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Immune-mediated Disorders among Women Carrier of Fragile X Premutation Alleles

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Abstract

The relative risk of immune-mediated disorders (IMDs) among women carrier of premutation alleles is estimated by a survey for IMDs among 344 carrier women (age 19 to 81 years; mean 46.35 and SD 12.60) and 72 controls (age 18 to 87 years; mean 52.40 and SD 15.40). One hundred fifty four (44.77%) women carrier had at least one IMD, as did 20 controls (27.78%). Among women carrier, autoimmune thyroid disorder was the most common (24.4%), then fibromyalgia (10.2%), irritable bowel syndrome (IBS; 9.9%), Raynaud's phenomenon (7.6%), rheumatoid arthritis (RA; 3.8%), Sjögren syndrome (2.6%), systemic lupus erythematosus (SLE; 2.03%), multiple sclerosis (1.74%). Of 55 carriers age 40 or older with FXTAS, 72.73% had at least one IMD, compared to 46.54% of those without FXTAS (n=159), and 31.58% of controls (n=57). The estimated odds ratio (OR) for IMD is 2.6 (95% CI 1.2–5.6, p = 0.015) for women with FXTAS relative to those without FXTAS; the likelihood of IMD in carriers without or with FXTAS was also significantly higher than for controls (OR 2.1, 95% CI 1.1–4.2, p = 0.034; OR 5.5, 95% CI 2.4–12.5, p < 0.001 respectively). Similarly, the odds of having an IMD among carriers with FXPOI is about 2.4 times higher when compared to carriers without FXPOI (95% CI 1.1–5.0; p =

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Conflict of interest

Randi Hagerman has received funding from Seaside Therapeutics, Roche, Novartis, Forest, and Curemark to carry out treatment trials. Paul Hagerman and Flora Tassone are uncompensated collaborators with Asuragen, Inc., and hold a US patent for expanded-CGG screening. There are no other conflicts of interest from the authors.

0.021). The likelihood of IMD in carriers with or without FXPOI is greater (OR 2.4, 95% CI 1.1–5.0; p = 0.021) compared to that of controls.

Keywords

Autoimmune; FXTAS; RNA toxicity; ovarian insufficiency

INTRODUCTION

Premutation alleles of the fragile X mental retardation 1 (*FMR1*) gene comprise an expanded trinucleotide (CGG) repeat element in the 55–200 repeat range, which is located in the 5' untranslated region of the gene [Maddalena et al., 2001]. The prevalence of the premutation allele is 1 in 130–259 women and 1 in 250–813 men in the general population [Fernandez-Carvajal et al., 2009; Hagerman, 2008; Rousseau et al., 1995]. Several disorders are associated with the premutation allele; including fragile X-associated primary ovarian insufficiency (FXPOI; cessation of menses before age 40) [Murray A, 1995; Sullivan et al., 2005; Uzielli et al., 1999]; fragile X-associated tremor/ataxia syndrome (FXTAS) [Hagerman et al., 2001; Leehey et al., 2007]; psychiatric dysfunction, including depression and anxiety [Bourgeois et al., 2010; Roberts et al., 2009]; and hypertension [Coffey et al., 2008; Hamlin et al., 2012]. Recently, immune-mediated disorders (IMDs), specifically autoimmune thyroid disorder (AITD) and fibromyalgia, were found to be associated with the premutation in women with FXTAS compared to age-matched controls [Coffey et al., 2008]. Similar problems in women premutation carriers were reported by Rodriquez-Revenga et al. [Rodriguez-Revenga et al., 2009], where 18.6% had FXPOI, 15.9% had thyroid disease, and 24.4% had chronic muscle pain.

