NEUROCOGNITIVE AND NEUROMOTOR EVIDENCE FOR THE INVOLVEMENT OF THE *FMR1* GENE IN FEMALE CARRIERS OF FRAGILE X SYNDROME

Submitted by
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A thesis submitted in fulfilment of the degree of
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To Simon Gee, for his love and support

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ABSTRACT

Fragile X syndrome (FXS) is the most common single gene cause of intellectual disability and autism worldwide. FXS is caused by a long (>200) trinucleotide CGG-repeat expansion on the *fragile X mental retardation 1* (*FMR1*) gene located on the long arm of the X chromosome, and through epigenetic gene silencing and alterations to the production of the fragile X mental retardation protein (FMRP), leads to a distinct neurological profile of abnormal synaptic structure and neuroplasticity. While FXS itself affects ~4000 individuals, it is estimated that as many as 1 in 209 females and 1 in 400 males are premutation (PM) 'carriers' of the FXS. For PM-carriers, the expanded CGG-repeat expansion (55-199) can lead to neurotoxic effects and progress to a late onset neurodegenerative disorder associated with executive dysfunction, dementia, tremor and ataxia, called fragile X-associated tremor/ataxia syndrome (FXTAS). Furthermore, female PM-carriers are at increased risk of developing premature menopause associated with fragile X-associated primary ovarian insufficiency (FXPOI). While much of the research to date has focussed on male PM-carriers and late-onset neurodegenerative disorders, the presence of neurocognitive and neurobehavioural manifestations among asymptomatic female PM-carriers is less well understood, but has been controversial. Although recent studies have reported difficulties in executive function, visuospatial processing and psychiatric functioning, and greater than hitherto expected prevalence of later dementia and parkinsonism-related symptoms, it remains unclear the extent to which subtle cognitive and motor manifestations are a *forme fruste* for later onset of more severe neurodegenerative decline, or a stable developmental phenotype.

The overarching aim of this thesis was to investigate neurobehavioural profiles in adult female PM-carriers using hypothesis-driven neuromotor and neurocognitive measures that are known to be sensitive to subtle signs of dysfunction in prefrontal and cerebellar neural networks. Chapter 3 presented the first investigation of the effects of cognitive dual-task interference (counting backward by 3s or 7s) on spatiotemporal gait characteristics in female PM-carriers (22-55 years old) and age-matched controls with normal alleles, and explored relationships between dual-task gait interference and age and CGG-repeat length. The findings from Chapter 3 on gait control revealed significant dual-task costs on spatiotemporal gait characteristics in female PM-carriers compared to controls, and an interaction between age and CGG-repeat length for dual-task related gait variability. These

findings indicated CGG-dose dependent effects on gait automaticity during dual-task performance, suggestive of dysfunction in cerebellar cognitive and motor networks in female PM-carriers. In Chapter 4, the extent to which these neuromotor at-risk profiles extended to postural control were investigated using hypothesis-driven measures of postural stability in response to manipulation of visual, proprioceptive and cognitive input. The results from Chapter 4 revealed significantly increased medio-lateral sway during concurrent performance of an excluded-letter-verbal-fluency task in female PM-carriers compared to controls, with CGG repeat length moderating the relationship between age and postural instability. Together, these findings suggest that measures of gait and postural control under dual-task interference may show clinical utility as outcome measures in future pharmaceutical interventions in the female PM.

To further explore the role of cerebellar-cortico involvement in cognitive and psychiatric symptoms in female PM-carriers, the next section of the thesis (Chapter 5) examined the extent to which female PM-carriers showed deficits in specific subdomains of executive function, and their interrelationships with symptoms of ADHD, anxiety and depression. These findings demonstrated a core deficit in response inhibition alongside elevated symptoms of ADHD and social anxiety in females with the PM. Importantly, measures of response inhibition and working memory were significantly associated with self-reported psychiatric symptoms, and a large proportion of female carriers with poor executive functioning exceeded threshold markers for probable caseness of a mental disorder. While these findings raised the possibility that female PM-carriers may be at-risk of developing a cognitive-affective disorder, the range of cognitive, visuospatial and affective impairments is most consistent with cerebellar-cognitive affective syndrome. Chapter 6 explored implicit sequence learning impairments that may tie in a range of cognitive, visuospatial and affective symptoms. Although female PM-carriers showed preserved implicit learning, the slowed reaction time and poorer awareness of the repeating sequence were suggestive of reduced automaticity. Importantly, there were several important associations between sequence learning performance and a range of executive function, visuospatial and affective symptoms suggestive of cerebellar-cognitive affective syndrome in some females with the PM allele. The lack of age- or CGG-repeat length dependent associations with sequencing performance and cognitive-affective profiles suggests that this

profile may arise from other developmental, molecular (e.g., FMRP, epigenetics) and/or environmental (psychosocial stress, carer burden) factors.

The findings from this thesis converge to suggest at least two pathways, one in which developmental mechanisms may lead to a subtle cognitive-affective profile associated with disruption to cortico-cerebellar pathways, and the other to neurotoxic and ageing effects in those with long CGG-repeat lengths on neural regions underpinning stepping automaticity and postural stability. This novel hypothesis-driven approach to teasing apart cerebellar cognitive and motor profiles offers potential in identifying those PM-carrier women who are at increased risk for neuropsychiatric and neurodegenerative involvement. It will be important for future longitudinal studies to begin to isolate distinct subgroups and identify sensitive risk biomarkers in the female PM that might portend more severe neurological and neuropsychiatric impairments across the lifespan.

LIST OF AWARDS, PUBLICATIONS AND PRESENTATIONS DURING CANDIDATURE

Awards and distinctions

Excellence in research poster presentation at School of Psychological Sciences Research Day forum

Postgraduate travel grant award

National Fragile X Foundation's Rosen/Weingarden Summer Fellowship (Awarded April 2012)

Scholarship: Australian Postgraduate Award

Award for excellence in research poster presentation in Behavioural Neuroscience Honours

Journal publications

Kraan, C. M., Hocking, D. R., Bradshaw, J. L., Georgiou-Karistianis, N., Metcalfe, S. A., Archibald, A. D., Fielding, J., Trollor, J., Cohen, J., & Cornish, K. M. (accepted, 6/2/2014). Symbolic sequence learning is associated with cognitive-affective profiles in female FMR1 premutation carriers. *Genes, Brain and Behavior.*

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Shelton, A. L., Cornish, K., Kraan, C., Georgiou-Karistianis, N., Metcalfe, S. A., Bradshaw, J. L., Hocking, D. R., Archibald, A. J., Cohen, J., Trollor, J., & Fielding, J. (2014). Exploring inhibitory deficits in female premutation carriers of fragile X syndrome: Through eye movements. *Brain & Cognition, 11*, 201-208.

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Published conference abstracts

Kraan, C., Hocking, D., Bradshaw, J., Georgiou-Karistianis, N., Sylvia Metcalfe, Alison Archibald, Joanne Fielding, Julian Trollor, Jonathan Cohen & Cornish, K. (2012). Motor sequence learning in fragile X carrier females: insights into cerebellar dysfunction? *Front. Hum. Neurosci. Conference Abstract: ACNS-2013 Australasian Cognitive Neuroscience Conference.*

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Cornish, K & Kraan, C. (2013). Carriers of Fragile X. *Invited talk - National Fragile X Family Meeting, Birmingham UK* September 2013.

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Kraan, C., Hocking, D., Bradshaw, J., Georgiou-Karistianis, N., & Cornish, K. (2012). Postural control in young female *FMR1* premutation carriers. *Conference Abstract: ANS-2013 Australian Neuroscience Society Conference.*

Kraan, C., Hocking, D., Bradshaw, J., Georgiou-Karistianis, N., & Cornish, K. (2012). New evidence for a complex interaction between executive control and motor functioning in young female *FMR1* premutation carriers. *Oral Presentation: ACNS-2012 Australasian Cognitive Neuroscience Conference* December 1st 2012.

Shelton, A., Kraan, C., Cornish, K., & Fielding, J. (2012). The use of eye movements to detect cognitive changes in carriers of medium expansions of the *FMR1* gene. *Oral Presentation: ACNS-2012 Australasian Cognitive Neuroscience Conference* December 1st 2012.

Kraan, C., & Hocking, D. & Cornish, K., (2011). The Step Ahead Project: Developmental trajectories in male and female carriers of fragile X syndrome. *Oral presentation at the Fragile X Association of Australia Symposium*, Brisbane, October 4th 2011.

Societal contributions

Cognitive underpinnings of fragile X-associated tremor/ataxia syndrome (FXTAS). Specific contribution: Guest lecturer

ACNS-2013 The fourth Australasian Cognitive Neuroscience Conference November 28th to December 1st 2013. Specific contribution: Local Organising Committee member.

Fragile X Syndrome Educational booklet. Monash University, School of Psychological Sciences in conjunction with St. Catherine's School, Science Department. Specific contribution: Author.

Journal article published in Genes, Brain and Behaviour. Specific contribution: Reviewer.

General Declaration

Monash University

Declaration for thesis based or partially based on conjointly published or unpublished work

In accordance with Monash University Doctorate Regulation 17.2 Doctor of Philosophy and Research Master's regulations the following declarations are made:

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes five original papers published in peer reviewed journals or in press subject to minor revisions. The core theme of the thesis is neurocognitive and neuromotor profiles in female carriers of fragile X syndrome. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the School of Psychological Sciences under the supervision of Professor Kim Cornish and Dr Darren Hocking.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapter's One, Three, Four, Five and Six my contribution to the work involved the following:

Thesis chapter	Publication title	Publication status*	Nature and extent of candidate's contribution
One	Neurobehavioural evidence for the involvement of the FMR1 gene in female carriers of fragile X syndrome	Published*	80% contribution by candidate: Review of relevant literature and writing of literature review
Three	Age and CGG-repeat length are associated with neuromotor impairments in at-risk females with the *FMR1* premutation	In press subject to minor revisions*	50% contribution by candidate: Project design, review of relevant literature, recruitment and testing of participants, analysis of data and writing of manuscript.
Four	Cognitive-motor interference during postural control indicates at-risk cerebellar profiles in females with the *FMR1* premutation	Published*	70% contribution by candidate: Project design, review of relevant literature, recruitment and testing of participants, analysis of data and writing of manuscript.
Five	Impaired response inhibition is associated with self-reported symptoms of depression, anxiety, and ADHD in female *FMR1* premutation carriers.	Published*	70% contribution by candidate: Project design, review of relevant literature, recruitment and testing of participants, analysis of data and writing of manuscript.
Six	Symbolic sequence learning is associated with cognitive-affective profiles in female *FMR1* premutation carriers.	In Press*	70% contribution by candidate: Project design, review of relevant literature, recruitment and testing of participants, analysis of data and writing of manuscript.

I have / have not (circle that which applies) renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Signed: ……

Date: ……

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I first extend my deepest thanks to the supervisory team: Professor Kim Cornish an illustrious scientist that has provided me with an unparalleled source of inspiration to help those afflicted by anomalies of the mind; and Dr Darren Hocking, a discerning expert on neuromotor functions and behavioural neuroscience. I especially thank both supervisor's for their honesty, guidance and encouragement throughout the completion of this doctorate.

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OVERVIEW

Fragile X syndrome (FXS) is a widely investigated neurodevelopmental genetic disorder that can affect individuals with variable penetrance and across multiple generations. FXS affects approximately 1 in 4000 males and 1 in 8000 females, and through a defect on the *fragile X mental retardation 1* (*FMR1*) gene, located at Xq27.3 on the long arm of the X-chromosome, causes intellectual disability and autism (Cornish, Turk, & Hagerman, 2008). In FXS, a long repeating trinucleotide CGG sequence (>200 repeats) typically leads to epigenetic *FMR1* gene silencing, and low or absent *fragile X mental retardation protein* (FMRP), a translational repressor important for synaptic structure, plasticity and function (Hagerman, Lauterborn, Au, & Berry-Kravis, 2012). Although the features of FXS (full mutation) have been well characterised at the genetic, cognitive and behavioural levels, it is only in recent years that attention has been focussed on individuals who have a medium expansion of the *FMR1* gene between 55 to 200 CGG repeats, known as premutation carriers (PM-carriers). It is estimated that 1 in 209 females and 1 in 430 males are PM-carriers (Tassone, Long, et al., 2012). Two clinical conditions have been discovered in PM-carriers. First, between 8-16% of females and 45% of males over the age of 50 develop fragile X-associated tremor/ataxia syndrome (FXTAS), a neurodegenerative disorder that is associated with a continuum of executive dysfunction, dementia, tremor and ataxia symptoms (Hagerman et al., 2001; Rodriguez-Revenga et al., 2009; Tassone & Hagerman, 2012). Second, approximately 20% of female PM-carriers will develop premature menopause associated with fragile X-associated primary ovarian insufficiency (FXPOI: (Sherman, 2000; Wittenberger et al., 2007). Furthermore, there is mounting evidence that young females with the PM also show anxious and depressive symptomatology, alongside subtle problems in cognitive functioning, visuospatial processing, and inattention (Keri & Benedek, 2009; Lachiewicz et al., 2010; Lachiewicz, Dawson, Spiridigliozzi, & McConkie-Rosell, 2006; Roberts et al., 2009). However, due to inconsistent findings in the literature these profiles in PM-carrier women are considered controversial (Hunter, Abramowitz, Rusin, & Sherman, 2009; Hunter et al., 2008). While the neurobehavioural profile of male PM-carriers has been relatively well characterised to include weaknesses in executive functions, such as inhibitory control and working memory, that grow worse with increasing age (Cornish, Hocking, Moss, & Kogan, 2011; Cornish et al., 2009; Cornish et al., 2008), the extent to which such cognitive phenotypes in female PM-carriers may portend later onset of dementia and motor symptoms in FXTAS remains to be determined.

FXTAS is a progressive neurodegenerative disorder thought to arise from *FMR1* mRNA gain of function neurotoxicity (Tassone et al., 2004; Tassone et al., 2000). In male PM-carriers, cerebral atrophy, white matter disease, and cerebellar dysfunction are common radiological findings, with white matter lesions in the middle cerebellar peduncles (MCP sign) a major diagnostic criteria (Apartis et al., 2012; Battistella et al., 2013; Hashimoto, Srivastava, Tassone, Hagerman, & Rivera, 2011). Intention tremor and/or cerebellar gait ataxia reflect the core clinical features of FXTAS and these can be accompanied by executive dysfunction (Berry-Kravis et al., 2007). Compared to males, females have relatively low penetrance for FXTAS with less severe neurological involvement (Adams et al., 2007; Apartis, et al., 2012), which is thought to be due to the protective effects of the second X-chromosome (Berry-Kravis et al., 2003; Jacquemont et al., 2003). However, recent studies with females PM-carriers have identified subtle difficulties in neuromotor control indicative of cerebellar involvement (Chonchaiya et al., 2010; Conde et al., 2012; Narcisa et al., 2011) and increased risk for other neurodegenerative disorders such as Parkinsonism and Alzheimer disease (Hall et al., 2011; Tassone, Greco, et al., 2012). Further indicating a broader spectrum of involvement, female PM-carriers both with and without FXTAS have been shown to have a continuum of health problems, encompassing thyroid dysfunction, fibromyalgia, autoimmune diseases, and peripheral neuropathy (Coffey et al., 2008; Rodriguez-Revenga, et al., 2009; Winarni et al., 2012), and at the neuropsychological level, they are at increased risk for both cognitive and psychiatric dysfunction (Adams et al., 2009; Yang et al., 2013). Together, this research literature indicates a diverse and as-yet poorly understood manifestation of *FMR1* related disorders in females with the PM allele. Further investigation of the synergistic effects of FXTAS-genetic risk factors (i.e., CGG-repeat length) and age upon cognitive, psychiatric and neuromotor functioning in females with the PM allele would lead to a better understanding of the risk-factors associated with such deleterious phenotypic impact.

RESEARCH AIMS

The overarching aim of this thesis is to investigate neurobehavioural profiles in adult female PM-carriers using hypothesis-driven neuromotor and neurocognitive measures that are known to be sensitive to subtle signs of dysfunction in prefrontal and cerebellar neural networks. To capture cortico-cerebellar involvement which may underlie subtle neuromotor profiles in females with the PM, this thesis provides a systematic examination of the control of gait and posture under various degrees of cognitive and attentional load. To understand the inter-relationships between executive dysfunction and psychiatric symptoms in female PM-carriers, this thesis will first establish the extent to which female PM-carriers have deficits in specific subdomains of executive function, and then examine the extent to which they are associated with self-reported symptoms of ADHD, anxiety and depression. Finally, to investigate the role of cortico-cerebellar involvement in cognitive phenotypes, this thesis also explores the extent to which deficits in cerebellar sequence learning may tie in a range of cognitive, visuospatial and affective abnormalities in female PM-carriers.

A second aim of this study is to investigate for the first time whether performance on neurocognitive and neuromotor tasks is associated with age- and CGG-repeat length in females with the PM allele. Given that previous research has shown that both age- and CGG-repeat length are associated with FXTAS risk (Leehey et al., 2008; Tassone et al., 2007), this hypothesis-driven approach will be employed to tease out stable neurodevelopmentally based profiles from symptoms of FXTAS and other FXTAS-associated neurodegenerative disorders.

RESEARCH OUTLINE

To achieve these overall aims, this research examines neurobehavioural performance across multiple hypothesis-driven neuromotor and neurocognitive assessments in a cohort of 22-55 year old female PM-carriers and age- and IQ-matched controls with normal alleles. In addition, the thesis will investigate whether neurobehavioural performance is associated with age- and CGG-repeat length in females with the PM.

This thesis is structured as a series of published papers, chapters or manuscripts under review. There are additional sections of joining text were appropriate to ensure that this thesis is coherent. Chapter one will review the existing evidence for a neurobehavioural profile in the female PM, while chapter two will review the role of the cerebellum in cognitive and neuromotor function, and appropriate methodologies for assessment. The empirical papers on gait and postural control are contained within Chapters three and four, while chapter five will examine whether specific subcomponents of executive function are impaired, and if so, whether executive dysfunction is associated with other aspects of the distinctive phenotype such as ADHD and emotional symptoms. The final empirical paper from chapter six will explore cerebellar symbolic sequence learning, and its association with ‘signature' female PM-carrier weaknesses across cognitive-affective domains. Given that the thesis is presented in formatted journal style, there will be unavoidable repetition in some sections. Finally, the General Discussion will evaluate the main findings in view of the overarching aims of this thesis. Given that all participants will be assessed in the same sitting (with cognitive tasks counterbalanced) there will be unavoidable overlap between the cohorts used across the four empirical papers in this thesis. Due to ongoing recruitment and the need for timely publication, chapters three and four will be based on a slightly smaller cohort than chapters five and six.

Declaration for Thesis Chapter 1

Declaration by candidate

In the case of Chapter 1, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Review of relevant literature and writing of literature review	80%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Dr Darren R Hocking	Contributed to discussion of theoretical issues, provided expertise on drafting and a critical review of the manuscript	
E/Prof. John L. Bradshaw	Critical review of manuscript	
Dr Joanne Fielding	Critical review of manuscript	
Dr Jonathan Cohen	Critical review of manuscript	
Prof. Nellie Georgiou-Karistianis	Critical review of manuscript	
Prof. Kim. M. Cornish *	Contributed to discussion of theoretical issues, provided expertise on drafting and a critical review of the manuscript	

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	**Date** 15/2/14
Main Supervisor's Signature	[redacted]	**Date** 15/2/14

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

CHAPTER 1

NEUROBEHAVIOURAL EVIDENCE FOR THE INVOLVEMENT OF THE *FMR1* GENE IN FEMALE CARRIERS OF FRAGILE X SYNDROME

1.1 Preamble to review paper 1: Neurobehavioural evidence for the involvement of the *FMR1* gene in female carriers of fragile X syndrome.

This published review paper explores the extant neurobehavioural evidence for the involvement of the *FMR1* gene in female PM-carriers. This paper covers a range of pertinent topics in the field, including genetics, endocrine function and FXPOI, neurocognitive impact, psychopathological symptoms, neuromotor control, and FXTAS. The specific focus is to generate research questions and hypotheses about potential neural correlates of neurocognitive and neuromotor dysfunction in the female PM. A second focus is to consider for females the importance of both CGG-repeat length and developmental mechanisms that may underlie phenotypic expression. This is the first and most comprehensive review to consider the scope of neurobehavioural involvement in females with the PM allele.

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Review

Neurobehavioural evidence for the involvement of the *FMR1* gene in female carriers of fragile X syndrome

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FMR1 mRNA [42]

ABSTRACT

For years, premutation-carriers of fragile X-syndrome (FXS) were assumed free from any deleterious phenotype. In this review, we discuss the current literature on neurocognitive, emotional and neuromotor profiles emerging in females with the fragile-X premutation, and discuss phenotypic profiles in male premutation-carriers to gain insights into possible underlying mechanisms associated with *FMR1* gene expression. We contend that this emerging phenotypic profile in females with the fragile-X premutation needs further investigation using experimentally-driven tasks sensitive to neural networks especially vulnerable to *FMR1* gene expression. Further investigation of developmental aspects of the female carrier profile is needed to determine the extent to which emotional, cognitive and neurobehavioural challenges indicate at-risk profiles for later degenerative decline, or rather a stable developmental phenotype. These future research avenues will provide critical new information which will enable identification of women at greatest risk for subtle age-dependent neurobehavioural changes well before the onset of more serious clinical consequences alongside the identification of biomarkers which may be useful in establishing the efficacy of future therapeutic interventions.

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Abbreviations: FXS, fragile X syndrome; PM-carrier, premutation carrier; FX-premutation, fragile X premutation; FMRP, fragile X mental retardation protein; mRNA, messenger RNA.

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1. Introduction

Linking genes to behaviour is never easy. It is complex work that relies on finding associations between gene-protein networks, neural circuits in the brain and neurobehavioural performance. Fragile X syndrome (FXS) is an example of a single gene disorder affecting approximately 1 in 3600 males and 1 in 4000–6000 females (American College of & Gynecologists Committee on, 2010), and one of the most investigated worldwide. It is caused by a large (>200) CGG trinucleotide expansion in the 5′ UTR region of chromosome X, whereby concomitant methylation results in allelic inactivation and a complete loss of the fragile X mental retardation protein (FMRP) (Auerbach and Bear, 2010; Tassone and Hagerman, 2012). Without FMRP, a selective RNA-binding protein, translational regulation of target mRNAs at dendritic spines is impaired during development, and this significantly alters the structure and function of neural networks in the brain; this results in FXS which is associated with a distinct neurobehavioural profile of impaired cognitive function, attentional regulation, high anxiety levels, and autism (Cornish et al., 2008a; Tassone and Hagerman, 2012; Wang et al., 2010).

For females who have a CGG expansion between 55 and 200 repeats in the gene associated with FXS, there is a 50% chance that this gene will expand to >200 causing FXS in future generations. These women have a fragile X premutation (FX-premutation) and are often described as carriers of the premutation (PM-carriers). For many years PM-carriers were assumed free from any deleterious phenotypic effect. We now know that FMRP translation can be slightly reduced in high repeat carriers (Tassone et al., 2000). It has also been shown that difficulties in inhibitory control, working memory and visual-spatial processing, alongside higher rates of autism (68–79% of premutation male probands, with 8–28% in non-probands) and ADHD (93% of premutation male probands, but 38% in non-probands), are core phenotypic features in male PM-carriers (Aziz et al., 2003; Cornish et al., 2005, 2008b, 2009, 2011; Farzin et al., 2006; Chonchaiya et al., 2012; Loesch et al., 2007). Furthermore, approximately 40% of male PM-carriers over 50 years and a lower percentage of 8–16% of older female PM-carriers are at risk of severe cognitive and neuromotor dysfunction associated with Fragile X-associated tremor/ataxia syndrome (FXTAS) (Hagerman et al., 2001; Rodriguez-Revenga et al., 2009a). FXTAS is a neurological disorder characterised neuropathologically by the presence of intranuclear inclusion bodies (e.g., in hippocampal pyramidal neurons) and behaviourally by cerebellar gait ataxia, intention tremor, Parkinsonism and executive dysfunction. FXTAS is thought to be caused by messenger RNA gain-of-function mechanisms, whereby elevated *FMR1* mRNA transcripts sequester proteins (e.g., Pur alpha, hnRNP) from their designated role; this leads to cellular stress, disruptions to the integrity of Lamin A/C nuclear wall architecture, loss of specific cerebellar Purkinje cells, and wide-spread brain atrophy that is most severe in the hippocampus, cerebellum and cortex (Brouwer et al., 2009; Garcia-Arocena et al., 2010; Hagerman and Hagerman, 2004a,b; Jin et al., 2007; Tan et al., 2009; Greco et al., 2002).

The discovery of FXTAS led to a new generation of research focused on premutation status and its impact across the lifespan and across multiple levels: molecular, cognitive, brain and behavioural levels. However, many of these studies have used male cohorts, and despite the greater proportion of premutation women (approximately 1 in 259 women and 1 in 813 men), there is only an emerging body of evidence pointing to a female phenotype. In fact, for female PM-carriers, who have a lower chance of developing FXTAS (possibly due to protective X-chromosome inactivation) and a higher (approximately 20%) chance of developing early-onset menopausal signs in Fragile X Primary Ovarian Insufficiency (FXPOI) (highest risk posed to those harbouring between 80 and 100 repeats) (De Caro et al., 2008; Jacquemont et al., 2007; Wittenberger et al., 2007; Murray, 2000; Sullivan et al., 2005), there has been relatively less attention on the female phenotypic profile.

Currently, the neurobiology underpinning an emerging female-specific cognitive and behavioural phenotype including higher rates of social phobia, depression, arithmetic and visuospatial difficulties, and self-reported attentional problems, is poorly understood (Franke et al., 1998; Hunter et al., 2008b; Lachiewicz et al., 2006, 2010). To date, subtle cognitive impairments on targeted visuospatial tasks are suggestive of parietal involvement, potentially affecting spatial, numerical and other comparative processes (Goodrich-Hunsaker et al., 2011a; Keri and Benedek, 2009, 2010). Similarly, recent studies have shown abnormal functional brain activation providing support for frontal involvement in working memory difficulties (Hashimoto et al., 2011a) and cerebellar involvement is indicated by findings of increased psychomotor ability, tremor and poor balance (Goodrich-Hunsaker et al., 2011b; Narcisa et al., 2011; Steyaert et al., 1994). In this review, we postulate that this subtle cognitive and neuromotor profile in the female premutation needs further investigation, and that targeted experimentally-driven tests that are sensitive to prefrontal, frontoparietal and cerebellar functioning are most appropriate. Moreover, we conjecture that further investigation of CGG-repeat sensitive deficits will play a crucial role in distinguishing between *developmental* profiles that resemble in a milder form the full mutation (e.g., attentional difficulties) and result from reduced FMRP, and *degenerative* mechanisms associated with elevated *FMR1* mRNA related to FXTAS. These future research avenues will provide critical new information which will enable informed targeted therapeutic intervention down the track.

2. The genetics of fragile X syndrome and fragile X-associated disorders

There is substantial variation in the clinical features of FXS and overlap between CGG-repeat size boundaries. As shown in Table 1, individuals with FXS can have variation in CGG-repeat between cells (CGG-size mosaicism) and variation in the methylation of the *FMR1* gene between cells (methylation mosaicism). In combination with the diluting effect of X-inactivation (XCI) skew, these factors influence phenotypic expression in females with the full mutation (Godler et al., 2010). In this section, we highlight the sites of *FMR1*

Table 1
CGG-repeat size boundaries and clinical features of fragile X syndrome and the *FMR1* premutation.

Name	CGG length	FMRP	FMR1 mRNA transcript levels	Phenotype
Full mutation	Over 200 CGG repeats	Low-absent	Very low	Severe to mild features of fragile X syndrome
Size mosaicism	Repeat sizes vary between cells with some in the full mutation range (over 200 CGG repeats) and some in the premutation range (between 55 and 200 CGG repeats)	Low-absent	Low in cells expressing full mutation expansion and elevated in cells that express the premutation expansion	Severe to very mild features of fragile X syndrome
Methylation mosaicism	Repeat sizes in the full mutation range (over 200 CGG repeats) with variable methylation	Low-absent	Very low	Severe to mild features of fragile X syndrome
Premutation	Between 55 and 200	Slight reduction in higher repeat holders	Elevated 2–8 times normal levels	Very mild/absent features of fragile X syndrome Risk for late-onset FXTAS and FXPOI
Grey Zone	Between 45 and 55	Unknown	Elevated	Very mild/absent features of fragile X syndrome Risk for FXPOI Possible risk for late-onset FXTAS and parkinsonism

Note: different phenotypes are associated with CGG-repeat length (Tassone and Hagerman, 2012; Godler et al., 2010, 2012; McConkie-Rosell et al., 2005; Loesch et al., 2011; Finucane et al., 2012). The additional impact of X-inactivation on phenotypic expression in females with FXS is not shown in this table.

expression and epigenetic effects underpinning methylation of the *FMR1* promotor. Following this, we focus on the premutation and discuss the molecular impact of FMRP reductions, mRNA gain-of-function toxicity and X-inactivation. We conclude by describing how CGG-length relates to FMRP and *FMR1* mRNA levels in the premutation.

2.1. Sites of genetic expression

FMR1 expression is cell-specific, occurring at different stages throughout development. In the seminal work of Abitbol et al. (1993), 8–9 week old human foetuses studied with in situ hybridisation were found to express *FMR1* mRNA in mesenchymal cells (human cartilage), neural retina, and the first migrating cells. At 25 weeks, *FMR1* mRNA was found in cortical, subcortical and cerebellar structures, with the largest expression in the pyramidal neurons of the hippocampus, and high expression in cells of the nucleus basalis magnocellularis having a significant impact on cholinergic transmission into cortical and limbic regions. More recently, Zangenehpour et al. (2009) used whole-brain expression analysis of FMRP in an Old-World monkey to further demonstrate high FMRP expression in the cerebellar cortex, cerebellar vermis, striatum, medial temporal lobe including the hippocampal formation, frontal cortical regions and the anterior portion of the anterior cingulate gyrus. These sites of *FMR1* expression are likely the most vulnerable to loss of FMRP in the full mutation, and mild FMRP-reductions and elevated *FMR1* mRNA levels within the premutation range.

2.2. Epigenetics

Methylation is an important biomarker of the impact of FMRP and X-inactivation on cognitive function in females with FXS. Promotor region-FREE 1 methylation has been shown to significantly and negatively correlate with the number of FMRP-positive lymphocytes and *FMR1* X-activation in a cohort of 22 FXS-affected females (Godler et al., 2010). Furthermore, hypermethylation of the *FMR1* intron 1 sites has been shown to predict verbal cognitive impairment on the Weschler Abbreviated Intelligence Scale-III (WAIS-III) in a cohort of premutation and full mutation females (Godler et al., 2012). Together these findings have implications for female PM-carriers who may show similar variations in the patterns of methylation on this promoter region.

2.3. The fragile X-mental retardation protein (FMRP) and the premutation

FMRP is a selective mRNA-binding protein that has an important role in regulating/repressing the translation of other non-*FMR1* mRNA cellular transcripts (Cornish et al., 2008a; Tassone and Hagerman, 2012); this process is called translational repression. In the absence of FMRP, translation of cellular mRNA is poorly controlled and this leads to aberrant translation of proteins at the site (Gross et al., 2011; Sokol et al., 2011). For example, translation of the amyloid-β (A-β) peptide precursor protein (APP) is repressed by FMRP; therefore in the absence of FMRP, APP secretion increases (Sokol et al., 2011). FMRP is also important for the elongation, morphology and density of dendritic spines (Comery et al., 1997; Galvez and Greenough, 2005; Irwin et al., 2001; Willemsen et al., 2011), the development of cortical circuits (Callan and Zarnescu, 2011; Till, 2010) and group-1 metabotropic-glutamate-receptor-5 (Gp 1 mGluR5) dependent N-Methyl-D-aspartate (NMDA) receptor internalisation and synaptic plasticity (Bear, 2005).

Insight into the role of FMRP in the emerging FXS-like "signature" in the premutation has come from studies of the knock-in (KI) mouse-model. These findings indicate that mild FMRP-reductions impact upon common signalling pathways that are active during neuronal migration in embryonic development, suggestive of direct effects on brain morphology within the premutation range (Chen et al., 2010; Cunningham et al., 2011; D'Hulst et al., 2009). This has important implications for studies reporting that FMRP significantly correlates with psychiatric levels, neurobehavioural performance and brain activation in humans (Fatemi and Folsom, 2011a; Fatemi et al., 2011b; Hessl et al., 2011; Keri and Benedek, 2011, 2012; Loesch et al., 2003a).

2.4. FMR1 messenger RNA and the premutation

In PM-carriers, *FMR1* mRNA transcripts are significantly increased (2–8 fold) (Tassone et al., 2007; Tassone and Hagerman, 2012). This is thought to produce a toxic-gain-of-function mechanism whereby different mRNA-protein interactions lead to

Table 2
Fragile X primary ovarian insufficiency studies.

Study	Participants	Measurements/methodology	Significant findings
Sullivan et al. (2005)	183 female premutation carriers (CGG: 59 to >100) aged 18–50 years and 324 age-matched controls	Medical and reproductive history questionnaire. Buccal brush samples analyses for CGG repeat length, FSH levels XCI	Female premutation carriers with 80–99 CGG-repeats have the highest risk for developing FXPOI. Specifically, women with repeats of 59–79 and 80–99 repeats entered menopause 2.5 and 4 years earlier than matched controls, respectively. This data does not support a parent of origin effect in the female premutation, and the sample was too small to discuss X-chromosome inactivation patterns. *The mid-range repeat (CGG: 80–99) is a strong risk factor for FXPOI*
Allen et al. (2007)	437 female premutation carriers (CGG: 55–199) aged 18–75 years and 520 age-matched controls. Premutation carriers split into groups of low (CGG: 59–79), mid (CGG: 80–99) and high (CGG: 100–199) repeat-length.	Medical and reproductive history questionnaire. Buccal brush samples or blood analysed for CGG repeat length.	Premature ovarian insufficiency indicated by menstrual dysfunction, infertility and twinning is more common in premutation carriers compared to controls. The earliest onset for menopause and greatest risk is posed to premutation carriers with mid-range repeat lengths (CGG: 80–99). *A non-linear CGG-dependent threshold of 80–99 repeats for FXPOI is supported.*
Spath et al., 2011	Large cohort of 1068 premutation (CGG: 55–200) and control women aged 18–75. * *Cohorts from Nijegen and Atlanta combined*	Medical and reproductive history questionnaire. Buccal brush samples or blood analysed for CGG repeat length.	Menopausal risk is for the first time gauged within a FXPOI prediction model using CGG repeat-length, smoking and background genetics (indexed by menopausal age of close relative). Importantly, CGG-repeat length was the greatest 'risk-factor' and ascertainment location had a significant effect on the model. *CGG-repeat length, smoking, background genetics and ascertainment bias significantly impact upon risk for FXPOI.*
Rodriguez-Revenga et al. (2009b)	260 female premutation carriers (CGG: range not available) aged over 40 years and 220 age-matched controls.	Analyses of peripheral blood for CGG repeat length and XCI.	No difference was found in XCI patterns between those with and without FXPOI in the premutation cohort. There was, however, a decrease in XCI in premutation carriers with 55–99 repeats, and an increase in those with >100 repeats. *Alongside other genetic mechanisms, XCI might contribute to FXPOI.*
Welt et al. (2004)	11 regularly cycling female premutation carriers (CGG: 56–135) aged 23–41 years and 22 age-matched controls.	Markers of ovarian function (LH, FSH, estradiol, progesterone, inhibin A and inhibin B) were measured in blood once per day across an entire menstrual cycle.	Due to a decreased follicular phase length, premutation women presented with a shorter menstrual cycle compared to controls. Differences in hormone levels, such as increased FSH (both follicular and luteal phase) and reduced inhibin A (follicular phase), inhibin B (luteal phase) and progesterone (luteal phase) support a model of reduced function and number of follicles in normally cycling premutation carriers. *Early signs of ovarian ageing can be detected in normally cycling premutation carriers.*
Rohr et al. (2008)	115 female premutation carriers (CGG: 79–100) aged 18–50 years with low (<70) and high (>70) CGG repeat lengths and 113 age-matched controls.	Examination of serum FSH (marker of ovarian senescence) and AMH (marker of ovarian follicle reserve), alongside PCR CGG-repeat analyses determined from buccal samples or venous blood and across three age groups.	In a combined cohort of carrier and non-carrier women, AMH was lower in high repeat women and this was shown early in the young (18–30 years old) group. This same effect was observed for FHS only in women 31–40 years old. In the premutation cohort that was aged 31–40 years, AMH levels were lower in those with greater than 70 CGG repeats compared to those with less than 70 repeats. *AMH has better sensitivity than FSH to reduced ovarian reserve in high repeat premutation carriers at risk for FXPOI.*

Table 2 (*Continued*)

Study	Participants	Measurements/methodology	Significant findings
Spath et al. (2011)	127 female premutation carriers (CGG: 79–100) aged 18–55 years and 113 age-matched controls.	Statistical methods used to ascertain the effect of age and premutation status on levels of serum AMH, a marker of ovarian follicle reserve. CGG repeat was determined by standard PCR methods on blood.	AMH levels in the combined cohort decreased by 10% each year, and, at any age, AMH levels were approximately 46% lower in premutation carriers compared to controls. Using statistical modelling, both age and premutation status significantly predicted AMH levels. *AMH levels could serve as a potential biomarker for FXPOI; however, larger longitudinal studies are first needed to establish certainty.*
Hunter et al. (2010)	334 female premutation carriers (CGG: 55–160) aged 18–50 years and 203 age-matched controls. 37 male premutation carriers (CGG: 55–180) aged 18–50 years and 114 age-matched controls.	Medical history and behavioural questionnaires.	Mental health problems including ADHD, anxiety and depression were more prevalent in females compared to males. Interestingly, ovarian insufficiency (indicated by self-reported irregular menses) was associated with both thyroid problems and depression/anxiousness, but not ADHD or any other cognitive profile. *Menstrual abnormalities should be considered in future studies examining mood disorders in the female premutation.*
Hoffman et al. (2012) **First mouse model study of ovaries*	45 female CGG KI mice (CGG: 130) aged 4 (reproductive maturity), 7–9 (reproductive peak) and 10–12 (peri-menopausal) months and age-matched female WT mice. Mice backcrossed at least 15 generations where possible.	Histological methods were used to index *FMR1* mRNA, FMRP and ubiquitin in the ovaries. Further analyses were used to determine follicle number and the presence of cysts.	Establishment of primordial follicle pools in ovary is normal, but follicles of all class rapidly decline in number in premutation mice after the age of 4mths. This is attributed to reduced follicle survival. High levels of *FMR1* mRNA (interactions noted to both age and genotype) were also observed in the ovaries of premutation mice alongside surprisingly high levels of displaced FMRP in the oocyte nucleus. Other novel findings include increased ubiquitin in cells with displaced FMRP, coronal abnormalities in many premutation follicles advancing in meiosis, more atretric follicles in premutation mice, a CGG repeat effect on atresia, and the detection of ovarian cysts. *This study supports the ovaries as the primary site of effect with its aetiology associated with a rapid decline in developing follicle pool number (not original pool number). The mechanisms of involvement could not be determined; for example, although findings fit the FMR1 RNA gain of function model, displaced FMRP in the cytoplasmic nucleus supports a previously unknown role for aberrant protein accumulation/folding/degradation in FXPOI.*
Lu et al. (2012)	Female CGG KI mice (CGG: 90) aged 8 days, 25 days and 9 weeks	Histological methods were used to quantify FMRP, *FMR1* mRNA, follicle number, hormone levels, and, Akt and mTOR protein phosphorylation.	Inconsistent with other mouse model studies the present study did not detect reduced FMRP levels. This allowed a unique investigation of independent effects of elevated *FMR1* mRNA transcripts. Results show a reduced number of developing follicle cells next to normal levels of primordial follicle, abnormal hormone levels, signs of increased apoptosis, downregulation of ovulation-related genes, and, reduced phosphorylation of Akt and mTOR proteins. *Although follicle establishment is normal in FXPOI, the development and survival of cells are seriously compromised. The present results associate the aetiology of FXPOI with FMR1 mRNA alone, and for the first time, implicate the Akt/mTOR pathway.*
Chang et al. (2011)	5 ovaries from female premutation carriers (CGG: 62–100) aged 31–72 years old and 4 from age- and menstrual-cycle matched controls. One control FXTAS brain (to compare inclusion bodies)	Histological methods were used to quantify ubiquitin-positive inclusion bodies.	Ubiquitin positive inclusion bodies that were similar (albeit smaller) to those seen in a donated FXTAS-positive brain were found in the stromal cells of premutation ovaries. Follicle number was comparable between the premutation and control cohort. *Inclusion bodies in FXPOI are unlikely to impact ovarian structural development, but may exert degradation through toxic gain of function mechanisms.*

Abbreviations-ADHD: attention deficit hyperactivity disorder; Akt: protein kinase B; AMH: anti-mullerian hormone; FSH: follicle stimulating hormone; FXPOI: fragile X premature ovarian insufficiency; FXTAS: fragile X tremor ataxia syndrome; LH: PCR: polymerase chain reaction; luteinising hormone: LH; mTOR: mammalian target of rapamycin; XCI: X-chromosome inactivation.

aberrant protein expression and sequestration of cytoplasmic proteins from their pre-determined role (Garcia-Arocena et al., 2010; Hagerman et al., 2004; Willemsen et al., 2011). Animal models support mRNA-gain of function as the cause of FXTAS neuropathology; for example, *FMR1* mRNA biochemically separated from the *FMR1* allele has been shown to induce inclusion formation and neurodegeneration in Purkinje neuron-specific transgenic mice (Hashem et al., 2009).

