

1 **Relationships between motor scores and cognitive functioning in**
2 **FMR1 female premutation X carriers indicate early involvement of**
3 **cerebello-cerebral pathways.**

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21 **Abstract**

22 **Background**

23 Smaller expansions of CGG trinucleotide repeats in the *FMR1* X-linked gene termed
24 'premutation' lead to a neurodegenerative disorder: Fragile X Associated Tremor/Ataxia
25 Syndrome (FXTAS) in nearly half of aged carrier males, and 8-16% females. Core features
26 include intention tremor, ataxia, and cognitive decline, and white matter lesions especially
27 in cerebellar and periventricular locations. A 'toxic' role of elevated and expanded *FMR1*
28 mRNA has been linked to the pathogenesis of this disorder. The emerging issue concerns
29 the trajectory of the neurodegenerative changes: is the pathogenetic effect confined to
30 overt clinical manifestations? Here we explore the relationships between motor and
31 cognitive scale scores in a sample of 57 asymptomatic adult female premutation carriers of
32 broad age range.

33 **Methods**

34 Three motor scale scores (ICARS-for tremor/ataxia, UPDRS-for parkinsonism, and the Clinical
35 Tremor) were related to 11 cognitive tests and two psychiatric pathology scores - DASS and
36 SCL-90 - using Spearman's rank correlations. Robust regression, applied in relationships
37 between all phenotypic measures, and genetic molecular and demographic data, identified
38 age and educational levels as common correlates of these measures, which were
39 incorporated as confounders in correlation analysis.

40 **Results**

41 Cognitive tests demonstrating significant correlations with motor scores were those
42 assessing psychomotor speed/visual attention (SDMT; TMT-A) and those dependent on

43 various aspects of executive functioning for their execution: sequencing and alternation
44 (TMTB-A); working memory (DS backwards); and non-verbal reasoning (MR). The largest
45 number of motor x cognitive correlations involved ICARS and Tremor scales, which reflect
46 the type of motor dysfunctions seen in FXTAS.

47 **Conclusions**

48 Subtle motor impairments correlating with cognitive deficits may occur in female
49 premutation carriers not meeting diagnostic criteria for FXTAS. This pattern of cognitive
50 deficits is consistent with those seen in other cerebellar disorders. Our results provide
51 evidence that more than one category of clinical manifestation reflecting cerebellar changes
52 – motor and cognitive - may be simultaneously affected by premutation carriage across a
53 broad age range in asymptomatic carriers. Future longitudinal studies should determine
54 whether cognitive dysfunction tracking motor impairments is driven by the premutation
55 status common to all carriers, or only to a subset of carriers who will develop FXTAS in older
56 age.

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58 **Key words:** *FMR1* premutation; CGG repeats; female premutation carriers; tremor/ataxia
59 scales; cognitive tests; motor-cognitive scores relationships; fragile X-associated tremor
60 ataxia.

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65 **Introduction**

66 Large CGG repeat expansions (>200 repeats) in the fragile X mental retardation 1 (*FMR1*) X-
67 linked gene, labelled ‘full mutations’, cause the Fragile X syndrome (FXS), a
68 neurodevelopmental disorder resulting from hypermethylation of this gene’s promoter,
69 resulting in decreased translation. Since this mutation is unstable across generations if
70 transmitted through the female parent, the mothers of FXS children are usually carriers of
71 smaller expansions ranging from 55-200 CGG repeats, labelled ‘premutations’. Yet smaller
72 expansions (40 to 54 repeats), which do not expand into the full mutation range within two
73 generations but which are nevertheless linked to neurodegenerative changes, are termed
74 “grey zone” expansions [1, 2]. *FMR1* premutations are common in the general population,
75 with the population prevalence ranging from 1 in 130 to 1 in 250 females, and from 1 in 250
76 to 1 in 810 males [3-4]. The premutation causes premature ovarian failure (FXPOI -
77 menopause before the age of 40) in approximately 20% of female carriers [5]. Both male
78 and female premutation carriers may develop a progressive neurodegenerative condition
79 termed Fragile X Associated Tremor/Ataxia Syndrome (FXTAS) as they age, with a much
80 higher rate in males (between 40-50% after the age of 55) than in females in the same age
81 group (approximately 15%) [6-9]. Typical FXTAS neuropathological changes are of
82 widespread intranuclear inclusions abundant in neurones and astrocytes [10], extending to
83 autonomic nervous and neuroendocrine systems and myocardial cells [11-13].
84 One component of the nuclear inclusions is the *FMR1* mRNA [14], which has previously been
85 found to be elevated in the blood of premutation carriers as a function of increased CGG
86 repeat number [15]. These findings have led to an hypothesized pathogenetic mechanism
87 that involves a toxic gain-of-function of the expanded CGG-repeat mRNA, which arises

88 through the adventitious binding/sequestration by the CGG repeat of one or more proteins,
89 contributing to dysfunction and/or death of the cell [16, 17].