The pathogenesis of premutation disorders is related to a gain-of-function effect from elevated levels of the expanded CGG-repeat *FMR1* mRNA, which is present in all carriers [Garcia-Arocena and Hagerman, 2010; Greco et al., 2002; Shan et al., 2008; Tassone et al., 2007; Tassone et al., 2000]. Consistent with this pathogenic model, *FMR1* mRNA is found in the intranuclear inclusions in carriers with FXTAS [Tassone et al., 2004]; such inclusions are found not only in the CNS, but also in the peripheral nervous system and in many organs including the adrenal, heart, testes and islets of Langerhans [Hunsaker et al., 2011]. The expanded-CGG-repeat mRNA is thought to partially sequester proteins (e.g., Sam68 [Sellier et al., 2010b] and Drosha/DGCR8 [Sellier et al., 2010a] that are important for diverse cellular functions, which leads to a functional insufficiency as is the case for muscleblind-like 1 (*MBNL1*) in myotonic dystrophy [Garcia-Arocena and Hagerman, 2010; Lee and Cooper, 2009; Wheeler and Thornton, 2007].

In the current study, we sought to determine the type and frequency of IMDs present in premutation women who have participated in our research studies. We also examine the relative likelihood of IMD in premutation carriers with and without FXTAS, as well as those with and without FXPOI.

MATERIAL AND METHODS

Participants

Participants comprised 344 premutation women age 19 to 81 years (mean 46.35 and SD 12.60) who were recruited through the Fragile X Treatment and Research Center at the MIND Institute at University of California, Davis, and who participated in our Genotype-Phenotype study of families with fragile X, or our study of individuals with FXTAS, between the years 2000 to 2011. The study also includes 72 women control age 18 to 87 years (mean 52.40 and SD 15.40). Some of the subjects were included in a previous article that described results of their medical evaluation (128 premutation carriers and 19 controls) [Coffey et al., 2008]. Fifty-six of 344 premutation women (16.3%) were diagnosed with FXTAS utilizing criteria reported by Jacquemont et al [2003].

METHODS

We surveyed the frequency of IMDs through medical history and, in some cases, the review of medical records, specifically about the occurrence of AITD, multiple sclerosis (MS), Sjögren syndrome, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Raynaud's phenomenon, irritable bowel syndrome (IBS), and optic neuritis. Table I provides a descriptive reference to these disorders in reference populations and our control group.

Our medical assessment is described in Coffey et al [2008], and includes demographic data, birth history, early developmental milestones, behavior problems, psychological history, medical history, neurological history, medications, and medical examination and neurological examination by physicians (Dr. R. Hagerman or physicians trained and supervised by her). A subject was considered to have an IMD if diagnosed and treated by a physician for that condition. Medical records were requested when an IMD was identified. Informed consent was obtained from all subjects who agreed to participate in our study; the protocol and consent form are approved annually by the University of California, Davis, Institutional Review Board.

Molecular Studies

A blood sample for measurement of CGG repeat size, methylation status, and *FMR1* mRNA levels was obtained from each subject. *FMR1* mRNA quantification, Southern blot, and PCR-based genotyping were performed as described previously [Tassone et al., 2000; 2008].

Statistical Analysis

We included all 416 subjects (344 premutation carriers and 72 controls) in our descriptive analysis to fully summarize IMDs involved in premutation carriers both with and without FXTAS (Table II). However, to examine the association between IMDs and FXTAS, we report results using logistic regression models (adjusted for age) based on individuals aged 40 or greater. This analysis was done to match the relevant age range for FXTAS, since FXTAS is not present in teens, and FXPOI by definition cannot be fully defined until age 40, resulting in data for 271 subjects. We report odds ratio (OR) estimates and their

associated 95% confidence interval (CI). The primary outcome in the logistic regression models is defined as the presence/absence of (any) IMDs. Logistic regression was also used to assess the association with molecular variables (CGG repeats, mRNA levels, and activation ratio/AR), as well as the association of FXPOI with IMDs. Univariate (unadjusted) analysis, stratified by FXTAS/non-FXTAS status, is based on Fisher's exact test.

