for age which was performed post-hoc. There was no significant correlation with endocrinopathy, sleep, BDS, neuropathy and memory. CGG length was positively associated with neuropathy (p=0.03) due to one observation of a neuropathy score of 10 and CGG of 200, and when removed, the association was no longer significant (p=0.07).

Prominent unexpected discrepancies were noted between the report of symptoms from the PMC subjects and the results on examination or questionnaires, with the following as examples. Thirty percent (10/33) of PMC women reported the presence of a memory disorder, but had normal memory on the Logical Memory Test, while 6% (2/33) of PMC had abnormal results but did not report memory issues (p=0.04). Thirty-three percent (11/33) of PMC reported the presence of an anxiety disorder despite normal scores on the BAI (although two of these women were on anti-anxiety medications), while all subjects with an abnormal BAI reported anxiety (p=0.001). Thirty-nine percent (13/33) of women denied any issues with sleep, despite having an abnormal high score on either the Epworth or Pittsburgh scales while only 6% (2/33) reported sleep issues but did not have abnormal scores (p=0.007). None of these women were on sleep aids. Seventy-two percent (24/33) of women denied a history of migraine headaches during the interview of past medical illness, but met criteria for migraine when evaluated by the study neurologist or reported having been diagnosed with migraine in the past by a physician on a questionnaire and no subject who did not have a diagnosis or meet diagnostic criteria reported a history of migraine (p<0.0001).

DISCUSSION

The results of this study show that *FMR1* PMC women have a neurological phenotype which overlaps with the phenotype seen in FXTAS. In addition, PMC women have a higher likelihood of central sensitivity syndromes, higher scores on the Sickness Impact Scale, more anxiety, and higher scores on the neuroticism domain of the NEO. However, they did not have confirmed endocrine dysfunction, depression, nor cognitive issues at higher rates than controls on examination. In addition, the molecular measures did not predict phenotype in this study.

One of the primary outcome measures chosen for this study was the FXTAS Rating Scale which was significantly different between PMC women and controls. Our results showing higher rates of neuropathy and tandem gait abnormalities in PMC are consistent with prior studies in this population.(4, 38) The FXTAS Rating Scale emphasizes those movement disorder features that are prominent in FXTAS, including tremor, ataxia, and parkinsonism,

and the higher scores on this scale seen in the PMC women is an important result of this study. The results suggest that detailed neuro-pathological studies of these women are needed to determine if these women are on a spectrum with individuals with FXTAS (only milder) or whether there is an entirely different pathophysiology involved. MRI was not performed as part of this pilot study and it is likely that more than two women would meet diagnostic criteria if this were added.

Endocrinopathies were not confirmed in our population, despite multiple questionnaire studies reporting them in PMC women in the past. The presence of thyroid disease in women in the general population is approximately 3.3% to 7%, which is less than that seen in our study.(39) However, in the United States National Health and Nutrition Examination Survey (NHANES III), 3702 women without known thyroid disease had thyroid antibodies and these levels rise from 14% to 30% for both anti-thyroid peroxidase and anti-thyroglobulin antibodies as women age (>30 years).(40) These numbers may be consistent with those seen in our study. Our control population had two women with reduced ovarian reserve, which is higher than would be expected in a typical control population and probably due to recruitment bias, likely caused a lack of significance between cases and controls. Adrenal disorders are rare and not ascertained in our study, with reported prevalence rates of 6/100,000 people for adrenal insufficiency (41) and 1/100,000 for Cushing syndrome.(42) To our knowledge, this is the first study utilizing both endocrine consultation and diagnostic endocrine laboratory testing in a blinded manner in PMC women. Although the results did not show differences, a larger follow up study is warranted to evaluate thyroid disease in PMC.