90 An alternative model for FXTAS pathogenesis has been proposed, in which “toxic” peptides
91 are generated by initiating translation at non-AUG codons located upstream of the CGG-
92 repeat element. It has been shown that that this process, known as RAN translation, is
93 attributable to the elevated and expanded *FMR1* mRNA , which generates a poly-glycine
94 peptide that is toxic to cells and is detectable in both the intranuclear inclusions of subjects
95 with FXTAS and in the inclusions of the Dutch premutation CGG-repeat mouse model [18,
96 19].

97 These potential mechanisms, associated with CGG expansions within the premutation range
98 (as reviewed in Hagerman & Hagerman 2015)[20] lead to the prominent central nervous
99 system pathological changes underlying FXTAS. The major changes identified on MR imaging
100 are cerebral atrophy and white matter disease, most prominent in the middle cerebellar
101 peduncles (‘MCP sign’) in affected males [21, 22], and in the splenium of the corpus
102 callosum in either sex [23].

103 The penetrance of FXTAS is reduced in female premutation carriers, which can be, at least
104 partly, attributed to the protective effect of the normal *FMR1* allele on the second X
105 chromosome [24]. However, despite evident neuroprotective effects in the female carriers
106 suggested by the data on FXTAS penetrance, female carriers may present with a number of
107 other clinical changes in addition to FXPOI, such as fibromyalgia, autoimmune thyroid
108 disease and other immune-related disorders, and hypertension, as well as psychiatric
109 problems including social phobia, hostility, obsessive/compulsive behaviour, and
110 anxiety/depression [25-33].

111 Apart from general health and psychiatric issues, few studies have explored subtle
112 impairments that might be directly related to brain changes in carrier PM females in the
113 absence of overt neurological symptoms or signs. These studies, based on small samples,
114 demonstrated deficits on a range of tasks of executive functioning requiring rapid temporal
115 responses [34], or subtle impairment of postural stability [35], compared with control non-
116 carriers. These results suggested that there may be a slow subclinical decline in executive
117 functioning and/or degradation of motor functioning, combined with (and perhaps
118 augmented by) stress and diminished adaptive capacity, in apparently asymptomatic
119 premutation females. However, most of the results that have been obtained by comparing
120 samples of carriers with non-carriers have yielded inconsistent results, in part attributable
121 to selection bias [36, 37], and thus provide limited insight into the effects of premutation
122 alleles on female phenotypes. Likewise, the results of correlations between phenotypic
123 (especially neuropsychiatric) findings and fragile X genotype, including CGG repeat size,
124 *FMR1*mRNA levels and the *FMR1* activation ratio, have been inconsistent [38], so that the
125 issue of the dynamics of brain changes associated with PM alleles other than the recognised
126 FXTAS manifestations still remains to be addressed.

127 Here we have taken a novel approach to this issue by exploring the relationships of three
128 motor scale scores - for tremor/ataxia, parkinsonism, and tremor, respectively - with the
129 results of a range of neurocognitive and psychiatric tests, in a sample of neurologically
130 asymptomatic, apparently unaffected adult (mainly postmenopausal) females carrying small
131 expansion *FMR1* alleles within the premutation range (53 cases) or the grey zone range (4
132 cases). We hypothesise that performance on these two broad clinical domains – motor and
133 cognitive - will be correlated, and that the presence of such correlations will be attributable
134 to an underlying pathological process simultaneously affecting motor and cognitive

135 performance at a sub-symptomatic level, and linked to premutation carriage across a broad
136 range of age and CGG small expansion sizes.

137 **MATERIALS AND METHODS**

138 **Subjects.**

139 The results of this study are based on retrospective analysis of 57 adult females (including
140 53 premutation and 4 grey zone carriers), originally ascertained through cascade testing of
141 the large cohort of fragile X families described in our earlier publications [39-45], who also
142 volunteered to participate in our 2008-2010 project supported by research grants from the
143 National Health and Medical Research of Australia (NHMRC) and the National Institute of
144 Health, US, to DZL & ES. Except for one East Asian, all participants were white Caucasian.
145 They provided informed consent according to protocols approved by the La Trobe University
146 and Monash University Human Research Ethics Committees. They underwent
147 comprehensive neurological testing between 2008 and 2010, conducted by two specialist
148 neurologists (ES and DZL-authors of this manuscript). They also underwent a battery of
149 neuropsychological tests under the supervision of ES, using standard protocols. Partial
150 retrospective analysis of SCL-90 data collected in this sample was described in our earlier
151 publication [46].