RESULTS

Summary of IMDs in premutation carriers and controls

Among 344 premutation carrier women, 154 (44.77%) had at least one IMD. AITD was the most common (24.4% of study participants), followed by fibromyalgia (10.2%), IBS (9.9%), Raynaud's phenomenon (7.6%), RA (3.8%), Sjögren syndrome (2.6%), SLE(2.03%), MS (1.74%), and optic neuritis (0.58%). We also compared the frequencies of IMDs in premutation carriers compared to controls. (Tables II, III, and IV). In Table I, the frequencies are compared with previous estimates in reference populations. Only fibromyalgia and AITD have been shown to be significantly different between carriers and controls in a previous study [Coffey et al., 2008]. Table II summarizes the observed proportions of specific IMDs by genotype and FXTAS/non-FXTAS status for carriers, as well as for controls. We observed significant differences between carriers with and without FXTAS, and between carriers with FXTAS and controls, only for AITD and fibromyalgia. The observed prevalences for these IMDs are highest for carriers with FXTAS, followed by carriers without FXTAS, and then controls; however, the higher observed prevalences in carriers without FXTAS were not statistically different from those of controls. A similar pattern of results was observed among subjects aged 40 or older (Table III). We note that the results described here (from Table II) should be interpreted descriptively. Table III, in conjunction with the results of the next section, provide the basis for inferring the association between IMDs and FXTAS status.

Association of FXTAS and FXPOI with immune-mediated disorders

To assess the possible association of IMDs with FXTAS, FXPOI, and *FMR1* molecular variables, we consider the cohort of women of age 40 or older (cohort summary in Table III; n=271). In this cohort, 40 of 55 (72.73%) of premutation women with FXTAS had IMDs, compared with 74/159 (46.54%) of carriers without FXTAS, and 18/57 (31.58%) of control. The observed frequencies of IMDs for carrierss of age 40 or older, with and without FXPOI (cohort summary in Table IV; n=238) are 27/41 (65.85%) for those with FXPOI, 67/147 (45.58%) for carriers without FXPOI; IMDs were present in 17/50 (34.00%) of women control. Although the age ranges are similar for women of age 40 or older, the mean ages differ among the three study groups: controls (mean 58.98, SD 8.88) vs. the FXTAS group (mean 63.44, SD 9.80), $p = 0.006$; controls vs. the non-FXTAS carrier group (mean 50.33, SD 7.82) $P<0.001$; and FXTAS vs. non-FXTAS groups ($p<0.001$). Thus, we determined the age-adjusted odds ratio for IMDs using logistic regression. The estimated odds ratio (OR) for IMD is 2.6 (95% CI 1.2–5.6, $p = 0.015$) for women premutation carriers with FXTAS relative to carriers without FXTAS. Similarly, the likelihood of IMD in carriers without or with FXTAS was also significantly higher than controls (OR 2.1, 95% CI 1.1–4.2, p =

0.034; OR 5.5, 95% CI 2.4–12.5, p < 0.001 respectively). With respect to FXPOI, the odds ratio of IMDs among women premutation carriers with FXPOI is about 2.4, higher when compared to carriers without FXPOI (95% CI 1.1–5.0; p = 0.021); similarly, compared to controls without POI, the likelihood of IMD is higher in both carriers without and with FXPOI (OR 2.0, 95% CI 1.0–4.0, p = 0.050; OR 4.8, 95% CI 1.9–11.8, p < 0.001 respectively).

We also examined the association/effects of CGG expansion size, *FMR1* mRNA level, and activation ratio with IMDs, and we found that none of these measures were associated with IMDs in women premutation carrier.

