Evidence for specific neurobehavioural signatures in male carriers of the *FMR1* premutation

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BPsych(Hons), MBMSci

A thesis in fulfilment of the requirements for the degree of

Doctor of Philosophy

School of Psychiatry
Faculty of Medicine

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Abstract 350 words maximum: (PLEASE TYPE)
Premutation (PM) expansions (55–200 CGG repeats) of the Fragile X Mental Retardation 1 (FMR1) gene confer risk for the development of fragile X-associated tremor ataxia syndrome (FXTAS), a neurodegenerative disorder affecting up to 45% of PM males aged over 50 years. Clinical and radiological features include intention tremor, gait ataxia, parkinsonism, cognitive dysfunction, diffuse white matter pathology and cerebral/cerebellar atrophy. The development of measures sensitive to the earliest indicators of FXTAS would have significant implications for determining risk, tracking progression, and monitoring treatment response. This thesis examined the interrelationships between neurobehavioural, radiological and FMR1 molecular measures among a final sample of 22 PM males (ages 26–80, seven with FXTAS) and 24 matched controls (ages 26–77). Comprehensive assessments included measures of cognitive function, psychiatric symptomatology, neuromotor function, structural brain imaging and FMR1 quantification. A number of clinical features including hearing loss, history of psychiatric disorder and psychomotor slowing were overrepresented among PM males compared to controls. Males with the PM also exhibited greater increases in motor symptom severity and greater reductions in cerebellar and subcortical volume with increasing age. Within the PM group, smaller thalamus and pallidum volumes were associated with poorer fine motor function. Significantly greater postural sway among PM males was associated with increasing CGG repeat length and decreasing cerebellar volume. The relationship between CGG repeat length and postural sway was mediated by a negative association between CGG repeat size and cerebellar volume. There was also preliminary evidence to suggest that reductions in cerebellar volume were associated with greater cognitive-motor interference of intra-individual variability in step width among PM males while performing a serial subtractions task. Further, increases in step width variability became more prominent among PM males with increasing age and CGG repeat length. Overall, this thesis provides strong evidence that subcortical pathology and CGG-related reductions in cerebellar volume contribute to specific decrements in neurobehavioural function among PM males. These findings have significant implications for guiding future research exploring the use of sensitive measures to track symptom progression and response to treatment.

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Acknowledgements

This thesis represents the end result of a journey so much greater than what could be captured between the covers of a book. It goes without saying that my experience would not have been nearly as rewarding without the diverse range of skills and extensive support made available to me through various aspects of my candidature.

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<tr>
<td>3D</td>
<td>Three-dimensional</td>
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<tr>
<td>ADHD</td>
<td>Attention deficit hyperactivity disorder</td>
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<td>ADLs</td>
<td>Activities of Daily Living</td>
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<td>AQ</td>
<td>Autism Quotient</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
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<tr>
<td>MANCOVA</td>
<td>Multivariate analysis of covariance</td>
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<tr>
<td>BDS-2</td>
<td>Behavioral Dyscontrol Scale 2</td>
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<td>COWAT</td>
<td>Controlled Oral Word Association Test</td>
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<td>CRST</td>
<td>Clinical Rating Scale for Tremor</td>
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<td>CoV</td>
<td>Coefficient of variation</td>
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<td>DASS</td>
<td>Depression</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>DSM-IV-TR</td>
<td>Diagnostic and Statistical Manual for Mental Health Disorders, 4th edition, Text Revised</td>
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<tr>
<td>DTC</td>
<td>Dual-task cost</td>
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<tr>
<td>FIRST</td>
<td>FMIRB’s Integrated Registration and Segmentation Tool</td>
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<tr>
<td>FLAIR</td>
<td>Fluid attenuation inversion recovery</td>
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<tr>
<td>FMR1</td>
<td>Fragile X Mental Retardation 1</td>
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<tr>
<td>FMRIB</td>
<td>Functional MRI of the Brain</td>
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<tr>
<td>FMRP</td>
<td>Fragile X Mental Retardation Protein</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>FSIQ</td>
<td>Full scale intelligence quotient</td>
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<td>FSL</td>
<td>FMRIB Software Library</td>
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<td>FX-</td>
<td>Premutation carrier without FXTAS</td>
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<td>FX+</td>
<td>Premutation carrier with FXTAS</td>
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<td>FXS</td>
<td>Fragile X syndrome</td>
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<td>FXTAS</td>
<td>Fragile X-associated tremor ataxia syndrome</td>
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<tr>
<td>GOLD</td>
<td>Genetics of Learning Disability Service</td>
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<tr>
<td>HC</td>
<td>Healthy control</td>
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<td>HDL</td>
<td>High-density lipoprotein</td>
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<tr>
<td>ICARS</td>
<td>International Cooperative Ataxia Rating Scale</td>
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<td>ICV</td>
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<td>LSAS-SR</td>
<td>Self report Liebowitz Social Anxiety Scale</td>
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<td>MCP</td>
<td>Middle cerebellar peduncles</td>
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<td>MCRI</td>
<td>Murdoch Childrens Research Institute</td>
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<td>MRI</td>
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<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
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<td>NART</td>
<td>National Adult Reading Test</td>
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<td>NESB</td>
<td>Non-English speaking background</td>
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<td>NeuRA</td>
<td>Neuroscience Research Australia</td>
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<td>NiL</td>
<td>Neuroimaging Lab, Centre for Healthy Brain Ageing, University of New South Wales</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
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<td>PBMC</td>
<td>Peripheral blood mononuclear cell</td>
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<td>PIQ</td>
<td>Performance IQ</td>
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<td>PM</td>
<td>Premutation</td>
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<td>RNA</td>
<td>Ribonucleic acid</td>
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<td>Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SEALS</td>
<td>South Eastern Area Laboratory Services</td>
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<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
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<tr>
<td>UPDRS</td>
<td>Unified Parkinson’s Disease Rating Scale</td>
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<tr>
<td>VIQ</td>
<td>Verbal IQ</td>
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<td>WAIS-III</td>
<td>Wechsler Adult Intelligence Scale, third edition</td>
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<tr>
<td>WASI</td>
<td>Wechsler Abbreviated Scale of Intelligence</td>
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<tr>
<td>WMH</td>
<td>White matter hyperintensity</td>
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<td>Wechsler Memory Scale, third edition</td>
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List of publications and presentations

Parts of this thesis have previously appeared in the following publications:

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Abstract

Premutation (PM) expansions (55–200 CGG repeats) of the Fragile X Mental Retardation 1 (FMR1) gene confer risk for the development of fragile X-associated tremor ataxia syndrome (FXTAS), a neurodegenerative disorder affecting up to 45% of PM males aged over 50 years. Clinical and radiological features include intention tremor, gait ataxia, parkinsonism, cognitive dysfunction, diffuse white matter pathology and cerebral/cerebellar atrophy. The development of measures sensitive to the earliest indicators of FXTAS would have significant implications for determining risk, tracking progression, and monitoring treatment response. This thesis examined the interrelationships between neurobehavioural, radiological and FMR1 molecular measures among a final sample of 22 PM males (ages 26–80, seven with FXTAS) and 24 matched controls (ages 26–77). Comprehensive assessments included measures of cognitive function, psychiatric symptomatology, neuromotor function, structural brain imaging and FMR1 quantification. A number of clinical features including hearing loss, history of psychiatric disorder and psychomotor slowing were overrepresented among PM males compared to controls. Males with the PM also exhibited greater increases in motor symptom severity and greater reductions in cerebellar and subcortical volume with increasing age. Within the PM group, smaller thalamus and pallidum volumes were associated with poorer fine motor function. Significantly greater postural sway among PM males was associated with increasing CGG repeat length and decreasing cerebellar volume. The relationship between CGG repeat length and postural sway was mediated by a negative association between CGG repeat size and cerebellar volume. There was also preliminary evidence to suggest that reductions in cerebellar volume were associated with greater
cognitive-motor interference of intra-individual variability in step width among PM males while performing a serial subtractions task. Further, increases in step width variability became more prominent among PM males with increasing age and CGG repeat length. Overall, this thesis provides strong evidence that subcortical pathology and CGG-related reductions in cerebellar volume contribute to specific decrements in neurobehavioural function among PM males. These findings have significant implications for guiding future research exploring the use of sensitive measures to track symptom progression and response to treatment.
CHAPTER 1.

INTRODUCTION
1.1 The Fragile X Mental Retardation 1 (FMR1) gene

Fragile X syndrome (FXS) is the most common form of inherited intellectual disability, affecting approximately 1 in 5000 males (Coffee, Keith, Albizua, Malone, Mowrey et al., 2009) and 1 in 8000 females in the general population (Crawford, Acuna, & Sherman, 2001). FXS is caused by large expansions (>200 repeats) in a polymorphic CGG trinucleotide repetitive sequence at the 5’ untranslated region of the Fragile X Mental Retardation 1 (FMR1) gene, located on the X chromosome (Xq27.3) (Verkerk, Pieretti, Sutcliffe, Fu, Kuhl et al., 1991). The FMR1 gene codes for the Fragile X Mental Retardation Protein (FMRP), which has been found to play an important role in the regulation of a number of neuronal processes associated with healthy brain development, including synaptic development and neuroplasticity (Huber, Gallagher, Warren, & Bear, 2002; Zalfa & Bagni, 2004). Full mutation FMR1 expansions cause aberrant methylation and transcriptional silencing of the gene, disrupting the production of FMRP (Verkerk et al., 1991). The absence of FMRP in the FMR1 full mutation results in the phenotype of FXS (Verkerk et al., 1991), characterised by behavioural and emotional characteristics (e.g. anxiety, autistic behaviours, attention deficit hyperactivity disorder (ADHD)); developmental signs (e.g. intellectual disability, difficulties with speech and communication), and physical characteristics (e.g. long and narrow face with prominent ears, hypotonia and hypermobility) (Hagerman, 2002).

In addition to full mutation expansions, CGG repeat sizes may be classified as being within the normal (6–44 CGG repeats), grey zone (45–54 CGG repeats) or
premutation range (55–200 CGG repeats) (Human Genetics Society of Australasia, 2012). Normal repeats are typically stable when transmitted from parent to offspring, with 29–30 repeats being the most commonly found alleles (as reviewed in Peprah, 2012). Grey zone alleles are also common in the general population, found in approximately 1 in 66 females and 1 in 112 males (Tassone, Iong, Tong, Lo, Gane et al., 2012). Approximately 1 in 209 females and 1 in 430 males (Tassone et al., 2012) carry premutation (PM) expansions of the FMR1 gene; unstable alleles that during maternal transmission may expand into the full mutation range in subsequent generations (Nolin, Brown, Glicksman, Houck, Gargano et al., 2003).

Unlike the full mutation, PM expansions are associated with increased transcription and elevated levels of FMR1 messenger ribonucleic acid (mRNA), and normal or slightly reduced levels of FMRP (Tassone, Hagerman, Taylor, Gane, Godfrey et al., 2000). Early investigations failed to identify a distinct clinical phenotype in PM males, although a subset of carriers with larger PM expansions and/or reduced production of FMRP had physical and behavioural characteristics consistent with mild FXS (Hagerman, Staley, O’Conner, Lugenbeel, Nelson et al., 1996; Loesch, Hay, & Mulley, 1994). However, in 2001, Hagerman et al. (2001) described a case series of five elderly PM males all of whom showed a distinct profile of motor and cognitive features. These individuals presented with cerebellar and Parkinsonian features including intention and resting tremor, bradykinesia, wide based gait and inability to tandem walk. Neuropsychological evaluation indicated impairments on the Wisconsin Card Sorting Task, a measure of executive function tapping abstract reasoning and set-shifting ability (Berg, 1948). Cognitive deficits were described as progressive and two cases met diagnostic criteria for dementia. It was proposed that
this progressive neurological syndrome, termed fragile X-associated tremor ataxia syndrome (FXTAS), represented a previously unrecognised phenotype associated with the PM caused by elevated production of FMRI mRNA (Hagerman, Leehey, Heinrichs, Tassone, Wilson et al., 2001).

The purpose of this chapter is to provide an overview of FXTAS including pathophysiology, treatment, and current systems for diagnosis and clinical staging. This is followed by a summary of key neurobehavioural and radiological findings associated with the PM, which will be expanded upon in subsequent specific chapters where relevant. An emphasis on the characterisation of neuropsychiatric, motor and radiological features among PM males, their interrelationships, and utility in early detection and intervention is developed, and this theme is revisited as an implication of findings in later chapters. Although FXTAS is known to affect both genders, the focus of this thesis is on PM males. As such, literature pertaining to PM females will only be reviewed where doing so enhances the understanding of findings among PM males.

1.2 Fragile X-associated tremor ataxia syndrome

FXTAS affects approximately 45% of PM males over 50 years of age and 8–16% of PM females over 40 years of age (Rodriguez-Revenga, Madrigal, Pagonabarraga, Xuncla, Badenas et al., 2009). The penetrance increases with age, such that approximately 17% of PM males in their 50s will have FXTAS, but this number rises to 38% in their 60s, 47% in their 70s and 75% of PM males in their 80s (Jacquemont, Hagerman, Leehey, Hall, Levine et al., 2004). The syndrome is
characterised by progressive development of intention tremor, cerebellar ataxia, parkinsonism, peripheral neuropathy, autonomic dysfunction, psychiatric symptomatology and cognitive decline (Apartis, Blancher, Meissner, Guyant-Maréchal, Maltête et al., 2012; Berry-Kravis, Goetz, Leehey, Hagerman, Zhang et al., 2007; Hagerman et al., 2001; Jacquemont, Hagerman, Leehey, Grigsby, Zhang et al., 2003; Juncos, Lazarus, Graves-Allen, Shubeck, Rusin et al., 2011; Loesch, Churchyard, Brotchie, Marot, & Tassone, 2005). Core radiological features on brain magnetic resonance imaging (MRI) include hyperintensities in the middle cerebellar peduncles (MCP) and splenium of the corpus callosum, increased white matter hyperintensity volume in the whole brain, and cerebral and cerebellar volume loss (Apartis et al., 2012; Brunberg, Jacquemont, Hagerman, Berry-Kravis, Grigsby et al., 2002; Cohen, Masyn, Adams, Hessl, Rivera et al., 2006; Jacquemont et al., 2003; Juncos et al., 2011; Renaud, Perriard, Coudray, Sévin-Allouet, Marcel et al., 2015). Generally, neurological symptoms are more frequent and severe in males compared to females, possibly due to the random activation of a normal X chromosome, the neuroprotective effect of oestrogen (Berry-Kravis, Potanos, Weinberg, Zhou, & Goetz, 2005; Hagerman, Leavitt, Farzin, Jacquemont, Greco et al., 2004; Horvath, Burkhard, Morris, Bottani, Moix et al., 2007; Jacquemont, Orrico, Galli, Sahota, Brunberg et al., 2005), or other, as yet unknown factors.