Inclusion bodies found in the central nervous system of men with FXTAS stain positive for *FMR1* mRNA and a broad array of more than 20 proteins (Greco et al., 2002, 2006; Iwahashi et al., 2006; Tassone et al., 2004). Significant insights into the mechanisms that underlie mRNA induced toxicity have been achieved by studying the mRNA-binding proteins found in these inclusion bodies (Iwahashi et al., 2006). For example, over-expression of Pur alpha, hnRNP A2/B1 and CUG BP1 have been shown to rescue neurodegeneration in a FXTAS-Drosophila-fly-model (Jin et al., 2007; Sofola et al., 2007). Furthermore, examination of KO-mice has revealed that Pur alpha is involved in postnatal brain development (White et al., 2009) and neural drop-out in brain regions with high plasticity functions–that is, the hippocampus and cerebellum (Khalili et al., 2003; Tan et al., 2009). Further proteins thought to be sequestered via *FMR1* mRNA gain-of-function mechanisms include Sam68, an mRNA binding protein that is involved in the regulation of splicing of target mRNAs (Sellier et al., 2010), Lamin A and C isoforms which impact the integrity of cellular walls (Garcia-Arocena et al., 2010), and heat shock proteins such as HSP and alpha-B crystallin (Arocena et al., 2005). Finally, although *FMR1* mRNA gain-of-function is typically understood as a toxic degenerative process, mRNA dependent inclusion formations have been found in mice as early as 20 weeks (Willemsen et al., 2003), with wide distribution at 52–70 weeks (Wenzel et al., 2010), suggestive of early toxic effects.

2.5. X-chromosome inactivation and the premutation

X-chromosome inactivation (XCI) is a process whereby one of the two female X-chromosomes is randomly turned off in embryonic development. This has a 'diluting effect' leading to a less severe FXS-phenotype in females (Sobesky et al., 1996) and reduced risk for FXTAS (8–16%) in premutation females compared to premutation males (40%) (Rodriguez-Revenga et al., 2009b). XCI combined with methylation state of the *FMR1* promotor, and changes in FMRP production, strongly impact phenotypic outcome in females with FXS (Godler et al., 2010). Of interest are the results of Garcia-Alegria et al. (2007) who have shown that the relationship between *FMR1* mRNA and CGG length changes significantly after 100 repeats. Thus XCI may biologically counter this effect by increasing the percentage of cells with the normal allele. This suggests that future studies in the female premutation should control for the impact of XCI on phenotypic outcome.

2.6. CGG-repeat length

Increasing CGG repeat length is associated with increased mRNA levels and moderately reduced FMRP. There exists sound support for a linear relationship between *FMR1* mRNA transcript levels and CGG repeat size within the premutation range (Allen et al., 2004; Garcia-Alegria et al., 2007; Peprah et al., 2010). However, the relationship between FMRP translation and CGG-repeat length remains unclear. In a preliminary study with 74 male PM-carriers, Peprah et al. (2010) have shown that mean FMRP levels are significantly higher in males with between 80 and 89 repeats than in those with low (54–79) or high (90–120) repeat lengths. Moreover, a significant correlation between CGG length and translational efficiency in lymphoblastoid cell lines was found in the upper premutation range (>95 repeats) (Hoem et al., 2011; Primerano et al., 2002). Thus a decrease in FMRP as CGG length moves towards and above 100 is supported by the current literature, but more research is needed to understand reduced translational inefficiency within the low repeat range. In males with FXTAS, evidence points towards a CGG-sensitive effect on the severity of motor dysfunction (Leehey et al., 2008), which is likely due to high *FMR1* mRNA levels within the male upper premutation range (>100). Male phenotypic features developing in high repeat carriers (>100) early in life, however, may be due to both increased *FMR1* mRNA and lowered FMRP (Cornish et al., 2011; Keri and Benedek, 2012). This differs from the female situation where XCI may dilute *FMR1* mRNA levels when CGG-length is over 100 repeats (Garcia-Alegria et al., 2007). Further studies are needed to determine the extent to which toxic effects dominate in specific CGG repeat ranges similar to those seen in male PM-carriers.

3. The reproductive endocrine profile in fragile-X premutation carriers

Female PM-carriers are at-risk for Fragile X Primary Ovarian Insufficiency (FXPOI), defined as complete cessation of the menses prior to the age of 40 (Sherman, 2000). FXPOI is more common in PM-carriers (approx. 20%) than Premature Ovarian Insufficiency (POI) in the general population (approx. 1%) (De Caro et al., 2008; Jacquemont et al., 2007; Wittenberger et al., 2007). Although cessation of menses is required for a clinical diagnosis, it is now well accepted as a disorder that presents with a spectrum of involvement: ranging from low impact symptoms such as heavy bleeding, irregular periods or increased rates of twinning, to infertility, to major impact symptoms such as premature menopause (Allen et al., 2007; Sherman, 2000; Nelson, 2009; Wittenberger et al., 2007). Indeed, it has been shown that even women who do not have FXPOI experience menopausal signs on average 5 years earlier than non-premutation females (Murray, 2000). In this section, we summarise studies on the reproductive endocrine profile in female PM-carriers, and provide a brief discussion on hormonal and molecular mechanisms (see Table 2). However, a thorough discussion of these reproductive studies is beyond the scope of this review (see Sullivan et al., 2011; Sherman, 2000; Nelson, 2009; Wittenberger et al., 2007, for comprehensive reviews of this topic).

As seen in Table 2, the ovaries are the currently supported loci for primary molecular involvement in FXPOI. Support comes from recent animal models (Hoffman et al., 2012; Lu et al., 2012) and evidence that reproductive problems do not occur in male PM-carriers. Ovarian ageing is also known to impact hormonal levels in the premutation. For example, follicle stimulating hormone (FSH) is increased and Anti-Müllerian hormone (AMH) is lowered even in women still cycling (Hundscheid et al., 2001; Murray et al., 1999; Rohr et al., 2008; De Caro et al., 2008). These changes are likely to result from a primary defect at the level of the ovary, but although speculative, may be secondary to mechanistic issues within negative sex-hormone (oestrogen and androgen) feedback loops acting on the hypothalamic Gonadotropic Releasing Hormone (GnRH) pulse generator (Berga and Naftolin, 2012). Importantly, the significance of such hormonal change for overall brain function and behaviour in the female premutation is hitherto unknown.

At the molecular level, emerging evidence supports FXPOI as a disorder resulting from degenerative effects, but not to developmental mechanisms associated with the establishment of the original follicle pool (Hoffman et al., 2012; Lu et al., 2012; Chang et al., 2011). This notion is in line with the currently accepted RNA toxicity model for FXPOI (see Section 2.4). Further support comes from the fact that FXPOI is not detected in females with the full mutation who have little or no FMRP (Sherman, 2000), and evidence of compromised ovarian cell quality in mouse models presenting

with normal FMRP protein levels (Lu et al., 2012). Most interestingly, however, a non-linear CGG-repeat FXPOI 'high-risk' band of 80–100 repeats has been consistently reported (the range associated with the highest levels of *FMR1* mRNA in female carriers) (Sullivan et al., 2005; Allen et al., 2007; Spath et al., 2011), alongside findings of increased ubiquitin-positive inclusion bodies in the stromal cells of the ovaries (Chang et al., 2011), providing further support for this notion.

Currently, the existing data has not yet been able to distinguish between potential secondary effects of FXPOI symptoms (i.e., the effects of hormones on mood disorders) and CCG-dependent profiles (i.e., at-risk profiles found in the 80–100 CGG-repeat band). However, there is emerging evidence for an association between FXPOI and psychopathology (Hunter et al., 2010), suggesting that future cross-domain studies are needed to examine CGG-repeat length thresholds and associations between FXPOI and the neurobehavioural profile in the female fragile X-premutation.

Table 3
The behavioural dyscontrol scale (BDS).

Item	Description
1	Tap twice with the right hand and once with the left in series
2	Tap twice with the left hand and once with the right hand in series
3	If I say "red" squeeze my hand. If I say "green" do nothing
4	If I tap twice, you tap once. If I tap once, you tap twice
5	Alternate touching of thumb and fingers (learning of a motor sequence)
6	Fist – edge – palm (learning of a motor sequence)
7	Mirror image test
8	Alphanumeric sequencing (e.g., 1, a, 2, b, 3, c,. . .12, l)
9	Insight rating (how do you think you went?)
10	Time required to complete alphanumeric sequence from 1 to the letter 'l'

NB: the BDS is 9-step behaviourally administered test of executive function and behavioural regulation that has shown sensitivity in both male and female premutation cohorts.

4. The neurocognitive profile in the fragile X-premutation

A decade ago it was assumed that the FX-premutation was free from any deleterious phenotype, that is, without discernible cognitive or brain impairment. In recent years, it has been shown that some male PM-carriers have subtle profiles reflecting deficits in the areas of executive function and visuospatial cognition. It is yet to be determined whether a similar profile exists in female PM-carriers, however. In the next section, we review the cognitive profile in males with the premutation which may offer important insights into possible underlying mechanisms associated with the *FMR1* gene expansion in female PM-carriers.

4.1. Executive dysfunction in the premutation

Despite findings of subtle neurobehavioural and morphological features of FXS in the premutation (Aziz et al., 2003; Farzin et al., 2006; Mazzocco, 2000; Sobesky et al., 1996), standardised intelligence tests have failed to find a neurocognitive profile in PM-carriers. This may be because PM-carriers do not exhibit global neuropsychological decline and are of average (Allen et al., 2011; Cornish et al., 2008b, 2009, 2011; Hunter et al., 2012b) to above average intelligence (Allen et al., 2011; Grigsby et al., 2008; Hunter et al., 2008b; Moore et al., 2004a), performing similarly to age-matched controls on standardised tests of general intelligence (Allen et al., 2011; Hunter et al., 2008b). Recently, Allen et al. (2011) found that 23 male PM-carriers performed similarly to non-carrier sibling controls on all subdomains of the WAIS-III. PM-carriers also perform similarly to controls on many tests of executive function. For example, Hunter et al. (2008b) examined performance on standardised tasks of executive function in a large cohort of 30 male (CGG: 55–180) and 293 female (CGG: 55–160) PM-carriers aged between 18 and 50 years old. In this study, PM-carriers performed similarly to controls on all measures. This may be due to the selection of multi-factorial executive function tests (Strauss et al., 2006). For example, the Wisconsin card sorting test (WCST), trial making test (TMT) and verbal fluency rely on multiple cognitive constructs and activation of numerous brain regions (Kraan et al., 2013; Lie et al., 2006; Sanchez-Cubillo et al., 2009). General intelligence tests and multi-factorial executive function tests may not be sensitive to the subtle changes seen clinically in a subset of individuals with the FX-premutation (Hagerman and Hagerman, 2004a,b).

Evidence for a subgroup of PM-carriers presenting a subtle profile of the same strengths and weaknesses found in the FXS is over a decade old (Franke et al., 1998; Loesch et al., 1994; Mazzocco, 2000; Murphy et al., 1999; Riddle et al., 1998; Sobesky et al., 1994, 1996; Steyaert et al., 1994). Importantly, specific deficits on the behavioural dyscontrol scale (BDS) in a young-adult premutation cohort support the presenting neurocognitive profile as predominantly one of executive dysfunction (Brega et al., 2008; Grigsby et al., 2008; Loesch et al., 2003a). The BDS is a 10-step behaviourally administered test (see Table 3). Using logistical regression, Loesch et al. (2003a) have shown that in a male ($N = 32$) and male/female ($N = 179$) combined premutation cohort, premutation status and FMRP levels are significantly associated with FSIQ-adjusted total BDS performance. Furthermore, additional logistic regression analyses indicated that FMRP interacted with performance in the premutation on items of purposefully controlled movement and executive function (items 5, 6, 9, and 10 in males and items 6 and 10 in females). The effect of FMRP on BDS performance in the FX-premutation also did not mirror that of performance in the full mutation, which was more generally impaired (items 2–7 in males and items 1, 2, 3, 7, 9 in females). Importantly, although the BDS has been used to show that the core underlying feature of the cognitive profile in FXTAS is executive dysfunction (Brega et al., 2008), it is not yet clear if impaired BDS performance in the premutation (especially items 6 and 10) reflects pre-clinical FXTAS.

As shown in Table 4, more recent studies which have teased apart specific subcomponents of executive function point towards core inhibitory and working memory impairments in a subset of male PM-carriers. Executive functions mediate appropriate initiation, inhibition, shifting and sustenance of thoughts and behaviours (Strauss et al., 2006). Cornish et al. (2008b) first identified an inhibitory profile, growing increasingly severe with age after the 3rd decade of life, in a cohort of 34 male PM-carriers. Similarly, an age- and CGG-dependent central executive working memory profile was found in a cohort of 18–69 year old premutation carriers ($N = 40$) (Cornish et al., 2009; Kogan and Cornish, 2010). Further examination of selective executive function tasks revealed that high-load, domain-specific measures were more likely to reveal subtle age- and CGG-dependent deficits than domain-general tasks (Cornish et al., 2011). Indeed, the Hayling's sentence completion test exhibited superior sensitivity to inhibitory deficits in the premutation compared to the Stroop colour word test. Similarly, the letter-number-sequencing (LNS) test was more sensitive to working memory than the paced auditory serial addition (PASAT) test. Functional MRI (fMRI) studies have revealed broad neural activation on the Stroop colour word test and specific activation of the dorsolateral prefrontal cortex (DLPFC) during performance on the Hayling's test (Leung et al., 2000; Nathaniel-James and Frith, 2002). Moreover, the PASAT activates broadly and the LNS is specific to the DLPFC, orbital frontal cortex and posterior parietal cortex (Audoin et al., 2005; Haut et al., 2000). These core inhibitory and working memory deficits reflect a specific vulnerability along

Table 4
Executive function studies.

Study	Participants	Measurement/methodology	Correlations	Significant findings for premutation carriers without FXTAS
Moore et al. (2004a)	20 male premutation carriers with a mean age of 53.25 (CGG mean was 85.6) and 20 age- and IQ-matched controls. Recruited through genetic services. No exclusion for confounding effects of FXTAS.	General intellectual functioning (WAIS-R); executive function (COWAT, TMT, TOL, EGT); memory (DB, DF, VMF & VMB WMS-R); long term memory (DPT); learning (verbal and visual paired associates of WMS-R subtests); prose recall (LMS of WMS-R), semantic memory (CF); attention (CPT); visuospatial and perceptual processing (VOSP); and language and pragmatics.	No relationship between performance deficits and CGG length, FMRP level or mRNA level.	Significantly reduced performance on tests of executive function (COWAT, CF, TMT B-A, problem solving), attentional, long term memory, verbal and visual pair-learning performance was detected. Also, a trend towards anxiousness was found. *Premutation deficits that are specific to certain aspects of cognition may reflect very early signs of FXTAS.*
Hunter et al. (2008b)	30 males (CGG: 55–180) aged 18–50 years old, 293 female (CGG: 55–160) aged 18–50 years old, 75 non-carrier male and 117 non-carrier female controls	A total of 21 variables generated from performance across general IQ, memory, executive function and attention measures were used in a PCA model to construct six cognitive factors in a large non-gender specific cohort of carrier, non-carrier and intermediate carrier controls. Relationships between carrier group and CGG length were then examined.	No factor structure correlated with repeat in males. CGG repeat length was positively and significantly associated with the frequency of self-reported attention problems in females only.	Premutation status is not associated with global neuropsychological decline. However, female carriers self-report more problems on *inattention and memory* compared to controls and this is significantly related to CGG length. *Female carriers may exhibit a subtle CGG-dependent attentional profile.*
Hunter et al. (2009)	Review paper: inclusion criteria: PCR and/or southern blotting of CGG length; standardised, valid and reliable tests; participants 18+ years old (males over 50 excluded, women over 50 included); non-carrier comparison included; statistical P-values reported; article published in peer reviewed journal.	Analyses of 16 male and female premutation studies crossing domains of cognition, emotion, general intelligence, memory, executive functioning, spatial abilities and psychiatric symptoms.	n/a	No reports of any male or female premutation-specific impairment on executive function, memory or visuospatial ability. However OCD was increased in males and depression, anxiety and somatisation appear to be enhanced in females. *More research needed because included studies were limited by sample size, lack of proper controls and inconsistent study design (*e.g., *ascertainment, CGG threshold for premutation cut-off and CGG range).*
Grigsby et al. (2008)	28 male premutation carriers *without* FXTAS (CGG: 57–150) with a mean age of 59.1 (mean), 42 males *with* FXTAS (CGG: 60–142) with a mean age of 68.1 and 39 education-matched controls (CGG: 15–47) with a mean age of 63.5. FXTAS determined on *neurological screen*	Extensive cognitive testing: WAIS-III composite scores (FSIQ, VIS, PIQ), WAIS-III subtests (WMI, SS, BD, OA, PA, PCS) and BDS, COWAT, RALVT, SDMT and S-CW.	No correlations examined	A profile consistent with dysexecutive syndrome has been identified in male carriers with FXTAS. Male premutation carriers without FXTAS also performed worse than controls on a composite executive function measure created from performance on the BDS and COWAT. Moreover, asymptomatic carriers performed worse than controls on the immediate and delayed recall test of declarative verbal learning memory. *In a subset of male premutation carriers without FXTAS, some, but not all, executive functions are impaired.*
Cornish et al. (2008b)	34 male premutation carriers without FXTAS (CGG: 55–161) aged 20–69, 6 male premutation carriers with FXTAS (CGG: 63–160) and 67 male controls that were individually age-matched and did not differ on IQ, socioeconomic or employment status. *FXTAS determined on neurological exam.*	Cognitive testing: Response inhibition (Hayling's and S-CW), attention (TEA & SART), visual functions (VOSP & JOL), visual-memory function (DOT-test), DF WMS-III)	An age-dependent relationship between CGG length and inhibitory control was found in male premutation carriers that do not have FXTAS.	Selective attention was overall lower compared to controls but did not show any age-related decline. Premutation males aged 30+ show age-and CGG-dependent divergence from controls on tasks that are sensitive to inhibitory control. *Selective brain networks subserving inhibitory control, but not attention or visual functions, may be vulnerable to toxic effects of FMR1 mRNA found in male premutation carriers with high CGG repeats.*

Table 4 (*Continued*)

Study	Participants	Measurement/methodology	Correlations	Significant findings for premutation carriers without FXTAS
Cornish et al. (2009)	34 male premutation carriers without FXTAS (CGG: 55–161) aged 18–69, 6 male premutation carriers with FXTAS (CGG: 63–160), and 67 male controls that were individually age-matched. *FXTAS determined on neurological exam.*	Analysis of phonological loop, visuospatial sketchpad and central executive of working memory functioning.	CGG length significantly correlated with central executive of working memory in the premutation and the visual-spatial working memory in a combined cohort of premutatuion and non-premutation carriers.	Premutation males aged 40+ show age-dependent divergence from controls on tasks that are sensitive to central executive working memory. *The results of this study support a relationship between CGG-length and performance on high load domain-specific tasks of executive function. Moreover, two genetic pathways may subserve the male premutation phenotype; one whereby reduced FMRP leads to developmental change and another where reduced FMRP and increased FMR1 mRNA lead to toxicity and neural degeneration.*
Kogan and Cornish (2010)	34 male premutation carriers without FXTAS (CGG: 55–161) aged 18–69, 6 male premutation carriers with FXTAS (CGG: 63–160) with FXTAS, and 67 male controls that were individually age-matched and did not differ on IQ, socioeconomic or employment status	Comparison of performance on subjective (Brown ADD scale working memory cluster) and objective (LNS, SS–F, SS-B, S-CW & Hayling's) measures of working memory,	Brown ADD Cluster 5 T scores significantly correlated with LNS performance. Also, CGG length significantly correlated with digit span backward and LNS performance only.	The severity of clinically significant working memory complaints was related to performance on objective measures on central executive working memory performance. *The Brown ADD scale can be used to identify male premutation carriers at risk for working memory problems.*
Cornish et al. (2011)	40 male premutation carriers (55–161) aged 20–68	Cognitive tests sensitive to inhibition (i.e., Category B errors on Hayling's and S-CW) and working memory (i.e., LNS & PASAT).	Age-dependent relationship between CGG repeat length and both category B errors on the Hayling's test and performance on the LNS. No interaction between CGG length and S-CW or PASAT performance.	Inhibitory and working memory control may deteriorate with age in male carriers with over 100 CGG repeats. *The CGG- and age-dependent deficit observed in the male premutation appears to reflect vulnerability along central executive control circuits.*
Hunter et al. (2012b)	Multi-site collaboration of 100 male premutation carriers aged 18+ and 216 non-carrier controls	Executive function tests examined across all three cohorts were the COWAT, S-CW, BD from WAIS-III & LNS. Tasks available for examination in select cohorts were the BDS, Hayling's, & WCST	Only performance on the Hayling's test showed an age-dependent association with age and CGG length	Decreased performance on the S-CW, BDS and Hayling's was found in the premutation group compared to the control group, however only the latter declined with age. *There is no global executive function deficit in the male premutation. A domain specific deficit however, may exist in a subset of carriers.*
Allen et al. (2011)	23 male premutation carriers without FXTAS (CGG: 55–150) aged 50+, 66 male premutation carriers with FXTAS (CGG: 55–180) aged 50+, and 18 non-carrier siblings recruited through FXS probands. *FXTAS determined by CATSY 2000 motor test*	A total of 23 variables determined by extensive neuropsychological testing. PCA analyses performed on each domain of interest (i.e., memory, executive function, depression and anxiety) resulted in the generation of numerous factor scores. Each factor was then examined for any relationship to CGG length.	Relationship between increasing CGG size and performance impairment indicated, however effect was not significant (possibly due to a small sample size).	Men with FXTAS are impaired on tests of VIQ, PIQ and LMT, however no deficit in executive function was found. Male premutation carriers that do not have FXTAS performed similarly to controls on all measures. *Use of clinical executive function tests (WSCT, D-KEFS & COWAT), small sample size, and differences in ascertainment and exclusion criteria, may account for lack of significant findings.*

Table 4 (*Continued*)

Study	Participants	Measurement/methodology	Correlations	Significant findings for premutation carriers without FXTAS
Loesch et al. (2003b)	40 male premutation carriers, 148 female premutation carriers, 87 males with the full mutation, 58 females with the full mutation, 105 male controls and 57 female controls were recruited across Australia and America. All participants were aged 4–75 years old.	All participants completed age-appropriate WAIS tests	In the full mutation, the FMRP effect was mostly significant in domains of visual attention and information processing. Within the premutation cohort, the effect of FMRP was significant for FSIQ, PIQ, VIQ, arithmetic, comprehension, digit span and object assembly in males and this was reflective of the finding from the full mutation cohort. Alternatively FMRP interacted with just arithmetic, symbol search and processing speed in premutation females.	The effect of full mutation was significant on all subtests administered, thus was suggestive of a deficit in visual attention and information processing. Alternatively, the effect of premutation was significant mostly for performance skills (PIQ, PO, BD & OA) in the male cohort and symbol search in the female cohort. *FMRP dependent deficits in cognition may appear in a linear dose dependent fashion in the premutation, however only in specific domains of cognitive function.*

Abbreviations-ADD: attention deficit disorder; BDS: behavioural dyscontrol scale, JOL: benton judgement of line orientation test; BD: block design; CF: category fluency; CPT: continuous performance test; COWAT: controlled oral word association test; D-KEFS: Delis-Kaplan executive function system; DB: digits backwards; DF: digits forwards; DPT: doors and people test; DOT-test: dot test of Visuospatial working memory; EGT: executive gold task; Hayling's: Hayling's sentence completion test; FSIQ: full-scale IQ; LNS: letter-number sequencing test; LMS: logical memory subtest; LMT: logical memory test; OCD: obsessive compulsive disorder; OA: object assembly; PIQ: perceptual IQ; PAT: picture arrangement test; PCA: principal component analysis; PCT: picture completion test; PCR: polymerase chain reaction; RALVT: Rey auditory verbal learning test; SS-B: spatial span backwards; SS-F: spatial span forward; S-CW: Stroop colour word raw score: SDMT: symbol digit modalities test; SS: symbol search; SART: sustained attention to response test; TEA: test of everyday attention; TOL: tower of London task; TMT: trail making test; TMT-B-A: trial making test B-A; VIQ: verbal IQ; VMB: visual memory backwards; VMF: visual memory forward; WAIS-III: Weschler adult intelligence scale-III; WAIS-R: Weschler adult intelligence scale-revised; WMS-III: Weschler memory scale III; WMS-R: Weschler memory scale revised; WMI: working memory index; WCST: Wisconsin card sorting test.

executive function networks. In support of this suggestion, a recent fMRI study with 22 male and 22 female PM-carriers aged 33–75 years found reduced activation of the right ventral inferior frontal gyrus, DLPFC, and premotor cortices during performance on a verbal working memory task (Hashimoto et al., 2011a). Inhibitory and working memory deficits found in high repeat male PM-carriers, who are known to have the highest *FMR1* mRNA and the lowest FMRP levels, may, as suggested by Cornish et al. (2011), reflect the very first signs of a modality specific change to the integrity of prefrontal networks subserving executive function.

Unlike inhibitory and working memory problems which deteriorate as repeat size increased in PM-carriers, selective attention deficits have been shown to remain stable over time (Cornish et al., 2008b). Attention Deficit Hyperactivity Disorder (ADHD) is elevated in young males, with 98% of probands and 38% of non-proband PM-carriers found to have significantly elevated scores on the Conners' Global Index-Parent Version for assessing symptoms of ADHD. In females aged 18–50 ($N = 191$), attentional difficulties on self-reported Connors Adult ADHD rating scales (CAARS) were elevated on inattention and memory, impulsivity and emotional lability (Hunter et al., 2012a). Further examination revealed a non-linear association with CGG length, with relatively increased self-reported ADD signs in the low band (61–80) compared to the high band (>100), but no elevated levels of ADD in women within the 81–100 CGG repeat range. Due to known comorbidities between executive function and ADD (Castellanos et al., 2006), it is likely that a subset of females with increased ADD symptoms may similarly show subtle executive cognitive impairments.

In summary, traditional standardised tests are unlikely to have the sensitivity necessary to detect subtle changes in executive function in females, and similar to those studies in male PM-carriers, there may well be a subtle female premutation profile on tasks known to be subserved by local prefrontal brain regions, rather than those processed globally across multiple cortical areas. It is not yet clear if an executive function profile exists and possibly interacts with everyday attention-related behaviours in the female premutation.

4.2. Visuospatial dysfunction in the premutation

Visuospatial dysfunction is an area of significant interest in the full and premutation range of FXS (see Table 5). The premutation profile appears to be mediated, albeit to a lesser degree, by similar neurobiological mechanisms to those underpinning visuospatial dysfunction in the full mutation. Both appear to be vulnerable to low-level processing impairments associated with M-neuron developmental alterations and dysfunction of the retino-geniculo-occipital-precortical-magnocellular visual pathway (M-pathway) (Keri and Benedek, 2009, 2012; Kogan et al., 2004a,b). Secondary developmental effects on higher level domains including complex motion perception, visuospatial working memory and visuospatial perception have also been found in both groups (Goodrich-Hunsaker et al., 2011a; Hocking et al., 2012; Keri and Benedek, 2010). Moreover, in both cohorts, there is evidence for preserved parvocellular neuron (P-neuron) development, intact retino-geniculo-occipital precortical parvocellular visual pathway (P-pathway) functions, and normal higher level ventral stream function such as object and colour recognition (Goodrich-Hunsaker et al., 2011a; Hocking et al., 2012; Keri and Benedek, 2009, 2010, 2011, 2012; Kogan et al., 2004a,b). In this section, we first draw upon studies that employ psychophysical techniques to describe the neurobiology of visuospatial cognition and the vulnerability of magnocellular neurons (M-neurons) to reductions in FMRP in both full mutation and PM-carriers. Following this, we review findings in support of higher level cortical dysfunction as a result of M-neuron vulnerability in males with the premutation and the emerging profile in female PM-carriers.

Psychophysical and immunohistochemical techniques have been used to present converging evidence for a cell-specific vulnerability of M-neurons to *FMR1* expression (Kogan et al.,

Table 5
Visuospatial cognition studies.

Study	Participants	Measurements/methodology	Significant findings
Kogan et al. (2004b)	11 male full mutation carriers with a MA of 17.61, 11 age-matched control males and 11 developmental-matched controls (i.e., matched on PPVT-R verbal mental age).	First-order (luminance) motion perception, second-order (texture) motion perception, first-order (luminance) form perception, and second-order (texture) form perception.	A severe impairment in the detection of both first and second order motion stimuli found in males with fragile X syndrome indicates a processing deficit at both entry (i.e., at the level of the magnocellular neuron) and higher (i.e., at the cortical level of parietal lobe) levels. *The observed visual impairment is likely to stem from alterations at the level of the magnocellular neuron of the lateral geniculate nucleus (LGN).*
Kogan et al. (2004a)	9 male full mutation carriers with a MA of 20.3 and 9 age- and IQ-matched (PPVT-R) controls. 2 adult male vervet monkeys, 2 male brains donated from individuals with the full mutation and 1 brain donated to a non-carrier control. Samples accessed at the 'Brain and Tissue Bank for Developmental Disorders'	M-pathway tests (high-temporal low-spatial contrast sensitivity; motion coherence) and P-pathway tests (low-temporal high-spatial contrast sensitivity; chromatic contrast sensitivity; form coherence) were administered. FMRP immunohistochemical staining	A select deficit exists on tasks of entry and higher level M-pathway functioning in males with the full mutation that is not observable on tests of P-pathway functioning. This is consistent with the high FMRP expression found post-mortem in M-, and not P-neurons of the LGN. Moreover, significant alterations were found post-mortem when M-neurons of males with fragile X syndrome were examined (e.g., alaminar, smaller). *FMRP has a major role in the development of M-neurons of the LGN. In the absence of FMRP, as in FXS, profound alterations in entry level and higher level cortical processing are detectable*
Hocking et al. (2012)	33 male premutation carriers (CGG: 55–161) aged 20 to 68 years and 62 non-carrier age-matched male controls	Select tasks of visuospatial (i.e., VOSP & Dot Test) and visuoperceptual (i.e., Mooney faces & JOL) cognition.	A significant association between age and visuospatial working memory performance found in male premutation carriers with high CGG repeat lengths (>100) was not observable in male carriers with low repeats (<100) or non-carrier controls. *In male premutation carriers, specific neural networks may be vulnerable to CGG – repeat toxicity.*
Keri and Benedek (2012)	20 male premutation carriers (mean CGG: 110.4) with a MA of 32.3 and 20 age- and IQ-matched controls	Entry level M-pathway tests (i.e., contrast sensitivity), high level M-pathway (i.e., motion defined vernier threshold) tests, entry level P-pathway (i.e., contrast sensitivity) tests and high level P-pathway (i.e., static defined vernier threshold) tests.	Male premutation carriers have a selective impairment on M-pathway tests (entry and higher levels) and no deficit on P-pathway tests. Regression analysis shows that M-pathway test performance is predicted by FMRP and not *FMR1* mRNA. *FMRP may have an important role in the developmental visuospatial profiles detected in male premutation carriers.*
Keri and Benedek (2011)	100 non-affected males aged 18–54 Participants grouped as having high (90–100%), medium (80–89%) and low (70–79%) levels of FMRP expression.	Entry level M-pathway (i.e., low spatial/high temporal contrast sensitivity), high level M-pathway (i.e., motion coherence), entry level P-pathway (i.e., high spatial/low temporal contrast sensitivity) and high level P-pathway (i.e., form coherence) tests.	Non-carrier males found to have a low percentage of FMRP-positive cells (70–79%) performed worse than those with high levels (90–100%) of FMRP-positive cells on entry level M-pathway testing. Performance on both entry and high level processing was significantly associated with FMRP levels. *FMRP variation can impact visuospatial processing in a non-carrier population.*
Goodrich-Hunsaker et al. (2011a)	24 female premutation carriers (CGG: 67–143) aged 23–42 years old and 15 age- and IQ-matched controls	Magnitude comparison (distance effect) task	A CGG-and age-dependent deterioration in visuospatial performance was observed in female premutation carriers. *Visuospatial functioning in the female premutation may be CGG dependent.*
Keri and Benedek (2009)	22 female premutation carriers (CGG: range not available) with a MA of 24.3 years and 20 sisters of female carriers that were age-, education- and IQ-matched. *All participants underwent neurological and opthalmological examinations.	Entry level M-pathway (i.e., low spatial/high temporal contrast sensitivity), high level M-pathway (i.e., vernier threshold), entry level P-pathway (i.e., high spatial/low temporal contrast sensitivity) and high level P-pathway (i.e., vernier threshold) tests.	A select deficit on entry and higher level M-pathway tests was not found on comparable P-pathway tests. *Impaired M-pathway functioning in female premutation carriers may originate at the level of the LGN.*

Table 5 (*Continued*)

Study	Participants	Measurements/methodology	Significant findings
Keri and Benedek (2010)	25 female premutation carriers (CGG: range not available) with a MA of 24.7 years and 20 sisters of female carriers that were age-, education- and IQ-matched. *All participants underwent neurological and opthalmological examinations.	Higher level visuospatial functioning (easy and difficult biological motion; easy and difficult mechanical motion) and psychiatric function (SCID-CV, HAM-D & HAM-A) were tested.	A high level visuospatial impairment was found in female premutation carriers and not matched controls. Moreover, performance on the difficult biological motion task significantly correlated with self-reported depression. *A correlation found between visuospatial performance and depression in the female premutation may reflect low-level impairments in the detection of biological motion. More research is needed to find out if this secondarily impacts on social perception.*

Abbreviations: dot test: dot test of Visuospatial working memory; HAM-A: Hamilton Anxiety rating scale; Hamilton depression rating scale: Ham-D; JOL: Benton line orientation test; LGN: lateral geniculate nucleus; M: magnocellular: MA: mean age; P: parvocellular; PPVT-R: peabody picture vocabulary test; SCID-CV: structured clinical interview for DSM-IV axis I disorders VOSP: cube analysis task of visual object and space perception battery.

2004a,b; Zangenehpour et al., 2009). Post-mortem immunohistochemical studies with humans and primates have revealed significantly more Nissl-stained FMRP in M-neurons, alongside little positive reaction in P-neurons (Kogan et al., 2004a). In addition, when compared to P neurons, M-neurons in FXS-affected human males were significantly smaller, and did not show the expected six-layer laminar structure. This profile is also seen in primates prenatally exposed to ethanol toxicity (Papia et al., 2010). Typically developing M-neurons are suited to the transmission of rapidly changing information; for example they have expansive dendritic fields (indicating a high degree of dendritic integration) and are able to rapidly depolarise and repolarise (Kogan et al., 2004a). These evolutionary preserved features make M-neurons suited to the transmission of rapidly changing (high temporal) information, and regions that are predominantly innervated by M-neuron inputs (i.e. the dorsal visual stream) are ideal candidates for the computation of motion/action. The finding that performance is impaired but not abolished on psychophysical tasks that are sensitive to entry level processing indicates that morphologically different M-neurons function poorly in FXS. For example, Kogan et al. (2004b) found that, when compared to developmentally matched controls, 11 young males with FXS (mean age: 17.61) had lower sensitivity thresholds to low spatial-high temporal frequency sinusoidal gratings presented on a computer screen (i.e., M-pathway tests), and normal sensitivity to high spatial-low temporal frequency and chromatic (colour) change (i.e., P-pathway tests). Interestingly, Van der Molen et al. (2012a,b) have shown that higher level attentional problems may stem from lower level abnormalities in cortical activity. Together these studies indicate that information processing impairments can be traced back to subcortical anomalies in males with the full mutation.

Keri and Benedek (2012) report significantly worse performance in 21 males with the FX-premutation compared to 20 age- and IQ-matched control participants on select M-pathway tasks of subcortical and cortical processing (see Table 5). Supporting a 'FXS-like' profile, regression analyses with both *FMR1* mRNA and FMRP as independent predictors showed that FMRP levels significantly predicted performance on M-pathway psychophysical tests. The lack of correlation between performance on M-pathway tasks and *FMR1* mRNA may be explained by the disproportionately higher *FMR1* mRNA levels in samples taken direct from the brain compared to those from blood lymphocytes (Tassone et al., 2000). Keri and Benedek (2009) have also shown that 22 female PM-carriers performed significantly worse than 20 non-carrier controls on psychophysical M-pathway tests of both subcortical (i.e., low-spatial-high-temporal contrast sensitivity) and cortical (i.e., vernier sensitivity) processing alongside intact performance on all P-pathway tests. Similarly, Keri and Benedek (2010) found that female PM-carriers had reduced sensitivity to an M-pathway task of coherently moving dots within a random dot kinogram on a computer screen (coherent motion) alongside normal perceptual functions. Importantly, both this finding and a correlation to self-reported depression were unique to the more primitive subcortical recognition of biological (i.e., moving dots of a walking figure), but not mechanical (structured rotation), motion. Together these findings support a developmental vulnerability to M-pathway functioning in both male and female PM-carriers.

Further insights into the genetic mechanisms of M-pathway maturation have come from a study by Keri and Benedek (2011), who administered M- and P-pathway tests of contrast sensitivity to a cohort of 100 non-carrier males (CGG: 10–39) aged 18–54 years. FMRP-positive lymphocyte cells varied between 70 and 100%. Participants were grouped as having high (90–100%), medium (80–89%) or low (70–79%) percentages. Those with low FMRP-translation performed significantly worse than those with high FMRP-translation on M-pathway, but not P-pathway tests. These findings provide the first evidence for developmental effects of FMRP on visuospatial functioning. Furthermore, the finding that this relationship was not mediated by intelligence supports our contention that domain-specific tasks (e.g., core executive function and visuospatial processing) are more sensitive to FMRP translation than standardised tests. These findings in the normal allele range will have implications for understanding the specific role of FMRP in M-pathway vulnerability, and individual differences in the methylation of epigenetic regulatory sites on the *FMR1* gene (Godler et al., 2010, 2012).

These findings raise the possibility of a selective deficit in higher level visuospatial processing in the premutation range as a result of *reduced* FMRP levels in vulnerable neural networks. To assess the presence of subtle visuospatial impairments within a vulnerable subset of male PM-carriers, Hocking et al. (2012) employed M-pathway visuospatial (i.e., VOSP cube analysis, dot test of visuospatial working memory) and P-pathway visuoperceptual (e.g., Benton judgement of line orientation test, Mooney faces) tasks in a cohort of 33 male PM-carriers aged 20–68 years. On measures of visuospatial processing, high repeat PM-carriers (CGG > 100) performed significantly worse than age- and IQ-matched controls on visuospatial tasks, a specificity not observed in low repeat PM-carriers (CGG > 55≤ 100). As expected, a significant correlation was also observed between age and visuospatial working memory performance only in high repeat PM-carriers. Goodrich-Hunsaker et al., 2011a, who examined performance on the quantitative magnitude comparison task in 24 female PM-carriers aged between 21 and 42 and 15 age- and IQ-matched controls, have also demonstrated age- and CGG-dependent visuospatial deficits. The quantitative magnitude comparison task detects high-level

Table 6
Mouse model neuromotor studies.

