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Psychological status in female carriers of premutation FMR1 allele showing a complex relationship with the size of CGG expansion

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Abstract

We utilised a sample of 299 adult females aged between 19 and 86 years, carrying *FMR1* alleles with small CCG expansions ranging from 50 to 141 repeats to analyse the relationships between psychological symptoms as assessed by the Symptom Checklist-90-Revised (SCL-90-R) and the size of the CGG repeat in the *FMR1* gene. There were highly significant (negative) correlations between the size of the CGG repeat and a great majority of SCL-90-R subscale scores and all the global indices, suggesting that carriers of premutations in the mid-size CGG repeat range may be at greatest risk for development of psychiatric disorder.

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Conflict of Interest Statement: Nothing to declare

Keywords

FMR1 premutation; premutation female carriers; psychological symptoms; CGG repeat size; regression analysis

Introduction

Premutation (PM) is the recognized category of Fragile X Mental Retardation 1 (*FMR1*) alleles containing CGG expansions between 55 and 200 repeats [1]. In females these common alleles (1 per 113 to 259 individuals) [2–4] are associated with a risk of further expansion into the full mutation range (>200) over one generation, leading to severe developmental abnormality, Fragile X Syndrome (FXS) [5]; on the other hand, the PM carrier status itself is associated with many phenotypic abnormalities in either sex, as reviewed in:[6,7]. Fragile X-associated Primary Ovarian Insufficiency (FXPOI) manifesting with a spectrum of diminished ovarian functions including premature menopause was the first recognized disorder in ~20% of PM carriers [6,8]. Fragile X Associated Tremor-Ataxia Syndrome (FXTAS), highly prevalent (~40%) in male PM carriers, occurs in approximately 16% of female PM carriers, where neurological and cognitive impairments tend to be milder [7,9].

Although psychiatric disorder appears to be common in female PM carriers, the type and severity of these problems varies between different studies, ranging from the absence of significant disorder [10], to high rates (between 41 and ~56%) of lifetime diagnosis of all affective disorders, with anxiety, depression and obsessive compulsive disorders being most prevalent [11–14], with a tendency to cluster in the same individuals (15). Other confounding effects, biases related to pre-selection of the PM carrier or control samples, or low power, may account for conflicting results from comparative studies. Here we adopted a different approach by relating the primary symptom dimensions and global indices of SCL-90-R to the size of the CGG expansion within the PM range.

Materials and Methods

The SCL-90-R [16] is a self-report instrument that covers a broad range of relevant psychological symptom clusters, with good validity (0.77 – 0.90), and reliability (.80 – 0.90). The Wechsler Adult Intelligence Scale (WAIS-III; [17]), including a prorated short form based on the Block-Design and Vocabulary subtests, which has been shown to be comparable with FSIQ (except for extreme scores, which did not occur in our sample) [18], was used to assess cognitive status.

The PCRs and Southern Blot analysis were used to assess the size of CGG expansion (see Legend to Table 1), with all assays fully validated by internal and external quality assessment to provide precision of +/− one repeat

In Australia, 117 females aged 18–79 were ascertained through the affected probands or other relatives attending Victorian Clinical Genetic Services (VCGS); none of these females

manifested FXTAS. The size of the CGG repeat (in blood) ranged from 50 to 141, including several subjects with the larger intermediate size alleles ranging from 45–55 CGGs [1].

The US sample consisted of 182 females aged 19–86 ascertained through the affected probands seen at the Fragile X Research and Treatment Center, MIND Institute, UC Davis, 29 females were diagnosed with FXTAS spectrum. The size of the CGG repeat ranged from 56 to 138.

Shaprico-Wilk statistics at the 5% significance level was used to test for normality of distributions. Principal components were used to combine the SCL-90-R subscale scores into linear weight combination of the original (inter-correlated) variables. First principal component (PC1) accounted for 70% of the variation for the SCL-90-R measures, weights ranging from 0.3 to 0.36. The second PC (not considered further) accounted for an additional 8% of variation. The relationship between the cognitive and the SCL-90-R scores, and PC1 (outcome variables), and CGG repeat number (predictor), adjusting for age, country and VIQ, was assessed using multiple linear regressions. The least square estimation was used initially to calculate regression coefficients. Robust regression was applied to down-weight the effect of outliers.

All analyses were conducted using the STATA statistical package, version 11.2 (http://www.stata.com).

Results

There were significant differences in means (or medians) between the American and Australian samples for the majority of symptom scores, with all the means being within the normal range (Table 1). Pro-rated IQ was included as a potential predictor of SCL-90-R scores, and VIQ was significantly correlated with the size of the CGG repeat in the US sample.