1.2.1 Pathophysiology

Characteristic neuropathological changes associated with FXTAS include ubiquitin-positive intranuclear inclusions in neurons and astrocytes throughout the central and peripheral nervous systems (with the highest load found in the hippocampus), which
may also extend to the reproductive and neuroendocrine systems (Gokden, Al-Hinti, & Harik, 2009; Greco, Berman, Martin, Tassone, Schwartz et al., 2006; Greco, Hagerman, Tassone, Chudley, Del Bigio et al., 2002; Greco, Soontrapornchaisri, Wirojanan, Gould, Hagerman et al., 2007; Hunsaker, Greco, Spath, Smits, Navarro et al., 2011; Louis, Moskowitz, Friez, Amaya, & Vonsatell, 2006). Broadly distributed white matter disease may also be evident on post-mortem examination including loss of axons and myelin, mild to moderate cortical atrophy, perivascular widening, spongiosis of the MCP and white matter, and variable degrees of glial and Purkinje cell loss (Greco et al., 2006). The observation of significantly elevated levels of FMR1 mRNA and normal or slightly reduced levels of FMRP described in the PM (Kenneson, Zhang, Hagedorn, & Warren, 2001; Tassone, Hagerman, Taylor, Gane, et al., 2000) informed the development of the ribonucleic acid (RNA) toxicity pathogenic model for FXTAS (Hagerman et al., 2001). According to this model, RNA toxicity occurs as a result of expanded CGG repeats sequestering RNA binding proteins. Sequestration of these proteins interrupts their normal functions, and may lead to decreased cell viability or cell death (Galloway & Nelson, 2009; Jin, Duan, Qurashi, Qin, Tian et al., 2007; Sellier, Freyermuth, Tabet, Tran, He et al., 2013; Sellier, Rau, Liu, Tassone, Hukema et al., 2010; Sofola, Jin, Qin, Duan, Liu et al., 2007). Exactly which proteins are affected, and the downstream effects of interruption to their processes on clinical phenotypes, has yet to be completely understood (Hagerman, 2012). Similarly, the mechanism underlying the formation of intranuclear inclusions, the neuropathological hallmark of FXTAS, remains unknown. Post-mortem studies suggest that the percentage of intranuclear inclusions in neurons and astrocytes in the central nervous system correlates with CGG repeat expansion size (Greco et al., 2002). Moreover, FMR1 mRNA and RNA binding
proteins have been detected in inclusions in human brain tissue (Iwahashi, Yasui, An, Greco, Tassone et al., 2006; Tassone, Iwahashi, & Hagerman, 2004), further implicating RNA toxicity in the pathogenesis of FXTAS.

Our understanding of the pathophysiology of FXTAS is complicated by the incomplete penetrance, suggesting that currently unknown biological or environmental protective factors may mitigate risk in some PM carriers (Hagerman, 2012). Further, evidence of early neurodevelopmental effects in CGG knock-in mouse models of the PM, including changes in neuronal migration and differentiation during embryonic development (Cunningham, Martínez Cerdeño, Navarro Porras, Prakash, Angelastro et al., 2011), indicate that PM-associated effects on the central nervous system may occur much earlier than clinical manifestations of FXTAS are observed. Indeed, early neurobehavioural signs have been described in the CGG knock-in mouse model, including evidence of deficits in spatial processing (Hunsaker, Wenzel, Willemsen, & Berman, 2009) and motor function (Hunsaker, von Leden, Ta, Goodrich-Hunsaker, Arque et al., 2011). It therefore remains unclear whether FXTAS represents the later stages of progressive neural dysregulation associated with the PM (possibly beginning in late embryonic stages); or whether both neurodevelopmental and neurodegenerative effects of the PM reflect different trajectories of the PM across the lifespan (Hagerman, 2012). While a number of studies have begun to explore age-related trajectories of cognitive (Cornish, Hocking, Moss, & Kogan, 2011; Cornish, Li, Kogan, Jacquemont, Turk et al., 2008; Hunter, Sherman, Grigsby, Kogan, & Cornish, 2012), motor (Kraan, Hocking, Georgiou-Karistianis, Metcalfe, Archibald, Fielding, Bradshaw et al., 2014; Kraan, Hocking, Georgiou-Karistianis, Metcalfe, Archibald et al., 2013), and radiological
features (Battistella, Niederhauser, Fornari, Hippolyte, Perrin et al., 2013; Hashimoto, Srivastava, Tassone, Hagerman, & Rivera, 2011; Wang, Hessl, Schneider, Tassone, Hagerman et al., 2013; Wang, Hessl, Hagerman, Tassone, Rivera et al., 2012), existing studies are typically focused on a limited selection of neurobehavioural and/or radiological features. An integrative approach utilising multimodal measures would allow for a more complete examination of age-related trajectories associated with the PM across multiple neuropsychiatric, motor, radiological and genetic levels.

1.2.2 Treatment

Currently there is no treatment targeting the pathophysiological mechanisms underlying FXTAS. Treatment is symptomatic, addressing specific motor, cognitive or psychiatric signs (Hall, Berry-Kravis, Hagerman, Hagerman, Rice et al., 2006). A randomised controlled trial exploring treatment efficacy of Memantine, a glutamate receptor antagonist approved for use in the management of Alzheimer’s Disease, suggested no significant improvement in intention tremor or executive function (Seritan, Nguyen, Mu, Tassone, Bourgeois et al., 2014) and limited benefit for language and memory function (Hall, Birch, Anheim, Jonch, Pintado et al., 2014; Yang, Niu, Simon, Chen, Seritan et al., 2013) compared to placebo. It has been suggested that other therapeutic modalities may improve motor, cognitive or psychiatric symptoms (reviewed in Hagerman, Hall, Coffey, Leehey, Bourgeois et al., 2008), although these have not been the subject of clinical trials. Potentially beneficial interventions include cholinesterase inhibitors, levodopa, antidepressants, antipsychotics, N-methyl-D-aspartate (NMDA) receptor antagonists, dietary
supplements and aerobic exercise (Hagerman et al., 2008). Current clinical best practice involves implementing individual treatment programs comprising a combination of these management strategies (Hagerman, Berry-Kravis, Kaufmann, Ono, Tartaglia et al., 2009; Polussa, Schneider, & Hagerman, 2014). A greater understanding of the pathogenic mechanisms of FXTAS (e.g. CGG expansion, mRNA toxicity, protein dysregulation) and their relationships to clinical manifestations is required to inform the development of targeted disease-modifying therapies.

1.2.3 Diagnosis and clinical staging

Diagnostic criteria for FXTAS (Table 1.1) describing core clinical and radiological features were formulated in 2003 based on a study of 20 PM males presenting with at least one clinical sign (intention tremor, gait ataxia) and white matter lesions in the MCP (Jacquemont et al., 2003). Using these criteria, individuals were classified as having ‘definite’, ‘probable’, or ‘possible’ FXTAS according to clinical and radiological signs. Neuropathological features were subsequently included and may be used to make diagnoses of FXTAS where post-mortem brain tissue is available (Hagerman & Hagerman, 2004a). Most recently, diagnostic criteria were updated to include white matter lesions in the splenium of the corpus callosum as a major radiological criterion, and neuropathy was added as a minor clinical criterion (Apartis et al., 2012; Hall et al., 2014). Criteria were also extended to include not only carriers of the premutation, but also the rare situations in which FXTAS develops in individuals with grey zone and full mutation expansions (Hall et al., 2014).
While the development of a diagnostic classification system for FXTAS has certainly proven useful in clinical and research practice (for example, raising clinical awareness and increasing reliability of diagnoses), there are several issues that should be considered when classifications are based on a hierarchical combination of cross-sectional symptoms. First, the current system does not allow for the recognition or characterisation of possible prodromal features among PM carriers. Currently the earliest indicators of FXTAS and underlying pathological mechanisms remain largely unknown; yet the capacity to identify those most at risk or in the earliest stages of disease would afford the opportunity for earlier intervention as treatments for FXTAS become available. Secondly, the examination of possible phenotypes among ‘asymptomatic’ PM carriers based on these criteria is challenging and potentially misleading, as a proportion of these individuals may in fact be exhibiting subtle signs that may progress to FXTAS in later years. Finally, the hierarchical approach and greater emphasis on primary motor/imaging features, which formed the inclusion criteria for the original case-series upon which the diagnostic criteria were established, may lack sensitivity for classification of cases dominated by neuropsychiatric, rather than motor dysfunction.
Table 1.1

*Diagnostic criteria for FXTAS* (Hagerman & Hagerman, 2004a; Hall et al., 2014; Jacquemont et al., 2003)

<table>
<thead>
<tr>
<th>Examination</th>
<th>Degree</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiological</td>
<td>Major</td>
<td>MRI white matter lesions in MCPs and or brain stem</td>
</tr>
<tr>
<td></td>
<td>Major</td>
<td>MRI white matter lesions in the splenium of the corpus callosum</td>
</tr>
<tr>
<td></td>
<td>Minor</td>
<td>MRI white matter lesions in cerebral white matter</td>
</tr>
<tr>
<td></td>
<td>Minor</td>
<td>Moderate-to-severe generalised atrophy</td>
</tr>
<tr>
<td>Clinical</td>
<td>Major</td>
<td>Intention tremor</td>
</tr>
<tr>
<td></td>
<td>Major</td>
<td>Gait ataxia</td>
</tr>
<tr>
<td></td>
<td>Minor</td>
<td>Parkinsonism</td>
</tr>
<tr>
<td></td>
<td>Minor</td>
<td>Neuropathy</td>
</tr>
<tr>
<td></td>
<td>Minor</td>
<td>Moderate-to-severe short-term memory deficiency</td>
</tr>
<tr>
<td></td>
<td>Minor</td>
<td>Executive function deficit</td>
</tr>
</tbody>
</table>

Diagnostic categories:

**Definite:**

a) One major clinical + one major radiological sign, or

b) One major clinical sign + presence of intranuclear neuronal and astrocytic inclusions on post-mortem examination of brain tissue

**Probable:**

a) One major radiological sign + one minor clinical symptom, or

b) Two major clinical symptoms

**Possible:**

a) One major clinical + one minor radiological sign

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*Inclusion criteria= FMR1* grey zone, premutation or full mutation

An alternative approach to conventional diagnostic criteria is clinical staging.

Clinical staging is a heuristic framework for diagnosis, recognising the importance of early identification of subclinical symptoms in providing opportunities for earliest intervention, with the ultimate aim of forestalling or even preventing disease progression. This approach is most widely applied to cancer staging (e.g. the TNM
classification system for malignant tumours, Edge & Compton, 2010). However, its utility to mental health research and practice is being increasingly recognised, for example, in mood and psychotic disorders (McGorry, Hickie, Yung, Pantelis, & Jackson, 2006; McGorry, Killackey, & Yung, 2008; Scott, Leboyer, Hickie, Berk, Kapczinski et al., 2013; Wood, Yung, McGorry, & Pantelis, 2011; Yung & McGorry, 2007). A seven-point clinical staging scale for FXTAS has been developed whereby individuals are assigned a score according to the severity of motor symptoms (Bacalman, Farzin, Bourgeois, Cogswell, Goodlin-Jones et al., 2006). These clinical stages are defined as follows: 0 (normal); 1 (subtle or questionable signs (i.e., subtle tremor and/or mild balance problems) but no interference with activities of daily living (ADLs)); 2 (minor, but clear tremor and/or balance problems producing minor interference with ADLs); 3 (moderate tremor and/or balance problems and at least occasional falls); 4 (severe tremor and/or balance problems requiring the use of cane or walker); 5 (uses wheelchair on a daily basis); 6 (bedridden). Although this scale incorporates motor symptom severity, walking capacity, and impact of motor symptoms on ADLs, it is commonly used in conjunction with conventional FXTAS diagnostic criteria, and is therefore subject to similar limitations. Importantly, the scale is not sensitive to the emergence of cognitive or psychiatric symptoms at any point in the disease process, yet the presence of these features would likely impact upon quality of life (Brega, Reynolds, Bennett, Leehey, Bounds et al., 2009). Further, the scale does not incorporate potential subthreshold features among asymptomatic carriers which may represent the earliest markers of later decline.
An alternative approach, such as that used in similar disorders including spinocerebellar ataxia type 7 (SCA7), may provide a useful heuristic framework on which to characterise neurobehavioural features in the PM. SCA7 is a neurodegenerative trinucleotide (CAG) expansion disorder characterised by progressive cerebellar and retinal degeneration (Enevoldson, Sanders, & Harding, 1994). A four stage model for SCA7 has been proposed as follows: Stage 0 (gene-positive, asymptomatic, with normal physiology (deep tendon reflexes and/or electroretinogram)); Stage 1 (asymptomatic, with abnormal physiology); Stage 2 (symptomatic, with mild disease and slow, variable progression); and Stage 3 (rapid clinical progression) (Horton, Frosch, Vangel, Weigel-DiFranco, Berson et al., 2013). This approach facilitates the dynamic assessment of symptom severity and change over time, considering a range of clinical involvement including features within asymptomatic stages. Asymptomatic stages (0 and 1) are delineated by abnormal performance on physiological measures that have been shown to be sensitive to both early detection and progression of symptoms (Horton et al., 2013). A similar approach could be applied to PM carriers at risk of FXTAS once equivalent markers (e.g. neurobehavioural or radiological) sensitive to possible prodromal features and symptom progression are identified.

1.3 Evidence for specific neurobehavioural signatures in FMR1 premutation carriers

Investigations of neurobehavioural features among PM carriers suggest a spectrum of clinical involvement, including but not limited to features characteristic of FXTAS. An overview of key findings from studies exploring neurobehavioural
(motor, cognitive, psychiatric) features among PM carriers is provided here. These studies raise a number of methodological and conceptual issues which will be discussed in greater detail in subsequent chapters as indicated.

Motor signs associated with FXTAS typically manifest from the age of 50 (Jacquemont et al., 2004) and may include intention tremor (increasing in amplitude toward the end point of a movement), and cerebellar ataxia (wide-based, unsteady gait), with less pronounced signs of parkinsonism (rigidity, bradykinesia, hypomimia, resting tremor) (Jacquemont et al., 2003; Leehey, Berry-Kravis, Goetz, Zhang, Hall et al., 2008). There is also evidence to suggest that motor signs may be discernible among PM carriers who do not meet diagnostic criteria for FXTAS including decrements in finger tapping, reaction time, proprioception and postural control relative to matched controls (Hocking, Kraan, Godler, Bui, Li et al., 2015; Kraan, Hocking, Georgiou-Karistianis, Metcalfe, Archibald, Fielding, Bradshaw, et al., 2014; Kraan et al., 2013; Narcisa, Aguilar, Nguyen, Campos, Brodovsky et al., 2011; O'Keefe, Dunn, Berry-Kravis, Ouyang, & Hall, 2012; O'Keefe, Robertson-Dick, Dunn, Li, Deng et al., 2015). These studies, which will be reviewed in greater detail in the Introductions to Chapters 7 and 8, have highlighted a need for the development of highly sensitive gait and postural control measures to capture the earliest markers of symptom onset.

