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

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Research

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

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Abstract

Background

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

Methods

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

Results

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

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

Conclusions

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

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

Introduction

Large CGG repeat expansions (>200 repeats) in the fragile X mental retardation 1 (*FMR1*) X-linked gene, labelled 'full mutations', cause the Fragile X syndrome (FXS), a neurodevelopmental disorder resulting from hypermethylation of this gene's promoter, resulting in decreased translation Since this mutation is unstable across generations if transmitted through the female parent, the mothers of FXS children are usually carriers of smaller expansions ranging from 55-200 CGG repeats, labelled 'premutations'. Yet smaller expansions (40 to 54 repeats), which do not expand into the full mutation range within two generations but which are nevertheless linked to neurodegenerative changes, are termed "grey zone" expansions [1, 2]. *FMR1* premutations are common in the general population, with the population prevalence ranging from 1 in 130 to 1 in 250 females, and from 1 in 250 to 1 in 810 males [3-4]. The premutation causes premature ovarian failure (FXPOI - menopause before the age of 40) in approximately 20% of female carriers [5]. Both male and female premutation carriers may develop a progressive neurodegenerative condition termed Fragile X Associated Tremor/Ataxia Syndrome (FXTAS) as they age, with a much higher rate in males (between 40-50% after the age of 55) than in females in the same age group (approximately 15%) [6-9]. Typical FXTAS neuropathological changes are of widespread intranuclear inclusions abundant in neurones and astrocytes [10], extending to autonomic nervous and neuroendocrine systems and myocardial cells [11-13].

One component of the nuclear inclusions is the *FMR1* mRNA [14], which has previously been found to be elevated in the blood of premutation carriers as a function of increased CGG repeat number [15]. These findings have led to an hypothesized pathogenetic mechanism that involves a toxic gain-of-function of the expanded CGG-repeat mRNA, which arises

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

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

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

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

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

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

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

MATERIALS AND METHODS

Subjects.

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

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

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

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

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
DASS (raw scores)						
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
SCL90 GSI	57	49.8	11.48	50.0	19.0	30-69

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

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

Methods.

Motor and cognitive scale scores.

Structured medical history and standard neurological motor rating scales with established inter-rater reliabilities [47-49] were administered by two neurologists (ES & DZL) with relevant experience in these scales from previous studies. Motor rating scales consisted of the Unified Parkinson's Disease Rating Scale Part III-Motor (UPDRS-III [50]) the International Cooperative Ataxia Rating Scale (ICARS [51]), and the Clinical Rating Scale for Tremor [52].

The Vocabulary and Matrix Reasoning subtests of the Wechsler Adult Intelligence Scale (Third Edition; WAIS-III) were used to calculate a prorated Full-Scale IQ score [53], with Matrix Reasoning providing a measure of non-verbal reasoning. WAIS-III Digit Span component (forward and backward separately) were employed as measures of attention

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

Psychiatric pathology test scores.

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

Genetic molecular measures.