Study	Participants	Measurements/methodology	Significant findings
Diep et al. (2011)	12 female CGG KI mice (CGG:70–200) aged 6mths and 6 age-matched female WT mice.	Acquisition of skilled forelimb reaching task	An inverse association between CGG length and neuromotor performance has been found. In addition, high CGG KI mice (136–200) were more impaired than low CGG KI mice (70–116) and WT mice at day 8 of task. *Findings implicate basal-ganglia-cortico-collicular-cerebellar circuit dependent vulnerabilities in visuomotor integration in the female premutation.*
Hunsaker et al. (2011)	30 female KI (CGG: 72–240) mice aged 2–16 months and 21 age- and gender-matched WT mice. 42 male KI (CGG: 72–240) aged: 2-16mths and 41 male age- and gender-matched WT mice.	Number of footslips on a ladder rung task	A subtle neuromotor deficit occurring early in life (at 2 months) was significantly and inversely associated with CGG length, however it was not affected by age or gender. *Deficits in frontal-parietal network-dependent vector calculations may be characteristic of early developmental effects of the premutation in both males and females.*
Wenzel et al. (2010)	8 male KI (CGG: 128–198) mice aged 16–76 weeks and 5 male WT mice aged 22–62 weeks. 2 female KI (CGG: 150–162) mice aged 70 & 75 weeks and 2 age-matched female WT mice.	Post-mortem examination of ubiquitin inclusion bodies (the hallmark of FXTAS)	Ubiquitin inclusion bodies first show at 12–25 weeks and are significantly increased in CGG KI mice at 52 weeks with high frequency in both neurons and astrocytes across layers II/III and V, sensory and motor areas, subcortical regions, visual cortices, inferior colliculus, cerebellar nuclei (however not Purkinje cells), GABAergic neurons/interneurons & and neocortical pyramidal cells. No gender effect was observed. *Neuromotor circuits may be particularly vulnerable to ageing in the premutation.*
Hunsaker et al. (2010)	14 high repeat female CGG KI (CGG: 150–190) mice, 15 low repeat female CGG KI (80–100) mice and 14 WT mice.	Behavioural tests: locomotor activity, object exploration, temporal ordering (ratio values) visual object novelty detection	No difference between mice groups on locomotion, object exploration and visual object novelty detection. The high repeat group however, was significantly impaired on a measure of temporal ordering compared to both low CGG repeat and WT groups. *A CGG dependent deficit for sequential ordering may exist in the premutation outside of the impact of global memory function.*
Van Dam et al. (2005)	10 male KI (CGG: 106–123) mice aged 20, 52 & 72 weeks and 5 age-matched male WT mice.	Cognitive performance (*Morris water maze; passive avoidance learning*) Exploration/activity (*open field and cage activity*) Neuromotor performance (*Accelerating rotorod; wire suspension task; stationary beam; gait*)	Compared to WT mice, 52 week old CGG KI mice were impaired in visuospatial learning and memory and rotarod performance, and at 72 weeks, exploration reduced and additional impairments arose on the stationary beam task. *Visuospatial deficits may indicate early signs of neuromotor impairment in premutation males.*

Table 6 (*Continued*)

Study	Participants	Measurements/methodology	Significant findings
Qin et al. (2011)	8 male KI mice (CGG: 120–140) aged 16–20 weeks and 8 WT mice matched on age, body weight and other physiological variables.	Behavioural tests: open field test, elevated plus maze, elevated zero maze, social interaction, rotarod, passive avoidance. Morphological analyses in medial prefrontal cortex, hippocampus and basal lateral amygdala: Golgi-Cox-stained dendrites and dendritic spines, regional rate of protein synthesis, western blotting for mRNA, immunohistochemical staining for anti-FMRP	Young adult male CGG KI mice showed increased hyperactivity, social anxiety (subtle only), passive avoidance (learning and memory) and general anxiety. There was no effect of premutation status on motor learning. Increased protein synthesis, dendritic density and dendritic length in the amygdala, hippocampus and medial prefrontal cortex was found in the CGG KI mice compared to WT mice. In premutation mice *FMR1* mRNA was increased (2–6 times higher) and FMRP was largely reduced (70–91%, depending on brain region). *A phenotype has been identified in the male premutation mouse model, at both behavioural and cellular levels, as one that has striking similarity, albeit less in degree of severity, to the full mutation male mouse model.*

Abbreviations-KI: knock-in; WT: wild-type.

visuospatial deficits by measuring the time needed to detect subtle size differences between two parallel and vertically positioned rectangles. This emerging profile of impaired visuospatial processing in a vulnerable subset of PM-carriers supports a relationship between repeat-dependent molecular events and a subtle yet detectable premutation cognitive phenotype.

In summary, the observed deficit at both lower and higher levels of visuospatial processing and the developmental role of FMRP present hitherto unprecedented opportunity for exploring developmental mechanisms underpinning visuospatial processing in the female FX-premutation.

5. The psychopathological profile in the fragile X-premutation

An extensive discussion of the psychopathological mechanisms in the FX-premutation is beyond the scope of this review. In this section, we briefly examine the current literature on neuropsychiatric functioning in females with the FX-premutation. Depression (Bourgeois et al., 2009, 2011; Lachiewicz et al., 2010; Roberts et al., 2009; Rodriguez-Revenga et al., 2008) and social anxiety (Adams et al., 2010; Bourgeois et al., 2011; Franke et al., 1998; Roberts et al., 2009) have been found to be elevated in females with the premutation compared to individuals from the general population, with self-reported depressive symptomatology, but not anxiousness, showing a positive association with CGG length (Hunter et al., 2008a; Johnston et al., 2001). However, risk for emotional problems appears to be dependent on stressful life events. Seltzer et al. (2011) found that both a repeat size of 90–100 and an above-average number of negative life events were associated with risk for emotional problems and blunted cortisol response in 82 premutation mothers of children with FXS (mean age 51.4). These findings, which suggest that epigenetic mechanisms play a role in *FMR1* gene expression and emotional functioning in the female FX-premutation, should be interpreted in light of the additional impact of neighbouring genes (Chonchaiya et al., 2010), hormonal levels (Spath et al., 2011), marital satisfaction (McCarthy et al., 2006) and differences in severity of child behavioural problems associated with FXS (Abbeduto et al., 2004; Hall et al., 2007).

Recent studies suggest that the hippocampus may show sensitivity to the molecular effects of the premutation and may be related to psychiatric symptomatology. Koldewyn et al. (2008) have shown that despite no behavioural differences on an associative memory recall task, males with the FX-premutation showed reduced left hippocampal activation alongside increased activation of both frontal and parietal regions when compared to controls matched on age, education, IQ and psychiatric symptomatology. Of most significance was the finding that left hippocampal activation negatively correlated with *FMR1* mRNA levels and psychiatric symptomatology in men with the premutation. These findings are suggestive of RNA toxicity in vulnerable hippocampal regions which may contribute to memory problems and psychiatric symptoms in premutation carriers. More recently, evidence has emerged to indicate that anxiety-related psychological symptoms are negatively correlated with hippocampal volume in both a cohort of female premutation carriers *with FXTAS* (mean age: 57.5) and *without* FXTAS (mean age: 44.94), suggesting that anxiety-related problems associated with hippocampal volume may represent early at-risk profiles for the later development of FXTAS in females with the FX-premutation (Adams et al., 2010).

In summary, correlations detected between hippocampal volume and *FMR1* mRNA support a relationship between RNA gain-of-function and anxiety in females carriers. It is not yet clear whether there is a combined effect of *FMR1* RNA and FMRP on psychiatric symptoms and vulnerable hippocampal regions in carriers within the upper premutation range.

6. The emerging neuromotor profile in the fragile X-premutation

6.1. Mouse model studies

Convincing evidence exists for age- and CGG-dependent vulnerability along neuromotor circuitry in male and female premutation mouse models (see Table 6). In this section, three core

impairments are discussed. First, we discuss evidence for a subtle visuomotor integration deficit. Following this, we highlight studies that indicate a possible extension of visuospatial and temporal ordering deficits to the visuomotor domain. Importantly, we focus on the premutation mouse model outside of the influence of FXTAS so as to increase our understanding of the complex developmental relationships between genetic mechanisms, morphological change and neuromotor performance in the premutation mouse model.

Visuomotor integration problems have been observed in both male and female premutation mouse models. Van Dam et al. (2005) examined 10 male CGG knock in (KI) mice (CGG: 106–123) on cognitive performance, exploration/activity and neuromotor performance at ages 20, 52 and 72 weeks. A progressive neuromotor profile detected in KI mice, and not 5 age-matched WT mice, presented with impaired visuospatial and rotorod performance at 52 weeks, and additional exploratory changes and impairments on the stationary beam task at 72 weeks. The rotorod task examines gradual acquisition of motor coordination and motor skill. That is, mice practice walking on a rotating rod for four days (four times per day), and on the final test day, mice are scored on their ability to maintain stance on a rod that linearly increases in speed. The stationary beam task is more generally sensitive to distance travelled and falls of mice placed on a stationary beam. The findings of an age-related decline in neuromotor performance in the KI-premutation mouse model indicate a subtle deterioration in motor coordination which may eventually lead to more severe impairments in gross motor functioning.

The earliest change in neuromotor performance in KI mice has been found with the ladder rung task. Specifically, in the ladder rung task mice are freely allowed to walk back and forth along the apparatus for a total of 2 min and the number of times that the mouse traverses the apparatus (one end to the other) is used as an index of general activity, with paw slips taken as the dependent variable. Hunsaker et al. (2011) have reported a significant increase in footslips as early as two months of age in a large cohort of 42 male and 30 female CGG KI mice, compared to 41 male and 21 female age-matched WT mice. Further analysis showed that footslips were associated with frequent over- or under-estimation of the location of the stepping beams. Critically, there was also clear evidence of a significant increase in footslips with increasing CGG repeat length in both male and female premutation mice. These findings may well be related to a trajectory of subtle neuromotor impairments beginning early in development, and present throughout the lifespan, but not one that increases in severity of neurodegenerative decline associated with FXTAS.

Further evidence for CGG-dependent impairments in feedforward loops in the cerebellum and parietal cortex in the FX-premutation comes from a study of skilled forelimb reaching (e.g., reaching towards a sucrose food pellet) with 12 female CGG KI mice aged 6 months and six age-matched WT mice (Diep et al., 2011). High CGG mice (136–200) performed significantly worse than both low CGG mice (70–116) and WT mice on visuomotor integration. Moreover, CGG KI premutation mice made frequent clumsy nonlinear motions towards the pellet or closed their claws too early or late following successful linear motions towards the pellet. Recent models of adaptive motor learning have proposed that the cerebellum acts as an internal forward model, predicting the sensory consequences of motor commands based on estimates of the current state, and in conjunction with the parietal cortex, utilises an efference copy signal to estimate state and predict the sensory consequences of movement (Ito, 2008). Given that cerebellar feedforward loops are critical for both reaching and stepping (Morton and Bastian, 2004a; Morton et al., 2004b; Nitschke et al., 2005), the motor coordination deficits observed in the KI mouse model may well reflect impaired transmission in cerebellar-cortico and fronto-parietal circuits.

Recent studies suggest that the neuromotor profile in the KI mouse model may extend to the visuospatial domain. Van Dam et al. (2005) found impaired learning on both a spatial processing task (the Morris water maze task) and rotorod performance which preceded more severe neuromotor decline in CGG KI mice (Van Dam et al., 2005). Similarly, Hunsaker et al. (2009) report an age-dependent decline in visuospatial performance in ten CGG KI mice (CGG: 80–180) aged 12, 24 and 48 weeks. These authors found a subtle impairment in the processing of distance between two objects which was evident as early as 12 and 24 weeks of age. Most significantly, this spatial processing impairment was affected by age, with 48 week old mice unable to detect changes in object location. This subtle early-onset visuospatial impairment may precede later neuromotor dysfunction associated with FXTAS.

Another emerging CGG-dependent profile identified in KI mouse models that may well interact with neuromotor function is that of sequential temporal ordering. Hunsaker et al. (2010) examined 14 high (150–200) and 15 low (80–100) repeat CGG KI mice on their ability to process the temporal order of three subsequently presented visual slides. This task is based on the Simon (2008) hypothesis of 'spatiotemporal hypergranuality' in children with neurodevelopmental disorders. According to this theory, processing impairments occur across spatial and temporal domains leading to poorly resolved or 'granular' cognitive representations. This impairment is thought to be secondary to abnormal development of spatial and temporal attention networks in hippocampal, parietal and rostral cortices in CGG KI mice. Behaviourally, poor performance on a temporal ordering task indicates poor temporal efficiency and interference between processing of consequent events. Hunsaker et al. (2010) showed that high CGG-repeat female mice performed more poorly on this temporal ordering task, while low CGG KI mice performed similarly to WT mice. In a further study, Borthwell et al. (2012) have shown that female CGG KI mice are similarly impaired in spatial novelty detection under high levels of spatial interference when compared to WT mice, a process which appears to be modulated by CGG repeat size. Together these findings support the contention of CGG repeat-dependent 'spatiotemporal hypergranuality' impairments in the female premutation.

Mouse model studies provide a unique opportunity to explore gene-brain-behaviour relationships in the premutation. These findings support a growing body of evidence for distinct neuroanatomical and neurochemical changes occurring in young developing premutation mouse models which may well reflect CGG-dependent motor endophenotypes (Chen et al., 2010; Cunningham et al., 2011). Furthermore, recent studies on the KI mouse model are consistent with human premutation studies in so far as they suggest both developmental and degenerative mechanisms associated with CGG repeat expansions impacting upon neural networks important for visuospatial and neuromotor performance.

6.2. Imaging correlates in the premutation

Recent imaging studies point to both grey and white matter abnormalities in specific neural regions and significant structural alterations along cerebellar-cortico pathways in males that do not have FXTAS (see Table 7). Although significant morphological and functional differences have been found in several neural regions including the brainstem, cerebellar, hippocampal-amygdala, and prefrontal regions (Brunberg et al., 2002; Cohen et al., 2006; Hashimoto et al., 2011b,c; Jakala et al., 1997; Moore et al., 2004b), few studies have screened for the confounding effects of FXTAS-related symptoms. In this section, we focus on the limited evidence from imaging studies that have revealed both age- and

Table 7
Imaging neuromotor studies.

Study	Participants	Measurements/methodology	Correlations	Significant findings in FXTAS-affected cohort	Significant findings in PM cohort
Wang et al. (2012)	15 young male PMs *without* FXTAS (CGG: 55–157) aged 18–45, 11 older male PMs *without* FXTAS (CGG: 59–113) aged 47–76, 15 male PMs *with* FXTAS (CGG: 62–154) aged 50–79 years. 19 young and 15 age- and education matched non-carrier controls were also tested.			Widespread white matter changes in motor, limbic, association and callosal fibres. Symptom severity varied. Those with MCP lesions showed worse structural connectivity.	Young PMs show increased age-related decline in the extreme capsule, and greater tract volume in the right angular bundle, compared to controls.
Hashimoto et al. (2011b)	24 male PMs *without* FXTAS (CGG: 55–166) aged 41–78, 31 male PMs *with* FXTAS (CGG: 59–130) aged 47–79 years, and 28 non-carrier age and IQ-matched controls from the general population.	1.5 T GE Signa Horizon LX NV/I MRI with VBM toolbox to assess grey matter differences between groups. Region of interest = cerebellar subregions. The SCL-90-R was administered to gauge psychological function and the total BDS-2 and sum of WAIS working memory subscales was taken to index cognitive function.	A trend correlation was observed between BDS-2 performance and right Crus I/lobule VI volume. No grey matter voxel was significantly correlated to CGG length or mRNA levels.	Significant grey matter reduction was found in multiple brain regions (i.e., cerebellum, dorsomedial prefrontal cortex and precuneus) in male carriers with FXTAS compared to male carriers without FXTAS.	The only significant volumetric difference found between male carriers without FXTAS and matched controls was in lobule I/II of the anterior vermis and lobule III of the left lateral hemisphere. *Targeted testing of abilities subserved by anterior and lateral cerebellar regions may reveal the first signs of pre-clinical FXTAS or developmental differences in carriers.*
Hashimoto et al. (2011c)	16 male PMs *without* FXTAS (CGG: 55–166) aged 41–78, 35 male PMs *with* FXTAS (CGG: 59–133) aged 47–79 years, and 20 non-carrier age and IQ-matched controls from the general population.	1.5 T GE MR scanner. Tract of interest analysis to calculate FA and axial and radial diffusivity maps (i.e., white matter).	In a combined carrier cohort (both with and without FXTAS) a significant inverted U shaped relationship was found between CGG length and both axial and radial diffusivities. There was also a trend correlation between mRNA levels and axial (not radial) diffusivity.	Significant reduction in MCP, superior cerebellar peduncle, bilateral cerebral peduncle, fornix, and bilateral fornix/stria terminalis.	A significant increase in axial and radial diffusivities in the MCP and left cerebral peduncle was also identified in male carriers without FXTAS compared to matched controls. *Further investigation of cerebellar afferent and efferent projections will inform researchers on the design of targeted tests that detect pre-clinical FXTAS. These tests will be needed when drug trials begin.*
Hashimoto et al. (2011a)	15 FXTAS-affected male (6) and female (9) PMs aged 33–72 (CGG: 78–130), 15 non-FXTAS affected male (8) and female (7) PMs aged 34–73 (CGG: 52–130), and 12 male (7 and female (5) age and IQ matched controls.	Functional MRI: 1.5 T GE Signa Horizon Verbal working memory task.	Sig negative effect of *FMR1* mRNA levels on right ventral inferior frontal cortex in combined sample.	Reduced activation in right ventral inferior frontal cortex and bilateral dorsolateral prefrontal cortex/premotor cortex during working memory task	Reduced activation in right ventral inferior frontal cortex and left dorsolateral prefrontal cortex/premotor cortex during a working memory task. *The functional capacity of networks subserving working memory performance may be vulnerable to FMR1 CGG expansions in both males and females.*

Table 7 (*Continued*)

Study	Participants	Measurements/methodology	Correlations	Significant findings in FXTAS-affected cohort	Significant findings in PM cohort
Adams et al. (2007)	20 female PMs *without* FXTAS (CGG mean 93.3) MA 43.3, 15 female PMs *with* FXTAS (CGG mean 91.9) MA 59.5 years, and 11 non-carrier age and IQ-matched controls from the general population. 25 male PMs *without* FXTAS (CGG mean 86.2) MA 53.5, 36 male PMs *with* FXTAS (CGG mean 93.7) MA 65 years, and 39 non-carrier age and IQ-matched controls from the general population.	Volumetric MRI.	Also, a trend between mRNA levels and hippocampal volume was found in both sexes, and cerebellar volume significantly associated with FXTAS symptomatology and CGG length in male, but not female, fragile X carriers.	Females with FXTAS are less likely (13%) to show the characteristic MCP sign compared to males with FXTAS (58%). Also, females do not differ from males with respect to FXTAS-associated brain volume loss and white matter disease. No difference in hippocampal volume between PMs with FXTAS and PMs without FXTAS. *Female carrier with FXTAS show a different neurological profile of FXTAS compared to males*	No difference on whole brain, cerebellar, CSF ventricles, or white matter hypertrophy found between non-FXTAS affected PMs (male or female) and controls. *Gross volumetric differences may not be observable between PMs and matched controls. Changes may be too small (and this is supported by* Hashimoto et al. (2011b,c) *to be detected with MRI. Alternatively, changes may relate to functional status or metabolism instead of volumetric change.*
Cohen et al. (2006)	11 male PMs *without* FXTAS (CGG: 55–163) aged 51–79, 25 male PMs *with* FXTAS (CGG: 62–130) aged 51–79 years, and 21 non-carrier age and education-matched controls from the general population.	MRI and neurocognitive testing (WAIS-III FSIQ, VIQ, PIQ).	Moreover, in males with FXTAS CGG length correlated with PIQ, cerebellar volume, ventricle size and whole brain white mater hypertrophies.	Significantly increased whole-brain white matter hyperintensity in FXTAS-affected cohort.	Brainstem volume was smaller and trend towards increased ventricles was found in the non-affected FXTAS group compared to the control cohort. *It is not clear if this change is pre-clinical FXTAS or a developmental mechanism.*
Moore et al. (2004b)	20 male PMs (CGG: 55–137) aged 20–72 and 20 non-carrier age, IQ and handedness matched male controls from the general population. **not screened for FXTAS*	MRI: 1.5 T neuroptimised MR system.	FMRP and CGG length negatively associated with grey matter in the amygdala-hippocampal complex and left thalamus. FMRP also associated with brainstem grey matter volume and CGG additionally associated with the right caudate, pre- and post-central gyri and inferior parietal cortex.		Male PMs have significantly reduced grey matter in cerebellar, brainstem, amygdala-hippocampal, subcortical, thalamic and temporal brain regions. Moreover, white matter was significantly reduced in cerebellar, brainstem, pons, cingulate, genu of corpus callosum, frontal and temporal regions. CGG length was negatively associated with grey matter. *This study did not screen for FXTAS so results may reflect the confounding effects of FXTAS symptomatology.*

Murphy et al. (1999)	8 female PMs with MA of 39 and 32 age, sex and handedness matched controls. *Control group was significantly more intelligent*	MRI: 0.5 T scanner to quantify volumes of cranium, cerebral hemispheres, lobar brain, caudate, lenticular nuclei, thalamic nuclei, ventricular and subarachnoid CSF. MRI: 1.5 T GE used to measure hippocampal volume. PET: Scanditronix PC-1024-7B tomograph after injection of 5 mCi of 18 FDG	No relationships found between CGG length and MRI or PET data	Not examined	Volumetric MRI data shows that female PM carriers are significantly decreased in whole brain, caudate and thalamic nuclei. Hippocampus and peripheral CSF and third ventricle are increased. PET show hypometabolism in right parietal, temporal and occipital association areas. Hypermetabolism is recorded in hippocampus, left cerebellum. Right-left asymmetry of Broca and Wernicke's area was different in PM women. *Morphological signature found in female PM mirrors female full mutation profile. Numerous networks may be implicated in female premutation status (e.g., the parietal-ponto-cerebellar tract connecting left cerebellar and right parietal regions). It is possible that this study included mosaic premutation-full mutation individuals. Repetition with a larger sample is needed.*
Jakala et al. (1997)	10 male PMs MA 52.4, 10 female PMs MA 48.3, 10 male FMs MA 29.1 and 10 female FM MA 34.2.	MRI	In premutation (and not full mutation) performance on the delayed memory recall test correlated with left hippocampal volume	Not examined	Hippocampal volumes comparable between PM and FM groups. *No control group in this study makes it difficult to ascertain the implications of the findings.*

Abbreviations-BDS; behavioural dyscontrol scale; CSF: cerebrospinal fluid; DLPFC: dorsolateral prefrontal cortex; FSIQ: full-scale intelligence; MA: mean age; MCP: middle cerebellar peduncle; MRI: magnetic resonance imaging; PET: position emission tomography; PIQ: performance intelligence; PM: premutation carrier; PMC: premotor cortex; SCL-90-R: symptom checklist-90-revised; FA: functional anisotropy; VBM: VIQ: verbal intelligence; VBM: voxel based morphometry; VIFC: ventral inferior frontal cortex; WAIS: Weschler abbreviated intelligence scale.

Table 8
Behavioural neuromotor studies.

Study	Participants	Measurements/methodology	Significant findings
Goodrich-Hunsaker et al. (2011b)	30 female PMs (CGG: 67–143) aged 21–41 years old and 20 age- and IQ-matched non-PM females.	Oral (verbal) and motor reaction time to a visual stimulus.	Female PMs responded significantly faster that matched controls on both oral and motor reaction time tasks. There was no association between performance and age, CGG repeat or *FMR1* mRNA levels. *Motor excitability in cerebello-thalamo-cortical-pathways may be increased in female carriers.*
Allen et al. (2008)	62 male PMs (CGG: 70–199) aged >50 and 27 non-PM age-matched brothers of a FXS family proband. *Subjects not screened for FXTAS	CATYSYS 2000: a portable windows based test system. It provides scores for ataxia (eyes open 30 s and eyes closed 30 s on sway metre), postural tremor (stylus pen held stationary), intention tremor (stylus pen traces image on computer screen), manual coordination #1 (pronation-supination movement in time to metronome) and manual coordination #2 (Lafayette grooved pegboard test). Ref.: www.catsys.dk.	A high percentage of high repeat (70–199) male carriers (48%) performed poorly on the eyes-closed ataxia test, postural tremor, intention tremor and manual coordination. *The CATSYS is sensitive to clinical symptoms of FXTAS in male PMs*
Aguilar et al. (2008)	16 male PMs without FXTAS (mean repeat: 75.77) MA 62.56, 16 male PMs with FXTAS (mean repeat: 93.67) MA 64.56, and 14 age-matched non-carrier controls	CATSYS 2000	Men with FXTAS are significantly more impaired on measures of postural sway and intention tremor compared to matched non-FXTAS carriers and non-carriers controls. CATSYS has minimal sensitivity in PMs without FXTAS to hand coordination and reaction time. *The CATSYS is not sensitive to pre-clinical changes associated with FXTAS in the male PM.*
Chonchaiya et al. (2010)	110 female PM daughters (CGG: 70–199) aged 35–66 to PM fathers with definite FXTAS, 36 female PM daughters (CGG: 70–199) aged 30–65 to PM fathers that do not have FXTAS, and 43 non-PM females aged 30–55 years.	Telephone historical interview CATSYS 2000 to measure balance.	Multiple neurological signs were elevated in daughters of men with FXTAS compared to population controls (i.e., problems with tremor, balance, memory, dizziness, achieving orgasm, menopausal symptoms, sleep and anxiety). Only balance and menopausal problems were significantly higher in daughters of men with FXTAS compared to daughters of carrier men without FXTAS. *Background gene effects may impact balance in female PM carriers.*
Narcisa et al. (2011)	90 female PMs (mean CGG: 86.66) between 36 and 80 years old compared to 37 age-matched controls	CATSYS 2000: Postural sway, intention tremor and postural hand tremor	Premutation females performed significantly different to controls on finger tapping and reaction time tasks, and there was a trend towards increased postural sway with eyes closed in the premutation female group compared to controls. *A subtle neuromotor profile may exist in the female premutation.*
Grigsby et al. (2008)	28 male PMs (CGG: 57–150) aged 41–89 years old and 39 age-matched controls	BDS and COWAT performance combined	Male carriers asymptomatic for FXTAS scored worse than matched controls on executive function and behavioural regulation tasks. *A comorbid executive function and motor control profile may exist in the male PM.*
Loesch et al. (2003a)	32 male PMs with a MA of 34.4, 147 female PMs with MA of 40.8 years	BDS	Significant effect of premutation status on total BDS score that was independent of WAIS full-scale IQ performance *Specific motor control impairments may exist in some individuals with the PM irrespective of gender.*

Abbreviations-BDS: behavioural dyscontrol scale; CATSYS: coordination, tremor and balance test system; COWAT: controlled oral word association test; PM: premutation; WAIS: Weschler abbreviated intelligence scale.

CGG-dependent structural changes in regions known to subserve neuromotor functioning in the FX-premutation.

The structural imaging studies in PM-carriers that do not have FXTAS are summarised in Table 7. Hashimoto et al. (2011b) used voxel-based morphometry (VBM) to examine individual regions of the cerebellum for volumetric differences in grey matter in 24 male PM-carriers aged 41–78 years, 31 PM-carriers with FXTAS aged 47–79 years and 28 age- and IQ-matched non-carrier controls. As predicted, men with FXTAS showed significantly reduced brain volumes in all regions examined compared to controls with normal alleles. Males with FXTAS were also significantly different from PM-carriers without FXTAS in a limited number of specific regions (i.e., in lobules IV/V, VI and VII in vermis, lobules I/II IV/V, VI, Crus I and right Crus II in hemisphere). Interestingly, when compared to controls, males without FXTAS had significant reductions in lobule I/II of the anterior vermis and lobule III of the left hemisphere. Notably, the finding that these two regions were not significantly different between male PM-carriers with and without FXTAS suggests that change in these particular regions is not progressive. Therefore, it is not clear if grey matter changes to regions I/II of the anterior vermis and lobule III of the left hemisphere indicate (a) the beginning of FXTAS (with little to no further progressive degeneration associated with FXTAS) or (b) the developmental premutation phenotype having little to do with FXTAS. However, it is noteworthy that postural control, which has recently been found to be impaired in female PM-carriers compared to controls, is subserved by similar regions within the anterior vermis (Ouchi et al., 1999; Stoodley and Schmahmann, 2009).

One explanation for these neuroimaging findings is that cerebellar changes preceding FXTAS do not impact upon grey matter until the disease has progressed. In this same cohort, performance on the BDS (see section 3.1) significantly correlated, before adjustment for multiple testing, with grey matter volume in the right Crus I/lobule VI only (Hashimoto et al., 2011b). This region has previously been associated with visuospatial skills and working memory (Stoodley et al., 2010), and may play a role in functional coupling between the executive control circuit (i.e., dorsolateral prefrontal cortex, inferior parietal lobules and thalamus) and the default mode network (i.e., precuneus cortex, ventromedial prefrontal cortex, hippocampus) (Passamonti et al., 2011).

Recent studies indicate that white matter changes may be more sensitive to early cerebellar changes occurring well before the onset of FXTAS. In a recent imaging study using Diffusion Tensor Imaging (DTI), Hashimoto et al. (2011c) examined fractional anisotropy (FA) and axial and radial diffusivities in cerebellar-brain stem and limbic white matter tracts in premutation males with FXTAS aged 47–78, 16 premutation males without FXTAS aged 42–78 and 20 age- and IQ-matched controls. These authors report that males with FXTAS showed significant white matter changes in the middle cerebellar peduncle (MCP), superior cerebellar peduncle, cerebral peduncle, and fornix and stria terminalis. Notably, both males with and without FXTAS showed significant alterations in axial (indicative of axonal damage) and radial (indicative of demyelination) diffusivities in the MCPs (Hashimoto et al., 2011c). MCPs contain fronto-cerebellar tracts connecting the cerebellum with areas of the orbitofrontal and prefrontal cortex (i.e., the DLPFC) (Kamali et al., 2010). A significant inverted U shape relationship between CGG-length and axial and radial diffusivity found in this study indicates that the largest effect to white matter appears above 100 repeats. Moreover, a trend correlation detected between *FMR1* mRNA levels and axial (but not radial) diffusivity supports a direct relationship between *FMR1* mRNA toxicity and axonal damage.

In a further study of white matter structural connectivity, Wang et al. (2012) found that FXTAS symptomatology was variably associated with motor (i.e., cerebral peduncle and cerebellar peduncle tracts), limbic (i.e., extreme capsule, cingulum, fornix and angular bundle), association (i.e., arcuate fasciculus, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus) and callosal (i.e., corpus callosum) fibres, with the strongest association to the superior cerebellar peduncle, corpus callosum and left cingulum (Wang et al., 2012). Furthermore, age-related decline was significantly increased in males with FXTAS compared to controls in limbic, association and callosal fibres. Male PM-carriers with lesions in the MCP and corpus callosum were also found to have the most profound reductions in structural connectivity compared to other males with FXTAS. In younger males without FXTAS, tract volume in the right angular bundle was greater compared to age-and IQ- matched controls but there was no association with age. However, PM-carriers without FXTAS showed an age-related decline in connectivity in the right posterior lateral projections of the extreme capsule. It is noteworthy that the previous finding of demyelination in the MCP (Hashimoto et al., 2011c) was not replicated in this study. This apparent discrepancy may be related to differences in imaging methodologies; the Hashimoto et al., study reported changes to voxel and ROI-based analyses of axial and radial diffusivity, whereas the Wang et al. study examined FA diffusivity. Nevertheless, these findings point to widespread alterations in structural connectivity and an age-related decline in white matter connectivity in FXTAS, with the earliest changes occurring in the extreme capsule.

In summary, the current literature indicating age-dependent changes to white matter integrity of MCPs and in the extreme capsule may reflect preclinical changes in vulnerable neural regions well before the onset of FXTAS. Alternatively, grey matter changes to the anterior vermis and findings of increased tract volume in the right angular bundle may reflect developmental aspects of the *FMR1* premutation. Notably, novel imaging methodologies for tracking early decline in FXTAS will need to distinguish between degenerative and developmental mechanisms, both of which appear to impact transmission within cerebellar-cortico loops in the premutation.

6.3. Neuromotor studies in the premutation

The neuromotor profile in male PM-carriers may offer important insights into the subtle gender-specific profile in the female FX-premutation (see Table 8). However, due to a paucity of experimentally-driven research into the neuromotor profile in the male FX-premutation, very little is known about the subtle female-specific profile. In the next section, we discuss the neurological profile of FXTAS and outline the common neurological assessments designed to index neuromotor dysfunction in this population. Finally, we highlight neuromotor vulnerabilities in motor programming (see section 3.1 for the executive function profile) (Grigsby et al., 2008; Loesch et al., 2003a), balance (Chonchaiya et al., 2010; Narcisa et al., 2011), and reaction time (Goodrich-Hunsaker et al., 2011b) that have emerged to indicate a subtle neuromotor profile in the female FX-premutation.

The neurological profile in male FXTAS is variable, with gait ataxia and intention tremor predominating alongside parkinsonism, dysautonomia, peripheral neuropathy, psychiatric problems and executive dysfunction (Berry-Kravis et al., 2007; Leehey, 2009). FXTAS is a progressive disorder that starts as early as the 5th decade (Leehey et al., 2007), and the severity of deterioration is linearly associated with CGG-length (Leehey et al., 2008). With the aim of delineating the severity of tremor, ataxia and parkinsonism in an unbiased manner, Berry-Kravis et al. (2003) employed a blind videotape assessment based on the Clinical Rating Scale for Tremor (CRST), International Cooperative Ataxia Rating Scale (ICARS) and Unified Parkinson's disease rating scale (UPDRS) with 21 PM-carriers aged over 50 years (14 of whom were female) and 16 age-matched non-carrier controls. The male PM-carrier group

performed significantly worse on the CRST, ICARS and UPDRS, which reflected increased tremor and ataxia scores when compared to the control groups, but no differences were observed in the female PM-carrier group. The FXTAS Rating Scale was later developed on the basis of these clinical scales, and comprises three subdomain scores relevant to FXTAS—tremor, ataxia, and parkinsonism—and has been validated as a tool for detecting and screening out the presence of FXTAS-related symptoms (Leehey et al., 2008).

To date, most studies examining neuromotor dysfunction have employed standardised neurological assessments in older male premutation cohorts and few have administered the FXTAS rating scale (see Table 8 for a summary). The extant studies have predominantly focused on the coordination tremor balance test system (CATSYS), a clinical test battery designed to detect neurological features such as tremor (postural and intention), postural sway, manual coordination and reaction time. When applied to the neurological signs in FXTAS, the CATSYS is a sensitive tool. For example, in a large cohort of older males the CATSYS was able to detect tremor in 23% men who did not self-report tremor, and ataxia in 30% men that were not aware of any balance problems (Allen et al., 2008). Importantly, Aguilar et al. (2008) found that premutation males without FXTAS ($N = 16$) performed similarly to age-matched controls on all measures of the CATSYS. Thus, although the CATSYS is sensitive to the neurological features (i.e., tremor and ataxia) of FXTAS, it appears to lack sensitivity to subtle neuromotor changes in PM-carriers that do not have FXTAS.

FXTAS has also been reported in female PM-carriers (Berry-Kravis et al., 2005; Hagerman et al., 2004; Rodriguez-Revenga et al., 2010), however in a milder and less penetrant form (prevalence rates ranging from 8 to 16%) (Adams et al., 2007; Berry-Kravis et al., 2003; Jacquemont et al., 2004; Coffey et al., 2008). This difference is due to variable penetration of the mutant X-chromosome in females. Indeed, controlling for X-inactivation has been shown to increase the strength of the association between CGG length and ataxia in female PM-carriers aged over 50 years (Leehey et al., 2008). Although FXTAS is less common in females, elevated rates of hypertension (Coffey et al., 2008), chronic muscle pain and sensory loss (Coffey et al., 2008), autoimmune or immune related problems (e.g., fibromyalgia, thyroid disorders) (Coffey et al., 2008; Rodriguez-Revenga et al., 2009a), parkinsonism (Hall et al., 2011), multiple sclerosis (Zhang et al., 2009) and Alzheimer's disease (Tassone et al., 2012) have been reported in the female premutation. Such clinical presentations can affect cognitive/neuromotor function, and thus are important for understanding FXTAS in the female-PM. For example, higher rates of immune mediated problems recently documented in female PM-carriers with FXTAS compared to those without FXTAS and controls may reflect shared downstream effects of RNA-toxicity (e.g., an upregulated inflammatory response) (Winarni et al., 2012).

Currently, there is a dearth of studies examining neuromotor dysfunction in female PM-carriers, possibly because only 8–16% of older PM-carriers will show progressive decline associated with FXTAS. In one study using the CATSYS system, Narcisa et al. (2011) provided the first quantitative assessment of tremor and ataxia in a cohort of 90 PM-carrier females aged 36–80 and 37 age- and IQ-matched controls. Although there was limited support for neuromotor abnormalities in finger tapping and reaction time in females without FXTAS, there were subtle postural control difficulties on the eyes-closed postural sway task. Similarly, Chonchaiya et al. (2010) have shown that female PM-carriers whose fathers are positive for FXTAS are significantly more likely to perform poorly on postural sway with eyes closed when compared to daughters of PM-carriers whose fathers do not have FXTAS. The cerebellum, which is known to play a critical role in balance control (Morton and Bastian, 2004a), may well be involved in these gender-specific trajectories associated with subtle postural control difficulties.

The presence of a subtle neuromotor profile in the female premutation is also supported by a recent examination of oral (verbal) and motor reaction time in 30 female PM-carriers aged 21–42 (mean age of 34.08) and 20 age-matched controls with normal alleles (Goodrich-Hunsaker et al., 2011b). In this study, participants responded to visual stimuli by speaking the word "GO" or pressing a button. Consistent with previous research (Steyaert et al., 1994), premutation women outperformed controls in both conditions. The authors' suggestion that anxiety may have enhanced psychomotor abilities remains difficult to establish because anxiety levels were not recorded. Goodrich-Hunsaker et al. (2011b), state that enhanced white-matter change along corticospinal and corticopontine tracts characteristic of FXTAS have been found in this same cohort, and correlates with CGG repeat length (unpublished). However, it is not yet clear how white matter changes might lead to increased psychomotor performance. Increased motor excitability in cerebello-thalamo-cortical-pathways has been linked to impaired inhibitory signalling in the cerebellum (Fierro et al., 2007; Tamburin et al., 2004). Thus an initial increase in reaction time may reflect early problems in cerebellar inhibitory transmission and possibly 'overactive' glutamatergic signalling. Notably, reaction time has also been reported as being significantly slower in 90 older (mean age 52.86) female PM-carriers compared to controls (Narcisa et al., 2011). Thus early changes in inhibitory transmission in cerebellar neurons may over time lead to impaired neural function and motor slowness in the female premutation.

In summary, emerging evidence in the FX-premutation for impairments in motor programming, balance and psychomotor changes implicates cerebellar-cortico pathways and possibly gender-specific adult trajectories of neuromotor decline. Future studies will be needed that employ experimentally-driven tasks which are sensitive to subtle neuromotor changes in the female premutation, which could potentially identify surrogate markers of at-risk profiles associated with later development of FXTAS and other neurological conditions.

7. Discussion and future directions

7.1. The importance of targeted experimentally-driven tasks

The over reliance on traditional measures to assess gross cognitive function in premutation females has led to debate over the presence of a premutation phenotype (Allen et al., 2011; Hunter et al., 2008b, 2009; Moore et al., 2004a). This debate extends to studies of neuromotor functioning in young adult PM-carriers, where gross clinical assessments such as the CATSYS may have limited sensitivity to detecting subtle neuromotor decline in the premutation (Aguilar et al., 2008). While the role of the cerebellum in motor functions is well established, the cerebellum has recently gained attention for its role in several cognitive functions including attention, language, executive functions and visuospatial cognition (Ito, 2008; Schmahmann, 2004; Stoodley and Schmahmann, 2009). The cerebellum is also inextricably linked with multiple cortical regions (e.g., the parietal lobe, dorsal stream regions, premotor cortex, oribitofrontal cortex, DLPFC), with each cerebellar nucleus having its own topographical representation of the connected cortical region (Glickstein, 2000; Kamali et al., 2010; Uusisaari and De Schutter, 2011). Taken together with previous findings of white matter disruption in middle cerebellar peduncles (Hashimoto et al., 2011c), future studies need to incorporate tasks that are sensitive to capture subtle cerebellar-cortico deficits in the female FX-premutation; for example, by examining the cognitive control of gait, stepping, and

postural control alongside examination of the relationship between neuromotor functioning and core executive and spatiotemporal impairments.