Earlier in the candidacy the author published a systematic review examining the neuropsychiatric phenotype of FXTAS (Birch, Cornish, Hocking, & Trollor, 2014; Appendix A). Although a number of comprehensive reviews describing neuropsychiatric features of FXTAS have been published (e.g. Bourgeois, Coffey,
Rivera, Hessl, Gane et al., 2009; Grigsby, Cornish, Hocking, Kraan, Olichney et al., 2014; Hagerman & Hagerman, 2004b), this was the first review incorporating a systematic approach, with strict inclusion criteria and careful consideration of potential sample overlap between published reports. Findings of the systematic review suggested that cognitive features associated with FXTAS include poorer performance on measures of executive function (Brega, Goodrich, Bennett, Hessl, Engle et al., 2008; Cornish, Kogan, Li, Turk, Jacquemont et al., 2009; Cornish et al., 2008; Grigsby, Brega, Engle, Leehey, Hagerman et al., 2008; Grigsby, Brega, Leehey, Goodrich, Jacquemont et al., 2007; Schneider, Ballinger, Chavez, Tassone, Hagerman et al., 2011; Wang, Hessl, Iwahashi, Cheung, Schneider et al., 2013; Yang, Chan, Khan, Schneider, Nanakul et al., 2013; Yang, Simon, Niu, Bogost, Schneider et al., 2013), working memory (Brega et al., 2008; Cornish et al., 2009; Cornish et al., 2008; Grigsby et al., 2008; Grigsby et al., 2007; Hashimoto, Javan, Tassone, Hagerman, & Rivera, 2011; Schneider et al., 2011; Yang, Chan, et al., 2013), information processing speed (Brega et al., 2008; Grigsby et al., 2008; Grigsby et al., 2007; Schneider et al., 2011; Yang, Chan, et al., 2013), and fine motor function (Grigsby et al., 2008; Schneider et al., 2011; Wang, Hessl, Schneider, et al., 2013) relative to matched controls. Subtle deficits in executive function (Cornish et al., 2011; Cornish et al., 2008; Hunter et al., 2012; Kraan, Hocking, Georgiou-Karistianis, Metcalfe, Archibald, Fielding, Trollor et al., 2014), working memory (Cornish et al., 2009; Kogan & Cornish, 2010) and visuospatial processing (Goodrich-Hunsaker, Wong, McLennan, Srivastava, Tassone et al.; Hocking, Kogan, Cornish, Hocking, Kogan et al., 2012; Wong, Goodrich-Hunsaker, McLennan, Tassone, Harvey et al., 2012) may also be discernible among PM males and females who do not meet diagnostic criteria for FXTAS. Additional decrements in verbal
memory and motor sequence learning have been observed among PM males without FXTAS (Grigsby et al., 2008; Hippolyte, Battistella, Perrin, Fornari, Cornish et al., 2014). Although deficits in cognitive function among PM males without FXTAS are not consistently found (Hunter, Abramowitz, Rusin, & Sherman, 2009; Hunter, Allen, Abramowitz, Rusin, Leslie et al., 2008), there is mounting evidence to suggest that subtle deficits in cognitive function may in fact precede the onset of tremor and ataxia. Further detailed review of cognitive features associated with the PM is undertaken in the Introduction to Chapter 4.

Psychiatric features frequently described among PM males with FXTAS include increased rates of depression, anxiety and obsessive-compulsive symptoms (Adams, Adams, Nguyen, Hessl, Brunberg et al., 2010; Bacalman et al., 2006; Bourgeois et al., 2009; Bourgeois, Seritan, Casillas, Hessl, Schneider et al., 2011). Additional features may include agitation, aggression, irritability and disinhibition (Bacalman et al., 2006). Similarly, increased rates of psychiatric symptoms, which may represent early markers of neurodegenerative diseases such as Parkinson’s Disease (Shiba, Bower, Maraganore, McDonnell, Peterson et al., 2000) and Huntington’s Disease (Duff, Paulsen, Beglinger, Langbehn, & Stout, 2007), have also been reported among PM males and females without FXTAS. These include obsessive-compulsive symptoms (Dorn, Mazzocco, & Hagerman, 1994; Hessl, Tassone, Loesch, Berry-Kravis, Leehey et al., 2005), social phobia (Bourgeois et al., 2011), depressive symptoms (Johnston, Eliez, Dyer-Friedman, Hessl, Glaser et al., 2001), schizotypal features (Sobesky, Hull, & Hagerman, 1994), and abnormalities in social cognition (Cornish, Kogan, Turk, Manly, James et al., 2005). Literature examining psychiatric
manifestations among PM will be reviewed in greater detail in the Introduction to Chapter 3.

A number of associations between \textit{FMR1} molecular measures and neurobehavioural features have been described among PM carriers. Evidence suggesting effects of elevated \textit{FMR1} mRNA and reductions in FMRP on psychiatric symptoms (Hessl et al., 2005; Hessl, Wang, Schneider, Koldewyn, Le et al., 2011; Koldewyn, Hessl, Adams, Tassone, Hagerman et al., 2008), as well as negative associations between CGG repeat length and cognitive performance (Cohen et al., 2006; Cornish et al., 2011; Cornish et al., 2009; Grigsby, Brega, Jacquemont, Loesch, Leehey et al., 2006; Hessl et al., 2005; Hocking et al., 2012; Hunter et al., 2012; Kogan & Cornish, 2010; Sevin, Kutalik, Bergman, Vercelletto, Renou et al., 2009), will be discussed in the Introduction to Chapters 3 and 4 respectively. Studies suggesting detrimental effects of increasing CGG repeat length (Allen, Juncos, Letz, Rusin, Hamilton et al., 2008; Apartis et al., 2012; Grigsby et al., 2006; Kraan, Hocking, Georgiou-Karistianis, Metcalfe, Archibald, Fielding, Bradshaw, et al., 2014; Kraan et al., 2013; Leehey et al., 2008; Tassone, Adams, Berry-Kravis, Cohen, Brusco et al., 2007) and \textit{FMR1} mRNA (Hocking et al., 2015) on neuromotor function will be reviewed in Chapters 7 and 8. Collectively these findings point to possible roles of CGG and RNA toxicity, as well as reductions in FMRP, in neurobehavioural manifestations of the PM.
1.3.1 Neural correlates

Neurobehavioural features of FXTAS are consistent with a dysexecutive syndrome and indicative of disruption to fronto-subcortical and cortico-cerebellar neural networks (Bacalman et al., 2006; Brega et al., 2008). Alexander and colleagues (1986) proposed that three segregated fronto-subcortical loops are involved in cognitive processing and affect (Alexander, DeLong, & Strick, 1986). The dorsolateral prefrontal, lateral orbitofrontal, and anterior cingulate loops are thought to modulate executive functions, social cognition and volition, respectively (Alexander & Crutcher, 1990). Originating in the frontal lobes, these circuits share common structures including the striatum (caudate and putamen), and comprise a circuit of afferent and efferent fibres with open connections projecting through thalamic nuclei to multiple regions of the cerebral cortex (Alexander et al., 1986; Middleton & Strick, 2000). It is now recognised that the cerebellum, previously thought to subserve only motor function, also plays an important role in the regulation of non-motor systems (Bernard, Peltier, Wiggins, Jaeggi, Buschkuehl et al., 2013; Bernard, Seidler, Hassevoort, Benson, Welsh et al., 2012; Schmahmann & Sherman, 1998; Strick, Dum, & Fiez, 2009). Schmahmann et al. (1998) proposed that cerebellar pathology may disrupt circuits between the cerebellum and cerebral cortices (including prefrontal, temporal and parietal), giving rise to a ‘cerebellar cognitive affective syndrome’ characterised by deficits in executive function, working memory, and visuospatial processing, in addition to blunted affect and disinhibition (Schmahmann & Sherman, 1998).
A number of structural and functional brain changes have been observed in multiple regions involved in fronto-subcortical and cortico-cerebellar pathways in PM carriers with and without FXTAS. There is also evidence to suggest that among PM males, structural and functional brain changes may be associated with *FMR1* molecular measures including greater CGG repeat length and elevated *FMR1* mRNA levels. These findings will be discussed in the Introduction to Chapter 5. Among PM males, some specific observations which link particular brain regions with signs and symptoms have also been made. For example, atrophy in the orbitofrontal cortex, cerebellar lobules VI/VII, vermis (Hashimoto, Javan, et al., 2011), thalamus, putamen and left caudate (Wang, Hagerman, & Rivera, 2013) as well as white matter pathology in the MCP and fornix (Hashimoto, Srivastava, et al., 2011) have been associated with greater severity of FXTAS symptoms. These findings, which will be discussed in more depth in the Introduction to Chapter 5, suggest a complex interplay between molecular factors associated with the PM, structural and functional integrity of brain regions implicated in fronto-subcortical and cortico-cerebellar circuits, and impairments in associated processes among PM carriers with and without FXTAS.

**1.4 Research questions and thesis overview**

This thesis explores neurobehavioural features among adult PM males with and without FXTAS. The systematic review published by the author earlier in the candidacy (Birch et al., 2014; see Appendix A) identified a need for more rigorous studies exploring cognitive and psychiatric manifestations among PM carriers. In particular, it was highlighted that there is a need for additional studies with
independent PM cohorts using comprehensive multimodal measures (e.g. cognitive, motor, psychiatric, MRI) and the inclusion of appropriate controls as comparison groups. Although a number of studies have examined neurobehavioural and radiological features among PM males, few have incorporated an integrative approach across multiple neuropsychiatric, motor, radiological and genetic levels. For example, subcortical volume loss has been associated with greater severity of motor symptoms (Wang, Hagerman, et al., 2013), but the relationship between subcortical pathology and cognitive features has not yet been explored. Finally, little is known about the earliest indicators of gait and postural dysfunction, core features of neurodegenerative decline associated with FXTAS. This thesis aims to address these gaps using data collected from a recently established Australian cohort of adult PM males with and without FXTAS, incorporating comprehensive multimodal assessments of cognitive, motor and psychiatric function together with structural brain MRI and \( FMR1 \) molecular measures (CGG repeat length and \( FMR1 \) mRNA levels).

The overarching methodology, including participant recruitment, measures and descriptions of data extraction processes are described in Chapter 2. This is followed by a description of clinical (physical and mental health), cognitive, motor and radiological characteristics of the cohort. Due to the vast array of measures, cohort characteristics are presented across three chapters (3, 4 and 5) to enhance readability. Although largely descriptive, detailed cohort characteristics are provided for three main reasons: (i) to provide relevant information on demographic and medical characteristics that may impact performance on neurobehavioural measures; (ii) to comprehensively characterise neurobehavioural and radiological features among this
newly established cohort, allowing for comparison with previously published samples; and (iii) to identify specific neurobehavioural and radiological features that will form the focus of more targeted investigations in subsequent chapters. Chapter 3 provides a review of clinical features among PM males and describes demographic, lifestyle, physical and mental health characteristics of the cohort included in this thesis. Possible associations between FMR1 molecular measures (CGG repeat length, mRNA levels) and current psychiatric symptoms are also explored. Chapter 4 includes a review of cognitive features among PM males, and goes on to describe cognitive features among this cohort. This includes a cross-sectional examination of cognitive performance with increasing age. Associations between cognitive features and FMR1 molecular measures among PM males are also explored. Chapter 5 includes a review of key findings relating to motor and radiological features among PM males, and an examination of age-related changes in motor and radiological features among this cohort. The relationships between FMR1 molecular measures and these features are also explored. Chapter 6 examines the associations between specific subcortical volumes (thalamus, caudate, putamen, pallidum) and performance on cognitive measures identified in Chapter 4 as being impaired in PM males compared to controls. Chapter 7 explores the influence of cerebellar volume on changes in postural control. Specifically, these analyses aim to examine postural sway (maximal anterior-posterior and medio-lateral displacements) during perturbation of visual and proprioceptive input in PM males, and to explore the interrelationships between postural sway, cerebellar volume, CGG repeat length, and FMR1 mRNA levels. Chapter 8 investigates the effects of varying degrees of cognitive load on specific spatiotemporal gait characteristics. The relationships between gait function, cerebellar volume, and FMR1 molecular measures are also
explored. Finally, Chapter 9 integrates the findings arising from the thesis, includes a discussion of possible clinical and research implications of the results, considers the limitations of the study and suggests directions for future research.
CHAPTER 2.

OVERARCHING METHODOLOGY
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2.1 Participants

This study sought to establish and comprehensively characterise a representative cohort of adult PM males. Twenty five PM males (ages 26–80) and 25 age- and education-matched controls with normal FMR1 alleles (ages 26–77) were recruited into the study. PM males were recruited Australia-wide by mail-out and advertisements through the Genetics of Learning Disability Service (GOLD, Hunter Genetics, NSW), the Victorian Clinical Genetics Service (Royal Children’s Hospital, Vic), the Fragile X Alliance Inc., and the Fragile X Association of Australia. Control participants were recruited from the general population by advertisements. Exclusion criteria for all participants included a history of enduring psychotic illness or neurological disorder other than FXTAS, current alcohol and other drug abuse, and progressive malignancy. Additional exclusion criteria for controls included self-reported symptoms of tremor or gait ataxia, current psychiatric disorder, and family history of fragile X-associated disorders. Three PM males were subsequently excluded due to PM/full mutation (>200 CGG repeats) mosaicism. One control was excluded due to cognitive dysfunction resulting from a vitamin B12 deficiency. This resulted in a final sample of 22 PM males and 24 controls. Informed consent was obtained from all participants and all procedures followed were in accordance with ethical requirements of the National Statement on Ethical Conduct in Human Research. Ethics approval for the study was obtained from Human Research Ethics Committees based at the University of New South Wales (approval number HC10311), Hunter New England Health (approval number 11/02/16/5.02), New
South Wales Institute of Psychiatry (approval number 052), and Monash University (approval number 10147B).

2.2 Materials and methods

2.2.1 Questionnaires

As with any new cohort it is important to establish baseline characteristics including demographic and relevant health information that may impact performance on neurobehavioural measures. Detailed information including participant demographics and medical history was obtained from self-report questionnaires (for a summary of data points, see Appendix B). Demographic items included date and country of birth, ethnicity, relationship status, education, primary language spoken at home and income. History of stroke, transient ischemic attack, cardiovascular disorder, thyroid problems, autoimmune conditions, seizures, neurodevelopmental disorders, psychiatric disorder, hearing loss, subjective cognitive complaints, smoking, alcohol and other substance use, and current medications were obtained by self-report. Given recent evidence to suggest higher prevalence of migraine in the PM (Au, Akins, Berkowitz-Sutherland, Tang, Chen et al., 2013), presence of migraines was assessed using the ID-Migraine, a valid and reliable screening measure comprising of three self-report items (Lipton, Dodick, Sadovsky, Kolodner, Endicott et al., 2003).

Psyciatric and behavioural features

A range of self-report scales were administered to measure psychiatric and behavioural features previously described as being elevated among PM males.
(reviewed in Chapter 3), including depression, anxiety, social anxiety and autistic traits. These features were assessed using the Depression, Anxiety and Stress Scales (DASS) (Lovibond & Lovibond, 1995b), self-report Liebowitz Social Anxiety Scale (LSAS-SR) (Liebowitz, 1987), and Autism Quotient (AQ) (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001).

The DASS (Lovibond & Lovibond, 1995b) comprises 42 self-report items forming three scales assessing symptoms of depression, anxiety and stress (14 items per scale). The depression scale explores feelings of hopelessness, anhedonia, dysphoria and worthlessness. The anxiety scale assesses physiological indicators of autonomic arousal (e.g. rapid heart rate, breathing difficulties, dryness in mouth) and anxious affect. Items on the stress scale examine effects of chronic stress including irritability, agitation and difficulties relaxing (Lovibond & Lovibond, 1995a; Lovibond & Lovibond, 1995b). Participants are asked to indicate the degree to which each statement has applied to them over the past week using a four-point scale of symptom frequency severity. Scores for depression, anxiety and stress are calculated by summing the scores of items for each scale, and are classified as normal (<9, <7; <14), mild (10–13, 8–9, 15–18), moderate (14–20, 10–14, 19–25), severe (21–27, 15–19, 26–33) or extremely severe (>28, >20, >34) for depression, anxiety and stress, respectively (Lovibond & Lovibond, 1995b). The DASS has been shown to have satisfactory psychometric properties including good discriminant and convergent validity, internal consistency and reliability in both normative and clinical samples (Crawford & Henry, 2003; Lovibond & Lovibond, 1995a).
The LSAS-SR (Liebowitz, 1987) is a self-report questionnaire comprising 24 items assessing symptoms of social anxiety and phobia across different situations. For each item the participant is asked to rate their level of fear and avoidance specific to that situation within the past week. Responses are scored on a four-point Likert scale, with higher scores denoting greater fear or more frequent avoidance. The scale is divided into subscales for social interaction (11 items) and performance situations (13 items). An overall score and subscale scores for fear and avoidance are calculated by summing the relevant responses (total fear, fear of social interaction, fear of performance, total avoidance, avoidance of social interaction, avoidance of performance). A total score >50 indicates social anxiety, which may be classed as moderate (50-65), marked (65-80), severe (80-95), or extremely severe (>95) (Liebowitz, 1987). Psychometric properties are acceptable with good test-retest reliability, internal consistency, and convergent and discriminant validity (Baker, Heinrichs, Kim, & Hofmann, 2002).