Initial regression analyses revealed significant interactions between CGG repeat size and site, resulting in differences between the two samples in the correlations between Pro-rated IQ, VIQ, Obsessive-Compulsive subscale and CGG size, but after adjusting for age and/or VIQ, were no longer significant ($p>0.05$). The results of regression analysis for the two samples combined showed that the majority of SCL-90-R global and partial scores were significantly and inversely correlated with the size of CGG repeat (Table 2). The size effect of CGG expansion was the strongest for the global scores, especially PSDI and GSI, and for Somatization and Obsessive-Compulsive subscales.

The scatterplots (presented in Figure 1a and 1b for GSI and PC1) showed that the SCL-90-R scores and global indices were highest for alleles within the 60–80 repeat range and the most obvious and consistent downward trend was between 80–100 CGG repeats. We did not have enough data to observe this trend after passing their minimum at approximately 100–120 repeats.

We also compared SCL-90-R scores between the two categories of CGG sizes (Table 3). The rationale for using 100 rather than 120 repeats as the cut-off value between those

categories was the smallness of our subsample of females carrying larger PM alleles, especially considering that the range with significant effect could only be accurately determined using the non-linear regression models. The means (or medians) were significantly higher in the ≤ 100 category than in >100 category for most of the SCL-90-R subscale and global scores. After relevant adjustments, the differences remained significant for GSI and PDSI, Anxiety, Obsessive-Compulsive, and Somatization scores.

We also explored the relationship of menopausal age with the CGG size in a subsample of 110 post-menopausal females (Figure 1c) which showed the maximum decrease in menopausal age at 60–80 repeats. The (linear) relationship between menopausal age and either CGG repeat size or SCL-90-R scores in a small sample was significant for Depression only (slope=−0.302; p=0.030).

Discussion

In a sample of female PM carriers from Australia and US we have demonstrated significant correlations between CGG repeat size and the SCL-90-R, with two global indices (PSD1 and GSI) and the Somatization and Obsessive-Compulsive subscale scores showing the highest values for effect size. Although the two samples were significantly different in the number of relevant features, the results were consistent in both combined and individual samples, after adjustments for the respective sites (countries) and other confounders in the regression analysis. These adjustments also corrected for the difference in the frequency of FXTAS, which was diagnosed in nearly 15% of the US, compared with nil in the Australian sample. A scarcity of FXTAS in Australian females, and much less severe manifestations of this disorder in the affected males have been noticed previously (19, Loesch unpublished data), the reason for this discrepancy is still unexplained.

Our data have demonstrated a significant effect of PM alleles on psychological status; however, this effect is moderate, with the scores above clinically significant threshold of 63 [16] occurring in less than 10% (23) of all participants. Therefore we hypothesize that those alleles may contribute to shifting the distribution of psychiatric distress scores towards the clinically significant range rather than being the sole cause of psychiatric disorder in the majority of carriers. The earlier finding that the PM females have increased sensitivity to major life changes leading to depression and anxiety [20] strongly supports this view. Considering all the above, as well as the smallness of our subsample with clinically significant SCL-90-R scores, we used the total sample representing a broader range of those scores in correlation with the CGG repeat size.

The important finding was that all subscales or global scores were *negatively* correlated with CGGs, with the relevant scatterplots suggesting that these relationships may not be linear. However, we could not verify this claim because of limitation in the data which did not cover the full PM range of repeat sizes. Nevertheless, significant differences between the two CGG size categories in both global (PSI and PDS) and several subscale scores strongly support this claim.

Notably, the results of earlier studies [12, 21] in large independent samples of PM female carriers have been indicative of a predominant effect of the midsize CGG repeat range on mood disorders such as anxiety and depression. In the more recent study [20], this effect was found to be linked to the negative life events in mothers of children with FXS, thus reinforcing the concept of the elevated risk of psychiatric symptoms in the carriers of midsize expansion size exposed to the life stress. The reported midsize range may vary according the dependent variable being studied [20], the size and composition of the sample and the statistical model applied.

The phenomenon of the greatest risk of a condition being associated with the midsize CGG range in PM females was first reported for FXPOI using non-linear regression models [22], and later confirmed in several independent samples [23–25]. Our present results, though based on a much smaller sample with truncated distribution of CGG sizes, are clearly consistent with those earlier findings.