152 The age of participants ranged from 26 to 85 (mean 51.5 years); the size of CGG expansion
153 ranged from 40 -54 (for four grey zone carriers) and from 55 to 175 (for 53 premutation
154 carriers). Frequency distributions of age and CGG repeat numbers in the total sample of
155 carriers are presented in Figs 1a & b, respectively. Mean FMR1 mRNA level was 1.44,
156 ranging from 0.9 to 2.7. Mean activation ratio (assessed in a subgroup of 12 premutation
157 carriers) was 0.56. The demographics of the sample are presented in Table 1.

158 None of the female participants in this sample (classified as ‘unaffected’) were diagnosed
 159 with - or reported any symptoms of - FXTAS, or any serious health or psychiatric issues.
 160 However, we recorded that, among these 57 female carriers, 23.5% had hypertension, 3.9%
 161 had diabetes mellitus, 17.3% had treated thyroid disease, 7.8% had congenital heart defects
 162 (including mitral valve prolapse), 14% reported current depression, and 18.9 % had a history
 163 of depression.

164 Six out of 29 (20.7%) postmenopausal females had FXPOI. The average age of menopause in
 165 29 females was 43.7 years (28 participants were still menopausal), and the average number
 166 of children was 2.4. Nearly half (40.8%) of participants reported at least one miscarriage,
 167 18.4% - infertility problems and 18.0% - a history of irregular menses.

168 **Table 1: Characteristics of sample**

Variable	N	Mean	SD	Median	IQR	Min-Max
Characteristic						
Age	57	51.5	11.6	52.0	16.0	26-85
Age of menopause	29	43.7	6.78	45.0	8.0	27-53
Year of Education	57	12.6	3.2	12.0	4.0	4-22
CGG repeats	57	80.3	23.1	77.0	24.0	41-175
mRNA	45	1.43	0.49	1.20	0.70	0.9-2.7
Motor Score						
UPDRS	51	2.41	3.16	1.0	4.0	0-17
Clinical Tremor	52	7.48	4.73	5.0	5.5	1-23
ICARS Total	52	7.08	3.58	7.0	5.0	2-16
Cognitive measures						
Vocab SS	57	9.83	2.59	10.0	3.00	2-17
MR SS	57	11.4	3.29	12.0	3.00	2-17
DS Forwards	57	9.77	2.28	10.0	3.00	5-15
DS Backwards	57	6.50	2.26	6.00	3.00	3-14
Pro-rated IQ	57	104	14.0	106	15.5	54-137
TMT A (raw score)	52	36.9	17.2	35.0	11.5	17-114
TMT B (raw score)	52	83.2	39.1	73.0	42.0	37-255

TMT B-A	52	44.4	32.2	39.0	29.0	0-191
HVLT-R DR (t-score)	55	48.4	10.7	50.0	11.0	20-63
HVLT-R DRI (t-score)	55	50.8	9.25	52.0	13.0	20-60
SDMT (raw score)	58	48.9	11.8	49.0	14.0	18-74
<hr/>						
DASS (raw scores)						
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Stress	56	9.32	7.90	8.0	10.0	0-32
Anxiety	56	4.54	5.76	2.0	6.0	0-28
Depression	56	4.68	5.46	2.0	7.0	0-22
<hr/>						
SCL90 GSI	57	49.8	11.48	50.0	19.0	30-69

169 Max = maximum; Min = minimum; IQR = Interquartile range.

170 Vocab SS = WAIS-III Vocabulary Scaled Score; MR SS = WAIS-III Matrix Reasoning Scaled
171 Score; DS Forwards = WAIS-III forwards digit span subtotal; DS Backwards = WAIS-III
172 backwards digit span subtotal; TMT-A and -B = Trail-Making Test A and B; HVLT-R DR =
173 Hopkins Verbal Learning Test-Revised, Delayed Recall; HVLT-R DRI = Hopkins Verbal Learning
174 Test-Revised Delayed Recognition Index; SDMT = Symbol-Digit Modalities Test; DASS =
175 Depression, Anxiety and Stress Scale; SCL90 Global Severity Index.