7.2. The role of CGG repeat-sensitive impairments

Given the previous findings showing a decrease in the strength of association between mRNA and CGG length in PM-carriers above 90–100 repeats compared to those with lower repeat lengths (Garcia-Alegria et al., 2007), it will be critical for future studies to examine CGG-repeat thresholds in the female FX-premutation. Further research may well reveal greater sensitivity to mRNA toxicity within the 80–100 CGG repeat range, which is consistent with previously reported 'high-risk' CGG-bands associated with FXPOI and major depression (Seltzer et al., 2011; Sullivan et al., 2005). It will be important to examine the subtle cognitive/neuromotor profile in the female FX-premutation, alongside investigation of the association with CGG repeat length, XCI, *FMR1* RNA, methylation and FMRP levels using recently developed techniques (Godler et al., 2010, 2012; Iwahashi et al., 2009).

Previous studies have pointed to a genetic variation moderating the stress of raising a child with FXS, which can impact the severity of anxiety and depression in women with the *FMR1* premutation (Seltzer et al., 2011). These findings highlight a critical role of the interaction between genetic and environmental stressors which are likely to extend to the neurobehavioural and neuromotor profiles. Together with previous studies which have shown that levels of emotional problems in women who carry the *FMR1* premutation are impacted by the inherent stress of raising a child with FXS and other life stressors (Abbeduto et al., 2004; Hall et al., 2007), future studies are required to examine the impact of severity of stressors, genetic variations, and neurobehavioural outcomes in mothers of children with FXS compared to those who carry a premutation but without a child with FXS.

7.3. The role of developmental mechanisms in the female carrier profile

Disruption to developmental mechanisms may lead to subtle neurocognitive and neuromotor difficulties in the female FX-premutation. Given the evidence for gender-specific pathways in neural development alongside the effects of X-inactivation, this profile may differ from early onset developmental cognitive problems previously reported in boys with the FX-premutation such as autism and ADHD (Aziz et al., 2003; Farzin et al., 2006), and the social deficits which have been associated with autism in young adult PM-carrier males (Cornish et al., 2005). Until recently, FMRP was not considered as important for the premutation, with identified phenotypic differences attributed to methodological differences such as sample size, ascertainment bias and possible inclusion of participants with mosaic status. However, there is now emerging evidence for a developmental impact of FMRP on embryonic neural development, brain function and neurobehavioural performance (Chen et al., 2010; Cunningham et al., 2011). Further investigation of developmental aspects of the female carrier profile are needed to determine the extent to which emotional, cognitive and behavioural challenges indicate at-risk profiles for later neurodegenerative decline, or rather a stable developmental phenotype associated with subtle variations in FMRP. Specifically, to fully understand possible relationships between early vulnerabilities and later decline in the female FX-premutation, future longitudinal studies will be needed that cross multiple cognitive and neuromotor domains alongside imaging techniques and routine collection of gender-specific molecular measures.

8. Conclusion

In conclusion, we contend that subtle cognitive and neuromotor profiles need further systematic investigation using sensitive experimentally-driven tasks which tap neural networks especially vulnerable to *FMR1* gene expression. Given the high prevalence of the female FX-premutation in the general population, alongside the associated risk of fertility problems and increased likelihood of having a child with FXS, it will be critical for future studies to identify those women at greatest risk for subtle age-dependent neurobehavioural changes well before the onset of more serious clinical consequences. An important determinant in distinguishing between neurobehavioural profiles that precede later neurodegeneration from those that represent a more stable developmental profile will be to study these divergent pathways using interdisciplinary and longitudinal approaches. Given that "at-risk" clinical trajectories associated with FXTAS and FXPOI are present in the female FX-premutation, prospective longitudinal data across multiple neurobehavioural and neuromotor domains will be critically needed to identify reliable, valid and sensitive biomarkers of disease onset and progression, particularly as new targeted clinical therapies evolve.

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CHAPTER 2

NEUROBEHAVIOURAL METHODOLOGIES FOR MEASURING CEREBELLAR FUNCTION

One of the main outcomes from the published review paper was that the cerebellum may be particularly vulnerable to the influence of the *FMR1* gene in the *FMR1* premutation. Although early views of the role of the cerebellum proposed that functions were exclusively devoted to motor learning and control of voluntary movement (Ito, 2000; Stein & Glickstein, 1992; Wolpert & Kawato, 1998), there is now converging evidence highlighting the cerebellum as an important regulatory system that contributes to a broad range of impairments in executive, visuospatial and affective functioning (Ito, 2008; Molinari & Leggio, 2013; Schmahmann & Sherman, 1998a; Stoodley, Valera, & Schmahmann, 2012). In the first section of this chapter, the role of the cerebellum beyond its contribution to motor dysfunction will be outlined and summarised. In the second section, validated and reliable approaches to assessing the contribution of cerebellum to motor and higher order processes will be discussed.

2.1 Cerebellar regions involved in motor and cognitive functions

The cerebellum, or "little brain", is located at the base of the skull, and through its widespread connections with the central nervous system (CNS), plays a crucial role in enabling efficient cognitive performance, and smooth and accurate movements. Within the cerebellum, homogenous cyto-architectonic maps with repeating micro-complexes are thought to 'learn' through long term depression (LTD) (Cheron, Servais, & Dan, 2008; Ito, 1984), and enable consistent information processing of 'algorithms' that are important for regulatory functions including timing control, effects to cortical homeostasis, modifications of cerebral blood flow, and the modulation of cortical excitability and plasticity (Courchesne & Allen, 1997; Daskalakis et al., 2004; Ivry & Keele, 1989; Manto & Oulad Ben Taib, 2013; Molinari, Filippini, & Leggio, 2002; Salmi et al., 2010). Purkinje cells modulate the majority of these functions. Specifically, Purkinje cells innervate and inhibit the main output of the cerebellum, the deep cerebellar nuclei (DCN). Because the DCN are normally excitatory, changes to Purkinje neuron number, or functional integrity, can lead to an over-excitatory

cerebellar drive and spontaneous firing (de Solages et al., 2008; Shakkottai et al., 2004). Although computational processing within cerebellum is largely homogenous, different regions of the cerebellum are involved in different aspects of functioning. As shown in Table 1, studies of neuromotor function have ascribed gait impairment and postural instability to dysfunction of the medial vermal zone of the cerebellum (Morton & Bastian, 2007; Palliyath, Hallett, Thomas, & Lebiedowska, 1998). By contrast, the intermediate zone is thought to be important for modulating spatial and temporal patterns of inter- and intra-limb coordination (Ilg, Giese, Gizewski, Schoch, & Timmann, 2008; Ilg, Golla, Thier, & Giese, 2007), while the lateral zone modulates anticipatory mechanisms of postural control that facilitate adaptation to a changing environment (Ilg & Timmann, 2013; Morton & Bastian, 2007). Studies of patients with cerebellar lesions show that disruption to these mechanisms can severely impact motor coordination, and result in gait ataxia, tremor and dysmetria (Diener, Dichgans, Bacher, & Gompf, 1984; Ito, 1984).

Table 1

Zones of the cerebellum and associated motor functions

Zone	Deep cerebellar nuclei	Motor Function
Medial/Vermal	Dentate nucleus	Movement regulation, execution and correction
Intermediate	Interpositus nucleus	Limb placement, extensor and flexor activity
Lateral	Fastigial nucleus	Adaptation to changing environment/postural control anticipation/movement planning, timing

Taken from: Morton & Bastian, 2007; Ilg & Timman, 2013; Ilg et al., 2008

More recently, imaging studies have provided insights into the functional specificity of each cerebellar region, with these findings indicating that the function of each specific cerebellar lobule is uniquely related to the cortical region with which it is reciprocally connected (Middleton & Strick, 1998, 2000, 2001; Stoodley & Schmahmann, 2010; Stoodley, Valera, & Schmahmann, 2010). As shown in Table 2, the anterior region of the cerebellum (lobule IV and V, extension of lobule VI and parts of VIII, anterior vermis) is primarily connected with the motor cortex subserving predictive motor control, postural stability and

movement coordination (Coffman, Dum, & Strick, 2011; D'Agata et al., 2011; Ouchi, Okada, Yoshikawa, Futatsubashi, & Nobezawa, 2001; Ouchi, Okada, Yoshikawa, Nobezawa, & Futatsubashi, 1999; Stoodley & Schmahmann, 2010; Sullivan, Rose, & Pfefferbaum, 2006). By contrast, the posterior region of the cerebellum (lobule VI and VII) receives the majority of contralateral input from cerebro-ponto-cerebellar projections arising in prefrontal and parietal cortices (Kamali, Kramer, Frye, Butler, & Hasan, 2010; Middleton & Strick, 2001; Ramnani, 2012; Ramnani et al., 2006; Stoodley, et al., 2012). Because this enables contributions to executive function, language and visuospatial processing (Salmi, et al., 2010; Strick, Dum, & Fiez, 2009), subsequent damage localised to the posterior cerebellum can lead to a range of cognitive-affective impairments, which has been termed cerebellar cognitive affective syndrome (CCAS) (Schmahmann, 2004; Schmahmann & Sherman, 1998b). Importantly, the posterior cerebellum is thought to have a protracted maturation, which may increase vulnerability to genetic variations, and developmental and environmental stressors (Ciesielski, Harris, Hart, & Pabst, 1997; Tiemeier et al., 2010).

Table 2

Lobules of the cerebellum and associated functions

Lobule	Cerebellar region	Connected cortical areas	Function
Lobule IV	Anterior	Motor regions	Sensorimotor
Lobule V	Anterior	Motor regions	Sensorimotor
Lobule VI	Extends into anterior, mostly posterior	Motor regions (small involvement), prefrontal, posterior parietal	Sensorimotor (small involvement), language, verbal working memory, visuospatial, emotion, executive function
Lobule VII	Posterior	Prefrontal, posterior parietal	Emotion, executive function, language
Lobule VIII	Some anterior, mostly posterior	Prefrontal, posterior parietal	Sensorimotor
Crus I & II	Posterior	Prefrontal, posterior parietal	Language, verbal working memory, visuospatial, emotion, executive function

Vermis	Anterior	Motor regions	Sensorimotor
Vermis	Posterior	Limbic system	Emotion

Taken from: Stoodley et al., 2009; 2010, 2012; Ramnani, 2012; Salmi et al., 2010; Kamali et al., 2010

Theories of cerebellar functioning have proposed a significant role off forward and inverse internal models (Bastian, 2006; Ben-Yehudah, Guediche, & Fiez, 2007; Ito, 2005, 2008; Wolpert, Ghahramani, & Jordan, 1995; Wolpert, Miall, & Kawato, 1998). As outlined in Table 3, there are specific functional domains associated with forward and inverse models which converge to facilitate both sensorimotor and cognitive processing by optimising performance in connected cortical regions (Courchesne & Allen, 1997; Salmi, et al., 2010). Theoretical accounts based on computational modelling suggest that the cerebellum utilises internal models for processing routine aspects of a cognitive task to "free up" the prefrontal cortex for flexible processing of complex operations (Ramnani, 2006; Schweizer et al., 2007). Further, it has been proposed that the cerebellum plays an important role in timing of motor functions (Ivry & Keele, 1989; Ivry, Keele, & Diener, 1988). This temporal control is involved in maintaining the timing of forward models, and when damaged can result in disrupted coordination of agonist and antagonist muscles during voluntary movement (Keele, Ivry, & Pokorny, 1987; Keele, Pokorny, Corcos, & Ivry, 1985). For instance, if the forward model signalling cessation of an antagonist muscle burst is delayed during reaching, an overshoot of the analogous agonist muscle and an intention tremor oscillation may occur (Deuschl, Raethjen, Lindemann, & Krack, 2001). It has also been postulated that the primary role of the cerebellum is to detect change and deviation from predictable sequences (Leggio, Chiricozzi, Clausi, Tedesco, & Molinari, 2011). This interpretation is supported by studies indicating that patients with cerebellar damage show poor recognition of correct spatial, temporal and/or semantic relationships in reconstructing a logical sequence (Leggio et al., 2008; Molinari & Leggio, 2013; Tedesco et al., 2011).

Table 3

Forward and inverse models of the cerebellum

Model	Description	Functional outcome –motor	Functional outcome –cognitive
Forward model	Motor: A combination of the predicted sensory outcome and motor efference command. Cognitive: Predicted state and/or signal associated with mental model.	1. Overcomes the delay of true sensory feedback to allow fast movement. 2. Can cancel sensory re-afferent effects (highly predictable events only). 3. Enables computation of error codes for learning (through comparisons with sensory re-afferent). 4. Enables mental practice. 5. Facilitates motor learning. 6. Determines contribution of inverse model to final motor command (inverse model explained below).	1. Predicts action/outcomes of others. 2. Theory of mind (helps us understand how others think and feel). 3. Understanding the 'gist' of what's going on. 4. Preparatory learning allows efficient neural processing (e.g., may shift attention across cortex). 5. Facilitates unconscious thought (mental model is converted into a forward model). 6. Language and reading (grapheme-phoneme conversion). 7. Verbal working memory
Inverse model	Motor: Generates motor command (modular specific and multiple copies are made). Cognitive: Generates cognitive command (modular specific and multiple copies are made).	1. Sets the specific expectations of motor control (e.g., heavy can) and can change rapidly to a different model if the actual experience differs from the expected experience (e.g., can is actually light). 2. Codes primitive responses that enhance survival of organism (e.g., sucking reflex in newborn). 3. Multiple models enable efficient coding of environment. 4. By expanding upon a set of inverse models, the cerebellum contributes to development of ultimately more complex motor behaviours across the lifespan.	1. Inverse models are thought to work in conjunction with forward models. Together they contribute to the final cognitive command. 2. Store properties associated with cortically derived 'mental states.' 3. Intuition and implicit thought.

Taken from: Ito, 2005; 2008; Wolpert et al., 1995; 1998; Bastian, 2006; Schweizer, 2007; Courchesne & Allen, 1997; Ben-Yehudah et al., 2007.

2.2 Assessment of cerebellar motor and cognitive functioning

This section will outline the use of experimentally-driven measures of cerebellar motor and cognitive functioning that may be sensitive to subtle *FMR1* signatures not otherwise detectable through traditional neurological assessments and examination. For example, because these measures investigate neuromotor performance as an integrated system operating in conjunction with central processing systems, they are expected to be more sensitive than gross neurological tools previously investigated in PM-carrier cohorts, such as the CATSYS. In the following section, this thesis presents an analysis of gait, postural control and motor sequencing methodologies known to be sensitive to subtle alterations of functioning in the cerebellar motor and cognitive circuits.

2.2.1. Gait analysis

The independent components of gait

The study of locomotion (hereafter called gait analysis) refers to the investigation of step-to-step fluctuations in human spatial and temporal gait patterns. There are multiple neural networks traversing the motor cortex, cerebellum and basal ganglia that contribute to gait; and through the investigation of distinct components of gait, significant insights can be gleaned into the pattern of abnormality, and the source of pathology (Lord et al., 2012). For instance, studies have detected relationships between gait velocity and markers of brain function, including structural white matter connectivity, cholinergic neurotransmitter systems, and both sensorimotor and frontoparietal networks (Annweiler et al., 2013; de Laat et al., 2011; Lord, Baker, Nieuwboer, Burn, & Rochester, 2011; Rochester et al., 2012; Rosano et al., 2008; Rosano, Brach, Longstreth Jr, & Newman, 2006). Moreover, prefrontal cortex has been highlighted for its role in adapting gait to changing environmental demands (Harada, Miyai, Suzuki, & Kubota, 2009; Suzuki et al., 2004). By contrast, cadence (i.e., stepping frequency or rhythmicity) is thought to be independent from cortical input, and largely mediated by brainstem and spinal cord circuits (Al-Yahya et al., 2011). Indeed, investigations have revealed decreased cadence and increased variability in patients with cerebellar disease (Ilg & Timmann, 2013; Stolze et al., 2002), as well as increased cadence and decreased step length in Parkinson's disease patients with basal ganglia dysfunction (Cho et al., 2010). On the other hand, step width and double support time are thought to reflect different components

of gait control, with a specific role in the maintenance of upright stance during gait (Chamberlin, Fulwider, Sanders, & Medeiros, 2005; Gabell & Nayak, 1984).

As shown in Table 4, the components of gait are commonly investigated through analysis of the relationships between spatial and temporal parameters of gait. Step length and time are thought to be determined by the gait patterning mechanism, whereas step width and double support time are considered primarily balance control mechanisms (Gabell & Nayak, 1984). In many forms of neurodegenerative disease, gait velocity and intra-individual variability of gait have demonstrated clinical utility as a non-invasive approach to identifying the location and significance of neuropathology, and for tracking both disease progression and treatment response (Lord, Galna, & Rochester, 2013; Montero-Odasso, Verghese, Beauchet, & Hausdorff, 2012). As an example, increased intra-individual gait variability, which is predominantly a cerebellar sign and not a natural consequence of ageing (Gabell & Nayak, 1984), has shown potential as a sensitive risk biomarker in both pre-symptomatic carriers of the LRRK2-G2019S gene mutation implicated in Parkinson's disease (Mirelman et al., 2011), and individuals in the pre-manifest stage of Huntington's disease (Rao, Mazzoni, Wasserman, & Marder, 2011). Gait variability or fluctuations in gait patterns is a core feature in a range of neurodegenerative conditions including Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis and inherited cerebellar ataxias (Hausdorff, Cudkowicz, Firtion, Wei, & Goldberger, 1998; Hausdorff et al., 2000; Serrao et al., 2012). Furthermore, recent studies in neurodegenerative disorders have revealed an association between gait velocity and the integrity of higher-order cortical systems (Al-Yahya, et al., 2011; Annweiler, Beauchet, Bartha, & Montero-Odasso, 2012), with longitudinal investigations showing that reductions of gait velocity are also reliable changes preceding mild cognitive impairment (MCI), dementia, Parkinson's disease and Huntington's disease (Buracchio, Dodge, Howieson, Wasserman, & Kaye, 2010; Camicioli, Howieson, Oken, Sexton, & Kaye, 1998; Hausdorff & Buchman, 2013; Mielke et al., 2013; Rao, Muratori, Louis, Moskowitz, & Marder, 2008; Waite et al., 2005).

Table 4

The spatiotemporal components of gait

Component	Description
Velocity	Distance traversed divided by speed of gait
Cadence	Steps per minute
Double support time	Time both feet are simultaneously on ground
Step length	Distance in anterior-posterior direction between two steps
Step width	Distance in medio-lateral direction between two steps
Base of support	Distance in diagonally direction between consecutive steps
Variability	Calculated coefficient of variation (or standard deviation) of one of the above temporal or spatial parameters

Recent factor analyses studies have shown that the large number of gait components can be reduced to independent domains of gait that guide outcome selection for analyses of gait in neurodegenerative disorders (Hollman, McDade, & Petersen, 2011; Lord, et al., 2012; Verghese, Wang, Lipton, Holtzer, & Xue, 2007). As shown in Table 5, principal component analysis performed within these studies has identified five independent domains of gait: Pace, Rhythm, Variability, Asymmetry, and Postural Control.

Table 5

The independent domains of gait

Domain	Parameter
Pace	Velocity, stride length
Rhythm	Cadence, swing time, stance time
Variability	Stride length variability
Asymmetry	Step swing time asymmetry
Postural Control	Step length asymmetry

Taken from: Verghese et al., 2007; Lord et al., 2012.

There are a range of methodologies for quantifying the spatiotemporal characteristics of gait including pressure sensitive footswitches, three-dimensional (3D) motion analysis and GAITrite walkways (Hausdorff, 2007; Lord, et al., 2013). Each method has its own advantages and limitations; for example, although 3D motion analysis offers excellent reliability and validity, it is too expensive and complicated for a typical clinical setting (Lord,

et al., 2013). While gait walkways are limited only by two dimensional analysis of gait characteristics, the GAITRite system provides ease of measurement and clinical utility as a method of gait analysis, with excellent agreement between GAITRite and 3D motion analysis systems (Webster, Wittwer, & Feller, 2005) alongside good reliability and validity (Bilney, Morris, & Webster, 2003; Menz, Latt, Tiedemann, Mun San Kwan, & Lord, 2004).

The effect of cognitive interference on gait

Gait is increasingly being understood as a complex task that involves both higher-order and lower-order systems, as well as the temporal organisation of sensory, executive function and attentional systems (Hausdorff, Peng, Ladin, Wei, & Goldberger, 1985; Hausdorff, Yogev, Springer, Simon, & Giladi, 2005). Mechanistically, it is thought that cognitive input modulates the forward model that codes the anticipatory postural responses associated with gait (Drew, Prentice, & Schepens, 2004; Massion, 1992). This is to ensure consistent and adaptable gait within changing environments. At the same time, executive functions play an important role in regulating attentional allocation, and in cases of subtle neuropathology, such as the prodromal stage of neurodegeneration, may compensate for the underlying dysfunction (Boisgontier et al., 2013). It is well established clinically that that sensitivity to locating the source of neuropathology is enhanced when examining gait function with concurrent performance of a secondary cognitive task (hereafter referred to as a dual-task) which may interfere with higher-order compensatory neural networks [for review of dual-task literature see (Al-Yahya, et al., 2011; Fraizer & Mitra, 2008; Kelly, Eusterbrock, & Shumway-Cook, 2012; Scherder et al., 2007; Woollacott & Shumway-Cook, 2002)]. In particular, converging evidence suggests that deficits in motor automaticity arising from cerebellar or basal ganglia dysfunction lead to increased attentional allocation to the motor task, which can result in reduced attention towards gait as a result of reaching the capacity limits of attentional resources (Iansek, Danoudis, & Bradfield, 2013; Lajoie, Teasdale, Bard, & Fleury, 1996; Lord, et al., 2011). This dual-task cost has been attributed to cognitive load on the attentional system and has been termed capacity interference (Hausdorff, et al., 2005; Holtzer, Verghese, Xue, & Lipton, 2006; Shumway-Cook & Woollacott, 2000).

While the distinct neural substrate of dual-task interference has been difficult to isolate due to inherent difficulties of measuring brain activity during gait (Klingberg, 2000), a recent network connectivity study has highlighted that the cerebellum plays a crucial role in

integrating the synergistic neural networks required to perform dual-tasks (Wu, Liu, Hallett, Zheng, & Chan, 2013). These findings shed light on several dual-task gait studies in which signs of cerebellar dysfunction, such as increased temporal variability of gait, emerge in normal ageing, and in those with dementia and at increased risk of falling (Allali et al., 2007; Hausdorff, Rios, & Edelberg, 2001; Hollman, Kovash, Kubik, & Linbo, 2007; Maki, 1997; Menz, Lord, & Fitzpatrick, 2003; Snijders, van de Warrenburg, Giladi, & Bloem, 2007). However, the dual-task approach has not yet been firmly established as a routine part of gait studies and clinical practice, and there is a lack of consistent findings as to which concurrent cognitive tasks offer the most sensitivity. Nonetheless, those studies that have specifically investigated the impact of the type of dual-task have shown that high load executive function tasks (i.e., serial backward counting, verbal fluency, stroop inhibition) appear to be more sensitive than memory or visuospatial tests (Hausdorff, Schweiger, Herman, Yogev-Seligmann, & Giladi, 2008; Liu-Ambrose, Katarynych, Ashe, Nagamatsu, & Hsu, 2009; Patel, Lamar, & Bhatt, 2013). Importantly, internal mental tracking tasks were found through meta-analysis to be more sensitive to subtle spatiotemporal fluctuation of gait than tasks related to external factors such as reaction time tasks (Al-Yahya, et al., 2011).

2.2.2 Analysis of postural control

The postural control system of the central nervous system maintains and informs on the position of our 'body in space'. It is a complex multifactorial system in which visual, vestibular and somatosensory inputs are centrally integrated by attentional systems (Massion, 1994), and across both higher order cortical and lower order sub-cortical systems. It is thought that the postural control system is highly adaptable in that the degree of sensory input is modulated online in response to changing environmental inputs (Horak, 2006). This process, which requires attentional resources, is called sensory re-weighting (Teasdale & Simoneau, 2001). The sensory re-weighting hypothesis portends that the central nervous system can select and adjust the relative contribution of sensory inputs, to achieve optimal postural stability during changing developmental time-points, (Bair, Kiemel, Jeka, & Clark, 2012; Mallau, Vaugoyeau, & Assaiante, 2010); for example, the down-weighting of proprioceptive input during the accelerated growth period of adolescence (Viel, Vaugoyeau, & Assaiante, 2009). Experimental paradigms that manipulate sensory input by modifying visual, vestibular or somatosensory experiences may illicit the requirement for sensory re-

weighting, which in turn increases attentional load (Patel et al., 2008; Teasdale & Simoneau, 2001; Teasdale, Stelmach, & Breunig, 1991). This approach is more ecologically valid as postural control is not merely an automatic process, but relies on cognitive input from central processing systems.

One of the most sensitive methods to investigate multi-sensory integration during postural control is to employ dual-task paradigms. The attentional cost associated with postural dual-tasks has been attributed to capacity interference when two tasks exceed the capability of limited attentional resources (Sheridan, Solomont, Kowall, & Hausdorff, 2003; Yogev-Seligmann, Hausdorff, & Giladi, 2008). The attentional demands on postural control may depend on the attentional load of the cognitive or motor task, and individual differences in executive control (Montero-Odasso, et al., 2012). Importantly, it has been shown that concurrent cognitive tasks with low cognitive demand may facilitate a more automatic state by shifting attention away from postural performance (Huxhold, Li, Schmiedek, & Lindenberger, 2006; Swan, Otani, Loubert, Sheffert, & Dunbar, 2004; Woollacott & Shumway-Cook, 2002). This improvement is associated with the selection of automated subcortical networks over higher-order cortical systems (Boisgontier, et al., 2013). Thus dual-task paradigms that load both attentional and sensory systems are considered the most sensitive for assessing the attentional demands on postural control studies (Woollacott & Shumway-Cook, 2002).

The electronic force plate is well-established for measuring postural stability (Golriz, Hebert, Foreman, & Walker, 2012; Prosperini & Pozzilli, 2013). However, force platforms are limited due to the significant cost and expertise required for data analysis. Alternatively, the Physiological Profile Assessment (PPA) is a low cost and less time consuming validated measure for examining sensory and motor physiological function which may be a more appropriate choice for clinical and research settings (Lord, Menz, & Tiedemann, 2003). In addition, postural displacement in the anterior-posterior and medio-lateral directions can be further assessed with a sway metre, which has been well validated with centre of pressure measures using a force plate (Lord, Ward, & Williams, 1996; Sherrington, 2000; Sturnieks, Arnold, & Lord, 2011).

2.2.3. Motor sequence learning

A large body of clinical and imaging studies have converged to indicate a critical role of the cerebellum in sequencing ability (Bellebaum & Daum, 2011; Braitenberg, Heck, & Sultan, 1997; Clegg, DiGirolamo, & Keele, 1998; Ito, 2000; Molinari & Leggio, 2013). At its most fundamental level, sequencing relies on establishing or detecting associative relationships between two stimuli to allow precise prediction of an upcoming response or action (Rah, Reber, & Hsiao, 2000; Schmidtke & Heuer, 1997; Timmann et al., 2010; Timmann et al., 2002). This notion arose from computational models of cerebellar function which propose that the cerebellum utilises forward models to optimise performance based on previous predictable events (Nixon & Passingham, 2001; Nixon, 2003). Accordingly, physiological studies have demonstrated increased spectral cerebellar activity just prior to sequenced somatosensory stimulation, and even greater activity in response to a random omission (Ivry, 2000; Tesche & Karhu, 2000). Similarly, cerebellar patients perform poorly on sequence detection tasks (Gomez-Beldarrain, Garcia-Monco, Rubio, & Pascual-Leone, 1998; Molinari et al., 1997; Shin & Ivry, 2003).

Sequence learning can occur explicitly through planned voluntary effort or implicitly and outside of conscious awareness. The neural networks of explicit and implicit learning can be differentiated, with the former associated with additional activation of prefrontal cortical regions, and the latter with mostly motor and sub-cortical circuits (Aizenstein et al., 2004; Hazeltine, Grafton, & Ivry, 1997; Russeler, Kuhlicke, & Munte, 2003). There are a number of approaches to measuring motor sequencing functions, including card sequencing tasks based on recognition of spatial and temporal relationships (Leggio, et al., 2008), and those that measure procedural learning of a motor sequence (Molinari, et al., 1997). However, the 'gold standard' approach is to employ serial reaction time tasks, which commonly examine the ability to implicitly adapt to a repeating sequence (Clegg, et al., 1998; Nissen & Bullemer, 1987). Although traditional versions employed sequenced presentation of stimuli across four horizontal bars, more recent imaging studies have emphasised the need for a more complex version in which non-spatial abstract or symbolic primes are imbedded in the task (Balsters & Ramnani, 2008; Bo, Peltier, Noll, & Seidler, 2011; Orban et al., 2010; Spencer & Ivry, 2009). Recent sequencing studies with symbolic cue information have shown that cerebellar lobule VI is crucial for symbolic sequence learning. These findings suggest that the cerebellar activation during sequence learning with symbolic cue information may reflect the mapping of stimulus to response code which enables the formation of automated

sequenced responses. Importantly, for the investigations in this thesis, it has been suggested that cerebellar contributions to cognition are mediated through symbolic sequencing mechanisms, which include working memory, language, visuospatial and cognitive-affective functioning (Bo, Jennett, & Seidler, 2012; Keele, Ivry, Mayr, Hazeltine, & Heuer, 2003; Molinari & Leggio, 2007).

Declaration for Thesis Chapter 3

Declaration by candidate

In the case of Chapter 3, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Project design, review of relevant literature, recruitment and testing of participants, analysis of data and writing of manuscript.	50%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Dr Darren R Hocking	Contributed to project design, review of relevant literature, and writing of manuscript.	
E/Prof. John L. Bradshaw	Critical review of manuscript	
Dr Joanne Fielding	Critical review of manuscript	
Dr Jonathan Cohen	Critical review of manuscript	
Prof. Nellie Georgiou-Karistianis	Critical review of manuscript	
Prof. Kim. M. Cornish *	Contributed to project design and writing of manuscript.	

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	**Date** 15/2/14
Main Supervisor's Signature	[redacted]	**Date** 15/2/14.

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

CHAPTER 3

GAIT CONTROL IN FEMALE CARRIERS OF FRAGILE X SYNDROME

3.1. Preamble to empirical paper 1: Age and CGG-repeat length are associated with neuromotor impairments in at-risk females with the *FMR1* premutation

Although the published review on female PM-carriers has raised the possibility of early onset cerebellar cognitive and motor impairments, the extent to which such cerebellar profiles represent a *forme fruste* for later neurodegenerative decline or a stable developmental profile is hitherto unknown. The following empirical paper presents the first investigation of the effects of cognitive dual-task interference on spatiotemporal gait characteristics in female PM-carriers. The GAITrite system can provide both spatiotemporal gait and step-to-step variability of fluctuations in gait, and has previously shown sensitivity to distinguishing neuropathological involvement across cerebellar, Parkinson and Alzheimer disease patients (Hausdorff, et al., 1998; Serrao, et al., 2012; Sheridan, et al., 2003). To explore the potential of employing gait analysis with dual-task methodology for prospective clinical interventions for female PM-carriers, this study examined spatiotemporal gait characteristics and intra-individual variability of gait during dual-task interference, and whether selective gait parameters showed age- and CGG-repeat length dependent impairments in females with the PM allele.

Age and CGG-repeat length are associated with neuromotor impairments in at-risk females with the *FMR1* premutation

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Glossary: DTC = dual task cost; *FMR1* gene = fragile X mental retardation 1 gene; FMRP = Fragile X Mental Retardation Protein; FXTAS = Fragile X Tremor Ataxia Syndrome; PM-carrier = Premutation carrier.

ABSTRACT

Recent studies report a higher risk of dementia and motor symptoms in females with the *fragile X mental retardation 1* (*FMR1*) premutation (PM-carriers) than has hitherto been appreciated. Here we employ dual-task gait paradigms to identify potential markers of cognitive and motor decline in female PM-carriers. Spatiotemporal gait characteristics and variability of gait were assessed during single- and dual-task conditions in 28 female PM-carriers (mean age 41.32 ± 8.03) and 31 female controls with normal *FMR1* alleles (mean age 41.61 ± 8.3). Despite comparable gait characteristics at baseline, gait performance was significantly poorer for PM-carriers when performing concurrent working memory tasks (counting backwards by 3's or 7's) when compared to controls. Correlational analyses showed that low working memory capacity was significantly associated with dual-task interference for the gait domains of *pace* (speed, step length) and *variability* (step time, swing time) in PM-carriers. Multiple regression analyses further showed that the interaction between age and CGG repeat length was strongly predictive of gait *variability* during dual-task performance. These findings indicate for the first time that vulnerability in a specific domain of gait control may act as a sensitive surrogate marker of future decline in female PM-carriers.

1. Introduction

Fragile X-related disorders (FXD) are associated with a CGG repeat expansion in the 5'untranslated region of the fragile X mental retardation 1 (*FMR1*) gene and include both developmental and degenerative neurologic manifestations. *FMR1* gene expansion can occur across successive generations, with individuals classified as demonstrating normal (<41), grey zone/intermediate (45-54) and premutation (55-200) CGG repeat expansions, or, the full fragile X mutation (>200). Fragile X syndrome (FXS), the most common single-gene cause of intellectual disability and autism, results from transcriptional silencing of the *FMR1* gene and absence of the fragile X mental retardation protein (FMRP) (Cornish et al., 2008). Although premutation carriers (PM-carriers) were assumed for many years to be free from any deleterious phenotype, it is now well-documented that approximately 40% of male PM-carriers (>50 years) and 8-17% of female PM-carriers will develop late-onset neurodegenerative decline associated with the fragile X-associated tremor ataxia syndrome (FXTAS) (Rodriguez-Revenga et al., 2009). Although a higher than expected risk of dementia and motor symptoms have been recently reported in females with the *FMR1* premutation (Chonchaiya et al., 2010; Hall et al., 2011; Narcisa et al., 2011; Paul et al., 2010; Tassone et al., 2012; Kraan et al., 2013), the extent to which early cognitive and motor changes represent a *forme fruste* for later neurodegenerative involvement is currently unknown.

In FXTAS, it is the increased levels of *FMR1* mRNA that result in a gain-of-function toxicity, whereby proteins that normally bind to mRNA are sequestered from their pre-determined role thereby compromising their function (Tassone and Hagerman, 2012). These pathologic mechanisms appear to be the cause of FXTAS, which is associated with progressive dementia (>55 years and predominantly in men), intention tremor, gait ataxia, parkinsonism and executive function impairments (Hagerman and Hagerman, 2007; Hagerman et al., 2001). In PM-carriers with and without FXTAS, larger CGG repeat expansions are thought to result in more severe phenotype as a result of increased *FMR1* mRNA toxicity and slightly reduced levels of FMRP (Kenneson et al., 2001; Tassone et al., 2000a). For female PM-carriers, there is a protective effect from the normal allele on the second X chromosome, and this is thought to result in milder clinical presentation than in male PM-carriers with and without FXTAS (Leehey et al., 2008). However, recent studies with female PM-carriers have identified abnormal psychomotor ability (Goodrich-Hunsaker et al., 2011), motor coordination deficits associated with higher postural sway (Narcisa et al.,

2011), the presence of both intranuclear inclusions and Alzheimer's pathology throughout the brain (Tassone et al., 2012), and elevated rates of Parkinson's disease in *FMR1* grey-zone alleles (Hall et al., 2011). Taken together, these findings raise the possibility of a neuromotor at-risk profile in adult female PM-carriers, which may represent the earliest changes in motor control, prior to the onset of more severe decline.

One of the most sensitive paradigms for investigating preclinical signs is that of dual-task related changes in gait and balance control. Dual-task impairments are primarily attributable to capacity interference caused by competing demands for limited attentional resources (Sheridan et al., 2003; Yogev-Seligmann et al., 2008). Gait fluctuation or variability in gait from stride-to-stride is thought to reflect the ability to consistently generate a rhythmic stepping pattern (Gabell and Nayak, 1984), and is considered a sensitive indicator of gait control and stability. Previous reports have also shown that increased variability of gait timing under dual-task conditions is associated with executive and attentional deficits in patients with Alzheimer's disease (Sheridan et al., 2003), and may be predictive of restricted mobility, falls and progression to dementia (Montero-Odasso et al., 2012). These findings highlight the possibility that poor attentional control, previously reported in females with the *FMR1* premutation (Hunter et al., 2011), may be associated with age-dependent changes in dual-task gait performance.

Here we explore for the first time the interrelationships between working memory and gait characteristics in female PM-carriers prior to the potential onset of FXTAS. Specifically, we aimed to characterize the effect of cognitive dual tasks on spatiotemporal gait characteristics and temporal gait variability in female PM-carriers. In addition, we investigate whether specific dual-task gait and variability parameters are associated with age- and CGG-repeat length, possibly indicative of more severe forms of cognitive and motor decline previously reported in a subset of older female PM-carriers (Berry-Kravis et al., 2007; Leehey, 2009; Tassone et al., 2012).

2. Methods

2.1 Participants

Female PM-carriers were recruited through local and national FXS support groups, via a population-based fragile X carrier screening pilot study (Metcalfe et al., 2008), and a

larger carrier screening study currently underway in Victoria and Western Australia (unpublished). Control participants were recruited through the current population-based fragile X carrier screening study, local networks and via online advertisements. All study participants provided signed informed consent and the study procedures were consistent with the Declaration of Helsinki and approved by the relevant institutional review boards. The comparisons between female PM-carriers and controls showed that the two groups were well-matched on age, IQ and anthropometric characteristics. The descriptive statistics are shown in Table 1. All participants were English speaking with no previous diagnosis of a neuropathy or fibromyalgia, and no history of epilepsy or of a serious head injury. Participants also had normal (or corrected) vision and hearing, and no sign of intellectual disability as assessed using the Wechsler Abbreviated Scale of Intelligence (FSIQ<70). The Letter-Number Sequencing (LNS) subtest from the Wechsler Adult Intelligence Scale (WAIS-IV) was selected to assess working memory performance. A total of 11 participants reported that a blood PM-carrier relative had been diagnosed with FXTAS or dementia: 5 had fathers with FXTAS, 1 had a father with Alzheimer's dementia, 2 had an uncle with FXTAS, and 3 reported Alzheimer's or dementia in the FXS-affected bloodline (aunt, grandmother and grandfather). The administration of the FXTAS Rating Scale (Leehey, 2009) was used to screen female PM-carriers for features related to FXTAS—that is, tremor, ataxia or parkinsonism—or any other neuromotor disorder.

Table 1

Sample characteristics for female FMR1 PM-carriers and control participants

	FMR1 PM-carriers (N=28)	Controls (N=31)	*p* value
	M ± SD (range)	M ± SD (range)	
Age (years)	41.32 ± 8.03 (22-53)	41.61 ± 8.3 (22-55)	.892
FSIQ	109.41 ±11.16 (88-128)	113.16 ± 7.86 (89-129)	.151
VIQ	105.07 ±14.38 (73-126)	109.74 ± 10.41 (88-136)	.159
PIQ	110.71 ±10.78 (87-133)	114.29 ± 9.31 (93-133)	.177
Height (cm)	163.46 ±6.91 (145-174.50)	166.48 ± 6.85 (151-181)	.100
Weight (kg)	73.71 ± 19.13 (48-129)	73.35 ± 15.11 (52-117)	.938
CGG-repeat length	84.79 ± 14.67 (61-122)	31.30 ± 3.14 (28-42)	<.001**

Abbreviations: FSIQ = Full Scale Intelligence Quotient; PIQ = Performance Intelligence Quotient; SD = Standard Deviation; VIQ = Verbal Intelligence Quotient.
**p<.01

2.2 Molecular genetic data

DNA was extracted from 2ml whole blood from all participants using the Promega Maxwell® 16 Instrument and associated Maxwell® 16 Blood DNA Purification Kit (Promega Cat No.: AS1010). PCR was performed using the Asuragen® AmplideX™ *FMR1* PCR Kit as this assay has been shown to detect a full range of fragile X expanded alleles (Chen et al., 2010). PCR products were assessed via capillary electrophoresis on an Applied Biosytems 3130 Genetic Analyzer with electropherogram analysis conducted using GeneMapper® software. All procedures were performed in accordance with manufacturer's instructions.