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

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

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

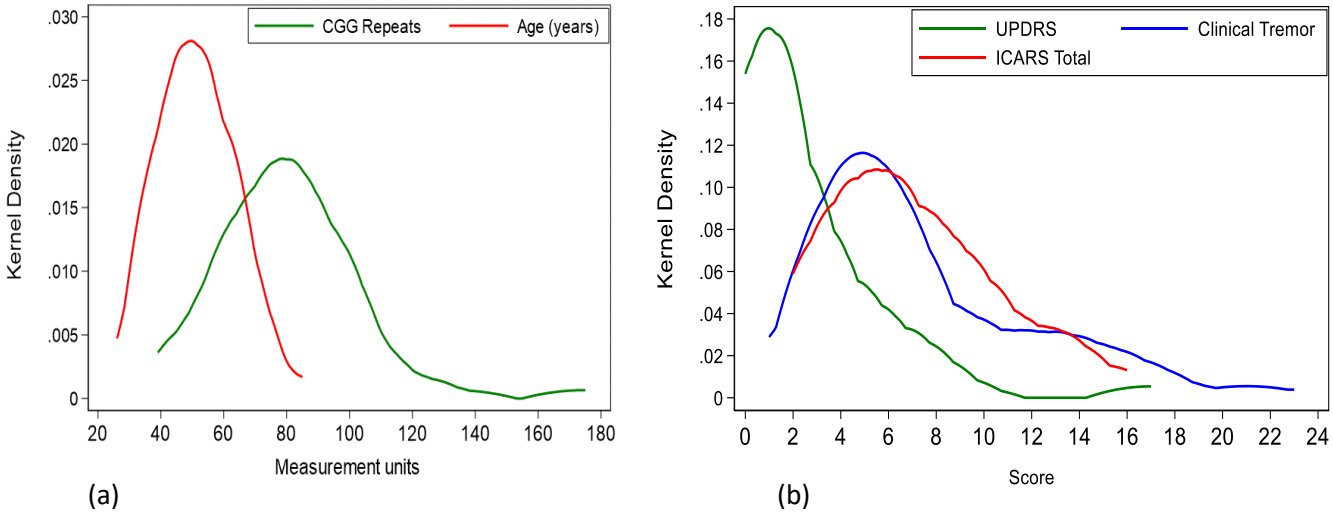
Statistical analysis

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

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

Results.

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



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

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

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

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

Table 2: Spearman's rank correlation (ρ) between each motor score and psychological score, 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*

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

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

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

Discussion

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

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

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

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

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

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

We have not observed significant relationships between either the motor or the cognitive scores included in our analysis with either CGG repeat size or FMR1 mRNA levels. This is consistent with earlier similar results in female premutation carriers [36, 38, 76], which have been interpreted as resulting from small sample sizes impacting adversely an analytic power, particularly in the setting of the potentially weaker and more complex genetic effect 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***

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

Consent for publication

Not applicable-only group data will be published.

Availability of data and materials

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

Competing interests

No competing interests.

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Authors' contributions

ES: Conception and partial execution of research project; neurological assessments and motor scales scoring; neuropsychological assessments or supervision of assessments; co-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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Figures

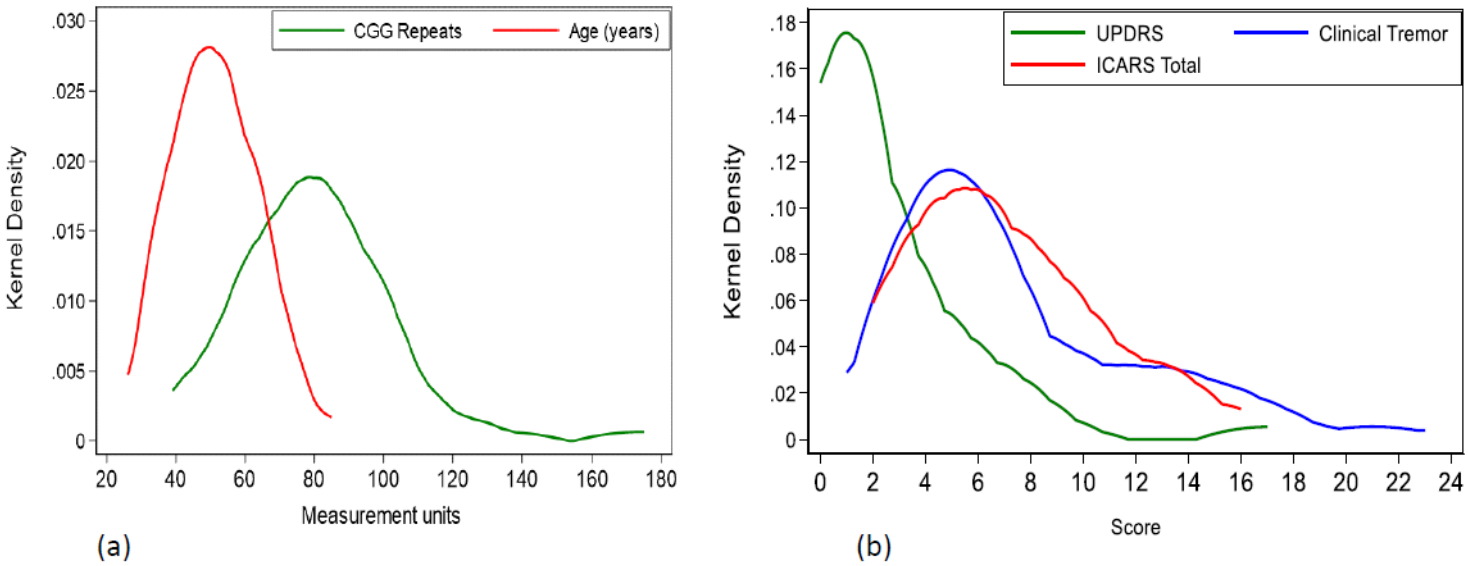

Figure 1

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