The AQ (Baron-Cohen et al., 2001) comprises 50 self-report items assessing social and communicative traits associated with the autistic spectrum. Items are phrased statements and are divided into five subscales: social skills, communication, imagination, attention to detail and attention switching. Participants are asked to respond on a Likert scale the extent to which they agree with each statement, with total scores ranging from 0–50. Higher scores denote a higher degree of traits associated with the autistic spectrum; with a total score of ≥ 32 being an indicator of caseness (Baron-Cohen et al., 2001). The AQ effectively discriminates between high-functioning individuals with autism and unaffected adults with normal intelligence, with good test-retest reliability (Baron-Cohen et al., 2001).
Chapter 2. Overarching methodology

Questionnaires were mailed out to participants prior to completing the clinical assessments.

2.2.2 Clinical assessment

Assessments were completed from 2011–2013 in New South Wales and Victoria at Neuroscience Research Australia (NeuRA), the GOLD Service, Monash Biomedical Imaging, and participants’ homes. Standardised cognitive, motor and psychiatric measures were administered as per test manuals. Following clinical assessments, diagnostic classifications for FXTAS in the PM group were based on clinical and radiological features according to the original consensus criteria (Jacquemont et al., 2003) so as to be comparable to existing literature.

Neuropsychological assessment

Although a number of controlled studies have examined specific areas of cognitive function in the PM (reviewed in Chapter 4), findings from the author’s previously published systematic review (Birch et al., 2014) suggested that further research examining a broad range of cognitive domains in independent PM cohorts is required. In line with the most comprehensive controlled study of cognitive function among PM males with and without FXTAS to date (Grigsby et al., 2008), a range of neuropsychological measures were selected in order to assess performance across multiple cognitive domains. Individual test results were grouped into cognitive domains based on clinical recommendations (Groth-Marnat, 2003; Hebben & Milberg, 2002; Lezak, Howieson, & Loring, 2004; Vanderploeg, 1994). These domains included general intelligence (current and premorbid), working memory and
attention, information processing speed, executive functions, verbal memory, visuospatial function, language, and fine motor function.

*General intelligence* was assessed using the four subtest version of Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999). This includes verbal measures of Vocabulary and Similarities, in addition to performance based measures of Matrix Reasoning and Block Design. Age-scaled scores are derived for Full Scale Intelligence Quotient (FSIQ), Verbal IQ (VIQ), and Performance IQ (PIQ). Estimates of *premorbid intellectual function* were obtained using the National Adult Reading Test (NART) (Nelson & Willison, 1991), an untimed reading task comprising unusual words. Participant responses were recorded and scored according to Australian pronunciation (NART for Australians, Department of Cognitive Science, Macquarie University, [http://www.cogsci.mq.edu.au/research/resources/nart/](http://www.cogsci.mq.edu.au/research/resources/nart/)).

*Working memory and attention* was examined using the Digit Span subtest of the Wechsler Adult Intelligence Scale III (WAIS-III) (Wechsler, 1997a), and the Letter Number Sequencing subtest of the Wechsler Memory Scale III (WMS-III) (Wechsler, 1997b). In the Digit Span task there are two conditions in which participants are required to repeat a series of digits read by the examiner. In the first condition (Digits Forwards), the digits are to be repeated in the same order as they were read to the participant. In the second condition, the digits must be recalled in reverse order (Digits Backwards). For Letter Number Sequencing, participants are required to recall a series of numbers and letters; but must recount them back to the examiner in a specific order (numbers first from smallest to largest, followed by the
letters in alphabetical order). In both tasks, the number of digits/letters included in each trial is gradually increased, thereby increasing the difficulty of the task, until the pre-defined stop point is reached after a specified number of incorrect trials.

*Information processing speed* was assessed using the Digit Symbol subtest of the WAIS-III (Wechsler, 1997a) and Trail Making Test (Part A) (Reitan, 1992). In the Digit Symbol task, participants are provided with a worksheet comprising a series of boxes containing numbers and paired symbols. Participants are required to fill in the relevant symbol within each box corresponding to the number provided, one at a time using reference stimuli as a guide. Participants are instructed to complete the task as quickly as possible, with the final score recorded as the number of correct responses made within 120 seconds. In Trails A, participants are required to join together a series of numbered circles (1–25) with a continuous line, in order, as quickly as they can. The number of seconds taken to complete the task is recorded as the final score.

*Assessment of executive functions* included measures of response inhibition (Hayling Sentence Completion Test (Burgess & Shallice, 1997)); dynamic behavioural control and regulation (Behavioral Dyscontrol Scale 2 (BDS-2) (Grigsby & Kaye, 1996)), phonemic fluency (FAS from the Controlled Oral Word Association Test (COWAT) (Spreen & Benton, 1977)) and cognitive flexibility (Trail Making Test (Part B) (Reitan, 1992)). The Hayling Sentence Completion Test is a timed task measuring simple response time and response inhibition. The task involves two conditions, each including a set of 15 sentences with the last word missing. In the first condition, participants are asked to provide a word that meaningfully completes the sentence
(measuring response time). In the second condition, participants are asked to suppress a meaningful response and produce a nonsense word (measuring response inhibition). An overall score incorporates scaled scores for the time taken to complete each condition in addition to the number and nature of errors in the response inhibition condition. The BDS-2 consists of nine items assessing various aspects of behavioural control including motor learning, go/no-go performance, and insight into performance. Responses are scored according to a standardised scoring system (0–3), with higher scores denoting better performance. In the COWAT, participants are asked to provide as many words as they can think of that begin with a particular letter. Each trial was 60 seconds, and participants completed three trials (F, A, S). The sum of the number of correct responses provided within each trial was recorded as the final score. Cognitive flexibility was assessed using the Trail Making Test Part B, a timed task which is similar to Part A (a measure of information processing speed), but includes both letters and numbers. Participants are asked to switch between letters and numbers as they join each circle with a continuous line in numerical/alphabetical order (1, A, 2, B, 3, C, etc). The time taken to complete the task is recorded as the final score.

*Verbal memory* was assessed using the Logical Memory Test from WMS-III (immediate and delayed recall trials) (Wechsler, 1997b). During this test, participants are read two short stories, each containing 25 elements. Free recall is assessed immediately (immediate recall) and again after 25–35 minutes (delayed recall).
Visuospatial function was examined using the Block Design subtest of the WASI (Wechsler, 1999). In this test, participants are asked to copy designs presented in a stimulus book using small blocks. Scores are based on the time taken to complete each design, with each trial increasing in difficulty until the predefined stop point (based on failure to complete) is reached.

Assessment of language function included measures of confrontation naming (Boston Naming Test– Half Form B (Fastenau, Denburg, & Mauer, 1998; Kaplan, Goodglass, & Weintraub, 2001)) and semantic fluency (Animal Naming (Borod, Goodglass, & Kaplan, 1980)). The Boston Naming Test (Half Form B) consists of 30 line drawings that participants are requested to name. The Half Form version has adequate internal consistency and correlates with performance on the complete version of the scale (60 items) (Franzen, Haut, Rankin, & Keefover, 1995). Scores are based on the number of correct responses, including responses given after providing a stimulus cue. On the Animal Naming task, participants are asked to name as many different animals as they can think of within 60 seconds. The total number of animals, minus repetitions, is recorded as the final score.

Fine motor function was assessed using the Lafayette Grooved Pegboard (Model 32025). On this task, participants are required to insert 25 grooved pegs into holes, one at a time, using only one hand. Dominant and non-dominant hands are tested separately. The total time taken to complete the board is recorded as the final score for each hand.
General medical and neurological exam

A general medical examination was performed to collect baseline characteristics of physical health in addition to anthropometric measures that may affect performance on neurobehavioural measures. The general medical examination included measures of systolic and diastolic blood pressure (sitting and standing), heart rate, and anthropometric measures (height (cm), weight (kg), and leg length (cm)). Leg length was measured using a tape measure while standing as the distance from the lateral malleolus to the greater trochanter. Non-fasting blood tests were performed at the South Eastern Area Laboratory Services (SEALS) Pathology centre based at the Prince of Wales Hospital, adjacent to where assessments took place. Bloods were tested to exclude medical conditions that may impact on neuropsychiatric function including reversible causes of cognitive dysfunction. Biochemical analyses performed by SEALS included measures of thyroid stimulating hormone, vitamin B12, folate, lipid profile, C-reactive protein, creatinine, urate, and homocysteine. Results were interpreted by an experienced clinician and none obtained from included participants were deemed likely to have significant clinical impact. A cardiovascular risk factor index was quantified based on the Framingham Heart Study (D’Agostino, Vasan, Pencina, Wolf, Cobain et al., 2008). The index is calculated using a regression model based on age, gender, total cholesterol, high-density lipoprotein (HDL) cholesterol, smoking status, and systolic blood pressure. Higher scores denote greater 10 year risk for a cardiovascular event.

Motor abnormalities and neurological soft signs were assessed using the FXTAS Rating Scale (Version 1.0) (Leehey et al., 2008), a composite scale comprising 44
items from the Clinical Rating Scale for Tremor (CRST) (Fahn, Tolosa, & Marin, 1987), International Cooperative Ataxia Rating Scale (ICARS) (Trouillas, Takayanagi, Hallet, Currier, Subramony et al., 1997), Unified Parkinson’s Disease Rating Scale (UPDRS) (Fahn, Elton, & UPDRS program members, 1987), and Unified Huntington’s Disease Rating Scale (Huntington Study Group, 1996). The scale assesses the presence and severity of tremor (rest, postural and intention), gait disturbance, postural instability, limb ataxia, oculomotor abnormalities, dysarthria, rigidity and bradykinesia (Allen, Leehey, Tassone, & Sherman, 2012). A total score (maximum 226) is calculated by summing individual item scores, with higher scores denoting greater severity of motor symptoms. The scale is effective in discriminating between PM males with motor signs associated with FXTAS and controls with normal FMR1 alleles (Leehey et al., 2008) with acceptable internal consistency (Berry-Kravis, Lewin, Wuu, Leehey, Hagerman et al., 2003; Hall et al., 2014). All PM males were staged according to the FXTAS rating scale based on their presenting symptoms and functional status (Bacalman et al., 2006).

Psychiatric interview

The Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition (SCID-I/NP) (First, Spitzer, Gibbon, & Williams, 2002) was administered in order to establish whether psychiatric symptoms met diagnostic thresholds for current and lifetime history of DSM-defined disorders. The SCID-I/NP is a semi-structured interview used to make diagnoses of Axis I psychiatric disorders described in the fourth edition of the Diagnostic and Statistical Manual for Mental Health Disorders (DSM-IV-TR) (American Psychiatric
Modules A, D and J were administered to assess current and lifetime history of mood disorders including major/minor depressive disorder, bipolar disorder, mania, hypomania, and dysthymia. Module F was administered to assess current and lifetime history of anxiety disorders including panic disorder, agoraphobia without history of panic disorder, social phobia, specific phobia, obsessive compulsive disorder, posttraumatic stress disorder and generalised anxiety disorder. During the psychiatric interview, two PM males reported a history of drug induced psychosis. The psychotic episodes were brief, self-limiting and related to substance intoxication. These individuals were retained in analyses as exclusion criteria included history or presence of only enduring psychotic disorder. These participants were not using illicit substances at the time they were recruited into the study.

*Postural sway*

The current study employed targeted measures of postural control following recent reports proposing that decrements in this area of motor function may provide a sensitive marker of cerebellar dysfunction in males and females with the PM (Aguilar, Sigford, Soontaraporncchai, Nguyen, Adams et al., 2008; Allen et al., 2008; Kraan et al., 2013; Narcisa et al., 2011; O'Keefe et al., 2012; O'Keefe et al., 2015; see Chapter 7 for further detail). However, previous studies of postural sway in the PM have not included MRI, so the possible relationships between postural sway and cerebellar integrity have not yet been specifically examined. In order to explore whether decrements in postural control relate to cerebellar structure in PM males, postural sway in the current study was quantified using a swaymeter, affixed via a
belt that is fitted at waist level (Lord et al., 2003). The swaymeter comprises a rod (40 cm long) projecting from the back of the participant, with a pen mounted on the end. Body displacements are recorded by the pen on a piece of graph paper aligned on an adjustable table, with the table height set so that the swaymeter rod is parallel to the floor and approximately at the level of the centre of mass. The swaymeter has demonstrated good concurrent and convergent validity for the assessment of postural sway under different sensory conditions. It has been shown to effectively discriminate between younger and older adults (Sturnieks, Arnold, & Lord, 2011), older fallers and non-fallers (Lord, Ward, Williams, & Anstey, 1994), and demonstrated good agreement and moderate-to-strong correlations ($r=0.56–0.87$) with anterior-posterior and medio-lateral sway measures obtained simultaneously from a forceplate (Sturnieks et al., 2011). The swaymeter has also shown good immediate test-retest repeatability across multiple trials ($r=0.65–0.94$), indicating that it provides a reliable measure of postural sway (Sturnieks et al., 2011). The swaymeter is a portable tool with short administration time that has been used extensively in studies of balance and falls risk assessment in older people and clinical populations (e.g. Brooke-Wavell, Prelevic, Bakridan, & Ginsburg, 2001; Hinman, Bennell, Metcalf, & Crossley, 2002; Lord, Clark, & Webster, 1991; Lord & Ward, 1994), and provides a cost-effective and simple way to assess postural sway during standing.

Postural control relies on input from multiple sensory, cognitive, and neuromuscular systems (Lord et al., 1991). A number of studies have utilised protocols designed to challenge input from these systems, proposing that this approach may yield more sensitive measures of postural sway compared to normal quiet stance (Kuo, Speers,
Peterka, & Horak, 1998; Lord, Menz, & Tiedemann, 2003; Maki, Holliday, & Topper, 1994; Pellecchia, 2003; Sullivan, Rose, & Pfefferbaum, 2006). A simple way of perturbing sensory input is completing sway trials with eyes closed (limiting visual input), or standing on a surface that disrupts proprioception (such as a foam mat) (Lord et al., 1991; Lord et al., 2003). In the current study, participants were instructed to stand quietly and as still as possible, with their arms by their side and feet shoulder-width apart, looking ahead and slightly below eye level at a blank wall approximately 3 metres away. Sway displacement was measured in the anterior-posterior and medio-lateral directions in millimetres for 30 seconds across four different conditions in the following specific sequence: (1) standing on the floor with eyes open; (2) standing on the floor with eyes closed; (3) standing on a foam mat (15cm thick) with eyes open; and (4) standing on foam with eyes closed. The administration of trials in this fixed order of increasing difficulty was consistent with previous studies using the swaymeter device (Kraan et al., 2013; Lord et al., 1994). Participants who were not able to complete an easier trial did not progress on to the more difficult conditions.