2.3 Quantitative gait analysis

Spatiotemporal gait characteristics were assessed by a 593 cm long × 89 cm wide instrumented walkway (GAITRite, CIR Systems Inc., Clifton, NJ, USA), which captured step-to-step variations or fluctuations in gait via embedded pressure sensors. The GAITRite is a valid and reliable method for assessing spatial and temporal parameters of gait, and has demonstrated high validity when compared to the 'gold standard' 3-dimensional motion analysis system (Menz et al., 2004). In accordance with published recommendations for improving the reliability of measuring gait variability, participants traversed the GAITrite walkway across six walking trials for each condition with minimal interruption between walks (>30 steps) (Galna et al., 2012). The total distance of the walkway was 4.9 m, even though participants initiated and ended walking 1.5m before and after the mat to reduce the effects of acceleration and deceleration during each walk.

Participants were required to complete the walks along the GAITrite walkway under three conditions: (1) walking at preferred speed without counting (single-task condition), (2) walking at preferred speed while counting backward aloud by 3's, and (3) walking at preferred speed while counting backward aloud by 7's (henceforth referred to as the dual-task conditions). For the dual-task conditions, participants were instructed to not specifically prioritise one task over another, but were asked to combine both tasks during performance. Before commencing each walk along the walkway, a trained evaluator provided participants

with a starting number which remained consistent across all participants. Cognitive performance was measured as the total number of correctly counted numbers while walking.

2.4 Statistical analyses

The GAITRite computer software recorded average speed, cadence, step length, and double support time for each walk. Step-to-step variability for step width, step time and swing time were calculated as coefficient of variation [CoV = (SD/mean) x 100)]. Dual-task costs (henceforth referred to as DTC) were defined as a change in performance using the following formula: DTC = [(dual-task score – single-task score)/single-task score] × 100. For each gait parameter, DTCs were calculated for both the count backwards by 3's and 7's dual-task conditions. This was calculated across the 6 walking trials and step-to-step variability measures for both single and dual-task conditions. Normality was assessed according to the Shapiro-Wilk test for normality, and parametric tests were employed to explore group differences, or nonparametric equivalents where violations of normality distribution or equality of variance between groups co-occurred.

Data were analysed using IBM SPSS Statistics 20.0. Independent groups *t*-tests (or nonparametic equivalent) were used to compare group differences in gait performance at baseline, and compare DTC scores between the premutation and control groups. Based on previous studies using factor analysis which have identified independent domains of gait associated with pace (speed, step length), rhythm (cadence) and variability (step width, step time and swing time), we calculated these three independent gait domains (Lord et al., 2012; Verghese et al., 2008). We computed z-scores for each gait parameter, combining them for each representative gait domain. To ensure consistency of interpretation, where higher scores on some gait variables (e.g., velocity, step length) and lower scores on other gait variables (e.g., double support time) represented better gait performance, we multiplied the z-score by -1. To examine the interrelationships between working memory and dual-task interference on each of these gait domains, correlational analyses were performed using Pearson correlation. Following this we performed a series of multiple linear regression with interaction terms, with each dual-task condition across the gait domains selected as the primary criterion measures, and the predictor variables as age, CGG repeat and their interaction. In accordance with previously established guidelines (Aiken and West, 1991), CGG repeat and age were centred prior to regression analyses and multiplied together to represent the interaction. The

assumptions of multiple regression (i.e., independence of residuals, normality of residuals, linearity, and homoscedasticity) were met for these analyses. A significance value of $p < 0.05$ was set for all regression analyses.

3. Results

3.1 Baseline characteristics

Our analysis included 29 female PM-carriers aged between 22 and 53 years of age, and 31 female controls with normal *FMR1* alleles aged between 22 and 55 years of age. One PM-carrier tested positive as a full mutation after *FMR1* DNA testing and hence was excluded from the final analysis leaving 28 females in this group. All participants completed the gait paradigm, although one PM-carrier was unwilling to complete the counting backwards by 7's task. Hence, we removed this participant from our analyses for this condition only. Performance on the concurrent counting tasks (both counting backward by 3's and 7's) did not differ between the PM-carriers and controls (Mann-Whitney: p=.442 and p=.945, respectively). There were also no differences between PM-carriers and controls on the letter-number sequencing test (t-test: p=.110) or any of the spatiotemporal gait characteristics and variability measures (CoV) during the single-task condition: velocity (t (57) = -.394, p=.695), step length (t (57) = -.980, p=.331), double support time (U = 428, z = -.091, p = .927), cadence (t (57) = .613, p=.542), step time variability (U = 367, z = -1.017, p = .309), swing time variability (t (57) = -.029, p=.977) and step width variability (U = 416, z = -.273, p = .785). The baseline gait characteristics are presented in Table 2.

3. 2 Dual-task gait characteristics

Between-group comparisons of each calculated DTC value (i.e., the change in dual-task performance as a proportion of baseline gait characteristics) showed significantly greater DTCs across a range of spatiotemporal gait parameters for the PM-carriers. Compared to controls, the PM-carriers walked slower (count back by 3's: U = 285, z = --2.262, p = .024; count back by 7's: U = 288, z = -2.034, p = .042) and with a reduced step length (count back by 3's: t (57) = -2.109, p=.039; count back by 7's: t (56) = -2.240, p=.029) during the concurrent cognitive tasks (see Table 2).

Table 2

Means and standard deviations for spatiotemporal gait parameters and intra-individual variability of gait at baseline, and during the dual-task conditions (dual-task costs) for FMR1 PM-carriers and controls.

Gait parameter	*FMR1* PM-carriers (n = 28)		Controls (n = 31)		
	Spatiotemporal measures (mean ± SD)	Dual task cost (%)	Spatiotemporal measures (mean ± SD)	Dual task cost (%)	*p* value (DTC)
Pace					
Velocity (cm/s)					
Single-task	140.32 ±20.04	n/a	142.34 ±19.43	n/a	
3CB	119.05 ±25.60	-14.96 ± 11.41	130.58 ± 20.95	-8.17 ± 8.99	.024*
7CB	108.22 ± 25.53	-22.87 ± 14.04	120.99 ± 21.73	-14.78 ± 11.65	.042*
Step length (cm)					
Single-task	70.44 ± 7.90	n/a	72.29 ± 6.59	n/a	
3CB	66.15 ±8.61	-6.16 ± 5.30	69.85 ± 7.17	-3.34 ± 4.98	.039*
7CB	63.69 ± 8.87	-9.60 ± 7.04	68.05 ± 7.28	-5.82 ± 5.81	.029*
Double Support Time (s)					
Single-task	.27 ±.05	n/a	.26 ± .05	n/a	
3CB	.33 ±.09	20.73 ± 21.44	.29 ± .05	10.32 ± 12.79	.062
7CB	.36 ± .10	36.16 ± 36.60	.32 ± .06	20.96 ± 19.28	.082
Rhythm					
Cadence (steps/min)					
Single-task	119.08 ±8.82	n/a	117.69 ± 8.59	n/a	
3CB	108.02 ±13.95	-22.45 ± 13.39	111.70 ± 10.11	-20.54 ± 10.02	.633
7CB	101.53 ± 16.62	-25.75 ± 15.19	106.09 ± 11.87	-24.44 ± 11.23	.536
Variability					
Step width variability (CoV)					
Single-task	3.54 ± 1.26	n/a	3.47 ± 1.05		
3CB	3.68 ± 1.16	13.30 ± 49.17	3.35 ± 1.04	.92 ± 32.77	.504
7CB	4.50 ± 1.66	28.99 ± 48.23	3.99 ± 1.31	25.14 .92 ± 55.27	.668
Step time variability (CoV)					
Single-task	3.39 ± 1.05		3.10 ± .87		
3CB	4.41 ± 1.88	35.33 ± 59.95	3.64 ± 1.42	24.23 ± 48.72	.606
7CB	5.69 ± 3.80	71.53 ± 107.31	4.49 ± 2.95	52.18 ± 101.92	.387
Swing time variability (CoV)					
Single-task	3.16 ± .84	n/a	3.17 ± .76	n/a	
3CB	4.81 ± 2.61	55.25 ± 76.26	4.05 ± 1.91	1.51 ± 43.62	.177
7CB	5.85 ± 3.66	94.14 ± 122.30	4.65 ± 2.76	52.59 ± 107.00	.077

* p<.05 level.

NB: 3CB = count backwards by 3's dual-task; 7CB = count backwards by 7's dual-task

3.3 Associations between working memory and DTC gait domains

The associations between performance on Letter-Number Sequencing and DTC gait domains were examined through correlational analyses. As shown in Table 3, there were several significant correlations between working memory performance and a range of gait domains only in PM-carriers. These associations showed that low working memory capacity was significantly correlated with reduced pace (count backwards by 3's: r = .460, p = .014; count backwards by 7's: r= 0.502, p = .008) and increased variability (count backwards by 3's: r = -.419, p = .027; count backwards by 7's: r= -.472, p = .013). No significant associations were revealed for controls with normal alleles.

Table 3

Correlational associations between DTC gait domain and working memory performance for FMR1 PM-carriers and controls.

	FMR1 premutation carriers	Control participants
Pace		
3CB	.460*	.217
7CB	.502**	.105
Rhythm		
3CB	.227	.139
7CB	.252	.142
Variability		
3CB	-.419*	-.161
7CB	-.472*	-.160

**p< .01, *p<.05 level

NB: 3CB = count backwards by 3's dual-task; 7CB = count backwards by 7's dual-task

3.3 Moderating role of CGG-repeat length

We employed multiple regression analyses to examine the moderating role of CGG-repeat length on the relationship between age and each DTC gait domain. As shown in Table 4, there was no interaction between age and CGG repeat length for the gait domains of pace (count backwards by 3's: beta = .008, p = .969; count backwards by 7's: beta = .136, p = .505) and rhythm (count backwards by 3's: beta = -.363, p = .064; count backwards by 7's: beta = .020, p = .917). There was a significant interaction between age and CGG repeat

length for the gait domain of variability during the dual-task condition (counting backwards by 3's; beta = .506, $p = .009$).

Table 4

Standardised β and t values for multiple linear regression analyses with interaction terms and the DTCs for each gait domain as the dependent variable

	Count backwards by 3's dual-task						Count backwards by 7's dual-task					
	Pace		Rhythm		Variability		Pace		Rhythm		Variability	
	Sβ	t	Sβ	t	Sβ	t	Sβ	t	Sβ	t	Sβ	t
Constant		-.753		.880		-.294		.279		1.957		-1.765
CGG length	-.045	-.231	-.183	-.933	-.047	-.256	-.113	-.523	-.266	-1.337	.279	1.410
Age	.200	1.017	-.005	-.027	.123	.665	.000	.001	-.204	-1.026	.139	.702
Age X CGG	-.338	-1.797	-.363	-1.942	.506	2.857**	-.116	-.559	.020	.105	.259	1.373
R2		.057		.173		.258		.028		.146		.191
Adjusted R2		.228		.070		.165		-.099		.039		.086

**$p<.01$

NB: In the above table there are 3 multiple linear regression analyses for the count backwards by 3's dual-task condition and 3 for the count backwards by 7's dual-task condition. The dependent variable for each analysis is the combined z-score for each select gait domain examined: pace = Z-velocity, Z-step-length and Z-double-support-time (reversed); rhythm = Z-cadence; variability = Z-step time-variability, Z-swing-time-variability and Z-step-width-variability. The independent variables are CGG-repeat length, age and the interaction term of age and CGG-repeat length. Sβ = Standardised beta value; t = t-score.

4. Discussion

This study is the first to investigate the interrelationships between working memory and gait control in adult females with the *FMR1* premutation. Our findings clearly demonstrate the differential impact of increasing cognitive load on high level gait characteristics in female PM-carriers. Furthermore, we show that an age-related decline in gait variability may represent a CGG repeat toxicity "signature" in an at-risk subset of females with the *FMR1*

premutation allele. Although future longitudinal studies will be critical in distinguishing the relative contribution of both developmental and neurodegenerative processes, these findings suggest that gait variability could provide an indicator of subtle neuromotor changes prior to the onset of dementia and motor symptoms previously reported in female PM-carriers.

Our findings of significantly greater dual-task effects on gait control in female PM-carriers when performing a concurrent counting task are consistent with capacity interference caused by competing demands for limited attentional resources (Hausdorff et al., 2008; Sheridan et al., 2003; Woollacott and Shumway-Cook, 2002; Yogev-Seligmann et al., 2008). The current findings revealed greater dual-task costs on gait parameters commonly associated with high level cortical control of walking including gait speed and step length in female PM-carriers (Sheridan et al., 2003; Springer et al., 2006; Verghese, et al., 2007). However, dual-task costs for cadence (stepping frequency) were comparable between PM-carriers and controls. This pattern of findings is in line with evidence that cadence control is not tightly linked to gait speed but rather involves lower order circuits associated with brain stem and spinal cord locomotor regions (Osaki, Kunin, Cohen, & Raphan, 2008). Significantly, PM-specific alterations to high level gait control were shown only in the counting backwards dual-task conditions, where there was concurrent performance of a working memory task. Together with the significant associations between working memory and dual-task gait performance, these findings indicate that working memory may be a specific executive subdomain associated with high level gait control in female PM-carriers.

We further present novel data which demonstrates an interaction between age and CGG-repeat length in dual-task related gait variability. The observed vulnerabilities in intra-individual variability of gait support a core age- and CGG-repeat dependent problem with gait automaticity and dynamic stability under dual-tasking in female PM-carriers. From a biomechanical view, increased gait variability suggests compromised stepping automaticity and impaired balance control mechanisms (Hausdorff et al., 2008). Indeed, this increased temporal variability is consistent with clinical observations of ataxic gait seen in cerebellar dysfunction (Ebersbach et al., 1999). Moreover, changes across these measures during complex dual-tasks, such as counting backward, which depends on working memory and attention, have been shown to predict balance problems and increased risk of falling in older adults (Maki, 1997; Nordin et al., 2010; Springer et al., 2006). Cerebellar involvement in female PM-carriers is consistent with evidence showing that the cerebellar motor networks are one of the earliest affected brain regions in PM-carriers with and without FXTAS

(Battistella et al., 2013; Berry-Kravis et al., 2007; Conde et al., 2012; Hashimoto et al., 2011a, b; Wang et al., 2013). Thus with increasing CGG-repeat length and age, female PM-carriers may be at-risk for subtle increases in variability and reduced control of gait stability, suggestive of cerebellar dysfunction.

The current findings indicate that there may be vulnerabilities of gait automaticity and stability in female PM-carriers with CGG-repeat lengths in the upper premutation range. Previous studies suggest that there is both increased *FMR1* mRNA toxicity and a reduction in the percentage of histochemically FMRP-positive cells in high repeat carriers when compared to low repeat carriers (Kenneson et al., 2001; Tassone et al., 2000b). *FMR1* mRNA toxicity is the purported cause of FXTAS and associated neurodegenerative disease (Tassone and Hagerman, 2012). Alternatively, FMRP has been assigned a stable developmental role and is suggested to be associated with a number of phenotypes found in the premutation including impairments in executive cognitive functioning (Loesch et al., 2003a), and motor programming (Loesch et al., 2003b). Thus we speculate that the deleterious effects of the *FMR1* premutation on the neuromotor phenotype are not simply the result of mRNA toxicity, but may also reflect reduced FMRP levels and greater neural susceptibility of cerebellar networks to the differential expression of *FMR1* protein.

There were several limitations that should be acknowledged. First, this study employed a cross-sectional design, so the extent to which dual-task related gait changes represent a *forme fruste* of FXTAS or a stable developmental phenotype remains a question to be addressed in prospective longitudinal studies with larger sample sizes. Second, the lack of examination of FMRP and *FMR1* mRNA leucocyte levels restricts a definitive explanation for the differential effects of RNA-mediated toxicity and FMRP expression on the cognitive-motor phenotype in females with the premutation allele.

Our results present the first cross-sectional evidence for a subtle cognitive-motor interference effect on gait variability and stability in females with the *FMR1* premutation. This age- and CGG repeat-dependent profile of gait variability may reflect the effects of distinct molecular events occurring in the upper permutation range on specific neural regions with greater vulnerability to the *FMR1* premutation. Prospective longitudinal studies will be critical in determining whether dual-task gait assessments offer potential as surrogate markers of age-related changes prior to the onset of more severe neurodegenerative decline.

Disclosure statement

Should a significant conflict of interest be present, the Editors reserve the right to reject the article on that basis.

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Author contributions

Claudine Kraan, Darren Hocking and Kim Cornish (first, second and last author) conceptualized and designed the study, provided intellectual input into the interpretation of the data and co-wrote the first draft of the manuscript. Nellie Georgiou-Karistianis (3rd author) provided intellectual input into the interpretation of the data, as well as input into drafts of the manuscript. Sylvia Metcalfe and Alison Archibald (4th and 5th authors) contributed to study design, assisted with recruitment, facilitated genetic testing of participants, and contributed to the manuscript. Joanne Fielding (6th author) provided intellectual input into the drafting of the manuscript. Julian Troller (7th author) assisted the design of the study, critical review and revision of the manuscript and interpretation of results. John Bradshaw (8th author) provided intellectual input into the drafting of the

manuscript and Jonathon Cohen (9th author) assisted with recruitment and contributed to drafts of the manuscript.

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Declaration for Thesis Chapter 4

Declaration by candidate

In the case of Chapter 4, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Project design, review of relevant literature, recruitment and testing of participants, analysis of data and writing of manuscript.	70%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Dr Darren R Hocking	Contributed to project design, critical review of manuscript	
E/Prof. John L. Bradshaw	Critical review of manuscript	
Dr Joanne Fielding	Critical review of manuscript	
Dr Jonathan Cohen	Critical review of manuscript	
Prof. Nellie Georgiou-Karistianis	Critical review of manuscript	
Prof. Kim. M. Cornish *	Contributed to project design, critical review of manuscript	

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	**Date** 15/2/14
Main Supervisor's Signature		**Date** 15/2/14

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

CHAPTER 4

POSTURAL CONTROL IN FEMALE CARRIERS OF FRAGILE X SYNDROME

4.1. Preamble to empirical paper 2: Cognitive-motor interference during postural control indicates at-risk cerebellar profiles in females with the *FMR1* premutation

The findings from Chapter 3 on gait control revealed significantly greater reductions in velocity and step length in female PM-carriers compared to controls, and an interaction between age and CGG-repeat length for dual-task related gait variability. This latter finding, which suggests CGG-dose dependent effects to gait automaticity during dual-task performance, is consistent with neuropathological involvement in subcortical systems, suggestive of a cerebellar origin (Boisgontier, et al., 2013; Wu, et al., 2013). Currently, the extent to which neuromotor profiles extend to postural control in female PM-carriers is unknown. Previous investigations of postural control in female PM-carriers have pointed towards mild difficulties in maintaining quiet stance with eyes closed (Narcisa, et al., 2011), and self-reported balance problems (Chonchaiya, et al., 2010). However, these studies were limited by a lack of systematic investigation into the effects of attentional load and sensory conflict on central processing systems important for maintaining postural stability. In adult women with the PM, the following chapter examines postural control in response to manipulation of visual and proprioceptive input, and during concurrent performance of an *excluded-letter-verbal-fluency* task. The same statistical approach from Chapter 3 will be adopted to explore both between group differences and inter-relationships between dual-task postural control and age- and CGG-repeat dependent changes.

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Research report

Cognitive-motor interference during postural control indicates at-risk cerebellar profiles in females with the *FMR1* premutation

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HIGHLIGHTS

- Female premutation carriers have a defective CGG trinucleotide expansion on the *FMR1* gene.
- This CGG expansion is associated with a high risk for ataxia motor symptoms.
- We showed enhanced dual-task postural displacement in asymptomatic female carriers.
- Dual-task postural displacement also associated with age- and CGG-length.
- Postural control paradigms may identify early cerebellar changes in female carriers.

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ABSTRACT

Recent investigations report a higher risk of motor symptoms in females with the *FMR1* premutation (PM-carriers) than has hitherto been appreciated. Here we examined basic sensorimotor and postural control under different sensory and attentional dual-task demands. Physiological performance and postural sway measures from the Physiological Profile Assessment (Lord et al., 2003 [39]) were conducted in 28 female PM-carriers (mean age: 41.32 ± 8.03) and 31 female controls with normal *FMR1* alleles (mean age: 41.61 ± 8.3). Multiple regression analyses were conducted to examine the moderating role of CGG-repeat length on the relation between age and postural sway under dual-task interference. In female PM-carriers, our results showed significantly poorer proprioceptive awareness, slower reaction time, and greater postural displacement when performing a concurrent verbal fluency task. Significantly, these findings showed age- and genetically-modulated changes in dual-task postural displacement in the medio-lateral direction in female PM-carriers. These findings highlight the sensitivity of postural control paradigms in identifying early cerebellar postural changes that may act as surrogate markers of future decline in female PM-carriers.

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1. Introduction

Fragile X syndrome (FXS) is a neurodevelopmental disorder resulting from transcriptional silencing of the *FMR1* gene on the X chromosome. This silencing is associated with a large expansion of a CGG trinucleotide repeat in the 5′ untranslated region and varying sizes of expansion can result in a continuum of fragile X-associated disorders. In FXS, there is a large expansion of the

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CGG repeat sequence (>200) and low production of the fragile X mental retardation protein (FMRP). Because FMRP is involved in normal brain development, through its impact on synaptic formation and function, reduced FMRP levels result in the characteristic intellectual impairment, autism and neurobehavioural profile associated with this condition [1,2]. In recent years, much research has focused on individuals who have a medium-sized CGG-expansion on their *FMR1* gene (CGG > 55–200), known as premutation carriers (PM-carriers). Emphasising the need to investigate any neurobehavioural consequences in PM-carriers, the population incidence of PM-carriers is high with an estimated 1 in 209 females and 1 in 430 males with the PM in North American populations [3].

Emerging evidence now indicates that the carrier status is not "phenotypic free" as previously assumed, but rather has clinical and behavioural phenotypes associated with an increased risk for the late onset of a neurodegenerative disorder, fragile X-associated tremor ataxia syndrome (FXTAS). FXTAS occurs in approximately 45% of male PM-carriers and 8–16% of female PM-carriers over the age of 50 [4,5]. FXTAS is associated with progressive dementia, intention tremor and ataxia alongside mood and global executive function deficits [4,6,7]. The protective effect of a normal allele on the second X chromosome in females can result in a milder clinical and pathological presentation than in male carriers. Female carriers may develop fragile X-associated primary ovarian insufficiency (FXPOI) a term encompassing irregular periods, fertility problems, elevated follicle stimulating hormone (FSH) and early menopause. Approximately 20% of female PM carriers will have premature menopause (<40 years) [8]. There are also increasing reports of neurodegenerative disorders including Parkinsonism and Alzheimer's disease [9–10]. Recent findings indicate that impairments in executive working memory, inhibitory control and visuospatial processing begin as early as middle adulthood, and progressively deteriorate with increasing age and CGG repeat size in male PM-carriers [11–15]. However, it remains unclear whether female PM-carriers show age-related neurocognitive impairments, resulting in neurodegenerative decline or a milder phenotype. Interestingly, recent investigations have identified attentional impairments, abnormal psychomotor ability, age- and genetically modulated visuospatial impairments, and greater deterioration with age in executive aspects of organisation and planning in female PM-carriers [16–21].

In addition to neurocognitive impairments in female *FMR1* premutation carriers, there is emerging behavioural and imaging evidence of dysfunction along neuromotor circuitry in both male and female PM-carriers (see [22] for a review). Although structural changes associated with reduced grey matter volume in cerebellum, cortical and subcortical areas are a prominent feature in FXTAS, recent studies have revealed cerebellar abnormalities in anterior vermis and middle cerebellar peduncles in male PM-carriers who are asymptomatic for FXTAS [23,24]. The anterior vermis, known to play a critical role in postural control [25,26], may represent the earliest changes marking the imminent onset of ataxia, a core feature of FXTAS. Behavioural studies, using the coordination tremor balance test system (CATSYS), a quantitative assessment of tremor and postural sway, have shown that ataxia can be detected in 30% of male PM-carriers who were not aware of any balance problems [27]. However, a limitation of the CATSYS is the lack of sensitivity to subtle changes in tremor (intention and postural) and postural sway in asymptomatic PM-carriers that may serve as surrogate markers of the imminent onset of neurodegenerative decline [19,28]. Significantly, daughters of men with FXTAS self-report more balance and memory problems when compared to carriers without a family history of FXTAS [18], and on the CATSYS female PM-carriers show higher postural sway with eyes closed when compared to controls, albeit the differences do not remain significant after correction for multiple comparisons [19]. There is also evidence from both male and female PM-carriers to show age- and CGG-dependent impairments on tasks that are sensitive to processing within dorsal stream networks (known to subserve visuomotor processing), alongside preserved performance on tasks sensitive to processing in ventral stream networks (known to subserve object recognition) [14,17,29]. Because the parietal dorsal stream and cerebellar networks have been implicated in 'internal models' which are important for maintaining and monitoring conceptualisations of our 'body parts in space' [30], maturational problems within these networks may lead to abnormalities in the development of the postural control system [31]. Taken together, these findings raise the possibility of a subtle neuromotor profile in female PM-carriers, which may indicate the earliest changes to vulnerable neural circuits, prior to the onset of more severe clinical decline.

However, postural control requires maintenance by higher order attentional networks and involves complex multisensory integration based on vestibular, visual and somatosensory input [30,32,33]. The attentional demands on postural control are commonly examined using the dual-task paradigm, which presumes that performing a secondary cognitive task during the regulation of postural control will compete for limited attentional resources between the two tasks [30,34]. Dual-task interference effects on postural control may depend on the nature and complexity of the cognitive or motor task, and individual differences in cognitive and sensorimotor control. Previous reports have shown that elderly individuals with Alzheimer's disease show greater dual-task effects on postural control when compared to older people without cognitive impairment [35], which may also be related to an increased risk for falls [36]. Given the emerging profile of subtle attentional control difficulties across several domains in female PM-carriers, dual-task paradigms may offer a sensitive approach for detecting early neuromotor changes which may indicate the onset of more severe neurodegenerative decline.

In this study, we explore for the first time postural control under different sensory and attentional demands through manipulation of visual, proprioceptive and cognitive input in young female PM-carriers. To assess physiological functions involved in postural stability, we employed a well-validated measure of physiological performance across different sensory and motor systems including vision, proprioception, muscle strength, reaction time and balance [37–39]. We examined postural sway (along both anterior-posterior and medio-lateral directions) during steady stance while manipulating proprioceptive and visual input in female PM-carriers when compared to age-matched controls with normal alleles. The impact of performing a secondary excluded-letter verbal fluency task [40,41] on postural sway was also explored to examine the interrelationships between weaknesses in inhibitory control, processing speed and postural stability in female PM-carriers. Further, we examined whether dual-task interference effects on postural control show age- and CGG-repeat dependent changes in a subset of at-risk females with the *FMR1* premutation.

2. Methods

2.1. Subjects

Female PM-carriers were recruited through support groups and population-based fragile X carrier screening studies. Female control participants were also recruited through population-based fragile X carrier screening studies, and through local networks and via online advertisements. All participants were English speaking and had normal (or corrected) vision and hearing. Participants were excluded if there was a history of epilepsy or of a serious head injury, a previous diagnosis of a neuropathy or fibromyalgia, or any sign of intellectual disability as assessed using the Wechsler Abbreviated Scale of Intelligence (FSIQ < 70). One PM-carrier tested positive as a full mutation after *FMR1* DNA testing and hence was excluded from the final analysis. The FXTAS Rating Scale [42] was used to screen all participants for features related to FXTAS—that is, tremor, ataxia or Parkinsonism—or any other neuromotor disorder. The final analysis included 28 female PM-carriers aged between

Table 1
Means and standard deviations for sample characteristics for female *FMR1* PM-carriers and control participants.

	FMR1 PM-carriers ($N = 28$) M ± SD (range)	Controls ($N = 31$) M ± SD (range)	*p*-value
Age (years)	41.32 ± 8.03 (22–53)	41.61 ± 8.3 (22–55)	.892
FSIQ	109.41 ± 11.16 (88–128)	113.16 ± 7.86 (89–129)	.151
VIQ	105.07 ± 14.38 (73–126)	109.74 ± 10.41 (88–136)	.159
PIQ	110.71 ± 10.78 (87–133)	114.29 ± 9.31 (93–133)	.177
Height (cm)	163.46 ± 6.91 (145–174.50)	166.48 ± 6.85 (151–181)	.100
Weight (kg)	73.71 ± 19.13 (48–129)	73.35 ± 15.11 (52–117)	.938
CGG-repeat length	84.79 ± 14.67 (61–122)	31.30 ± 3.14 (28–42)	<.001**

Abbreviations: FSIQ = Full Scale Intelligence Quotient; PIQ = Performance Intelligence Quotient; SD = Standard Deviation; VIQ = Verbal Intelligence Quotient.
**$p < .01$.

22 and 53 years of age (mean age: 41.32), and 31 female controls with normal *FMR1* alleles aged between 22 and 55 years of age (mean age: 41.61). Both groups were matched on age, IQ and anthropometric characteristics. The descriptive statistics are shown in Table 1. All study participants provided signed informed consent and the study procedures were consistent with the Declaration of Helsinki and approved by the Southern Health Ethics Committee (project 10147B).

2.2. Molecular analyses

DNA was extracted from 2 ml whole blood from all participants using the Promega Maxwell® 16 Instrument and associated Maxwell® 16 Blood DNA Purification Kit (Promega Cat No.: AS1010). PCR was performed using the Asuragen® AmplideX™ *FMR1* PCR Kit as this assay has been shown to detect a full range of fragile X expanded alleles [43]. PCR products were assessed via capillary electrophoresis on an Applied Biosytems 3130 Genetic Analyzer with electropherogram analysis conducted using GeneMapper® software. All procedures were performed in accordance with manufacturer's instructions.

2.3. Short-form Physiological Profile Assessment (PPA)

The short form PPA comprises validated measures of sensory and motor physiological function [39,44]. First, *visual contrast sensitivity* was assessed with the Melbourne Edge Test [45], an A4 page of 20 circles split with two different shades of grey and at varying angles. The contrast between the two shades was increasingly difficult to ascertain, the highest level of difficulty (lowest contrast) correctly assessed was taken for further analysis. To assess simple *reaction time* participants responded to a red light located on a computer mouse, using their dominant hand to rapidly click the mouse button (5 practise and 10 test responses). We selected mean response time for further analysis. *Proprioception*, the sensory awareness of body parts in space, was assessed with a lower limb matching task [46]. Participants were seated on a tall chair with their eyes closed and made five successive attempts at raising and matching their big toes on either side of a large protractor (See Fig. 1). The number of degrees between each big toe was recorded with the mean selected for further analysis. To examine *muscle force*, we gauged only knee flexion, where the participant sat on a tall chair and extended their dominant leg which had a Velcro strapped across the calf area and a weight attached. Of three trials, the greatest force was recorded (in kilograms).

We further assessed *postural sway* with a sway metre, which is a mechanical device that records postural displacement at the level of the waist (see Fig. 2). We recorded anterior-posterior (AP) and medio-lateral (ML) postural displacement in millimetres. The sway metre has been well validated with strong correlations with centre of pressure measures taken on a force plate [39,47]. For the present study, we employed an extended version of the original 30-s sway metre task and manipulated visual, proprioceptive and attentional input. Specifically, participants completed four 60-s single-task trials–standing on the floor with eyes open and eyes closed, standing on 15 cm thick high density foam to additionally reduce proprioceptive feedback with eyes open and eyes closed. For the postural sway dual-task, we examined the effect of a concurrent *excluded-letter-verbal-fluency* (ELVF) task which required generating as many words as possible within 60 s while inhibiting words containing a select vowel, proper nouns and repeats [48,49]. The first fluency task was completed at baseline (while seated) (words with no "A"), and then while balancing on the floor (words with no "E") and foam (words with no "I") with performance measured as the number of correctly enumerated words. All postural sway tasks were in order of increasing difficulty as per previous recommendations [39].

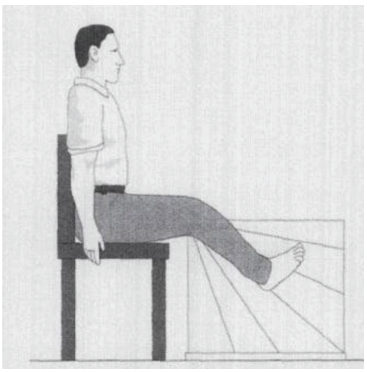

Fig. 1. Adapted from [39] with permission of the American Physical Therapy Association. Copyright © 2003 American Physical Therapy Association. All rights reserved.

2.4. Statistical analyses

Data were analysed using IBM SPSS Statistics 20.0. Normality was checked with the Shapiro–Wilk test and all outliers were adjusted to one value point above or below two standard deviations from the group mean [50]. Independent group *t*-tests were first used to compare group differences for the entire short-form PPA assessment and additional dual-task conditions. A Bonferroni correction was made for each sensorimotor condition ($p < .013$), while *p*-values for postural control tasks were corrected on the number of levels within single-task ($p < 013$) and dual-task ($p < .025$) conditions. Following this we performed a series of multiple linear regression with interaction terms, with each dual-task postural control condition selected as the primary criterion measure, and the predictor variables as age, CGG repeat length and their interaction. In accordance with previously established guidelines [51], CGG repeat and age were centred prior to regression analyses and multiplied together to represent the interaction. The assumptions of multiple regression (i.e., independence of residuals, normality of residuals, linearity, and homoscedasticity) were met for these analyses.

3. Results

All participants completed the physiological profile assessment (PPA) and dual-task conditions. Due to technical issues, which resulted in non-recording of results, performance data were only available on the excluded-letter-fluency-dual-task for 25 PM-carriers and 30 controls.

3.1. Sensorimotor function assessment

As evident in Table 2, between-group comparisons showed that when compared to controls, the PM-carrier cohort was comparable on measures of contrast sensitivity and leg strength, but showed

Table 2
Means and standard deviations for sensorimotor assessments for PM-carriers and control participants.

	FMR1 PM-carrier M ± SD	Control M ± SD	*p*-value
Contrast sensitivity	22.43 ± 1.26	22.06 ± 1.32	.283
Reaction time (milliseconds)	222.37 ± 34.26	200.77 ± 26.65	.009*
Proprioception (degrees)	.90 ± .52	.56 ± .47	.010*
Leg strength (kg)	20.68 ± 5.72	20.65 ± 5.60	.982

*$p < .013$.

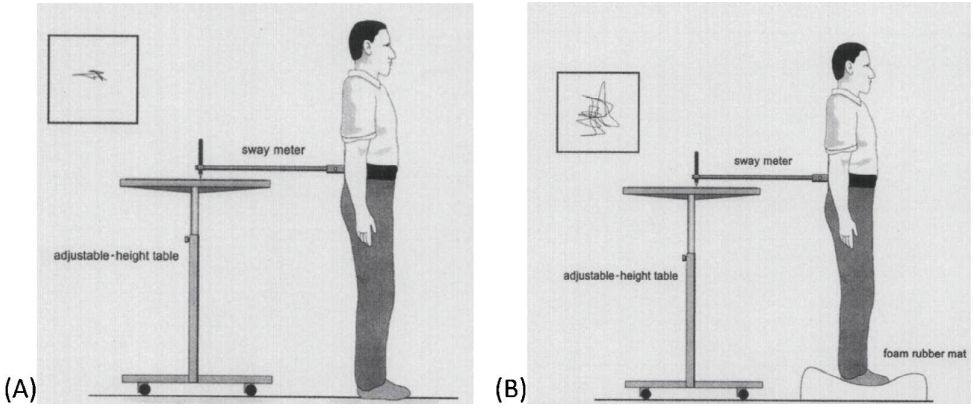

Fig. 2. The sway metre task from the short form PPA consisted of a waist high 40 cm-long-rod attached to a vertically mounted pen. This pen was placed on graph paper and postural displacement in both anterior posterior and medio-lateral directions was taken for further analyses (as indexed by movement of the pen). We examined postural displacement during stance on the floor (A) and stance on a high-density foam surface (B). Adapted from [39] with permission of the American Physical Therapy Association. Copyright © 2003 American Physical Therapy Association. All rights reserved.

slower motor reaction time ($p = .009$) and worse proprioceptive awareness ($p = .010$).

3.2. Performance of the verbal fluency task

PM-carriers performed significantly worse than controls on all three versions of the verbal fluency task (baseline: $t(57) = -4.066$, $p = .001$; verbal fluency-task standing on floor: $t(56) = -2.723$, $p = .009$; verbal fluency-task standing on foam: $t(53) = -2.435$, $p = .018$).

3.3. Balance performance

As shown in Table 3, PM-carriers showed significantly increased postural displacement when performing the concurrent verbal fluency task and standing on foam and on the floor when compared to controls. The higher postural sway in female PM-carriers was evident for both AP ($p = .012$) and ML ($p = .005$) directions relative to controls when standing on foam, and in the ML direction only when standing on the floor ($p = .024$). As shown in Fig. 3, the control group showed improved postural performance in AP and ML directions for all dual-task conditions. Alternatively, PM-carriers showed improved postural performance in the AP direction for both dual-task conditions, however poorer performance was indicated by increased displacement in the ML direction during stance on the foam surface.

3.4. Associations with CGG-repeat length and age

We employed regression analyses to examine the moderating role of CGG-repeat length on the relation between age and dual-task effects on postural control (see Table 4).

When standing on the floor, age showed a negative association with increased AP displacement during the verbal fluency dual-task condition ($p = .020$). There was also a significant positive association between CGG repeat length and ML displacement during the secondary task ($p = .029$).

For the standing on foam condition, there were no associations between age or CGG-repeat repeat and postural displacement in AP or ML directions. As shown in Table 4, there was a significant interaction between age and CGG repeat length for postural ML displacement (with eyes open) during the secondary verbal fluency task. This shows that CGG repeat length moderates the relationship between age and greater postural instability for ML displacement during dual-task performance in PM-carriers. For baseline verbal fluency, basic sensory and motor functions, there were no associations with age or interactions between age and CGG repeat length (please refer to supplementary Table e-1).

4. Discussion

This study is the first to examine postural control during concurrent manipulation of visual, proprioceptive and attentional demands in young female PM-carriers. Our findings indicate core impairments associated with dual-task interference effects on postural control in females with the *FMR1* premutation. The findings also emphasise the utility of dual-task paradigms in identifying age- and genetically modulated changes in postural control which may be associated with at-risk profiles in female PM-carriers. These results extend previous studies which have employed self-report and standardised assessments of postural control in female PM-carriers [18,19], to include a more sensitive experimental approach to dual-task effects on complex multisensory integration and postural stability. Although future longitudinal studies will be critical in distinguishing between neurodegenerative and neurodevelopmental processes, these findings suggest that cerebellar networks underlying postural control may be especially vulnerable to the effects of the *FMR1* premutation.

Our results showing significantly increased dual-task effects on postural sway in female PM-carriers compared to controls are consistent with capacity interference caused by competition for limited attentional resources [30,52,53]. The contrasting pattern of findings in the control group, who showed improved postural displacement during performance of a concurrent verbal fluency task, is consistent with the contention that secondary cognitive tasks with low attentional demands may direct attention away from balance control, and shift postural performance to a more automatic state [52,54]. In contrast, the poorer performance on verbal fluency and postural sway in female PM-carriers suggests that reduced attentional (inhibitory) control could result in performance that is closer to their stability boundaries. This interpretation is consistent with the pattern seen in patients with Parkinson's disease, where poor performance on secondary cognitive tasks typically compounds already compromised cognitive and postural control systems [55]. Consistent with this interpretation, we show that even though female PM-carriers show intact postural control during multisensory integration, the dual-task effects on increased medio-lateral sway may only become apparent under increasing demands on cognitive control. Given that early disturbances of executive control associated with postural instability may predict increased risk of

Table 3
Means and standard deviations for postural control displacement in millimetres (mm) with sensory and cognitive interference manipulated for *FMR1* PM-carriers and controls.