176 **Methods.**

177 ***Motor and cognitive scale scores.***

178 Structured medical history and standard neurological motor rating scales with established
179 inter-rater reliabilities [47-49] were administered by two neurologists (ES & DZL) with
180 relevant experience in these scales from previous studies. Motor rating scales consisted of
181 the Unified Parkinson's Disease Rating Scale Part III-Motor (UPDRS-III [50]) the International
182 Cooperative Ataxia Rating Scale (ICARS [51]), and the Clinical Rating Scale for Tremor [52].
183 The Vocabulary and Matrix Reasoning subtests of the Wechsler Adult Intelligence Scale
184 (Third Edition; WAIS-III) were used to calculate a prorated Full-Scale IQ score [53], with
185 Matrix Reasoning providing a measure of non-verbal reasoning. WAIS-III Digit Span
186 component (forward and backward separately) were employed as measures of attention

187 and working memory, respectively [53]. Executive functioning was also assessed using a
188 measure of divided attention/set shifting, the Trail Making Test (TMT)[54]. The Symbol Digit
189 Modalities Test was used as a measure of psychomotor processing speed [55]. The Hopkins
190 Verbal Learning Test-Revised, (HVLTR)[56] is a standard measure of verbal anterograde
191 episodic learning. The HVLTR delayed recall and discrimination recognition indices were
192 employed as measures of recall and recognition memory, respectively.

193 ***Psychiatric pathology test scores.***

194 The SCL-90-R [57] – a 90 item self-administered questionnaire- was chosen as an
195 instrument that can efficiently provide information on a broad range of relevant symptom
196 clusters and psychological concerns. It has good validity and reliability (range: 0.77
197 [Psychoticism index] to 0.90 [Depression index], test-retest: 0.80 to 0.90). Each of the 90
198 items is rated on a five-point Likert scale of distress, ranging from “not at all” (0) to
199 “extremely” (4). The subject is asked to respond to questions based on how much a given
200 problem has “distressed or bothered” him or her during the past 7 days, including the
201 present day. It is typically completed in about 12 to 15 minutes. Here we report a summary
202 score providing a measure of overall psychological distress - the Global Severity Index (GSI) -
203 but the questions relate to nine primary symptom dimensions: Somatization, Obsessive-
204 compulsive, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic Anxiety,
205 Paranoid Ideation and Psychoticism. We also used the DASS (Depression, Anxiety and Stress
206 scale), which is a 21-question subject-self-completed five-point Likert scale measure of their
207 symptoms in these three domains over the preceding week [58].

208 ***Genetic molecular measures.***

209 CGG sizing: Genomic DNA was isolated from peripheral blood lymphocytes using standard
210 methods (Purygene Kit; Gentra, Inc., Minneapolis, MN). For Southern blot analysis, 10
211 micrograms (μg) of isolated DNA was digested with EcoRI and NruI. Hybridization was
212 performed using the specific FMR1 genomic dig labelled StB12.3 probe as previously
213 described [59]. Genomic DNA was also amplified by PCR [60]. DNA analysis was performed
214 in the Laboratory of Dr. Tassone at the MIND Institute, UC Davis.

215 FMR1 mRNA expression level measurements. This assay was conducted at the MIND
216 Institute, University of California Davis Medical Center, Sacramento, CA, USA. Total RNA was
217 isolated from 3mL of blood collected in Tempus tubes (Applied Biosystems, Foster City,
218 California, USA). The measurement of FMR1 mRNA expression levels was carried out by
219 quantitative Real Time qRT-PCR using custom-designed Taqman gene expression assays
220 (Applied Biosystems) as previously described [15].

221 Activation ratio. Activation ratio (AR) indicates the proportion of cells that carry the normal
222 allele on the active X chromosome, so that $AR=1.00$ indicates a normal allele active in 100%
223 of the cells. It was measured based on the intensity of the appropriate bands on Southern
224 blots as described in Tassone et al 1999 [61].