DISCUSSION

Premutation alleles of the *FMR1* gene were originally thought not to be associated with clinical symptoms; their significance being their propensity for expansion to a full mutation allele (>200 CGG repeats) when transmitted by a woman carrier to the next generation. However, since 2000, it has been recognized that premutation alleles generate 1.5 to 8-fold elevated levels of *FMR1* mRNA [Tassone et al., 2000]. The excessive levels of the expanded CGG-repeat *FMR1* mRNA result in a gain-of-function “toxicity”, which is thought to cause psychiatric problems in mid-life, including depression and anxiety [Bourgeois et al., 2010; Roberts et al., 2009]; FXPOI [Sullivan et al., 2005]; autonomic dysfunction, including hypertension [Coffey et al., 2008; Hamlin et al., 2012]; and the progressive neurological disorder, FXTAS, which is usually milder in women than in men [Adams et al., 2007; Hagerman et al., 2008].

Prior reports by Coffey et al. [2008] and Rodriquez-Revenga et al. [2009], of IMDs in women premutation carrier provided the stimulus for the current study, where a variety of IMDs were surveyed in the medical history of 344 women carrier and 72 controls, who participated in our studies, either because their children had fragile X syndrome, or because they themselves were having some form of clinical involvement (e.g., FXTAS). Among women (age ≥ 40) in the current study, we found that 46.54% of those without FXTAS experienced one or more of the IMDs surveyed, and the prevalence increased to about 72.73% for those with FXTAS (compared to 31.58% for the control group).

As *FMR1* premutation alleles are common in the general population, our observation of the frequent occurrence of IMDs among this group implies that there will be a substantial societal impact of the premutation via the IMD burden, particularly AITD or fibromyalgia. Therefore, we suggest that women with the symptoms described here should be considered for screening using the fragile X DNA diagnostic test. If the test identifies a premutation allele, there are treatment implications not only for the patient [Hagerman et al., 2008], but also for the extended family as other members of the family tree will typically be positive for the premutation or full mutation alleles.

Why women with the premutation have an increased propensity for fibromyalgia and AITD [Coffey et al., 2008] is not known. RNA toxicity leads (directly or indirectly) to up-regulation of heat shock proteins (e.g., Hsp70 and αB-crystallin [Arocena et al., 2005; Wu et

al., 2004], which themselves may stimulate immune dysregulation [Georgopoulos and McFarland, 1993]. Hsp70 is involved in binding antigens and presenting them to the immune system [Nishikawa et al., 2008]. Autoantibodies to a number of stress proteins have been identified in SLE and RA, but their pathogenic significance remains to be established [Winfield and Jarjour, 1991]. In MS, autoantibodies against αB-crystallin (a small heat shock protein) are detected while αB-Cry transcript levels are found to be up regulated in FXTAS [Arocena et al., 2005].

The expanded CGG-repeat *FMR1* mRNA in premutation carriers and, in particular, those with FXTAS, is thought to exert its toxicity principally through sequestration of RNA-binding proteins, including DGCR8, which is critical for processing miRNAs [Garcia-Arocena and Hagerman, 2010; Sellier et al., 2010a; 2010b]. Indeed a dysregulation of the miRNA processing machinery has been reported in FXTAS premutation carriers [Sellier et al., 2010b]. Targeted deletions of individual miRNA genes result in mice with various immune deficiencies [Rodriguez et al., 2007; Thai et al., 2007]. In 2007, the role of miRNA (miRNA precursor family 101/miR-101) in regulating autoimmunity was discovered in T-cell lymphocytes in the *sanroque* mouse. Potential consequences of miRNA dysregulation in developing IMDs has been investigated through two pathways, namely, through dysregulation of T cell function and up-regulation of the innate immune response, the latter through increased or prolonged inflammatory cytokine production [Dai and Ahmed, 2011; Lindsay, 2008; Pauley et al., 2009; Tomankova et al., 2011]. The sequestration of proteins important for splicing messages including Sam 68 and the subsequent mis-splicing of a variety of message is also known to lead to many forms of autoimmune disease including MS, SLE, RA and others [Evsyukova et al., 2010]. Finally, a recent report of miRNA dysregulation was found associated with infertility and corpus luteum failure in a mouse that is hypomorphic for the Dicer allele [Otsuka et al., 2008]. Therefore, it is possible that FXPOI may also be related to miRNA dysregulation secondary to RNA toxicity; however this hypothesis needs experimental confirmation.