	FMR1 PM-carrier M ± SD	Control M ± SD	*p*-value
Postural sway in mm			
Single-task conditions			
Eyes open on floor AP	20.46 ± 7.32	19.44 ± 5.52	.542
Eyes open on floor ML	19.38 ± 13.15	19.07 ± 11.91	.925
Eyes closed on floor AP	22.00 ± 7.76	21.90 ± 6.96	.960
Eyes closed on floor ML	14.41 ± 10.11	14.05 ± 9.41	.887
Eyes open on foam AP	33.43 ± 14.47	29.61 ± 9.00	.224
Eyes open on foam ML	27.70 ± 16.53	25.00 ± 11.93	.472
Eyes closed on foam AP	44.71 ± 9.35	42.23 ± 13.74	.425
Eyes closed on foam ML	42.89 ± 24.40	37.81 ± 15.51	.339
Dual-task conditions			
Eyes open dual-task floor AP	19.46 ± 7.16	19.13 ± 6.34	.849
Eyes open dual-task floor ML	19.46 ± 11.77	13.48 ± 7.04	.024*
Eyes open dual- task foam AP	33.27 ± 11.14	26.86 ± 7.05	.012*
Eyes open dual-task foam ML	33.32 ± 18.97	21.37 ± 11.63	.005**

Abbreviations: AP = Anterior-posterior direction; *FMR1* = Fragile X Mental Retardation 1 gene; ML = Medio-lateral direction.
*$p < .025$, **$p < .01$.

falls, restricted mobility and progression to dementia [36,56], the increased interference of an attention-demanding task on postural control in female PM-carriers may represent the earliest indicator of the onset of more severe age-related cognitive and motor decline.

In terms of the neural basis for dual-task effects on postural control in female PM-carriers, existing imaging studies suggest that cortio-cerebellar pathways which functionally connect with higher level cognitive networks are disrupted in male PM-carriers with and without FXTAS [23,24,57,58]. Previous imaging studies

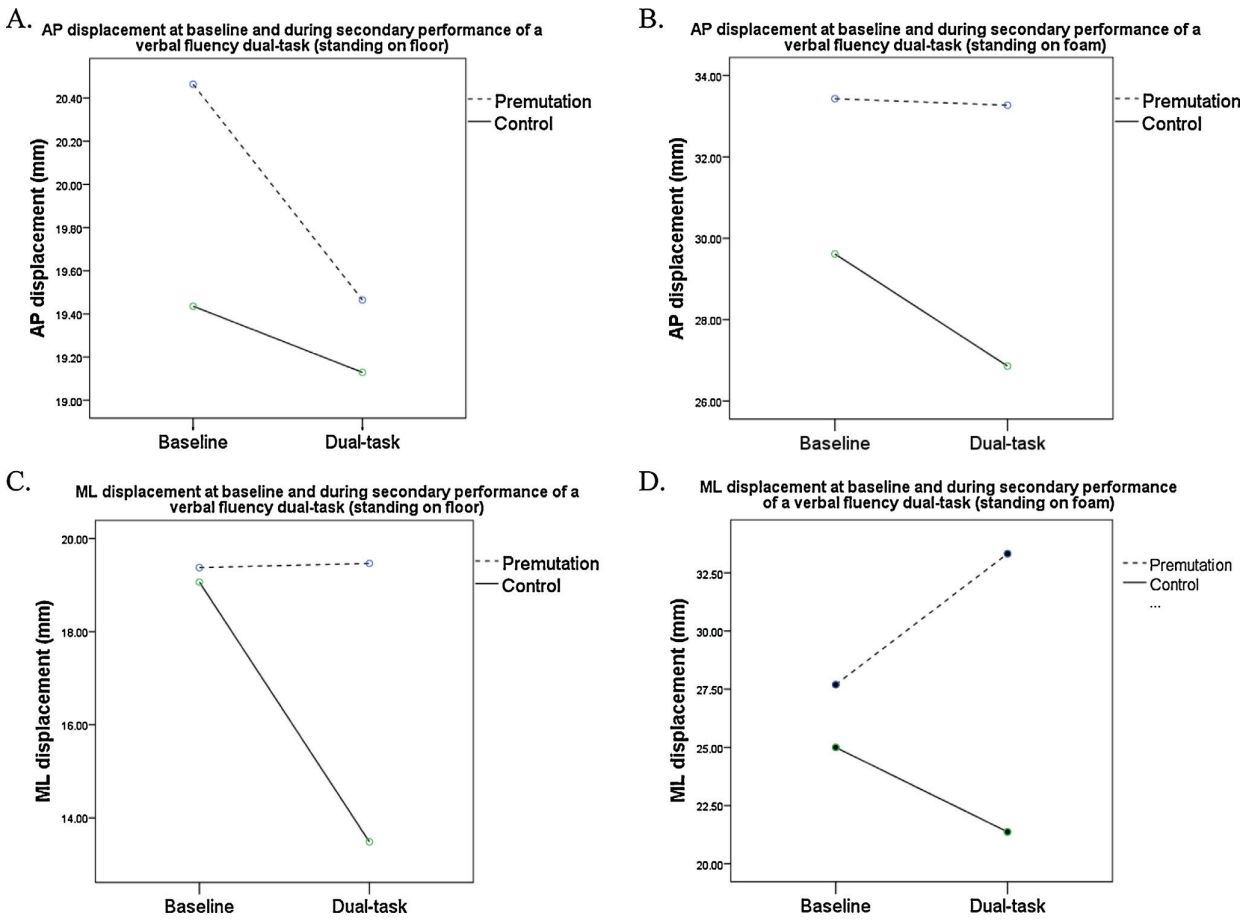

Fig. 3. Postural control displacement in AP direction at baseline and under dual-task performance while standing on the floor with eyes open (A) and on foam with eyes open (B) and postural control displacement in ML direction at baseline and under dual-task performance while standing on the floor with eyes open (C) and on foam with eyes open (D) for PM-carriers and control participants. These graphs show that for AP spatial displacement, both controls and PM-carriers showed maintenance or even improved performance in the dual-task condition. In the ML direction controls showed maintenance or improvement of performance when the dual-task was added, and by contrast, PM-carriers showed an increase in postural displacement reflective of a worsening in performance.

Table 4
Standardised β and *t* values for the continuous moderator variable of CGG repeat length in the moderator regression analyses for the relation between age and postural displacement under cognitive interference for PM-carriers.

	On floor				On foam			
	Eyes open AP		Eyes open ML		Eyes open AP		Eyes open ML	
	Sβ	*t*	Sβ	*t*	Sβ	*t*	Sβ	*t*
Constant		2.650		.331		1.358		.590
CGG length	.328	1.744	.433	2.330*	.129	.617	.349	1.924
Age	−.470	−2.498*	−.256	−1.377	.066	.315	−.209	−1.150
ageXCGG	−.010	−.057	.222	1.251	.161	.806	.376	2.173*
R^2		.234		.254		.053		.289*
Adjusted R^2		.138		.161		-.065		.201*

Abbreviations: AP = Anterior-posterior direction; ML = Medio-lateral direction; Sβ = Standardised Beta; *t* = test statistic.
*$p < .05$.

in asymptomatic *FMR1* premutation carriers show white matter alterations (demyelination and axonal damage) of the afferent projections of middle cerebellar peduncles (MCPs) and superior cerebellar peduncles [22,57,58]. These may be the earliest neuroanatomical markers of the onset of cognitive and motor symptoms associated with FXTAS. The MCPs include fronto-cerebellar tracts connecting to orbitofrontal and dorsolateral prefrontal cortices that are critical for cognitive control [59]. Moreover, a recent Transcranial Magnetic Stimulation (TMS) study has detected an absence of cerebellar inhibition over the primary motor cortex alongside deficient GABAergic mechanisms (reduced GABA-A mediated intracortical inhibition) in young female PM-carriers [60]. These alterations to cerebellar-cortico and cerebello-motor connectivity may well disrupt extensive brain networks subserving dual motor and cognitive performance in female PM-carriers. Indeed, network connectivity studies have shown that the cerebellum is critical for functional connectivity within cognitive- and motor-related regions necessary for efficient cognitive control during dual-task performance [61]. Together, these findings imply that cerebellar abnormalities may disrupt the integration and efficiency of widespread cognitive and motor networks critical for stable postural control in female PM-carriers.

The current findings on measures of sensory and motor physiological function suggest that female PM-carriers exhibit slowed psychomotor speed and reduced proprioceptive awareness but with no evidence for age-related decline. The presence of slowed motor reaction time is inconsistent with previous studies which have reported enhanced basic psychomotor speed in female PM-carriers [16,62]. One possible explanation for the discrepancy between these studies and our findings could be the higher mean age and range (mean age 41.32; range: 22–53) of the female-PM carriers in the current study. Consistent with this, simple reaction time to an auditory stimulus and finger tapping has been reported as being significantly slower in older (mean age 52.86) female PM-carriers without FXTAS [19]. Taken together with the findings of poorer proprioceptive awareness in female PM-carriers, the subtle proprioceptive and motor coordination deficits are consistent with disrupted cerebellar functioning. Proprioceptive information, which informs our sense of self-position and movement [63], is integrated within lower levels of the spinal cord and the cerebellum, where internal models for guiding movement are developed and regulated [64]. Significantly, although the motor coordination deficits are consistent with previous imaging studies showing cerebellar abnormalities in PM-carriers [24,58], the poor integration of proprioceptive sensory input may well be associated with the previously reported peripheral and sensory neuropathies in female PM-carriers [65]. Given the lack of association with age and CGG repeat length or their interaction, we interpret the impairments in low level sensorimotor integration as reflecting likely neurodevelopmental changes that may present early in the developmental trajectory, possibly causing lifelong alterations to sensory and motor networks.

Our results showing age- and genetically modulated cognitive and postural dual-task impairments suggest distinct molecular events operate in female PM-carriers with expanded repeats. This is consistent with previous studies which have indicated increased CGG-repeat vulnerability along neuromotor circuitry in female premutation knock-in (KI) mouse models [66,67]. Furthermore, it highlights molecular evidence showing that increased levels of *FMR1* mRNA in high CGG repeat carriers is associated with moderately reduced FMRP levels presumably due to a deficit in translational efficiency [68,69]. Although the *FMR1* mRNA elevations are the purported cause of FXTAS neuropathology [70], the RNA-binding protein FMRP, which is essential for synaptic maturation and neuronal plasticity, has been proposed to underlie a range of developmental cognitive problems associated with the *FMR1* premutation [71–74]. In female PM-carriers, the diluting effect of X-inactivation skews the positive correlation between CGG repeat length and total *FMR1* mRNA levels [75]. In terms of risk of mild cognitive impairment, it has been shown that with increasing CGG repeat size (CGG >100) and reduced FMRP expression (<60% in hair roots), there is an increased decline in IQ scores in female *FMR1* premutation carriers [76], and hypermethylation of *FMR1* intron sites has been shown to predict verbal cognitive impairment [77]. Thus, we speculate that the present findings of enhanced sensitivity to attentional demands on postural control in female PM-carriers may not only be associated with *FMR1* mRNA toxicity, but may also reflect greater neural susceptibility of cerebellar networks to the differential expression of the *FMR1* protein.

The use of a cross-sectional design is a limitation of the present investigation and longitudinal imaging studies examining structural and functional connectivity will be needed to identify cerebellar profiles that differentiate between neurodegenerative processes and subtle neurodevelopmental changes. Furthermore, it is not yet clear if these sensorimotor abnormalities and dual-task costs in postural control would show greater age-related decline in male PM-carriers with an increased risk for FXTAS. Future studies should manipulate attentional load across both cognitive and motor tasks to provide a more sensitive marker of age-related decline than otherwise detectable using gross neuropsychological measures in female PM-carriers. Finally, given that methylation patterns and X-inactivation levels have been shown to skew relationships between CGG repeat length and both FMRP and *FMR1* mRNA levels [78], further investigations of epigenetic markers and the molecular events downstream of the CGG repeat expansion on the *FMR1* allele are warranted.

In conclusion, the current findings highlight the sensitivity of dual-task postural control paradigms in examining the attentional demands on postural sway in young female PM-carriers. We contend that the cerebellar motor and cognitive networks are

implicated in postural control difficulties, possibly as a result of *FMR1* mRNA toxicity and/or differential expression of the *FMR1* protein. The lack of an age-related decline in verbal fluency and low-level sensory and motor physiological function may well reflect a stable developmental phenotype in female PM-carriers. Prospective longitudinal studies will be needed to evaluate the utility of dual-task postural paradigms in detecting subtle age-related decline associated with the onset of degenerative processes or more stable developmental changes. These investigations will be critical for the development of surrogate markers in both male and female PM-carriers for future use in therapeutic trials for FXS-associated disorders.

Author contributions

Claudine Kraan, Darren Hocking and Kim Cornish (first, second and last author) conceptualised and designed the study, provided intellectual input into the interpretation of the data and co-wrote the first draft of the manuscript. Nellie Georgiou-Karistianis (3rd author) provided intellectual input into the interpretation of the data, as well as input into drafts of the manuscript. Sylvia Metcalfe and Alison Archibald (4th and 5th authors) contributed to study design, assisted with recruitment, facilitated genetic testing of participants, and contributed to the manuscript. Joanne Fielding (6th author) provided intellectual input into the drafting of the manuscript. Julian Troller (7th author) assisted the design of the study, critical review and revision of the manuscript and interpretation of results. John Bradshaw (8th author) provided intellectual input into the drafting of the manuscript and Jonathon Cohen (9th author) assisted with recruitment and contributed to drafts of the manuscript.

Disclosures

The authors report no disclosures.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2013.07.033.

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Glossary

FMR1 gene: Fragile X mental retardation 1 gene
FMRP: Fragile X Mental Retardation Protein
FXTAS: Fragile X Tremor Ataxia Syndrome
PM-carrier: Premutation carrier

Declaration for Thesis Chapter 5

Declaration by candidate

In the case of Chapter 5, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Project design, review of relevant literature, recruitment and testing of participants, analysis of data and writing of manuscript.	70%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Dr Darren R Hocking	Contributed to project design, critical review of manuscript	
E/Prof. John L. Bradshaw	Critical review of manuscript	
Dr Joanne Fielding	Critical review of manuscript	
Dr Jonathan Cohen	Critical review of manuscript	
Prof. Nellie Georgiou-Karistianis	Critical review of manuscript	
Prof. Kim. M. Cornish *	Contributed to project design, critical review of manuscript	

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	**Date** 15/2/14
Main Supervisor's Signature	[redacted]	**Date** 15/2/14

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

CHAPTER 5

EXECUTIVE FUNCTION AND PSYCHOLOGICAL SYMPTOMS IN FEMALE CARRIERS OF FRAGILE X SYNDROME

5.1. Preamble to empirical paper 3: Impaired response inhibition is associated with self-reported symptoms of depression, anxiety, and ADHD in female *FMR1* premutation carriers.

The published review (Chapter 1) has raised the possibility that female PM-carriers have a core executive function deficit. Following submission of the review for publication, several studies have directly addressed this question. Specifically, in a cohort of older female PM-carriers, executive function deficits were revealed using event related potential (ERP) techniques and performance on the BDS (Yang, et al., 2013). Further, an examination of language dysfluencies in the female PM has shown significant age-related impairments of organisation and planning, suggestive of executive dysfunction (Sterling, Mailick, Greenberg, Warren, & Brady, 2013). However, these studies were limited by an over-reliance on gross neuropsychological tests that measure multiple subcomponents of executive function. Previous investigations that have isolated specific components of executive function have revealed response inhibition and working memory deficits in the male PM starting as early as the third decade (Cornish, et al., 2011; Cornish, et al., 2009; Cornish, Li, et al., 2008). This profile may also be present in female PM-carriers. The following published empirical paper examined whether distinct component processes such as response inhibition and working memory are associated with self-reported symptoms of depression, social anxiety and ADHD. Due to ongoing recruitment, this paper comprised a larger cohort than what was available for neuromotor function in Chapter's 3 and 4.

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

Impaired Response Inhibition Is Associated With Self-Reported Symptoms of Depression, Anxiety, and ADHD in Female *FMR1* Premutation Carriers

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***Fragile X Mental Retardation 1* (*FMR1*) premutation carriers (PM-carriers) have a defective trinucleotide expansion on the *FMR1* gene that is associated with continuum of neuropsychological and mental disorders. Currently, little is known about the distinct subcomponents of executive function potentially impaired in female PM-carriers, and there have been no investigations into associations between executive function and incidences of mental disorders. A total of 35 female PM-carriers confirmed by Asuragen triple primed PCR DNA testing and 35 age- and intelligence-matched controls completed tests of executive function (i.e., response inhibition and working memory) and self-reported on social anxiety, depression, and ADHD predominantly inattentive (ADHD-PI) symptoms. Compared to controls, PM-carriers were significantly elevated on self-reported social anxiety and ADHD-PI symptoms. Irrespective of mental symptoms, female PM-carries performed significantly worse than controls on a response inhibition test, and further investigations revealed significant correlations between executive function performance and self-reported symptoms of anxiety, depression and ADHD-PI. Critically, among PM-carriers with good executive function performance, no women exceeded threshold markers for probable caseness of mental disorder. However, rates of probable caseness were elevated in those with average performance (response inhibition: social anxiety: 41.7%;**

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Abbreviations: *FMR1* gene, Fragile X Mental Retardation 1 gene; FMRP, Fragile X Mental Retardation Protein; FXTAS, fragile x-associated tremor/ataxia syndrome; PM-carrier, premutation carrier.

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depression: 20%; ADHD: 44.4%; working memory: social anxiety: 27.3%; depression: 9.1%; ADHD: 18.2%) and highly elevated for those with poor executive function performance (response inhibition: social anxiety: 58.3%; depression: 80%; ADHD: 55.6%; working memory: social anxiety: 100%; depression: 50%; ADHD: 83.3%). These data suggest that subtle executive dysfunction may be a useful neuropsychological indicator for a range of mental disorders previously reported in female PM-carriers. © 2013 Wiley Periodicals, Inc.

Key words: Fragile X Mental Retardation Protein (FMRP); fragile x-associated tremor/ataxia syndrome (FXTAS); attention deficit-hyperactivity disorder (ADHD); depression; anxiety; executive function; premutation; Fragile X Mental Retardation 1 (FMR1)

INTRODUCTION

The *fragile X mental retardation 1* (*FMR1*) gene contains a trinucleotide expansion of a CGG repeat sequence in the 5′ untranslated region at the Xq27.3 locus. This gene can be defective and is associated with a continuum of neurodevelopmental, neurodegenerative, and neuropsychological involvement [Jacquemont et al., 2011]. At full expansions of >200 CGG repeats, epigenetic mechanisms "silence" transcription of the *FMR1* allele, and through low production of the Fragile X Mental Retardation Protein (FMRP), this leads to the neurodevelopmental delay and autistic features that are characteristic of fragile X syndrome (FXS) [Cornish et al., 2008a]. In recent years, much research has focused on premutation (PM) expansions of between 55 and 200 CGG-repeats, which can lead to variable, gender-specific phenotypes. For male PM-carriers, research suggests a subtle FXS "signature" of weaknesses in executive function, visuospatial processing and attentional control [Cornish et al., 2005, 2011; Hocking et al., 2012]. In contrast, female PM-carriers show evidence for anxious and depressive symptomatology, subtle problems in executive function, and mild difficulties with mathematical reasoning and inattention [Loesch et al., 2003a; Lachiewicz et al., 2006; Roberts et al., 2009; Hunter et al., 2012a]. However, the presence of a female phenotype is controversial, and there are gaps in our understanding of the executive function deficits that may be associated with the female *FMR1* premutation. Although protective effects from the second X chromosome result in a milder phenotype [Leehey et al., 2008], mental disorders in females may be more prevalent than in male PM-carriers [Hunter et al., 2010], and this emphasizes the importance of identifying neuropsychological indicators of at-risk profiles in female PM-carriers.

Existing studies have shown an age-related deterioration in cognitive, motor and anxiety symptoms [Adams et al., 2010; Narcisa et al., 2011; Sterling et al., 2013], as well as impairments on frontally mediated tasks of processing speed and executive function in female PM-carriers [Yang et al., 2013]. These clinical and behavioral phenotypes may be associated with an increased risk for neurodegenerative decline associated with fragile X-associated tremor/ataxia syndrome (FXTAS). Specifically, approximately 8–16% of females (and 45% males) over the age of 50 go on to develop FXTAS, which is primarily characterized by ataxia and intention tremor, dementia, mood, and global executive function deficits [Jacquemont et al., 2003; Berry-Kravis et al., 2007; Rodriguez-Revenga et al., 2009]. There are also cellular and volumetric changes in FXTAS patients, including the presence of intranuclear inclusion bodies, and widespread cortical and cerebellar structural abnormalities [Greco et al., 2002, 2006; Hashimoto et al., 2011b,c]. Despite the lower prevalence and penetrance of FXTAS in females, there is evidence from post-mortem examinations of an increased risk for *FMR1*-dependent neuro-pathological changes (amyloid plaques and intranuclear inclusion bodies) in older female PM-carriers [Tassone et al., 2012]. Females may also develop fragile X primary ovarian insufficiency (FXPOI), which encompasses irregular menses, fertility problems, hormonal changes and a 20% risk for premature menopause [Sherman, 2000; Wittenberger et al., 2007]. These clinical consequences are thought to result from mRNA gain-of-function toxicity, where over-expression of expanded mRNA transcripts leads to sequestration of RNA binding proteins from their pre-determined role [Tassone et al., 2000; Hagerman et al., 2001; Sellier et al., 2013]. Together these studies indicate synergistic effects of increasing age and *FMR1* molecular events in female PM-carriers well before the presence of more severe age-related decline.

The presence of a cognitive phenotype in adult female PM-carriers is controversial, with standardized neuropsychological tests revealing no evidence for global cognitive decline [Hunter et al., 2008b, 2009; Allen et al., 2011]. For example, when Hunter et al. [2008b] examined performance on standardized tasks of executive function in a large cohort of male and female PM-carriers aged 18–50 years old, PM-carriers performed similarly to controls on all measures. However, a limitation of these previous studies has been the over-reliance on gross neuropsychological tests that tap into multiple cognitive constructs. It is well established that executive function is a multifactorial construct that can be fractionated into separable but not completely independent component processes—that is, mental set-shifting, working memory, and inhibition of prepotent thoughts and behaviors [Miyake et al., 2000; Miyake and Friedman, 2012]. These component processes, which guide and maintain cognitive control- and goal-directed behaviors, are subserved by complex functional links between prefrontal, basal ganglia, and cerebellar neural circuits [Heyder et al., 2004]. Thus impairments in one or more of these component processes may not severely disrupt gross executive functioning [Chan et al., 2008], yet when teased apart they may reveal subtle cognitive profiles that represent the earliest changes in vulnerable neural networks [Christodoulou et al., 2012]. For example, Cornish et al. [2011] showed that in male PM-carriers there were subtle deficits in inhibitory control and executive working memory, beginning as early as middle adulthood, and progressively deteriorating with increasing age and CGG repeat size. There is only limited evidence for a profile of poor executive functioning in adult female PM-carriers, which has revealed specific difficulties in mathematical reasoning and self-regulation of behavior and attention [Loesch et al., 2003a; Steyaert et al., 2003; Lachiewicz et al., 2006]. More recent studies in older female PM-carriers without FXTAS have shown age-related decline in linguistic aspects of organization and planning [Sterling et al., 2013], and frontally mediated impairments in inhibition, working memory, and performance monitoring [Yang et al., 2013]. Given

that the majority of recent studies have been conducted in older PM-carriers, to date little is known about how early changes in specific subcomponents of executive function are associated with other aspects of the distinctive female phenotype.

In addition to emerging evidence for a subtle profile of executive dysfunction, female PM-carriers may suffer from a range of mental disorders including anxiety, depression, and attention deficit-hyperactivity disorder (ADHD) [Franke et al., 1998; Johnston et al., 2001; Roberts et al., 2009; Hunter et al., 2010, 2012a]. Indeed, extant studies have shown that executive dysfunction is frequently comorbid with an ADHD diagnosis [Barkley, 1997; Willcutt et al., 2005]. This may result from a common dysfunction of fronto-subcortical-cerebellar pathways or alterations to transmission in the prefrontal cortex system [Seamans and Yang, 2004; Faraone, 2005]. There is also accumulating evidence to suggest that executive dysfunction is a prevailing characteristic of both anxiety and depression [Ottowitz et al., 2002; Micco et al., 2009; Hosenbocus and Chahal, 2012]. Furthermore, executive function is associated with performance on experimentally based neuropsychological investigations of emotional regulation [Schmeichel et al., 2008; Gyurak et al., 2009] and physiological measures of acute stress reactivity [Hendrawan et al., 2012]. It has been suggested that a disruption of "frontally located" executive functions may actively reduce the inhibition of more posterior emotion systems, resulting in overactive transmission within amygdala and hippocampal regions underlying anxiety and depressive symptoms [Galynker et al., 1998; Ohta et al., 2008; Ray and Zald, 2012]. Taken together with previous evidence for impairments in frontal executive functions in older female-PM carriers [Hashimoto et al., 2011a; Yang et al., 2013], these findings raise the possibility that poorly regulated frontal activation may be concomitantly associated with subtle executive dysfunction and symptoms of mental disorders. Furthermore, in light of recent data showing a later than expected age-of-onset for psychiatric problems in PM-carriers [Seritan et al., 2013], these suggested cognitive and psychiatric changes may well reflect the first signs of more severe age-related neurological change.

In the current study, we investigated for the first time whether distinct subcomponents of executive function are impaired and, if so, whether executive function deficits are associated with psychological symptoms in adult females with the *FMR1* premutation. In line with our previous investigations of the executive function profile in male PM-carriers [Cornish et al., 2008b, 2009, 2011], we hypothesized that female PM-carriers would show subtle deficits in the areas of inhibition and working memory, resembling in a milder form the executive profile in male PM-carriers. Furthermore, in light of the high rates of behavioral and emotional profiles in female PM-carriers, we expected that specific subcomponents of executive function would be associated with symptoms of ADHD, anxiety, and depression.

METHOD

Study Participants

A total of 35 female PM-carriers aged between 22 and 55 were recruited through support groups and population-based fragile X carrier screening studies [Metcalfe et al., 2008]. Female control participants (n = 35) aged between 22 and 55 years old were also recruited through population-based fragile X carrier screening studies, and through local networks and via online advertisements. All participants were English speaking with no history of epilepsy or of a serious head injury and had normal (or corrected) vision and hearing, and no sign of color blindness or intellectual disability as assessed using the Wechsler Abbreviated Scale of Intelligence (FSIQ < 70). The female PM-carriers and controls were well-matched on age, IQ, socioeconomic status, and current use of psychotropic medication(s), but female PM-carriers were significantly more likely to be caring for a child with special needs ($P \leq 0.001$). The small portion of medicated women in this study were taking anti-depressants, with two female PM-carriers additionally taking anti-psychotic medications and one PM-carrier additionally taking a stimulant. All study participants provided signed informed consent and the study procedures were consistent with the Declaration of Helsinki and approved by the Southern Health Ethics Committee (project 10147B). The descriptive information and statistics are shown in Table I.

TABLE I. Means and Standard Deviations of Sample Characteristics for Female *FMR1* PM-Carriers and Control Participants

	***FMR1* PM-carriers (N = 35)**	**Control (N = 35)**	
	M ± SD (range)	**M ± SD (range)**	***P*-value**
Age (years)	41.14 ± 8.34 (22–55)	41.11 ± 8.64 (22–55)	0.989
FSIQ	110.26 ± 10.94 (88–128)	111.91 ± 9.54 (79–129)	0.506
VIQ	106.59 ± 13.90 (73–128)	108.11 ± 11.36 (84–136)	0.619
PIQ	110.86 ± 11.34 (87–133)	113.89 ± 11.15 (79–133)	0.260
Socioeconomic disadvantage (% < AUD $51,999)	8.6%	14.3%	0.869[a]
Use of psychotropic medications (% yes)	14.3%	5.7%	0.232[a]
Child special needs (% yes)	65.7%	17.1%	<0.001[a,**]
CGG-repeat length	86 ± 15.07 (59–122)	26 ± 3.52 (20–42)	<0.001[**]

FSIQ, Full Scale Intelligence Quotient; PIQ, Performance Intelligence Quotient; SD, standard deviation; VIQ, Verbal Intelligence Quotient.
[a]Chi square test for independence.
**$P < 0.01$.

Molecular Analyses

To ascertain CGG repeat size, DNA was extracted from 2 ml whole blood from all participants using the Promega Maxwell® 16 Instrument and associated Maxwell® 16 Blood DNA Purification Kit (Promega, Maddison, WI; Cat No.: AS1010). PCR was performed using the Asuragen® AmplideX FMR1 PCR Kit as this assay has been shown to detect a full range of fragile X expanded alleles [Chen et al., 2010]. PCR products were assessed via capillary electrophoresis on an Applied Biosytems 3130 Genetic Analyzer (Life Technologies, Carlsbad, CA) with electropherogram analysis conducted using GeneMapper® software. All procedures were performed in accordance with manufacturer's instructions.

MEASURES

Neuropsychological Tests

The following tests of executive function were selected on the basis of the age- and CGG-repeat vulnerability profile in male PM-carriers [Cornish et al., 2011]: Hayling Sentence Completion Test, Stroop Color and Word Test, and Letter-Number Sequencing.

Hayling Sentence Completion Test. The *Hayling Test* [Burgess and Shallice, 1997] involves a sentence completion task in which participants firstly have to complete sentences with a related or connected word that is meaningful to the sentence (Section A), and secondly (Section B) complete sentences with words that are unconnected to the sentence. The latter requires inhibiting a prepotent response that has been previously established. The second section (i.e., unconnected completion) of the Hayling Test was scored for Category A (connected) and Category B (somewhat connected) errors, yielding a converted A + B error score. A higher score indicated a greater number of errors and worse performance.

Stroop Color and Word Test [Golden and Freshwater, 2002]. The Stroop is based on the principle that a participant must respond to the color of the word whilst selectively inhibiting the more automatic response of reading the printed word. Performance was determined by the number of correctly named words within a 45 sec timeframe and the calculated Stroop Interference score, in which a negative value indicated an interference effect, was taken for further analyses.

Letter-Number Sequencing (LNS) Test. The LNS is a subtest from the Wechsler Adult Intelligence Scale-IV (WAIS-IV) and is designed to assess the participant's ability to temporarily store and manipulate information. Participants listened to a combination of letters and numbers of increasing lengths, and were required to repeat each letter-number sequence, however with the numbers in ascending order followed by the letters in alphabetical order. The scores from the LNS were calculated after three consecutive trial failures on a given level, with the total score calculated by adding all correct items. LNS scores 1 SD below the group mean were indicative of poor performance.

Behavioral Measures of Psychological Symptoms

The following self-report questionnaires were selected on the basis of their sensitivity to psychological symptoms previously reported in female PM-carriers [Hunter et al., 2010]: The self-report Liebowitz Social Anxiety Scale (SR-LSAS), Depression Anxiety Stress Scales (DASS), and Brown Attention Deficit Disorder Scales (Brown ADD).

The SR-LSAS is a widely used 24-item Likert-scale self-report questionnaire that measures the impact of *social anxiety* symptoms in the preceding week across a range of social situations adapted from day-to-day life. Based on a clinician's administered Liebowitz Social Anxiety Scale (CA-LSAS), the SR-LSAS has excellent psychometric properties including high internal consistency (0.94), high convergent validity with the CA-LSAS, and discriminant validity for social anxiety [Liebowitz, 1987; Fresco et al., 2001; Rytwinski et al., 2009]. Caseness for social anxiety is indicated by a score >50 and can be classified as moderate (50–65), marked (65–80), severe (80–95), or extremely severe (>95).

To assess symptoms of *depression* we administered the DASS [Lovibond and Lovibond, 1995], a 42 item self-report questionnaire that measures the frequency and severity of anxiety, depression, and stress experienced during the preceding week. The psychometric properties of the DASS are good, with high internal consistency for the subscales depression (0.96), anxiety (0.92), and stress (0.95) alongside adequate reliability, and excellent convergent validity and discriminant validity [Crawford and Henry, 2003; Page et al., 2007]. We examined scores on the depression sub-scale only. A score of greater than 10 indicated caseness for depression, and the degree of depression was indexed as mild (10–13), moderate (14–20), severe (21–27), or extremely severe (28 +).

To measure behavioral ADHD symptoms, we administered the adult Brown ADD scale (18+ years), a 40 item self-report questionnaire that assesses the impact of inattentive behavioral symptoms over the preceding 6 months. This scale scores high for internal consistency reliability (0.96) and has excellent specificity for ADHD [Brown, 1996; Muniz, 1996; Rucklidge and Tannock, 2002]. The term ADD has been re-classified as ADHD-Predominantly Inattentive (ADHD-PI) since the creation of this scale, and thus it was deemed appropriate to interpret data from this scale as indicative of cognitive and affective symptoms associated with the inattentive criterion of ADHD (i.e., ADHD-PI). We selected the total score and used it for ADHD-PI determination of caseness by examining the Threshold Interpretation Scale (e.g., ADHD-PI possible but not likely: 0–40; ADHD-PI probable but not certain: 40–55; ADHD-PI highly probable: 55–120).

STATISTICAL ANALYSES

Data were analysed using IBM SPSS Statistics 20.0. Normality was checked with the Shapiro–Wilk test. We first conducted independent *t* tests to compare group differences for raw scores on the neuropsychological assessments of executive function and self-reported psychological symptoms. Further comparisons of executive function performance were conducted after excluding those participants meeting threshold for caseness for probable mental disorder. Between group comparisons of executive function performance and psychological symptoms were further analyzed after removing participants caring for a child with special needs. However, given unequal numbers of participants aged over 40 and not caring for a child with special needs (PM-carriers = 2;

controls = 15), we compared only women below the age of 40 where numbers were relatively equal (PM-carriers = 10; controls = 14). This approach ensured comparable groups and reduced confounding effects due to age. To mark thresholds for neuropsychological involvement, we converted Hayling error scores to scaled scores (range 1–8, with a score of 1 marking impaired performance) so that a scaled score between 4 and 1 categorically signified "poor" performance. Similarly, a negative Stroop Color Word Interference Score indicated interference. Threshold markers for working memory dysfunction were not included in the task, and thus we marked all participants performing less than 1 SD from the mean as having "poor" working memory performance. Using Chi square test for independence, we further compared PM-carriers and controls on percentages of caseness for a probable mental disorder as defined by threshold criterion markers within self-report scales. This was repeated for a self-reported lifetime diagnosis (any previous diagnosis) of depression, anxiety and ADHD. Correlational analyses with Pearson's coefficients were conducted between executive function performance, psychological symptoms, age, and CGG-repeat length. Finally, to examine the percentage of our PM-carrier cohort meeting threshold for caseness (i.e., those with probable anxiety, depression, or ADHD-PI) as a function of executive function performance, we stratified percentages of PM-carrier women meeting threshold for probable caseness by executive function impairment. A Bonferroni correction across each domain (i.e., executive function and psychological symptoms) allowed for a more conservative P value ($P < 0.017$) for all analyses.

RESULTS

Neuropsychological Tests of Executive Function and Self-Reported Symptoms of Social Anxiety, Depression, and ADHD-PI

As evident in Table II, between group comparisons reveal that when compared to controls, the PM-carriers were comparable on Stroop interference and working memory performance on LNS, but showed significantly poorer response inhibition as measured by errors on the Hayling Test ($P \leq 0.001$). After excluding those participants meeting criteria for caseness for probable mental disorder, the differences in response inhibition between PM-carriers and controls did not survive Bonferroni correction ($t(49) = 2.390$, $P = 0.023$). Similarly, after excluding those women caring for children with special needs, the poorer response inhibition in PM carriers relative to controls did not reach significance after Bonferroni correction ($t(22) = 2.137$, $P = 0.044$).

With regard to levels of self-reported behavioral problems, there were no significant differences between groups for self-reported depression, but levels of both social anxiety ($P = 0.023$) and ADHD-PI ($P = 0.005$) were elevated in PM-carriers compared to controls. Further analyses with participants *not* caring for a child with special needs revealed increased behavioral problems in PM-carrier women compared to controls for social anxiety (PM-carrier: 43.70; control: 35.50), depression (PM-carrier: 2.70; control: 2.43), and ADHD-PI (PM-carrier: 40.30; control: 25.57); however, these group differences were not statistically significant (LSAS: $t(22) = 0.939$, $P = 0.366$; DASS: $t(22) = 0.185$, $P = 0.855$; ADHD-PI: $t(22) = 1.374$, $P = 0.183$).

Chi-square test for independence (with Yates Continuity Correction) revealed that in comparison to controls, there was a higher proportion of PM-carriers with poor response inhibition (PM-carrier: 31.4%; control: 8.6%; χ^2 (1, n = 70) = 4.375, $P = 0.036$, phi = 0.286) and caseness for social anxiety (PM-carrier: 34.3%; control: 11.4%; χ^2 (1, n = 70) = 3.970, $P = 0.046$, phi = 0.272), but the group differences did not survive adjustment for multiple testing (see Table III).

The proportion of PM-carriers meeting threshold criteria for Stroop Interference, poor working memory, caseness for depression and ADHD-PI did not exceed that observed for control participants. When compared to control participants, PM-carriers were not significantly more likely to self-report a lifetime diagnosis

TABLE II. Means and Standard Deviations for Raw Scores on Neuropsychological Assessments of Executive Function and Self-Report Scales for PM-Carriers and Control Participants

	FMR1 PM-carrier (N = 35)	Control (N = 35)	
	M ± SD (range)	M ± SD (range)	*P*-value
Executive function			
Hay	10.49 ± 10.26 (0 to 44)	4.0 ± 6.93 (0 to 38)	<0.001**
StroopI	5.89 ± 12.76 (−10.70 to 47.06)	4.99 ± 7.18 (−10 to 20.87)	0.719
LNS	12.60 ± 3.47 (5 to 20)	13.89 ± 3.21 (9 to 21)	0.112
Self-report scale			
LSAS	46.34 ± 28.53 (6 to 106)	33.03 ± 17.90 (4 to 74)	0.023
DASS_D	3.97 ± 5.70 (0 to 21)	2.37 ± 3.66 (0 to 20)	0.067
ADHD-PI	38.23 ± 27.80 (1 to 101)	22.34 ± 16.21 (0 to 66)	0.005*

ADHD-PI, ADHD-Predominantly Inattentive from the Brown ADD scale; DASS_D, Depression subscale from the Depression Anxiety Stress Scales; Hay, Hayling A + B error score; LSAS, Liebowitz Social Anxiety Scale; LNS: Letter Number Sequencing; SD, standard deviation; Stroop I, Stroop Interference.
Higher scores for LSAS, DASS_D, ADHD-PI, and Hayling's errors reflect an increase in symptoms and worse performance. Alternatively, higher scores on the Stroop Interference measure and LNS suggest better performance.
*$P < 0.017$.
**$P < 0.001$.

TABLE III. Chi Square Test for Independence Comparing Percentages of PM-Carrier and Control Participants With Poor Performances on Executive Function Tests and Probablecaseness for a Mental Disorder

	PM-carrier (N = 35)	Control (N = 35)			
	Number (%)	Number (%)	Phi coefficient	Chi square	*P*-value
Poor performance on neuropsychological test					
Inhibitory dysfunction likely	11 (31.4%)	3 (8.6%)	0.286	4.375	0.036
Interference likely	10 (28.6%)	9 (25.7%)	0.032	0	0.100
Working memory problems likely	6 (17.1%)	4 (11.4%)	−0.082	0.117	0.733
Threshold for probable caseness met					
ADHD-PI highly probable	9 (25.7%)	3 (8.6%)	0.227	2.514	0.113
Social phobia probable	12 (34.3%)	4 (11.4)	0.272	3.970	0.046
Depression probable	5 (14.3%)	0 (0%)	0.277	3.446	0.063
Lifetime diagnosis of a mental disorder (SR)					
ADHD[a]	0%[a]	0%	—	—	—
Anxiety[b]	31.4%	22.9%	0.096	0.650	0.420
Depression[b]	37.1%	25.7%	0.123	1.061	0.303

SR, self-report.
[a]Chi square test could not be performed due to zero frequency of PM-carrier ADHD diagnoses.
[b]Further comparisons of proportions of a lifetime diagnosis between PM-carrier women and Australian statistics (any anxiety disorder: 18% women; any affective disorder (i.e., depression): 7.1% women) show significant elevations in diagnostic rates for the presently examined PM-carriers (anxiety: χ^2 (1, n = 35) = 4.276, $P = 0.039$; depression: χ^2 (1, n = 35) = 47.893, $P = 0.001$). In contrast, the proportion of lifetime diagnoses in our control group was comparable to Australian statistics for anxiety (χ^2 (1, n = 35) = 0.559, $P = 0.454$), yet elevated for depression (χ^2 (1, n = 35) = 18.386, $P = 0.001$).