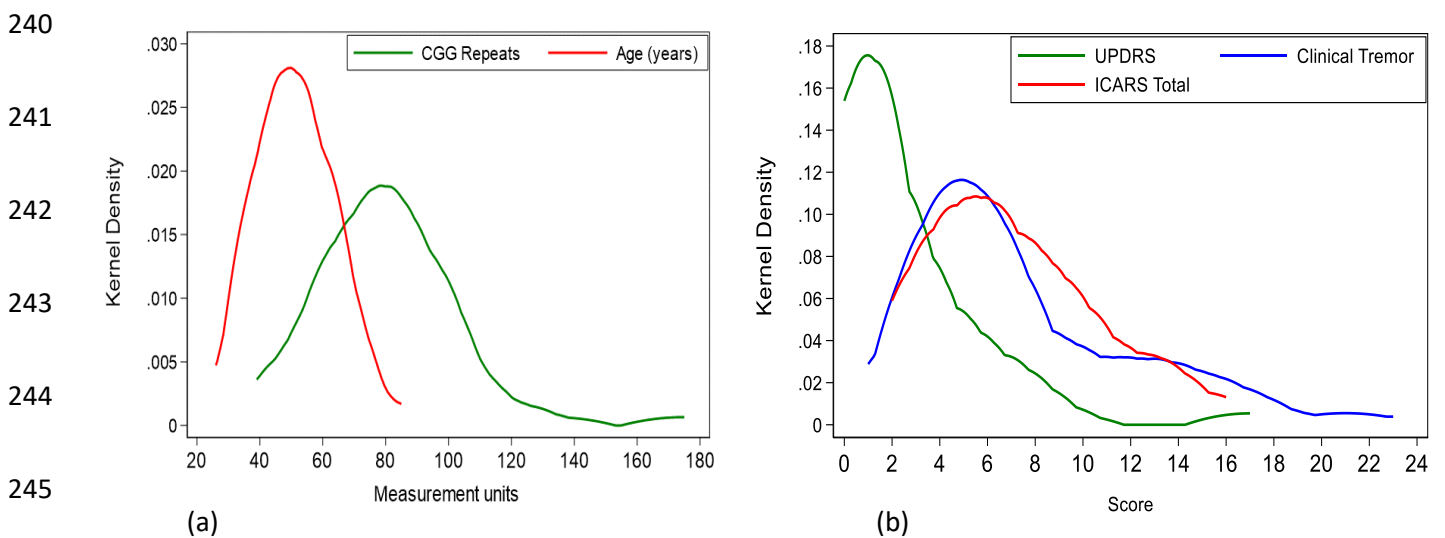
225 ***Statistical analysis***

226 Summary statistics for sample characteristics, cognitive, psychiatric and motor scores, are
227 presented by both mean and standard deviation (SD), and median and interquartile (IQR).
228 Distribution of age, CGG repeat size and motor scores were estimated using the Kernel
229 density method. The relationship between each of the phenotypic scores (as outcome), and
230 CGG repeat size, FMR1 mRNA, age and the level of education (as predictors), was assessed
231 using robust regression. This method down-weights the effect of outliers and influential

232 observations when present. If a relationship was significant, adjustment was made for these
233 predictors by using the residual score (the difference between actual and predicted score)
234 to assess relationships among these outcomes, using Spearman's rank correlation. False
235 Discovery Rate (FDR) was used to adjust for multiple testing. All analyses were carried using
236 commercial software STATA, version 16 (<http://www.stata.com>).

237 **Results.**

238 **Fig 1 Kernel density distribution of age and CGG repeat size (a) and three motor scores (b) in the**
239 **total sample of female carriers.**



246
247 The descriptive statistics (**in Table 1**) include all the core measures used in the correlations
248 presented in **Table 2**. There was a single individual aged 26, and about one third of the
249 participants were aged between 35 and 50, but, as shown in **Fig 1a**, the majority was >50
250 years of age. CGG repeat sizes ranged from 40 to 180, with the mode at approximately 80.
251 A somewhat unexpected result concerned all three motor scales (**Fig 1b**), where one
252 individual (out of 49) scored 17 on the UPDRS; one scored 23 and ten others scored
253 between 11 and 15 on the Clinical Tremor Scale, and nine individuals scored between 11

254 and 16 on the ICARS. Rare individuals scored highly on more than one score. The average
255 scores for ICARS and UPDRS also appear elevated compared with normal control values
256 from the literature for similar age groups but for sexes combined (mean 4.07 ± 2.19 , range
257 1-9 –for ICARS in: Fitzpatrick et al, 2012 [62]; and mean $1.9+2.0$ -for UPDRS in: Postuma et
258 al, 2012 [63]). There is an absence of any control data for the Clinical Tremor Scale in the
259 literature. It is obvious from the data in **Table 1** that the ranges are also increased relative to
260 the normative values, and the mean values are towards the upper end of available control
261 values. We categorized this increase as sub-symptomatic since it had not generated any
262 specific medical diagnoses or realization of abnormality on the part of those individuals
263 presenting with evidently abnormal scores. The wide range of scores is also noticeable for
264 the cognitive test results.

265 The major focus of this study, however, was to ascertain if there is a systematic trend
266 towards parallel motor and cognitive dysfunctions in apparently unaffected female
267 premutation carriers across a wide age range. Firstly, using robust regression, we
268 established that there were no significant relationships between any motor or cognitive
269 scores and either the CGG repeat size or mRNA level (except one significant correlation of
270 the latter with HVLT-R DR, which did not survive adjustment for FDR). However, a majority
271 of both motor and cognitive scores were significantly related to age and level of education.
272 More specifically: UPDRS, DS Forwards, TMT B and TMT B-A, and SDMT scores were
273 significantly affected by both Age and Years in Education; ICARS -by Age only; and Clinical
274 Tremor, Vocab, MR, Pro-rated IQ and HVLT-R DR -by Years in Education only. Consequently,
275 these two confounders have been included in correlations between the motor and
276 cognitive/psychiatric scores, respectively, if required.