Central sensitivity syndromes (CCS), which comprise an overlapping and similar group of syndromes without structural pathology, are believed to be bound by a common mechanism of central sensitization or hyper-excitement of central neurons.(43) Genetic factors may play a role in these syndromes as evidenced by twin and family studies. Neuroendocrine and immune dysfunction are postulated to be causal.(43) PMC women in this study had higher numbers of CCS diagnoses and symptoms compared to controls, in addition to having higher scores on the Sickness Impact Scale. Hypersensitivity in these women to various phenomena, such as somatic and visceral stimuli, may not only be accounting for the increase in CCS diagnoses, but may also be accounting for some of the discrepancy in reporting of illness. A rheumatologist who specializes in these disorders was not included in this study, but would be helpful to examine this relationship further.

The NEO Personality Inventory tests five domains to understand personality at the broadest level. The domains are neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness. The scores on the neuroticism domain were elevated in the PMC women compared to controls. This domain contains items measuring anger, depression, self-consciousness, impulsiveness, anxiety, and vulnerability to stress. Each of the facets of the neuroticism domain independently contribute to negative affect and lower life satisfaction.(44) Additionally, clinicians who see patients with high anxiety, hostility, self-consciousness, and depression can be confident that they has pervasive psychological distress. This result is consistent with prior studies in this area that show mothers of children with FXS are at elevated risk of high parenting stress and poor psychological health.(12, 45) There also

appears to be a direct relationship between CGG repeat size, number of negative life events, and depressive and anxiety symptoms.(46) Our data does not clarify whether the neuroticism profile of the PMC is caused from the environment or a genetic vulnerability of these women, but may contribute to over-reporting of symptoms in the psychological realm.

Throughout the course of the study, the specialists examining the subjects noticed discrepancies between historical information from the women and findings on examination and testing. Indeed, on analysis, PMC women over-reported symptoms of anxiety and the presence of memory disorders, but at the same time under-reported sleep problems and a history of migraines. The control women did not have the same discrepancies. This finding may explain discrepant questionnaire study results in the literature in the past and suggests the importance of using objective measures of diagnosis of neurological or endocrine disorders in this patient population.

It was predicted that the molecular results would show that secondary gene effects play a role in neurological and endocrine phenotypes in PMC women. Secondary gene effects or other molecular factors are a likely explanation for the wide phenotypic variability seen in families with multiple PMC sisters who all have similar CGG repeat sizes. In our study, we did not discover any associations, but these results should be interpreted with caution given the small sample size of this study.

This study was designed as a pilot study in order to plan a larger phenotype study in this patient population. The main limitation of the study was a small sample size, especially in the control group. The low sample size prevented the use of multivariate analysis techniques and investigation of confounders in our results. However, our results suggest that a larger project, ideally multi-center, with direct specialist examinations would be ideal to define phenotypes related to being a PMC woman. The addition of an evaluation with a rheumatologist and psychiatrist would also be critical based on the results of prior studies and our pilot data of poor self-report of illnesses in these areas.

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Table 1

Demographics of Study Participants

	Carriers (n=33)	Controls (n=13)	p value
Age	54.2 ± 16.8	47 ± 10.1	0.18
Race	96% white	92% white	0.22
Ethnicity	12% Hispanic	0 Hispanic	0.19
Educational Level	15.5 ± 2.3	15 ± 2.7	0.9
Marital status	57% married	76% married	0.6
CGG longest	91 ± 24.9	31 ± 6.4	0.001
Family history FXS	90%	15%	0.001
Family history FXTAS	78%	0	0.001
Family history FXPOI	12%	0	0.2