(any previous diagnosis) of anxiety (31.4%; χ^2 (1, n = 70) = 0.096, $P = 0.420$) or depression (37.1%; χ^2 (1, n = 70) = 1.061, $P = 0.303$). Given that these percentages from controls may not be reflective of true caseness in a general population, we further compared proportions of a lifetime diagnosis between PM-carrier women and Australian statistics (ABS: National Survey of Mental Health and Wellbeing) to show significant elevations in diagnostic rates for PM-carriers in the present cohort (anxiety: χ^2 (1, n = 35) = 4.276, $P = 0.039$; depression: χ^2 (1, n = 35) = 47.893, $P = 0.001$). In contrast, the proportion of lifetime diagnoses in our control group were comparable to Australian statistics for anxiety (χ^2 (1, n = 35) = 0.559, $P = 0.454$), but were elevated for depression (χ^2 (1, n = 35) = 18.386, $P = 0.001$). Furthermore, a comparable percentage of women with and without children with special needs reported a lifetime diagnosis of depression (with: 39.1%; without: 33.3%) and anxiety (with: 30.4%; without: 33.3%). With regard to ADHD, despite elevated rates of probable ADHD-PI in female PM-carriers (25.7%), the medical history questionnaire showed that no women in this study had previously received a medical diagnosis of ADHD-PI.

Associations Between Executive Function Performance and Self-Reported Symptoms of Social Anxiety, Depression, and ADHD-PI

The associations between performance on tests of executive function and self-reported psychological symptoms were examined through correlational analyses. As shown in Table IV, there were

TABLE IV. Correlational Associations Between Self-Reported Symptoms of a Mental Disorder and Performance on Neuropsychological Assessments of Executive Function for *FMR1* PM-Carriers and Controls

	PM-carrier (N = 35)			Control (N = 35)		
	Hay	Stroop-I	LNS	Hay	Stroop-I	LNS
LSAS	0.440*	0.089	−0.619**	0.092	−0.055	0.160
DASS_D	0.627**	−0.020	−0.377	−0.253	−0.010	−0.178
ADHD-PI	0.368	0.264	−0.496*	−0.258	−0.149	−0.038

DASS_D, Depression subscale from the Depression Anxiety Stress Scales; Hay, Hayling's Test; LSAS, Liebowitz Social Anxiety Scale; LNS, Letter Number Sequencing; SD, standard deviation; Stroop-I, Stroop Interference.
Partial correlation controlling for use of medications with a Bonferroni corrected *P*-value of 0.017 selected. Higher scores for LSAS, DASS_D, ADD-PI, and Hayling's errors reflect an increase in symptoms and worse performance. Alternatively, higher scores on the Stroop Interference measure and LNS suggest better performance.
*$P < 0.017$.
**$P < 0.001$.

several significant correlations between executive function performance and self-reported symptoms in PM-carriers. After controlling for medication use, performance as indexed by errors on the Hayling and working memory performance on LNS were significantly correlated with elevated levels of social anxiety (Hayling: $r = 0.440$, $P = 0.009$; LNS: $r = -0.619$, $P \leq 0.001$), depression (Hayling: $r = 0.627$, $P \leq 0.001$), and ADHD-PI (LNS: $r = -0.496$, $P = 0.003$). Associations between both symptoms of depression and working memory performance ($r = -0.377$, $P = 0.028$) and symptoms of ADHD-PI and inhibitory control ($r = 0.368$, $P = 0.032$) did not survive the Bonferroni correction. No significant correlations between scores on self-report scales and the Stroop Interference task were observed, nor were there correlations between neuropsychological performance and self-reported symptoms for controls with normal alleles.

Likelihood of Caseness for a Mental Disorder Stratified by Executive Function Profile

To determine which threshold levels of executive dysfunction were most associated with caseness for a mental disorder, we stratified the proportion of individuals meeting probable caseness for social anxiety, depression and ADHD-PI by their executive function profile (see Table V). This analysis showed that caseness was completely absent in those with excellent Hayling Test and LNS performances. In contrast, rates of behavioral problems were moderately elevated in those with average performance on both the Hayling Test (social anxiety: 41.7%; depression: 20%; ADHD-PI: 44.4%) and LNS (social anxiety: 27.3%; depression: 9.1%; ADHD-PI: 18.2%). Finally, psychological symptoms were very highly elevated for those with poor performance on the Hayling Test (social anxiety: 58.3%; depression: 80%; ADHD-PI: 55.6%) and LNS (social anxiety: 100%; depression: 50%; ADHD-PI: 83.3%). Stroop performance was not sensitive to caseness for a mental disorder.

TABLE V. Proportion of PM-Carriers Meeting Criteria for Probable Caseness for a Mental Disorder Stratified by Executive Function Profile

	Probable social anxiety (%)	Probable depression (%)	Probable ADHD-PI (%)
Hayling Test[a]			
Poor (N = 11)	58.3	80.0	55.6
Average (N = 16)	41.7	20.0	44.4
Excellent (N = 8)	0	0	0
Stroop Inhibition[b]			
Interference (N = 10)	41.7	20	22.2
No Interference (N = 25)	58.3	80	77.8
Letter number seq.[c]			
Poor (N = 6)	100	50	83.3
Average (N = 22)	27.3	9.1	18.2
Excellent (N = 7)	0	0	0

[a]Threshold determinant: poor = scaled score 1–4; average = scaled score 5–6; excellent = scaled score 7–8.
[b]Threshold determinant: Interference = score <0; No interference = score >0.
[c]Threshold determinant: poor = 1 SD or more below the mean; average = from 1 SD below mean to 1 SD above mean; excellent = 1 SD or more above mean.

Correlations Between Age/CGG-Repeat Length and Executive Function/Self-Reported Psychological Symptoms

Correlational analyses showed that there were no significant associations between CGG-repeat length and performance on tests of executive function and self-reported behavioral symptoms. The correlations between increasing age and poorer performance across the neuropsychological tests of executive function (Hayling Test: $r = 0.357$, $P = 0.035$; Stroop Interference task: $r = -0.383$, $P = 0.023$; LNS: $r = -0.327$, $P = 0.055$) did not reach significance after the Bonferroni correction (see Table VI).

DISCUSSION

In this study we investigated the utility of executive function profiles as neuropsychological indicators of symptoms of psychological disorders in females with the *FMR1* premutation. First, our results showed elevations in self-reported symptoms of social anxiety (34.3%) and ADHD-PI (25.7%), alongside core impairments in response inhibition. As expected, poor executive functions (both in response inhibition and working memory) were strongly associated with self-reported symptoms of mental disorders for female PM-carriers which differentiated them from controls with normal alleles. Further analysis revealed that probable caseness for a mental disorder was most common amongst female PM-carriers that performed poorly on neuropsychological tests of executive function, but PM carriers with preserved executive function were relatively risk-free from psychological symptoms. This suggests

TABLE VI. Correlational Associations Between Both Age and CGG Repeat Length With Self-Reported Behavioral Symptoms and Performance on Neuropsychological Assessments of Executive Function for *FMR1* PM-Carriers and Controls

	PM-carrier		Control	
	CGG- length	Age	CGG-length	Age
Hay	0.178	0.357	−0.098	0.154
Stroop-I	−0.107	−0.383	−0.059	−0.223
LNS	−0.142	−0.327	0.177	0.053
LSAS	−0.040	0.168	−0.181	−0.268
DASS_D	0.050	0.147	0.170	−0.246
ADHD-PI	−0.032	−0.047	0.028	−0.241

ADHD-PI, ADHD-Predominantly Inattentive; DASS_D, Depression subscale from the Depression Anxiety Stress Scales; Hay, Hayling's Test; LSAS, Liebowitz Social Anxiety Scale; LNS, Letter Number Sequencing; SD, standard deviation; Stroop-I, Stroop Interference.
Higher scores for LSAS, DASS_D, ADD-PI, and Hayling's errors reflect an increase in symptoms and worse performance. Alternatively, higher scores on the Stroop Interference measure and LNS suggest better performance.

that in adult female PM-carriers, executive function may tie in a range of symptoms of psychological disorders including social anxiety, depression and ADHD-PI, with the likelihood of involvement decreasing as a function of executive function ability. These findings have important clinical implications as neuropsychological tests of executive function could identify probable caseness for symptoms of psychological disorders in adult female PM-carriers.

The poorer performance on the Hayling Test indicated a core inhibitory control problem even after excluding women with a probable mental disorder and carer burden associated with raising a child with special needs. This is consistent with our previous studies showing inhibitory control deficits in male PM-carriers [Cornish et al., 2008b, 2011], and suggests a general *FMR1* premutation deficit in overcoming habitual word association responses. This implies that the earliest molecular events associated with the expanded CGG sequence (i.e., RNA toxicity, reductions of FMRP) may be domain-specific, with vulnerability only in specific neural circuits. Crawford and Henry [2005] showed that Hayling's test performance is more specific for suppression/inhibition in anterior lesion cases than for posterior lesion cases in brain injured patients. In line with this, Hayling's Test performance correlated with gray matter atrophy of the orbitofrontal cortex in patients with frontotemporal dementia [Hornberger et al., 2011]. This is consistent with findings of reduced prefrontal activation during performance of executive working memory tasks in male PM-carriers [Hashimoto et al., 2011a; Yang et al., 2013], and reduced functional connectivity between prefrontal and more posterior brain regions during memory encoding [Hashimoto et al., 2011c; Wang et al., 2012; Battistella et al., 2013]. These neural alterations may provide an explanation for the association between executive function and psychological symptoms reported here in female PM-carriers. We have previously suggested that selective executive deficits in inhibitory control and working memory may reflect early signs of toxicity to frontal regions in male PM-carriers [Cornish et al., 2011]. Although executive working memory does not appear to be a relative weakness in female PM-carriers, our results suggest frontally mediated inhibitory control impairments may be a core feature of the phenotype in at least a subgroup of at-risk women with the premutation.

The current findings of detected rates of probable caseness for mental disorders in female PM-carriers were relatively comparable to those suggested in previous studies. For example, we report that 34.3% of our participants met threshold markers within the LSAS scale for probable social anxiety (31.4% self-reported a previous clinical diagnosis), while previous studies using different measures of anxiety have documented involvement at 20.9% [Hunter et al., 2008a], 30% [Lachiewicz et al., 2010], and 29.03% [Roberts et al., 2009]. Similarly, while we report that 14% of participants met criteria for probable depression on the DASS (37.1% self-reported a previous clinical diagnosis), other studies using different scales for depression suggest prevalence rates of 26.3% [Hunter et al., 2008a], 33% [Lachiewicz et al., 2010], and 47.3% [Roberts et al., 2009]. Although our findings showed no participants reported a previous clinical diagnosis of ADHD, as many as 25% of female PM-carriers exceeded the threshold marker for highly probable ADHD-PI. This prevalence rate far exceeds a previously reported proportion of ADHD involvement (4.5%) based on an American female PM-carrier sample [Hunter et al., 2010]; however, it is consistent with neuropsychological investigations showing impaired attentional control in female PM-carriers [Loesch et al., 2003b; Steyaert et al., 2003]. Given that ADHD-PI in women is under-recognized in the general population [Biederman et al., 2002], it is entirely conceivable that female PM-carriers with clinical levels of ADHD-PI remain undiagnosed which may be associated with an increased risk for anxiety disorders and depression [Barkley and Brown, 2008].

The considerable variability in women meeting the threshold for a probable mental disorder is another important finding of the current study. One explanation for heterogeneity in psychological symptoms might be the greater carer burden associated with rearing a child with special needs [Abbeduto et al., 2004]. However, the current findings showed elevated levels of psychological symptoms even in PM-carrier women *not* caring for a child with special needs. Alternatively, other molecular events such as increased mRNA levels and moderately reduced FMRP may play an important role in the variable expression of the psychiatric phenotype in female PM-carriers. With regard to CGG repeat length, we found no relationships with executive functioning and self-reported psychological symptoms. This is consistent with studies suggesting elevated depressiveness in women with both high (>100) and low (<100) CGG repeats [Johnston et al., 2001; Roberts et al., 2009; Seltzer et al., 2012], and lack of association with social anxiety [Adams et al., 2010]. We speculate that a complex range of factors may contribute to increased risk for symptoms of mental disorders in female PM-carriers, involving residual genetic factors, lifetime experiences, hormones, child behavior, and marital satisfaction [Abbeduto et al., 2004; McCarthy et al., 2006; Hunter et al., 2010, 2012b; Seltzer et al., 2012]. We contend that significant associations between executive functions and psychological symptoms provide support for both at-risk profiles in some female PM-carriers, and in a subgroup with preserved executive functioning, some protective effects resulting in a much lower risk for a mental disorder.

A limitation of this research is the correlational nature of the impact of executive function impairments on psychological symptoms detected in female PM-carriers. Prospective longitudinal studies should be conducted to determine whether the observed relationships may be mediated by other factors, since correlations are insufficient to determine the direction of causality between executive function and mental disorders. A second limitation is the use of self-report measures of anxious and depressive symptomatology; however, it should be acknowledged that our conclusions are strengthened by consistency between self-report scales, rates of lifetime diagnoses and prevalence rates reported in previous studies. Future studies should include more detailed clinical interviews such as the Structured Clinical Interview for DSM-IV-TR (SCID) to confirm these relationships in larger samples of female PM-carriers. Future studies would also need to consider variations in residual polygenic effects previously shown to be associated with carer-stress, and symptoms of depression and anxiety [Hunter et al., 2012b; Seltzer et al., 2012]. Finally, this cross-sectional design will need to be extended to a larger sample and follow-up longitudinal analyses to ascertain the lifetime impact of executive dysfunction and symptoms of mental disorders in the same cohort of *FMR1* premutation carriers.

In conclusion, this is the first study to examine neuropsychological indicators of at-risk profiles associated with symptoms of mental disorders in females with *FMR1* premutation. The present data, showing significant associations between psychological symptoms and executive function profiles, may have important clinical implications for the assessment of female PM-carriers at-risk for more severe anxious and depressive symptomatology. Prospective longitudinal studies will be important in determining whether early problems within the frontally mediated networks that subserve response inhibition lead to increased risk for a range of symptoms associated with mental disorders. Follow-up studies will be critical for the development of sensitive neuropsychological markers to identify those female PM-carriers at greatest risk of developing a range of mental disorders across the lifespan.

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Declaration for Thesis Chapter 6

Declaration by candidate

In the case of Chapter 6, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Project design, review of relevant literature, recruitment and testing of participants, analysis of data and writing of manuscript.	70%

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Dr Darren R Hocking	Contributed to project design, critical review of manuscript	
E/Prof. John L. Bradshaw	Critical review of manuscript	
Dr Joanne Fielding	Critical review of manuscript	
Dr Jonathan Cohen	Critical review of manuscript	
Prof. Nellie Georgiou-Karistianis	Critical review of manuscript	
Prof. Kim. M. Cornish *	Contributed to project design, critical review of manuscript	

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	**Date** 15/2/14
Main Supervisor's Signature	[redacted]	**Date** 15/2/14

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

CHAPTER 6

MOTOR SEQUENCE LEARNING IN FEMALE CARRIERS OF FRAGILE X SYNDROME

6.1. Preamble to empirical paper 4: Symbolic sequence learning is associated with cognitive-affective profiles in female *FMR1* premutation carriers.

The findings from Chapter 3 have demonstrated a core deficit in response inhibition, alongside elevated symptoms of ADHD and social anxiety, in young adult female PM-carriers. Importantly, the associations detected between executive deficits and self-reported psychological symptoms raised the possibility that female PM-carriers may be at-risk of developing a cognitive-affective disorder. While the frontal cortex is an important neural substrate for executive function difficulties, the range of problems observed in female PM-carriers, including visuospatial, motor, executive and psychiatric difficulties, is most consistent with the cerebellar cognitive-affective syndrome (Schmahmann, 2004; Schmahmann & Sherman, 1998a). This has not been previously considered in the literature. The following published empirical paper examined for the first time performance by female PM-carriers on well-established task of cerebellar motor sequencing, the symbolically primed serial reaction time task (SRTT). Following this, associations were examined to elucidate whether performance on the SRTT may tie in a range of cognitive and affective symptoms in females with the PM allele.

Symbolic sequence learning is associated with cognitive-affective profiles in female *FMR1* premutation carriers

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This study examines implicit sequence learning impairments that may indicate at-risk cerebellar profiles proposed to underlie some aspects of subtle cognitive and affective dysfunctions found amongst female *FMR1* premutation carriers (PM-carriers). A total of 34 female PM-carriers and 33 age- and intelligence-matched controls completed an implicit symbolically primed serial reaction time task (SRTT) previously shown to be sensitive to cerebellar involvement. Implicit learning scores indicated a preservation of learning in both groups; however, PM-carriers demonstrated poorer learning through significantly elevated response latencies overall and at each specific block within the symbolic SRTT. Group comparisons also revealed a core deficit in response inhibition, alongside elevated inattentive symptoms in female PM-carriers. Finally, strong and significant associations were observed between poor symbolic SRTT performance and executive, visuospatial and affective deficits in the PM-carrier group. These associations remained strong even after controlling motor speed, and were not observed in age- and IQ-matched participants. The findings implicate cerebellar non-motor networks subserving the implicit sequencing of responses in cognitive-affective phenotypes previously observed in female PM-carriers. We contend that symbolic SRTT performance may offer clinical utility in future pharmaceutical interventions in female PM-carriers.

Keywords

Fragile X mental retardation 1 gene (*FMR1*) gene; fragile X mental retardation protein (FMRP); fragile-associated X tremor/ataxia syndrome (FXTAS); premutation carrier; cerebellar cognitive affective syndrome; cortico-cerebellar networks; phenotype

Abbreviations

ADHD-PI = attention deficit hyperactivity disorder-predominantly inattentive; CCAS = cerebellar cognitive affective syndrome; *FMR1* gene = fragile X mental retardation 1 gene; FMRP = fragile X mental retardation protein; FXTAS = fragile-associated X tremor/ataxia syndrome; MCPs = middle cerebellar peduncles; PM-carrier = Premutation carrier. SRTT = serial reaction time task;

Introduction

Fragile X syndrome (FXS) is the most common single gene cause of intellectual disability and autism and is caused by an expanded CGG-repeat sequence (>200) on the fragile X mental retardation 1 (*FMR1)* gene. Approximately 1 in 209 females carry a premutation expansion (CGG: 55-200) that can result in a deleterious phenotype through elevated levels of *FMR1* mRNA and reduced fragile X mental retardation protein (FMRP) (Tassone *et al.*, 2000, Tassone *et al.*, 2012). Females with the PM allele are at increased risk for developing a late onset neurodegenerative disorder, fragile X–associated tremor/ataxia syndrome (FXTAS) (Hagerman *et al.*, 2001). Although there are some protective effects of the second X chromosome, recent studies have documented subtle motor and postural control abnormalities, alongside poor response inhibition and psychiatric dysfunction (Kraan *et al.*, 2013a). Given more recent emphasis on the involvement of the cerebellum in higher order cognitive functions, it remains to be determined the extent of influence of *FMR1* gene expansion on subtle cognitive and affective manifestations.

Recent studies in young adult female carriers have provided evidence to suggest a role of the cerebellum in motor and balance impairments (Chonchaiya *et al.*, 2010, Goodrich-Hunsaker *et al.*, 2011b, Kraan *et al.*, 2013b, Narcisa *et al.*, 2011), motor sequencing deficits (Loesch *et al.*, 2003), and subtle impairments in response inhibition (Kraan *et al.*, 2013c), visuospatial processing (Goodrich-Hunsaker *et al.*, 2011a, Keri & Benedek, 2009, Keri & Benedek, 2010) and psychiatric functioning (Lachiewicz *et al.*, 2010, Roberts *et al.*, 2009). This is consistent with the features of cerebellar cognitive affective syndrome (CCAS) which is characterized by a range of impairments in executive, visuospatial and affective functions arising from disrupted cerebellar-cortico connectivity (Molinari & Leggio, 2013, Schmahmann, 2004, Tedesco *et al.*, 2011).

Symbolic sequence learning may provide a sensitive paradigm to explore the role of the cerebellum in these at-risk profiles in the female PM. It is thought that the cerebellum is important for associating the 'stimulus' with the 'response' command (Bo *et al.*, 2011b, Spencer & Ivry, 2009). This is consistent with inverse internal models which propose that cerebellar predictions are compared with actual feedback and performance error is adjusted accordingly (Ito, 2008, Koziol *et al.*, 2010). The imaging evidence for dysfunction along cerebellar-cortico circuitry (middle cerebellar peduncles) in asymptomatic male PM carriers (Battistella *et al.*, 2013, Hashimoto *et al.*, 2011, Wang *et al.*, 2013), alongside increased

activation of the cerebellum during symbolic sequence learning (Bo *et al.*, 2011a, Bo *et al.*, 2011b), raises the possibility of a cerebellar role in the previously reported cognitive and affective profiles in the female PM.

Here we examine symbolic sequence learning to explore the ability to acquire and flexibly adapt to symbolic cue information, and its association with 'signature' female PM weaknesses in executive function, visuospatial processing and psychological symptoms. We predict that female poor symbolic sequence learning will be related to cognitive and affective manifestations associated with expansion of the *FMR1* gene.

Methods

Participants

A total of 35 females with the PM (hereafter named PM-carriers) aged between 22 and 55 were recruited through support groups and population-based fragile X carrier screening studies (Martyn *et al.*, 2013, Metcalfe *et al.*, 2008). The specific announcement was entitled, 'An Australian study of families who have expansions in the gene associated with fragile X'. A further 35 female control participants aged between 22 and 55 years were also recruited through population-based fragile X carrier screening studies, and through local networks and via online advertisements. The final analysis included 34 PM-carriers and 33 controls due to three incidences in which the software did not record SRTT performance. All participants were English speaking with no history of epilepsy or of a serious head injury and had normal (or corrected) vision and hearing, and no sign of color blindness or intellectual disability as assessed using the Wechsler Abbreviated Scale of Intelligence (FSIQ<70). The FXTAS Rating Scale (Leehey, 2009) was used to screen all participants for features related to FXTAS—that is, tremor, ataxia or parkinsonism—or any other neuromotor disorder. The comparisons between female PM-carriers and controls showed that the two groups were well-matched on age, IQ, socioeconomic status and current use of psychotropic medication(s); however, female PM-carriers were significantly more likely to be caring for a child with special needs (p = .001). The small portion of medicated women in this study were taking anti-depressants at the time of testing, with two female PM-carriers additionally taking anti-psychotic medications and one PM-carrier additionally taking a stimulant. All study participants provided signed informed consent and the study procedures were consistent with

the Declaration of Helsinki and approved by the Southern Health Ethics Committee (project 10147B). The descriptive demographics and related statistics are shown in Table I.

Table 1. Means and standard deviations of sample characteristics for female *FMR1* PM-carriers and control participants

	FMR1 PM-carriers (N = 34)	Controls (N = 33)	*t*-value	*p*-value
	M ± SD (range)	M ± SD (range)		
Age (years)	40.88 ± 8.32 (22-55)	41.18 ± 8.18 (22-55)	-.149	.882
FSIQ	110.61 ± 10.92 (88-128)	113.00 ± 7.83 (89-129)	-1.023	.310
VIQ	107.06 ± 13.84 (73-128)	108.70 ± 10.86 (88-136)	-.534	.595
PIQ	111.00 ± 11.27 (87-133)	115.27 ± 9.40 (93-133)	-1.682	.097
CGG-repeat length	86 ± 15.14 (59-122)	31 ± 3.63 (20-42)	19.98	<.001**
Socioeconomic disadvantage (% < AUD $51,999)	8.8%	15.2%		.785[b]
Use of psychotropic medications (% yes)	17.6%	9.1%		.305[b]
Child special needs (% yes)	64.7%	18.2%		<.001**[b]

FSIQ = Full Scale Intelligence Quotient; PIQ = Performance Intelligence Quotient; SD = Standard Deviation; VIQ = Verbal Intelligence Quotient.

[b]Chi square test for independence.

***p*<.001

Molecular analyses

To ascertain CGG repeat size, DNA was extracted from 2ml whole blood from all participants using the Promega Maxwell® 16 Instrument and associated Maxwell® 16 Blood DNA Purification Kit (Promega Cat No.: AS1010). PCR was performed using the Asuragen® AmplideX™ *FMR1* PCR Kit as this assay has been shown to detect a full range of fragile X expanded alleles (Chen *et al.*, 2010). PCR products were assessed via capillary electrophoresis on an Applied Biosytems 3130 Genetic Analyzer with electropherogram

analysis conducted using GeneMapper® software. All procedures were performed in accordance with manufacturer's instructions.

Neurobehavioral measures

Implicit sequence learning

A computer based symbolic serial reaction time task (SRTT) was employed to measure implicit learning. On the basis of previous studies which have shown that non-spatial 'symbolic' cues were most sensitive to cerebellar dysfunctions and functions, we adopted stationary colored circles (red, green, blue, yellow) in favor of the traditional non-colored parallel and spatially located cues used in classic SRTT paradigms (Nissen & Bullemer, 1987). Participants were instructed to place middle and index fingers of the left hand on keys 'Z' and 'X', and middle and index fingers of the right hand over keys 'N' and 'M'. Each key corresponded to a circle color (Z: blue; X red; N: green; M: yellow). On screen, a colored circle was presented. Participants were told to respond by pressing down on the matching key as fast as possible. If the key press did not correspond to the associated color presented on screen, an 'X' cross over the colored circle prompted the participant to try again. When the key-press was correct, the colored circle stimulus was removed and replaced with a white circle for 200 milliseconds; following this, the next successive colored circle in the sequence was presented. The overall aim of this task is to detect whether participants would learn the repeating sequence. A total of 8 blocks were presented. Within each block there were 9 runs of an 8-step sequence. Thus one block required 72 key-presses. For blocks 1-6 and block 8, the repeating 8-step sequence was presented (i.e., red, green, red, blue, blue, green, yellow, yellow). For block 7, each sequence was random. The software recorded the response latency corresponding to each key-press across the 8 blocks. We exported both the median response time and the total number of errors at each block for further statistical analysis.

Executive function

The following tests of executive function were selected on the basis of previously demonstrated sensitivity to profiles within an adult PM-carrier cohort: Hayling Sentence Completion Test, Letter-Number Sequencing and Excluded Letter Verbal Fluency. These

tasks were explained in previous publications (Cornish *et al.*, 2011, Kraan *et al.*, 2013a, Kraan *et al.*, 2013b).

Visuospatial function

The tasks selected to measure visuospatial function were selected on the basis of previous demonstrated vulnerabilities in PM-carrier cohorts to tasks that tap into both dorsal stream visuospatial functions and visuospatial working memory (Hocking *et al.*, 2012, Keri & Benedek, 2009): Clock Test, Mental Rotation task and the Block Design sub-test from the WASI-IV scale.

We employed a written adaptation of the auditory "Visuospatial Decision Test" (otherwise known as the "Clock Test") previously employed in dual-task investigations (Haggard *et al.*, 2000, Plummer-D'amato *et al.*, 2008). Participants were provided a list of 60 digital times (e.g., 2.45pm, 1.34pm) and told to imagine the analog time (i.e., an analog clock face). From the imagined analogue image, participants circled one of two answers on a record sheet ascribing the two clock hands as (a) on the same side or (b) on different sides of the clock face. With no access to drawing and reference materials, participants relied solely on the activation of mental imagery to complete this task. Four practice items were completed prior to commencement and the outcome variable was the number of correctly answered items within 60-seconds.

The Mental Rotation test (Cooper & Shepard, 1973) employed for this study was designed to test the participant's ability to detect whether a rotated letter (letter "R") was in its normal form or a mirror image. The task was administered as a flip-book in which two letters (both "R") were presented side by side. The left-side "R" was always in its proper form, while the right-side 'R" was always rotated and sometimes also a mirror image. Participants were timed and a higher value of correctly answered items indicated better performance. Due to an unexpected ceiling effect in performance, we took total time as the variable for further analyses.

Block Design is a sub-test from the WASI that measures visuospatial skills. To complete the task, participants are required to re-construct a series of increasingly complicated visual designs with colored blocks. This task was timed and a higher score

indicated better performance. We interpreted the Block Design WASI *T* score for this investigation.

Psychological symptoms

The following self-report questionnaires were administered on the basis of their sensitivity to psychological symptoms previously reported in female PM-carriers : The self-report Liebowitz Social Anxiety Scale (SR-LSAS), Depression Anxiety Stress Scales (DASS) and Brown Attention Deficit Disorder Scales (Brown ADD). These tasks were explained in our previous publication (Kraan *et al.*, 2013c).

Procedure

All participants completed the battery of tests in a single session. Neuropsychological tests of executive function and visuospatial ability were administered in random sequence, and symbolic SRTT was administered first. To ensure implicit learning, participants were not provided with training on the symbolic SRTT task. A card showing the key-color associations was placed next to the computer and the participants were told that although they may use this as a guide, the goal was to learn these associations and respond as fast as possible. This task was performed continuously with no breaks in between blocks, and participants were informed prior to the task that it would entail approximately 10 minutes of attentive key-pressing. Prior to performance there was no mention of any repeating sequence. However, immediately following completion of the task, participants were asked a series of structured questions about their explicit knowledge of the repeating sequence. Specifically, participants were asked the following questions: (1) Did you notice anything different/unusual? (2) Did you notice any patterns or repetitions? (3) Did you notice a repeating sequence? (4) Can you tell me what the repeating sequence was?

Statistical analysis

Data were analyzed using IBM SPSS Statistics 22.0. Normality was checked with the Shapiro-Wilk test. We firstly conducted independent *t*-test to compare raw scores for

differences between PM-carriers and controls on neuropsychological measures and self-reported psychological symptoms (i.e., affect). To analyze awareness of the repeating sequence on the symbolic SRTT, we employed Chi square analysis to explore group differences in the percentage of individuals noting awareness of the sequence, and for those successfully able to recall >3/4 of the repeating sequence. Mixed model ANOVA was conducted to ascertain group effects on symbolic SRTT response latency, with further *t*-tests to explore differences in errors, and time at each block. To index implicit learning we also computed slope (rise over run from B2 to B6), and consistent with previous examinations of performance on the SRTT (Bussy *et al.*, 2011), we compared response latency on the random block (B7) with its preceding (B6) and successive (B8) sequenced blocks. This enabled three scores in which a higher score indicated good implicit learning and a preservation of the previously learned sequence following the random block interruption: slope, B7 minus B6, and B7 minus B8. Finally, we analyzed the strengths of correlational associations between SRTT completion time and implicit learning scores with performance on tests of executive function (Hayling's, LNS and EL verbal fluency), visuospatial processing (visuospatial clock test, mental rotation, block design test) and self-reported psychological symptoms (LSAS, DASS and Brown ADD scale). Relationships to age- and CGG-repeat length were also examined. To reduce multiple correlations between neurobehavioral measures and each block of the SRTT we took total log transformed SRTT performance (corrected for slight positive skew in PM-carrier cohort) as a maker of sequence learning. This decision was based on very strong and significant correlations between each block and total SRTT time. Importantly, these correlations were conducted controlling for simple motor reaction time. This task is part of the Physiological Profile Assessment (PPA) and is a measure of the time taken to click on a computer mouse in response to an illuminated red light (Kraan *et al.*, 2013b). Due to the large number of comparisons, a relatively stringent Bonferroni correction of $p<.01$ was selected for all analyses.

Results

Neuropsychological performance and self-reported psychological symptoms

The analyses revealed significant performance decrements in PM-carriers compared to controls in areas of response inhibition ($t(65) = 3.656$, $p = .001$) and EL verbal fluency ($t(65) = -3.482$, $p = .001$), and there was a trend towards a PM-carrier deficit in the areas of block

design ($t(65) = -2.172$, $p = .034$). Compared with controls, PM-carriers demonstrated elevated symptoms of ADHD-PI ($t(65) = 2.828$, $p = .007$).and increased symptoms of anxiety ($t(65) = 2.202$, $p = .032$).

Table 2. Means and standard deviations for scores on neuropsychological assessments and self-report psychological scales for *FMR1* PM-carriers and control participants

	FMR1 PM-carrier (N=34)	Control (N=33)	*p*-value
	M ± SD (range)	M ± SD (range)	
Executive function			
Response Inhibition (Hayling's)	9.80 ± 9.06 (0-29)	3.49 ± 4.31 (0-16)	.001**
Working memory (LNS)	12.65 ± 3.52 (5-20)	13.88 ± 3.22 (9-21)	.140
EL verbal fluency)	15.41 ± 5.36 (3-26)	19.97 ± 5.35 (9-32)	.001**
Visuospatial function			
Visuospatial decision	20.09 ± 8.88 (5-44)	22.33 ± 6.89 (8-40)	.253
Mental rotation (msec)	1049.70 ± 384.84 (460-2130)	905.80 ± 405.06 (430-2040)	.154
WASI block design	55.59 ± 7.11 (42-73)	59.45 ± 7.46 (40-72)	.034[a]
Psychological symptoms			
Anxiety (LSAS)	45.94 ± 28.86 (6-106)	33.03 ± 18.07 (4-74)	.032[a]
Depression (DASS_D)	3.91 ± 5.77 (0-21)	2.03 ± 2.27 (0-8)	.085
ADHD-PI (Brown ADD)	38.32 ± 28.21 (1-101)	22.48 ± 16.21 (0-66)	.007*

ADHD-PI = ADHD-Predominantly Inattentive from the Brown ADD scale; DASS_D = Depression subscale from the *Depression Anxiety Stress Scales; Hayling's = Hayling A + B error score;* LSAS = *Liebowitz Social Anxiety Scale*; LNS: Letter Number Sequencing; SD =

Standard Deviation; Stroop I = Stroop Interference. *Note:* Higher scores for Hayling's errors, mental rotation, LSAS, DASS_D and ADHD-PI and reflect an increase in symptoms and worse performance. Alternatively, higher scores on the LNS, EL verbal fluency, visuospatial decision and block design suggest better performance.

$^{**}p<.001$, $^{*}p<.01$, $p < .05^{a}$,

Symbolic SRTT performance

PM-carriers were significantly less likely than control participants to report that they had noticed the repeating sequence (PM-carrier: 52.9%; control: 93.9%; χ^2 (1, $n = 67$) = 14.326, $p < .001$, phi =-.462). When asked to demonstrate this knowledge, there were no significant differences between the proportion of PM-carriers and controls that successfully recalled more than ¾ of the pattern. (PM-carrier: 20.6%%; control: 39.4%; χ^2 (1, $n = 67$) = 2.828, $p = .093$, phi = -.205). Refer to Table 3.

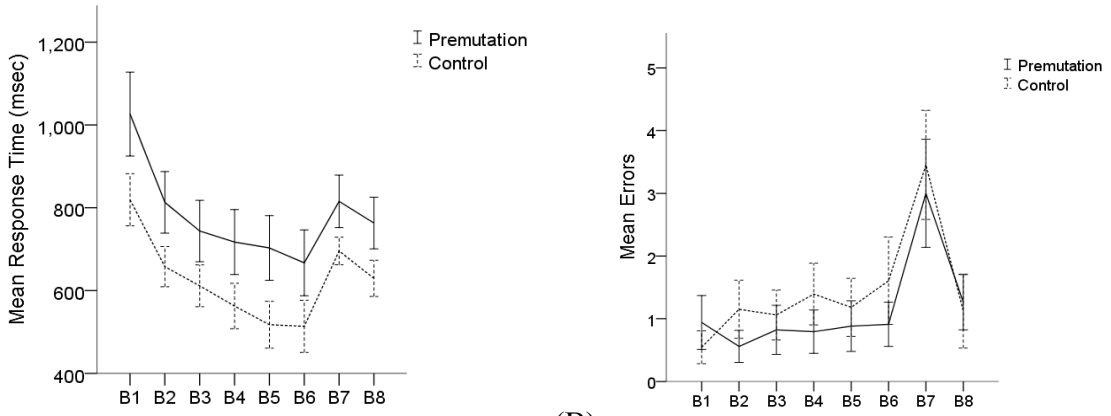
Table 3. Recognition and successful recall of repeating pattern for female PM-carrier and control participants

	PM-carrier (N = 34)	Control (N = 33)	Phi coefficient	Chi Square	*p*-value
	N (%)	N (%)			
Noticed pattern	18 (52.9%)	31 (93.9%)	-.462	14.326	<.001**
Recalled >3/4 of pattern	7 (20.6%)	13 (39.4%)	-.205	2.828	.093

$p < .001$**

A mixed model ANOVA was conducted to investigate group difference in response time across the 8 blocks. This analysis revealed a significant interaction between group and response time, Wilks Lambda = .780, $F(7,59) = 2.377$, $p = .033$, partial eta squared = .220. Inspection of the graphical output revealed that both groups showed a gradual increase of efficiency up to block 6, and an interruption to this 'learning' at block 7 where the random sequence was introduced. However, while PM-carriers showed little change between B3 and B5 (40.88msec), control participants demonstrated a steep learning curve during this time-period (93.91msec) and this difference was statistically significant ($t(65) = 2.453$, $p = .017$).

Further analyses showed a significant main effect for time (Wilks Lambda = .207, $F(7,59)$ =32.272, p = .001, partial eta squared = .793). The main effect of group was also significant ($F(1,65)$ =13.490, p = .001, partial eta squared = .172), with female PM-carriers showing significantly prolonged response latencies across each block relative to controls with normal alleles (B1: $t(65)$ = 3.596, p = .001; B2: $t(65)$ = 3.474, p = .001; B3: $t(65)$ = 2.937, p = .005; B4: $t(65)$ = 3.211, p = .002; B5: $t(65)$ = 3.831, p < .001; B6: $t(65)$ = 3.013, p = .004; B7: $t(65)$ = 3.344, p = .002; B8: $t(65)$ = 3.573, p = .001). Please see supplementary Table e-1 for specific details.

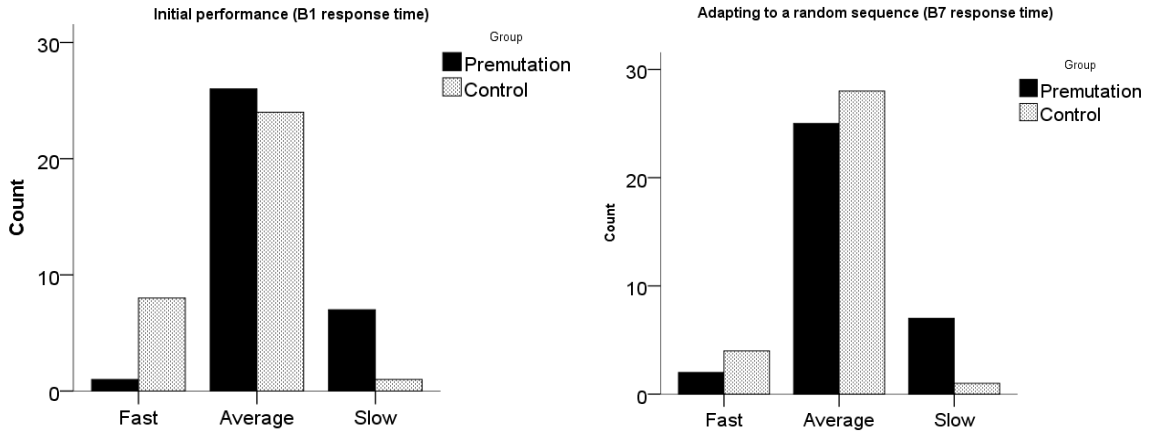


(A) (B)

Figure 1. Figures show (A) mean response time and (B) errors for PM-carrier and control participants across 8 task blocks. A repeating sequence was presented from B1 to B6, and again at B8. B7 reflects the introduction of a random sequence. Error bars represent ±2 standard error.