Quantitative gait assessment

Spatiotemporal gait measures (such as speed and step-to-step variability) provide useful markers of neurological dysfunction in ageing and neurodegenerative disease (Lord, Galna, & Rochester, 2013), with utility in tracking progression of symptoms, response to treatment, and risk for adverse outcomes including disability and falls (Abellan Van Kan, Rolland, Andrieu, Bauer, Beauchet et al., 2009; Mirelman, Gurevich, Giladi, Bar-Shira, Orr-Urtreger et al., 2011; Nieuwboer, Kwakkel, Rochester, Jones, van Wegen et al., 2007; Verghese, Wang, Lipton, Holtzer, & Xue,
The examination of cognitive-motor interference in gait control – that is, the magnitude of change in gait characteristics when performing a concurrent cognitive task – also provides a sensitive measure of motor function. As gait is a complex task requiring cognitive input from executive and attentional systems (Al-Yahya, Dawes, Smith, Dennis, Howells et al., 2011; Yogev-Seligmann, Hausdorff, & Giladi, 2008), dual-task related changes in performance are thought to arise due to the sharing of finite attentional resources between cognitive and motor tasks (Hausdorff, Yogev, Springer, Simon, & Giladi, 2005; Pashler, 1994). Although there is no consensus as to the optimal type of dual-task in terms of sensitivity to interference (Lord, Galna, & Rochester, 2013), there is evidence to suggest that cognitive tasks tapping executive function (e.g. serial subtractions) may be more sensitive than other measures (e.g. memory tests or reaction time tasks) (Al-Yahya et al., 2011; Hausdorff, Schweiger, Herman, Yogev-Seligmann, & Giladi, 2008; Patel, Lamar, & Bhatt, 2014; Yogev-Seligmann et al., 2008).

To explore whether dual-task related changes in gait function have utility as markers of neuromotor dysfunction among PM males, spatiotemporal gait characteristics in the current study were assessed using the GAITRite system (CIR Systems Inc., Clifton, NJ, USA). The GAITRite computer software calculates data relating to spatial and temporal parameters of gait in response to sensor activation as the participant crosses the walkway. The computer-based walkway measures 593 cm long × 89 cm wide containing embedded pressure sensors and has been shown to have high validity for spatial and temporal measurements of gait (van Uden & Besser, 2004). The GAITRite also shows good agreement with a three-dimensional motion analysis system, considered to be the gold standard in the measurement of
spatiotemporal gait parameters, and is suitable for use in both research and clinical settings (Webster, Wittwer, & Feller, 2005).

Participants completed six trials per condition\(^1\), with minimal interruption between walks as per recommended protocol (Galna, Lord, & Rochester, 2013). In the single-task condition, participants were asked to walk at their comfortable pace. During the two dual-task conditions, participants were required to count backwards by 3s, and then backwards by 7s, from a starting number provided by the examiner. All participants were instructed to count out aloud while walking and were given the same starting numbers. For the dual-task conditions, the number of correct responses to the cognitive task was recorded and participants provided a difficulty rating for the task (1–10, higher rating denotes greater difficulty). Participants started and finished each walk 1.5 metres before and after the mat to minimise effects of acceleration and deceleration. Two PM males did not complete the gait assessments as their natural gait was obscured due to inability to walk without the assistance of a walking frame (one PM male with FXTAS), or acute knee injury (one PM male without FXTAS). One PM male with FXTAS did not complete the dual-task conditions due to balance instability putting him at risk of a fall during the assessment. Data from the -7s condition for an additional PM male with FXTAS was collected but could not be used due to scuffing of the feet and veering off the mat.

\(^{1}\) Five PM males completed only three trials per condition before the protocol was amended.
2.2.3 Structural brain MRI scans

The examination of associations between structural MRI changes and neurobehavioural measures is a widely used approach with recognised utility in discerning the underlying pathology of clinical features, and also in identifying early markers of dysfunction in prodromal stages of disease. The utility of this approach has been recognised in a range of populations including non-demented older adults (Zhang, Sachdev, Wen, Kochan, Crawford et al., 2013); prodromal groups such as pre-manifest Huntington’s Disease (Scahill, Hobbs, Say, Bechtel, Henley et al., 2013) and mild cognitive impairment (Annweiler, Beauchet, Bartha, & Montero-Odasso, 2013); and clinical groups such as depression (Frodl, Schaub, Banac, Charypar, Jäger et al., 2006) and Alzheimer’s disease (de Jong, van der Hiele, Veer, Houwing, Westendorp et al., 2008). In the current study, brain MRI scans were conducted using a Phillips 3T Achieva Quasar Dual Scanner (Phillips Medical Systems, Best, The Netherlands) located at NeuRA, Sydney. One PM male assessed interstate was not able to travel to the scanner location. MRI was contraindicated for a second PM male due to the presence of shrapnel within his body. All other participants underwent T1-weighted and T2-weighted fluid attenuated inversion recovery (FLAIR) imaging. Three-dimensional (3D) T1-weighted scans were acquired as follows: TR = 6.39 ms, TE = 2.9 ms, flip angle = 8°, matrix size = 256 × 256, FOV = 256 × 256 × 190, and slice thickness = 1 mm with no inter-slice gap, yielding 1 × 1 × 1 mm$^3$ isotropic voxels. The FLAIR sequences were acquired using the following parameters: TR = 10000 ms, TE = 110 ms, T1 = 2800, matrix size = 512 × 512, and slice thickness = 3.5 mm with no inter-slice gap, yielding a spatial resolution of 0.488 × 0.488 × 3.5 mm$^3$/voxel.
2.2.4 *FMR1* molecular analyses

Twenty millilitres of fresh whole blood collected in EDTA-treated tubes was sent to collaborators based at the Cyto-molecular Diagnostics Research Group, Murdoch Children’s Research Institute (MCRI). All *FMR1* molecular processing was performed by the Cyto-molecular Diagnostics Research Group (MCRI), with the exception of CGG sizing, which was performed by HealthScope Pathology. The Asuragen® AmplideX™ *FMR1* PCR Kit was used by HealthScope Pathology to perform CGG sizing on blood Deoxyribonucleic acid (DNA) as previously published (Kraan, Hocking, Georgiou-Karistianis, Metcalfe, Archibald, Fielding, Trollor, et al., 2014). The PM or control status was confirmed by the Cyto-molecular Diagnostics Research Group (MCRI) using methylation sensitive Southern blot analysis as described in Kaufmann et al. (1999). Presence of methylated *FMR1* alleles in this cohort was further ruled out using the EpiTYPER system methylation analysis as described in Godler et al. (2010). Peripheral blood mononuclear cell (PBMC) isolation was performed on up to 15 ml of blood using Ficoll gradient separation, as per manufacturer’s instructions (Amersham Pharmacia Biotech, Uppsala, Sweden). Reverse transcription real-time PCR was performed on RNA extracted from one million PBMCs as previously published (Loesch, Godler, Evans, Bui, Gehling et al., 2011). The relative standard curve method was used to quantify *FMR1-5′* and *FMR1-3′* mRNA standardised to three internal control genes (GUS, EIF4A2, and SDHA) using the ViiA™ 7 Real-Time PCR System (Life technologies, Foster City, CA). This approach of combining assays targeting conserved portions of *FMR1 5′* and 3′ mRNA has been shown to minimise confounding effects of mRNA degradation (Godler, Loesch, Huggins, Gordon, Slater et al., 2009). Reverse-
transcription for each RNA sample was performed in two separate cDNA reactions, with each cDNA analysed in two separate real-time PCR reactions. The mean of the four outputs was used as a summary measure for *FMR1* mRNA expression for each participant.

The method for assessment of *FMR1* mRNA levels was normalised differently from that of previous studies of *FMR1* expression which have mostly used a real-time PCR method targeting 5′ mRNA normalised to a single internal control gene (GUS) (e.g. Tassone, Hagerman, Taylor, Gane, et al., 2000). The method developed by Godler et al. (Godler et al., 2009; Loesch et al., 2011) was selected for use in the current study following recommendations that normalisation to multiple control genes provides more accurate and reliable measures of gene expression from real-time PCR (Vandesompele, De Preter, Pattyn, Poppe, Van Roy et al., 2002). Internal genes are used to control for variables including the amount and quality of the starting material, differences in enzymatic efficiencies of amplification, and variation between tissues or cell types (Vandesompele et al., 2002). Appropriate normalisation of these variables ensures that real-time PCR results reflect biological variation of *FMR1* mRNA expression and are not due to variation in the expression of the internal control gene/s or issues surrounding RNA quality. The internal control genes used in the current study were determined to be the most stably expressed in PBMCs between PM males and controls using the geNorm approach (Godler et al., 2009; Loesch et al., 2011; Vandesompele et al., 2002) and therefore provide a suitable reference.
2.3 Data extraction and preparation

Data were analysed using IBM Statistical Package for the Social Sciences (SPSS) Version 22. For neurobehavioural measures, with the exception of spatiotemporal gait characteristics, extreme data points (>3 standard deviations (SDs) from the relevant group mean) were visually inspected to ensure they were not due to data entry error. Given the wide range of age and clinical involvement in the sample, extreme scores were retained if they were found to be a true reflection of performance (e.g. greater symptom severity). For step-to-step spatiotemporal gait characteristics, observations that were beyond 2 SDs of the mean (within-subject) were winsorised (see section 2.3.5 Quantitative gait measures for full details). This approach is consistent with previous research (Kraan, Hocking, Georgiou-Karistianis, Metcalfe, Archibald, Fielding, Bradshaw, et al., 2014) and was adopted to avoid artificial inflation of intra-individual variability measures. Normality was assessed using the Shapiro-Wilk test, and where appropriate variables were transformed (as outlined in specific subsections below).

2.3.1 Deriving domain scores for cognitive function

For Chapter 3, domain scores for working memory, attention, information processing speed, executive function, verbal memory, visuospatial function, language, and fine motor function were derived by calculating the first principal component for each family of tests (outlined in Neuropsychological assessment). This was done to reduce the data into a single composite score for each cognitive domain. Domain scores for fine motor function and information processing were also included in Chapter 4. Calculation of domain scores first involved converting all raw scores into z-scores.
based on the entire sample (PM and controls combined). Z-scores were then entered into principal components analyses and the first principal component for each family of tests was extracted. Trails B scores were adjusted for fine motor function before being entered into the principal components analysis on which the executive function score was derived to control for the effect of motor dysfunction. Skewed cognitive domain scores (information processing speed, fine motor function, language) were then converted to normalised rank scores using Blom’s formula (Blom, 1958). This was done to minimise the effect of extreme scores due to the wide range of ages and clinical involvement in the cohort, and to meet assumptions of parametric statistics.

2.3.3 Overall motor function

Total FXTAS rating scale scores were transformed using a natural log transformation in order to meet assumptions of parametric statistics.

2.3.4 Postural sway

Missing postural sway scores were imputed based on performance on available sway trials using the missing values analysis function in SPSS (four PM males with FXTAS and one PM male without FXTAS did not complete eyes closed conditions as these were introduced in a later phase of the project; two PM males with FXTAS were unable to complete the foam conditions; and one PM male without FXTAS was unable to complete the eyes closed on foam condition). Missing data were imputed using the expectation-maximisation algorithm (Dempster, Laird, & Rubin, 1977). This method estimates the most likely missing value from available data, and has been shown to provide a more valid estimate reflexive of the true value than other
methods (Musil, Warner, Yobas, & Jones, 2002). Measures of anterior-posterior and medio-lateral postural displacement under each of the sway conditions were entered into a principal components analysis, and the first principal component was extracted to provide an overall sway score. This was done to reduce the data and avoid multiple comparisons across correlated measures. The overall sway score was then normalised using Blom’s rank-based transformation (Blom, 1958) in order to minimise effects of extreme scores and meet assumptions of parametric statistics. For all measures of sway, a higher score denotes greater postural displacement, i.e., poorer postural control.

2.3.5 Quantitative gait measures

Numerous individual spatial and temporal measures of gait function can be extracted from the GAITRite software. Examination of all of these would lead to issues with multiple testing across redundant variables that are highly correlated. Recent efforts in the wider literature have focussed on the grouping of gait parameters into independent domain scores (e.g. Lord, Galna, Verghese, Coleman, Burn et al., 2013; Verghese et al., 2007; Verlinden, van der Geest, Hoogendam, Hofman, Breteler et al., 2013), but these have only been validated in healthy populations. It therefore remains unknown the extent to which these factors would provide valid measures of independent gait domains in clinical samples. As the analyses presented in Chapter 8 are the first to explore dual-task related changes in function among males with the PM, quantitative gait measures were limited to those most commonly examined in the gait literature (Verlinden et al., 2013). Specifically, these measures were average gait speed (cm/s), step length (cm), step length variability, step width (cm), and step width variability. These variables and their definition are summarised in Table 2.1
(adapted from GAITRite Electronic Walkway Technical Reference; CIR Systems Inc., 2013). For step-to-step variables, data points for each participant were screened for outliers (>2 SDs) within each condition (walking only; counting backwards by 3s; counting backwards by 7s). Where identified, these values were winsorised to be within 2 SDs of the mean value for that variable across the six trials of the relevant condition. Intra-individual variability for step length, and step width were calculated as a coefficient of variation (CoV), where: CoV = ((SD / Mean) × 100). To quantify dual-task interference as a proportion of baseline performance, dual-task cost (DTC) was calculated using the following formula: DTC = ((dual-task score – single-task score) / single-task score) × 100. DTCs were calculated for both counting backwards by 3s and 7s for each gait variable. DTCs for variables with non-normal distributions (velocity, step length variability, step width variability) were then normalised using Blom’s rank-based transformation to reduce effects of extreme scores and meet the assumptions of parametric statistics (Blom, 1958).
Table 2.1

Definition of spatiotemporal gait variables included in Chapter 8 (adapted from GAITRite Electronic Walkway Technical Reference; CIR Systems Inc., 2013)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed</td>
<td>Distance travelled divided by ambulation time</td>
<td>cm/s</td>
</tr>
<tr>
<td>Step length</td>
<td>Distance from the centre of the heel of the current foot to the centre of the heel of the previous footprint on the opposite foot</td>
<td>cm</td>
</tr>
<tr>
<td>Step width</td>
<td>Distance from the midline midpoint of the current foot to the midline midpoint of the previous footprint on the opposite foot</td>
<td>cm</td>
</tr>
<tr>
<td>Step length variability</td>
<td>Intra-individual variability in step length</td>
<td>CoV</td>
</tr>
<tr>
<td>Step width variability</td>
<td>Intra-individual variability in step width</td>
<td>CoV</td>
</tr>
</tbody>
</table>

CoV = coefficient of variation

2.3.6 MRI processing

MRI scans were fed into a standard imaging analysis pipeline and processed by the Neuroimaging Lab based at the Centre for Healthy Brain Ageing, University of New South Wales (NiL). Cortical and cerebellar volumes were obtained by processing the T1-weighted scans of participants using FreeSurfer v5.3.0 (http://surfer.nmr.mgh.harvard.edu/). Image processing included motion correction and averaging of the T1-weighted images (Reuter, Rosas, & Fischl, 2010), removal of non-brain tissue (Ségonne, Dale, Busa, Glessner, Salat et al., 2004), automated Talairach transformation, segmentation of subcortical structures (Fischl, Salat, Busa, Albert, Dieterich et al., 2002; Fischl, Salat, van der Kouwe, Makris, Ségonne et al., 2004), intensity normalisation (Sled, Zijdenbos, & Evans, 1998), tessellation of grey matter and white matter boundary and automated topology correction (Fischl, Liu,
Dale, 2001; Ségonne, Pacheco, & Fischl, 2007), and surface deformation (Fischl & Dale, 2000). The cortical surface was reconstructed and parcellated into 34 cortical regions of interest (ROIs) using an automatic approach (Fischl et al., 2002; Fischl, van der Kouwe, Destrieux, Halgren, Ségonne et al., 2004) and the ‘Desikan-Killiany’ cortical atlas (Desikan, Ségonne, Fischl, Quinn, Dickerson et al., 2006). For analyses including cortical and cerebellar volumes derived from FreeSurfer, the automated value for estimated total intracranial volume (ICV) generated by the same software was used as a covariate. The automated MRI processing was visually inspected for quality. Common errors, often caused by failed intensity normalisation, wrongly segmented subcortical white matter hyper-intensities or inclusion of dura or vessel to the cortex, were manually corrected using the TKMEDIT toolbox in FreeSurfer on 17 participants (six PM, 11 HC). Cortical volumes for three PM males with FXTAS were excluded as these were not able to be manually corrected.