A hypothesis attributing the non-linear effect of CGG size within the PM range on severity of FXPOI manifestations to qualitative differences in the *FMR1* RNA transcript [23] may also be applicable to psychiatric symptoms. Those differences may result either from a deleterious structure of this transcript in the mid-PM range allowing inappropriate protein binding, or from the alternative transcription initiation sites for *FMR1* varying with repeat number as previously shown [26]. The finding of neurotoxic effects of *FMR1*mRNA over a repeat sizes <100 CGGs [27] supports the concept of maximal mRNA toxicity in the mid-PM range.

Because of already acknowledged limitations of this study, we recommend that the results are confirmed by data covering the whole range of PM expansion sizes, so that the non-linear regression models can be applied to better characterize its relationship with the phenotype, and to accurately determine the range with clinically significant effects. The data from subjects with the intermediate size alleles [6] should also be included in order to be able to identify the minimum size of repeat that may elevate the risk of psychiatric disorder.

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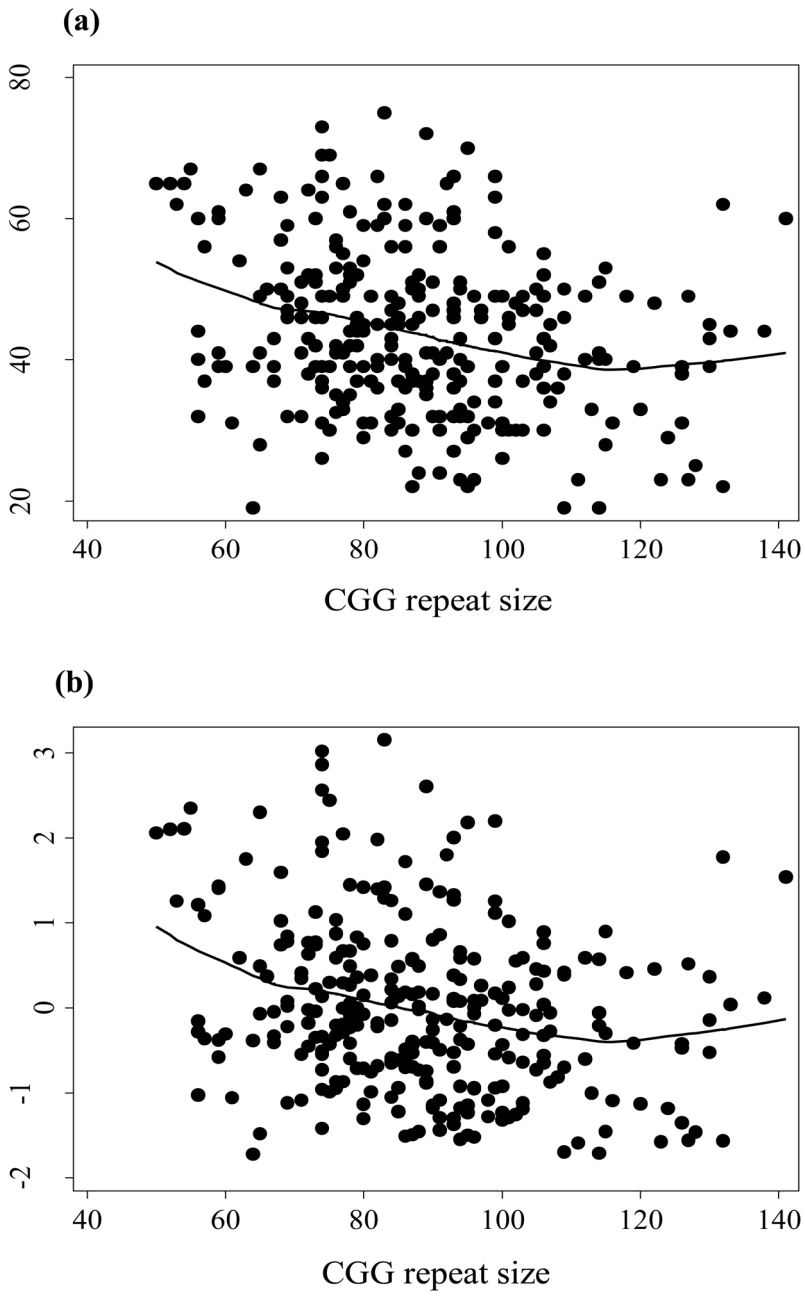