An additional mechanism through which the premutation may lead to IMDs is stress and emotional dysfunction. The *FMR1* premutation leads to dysregulation of the HPA axis, leading in turn to enhanced release of the stress hormone cortisol, as demonstrated in the premutation mouse [Brouwer et al., 2008]. Cortisol dysregulation and stress can lead to inflammation and activation of the immune system [Chang et al., 2009]. Levels of anxiety and depression are increased in premutation carriers [Bourgeois et al., 2010; Roberts et al., 2009], and magnetic resonance imaging (MRI) studies have shown that increased levels of anxiety are associated with a decrease in the size of the hippocampus in women carrier [Adams et al., 2009]. Diffusion tensor imaging (DTI) studies have also demonstrated early involvement of the insula and cingulate in premutation carriers well before the onset of FXTAS [Hashimoto et al., 2011].

The strong association between FXTAS, FXPOI, and IMDs suggests that the elements of the cellular pathology associated with FXTAS or FXPOI may stimulate IMDs, or perhaps the IMD could lead to CNS inflammation that could predispose the individual to develop FXTAS. This latter possibility may be the case in the women carrier, described by Greco et al [Greco et al., 2008], who died of MS after a 15-year history of disease; on

neuropathological study, there were demyelinating lesions of MS in addition to the inclusions of FXTAS, evidence of the co-occurrence of both disorders. It is also possible that the loss of neurons or astrocytes in FXTAS further stimulates an immune response, or that the elevated mRNA levels themselves might directly stimulate Toll receptors that lead to IMDs [Basu and Fenton, 2004; Dabbagh and Lewis, 2003]. Future studies are needed to address these questions.

IMDs have also been described in some mothers of children with autism [Croen et al., 2005; Keil et al., 2010] and we have recently become aware of IMD in many mothers of FG syndrome [Opitz et al., 2008]. The causes of IMDs in these other conditions deserve further study because some of the mechanisms leading to these problems may overlap with the mechanisms hypothesized here.

Although a principal strength of the current study is the large sample size, there are several limitations that should be noted. Factors/differences in women premutation carrier with and with out FXTAS (or with and without FXPOI) that could confound association with IMDs are not currently well understood and, therefore, not modeled in this work. Our summary of IMDs in this cohort (e.g., type and frequency of IMD) is descriptive and may not provide unbiased estimates of prevalence in the women premutation population. We studied only women with the premutation because in our experience we have not, with rare exceptions, observed IMD disease in men with the premutation. We also have an ascertainment bias toward individuals with FXTAS because they are recruited in two of our studies, and women with IMDs may be more likely to be seen in our clinical and research program. Well-controlled studies and further testing for biomarkers of immune dysregulation are needed to verify and/or provide more accurate estimates of IMD prevalence in this population. However, our findings here provide a basis for such studies.

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TABLE I

Prevalence of Immune-mediated Disorders among Women Carrier of *FMR1* Premutation Alleles Compared to the General Population

Autoimmune Disorders	Prevalence among 344 women premutation carrier (%)	Prevalence among 72 women control (%)	Prevalence in reference populations (%)
AITD	24.42	11.11	2.5 [Bjoro et al., 2000]
Fibromyalgia	10.17	4.17	2.7– 4.7 [Branco et al., 2010]
IBS	9.88	6.52	3–20 [Saito et al., 2002]
Raynaud's phenomenon	7.56	5.56	4.8 [Cakir et al., 2008]
RA	3.78	4.17	0.5 [Carmona et al., 2002]; 1.16 [Symmons et al., 2002]
Sjögren syndrome	2.62	N/A	0.5–3 [Gaubitz, 2006]
SLE	2.03	N/A	0.015–0.05 [Gaubitz, 2006]
MS	1.74	N/A	0.002–0.15 [Rosati, 2001]
Optic neuritis	0.58	N/A	0.12 [Rodriguez et al., 1995]