Subcortical volumes were obtained by processing T1-weighted scans of participants using the Functional MRI of the Brain (FMRIB) Software Library (FSL) v5.01 (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). Volumes of subcortical ROIs were generated using FMRIB’s Integrated Registration and Segmentation Tool (FIRST) (Patenaude, Smith, Kennedy, & Jenkinson, 2011). Estimated ICV was generated using an atlas scaling and covariance approach as previously published (Buckner, Head, Parker, Fotenos, Marcus et al., 2004). Quality checking was done following ENIGMA protocols (http://enigma.ini.usc.edu) to ensure accuracy of automatic segmentations. In short, this involved screening for outliers (beyond 1.96 SD of the overall cohort mean) and visual inspection of all scans. Values of
subcortical ROIs were excluded if they failed visual quality inspection. Missing values were mostly for the basal ganglia.

Volumetric estimation of white matter hyper-intensities (WMHs) was performed by NiL using an in-house method as previously published (Wen & Sachdev, 2004; Wen, Sachdev, Li, Chen, & Anstey, 2009). Both the T-1 weighted and FLAIR images were used, and WMHs were detected and segmented into volumes of periventricular and deep WMHs respectively, using an automated procedure. Accuracy of the WMH processing was visually inspected, and unsatisfactory segmentations were manually corrected using the FSLVIEW toolbox (Jenkinson et al., 2012). Correction for ICV was performed as per subcortical volumes as described above.

2.3.7 FMR1 molecular measures

Both CGG repeat length and FMR1 mRNA levels were positively skewed in the PM group. For analyses assuming a normal distribution, CGG repeat length and FMR1 mRNA values were transformed using natural log.
CHAPTER 3.

COHORT CHARACTERISTICS: CLINICAL
CHAPTER 3. COHORT CHARACTERISTICS: CLINICAL

3.1 Introduction

Investigations of neurobehavioural features among PM males suggest a spectrum of clinical involvement, including but not limited to features characteristic of FXTAS. As described in Chapter 1, phenotypes consistent with mild FXS have been described among young PM males with larger CGG expansions (Hagerman et al., 1996; Loesch et al., 1994; Tassone, Hagerman, Taylor, Mills, Harris et al., 2000). PM males may also show increased vulnerability to neurodevelopmental disorders including attention deficit hyperactivity disorder (ADHD) and autism (Clifford, Dissanayake, Bui, Huggins, Taylor et al., 2007; Dorn et al., 1994; Farzin, Perry, Hessl, Loesch, Cohen et al., 2006), which may be associated with FMRP deficits as seen in the full mutation. Limited evidence suggests that increased rates of other conditions, including hearing loss (Juncos et al., 2011), hypertension (Hamlin, Sukharev, Campos, Mu, Tassone et al., 2012), olfactory dysfunction (Juncos, Lazarus, Rohr, Allen, Shubeck et al., 2012), sleep apnoea (Hamlin, Liu, Nguyen, Tassone, Zhang et al., 2011) and migraine (Au et al., 2013), may be indicative of a broader spectrum of PM-associated disorders. However, these findings await replication in independent cohorts.

Studies suggesting increased vulnerability to psychiatric disorder among PM carriers also provide evidence that clinical features of the PM extend beyond the cognitive and motor characteristics of FXTAS. Psychiatric features frequently described among PM males with FXTAS include increased rates of depression, anxiety and
obsessive compulsive symptoms compared to matched controls or published norms 
(Adams et al., 2010; Bacalman et al., 2006; Bourgeois et al., 2009; Bourgeois et al., 2011). In addition, other features may include agitation, aggression, irritability and disinhibition (Bacalman et al., 2006). This psychiatric profile is similar to other neurodegenerative disorders with primary cognitive and motor features including Parkinson’s Disease and Dementia with Lewy Bodies, and is suggestive of dysfunction to fronto-subcortical neural circuits modulating affect and behaviour (Bacalman et al., 2006).

Little is known about the natural history of psychiatric manifestations in the PM in terms of onset and relationships with other neurobehavioural features. Findings of increased rates of obsessive-compulsive symptoms (Dorn et al., 1994; Hessl et al., 2005), social phobia (Bourgeois et al., 2011) and abnormalities in social cognition (Cornish et al., 2005) among PM males without motor symptoms suggest that psychiatric symptoms may be observable prior to the onset of FXTAS. There is only one study to date examining the ages of onset of psychiatric disorder, tremor and ataxia in PM males and females both with and without FXTAS (Seritan, Bourgeois, Schneider, Mu, Hagerman et al., 2013). In that cohort, psychiatric symptoms were found to precede the onset of motor symptoms. However, without prospective longitudinal follow-up of these PM cohorts, the extent to which psychiatric manifestations of the PM emerge from distinct pathological mechanisms independently of FXTAS, or represent early preclinical manifestations of the disease, remains poorly understood (Hagerman, 2012).
Although previously described in several studies, the empirical evidence of elevated rates of psychiatric symptoms among PM males have not always been consistently observed. For example, rates of depression and anxiety among a large cohort of PM males (n=89) comparable to matched controls have been reported using self-report questionnaire scales (Allen, Hunter, Rusin, Juncos, Novak et al., 2011). Possible explanations for discrepancies between studies may include differences in ascertainment method, the use of appropriate comparison groups (as opposed to population norms), and the types of psychiatric measures employed in each study. One limitation is that studies have often recruited PM carriers following contact with a clinical service, and it is therefore possible that symptoms among those presenting with health concerns may not be representative of those found in the general population (Besterman, Wilke, Mulligan, Allison, Hagerman et al., 2014). This is evidenced by studies suggesting higher rates of autism spectrum disorders and ADHD among young PM male probands (the first family member identified as clinically affected after presenting for medical treatment) compared to PM male non-probands (usually identified through cascade genetic testing) (Chonchaiya, Au, Schneider, Hessl, Harris et al., 2012; Farzin et al., 2006). Recruitment through clinical services may therefore result in an overrepresentation of psychiatric disorders among males with the PM. Further, studies have utilised a range of psychiatric measures varying from self- or informant-rated questionnaire scales to structured diagnostic interviews. Although scores derived from a number of questionnaire-based scales may give an indication of ‘caseness’, the proportion of participants meeting thresholds for clinically significant symptoms are not consistently described between studies. The presentation of this information, or alternatively results of structured diagnostic interviews based on criteria for DSM-
defined disorders, would provide more meaningful information in terms of the clinical (as opposed to statistical) significance of study findings. Further research considering the clinical significance of symptoms among representative samples of PM males and matched controls is required to determine the prevalence and course of psychiatric disorder associated with the PM.

There are several studies that have provided evidence to point to biological determinants of psychiatric symptomatology among PM carriers. Specifically, both elevated levels of *FMR1* mRNA (Hessl et al., 2005; Hessl et al., 2011; Koldewyn et al., 2008), and reductions in FMRP (Hessl et al., 2011) have been positively associated with psychiatric symptoms in the PM. These findings point to a role for both RNA toxicity and reductions in levels of FMRP in the development of psychiatric features in the PM.

The purpose of this chapter is to describe clinical characteristics among a newly established cohort of PM males and controls on which subsequent analyses included in this thesis will be based. The aims of the chapter are to (i) examine demographic, lifestyle, physical health, and mental health characteristics, including lifetime history of psychiatric disorder and current symptomatology among PM males and controls, and (ii) explore possible associations between current psychiatric symptomatology and *FMR1* molecular measures (CGG repeat length, mRNA) among PM males. It was hypothesised that: (1) PM males would exhibit higher rates of physical health conditions including hypertension, migraine and hearing loss compared to controls; (2) PM males would report greater psychiatric symptoms compared to controls and that symptoms would be most severe among PM males with FXTAS; and (3) among
PM males, given evidence that *FMR1* mRNA may be more related to psychiatric symptomatology than CGG repeat length (Hessl et al., 2005), greater current psychiatric symptomatology would be associated with elevated *FMR1* mRNA levels.

### 3.2 Assessment and measures

A detailed description of participant recruitment and measures was provided in Chapter 2 and is therefore not repeated in full here. Briefly, 22 PM males aged 26–80 years and 24 controls aged 26–77 years were included in this study. Detailed information including participant demographics and medical history was obtained using a self-report questionnaire. A general medical exam, including assessment of blood pressure, anthropometric measures, and a non-fasting blood test was performed. All PM males were staged according to the FXTAS rating scale based on their presenting symptoms and functional status (Bacalman et al., 2006). CGG repeat length and *FMR1* mRNA levels were quantified from blood. Cardiovascular risk was quantified using the Framingham Index, based on the Framingham Heart Study (D’Agostino et al., 2008). Psychiatric symptoms were assessed using a structured interview and self-report scales. Current and lifetime history of mood and anxiety disorders was assessed using the SCID-I/NP (First et al., 2002). Lifetime history was defined as having ever met diagnostic criteria for a given disorder and included current episodes. Self-reported psychiatric symptoms of depression, anxiety and stress were measured using the DASS (Lovibond & Lovibond, 1995b). Symptoms of social phobia were assessed using the LSAS-SR (Liebowitz, 1987). Autistic traits were measured using the AQ (Baron-Cohen et al., 2001).
3.3 Statistical analyses

3.3.1 Demographic and clinical characteristics

Independent $t$-tests, analysis of variance (ANOVA), or non-parametric equivalents where appropriate were used to explore group differences in continuous variables measuring demographic, lifestyle, physical and mental health characteristics. Categorical variables were analysed using chi-squares ($\chi^2$), except for in cases of small observations (i.e., where expected counts <5 per cell). Pairwise comparisons were conducted for: (a) all PM carriers vs controls (PM vs HC); (b) PM carriers with FXTAS vs controls (FX+ vs HC); (c) PM carriers without FXTAS vs controls (FX- vs HC); (d) PM carriers with FXTAS vs PM carriers without FXTAS (FX+ vs FX-).

3.3.2 Relationships between FMR1 molecular measures and current psychiatric symptomatology

Associations between $FMR1$ molecular measures (CGG repeat length, mRNA levels) and psychiatric symptoms (DASS, LSAS, and AQ scores) among PM males were explored using Spearman correlations.

3.4 Results

3.4.1 Demographic characteristics

Demographic characteristics of the sample are presented in Table 3.1. The combined PM group were well matched to controls with no significant differences in demographic variables of age, education, premorbid IQ, current IQ, relationship status (married/defacto) and gross annual income (total household). Observations
were not sufficient to perform statistical analyses for the proportions of those from a non-English speaking background (NESB) and ethnicity other than Caucasian, but it is clear from the descriptive statistics that the proportions did not differ by group. All participants reported English as being their primary language spoken at home. As expected, PM males had significantly greater CGG repeat length (Mann-Whitney \( U=0.000, p<.001 \)) and \( FMR1 \) mRNA levels (Mann-Whitney \( U=46.000, p<.001 \)) compared to controls.

After dividing the PM group into those with (FX+ n=7) and without (FX- n=15) FXTAS, no significant differences were detected between groups for education, premorbid IQ, relationship status and gross annual income. Significant differences emerged for age \( (F(2.43)=4.492, p=.017) \), current IQ (FSIQ: \( \chi^2=8.074, p=.018 \), VIQ: \( F(2.43)=6.167, p=.004 \), PIQ: \( \chi^2=7.142, p=.028 \)), CGG repeat length \( (\chi^2=34.842, p<.001) \), and \( FMR1 \) mRNA \( (\chi^2=23.247, p<.001) \). Post-hoc comparisons revealed that PM males with FXTAS were significantly older than PM males without FXTAS \( (p=.005) \). PM males with FXTAS also performed significantly worse than both controls and PM males without FXTAS on measures of current FSIQ (vs HC \( p=.005 \); vs FX- \( p=.014 \)), VIQ (vs HC \( p=.001 \); vs FX- \( p=.004 \)) and PIQ (vs HC \( p=.012 \); vs FX- \( p=.021 \)). CGG repeat length and \( FMR1 \) mRNA levels were significantly greater among PM males with and without FXTAS compared to controls (all \( ps<.001 \)). However, there were no significant differences between PM carriers with and without FXTAS for CGG repeat length \( (p=.162) \) or \( FMR1 \) mRNA levels \( (p=.731) \).
In terms of functional status, FXTAS stages in the PM group ranged from 0–4 (Bacalman et al., 2006). Fourteen PM males without FXTAS were classed at stage 0 (normal). One PM male without FXTAS and 2 PM males with FXTAS demonstrated mild tremor or balance problems but no interference with ADLs (stage 1). Two PM males with FXTAS demonstrated clear tremor producing minor interference with ADLs (stage 2). One PM male with FXTAS exhibited moderate tremor/balance problems with occasional falls (stage 3). Two PM males with FXTAS showed severe balance problems requiring the use of a cane or walker (stage 4).
## Table 3.1