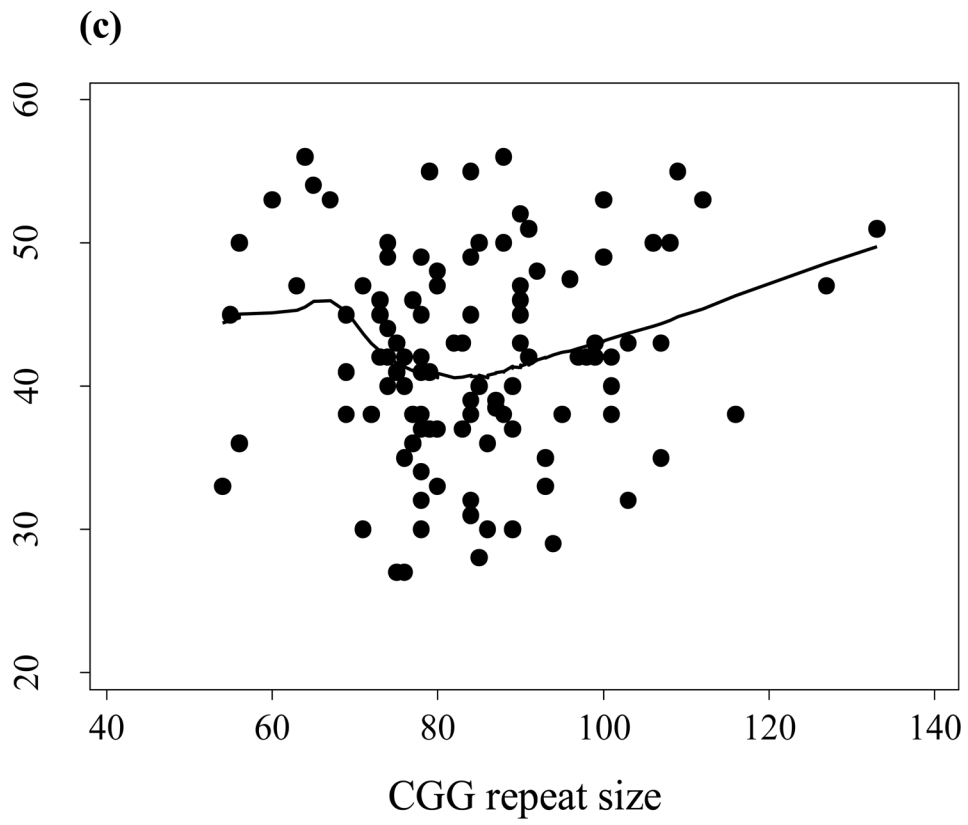

Figure 1.

Scatterplots and regression line representing the relationships between CGG repeat size and the Global Severity Index, GSIT (a), the First Principal Component, PC1, both based on the SCL-9-R self-report instrument (b); and menopausal age (c), using nonparametric locally weighted regression.

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Table 1

List of variables and summary statistics for the American (US) and Australian (AU) samples, separately and combined. Two-sample t-tests were used to test for the difference between means if the data was normally distributed; the nonparametric Mann-Whitney test was used to determine the difference in the medians (p-values).

	All			US			AU			
	n	Mean	SD	n	Mean	SD	n	Mean	SD	p-value
CGG repeat*	299	88.09	17.62	182	90.72	17.14	117	83.95	17.64	0.0013
Age	299	44.10	12.61	182	45.12	13.18	117	42.54	11.55	0.1330
Menopausal age	110	41.91	7.130	56	41.52	7.670	54	42.31	6.58	0.5605
Prorated IQ‡	260	105.9	13.73	144	108.9	12.85	116	102.02	13.87	< 0.0001
Anxiety†	294	42.69	10.52	182	40.02	8.380	112	47.02	12.13	< 0.0001
Depression†	294	43.70	11.39	182	39.27	9.480	112	50.89	10.56	< 0.0001
Interpersonal Sensitivity†	294	45.40	11.23	182	41.76	9.750	112	51.33	10.98	< 0.0001
Hostility†	294	45.87	9.490	182	43.81	8.200	112	49.22	10.46	< 0.0001
Phobic Anxiety†	294	45.25	8.250	182	43.98	7.060	112	47.31	9.57	< 0.0001
Paranoid Ideation †	294	44.16	9.570	182	41.79	8.440	112	48.02	10.07	< 0.0001
Obsessive-Compulsive†	294	48.47	11.09	182	45.71	9.870	112	52.95	11.53	< 0.0001
Psychotism†	294	43.59	10.98	182	39.04	8.780	112	50.97	10.16	< 0.0001
Somatization†	293	48.34	10.31	182	47.87	10.00	111	49.10	10.79	0.3243
GSI††	294	44.01	11.75	182	40.08	10.10	112	50.40	11.48	< 0.0001
PSDI††	294	43.18	11.36	182	39.81	10.30	112	48.66	10.89	< 0.0001
PST††	294	45.12	11.78	182	41.74	10.71	112	50.60	11.42	< 0.0001
VIQ ‡	215	104.0	14.65	144	107.0	13.26	71	97.86	15.48	< 0.0001

*.Genomic DNA was isolated from peripheral blood lymphocytes using standard methods (Qiagen). For Southern blot analysis, 5–10 micrograms of isolated DNA was digested with EcoRI and NruI. Hybridization was performed using the FMR1 genomic dig labelled StB12.3 probe. DNA was also amplified by PCR primers c and f [5]. PCRs were performed using the method previously described: in [Tassone F, et al. J Mol Diagn 2008;10: 43–9], for the American sample, and in: [Khaniani MS, et al. Mol Cytogenet 2008;1:5] for the Australian sample.