AITD=autoimmune thyroid disorders; IBS=irritable bowel syndrome; RA= rheumatoid arthritis; SLE=systemic lupus erythematosus; MS=multiple sclerosis

TABLE II

Summary of Immune-mediated Disorders for All Subjects

		Group A: Pre w/FXTAS		Group B: Pre w/o FXTAS		Group C: Controls		P-value			
Variable	**Response**	**Frequency**	**Percent**	**Frequency**	**Percent**	**Frequency**	**Percent**	**Overall**	**A vs C**	**B vs C**	**A vs B**
Immune-mediated disorders*	No	15	26.79	175	60.76	52	72.22	<.0001	<.0001	0.0771	<.0001
	Yes	41	73.21	113	39.24	20	27.78				
AITD	No	26	46.43	233	81.18	64	88.89	<.0001	<.0001	0.1621	<.0001
	Yes	30	53.57	54	18.82	8	11.11				
Fibromyalgia	No	42	75.00	267	92.71	69	95.83	0.0002	0.001	0.4366	0.0003
	Yes	14	25.00	21	7.29	3	4.17				
IBS	No	51	91.07	259	89.93	43	93.48	0.8469	0.7267	0.5945	1
	Yes	5	8.93	29	10.07	3	6.52				
Raynaud's phenomenon	No	49	87.50	269	93.40	68	94.44	0.2841	0.2094	1	0.1618
	Yes	7	12.50	19	6.60	4	5.56				
RA	No	53	94.64	278	96.53	69	95.83	0.6741	1	0.7288	0.4516
	Yes	3	5.36	10	3.47	3	4.17				
Sjögren syndrome	No	53	94.64	282	97.92	43	100.00	0.1906	0.2554	1	0.1669
	Yes	3	5.36	6	2.08	0	0.00				
SLE	No	56	100.00	280	97.56	72	100.00	0.3301	0.2554	0.3527	0.6042
	Yes	0	0.00	7	2.44	0	0.00				
MS	No	55	98.21	283	98.26	72	100.00	0.6716	0.4375	0.5875	1
	Yes	1	1.79	5	1.74	0	0.00				
Optic neuritis	No	55	98.21	287	99.65	72	100.00	0.281	0.4375	1	0.2995
	Yes	1	1.79	1	0.35	0	0.00				

*Any immune-mediated disorders

FXTAS= fragile X-associated tremor ataxia syndrome; AITD=autoimmune thyroid disorders; IBS=irritable bowel syndrome; RA= rheumatoid arthritis; SLE=systemic lupus erythematosus; MS=multiple sclerosis