As shown in Figure 1A, implicit learning of the sequence occurred for both groups, with a reduced reaction time in B8 compared to B7. The impact of the random sequence in B7 was not significantly different between controls and PM-carriers (B7-B6: PM = 148.44, PC = 182; $t(65)$ = -.874, p=.385). Similarly, controls returned to the sequence in B8 with comparable efficiency to PM-carriers (B7-B8: PM = 52.21, PC = 66.24; ($t(65)$ = -.609, p=.545). These results suggest that controls and PM-carriers overall retain preservation of the sequence. Analysis of slope produced similar patterns of results with no significant difference between groups on this variable (control: -28.81; PM-carrier: -29.21; $t(65)$ = -.060, .952). Because fast initial learning and good adaptability to the random B7 sequence could reduce slope and implicit learning scores we also examined the percentage of participants with fast, average or slow response latency during block one. As shown in Figure 2, fewer PM-carriers than

controls responded fast during B1 (χ(1, n=67) = 10.012, p = .005). There was also a trend towards a greater percentage of PM-carriers responding slowly on the random block (χ(1, n=67) = 5.323, p = .083). As shown in Figure 1B, there were no significant differences in errors made between PM-carriers and controls (B1: U = 492, p = .337; B2: U = 407, p = .037; B3: U = 485, p = .309; B4: U = 403, p = .037; B5: U = 498, p = .399; B6: U = 480, p = X.283; B7: U = 496, p = .411; B8: U = 474, p = .251), although there was a trend towards female PM-carriers making less errors than control participants at B2 and B4. Please refer to supplementary table e-2 for specific details.



(A) (B)

Figure 2. Proportion of PM-carriers and controls as a function of fast, average and slow response latency at (A) the initial block named B1 and (B) the random block termed B7.

Total symbolic SRTT response latency

Total time for SRTT completion was significantly longer in PM-carriers compared to controls (PM-carrier: 11395; control: 9474.24; t(65) = 3.889, p < .001). Given that psychiatric rating scales classified some women within a possible clinical range for a mental health disorder−9 meeting threshold for probable ADHD-PI, 12 probable social phobia, and 5 probable depression−we repeated our analyses for SRTT total time after excluding these women (refer to Kraan et al., 2013 for methodology). The results retained significance. Further analyses were computed to ensure there were no further confounding variables which may have impacted this result. For example, simple motor reaction time which was significantly slower in female PM-carriers compared to controls (PM-carrier: .222; control: .220; t(65) = 2.771, p

= .007) could have impacted SRTT performance. Importantly, re-computation of this statistic with univariate ANOVA whilst controlling for reaction time, FSIQ, mood stabilizing medication use and a probable mental health disorder maintained significance, with PM-carriers exhibiting slower overall performance on the SRTT ($F(1,65) = 8.765$, $p = .004$, partial eta squared = .127). To ensure that this result was not further mediated by attentional profiles as determined by the Brown ADD scale, we re-run this analyses additionally controlling for ADD symptoms. The significant difference remained ($F(1,65) = 4.954$, $p = .030$, partial eta squared = .077).

Correlations to neuropsychological measures and self-reported psychological symptoms

The associations for PM-carriers between performance on the symbolic SRTT and performance on neuropsychological tasks of executive function, visuospatial processing and self-reported psychological symptoms were examined using Pearson's correlational analyses. As shown in Table 4, after controlling for simple motor reaction time, we observed significant correlations between overall symbolic SRTT response latency and worse performance on executive measures (Hayling's: $r = .526$, $p = .002$; LNS: $r = -.403$, $p = .020$; verbal fluency: $r = -.308$, $p = .081$), deficits on visuospatial measures (visuospatial decision: $r = -.562$, $p = .001$; mental rotation: $r = .377$, $p = .044$; block design: $r = -.645$, $p < .001$) and higher incidences of self-reported psychological symptoms (anxiety: $r = .551$, $p = .001$; depression: $r = .584$, $p < .001$; ADHD-PI: $r = .456$, $p = .008$). For control participants, there were no correlations between symbolic SRTT performance and any of the selected neuropsychological measures or scales. To rule out the possible confound of a probable mental health disorder, we repeated the correlational analyses excluding participants meeting criteria for probable anxiety, depression or ADHD-PI (see Kraan et al., 2013 for methodologies). The strength of the correlational values remained significant for associations between symbolic SRTT response latency and performance on block design ($r = -.590$, $p = .003$) and visuospatial decision making tasks ($r = -.516$, $p = .012$), suggesting that worse SRTT performance was associated with poorer visuospatial ability irrespective of psychological status. A repeat of this correlational analyses with all individuals (N=35) and the implicit learning scores in place of total SRTT response latency showed a positive association between implicit learning and performance on the visuospatial decision task (B7-B6: $r = .453$, p =.004), indicating better implicit learning in PM-carriers with good

visuospatial ability. By contrast, for controls but not PM-carriers, there were associations between higher implicit learning scores indicative of good learning and better working memory performance (implicit learning B7-B6: $r = .371$, $p = .018$; implicit learning B7-B8: $r = .312$, $p = .041$). Further analyses revealed no correlational associations between any of the selected neuropsychological measures and both age and CGG-repeat length in PM-carriers. However, for controls we did observe a trend association between older age and both reduced SRTT errors (r = -.382, $p = .028$) and increased total SRTT response latency (r = .346, $p = .048$), indicating worse performance on the SRTT in older control participants.

Table 4. Correlational associations between symbolic SRTT response latency and performance on neuropsychological measures of executive function, visuospatial function and affective regulation (controlling for motor reaction time).

	PM-carrier	Control
Task	Total symbolic SRTT time (N=34)	Total symbolic SRTT time (N=33)
Executive function		
Response inhibition (Hayling's)	.526*	-.144
Working memory (LNS)	-.403ª	-.071
EL verbal fluency	-.308	.106
Visuospatial		
Visuospatial decision	-.562**	-.176
Mental rotation	.377ª	-.038
WASI block design	-.645**	-.256
Psychological symptoms		
Anxiety	.551**	-.068
Depression	.584**	-.130
ADHD-PI	.456*	.175

p<.001**, *p*<.01*, *p<.05*ª

Note: Higher scores for Hayling's errors, mental rotation, LSAS, DASS_D and ADHD-PI reflect an increase in symptoms and worse performance. Alternatively, higher scores on the LNS, EL verbal fluency, visuospatial decision and block design suggest better performance.

Discussion

Here we show that poorer performance in sequence learning is associated with a range of higher order functions previously shown to be impaired in some females with the expanded *FMR1* allele. Although female PM-carriers appear to have shown preserved implicit learning, the slowed response latency and poor awareness of the repeating sequence suggest a deficit in automaticity required for successful symbolic SRTT performance. Importantly, there were several interrelationships between poorer symbolic sequence learning and a range of cognitive, visuospatial and affective abnormalities in female PM-carriers that differentiated them from controls with normal alleles. The significant associations between working memory and symbolic SRTT implicit learning in controls suggested different strategies of working memory in female PM-carriers. We interpret these data to suggest that cortico-cerebellar involvement may be related to cognitive and affective dysfunction in some PM-carrier women.

Our results showing significantly poorer response latency on the symbolic SRTT in female PM-carriers compared to controls are consistent with a deficit of automaticity in motor sequencing with a symbolic prime. This finding is in line with previous studies of PM-carriers which have shown poorer performance on the BDS, a multifactorial measure that partially loads onto motor sequencing (Grigsby *et al.*, 2008). This is consistent with a previous study which reported that FMRP levels significantly associated with performance on the motor sequencing test from the BDS in female PM-carriers (Loesch *et al.*, 2003). Our data do not support an overall deficit in implicit, or unconscious, learning as reflected in the slope and effects from the random block to response latency. However, one possibility is that better performance and more efficient adaptability to B7 in the controls could have contributed to a reduction of slope and implicit learning scores. In line with this, our data from figure 2 showed that controls were also more efficient in responding to the random sequence at block 7. This may have inadvertently reduced the implicit learning scores for control participants performing well at the outset which could have affected our between group comparisons. Indeed, poor sequence learning performance by PM-carriers was supported by the fact that only 52.9% of PM-carriers were consciously aware of the repeating sequence when compared to 93.9% of controls. Furthermore, the associations between symbolic SRTT response latency and cognitive and affective dysfunction in PM-carriers, after controlling for motor speed, suggest a unique cognitive contribution to symbolic SRTT

performance. It has been suggested that the role of the cerebellum in SRTT performance is in setting up the associations between stimulus and response commands necessary for the development of automatic responding to a symbolic prime (Bo *et al.*, 2011a, Bo *et al.*, 2011b). This occurs in the earliest stages of performance, with the later stages characterized by a disengagement from cerebellar regions and the emergence of cortical and fronto-striatal involvement (Eliassen *et al.*, 2001, Honda *et al.*, 1998). Indeed, successful SRTT performance typically leads to explicit awareness over time and the recruitment of working memory systems that may enhance performance through strategy (i.e., enumerating colors of sequence). This notion is supported by our present data showing correlations between working memory and implicit SRTT learning in controls. Given the lack of such associations between SRTT implicit scores and working memory in PM-carriers, it is possible that their slowed performance is due to cerebellar-cortico disruption underlying implicit sequencing performance.

Another important finding of the study was that visuospatial functioning correlated with symbolic SRTT performance even after controlling for self-reported psychological symptoms (i.e., anxiety, depression, ADHD-PI). Our previous investigation with female PM-carriers showed that poor executive function was associated with self-reported symptoms of anxiety, depression and ADHD suggestive of a range of comorbid symptoms associated with core inhibitory control impairments (Kraan *et al.*, 2013c). The significant interrelationships between performance on SRTT and visuospatial functioning in female PM carriers are suggestive of dysfunction in fronto-parietal dorsal stream networks resembling that seen in the FXS full mutation (Kogan *et al.*, 2004a, Kogan *et al.*, 2004b). Indeed, studies with female PM-carriers have shown preserved performance on tasks of ventral stream visuoperceptual function, alongside age- and CGG-dependent deficits on tasks known to tap dorsal stream visuospatial functions (Goodrich-Hunsaker *et al.*, 2011a, Keri & Benedek, 2009). It is possible that the detected correlations between symbolic SRTT task and visuospatial performance reflect disrupted connectivity in networks important for the visual guidance of movement, such as the closed loops reciprocally connecting dorsal steam networks with the cerebellum (Glickstein, 2000).

These findings are consistent with the contention that cerebellar dysfunction may underlie a range of deficits in executive and visuospatial functioning, and psychological symptoms in female PM-carriers (Kraan *et al.*, 2013a). This interpretation is consistent with functionally distinct cerebro-cerebellar networks reciprocally connecting cerebellum with the

striatum (Bostan *et al.*, 2013), limbic system (Schmahmann, 2000, Snider & Maiti, 1976), and association areas within the prefrontal and parietal cortices (Krienen & Buckner, 2009, Middleton & Strick, 2001, Ramnani, 2006, Strick *et al.*, 2009). Moreover, investigations with clinical populations and functional neuroimaging studies have consistently demonstrated that posterior cerebellum (lobule VI and VII) is important for a range of executive, visuospatial, linguistic and affective abilities (Schmahmann, 2004, Stoodley *et al.*, 2010). The cerebellar cognitive affective syndrome (CCAS) has been described as dysfunction of posterior cerebellum which results in cognitive, visuospatial and affective deficits which resemble in a milder form that seen in female PM-carriers (Bernard *et al.*, 2012, Stoodley & Schmahmann, 2009, Stoodley *et al.*, 2010, Stoodley *et al.*, 2012, Tedesco *et al.*, 2011). It has been suggested that feed-forward models, such as those that enable smooth and accurate movement control (Wolpert *et al.*, 1995, Wolpert *et al.*, 1998), are also important for rapid unconscious information processing and automaticity in performance (Koziol et al., 2010). Because the posterior cerebellum has strong projections to prefrontal and parietal cortices, disruption to posterior cerebellum has been proposed to result in disorganization, or dysmetria, of cognition (Schmahmann, 2004). Overlap between symptoms of CCAS and female PM-carrier phenotypes, suggests that tasks with sensitivity to cognitive cerebellar operations may offer clinical utility in identifying women at-risk of FMR1-associated deleterious phenotypic impact.

The current findings do not support the hypothesis of age or CGG-repeat length effects on symbolic sequence learning in female PM-carriers. One interpretation is that molecular variables known to be associated with increased CGG-length such as elevated *FMR1* mRNA, and slight reductions in the percentage of histochemically stained FMRP-positive cells, are not interrelated in sequence learning and cognitive phenotypes in female PM-carriers (Kenneson *et al.*, 2001, Tassone *et al.*, 2000); however, this claim is tentative given that we were unable to obtain an extensive molecular profile. One explanation is that other molecular and environmental factors may be involved in the manifestation of cognitive and affective phenotypes; for example, psychosocial stressors, carer burden, hormones and X-chromosome inactivation (Abbeduto *et al.*, 2004, Hunter *et al.*, 2012, Hunter *et al.*, 2010, Mccarthy *et al.*, 2006). The lack of any association between sequencing performance and age in female PM-carriers is suggestive of developmental mechanisms. The posterior region of the cerebellum important for symbolic sequencing is phylogenetically recent, and alongside the prefrontal cortex, developmentally vulnerable with relatively late maturation (Altman &

Bayer, 1985, Ciesielski *et al.*, 1997). Longitudinal neurobehavioral and imaging studies will be important for ascertaining the extent to which impairments in sequencing ability in female PM-carriers are reflective of risk for a neurodegenerative disorder, or subtle and developmentally based phenotypes possibly associated with posterior cerebellum and cortico-cerbellar tracts.

Some limitations require acknowledgment. Firstly, due to the cross-sectional nature of this study, the extent to which problems in sequence learning reflect a stable or age-attenuated phenotype will need to be addressed in prospective longitudinal studies in which both FMRP and FMR1 mRNA levels are routinely collected. Secondly, the neural basis of the associations between sequencing and cognitive-affective phenotypes will need to be confirmed in future imaging investigations. They may include voxel based morphometry and diffusion tensor imaging, and fMRI activation patterns during symbolic SRTT performance and completion of paradigms sensitive to the observed cognitive and affective phenotypes. For example, researchers could use these neuroimaging techniques to examine structural and functional associations between dorsal stream and cerebellar networks in individuals with the expanded *FMR1* allele (Keri & Benedek, 2009).

In conclusion, this is the first study to use experimentally-driven measures of sequence learning to explore cerebellar involvement in cognitive and affective phenotypes previously reported in female PM-carriers. Our data show a clear slowing of symbolic SRTT performance which is reflective of a deficit in automaticity. We conclude that the cerebellum may play a critical role in a range of impairments in response inhibition, visuospatial function, and attentional and affective regulation in female PM-carriers of the *FMR1* gene. These data highlight the importance of identifying sensitive measures which may be useful indicators of at-risk profiles in females with the expanded *FMR1* allele.

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Supplementary results

Table e-1. Means and standard deviations for response time on the serial reaction time task for PM-carrier and control participants.

	PM-carrier (N=34) Mean ± SD	Control (N=33) Mean ± SD	t-value	*p*-value
Block 1	1026.27 ± 295.53	819.36 ± 180.61	3.596	.001**
Block 2	813.03 ± 216.98	657.67 ± 139.56	3.474	.001**
Block 3	743.82 ± 216.03	611.42 ± 144.98	2.937	.005*
Block 4	717.12 ± 228.70	562.58 ± 157.60	3.211	.002*
Block 5	702.94 ± 227.29	517.52 ± 162.55	3.831	<.001**
Block 6	666.97 ± 231.98	513.64 ± 180.48	3.013	.004*
Block 7	815.41 ± 184.83	695.64 ± 95.82	3.344	.002*
Block 8	763.21 ± 181.78	629.39 ± 125.76	3.573	.001**
Total time	11395.00 ± 2546.18	9474.24 ± 1325.28	3.889	<.001**

p<.001**, *p*<.01*

Table e-2. Means and standard deviations for errors on the serial reaction time task for PM-carrier and control participants.

	PM-carrier (N=34) Mean ± SD	Control (N=33) Mean ± SD	Mann-Whitney U	*p*-value
Block 1	.94 ± 1.25	.55 ± .75	492	.337
Block 2	.56 ± .75	1.15 ± 1.33	407	.037[a]
Block 3	.82 ± 1.14	1.06 ± 1.14	485	.309
Block 4	.79 ± 1.01	1.39 ± 1.41	403	.037[a]
Block 5	.88 ± 1.18	1.18 ± 1.33	498	.399
Block 6	.91 ± 1.03	1.61 ± 2.00	480	.283
Block 7	3.00 ± 2.51	3.45 ± 2.50	496	.411
Block 8	1.27 ± 1.29	1.12 ± 1.69	474	.251
Total Errors	8.94 ± 5.54	11.52 ± 8.42	469	.248

p < .05[a]

CHAPTER 7

GENERAL DISCUSSION

The overarching aim of this thesis was to investigate neurobehavioural profiles in adult female PM-carriers using hypothesis-driven neuromotor and neurocognitive measures that are known to be sensitive to subtle signs of dysfunction in prefrontal and cerebellar neural networks. This thesis also offers a systematic investigation into the importance of age- and CGG-repeat length to obtain a better understanding of the relative contribution of stable developmental manifestations of the *FMR1* gene, and changes arising from neurodegenerative processes. The findings from Chapter's 3 and 4 make an important contribution to the literature by suggesting that gait and postural control measures offer sensitivity to subtle *FMR1*-related changes, suggestive of dysfunction in cerebellar neuromotor networks. If validated longitudinally, these measures could be employed for tracking and monitoring treatment response in prospective pharmaceutical trials. A second contribution from this thesis is the novel identification of a possible cognitive-affective disorder in adult PM-carrier women. Although this would need validation, the present findings indicate that this is an early onset 'disorder' characterised by executive and/or cerebellar sequencing dysfunction. Importantly, this finding may explain the elevated rates of psychiatric problems found amongst female PM-carriers. This thesis chapter integrates the main findings, highlights theoretical implications for the understanding of the *FMR1* gene on brain function, and discusses their clinical implications. Finally, the limitations and future directions arising from this thesis will be discussed.

7.1. General overview

Chapter 3, which examined for the first time subtle spatiotemporal fluctuation of gait, offers two specific contributions. First, although there were no differences in baseline gait parameters between female PM-carriers and matched controls, females with the PM were found to have significantly reduced gait velocity and step length when performing a concurrent working memory task. This dual-task interference on the pace domain of gait is consistent with cortico-cerebellar, pre-frontal and higher level cortical involvement in gait (Boisgontier, et al., 2013; Hausdorff & Buchman, 2013; Woollacott & Shumway-Cook, 2002). The second finding was that CGG-repeat length moderated the relationship between

dysfunction (see Chapter 2), these findings suggest that subtle changes in the cortico-cerebellar networks may be present in female carriers with CGG-repeats within the upper PM range, possibly reflecting early signs of a late-onset neurodegenerative disorder.

Chapter 4 provided an investigation of sensorimotor functions and postural stability with several important findings. Firstly, female PM-carriers exhibited poorer proprioceptive awareness and slower reaction time when compared to matched control participants, although there was no evidence for any relationship with age- or CGG-repeat length. Secondly, PM-carriers showed increased mediolateral postural displacement only when performing a concurrent *excluded-letter-verbal-fluency* task under reduced sensory feedback. Consistent with the gait findings from Chapter 3, it was revealed that CGG-repeat length moderated the relation between age and dual-task mediolateral postural instability under reduced proprioceptive input. Given the well known role of the cerebellum in postural control mechanisms (Morton & Bastian, 2007), these findings are consistent with *FMR1* related disruption to cognitive and motor networks critical for postural stability.

Chapter 5 provides the first targeted investigation of the distinct sub-components of executive function that tie in a range of symptoms of anxiety, depression and ADHD. The main finding was that some female PM-carriers may present with an early onset, and relatively stable, cognitive-affective disorder. Importantly, when executive function profiles were teased apart in terms of response inhibition and working memory, executive dysfunction was significantly correlated with self-reported symptoms of ADHD inattentive symptoms, social anxiety and depression. Further, response inhibition and working memory deficits were highly likely to meet threshold criteria for probable caseness for ADHD (inhibition: 55.6%; working memory: 83.3%), social anxiety (inhibition: 58.3%; working memory: 100%) and depression (inhibition: 80%; working memory: 50%). These findings indicate that irrespective of age and the length of the CGG-repeat expansion, a core deficit in executive functioning may confer significant risk for symptoms of mental disorders in female PM-carriers. This raises the possibility that assessment of executive functioning may be useful for identifying a subgroup of female PM-carriers most likely to develop more severe anxious and depressive symptomatology.

Chapter 6 investigated motor sequencing through analyses of performance on the symbolic serial reaction time task (SRTT), and the associations between SRTT performance and cognitive-affective profiles in the female PM. The first finding was that when compared

to matched controls, female PM-carriers showed significantly slower SRTT performance, and poorer efficiency in detecting the repeating sequence suggestive of reduced automaticity. Importantly, even after simple motor reaction time was controlled for in the analyses, SRTT performance was correlated with a range of deficits in executive, visuospatial functioning and self-reported psychological symptoms in the female PM-carriers. Another important finding was that SRTT performance was significantly associated with working memory performance in female controls with normal alleles, but not in the female PM-carriers, suggestive of less recruitment of working memory systems to enhance performance in the female PM. This finding is consistent with the notion that early stages of performance involve engagement of working memory systems that in turn contribute to explicit awareness (Bo, Jennett, & Seidler, 2011). For PM-carriers, the findings from this chapter suggest that the presenting profile, in at least a subgroup of female PM-carriers, is one that resembles cerebellar cognitive-affective syndrome (CCAS) (Schmahmann, 2004; Schmahmann & Sherman, 1998a). The lack of age- and CGG-repeat length-related effects on SRTT performance and cognitive-affective impairments suggests that this profile may arise from molecular (e.g., FMRP, epigenetics) and environmental (psychosocial stress, carer burden) factors. Given the known role of cerebellum in motor sequencing (Molinari & Leggio, 2013; Tedesco, et al., 2011), these findings of a 'signature' female PM-specific weakness in executive functioning and psychological symptoms may well be of a cerebellar origin which disrupts connections to critical cortical regions.

7.2 Theoretical implications

7.2.1. Implications for the neural basis of neuromotor and neurocognitive profiles in female PM-carriers

The findings from this thesis converge to suggest cerebellar involvement in female PM-carriers, and for the first time, provide support for the contention that cerebellar dysfunction may tie in a range of deficits in executive, visuospatial and affective functioning in at least a subgroup with the PM allele. These findings are consistent with previous studies which have shown subtle neuromotor signatures and possible abnormalities in cortico-cerebellar connectivity (Chonchaiya, et al., 2010; Conde, et al., 2012; Narcisa, et al., 2011). The findings of this thesis are also in accordance with histological investigations showing high expression of FMRP in the cerebellum (Zangenehpour, Cornish, & Chaudhuri, 2009), and

neuroimaging investigations that have revealed structural volumetric abnormalities in the cerebellum in males with the full mutation (Greco et al., 2011; Wilson, Tregellas, Hagerman, Rogers, & Rojas, 2009), and dysfunction within cerebellar and cortico-cerebellar networks in older males with FXTAS (Wang, Hessl, Schneider, et al., 2013).

There are several possible explanations for specific neuronal consequences in cerebellar motor and cognitive networks which may show greater susceptibility to the *FMR1* gene expansion. First, through its regulatory role as a translational repressor protein, FMRP is required for regulating/repressing the protein synthesis implicated in post-synaptic Gp 1 mGluR1 and mGluR5 dependent long term depression (LTD), a form of neuroplasticity (Bhakar, Dolen, & Bear, 2012; Sidorov, Auerbach, & Bear, 2013). In FXS full mutation, the loss of FMRP is associated with poor control of protein synthesis subserving LTD, which can lead to internalisation of AMPA receptors, exaggerated mGluR-LTD signalling, and FXS-like behavioural and cognitive impairments (Bear, 2005). Developmental effects resulting from un-regulated LTD in the PM are supported by findings of aberrant dendritic growth and an upset to balances of GABA/Glutamate signalling in the cerebellum (Chen et al., 2010; D'Hulst et al., 2009). LTD processes play an important role in modulating the main cerebellar output through Purkinje cell activity (Shakkottai, et al., 2004). It could be speculated that slight to moderate reductions in FMRP may result in excessive LTD, and unregulated/spontaneous drive from cerebellum to connected cortical regions may occur. In this regard, Riluzole an FDA-approved neuroprotective agent shown to reduce deep cerebellar nuclei (DCN) hyperactivity may be useful (Ilg et al., 2013). Postmortem investigations of *FMR1* expression in the brains of patients previously diagnosed with major depression, schizophrenia, bipolar and autism also show significant reductions of FMRP in the cerebellum (Fatemi & Folsom, 2011; Fatemi, Folsom, Kneeland, & Liesch, 2011; Fatemi, Folsom, Rooney, & Thuras, 2013). This suggests that within cerebellar regions FMRP interacts and responds to alterations in other molecular pathways that have similar critical roles in synaptic function. Indeed, widespread overlap between many of the genes involved in autism and those regulated by FMRP has now been demonstrated (Steinberg & Webber, 2013). Furthermore, recent findings suggest that many of the genes regulated by FMRP are highly enriched in 5-Hydroxymethylcytosine (5-hmC) which is associated with epigenetic changes in the developing cerebellum (Wang et al., 2012). Together, these findings highlight the cerebellum as a region that is sensitive to alterations of FMRP translation and neurodevelopmental processes in PM affected individuals.

Recent imaging studies may provide valuable insights into the neural basis of motor and executive function impairments in female PM-carriers. These studies have shown associations between *FMR1* mRNA levels and disrupted structural connectivity of the middle cerebellar peduncles (MCPs) and superior cerebellar peduncles (SCPs) in males with and without FXTAS (Wang, Hessl, Schneider, et al., 2013). This may be associated with axonal swelling secondary to mitochrondrial dysfunction (Ross-Inta et al., 2010), or effects to the integrity of cellular walls arising from the sequestration of Lamin A and C isoforms (Arocena et al., 2005; Garcia-Arocena et al., 2010). Importantly, the *FMR1* gene appears to play a role in neural correlates of normal cognitive functioning in healthy adult males with normal alleles, with *FMR1* gene expression (as indexed by *FMR1* mRNA levels) significantly associated with the integrity of widespread structural white matter organisation (Wang, Hessl, Iwahashi, et al., 2013). The cerebellum is connected through widespread white matter tracts to several cortical regions (Stoodley & Schmahmann, 2010), and through mechanisms of diaschisis, damage in the cerebellum can lead to functional changes in connected cortical regions (Gold & Lauritzen, 2002; Sobesky et al., 2005). Indeed, a recent investigation with multiple system atrophy patients has demonstrated that white matter degeneration in the cerebellum can lead to reduced nodal efficiency between lobules within the cerebellum, and in network efficiency of connected cortical regions (Lu et al., 2013). These approaches which consider the influence of cerebellar dysfunction on brain network efficiency may be particularly important for understanding the development of FXTAS. As suggested by Battistella et al., (2013), the developmental effect of grey matter changes in the cerebellum of asymptomatic PM-carriers may reflect the *primum movens* for later structural network dysregulation leading to late-onset dementia and motor symptoms associated with FXTAS. The findings of this thesis showing subtle early onset cerebellar cognitive-motor profiles in the female PM support this contention, and in conjunction with other factors (i.e., epigenetic, psychosocial, caregiver burden and hormonal changes), may represent the earliest indicators for later neurodegenerative involvement in at least a subgroup of women with the PM allele.

7.2.2. Implications for neuromotor and neurocognitive pathways in female PM-carriers

The first pathway arising from this thesis is that of a possible cerebellar cognitive affective syndrome (CCAS) in the female PM (Schmahmann, 2004; Schmahmann & Sherman, 1998b). While this condition typically arises following acquired damage to posterior cerebellum, it

has also been implicated in neurodevelopmental disorders including schizophrenia, dyslexia and autism (Hoppenbrouwers, Schutter, Fitzgerald, Chen, & Daskalakis, 2008). The observed deficit in response inhibition and its association with slower response time for motor sequencing may implicate the fronto-ponto-cerebellar pathway which begins in orbitofrontal and prefrontal cortices, descends through the internal capsule, decussates at the pons and enters cerebellum through the MCPs (Kamali, et al., 2010). The finding that visuospatial difficulties were associated with the efficiency of motor sequencing implicates the parieto-ponto-cerebellar pathway, which reciprocally connects posterior parietal cortices with the cerebellum (Glickstein, 2000). Importantly, it is the early onset of subtle neurocognitive changes and the interrelationships between executive dysfunction, visuospatial difficulties and self-reported psychological symptoms that resemble in a milder form the cognitive-affective profile associated with cerebellar dysfunction. However, as there were no associations with age or CGG-repeat length in female PM-carriers, the CCAS may be influenced by variable penetrance from environmental, psychosocial or hormonal factors. It is also worth considering methylation of (*FMR1*) intron 1 CpG sites FREE1 and FREE2, as they have been shown to correlate significantly with FMRP, as well as *FMR1* antisense (or AS*FMR1* mRNA) previously implicated in mitochondrial function (Godler et al., 2011; Loesch et al., 2011). However, while the CpG sites correlated significantly with intellectual function in females with FXS, the relevance of CpG methylation for PM women is currently unclear (Godler et al., 2012). Nonetheless, CpG methylation offers, in conjunction with investigation of further environmental factors, significant potential for revealing the genetic factors underlying this cognitive-affective profile in females with the PM allele.

The second pathway in female PM-carriers appears to be associated with the effects from molecular events evident in the upper PM range to selective disruption of stepping automaticity and postural stability. In particular, the findings of reduced gait pace and postural stability under increased attentional load are suggestive of cerebellar or prefrontal involvement, or a combination of both (Hausdorff & Buchman, 2013; Morton & Bastian, 2004). Specifically, these findings indicate that female PM-carriers may employ additional attentional resources to compensate for underlying subcortical dysfunction (Boisgontier, et al., 2013). This implies a specific weakness in either the use or the selection of automatic motor components that normally operate outside conscious awareness (Boisgontier, et al., 2013). In light of previous research showing that *FMR1* mRNA −which is implicated in FXTAS and FXPOI−is positively associated CGG-repeat length (García-Alegría et al., 2007;

Peprah et al., 2010), these findings are consistent with poor automaticity in motor control as a possible early neural change preceding more severe neuropathology in PM-associated neurodegenerative disorders. However, these hypotheses for neurodegeneration must be considered with respect to further factors which may also be associated with FXTAS risk, including the presence of one apolipoprotein E epsilon4 (ApoE) allele (Silva et al., 2013), exposure to neurotoxins (Paul et al., 2010), hypertension (Hamlin et al., 2012), sleep apnea (Hamlin et al., 2011), migraine (Au et al., 2013) and autoimmune disorders (Winarni, et al., 2012).

One possible interpretation of these two pathways is that developmental mechanisms lead to disproportional impact upon vulnerable and/or late maturing brain regions, while molecular events evident in the upper CGG repeat range (i.e., *FMR1* mRNA toxicity) act in conjunction with environmental factors leading over time towards atypical development and an increased risk for late-onset degenerative disorders. A critical goal of this next generation of research will be to isolate distinct subgroups and sensitive risk biomarkers in the female PM that might portend more severe conditions associated with dementia and neurodegenerative decline.

7.3 Clinical implications

The most important clinical implication arising from this thesis is the identification of novel hypothesis-driven measures which offer potential for ascertaining which PM-carrier women are most at-risk for neuropsychiatric and neurodegenerative clinical impact. To date, there has been a paucity of appropriate measures employed in pharmaceutical trials with FXS families for tracking and monitoring treatment response. Although recent trials of Memantine, a drug used to treat Alzheimer disease, have employed the BDS and CATSYS as the primary outcome measures in patients with FXTAS (Ortigas et al., 2010; Seritan et al., 2013), these measures lack sensitivity to subtle motor and tremor symptoms in the early stages of disease manifestation (Aguilar et al., 2008). This thesis provides a new perspective for the development of sensitive outcome measures for use in future treatment interventions. The findings from this thesis suggest that the variability domain of gait, and postural sway under dual-task interference, may represent a sensitive risk marker for later neurodegenerative involvement in some women with *FMR1* PM. Furthermore, the findings highlight both sequence learning and executive function tasks as novel approaches to

identifying female PM-carriers most at-risk for developing psychiatric disorders (i.e., social anxiety, depression and ADHD). The length of the CGG-repeat transcript may also be useful in conjunction with other molecular measures (*FMR1* mRNA levels, FMRP) in identifying those most at-risk for further neurological involvement. Although beyond the scope of this thesis, future studies should examine neurocognitive and neuromotor profiles in women within the mid-size CGG-repeat range where there is an increased risk of developing FXPOI (Allen et al., 2007; Ennis, Ward, & Murray, 2006; Sullivan et al., 2005), and elevated mental and physical health problems (Roberts, et al., 2009; Seltzer et al., 2012).

7.4. Limitations

There are a number of limitations that require acknowledgment. Firstly, the neurobehavioural and neuromotor findings from this thesis should be confirmed in future imaging investigations using diffusion tensor imaging (DTI), and functional activation and connectivity analysis during imaging paradigms sensitive to *FMR1* gene expression. Secondly, due to the cross-sectional nature of the study design it remains to be determined whether core deficits in inhibitory control represent an early at-risk marker of more severe psychiatric dysfunction. The lack of a longitudinal design is a limitation in distinguishing those PM females at greatest risk of FXTAS and other neurodegenerative disorders. Thirdly, to distinguish between degenerative and developmental pathways in the female PM, prospective longitudinal studies using a range of epigenetic and genetic markers including *FMR1* mRNA levels, FMRP, X-inactivation, and FREE1 and FREE2 methylation patterns are warranted.

A further limitation relates to the small sample size and restricted CGG-repeat range of the female PM-carriers. It was therefore not possible to examine non-linear relationships between CGG-repeat length and neurobehavioural and neuromotor performance. Thus it is yet to be determined the extent to which CGG at-risk bands and sensitivity to life changes influence neurocognitive and affective symptoms (Seltzer et al., 2012). It is also possible that neurobehavioural and neuromotor impairments could be influenced by psychosocial stress or the presence of an affective disorder (although, as shown in appendix A this is unlikely to be the case). Future studies which include structured clinical interviews to explore these relationships in larger samples of females with the PM are clearly warranted.

7.5. Directions for future research

7.5.1 The importance of prospective longitudinal neuroimaging studies

Given that the ocular motor system is one of the most clearly understood neural circuits in the brain, future studies should utilise state-of-the-art imaging techniques with MRI-compatible ocular motor paradigms that enable examination of higher order cognitive control processes such as inhibition and working memory in females with the PM. As well as providing considerable practical advantages in terms of ease of measurement and analysis, eye movements are highly stereotyped and reproducible in response to specific stimuli, thereby permitting insights into patterns of abnormality that could provide important insights into the location and significance of subtle cognitive control impairments. Functional connectivity analysis in conjunction with ocular motor measures such as saccade accuracy and latency would provide a novel approach for investigating the neural correlates of cerebellar contributions to cognition (Baloh, Honrubia, & Sills, 1977; Pawlak-Osinska, Kazmierczak, & Kazmierczak, 2005). Future research examining the utility of these neural correlates would benefit from prospective longitudinal designs that may begin to disentangle the relative contribution of developmental and degenerative processes underlying the neural abnormalities in females with the *FMR1* premutation.

7.5.2. The role of molecular and hormonal factors

Sensitive molecular biomarkers that can accurately predict women most at-risk for clinical impact and most likely to respond to pharmaceutical treatment are now needed. While this thesis has examined only CGG-repeat length, there are further molecular parameters such as FMRP, X-inactivation patterns, *FMR1* mRNA and methylation of FREE1 and FREE2 sites, which may offer significant potential for informing clinicians about the risk of developing motor and/or cognitive-affective disorders in females with the PM allele. Future research should also systematically examine the relationships between FXPOI symptoms and neurobehavioural profiles. FXPOI is associated with abnormal hormonal levels including lowered anti-müllerian hormone and elevated follicle stimulating hormone which may have further impact upon hypothalamic-pituitary-adrenal axis and brain function (Hundscheid, Braat, Kiemeney, Smits, & Thomas, 2001; Rohr et al., 2008). Androgen levels, which are produced by both the ovaries and adrenal glands, also offer potential for explaining some

cognitive phenotypes in female PM-carriers. Indeed androgens appear to have neuroprotective properties (Pike, Carroll, Rosario, & Barron, 2009), and play an important role in cerebellar development (Dean & McCarthy, 2008). Future studies should also explore other factors ascribed a role in female PM neurobehavioural profiles, such as the interaction between CRHR1 polymorphoisms and carer stress which can influence levels of social anxiety (Hunter et al., 2012).

7.5.3. The importance of identifying at-risk cerebellar profiles

Sensory-motor adaptation paradigms combined with Transcranial Magnetic Stimulation (TMS) may offer an innovative approach to investigating cerebellar profiles in female PM-carriers. Regarding sensory-motor adaptation, both SCA pre-symptomatic gene carriers (Velazquez-Perez et al., 2009) and patients with cerebellar degeneration (Block & Bastian, 2012; Schlerf, Xu, Klemfuss, Griffiths, & Ivry, 2013) fail to appropriately adapt internal models required to successfully perform prism adaptation tasks. Of particular relevance to the findings of this thesis, imaging studies have shown posterior cerebellum and its connection with medial inferior parietal cortex (MIP) underlying performance of visuospatial adaptation tasks (Baizer, Kralj-Hans, & Glickstein, 1999; Prevosto, Graf, & Ugolini, 2010). However, while the prism adaptation task has demonstrated utility for elucidating regions of vulnerability within the cerebellum, it may also be useful for rehabilitation by exploring positive effects on cognition and visuospatial deficits through modulations to neuroplasticity. For example, prism adaptation training has been shown to enhance activity in brain regions important for spatial attention as well as improving performance on visuospatial neuropsychological tasks (Saj, Cojan, Vocat, Luaute, & Vuilleumier, 2013; Striemer & Danckert, 2010). Similarly, while TMS is useful for investigating structure-function relationships in the brain, studies have also highlighted it as a potential non-pharmaceutical treatment for psychiatric disorders, especially major depression (Berlim, Van den Eynde, & Jeff Daskalakis, 2013; Hovington, McGirr, Lepage, & Berlim, 2013). Given the potential of TMS as a sensitive risk biomarker and outcome measure of treatment effects, further studies should also explore TMS-related interventions in a larger cohort of female PM-carriers in conjunction with sophisticated imaging techniques.

7.6. Conclusion

This thesis has investigated neurobehavioural profiles in females with the PM allele across neurocognitive and neuromotor domains to reveal a subtle profile that is consistent with cerebellar involvement. While there has been controversy in past literature about the presence of deleterious phenotypic impact in female PM-carriers, the findings of impaired response inhibition, social anxiety and ADHD inattentive symptoms clearly demonstrates evidence for the involvement of the *FMR1* gene in women with the PM. Analysis of performance on tasks designed specifically to tap into cerebellar neurocognitive and neuromotor functions suggests that some women in this study may reflect an at-risk subgroup who may go on to develop later neurodegenerative symptoms. Further examination of what may reflect different pathways in the female PM is now needed to ascertain whether early cognitive symptoms represent a risk-factor for FXTAS and FXPOI. Given the high percentage of women in the general population and the fact that many also care for a child with FXS, there is a pushing need for greater understanding of the influence of the *FMR1* gene on neurodevelopmental and neurodegenerative processes in females with the PM allele.

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APPENDIX

Additional statistics were computed to ensure that the presence of a probable mental health disorder and carer burden did not impact upon the neuromotor findings from Chapter's 3 and 4. First, most of the results pertaining to slower gait under attentional interference remained when considering only those free from psychological symptoms (DT Vel 3CB: $U = 128$, z = -2.165, $p = .030$; DT Vel 7CB: $U = 131$, z = --2.088, $p = .037$; step length 3CB: $t(40) = -1.497$, $p = .142$; step length 7CB: $t(40) = -1.669$, $p = 1.03$) and without carer burden (DT Vel 3CB: $U = 62$, z = -2.076, $p = .038$; DT Vel 7CB: $U = 78$, z = -1.056, $p = .307$; step length 3CB: $t(33) = -1.942$, $p = .061$; step length 7CB: $t(33) = -.973$, $p = .337$). The findings of significantly greater postural displacement for PM-carriers compared to controls from chapter 4 also mostly remained after excluding those with probable psychological involvement (dual task-foam sway AP: $t(40)=2.099$, $p = .042$; dual-task foam sway ML: $t(40)=1.989$, $p = .054$) and those caring for a child with special needs (dual task-foam sway AP: $t(33)=1.458$, $p = .154$; dual-task foam sway ML: $t(33)=2.800$, $p = .008$).