†Nine primary symptoms dimensions are assessed by the 90 items rated on a five-point Likert scale of distress during the past 7 day, ranging from 0 to 4.

††Three global indices provide measures of overall psychological distress: the Global Severity Index (GSI), the Positive Symptom Total (PST) and the Positive Symptom Distress Index (PSDI).

Both primary symptoms and global indices are in the form of T scores with the normal range of 40–60.

‡Pro-rated IQ and the standard composite score VIQ (Verbal Intelligence Quotient); VIQ normal range of 90–110.

Table 2

Relationship between CGG repeats (predictor) and outcome variables: prorated IQ, VIQ, SCL-90-R global indices and domain scores, and PC1 assessed using linear regression method, adjusted for country, age and VIQ and country (whenever significant).

	N	Coef.	s.e	p-value	Std Coef.
Prorated IQ	171	0.008	0.034	0.807	0.011
VIQ	171	−0.086	0.064	0.178	−0.104
Anxiety	291	−0.092	0.034	0.008	−0.151
Depression	210	−0.111	0.039	0.005	−0.176
Interpersonal Sensitivity	210	−0.112	0.042	0.009	−0.179
Hostility	210	−0.063	0.040	0.115	−0.128
Phobic Anxiety	169	−0.028	0.032	0.385	−0.059
Paranoid Ideation	210	−0.102	0.036	0.005	−0.192
Obsessive Compulsive	210	−0.121	0.043	0.005	−0.197
Psychotism	210	−0.077	0.038	0.047	−0.129
Somatization	168	−0.150	0.045	0.001	−0.255
GSI	210	−0.131	0.043	0.002	−0.201
PSDI	291	−0.136	0.036	< 0.001	−0.210
PST	210	−0.111	0.044	0.012	−0.171
PC1	209	−0.010	0.004	0.006	−0.188

Std Coef. = estimated standardised coefficient representing the size effect of CGG expanded repeat, where outcome variable and predictors were standardised to have mean zero and variance of 1.

Table 3

Comparison of the prorated IQ, VIQ, SCL-90-R global indices and domain scores, and PC1 between samples with less than or equal to 100 CGG repeats, and with greater than 100 CGG repeats.

	≤100 CGG repeats			>100 CGG repeats				
	n	**Mean**	**SD**	**n**	**Mean**	**SD**	**p-value***	**p-value****
Prorated IQ‡	199	105.9	13.39	59	105.7	14.99	0.9051	0.644
Anxiety†	229	43.48	10.87	63	39.63	8.720	0.0217	0.038
Depression†	229	44.56	11.58	63	40.43	10.27	0.0153	0.089
Interpersonal Sensitivity†	229	46.24	11.43	63	42.00	9.820	0.0233	0.137
Hostility†	229	46.48	9.640	63	43.71	8.800	0.0737	0.177
Phobic Anxiety†	229	45.52	8.620	63	44.22	6.840	0.4589	0.473
Paranoid Ideation †	229	44.61	9.880	63	42.11	7.950	0.1099	0.256
Obsessive-Compulsive†	229	49.16	11.38	63	45.56	9.450	0.0220	0.047
Psychotism†	229	44.14	11.50	63	41.41	8.690	0.1795	0.398
Somatization†	228	49.23	10.40	63	44.84	9.280	0.0026	0.041
GSI†	229	45.12	11.94	63	39.70	10.11	0.0037	0.042
PSDI†	229	44.10	11.75	63	39.79	9.320	0.0076	0.007
PST†	229	46.09	11.78	63	41.17	11.04	0.0032	0.113
VIQ ‡	161	104.5	15.08	53	102.6	13.40	0.4119	0.307
PC1	228	0.080	1.030	63	−0.300	0.830	0.0083	0.108

*p-values were computed using either two-sample t-test or nonparametric Mann-Whitney tests.

**p-values using analysis of covariance (regression), adjusted for country, age and verbal IQ whenever appropriate.

†T score values, with a normal range of 40–60,

‡Index scores with a normal range of 90–110.