### Sociodemographic characteristics of sample

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HC (n=24)</th>
<th>PM (n=22)</th>
<th>FX+ (n=7)</th>
<th>FX- (n=15)</th>
<th>PM vs HC</th>
<th>FX+ vs FX- vs HC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>t</td>
<td>p</td>
</tr>
<tr>
<td>CGG repeat length</td>
<td>29.88 (4.09)</td>
<td>88.05 (15.69)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.57 (3.16)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.33 (19.06)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>FMR1 mRNA level</td>
<td>1.08 (.25)</td>
<td>2.16 (1.05)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.35 (1.06)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.08 (1.07)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Age</td>
<td>55.33 (14.60)</td>
<td>53.73 (15.15)</td>
<td>66.43 (8.14)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.80 (14.06)</td>
<td>.37</td>
<td>.72</td>
</tr>
<tr>
<td>Education (years)</td>
<td>13.50 (3.44)</td>
<td>13.18 (3.42)</td>
<td>12.29 (2.93)</td>
<td>13.60 (3.64)</td>
<td>.17</td>
<td>.68</td>
</tr>
<tr>
<td>Premorbid FSIQ</td>
<td>110.41 (5.92)</td>
<td>107.58 (6.99)</td>
<td>104.28 (8.31)</td>
<td>109.11 (5.97)</td>
<td>1.49&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.14</td>
</tr>
<tr>
<td>Premorbid VIQ</td>
<td>109.43 (6.59)</td>
<td>106.28 (7.77)</td>
<td>102.61 (9.25)</td>
<td>107.99 (6.64)</td>
<td>1.49&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.14</td>
</tr>
<tr>
<td>Premorbid PIQ</td>
<td>109.77 (4.63)</td>
<td>107.55 (5.46)</td>
<td>104.98 (6.49)</td>
<td>108.75 (4.66)</td>
<td>1.49&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.14</td>
</tr>
<tr>
<td>Current FSIQ</td>
<td>113.46 (11.43)</td>
<td>106.32 (14.44)</td>
<td>93.43 (16.58)&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>112.33 (8.54)</td>
<td>1.87&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.07</td>
</tr>
<tr>
<td>Current VIQ</td>
<td>112.00 (12.48)</td>
<td>105.95 (13.80)</td>
<td>94.43 (15.26)&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>111.33 (9.41)</td>
<td>1.56&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.13</td>
</tr>
<tr>
<td>Current PIQ</td>
<td>110.96 (12.19)</td>
<td>105.00 (14.35)</td>
<td>94.00 (14.59)&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>110.13 (11.36)</td>
<td>1.52&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.14</td>
</tr>
<tr>
<td>N (%)</td>
<td>2 (8.33)</td>
<td>2 (9.09)</td>
<td>1 (14.29)</td>
<td>1 (6.67)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NESB</td>
<td>24 (100)</td>
<td>22 (100)</td>
<td>7 (100)</td>
<td>15 (100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Primary language English</td>
<td>23 (95.83)</td>
<td>22 (100)</td>
<td>7 (100)</td>
<td>15 (100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Married/defacto</td>
<td>13 (54.17)</td>
<td>17 (77.27)</td>
<td>6 (85.71)</td>
<td>11 (73.33)</td>
<td>2.70</td>
<td>.09</td>
</tr>
<tr>
<td>Total household income ≥$52,000&lt;sup&gt;f&lt;/sup&gt;</td>
<td>16 (66.67)</td>
<td>13&lt;sup&gt;a&lt;/sup&gt; (59.09)</td>
<td>2 (28.57)</td>
<td>11 (73.33)</td>
<td>.34</td>
<td>.85</td>
</tr>
</tbody>
</table>

<sup>a</sup>HC; <sup>b</sup>FX-; <sup>c</sup><HC; <sup>d</sup><FX-; <sup>e</sup>non-parametric equivalent used; <sup>f</sup>four HC, three FX+ and one FX- did not disclose total household gross income
3.4.2 Lifestyle factors

Lifestyle factors among the sample are presented in Table 3.2. Categories for alcohol consumption were based on the Australian Guidelines to Reduce Health Risks from Drinking Alcohol (National Health and Medical Research Council, 2009), which recommend adult males and females consume no more than two standard drinks per day. The combined PM male reported higher rates of past or current regular smoking compared to controls ($\chi^2=4.224, p=.04$); however, this group difference did not emerge when carriers were divided according to FXTAS diagnosis. No significant differences emerged between PM males and controls for alcohol consumption. Observations were not sufficient to compare alcohol use across FXTAS diagnostic categories.

3.4.3 Physical health characteristics

Physical health characteristics of the sample are presented in Table 3.3. When all PM males were combined, there were no significant differences between groups for measures of height, weight, systolic blood pressure, body mass index (BMI), cardiovascular risk, and previous diagnosis of hypertension. Observations were not sufficient to compare the rates of self-reported migraine, and rates of previous diagnoses of diabetes, thyroid disorder, epilepsy, and ADHD. A greater proportion of PM males self-reported hearing loss ($\chi^2=4.207, p=.040$). Although a greater number of PM males reported having at least one fall in the previous 12 months compared to controls (5 PM vs 0 HC), the number of observations was insufficient to perform chi-square analyses.
Table 3.2

*Lifestyle factors of sample*

<table>
<thead>
<tr>
<th></th>
<th>HC (n=24)</th>
<th>PM (n=22)</th>
<th>FX+ (n=7)</th>
<th>FX- (n=15)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>PM vs HC</td>
</tr>
<tr>
<td>Past or current regular smoking</td>
<td>8 (33.33)</td>
<td>14 (63.64)</td>
<td>5 (71.43)</td>
<td>9 (60)</td>
<td>4.22</td>
</tr>
<tr>
<td>&gt;14 alcohol drinks per week(^a)</td>
<td>7 (29.17)</td>
<td>6 (30)</td>
<td>2 (40)</td>
<td>4 (26.67)</td>
<td>.00</td>
</tr>
</tbody>
</table>

\(^a\)missing for 2 FX+
After classifying PM males according to FXTAS diagnosis, no significant differences emerged between groups for measures of height, weight and systolic blood pressure. Observations were not sufficient to compare the proportion of those with BMI>30, self-reported migraine, number of falls and previous diagnoses of hypertension diabetes, thyroid disorder, epilepsy, and ADHD. Significant group effects emerged for cardiovascular risk \( F_{(2,43)} = 5.902, p=.005 \) and self-reported hearing loss \( \chi^2 = 13.112, p=.001 \). Post-hoc analyses identified significantly greater cardiovascular risk scores among PM males with FXTAS compared to controls \( p=.008 \) and PM males without FXTAS \( p=.001 \). Self-reported hearing problems were also more common among PM males with FXTAS compared to controls \( \chi^2 = 12.159, p<.001 \) and PM males without FXTAS \( \chi^2 = 8.556, p=.003 \). There were no significant differences between PM males without FXTAS and controls for any measure of physical health.
Table 3.3

Physical health characteristics of sample

<table>
<thead>
<tr>
<th></th>
<th>HC (n=24)</th>
<th>PM (n=22)</th>
<th>FX+ (n=7)</th>
<th>FX- (n=15)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>t</td>
</tr>
<tr>
<td>Height</td>
<td>173.18 (7.57)</td>
<td>174.86 (5.87)</td>
<td>173.46 (4.45)</td>
<td>175.52 (6.46)</td>
<td>-.84</td>
</tr>
<tr>
<td>Weight</td>
<td>80.35 (13.77)</td>
<td>87.90 (13.72)</td>
<td>84.93 (11.05)</td>
<td>89.28 (14.95)</td>
<td>-1.86</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>144.17 (21.45)</td>
<td>141.50 (16.64)</td>
<td>144.29 (18.08)</td>
<td>140.20 (15.96)</td>
<td>-.47</td>
</tr>
<tr>
<td>Framingham Index</td>
<td>11.38 (5.31)</td>
<td>12.09 (5.90)</td>
<td>17.43 (3.82)</td>
<td>9.60 (5.00)</td>
<td>-.43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
<th>N (%)</th>
<th>N (%)</th>
<th>N (%)</th>
<th>X²</th>
<th>p</th>
<th>X²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI ≥30</td>
<td>4 (16.66)</td>
<td>7 (31.82)</td>
<td>2 (28.57)</td>
<td>5 (33.33)</td>
<td>1.45</td>
<td>.23</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Previously diagnosed diabetes</td>
<td>0 (0)</td>
<td>2 (9.09)</td>
<td>1 (14.29)</td>
<td>1 (4.35)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hypertension: ever diagnosed or treated</td>
<td>6 (25)</td>
<td>5 (22)</td>
<td>4 (57.14)</td>
<td>1 (6.67)</td>
<td>.03</td>
<td>.86</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Previously diagnosed thyroid disorder</td>
<td>1 (4.17)</td>
<td>2 (9.09)</td>
<td>0</td>
<td>2 (13.33)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Previously diagnosed epilepsy</td>
<td>0 (0)</td>
<td>1 (4.55)</td>
<td>1 (14.30)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Previously diagnosed ADHD</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Self-reported hearing problems</td>
<td>6 (25)</td>
<td>12 (54.55)</td>
<td>7 (100)</td>
<td>5 (33.33)</td>
<td>4.21</td>
<td>.04</td>
<td>13.11</td>
<td>.001</td>
</tr>
<tr>
<td>Self-reported migraine</td>
<td>0 (0)</td>
<td>1 (4.55)</td>
<td>0 (0)</td>
<td>1 (6.66)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>≥1 fall in past 12 months</td>
<td>0 (0)</td>
<td>5 (22.73)</td>
<td>4 (57.14)</td>
<td>1 (6.66)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a>HC; b>FX-
3.4.4 Mental health characteristics

Current and lifetime history of psychiatric disorder

Mental health characteristics of the sample are presented in Table 3.4. A total of eight PM males and two controls met lifetime criteria for at least one DSM-IV defined psychiatric disorder, and in four PM males criteria for at least two disorders were met. Comorbid diagnoses were treated as separate cases. As such, the total number of cases may exceed the number of individuals meeting diagnostic criteria for at least one disorder. Diagnoses included dysthymic disorder (1 FX+, 1 FX-), minor depressive disorder (1 FX+, 1 FX-, 2 HC), major depressive disorder (2 FX-), obsessive-compulsive disorder (2 FX+), social phobia (2 FX-, 1 HC), posttraumatic stress disorder (2 FX+), agoraphobia without panic disorder (1 FX+) and specific phobia (1 FX+). As current psychiatric disorder formed part of the exclusion criteria for the recruitment of controls into the study (see Chapter 2, Participants), group comparisons of the rates of current psychiatric diagnoses among PM males and controls were not conducted. Comparisons of current psychiatric disorder between PM males with and without FXTAS were not performed due to the small number meeting diagnostic criteria within each group (one FX-, two FX+).

When the PM group were combined, a significantly greater proportion of PM males (38%) met diagnostic criteria for any mood or anxiety disorder during their lifetime compared to controls (8%) ($\chi^2=5.740, p=.017$). Due to the small number of observations it was not possible to assess the rates of mood and anxiety clusters separately. Similarly, the number of observations was not sufficient to allow for the separation of PM males according to FXTAS diagnosis.
Current psychiatric symptoms as measured by self-report scales

There were no significant differences between PM males and controls on measures of current psychiatric symptoms using the DASS, LSAS and AQ (Table 3.4). Similarly, no group differences were observed after PM males were classified according to FXTAS diagnosis. There were insufficient observations to statistically test the proportions of PM males and controls scoring above clinical cut-offs for caseness on self-report scales and those reporting psychotropic medication use.

3.4.5 Associations between current psychiatric symptoms and FMR1 molecular measures (CGG repeat length and mRNA levels) in PM males

Results of Spearman correlations demonstrated that CGG repeat length and FMR1 mRNA levels were not significantly associated with DASS subscales of depression (CGG: r=.031, p=.892; FMR1 mRNA: r=.049, p=.830), anxiety (CGG: r=-.003, p=.988; FMR1 mRNA: r=-.104, p=.647), and stress (CGG: r=-.055, p=.807; FMR1 mRNA: r=.017, p=.940); total LSAS-SR score (CGG: r=-.046, p=.838; FMR1 mRNA: r=.046, p=.838) or AQ total score (CGG: r=-.139, p=.537; FMR1 mRNA: r=-.046, p=.837).
Table 3.4

*Mental health characteristics of sample*

<table>
<thead>
<tr>
<th></th>
<th>HC (n=24)</th>
<th>PM (n=22)</th>
<th>FX+ (n=7)</th>
<th>FX- (n=15)</th>
<th>Statistics</th>
<th>PM vs HC</th>
<th>FX+ vs FX- vs HC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>X²</td>
<td>p</td>
<td>X²</td>
</tr>
<tr>
<td>Lifetime history of any disorder</td>
<td>2 (8.33)</td>
<td>8 (38.10)</td>
<td>3 (42.86)</td>
<td>5 (35.71)</td>
<td>5.74</td>
<td>.02</td>
<td>-</td>
</tr>
<tr>
<td>Lifetime history mood disorder</td>
<td>2 (8.33)</td>
<td>5 (23.81)</td>
<td>1 (14.29)</td>
<td>4 (28.57)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lifetime history anxiety disorder</td>
<td>1 (4.14)</td>
<td>5 (23.81)</td>
<td>3 (42.86)</td>
<td>2 (14.29)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Current mood disorder</td>
<td>0 (0)</td>
<td>1 (4.76)</td>
<td>1 (14.29)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Current anxiety disorder</td>
<td>0 (0)</td>
<td>3 (14.29)</td>
<td>2 (28.57)</td>
<td>1 (7.14)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>On psychotropic medication</td>
<td>0 (0)</td>
<td>2 (9.09)</td>
<td>0 (0)</td>
<td>2 (13.33)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DASS Depression score ≥ 9</td>
<td>1 (4.17)</td>
<td>2 (9.09)</td>
<td>2 (28.57)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DASS Anxiety score ≥ 7</td>
<td>1 (4.17)</td>
<td>3 (13.64)</td>
<td>3 (42.86)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DASS Stress score ≥ 14</td>
<td>0 (0)</td>
<td>4 (18.18)</td>
<td>2 (28.57)</td>
<td>2 (13.33)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LSAS Total (%) score &gt;50</td>
<td>0 (0)</td>
<td>3 (13.64)</td>
<td>1 (14.29)</td>
<td>2 (13.33)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AQ Total (%) score ≥ 32</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Median (range)</th>
<th>Median (range)</th>
<th>Median (range)</th>
<th>Median (range)</th>
<th>U</th>
<th>p</th>
<th>X²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DASS Depression</td>
<td>0 (0–15)</td>
<td>1 (0–23)</td>
<td>3 (0–23)</td>
<td>1 (0–8)</td>
<td>199.50</td>
<td>.14</td>
<td>3.87</td>
<td>.15</td>
</tr>
<tr>
<td>DASS Anxiety</td>
<td>1 (0–7)</td>
<td>1.50 (0–25)</td>
<td>5 (0–25)</td>
<td>1 (0–5)</td>
<td>182.50</td>
<td>.07</td>
<td>5.65</td>
<td>.06</td>
</tr>
<tr>
<td>DASS Stress</td>
<td>3 (0–10)</td>
<td>5 (0–31)</td>
<td>3 (0–31)</td>
<td>5 (0–21)</td>
<td>214.00</td>
<td>.27</td>
<td>1.31</td>
<td>.52</td>
</tr>
<tr>
<td>LSAS Total</td>
<td>22 (4–46)</td>
<td>27 (5–86)</td>
<td>38 (11–86)</td>
<td>20 (5–70)</td>
<td>220.50</td>
<td>.34</td>
<td>2.52</td>
<td>.28</td>
</tr>
<tr>
<td>AQ Total</td>
<td>17.50 (3–28)</td>
<td>19.50 (7–29)</td>
<td>21 (15–29)</td>
<td>18 (7–29)</td>
<td>1.45b</td>
<td>.15</td>
<td>1.93b</td>
<td>.16</td>
</tr>
</tbody>
</table>

*a one FX- missing; b parametric equivalent used*
3.5 Discussion

This chapter described the sociodemographic and clinical features among a newly established cohort of PM males and matched controls on which subsequent analyses in this thesis will be based. The key findings suggested that the combined PM male and control groups were well matched in terms of sociodemographic and lifestyle factors. When classified according to FXTAS diagnosis, it was shown that PM males with FXTAS were significantly older than PM males without FXTAS, and demonstrated poorer performance on measures of current IQ compared to asymptomatic carriers and controls. In terms of physical health, PM males reported higher rates of hearing loss compared to controls, only partially supporting the hypothesis that PM males would demonstrate greater vulnerability to physical health conditions identified in previous cohorts. Males with the PM were also more likely to have a lifetime history of any mood or anxiety disorder compared to controls, supporting the hypothesis of greater vulnerability to psychiatric disorder in the PM group. No group differences emerged for total scores on self-report scales of current psychiatric symptomatology, and among PM males current symptoms were not associated with FMR1 molecular measures. These findings are not consistent with the prediction that current psychiatric symptoms would be elevated among PM males and positively associated with FMR1 mRNA levels. Together, the findings provide some evidence to suggest that PM males demonstrate increased vulnerability to physical and mental health conditions. This raises possible clinical implications for the appropriate identification and management of physical and mental health concerns among PM males that extend beyond the characteristic features of FXTAS.
3.5.1 Sociodemographic and lifestyle characteristics

An examination of demographic and lifestyle characteristics revealed that the combined PM group were well matched to controls with regard to factors that may impact upon performance on neurobehavioural measures. After classifying PM males according to FXTAS diagnosis, it was shown that PM males with FXTAS were significantly older than PM males without FXTAS, consistent with the age-related penetrance of the disorder (Jacquemont et al., 2004). Importantly, no significant differences in age were found across the PM groups and controls.