277 The results of relationships between all three motor scores (UPDRS, Clinical Tremor and
 278 ICARS) and the 11 cognitive test scores are shown in **Table 2**. All three motor scores are
 279 significantly and consistently correlated with MR, DS Backwards, and TMT B, as well as with
 280 Pro-rated IQ (both for 2 - and 1-sided p values, and after FDR adjustment). Additionally,
 281 both Clinical Tremor and ICARS scores are highly correlated with SDMT. The ICARS score also
 282 correlated with TMT B-A.

283 **Table 2:** Spearman’s rank correlation (ρ) between each motor score and psychological score,
 284 adjusted for age and/or year of education (whenever appropriate).

	UPDRS			Clinical Tremor		ICARS Total	
	N	ρ	p-value	ρ	p-value	ρ	p-value
Vocab SS	48	-0.07	0.650	-0.24	0.099	-0.07	0.658
MR SS	49	-0.36	0.011*	-0.36	0.011*	-0.39	0.006*
DS Forwards	48	-0.07	0.634	-0.17	0.237	-0.20	0.185
DS Backwards	48	-0.29	0.049	-0.33	0.024*	-0.37	0.011*
PRO-rated IQ	48	-0.33	0.023*	-0.38	0.008*	-0.42	0.003*
TMT A (raw score)	46	0.21	0.168	0.53	<0.001*	0.46	0.001*
TMT B (raw score)	46	0.51	<0.001*	0.39	0.008*	0.50	<0.001*
TMT B-A	46	0.43	0.003*	0.21	0.159	0.46	0.001*
HVLT-R DR (t-score)	47	-0.14	0.364	0.11	0.460	0.02	0.891
HVLT-R DRI (t-score)	47	0.01	0.981	-0.01	0.946	0.17	0.262
SDMT (raw score)	47	-0.28	0.060	-0.43	0.002*	-0.36	0.014*

285 *p-values remain < 0.05 after adjustment for multiple testing using False Discovery Rate.

286 Two aspects of these correlation results are of special interest: first, the cognitive measures
 287 showing significant relationships with the motor scores reflect aspects of psychomotor
 288 speed and executive functioning; second, that the largest number of motor x cognitive
 289 correlations concern the ICARS and Tremor scale scores, which represent the type of motor
 290 dysfunctions typically occurring in FXTAS.

291 None of the three motor scale scores showed a significant relationship with the psychiatric
292 pathology test scores: DASS (stress, depression and anxiety domains), and SCL90 Global
293 Index (GSI) or indeed any of the 10 symptom dimensions of the latter (data not shown).
294 Consistent with this result, no significant relationships were encountered between these
295 scores and any of cognitive tests included in this study (after accounting for FDR).

296

297 **Discussion**

298 This is the first study relating performance on three motor clinical scales - assessing tremor,
299 ataxia and parkinsonism- to the level of cognitive performance in a sample of non-FXTAS
300 female premutation carriers. The study results confirm our hypothesis that the two broad
301 clinical domains – motor and cognitive - are correlated, indicating that early
302 neurodegenerative changes simultaneously affect motor and cognitive performance at a
303 sub-symptomatic but clinically detectable level. As this study is cross-sectional rather than
304 longitudinal, we can only infer from these correlations that mild cognitive decline in our
305 sample of premutation carriers tracks motor progression, suggesting a common underlying
306 pathological process. On the one hand, the relationships observed in our non-FXTAS sample
307 of females with premutations involve a set of motor and cognitive traits reflecting deficits
308 occurring in FXTAS. We may therefore suggest that this postulated sub-symptomatic
309 neuropathology may progress to a more extensive form of cerebellar white matter
310 degeneration, with overt clinical manifestations of tremor/ataxia-FXTAS in a proportion of
311 these carriers. On the other hand, these correlations correspond to a set of deficits which
312 has been reported to co-occur in various other conditions associated with lesions of the

313 cerebello-thalamic and thalamo-cortical efferent, and cortico-pontine and ponto-cerebellar
314 afferent tracts (see O'Halloran et al 2012 [64] for review).