Table 2

Results

Measure	Carriers (n=33)	Controls (n=13)	p value
Primary outcome measures			
FXTAS Rating Scale, median (IQR)	7 (8)	3 (5)	0.005
Endocrinopathy	54%	33%	0.21
Secondary outcome measures			
WASI, mean (sd)	110 (9.8)	114 (22.3)	0.35
Beck Anxiety Inventory, mean (sd)	7.8 (7.5)	3.6 (3.5)	0.01
CES-D, mean (sd)	10.4 (9.1)	6.4 (5.8)	0.15
Thyroid disease	38%	31%	0.67
Ovarian dysfunction	32%	16%	0.31
Pituitary/adrenal dysfunction	0	0	n/a
Exploratory outcome measures			
Abnormal neurological exam	73%	23%	0.002
Tandem gait	30%	0	0.02
Tremor	39%	23%	0.49
Dystonia	24%	0	0.08
Total Neuropathy Score, median (IQR)	2 (3)	0 (1)	0.02
Behavioral Dyscontrol Scale, mean (sd)	25.4 (1.3)	25.8 (1)	0.23
Logical Memory Test – D, mean (sd)	10.6(2.5)	12 (1.9)	0.08
Digit Span, mean (sd)	9.9(2.3)	9.8(2.3)	0.9
Symbol Digit Modalities Test, mean (sd)	98.2(12.9)	107.4(6.9)	0.003
Controlled Oral Word Association, mean (sd)	99.1(20)	99.7(13.2)	0.92
Thyroid Antibodies*	28%	23%	1
Low 8am Cortisol Level (<10μg/dl)	43%	64%	0.26
Pre-Diabetes (HbA1c 5.7–6.4%)	20%	0%	0.15
Central sensitivity syndromes, mean (sd)	3.4(1.8)	1.7(1.7)	0.005
Sickness Impact Scale, median (IQR)	34.6 (85.1)	0 (14.6)	0.005
NEO Personality Scale Domains, mean (sd)			
Neuroticism	92.2 (38)	72.4(18.3)	0.02
Extraversion	111.5 (25.8)	120.2 (20.7)	0.29
Openness to Experience	115.9 (15.4)	114.5 (14.9)	0.79
Agreeableness	127.9 (15.5)	129.3 (10.2)	0.77
Conscientiousness	123.7 (19.5)	121.1 (12)	0.65

Key: IQR, interquartile range; FXTAS, Fragile X-associated tremor/ataxia syndrome; WASI, Weschler Abbreviated Scale of Intelligence; CES-D, Center for Epidemiologic Studies Depression Scale; HbA1c, hemoglobin A1c;

*Thyroid peroxidase and thyroglobulin antibodies

Table 3

Central Sensitivity Syndromes Questionnaire Results.

	Carriers (n=33)		Controls (n=13)	
Central Sensitivity Syndrome	**Patient Reported**	**Physician Diagnosis**	**Patient Reported**	**Physician Diagnosis**
Chronic Fatigue Syndrome	0	1	0	0
Irritable Bowel Syndrome	4	7	0	0
Tension Headaches	10	11	5	2
Migraine Headaches	2	13*	1	1
Temporomandibular Disorder	8	11	2	1
Myofascial Pain Syndrome	3	0	0	1
Restless Legs Syndrome	5	4	1	0
Periodic Limb Movements in Sleep	6	1	0	0
Multiple Chemical Sensitivity	0	0	0	0
Primary Dysmenorrheal	10	5	5	0
Female Urethral Syndrome/Interstitial Cystitis	8	0	0	0
Post-traumatic Stress Disorder	0	1	1	1
Fibromyalgia	3	1	0	1

The patients were asked if they thought they had the disorder (Patient Reported Column) and if a physician had given them the diagnosis of the disorder (Physician Diagnosis Column). Unless noted, results were not statistically significant between groups.

Key:

* p=0.04 for physician diagnosis

Table 4

Molecular Measures.

Molecular Measure	FXTAS Rating Scale Score	Endocrinopathy
CGG Repeat Size	0.18	0.08
AGG Interspersions	−0.47*	0.89
ASFMR1 Splice Variant Levels	0.13	0.57
Activation Ratio	−0.17	0.89
FMRP	−0.08	0.81
FMR1 mRNA	−0.06	0.4

The FXTAS Rating Scale score is reported as a correlation coefficient with * indicating p-value < 0.05. Presence of an endocrinopathy (by Wilcoxon) is reported as a p-value.