TABLE III

Summary of Immune-mediated Disorders for Subjects Age 40 and Older

		Group A: Pre w/FXTAS		Group B: Pre w/o FXTAS		Group C: Control		P-value			
Variable	**Response**	**Frequency**	**Percent**	**Frequency**	**Percent**	**Frequency**	**Percent**	**Overall**	**A vs C**	**B vs C**	**A vs B**
Immune-mediated disorders*	No	15	27.27	85	53.46	39	68.42	<.0001	<.0001	0.0611	0.0009
	Yes	40	72.73	74	46.54	18	31.58				
AITD	No	26	47.27	123	77.85	50	87.72	<.0001	<.0001	0.122	<.0001
	Yes	29	52.73	35	22.15	7	12.28				
Fibromyalgia	No	41	74.55	144	90.57	54	94.74	0.0032	0.0034	0.4128	0.0052
	Yes	14	25.45	15	9.43	3	5.26				
IBS	No	50	90.91	138	86.79	34	91.89	0.6515	1	0.5786	0.4833
	Yes	5	9.09	21	13.21	3	8.11				
Raynaud's phenomenon	No	48	87.27	149	93.71	53	92.98	0.2903	0.3563	0.7646	0.1496
	Yes	7	12.73	10	6.29	4	7.02				
RA	No	52	94.55	152	95.60	55	96.49	0.8458	0.6761	1	0.7195
	Yes	3	5.45	7	4.40	2	3.51				
Sjögren syndrome	No	52	94.55	153	96.23	35	100.00	0.4658	0.279	0.5938	0.6973
	Yes	3	5.45	6	3.77	0	0.00				
SLE	No	55	100.00	152	96.20	57	100.00	0.2097	0.279	0.3449	0.3423
	Yes	0	0.00	6	3.80	0	0.00				
MS	No	54	98.18	158	99.37	57	100.00	0.4089	0.4911	1	0.4489
	Yes	1	1.82	1	0.63	0	0.00				
Optic neuritis	No	54	98.18	159	100.00	57	100.00	0.203	0.4911	1	0.257
	Yes	1	1.82	0	0.00	0	0.00				

*Any immune-mediated disorders

FXTAS= fragile X-associated tremor ataxia syndrome; AITD=autoimmune thyroid disorders; IBS=irritable bowel syndrome; RA= rheumatoid arthritis; SLE=systemic lupus erythematosus; MS=multiple sclerosis

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TABLE IV

Summary of Immune-mediated Disorders for Subjects Aged 40 and Older With and Without FXPOI

		Group A: Pre w/FXPOI		Group B: Pre w/o FXPOI		Group C: Control w/o FXPOI		P-value			
Variable	**Response**	**Frequency**	**Percent**	**Frequency**	**Percent**	**Frequency**	**Percent**	**Overall**	**A vs C**	**B vs C**	**A vs B**
Immune-mediated disorders*	No	14	34.15	80	54.42	33	66.00	0.0101	0.0032	0.186	0.0333
	Yes	27	65.85	67	45.58	17	34.00				
AITD	No	28	68.29	107	72.79	43	86.00	0.0982	0.0733	0.0826	0.5622
	Yes	13	31.71	40	27.21	7	14.00				
Fibromyalgia	No	34	82.93	127	86.39	47	94.00	0.2252	0.1758	0.2037	0.616
	Yes	7	17.07	20	13.61	3	6.00				
IBS	No	34	82.93	131	89.12	29	90.63	0.4907	0.4969	1	0.2883
	Yes	7	17.07	16	10.88	3	9.38				
Raynaud's phenomenon	No	38	92.68	137	93.20	46	92.00	0.9375	1	0.7555	1
	Yes	3	7.32	10	6.80	4	8.00				
RA	No	38	92.68	140	95.24	49	98.00	0.4996	0.3235	0.6822	0.4566
	Yes	3	7.32	7	4.76	1	2.00				
Sjögren syndrome	No	38	92.68	143	97.28	31	100.00	0.2278	0.2541	1	0.177
	Yes	3	7.32	4	2.72	0	0.00				
SLE	No	39	95.12	143	97.95	50	100.00	0.1965	0.2002	0.5717	0.3018
	Yes	2	4.88	3	2.05	0	0.00				
MS	No	40	97.56	146	99.32	50	100.00	0.3589	0.4505	1	0.3895
	Yes	1	2.44	1	0.68	0	0.00				
Optic neuritis	No	41	100.00	146	99.32	50	100.00	1	0.4505	1	1
	Yes	0	0.00	1	0.68	0	0.00				

*Any autoimmune disease

FXPOI= fragile X-associated premature ovarian insufficiency; AITD=autoimmune thyroid disorders; IBS=irritable bowel syndrome; RA= rheumatoid arthritis; SLE=systemic lupus erythematosus; MS=multiple sclerosis