315 While the association of the tremor-ataxia motor scales with cerebellar disorders has long
316 been recognized, evidence for the relevance of the cerebellum to a wide range of selected
317 cognitive functions, based on functional magnetic resonance imaging (fMRI) and positron
318 emission tomography (PET), was first presented in the last two decades [65], and indeed
319 these investigations are still in progress. Apart from specific cognitive impairments
320 (particularly affecting executive functions), cerebellar lesions have been implicated in
321 complex behavioural changes, such that ataxia, combined with multiple cognitive and
322 behavioural impairments specifically attributed to cerebellar damage, was originally termed
323 the Cerebellar Cognitive Affective Syndrome [66]. Disconnection of cerebello-cerebral tracts
324 was postulated as an underlying mechanism of this syndrome [67], with the wide range of
325 individual deficits determined by the specific localization(s) of the lesion(s). It is therefore
326 likely that our results, showing correlations between all three motor scores and several
327 cognitive scale scores in premutation carriers, may reflect a sub-symptomatic status
328 determined by incipient structural changes in the cerebellar peduncles, with the potential to
329 progress to more severe and clinically consequential damage. That the brain pathology
330 underlying this apparently sub-symptomatic disorder in the carriers included in our sample
331 was not macroscopically evident is indicated by the absence of MRI changes, including T2
332 hyperintensity in cerebellar peduncles, in a subsample of 32 females with routine clinical
333 MRI scans conducted at the time of neurological and cognitive assessment in this study.
334 However, other studies, by applying more advanced and sensitive MRI techniques - diffusion
335 tensor imaging (DTI) and magnetic resonance spectroscopy (MRS)- have shown that
336 microstructural white matter abnormalities in the middle cerebellar peduncles (MCP) and

337 the genu and splenium of the corpus callosum correlate with executive dysfunction and
338 slowed processing speed in a sample of male premutation carriers without FXTAS [68].

339 The ICARS and Clinical Tremor scales applied in this study are the measures of motor
340 deficits used as the main tools for extracting and rating cerebellar signs in tremor-ataxia
341 disorders, including the FXTAS spectrum, where they highlight core neurological
342 manifestations. Although the UPDRS scale used here largely reflects parkinsonian features,
343 it overlaps with the other two motor scales, particularly as regards tremor, and thus shows
344 significant correlations with the above scores in various cerebellar degenerative disorders
345 [69]. Concerning the motor scores' correlates, it is not unexpected that a variety of non-
346 motor cognitive processes attributed to discrete regions of the cerebellum has been
347 identified in our sample, given that the involved peduncular tracts project into multiple
348 areas throughout the cerebellum. Indeed, a broad range of cognitive deficits, including
349 executive functioning, visuospatial processing, linguistic abilities and affective processes
350 were linked with the impairment following cerebellar lesions in the original description [63],
351 and in subsequent studies (O'Halloran et al [64] see for review). Although the
352 neuropsychological variables considered in our relationship analysis only represent some
353 elements of executive dysfunction (in addition to psychomotor processing speed), those
354 cognitive tests showing significant correlations with motor scores are considered to be
355 dependent on executive processing or psychomotor speed for their execution. More
356 specifically, they consist of two psychomotor speed/visual attention tests (SDMT, TMT-A),
357 and three tests of aspects of executive function: sequencing and alternation (TMT B-A);
358 working memory (DS Backwards; and non-verbal reasoning (MR); with pro-rated IQ partially
359 determined by MR. Notably, the measures which are not as dependent on executive
360 functioning were not correlated with motor scores; they included Vocab (semantic

361 memory), DS Forward (attention/immediate memory) and HVL-R DR and RDI (verbal
362 anterograde episodic memory).

363 The two psychopathology scales, DASS and SCL-90, were included in correlation analysis
364 because of the not uncommon occurrence of psychopathology, especially in female
365 premutation carriers (25, Roberts et al 2016). Neither the DASS (assessing anxiety,
366 depression and stress) or the SCL-90 assessing a wide range of psychopathology domains) in
367 SCL-90 [25, 26] showed significant, or a consistent pattern of, correlations with any of the
368 three motor scores. This is not unexpected considering that the psychopathology tests
369 reflect relatively rudimentary knowledge of the processes underlying psychopathological
370 disorders. Moreover, the psychopathological scales are subjective and completed by the
371 participants themselves, such that there are potential biases related to subjectivity.
372 Moreover, in addition to subjectivity bias and the lack of confirmatory input from an
373 observer familiar with the subject, the tests were designed for clinical evaluation rather
374 than for rigorous statistical process.

375 In spite of the limitations of the study, including relatively small sample size, MR imaging
376 studies performed only in a subset of participants, and the lack of reports assessing post-
377 mortem evidence of structural damage to cerebellar peduncles in asymptomatic females,
378 the results are important at two levels. First, they provide further evidence of cerebellar
379 involvement in cognition, and especially in the executive function domain. Second, both the
380 wide range of tremor/ataxia scores in apparently asymptomatic female carriers, and the
381 relationships between these scores and decline in executive functioning, provide evidence
382 for the existence of an early, widespread disorder of the cerebellum that simultaneously

383 affects motor and higher cognitive performance in non-FXTAS younger premutation female
384 carriers.

385 In contrast to the common belief that neurological and cognitive decline are confined to the
386 FXTAS spectrum occurring in older, predominantly male, individuals, reports of neural
387 pathology in younger and apparently unaffected FMR1 premutation carriers have started to
388 emerge during the last decade. The findings of subtle changes in the integrity of white
389 matter in premutation male carriers without, or prior to, the occurrence of FXTAS [70-72],
390 and of abnormal trajectories in cerebellar and brain stem volumes from early adulthood in
391 these carriers [73] has provided direct evidence for preclinical brain changes. Indirect
392 evidence for the pathological process relevant to neural involvement in non-FXTAS male
393 carriers has also been obtained during the last decade [74, 75], by observing the relationship
394 between CGG expansion size and a modified combined measure of the three motor scales
395 used individually in this study. However, there has been less evidence so far of neural
396 involvement in non-FXTAS female carriers apart from a few reports of the presence of
397 executive dysfunction [34], and early changes in postural stability [35]. However, the most
398 remarkable earlier finding demonstrated the presence of intranuclear inclusions typical of
399 FXTAS in both FXTAS and non-FXTAS female premutation carriers [5].

400 We have not observed significant relationships between either the motor or the cognitive
401 scores included in our analysis with either CGG repeat size or FMR1 mRNA levels. This is
402 consistent with earlier similar results in female premutation carriers [36, 38, 76], which have
403 been interpreted as resulting from small sample sizes impacting adversely an analytic
404 power, particularly in the setting of the potentially weaker and more complex genetic effect
405 on the phenotype of women with X-linked mutations. In addition, it is known that there is a

406 brain-blood difference in *FMR1* mRNA expression, and potentially of the CGG expansion [14,
407 77-79]. Similar concerns apply to the activation ratio (AR), which did not show any relation
408 to any of the cognitive or other phenotypic test scores in the recent comprehensive study
409 [37]. Our analysis of this relationship in a small subsample using activation ratio based on
410 the intensity of the appropriate bands on Southern blots [59], also showed an extensive
411 variation between individuals without any correspondence to individual phenotypes and,
412 for this reason, activation ratio was not considered as a potential confounder in correlation
413 analysis.

414 **Conclusions**

415 Our data expands the evidence for neural involvement in non-FXTAS female premutation
416 carriers by showing that not one but two categories of clinical manifestations of early brain
417 changes – cognition and motor performance - may be simultaneously affected by
418 premutation carriage across a broad range age in asymptomatic individuals. These changes
419 have not been associated with macroscopic brain lesions on MRI in a FXTAS-typical location
420 or otherwise, but obviously more detailed examination of brain tissue, such as cerebral
421 volumes or analysis of incipient structural connectivity changes, might have proved
422 otherwise. Most importantly, future longitudinal studies should follow our findings to assess
423 progress in order to decide whether the elevated motor scores tracking cognitive
424 dysfunction is driven by the premutation status common to all carriers, or by neurological
425 process affecting a subset of carriers who will develop FXTAS spectrum in the future.

426 **Declarations**

427 ***Ethics approval***

428 Research was conducted following procedures approved by the La Trobe and Monash
429 Universities Human Research Ethics Committees in Melbourne, Australia, and UC Davis
430 Institutional Review Board, USA. All participants provided written consent for participating
431 in clinical testing and blood sample collection and analysis.

432 ***Consent for publication***

433 Not applicable-only group data will be published.

434 ***Availability of data and materials***

435 The datasets used and/or analysed during the current study are available from the
436 corresponding author on reasonable request.

437 ***Competing interests***

438 No competing interests.

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443 Loesch and Dr F Tassone.

444 ***Authors' contributions***

445 ES: Conception and partial execution of research project; neurological assessments and
446 motor scales scoring; neuropsychological assessments or supervision of assessments; co-
447 writing (with DZL) of manuscript.

448 DZL: Conception, organization and partial execution of research project; neurological
449 assessments and motor scales scoring; review of statistical analysis; co-writing (with ES) a
450 manuscript.

451 MQB: Design and execution of statistical analysis; review and critique of manuscript.

452 PS: Conduct and interpretation of neuropsychological and psychiatric pathology
453 assessments; organization and partial execution of research project.

454 FT: Conduct and interpretation of genetic molecular assays; review and critique of
455 manuscript.

456 AA: Contribution to cognitive testing and scoring, creating study database; partial execution
457 of statistical analysis; contribution to review and final editing of manuscript.

458

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