Assessment of premorbid general intelligence showed no significant differences between groups even though poorer performance on measures of current general intelligence were observed among PM males with FXTAS. Although within-subject comparisons of current and premorbid intelligence were not included in these analyses, this suggests that cognitive changes associated with FXTAS may contribute to lower current IQ scores within this group. Performance on more specific domains of cognitive function will be explored in Chapter 4. It is important to note that although scores for current FSIQ, VIQ and PIQ among PM males with FXTAS were significantly lower than both controls and PM males without FXTAS, mean scores for all groups were within the average range (100 ± 15). These findings highlight the importance of considering premorbid ability when assessing cognitive decline in individuals with FXTAS, even among those performing within the average range at the time of assessment. Thus, ‘average’ performance may in fact represent decline in cognitive function, particularly among those with high levels of education,
as previously proposed by others (Bacalman et al., 2006; Brega et al., 2008; Grigsby et al., 2008; Grigsby et al., 2007).

3.5.2 Physical health

A significantly greater proportion of PM males reported hearing loss compared to controls. This effect was largely driven by PM males with FXTAS. Although hearing loss is commonly associated with increasing age and is therefore not a surprising comorbid concern of PM males with FXTAS compared to younger asymptomatic carriers, the rate of hearing loss in PM males with FXTAS was also higher than that reported in age-matched controls. This suggests that the underlying cause of greater hearing loss among PM males with FXTAS may not be solely related to age-dependent changes. Hearing loss has previously been described among PM males with FXTAS and may reflect susceptibility of the vestibulocochlear nerve to pathogenic processes associated with FXTAS (Hagerman et al., 2001; Juncos et al., 2011; Paul, Pessah, Gane, Ono, Hagerman et al., 2010; Schneider et al., 2011).

Future studies incorporating assessments of vestibular and auditory function, as opposed to self-report measures, are required to determine the possible associations between FXTAS and damage to the vestibulocochlear nerve. This will have significant clinical implications, as hearing loss may contribute to negative physical, psychological and social outcomes resulting in overall poorer quality of life (Dalton, Cruickshanks, Klein, Klein, Wiley et al., 2003; Strawbridge, Wallhagen, Shema, & Kaplan, 2000).
Cardiovascular risk scores, as measured by the Framingham Index (D’Agostino et al., 2008), were also significantly higher among PM males with FXTAS compared to those without FXTAS and controls. This measure incorporates a number of variables including age, gender, total cholesterol, HDL cholesterol, smoking status, and hypertension. Although current guidelines for the management of FXTAS recommend modification of lifestyle factors that may contribute to increased cardiovascular risk (Hagerman et al., 2009; Polussa et al., 2014), previous studies have focused more specifically on the association between FXTAS and hypertension (Hamlin et al., 2012; Marek, Papin, Ellefsen, Niederhauser, Isidor et al., 2012), rather than a combination of risk factors as was used in the current study. Rates of hypertension between PM males and controls in the current study were comparable, in line with a previous study comparing blood pressure among asymptomatic PM males and controls (Marek et al., 2012). The small number of observations precluded statistical comparisons across FXTAS +/- diagnostic categories, and as such the possibility of higher rates of hypertension among those with FXTAS as previously reported by Hamlin et al. (2012) could not be explored. The association between cardiovascular risk factors in midlife and later onset of FXTAS symptoms among PM males may be an area worthy of further research, given the well-established associations between cardiovascular risk and cognitive decline (Knopman, Boland, Mosley, Howard, Liao et al., 2001; Meyer, Rauch, Rauch, Haque, & Crawford, 2000; Skoog, Nilsson, Persson, Lernfelt, Landahl et al., 1996; Whitmer, Sidney, Selby, Johnston, & Yaffe, 2005). This would provide important information regarding potential behavioural and biomedical risk factors for FXTAS that may be modifiable.
3.5.3 Mental health

A significantly larger proportion of PM males in this cohort met lifetime diagnostic criteria for any DSM-defined mood or anxiety disorder compared to controls. This is consistent with previous research suggesting that PM males exhibit increased vulnerability to psychiatric disorder (Bourgeois et al., 2011). These findings may reflect an increasing burden of neuropsychiatric disorders related to accumulating pathology or disruption within prefrontal and limbic systems. It has been proposed that dysregulation of connections between hippocampal-prefrontal cortex pathway (comprising efferent connections from the hippocampus to the prefrontal cortex) and the reciprocal connections of these structures with the amygdala may contribute to cognitive and emotional deficits common to a range of psychiatric disorders (Godsil, Kiss, Spedding, & Jay, 2013). Associations between FMR1 expression and functional connectivity of the prefrontal cortex (Hashimoto, Backer, Tassone, Hagerman, & Rivera, 2011), hippocampus (Koldewyn et al., 2008) and amygdala (Hessl, Rivera, Koldewyn, Cordeiro, Adams et al., 2007; Hessl et al., 2011) highlight a possible role of mRNA toxicity and reductions in FMRP in dysregulation of the hippocampal-prefrontal pathway among PM males with and without FXTAS. As features suggestive of disruption to this pathway, including deficits in memory (Grigsby et al., 2008; Hippolyte et al., 2014), working memory (Cornish et al., 2009; Kogan & Cornish, 2010), and elevated psychiatric symptoms (Bourgeois et al., 2011; Dorn et al., 1994; Hessl et al., 2005) have been described among PM males prior to the onset of FXTAS, it is possible that these features may serve as early indicators of later decline. Alternatively, these features may reflect an independent and diffuse vulnerability to an array of cognitive and emotional characteristics, which may then
become exacerbated by brain atrophy and white matter disease with the onset of FXTAS. Longitudinal studies will be vital in determining whether psychiatric symptoms among PM males without motor symptoms may represent an early indicator of later decline associated with FXTAS. Together, the available evidence suggesting greater vulnerability to psychiatric disorder in males with the PM, regardless of its association with FXTAS, has important clinical implications for screening, timely identification and management of symptoms that may impact upon quality of life.

Despite the elevated rates of lifetime psychiatric diagnoses among PM males compared to controls, no group differences were found on measures of current symptomatology and few participants met clinical cut-offs for probable caseness. The lack of group differences is in contrast to previous studies suggesting greater psychiatric involvement among PM males with and without FXTAS (Adams et al., 2010; Bacalman et al., 2006; Bourgeois et al., 2009; Bourgeois et al., 2011; Cornish et al., 2005; Dorn et al., 1994; Hessl et al., 2005; Seritan et al., 2013). This may relate to differences in the selection of measures used to assess psychiatric symptoms between studies. For example, increased rates of depression among PM males with FXTAS measured using the Symptom Checklist-90-Revised (SCL-90-R) were compared to an average T score of 50 (Hessl et al., 2005), while DASS scores in the current study were compared to matched controls. Although depression scores reported by Hessl et al. (2005) were significantly elevated among PM males with FXTAS relative to the comparison value, the mean (57.93) was still within the average range of the T-score distribution (50 ± 10). With regard to autistic traits, Cornish et al. (2005) reported group differences on the attention switching domain of
the AQ, but not the total score. Thus, the use of the total score in the current study may not have been sensitive to more specific features captured by the attention switching subscale, such as preference for routines and tendency to focus on details. Elevated rates of social phobia among PM males as described by Bourgeois et al. (2011) reflected lifetime history, and not necessarily current symptomatology. Indeed, estimates of current social anxiety symptoms and social phobia may be underestimated by research studies, as those with active symptoms may be less likely to volunteer to participate in research (Hunter et al., 2008). It is also possible that self-report scales may lead to an underestimation of psychiatric symptoms among PM males with cognitive impairment, and that informant-rated scales may provide more complete information regarding psychiatric symptoms (McAvay, Raue, Brown, & Bruce, 2005). This is in accordance with informant ratings of psychiatric symptoms among PM males with FXTAS that suggest clinically significant elevations in psychiatric symptoms including disinhibition, irritability, agitation and aggression (Bacalman et al., 2006).

Among PM males, current psychiatric symptoms were not associated with increased levels of FMR1 mRNA levels. This is not consistent with a previous study that demonstrated a positive association between FMR1 mRNA levels and multiple subscales of the SCL-90-R, including depression, anxiety and the global severity index (Hessl et al., 2005). A possible explanation for this difference between studies may relate to the larger sample of PM males with FMR1 mRNA available (n=55) reported in Hessl et al. (2005). Alternatively, the minimal current psychiatric involvement in this cohort may have resulted in an insufficient range of scores to be associated with mRNA levels. Another possible explanation is that the relationship
between mRNA toxicity and psychiatric symptoms in the PM is not a simple linear relationship, but rather reflects a complex interaction of genetic and environmental factors. These may include genetic vulnerabilities unrelated to the \textit{FMR1} gene, psychosocial stressors, chronic medical conditions other than FXTAS, exposure to traumatic events and substance abuse. Future studies in larger cohorts are needed to explore possible gene-environment interactions contributing to psychiatric disorder in the PM to gain a better understanding of specific risk and/or protective factors.

There are a number of limitations of this study that should be considered. Those that are relevant to multiple chapters throughout this thesis (for example, issues relating to sample size, ascertainment bias and cross-sectional study design) will be discussed in the summary and conclusions of this thesis (Chapter 9) to avoid unnecessary repetition. A further limitation relevant to this chapter is that the included measures relied on self-report measures (e.g. hearing loss, previous diagnoses, psychiatric symptoms), which may lead to error due to inaccurate recall of symptoms, particularly among individuals with cognitive impairment. Despite the small size of the current PM male cohort, the strategy of recruiting PM males outside the context of a clinical service can be considered a strength that may increase the representativeness of the sample, particularly when considering current physical and mental health status. Future research with larger PM cohorts and longitudinal follow-up will be required to determine the reliability of clinical characteristics among PM males compared to controls.

In conclusion, this chapter has described the basic clinical characteristics of a newly established cohort of PM males and matched controls on which the remainder of this
thesis will be based. The findings showed that PM males were well matched on demographic and lifestyle variables that may impact performance on neurobehavioural measures. Findings of significantly lower current, but not premorbid IQ among males with FXTAS suggest that IQ deficits likely arise as a consequence of cognitive disruption associated with FXTAS. The findings also lend some support to the notion that PM males may demonstrate increased vulnerability to physical and mental health conditions; however, the mechanisms underlying this vulnerability are unknown. Nonetheless, the findings raise important clinical implications, highlighting the importance of identification and appropriate management of a range of clinical features among PM males, including but not limited to those considered to be characteristic of FXTAS.
CHAPTER 4.

COHORT CHARACTERISTICS: COGNITIVE
CHAPTER 4. COHORT CHARACTERISTICS:

COGNITIVE

4.1 Introduction

Clinical characteristics of the cohort were described in Chapter 3. An examination of demographic and lifestyle characteristics revealed that the combined PM group were well matched to controls on variables that may impact performance on neurobehavioural measures. Although a greater proportion of PM males endorsed a lifetime history of psychiatric disorder there were no differences between groups for current symptomatology. The following chapter will continue to explore characteristics of the cohort with a focus on cognitive features among PM males and controls.

As described in Chapter 1, cognitive features associated with FXTAS include poorer performance on measures of executive function (Brega et al., 2008; Cornish et al., 2009; Cornish et al., 2008; Grigsby et al., 2008; Grigsby et al., 2007; Schneider et al., 2011; Yang, Chan, et al., 2013; Yang, Simon, et al., 2013), working memory (Brega et al., 2008; Cornish et al., 2009; Cornish et al., 2008; Grigsby et al., 2008; Grigsby et al., 2007; Hashimoto, Javan, et al., 2011; Schneider et al., 2011; Yang, Chan, et al., 2013), information processing speed (Brega et al., 2008; Grigsby et al., 2008; Grigsby et al., 2007; Schneider et al., 2011; Yang, Chan, et al., 2013), and fine motor function (Grigsby et al., 2008; Schneider et al., 2011; Wang, Hessl, Schneider, et al., 2013) relative to matched controls. These features suggest difficulties with problem solving, behavioural regulation, psychomotor speed and manual dexterity.
Deficits in verbal memory (Seritan, Nguyen, Farias, Hinton, Grigsby et al., 2008) and visuospatial function (Grigsby et al., 2008) have also been described, although controlled studies exploring these domains are lacking (Birch et al., 2014). It has been proposed that changes in cognitive function represent an early and progressive feature of FXTAS, with one study reporting cognitive impairment in 31% and 67% of possible and definite FXTAS cases, respectively (Juncos et al., 2011). Estimates suggest that in 40% of PM males with FXTAS, cognitive changes may be sufficiently severe to impact on ADLs, meeting diagnostic criteria for dementia under DSM-IV criteria (American Psychiatric Association, 2000; Seritan et al., 2008). Deficits in executive function in particular appear to contribute to functional disability (Brega et al., 2009).

Subtle deficits in cognitive function have also been described among adult PM males and females who do not meet diagnostic criteria for FXTAS. These include deficits in executive function (Cornish et al., 2011; Cornish et al., 2008; Kraan, Hocking, Georgiou-Karistianis, Metcalfe, Archibald, Fielding, Trollor, et al., 2014), working memory (Cornish et al., 2009; Kogan & Cornish, 2010) and visuospatial processing (Goodrich-Hunsaker et al.; Hocking et al., 2012; Wong et al., 2012) among both PM males and females, and decrements in verbal memory and motor sequence learning among PM males (Grigsby et al., 2008; Hippolyte et al., 2014). Although retrospective reports suggest that motor symptoms typically precede changes in cognitive function among PM males with FXTAS (Juncos et al., 2011; Leehey, Hall, Rice, Jacquemont, Zhang et al., 2005), findings of early cognitive changes in asymptomatic PM carriers suggest that subtle deficits in cognitive function may be observable prior to the onset of tremor and ataxia. Cornish et al. (2008) proposed that
PM males diverge into two separate pathways: a milder and stable phenotype, possibly reflecting neurodevelopmental effects of reduced FMRP; and progressive decline associated with mRNA toxicity, ultimately leading to FXTAS. The notion of subtle neurodevelopmental involvement is supported by a recent study demonstrating low-level visual processing deficits among infant and toddler PM males and females that resemble those seen in the full mutation (Gallego, Burris, & Rivera, 2014). Further longitudinal studies will be needed to distinguish neurodevelopmental versus neurodegenerative pathways associated with the PM as this will prove invaluable in informing the development of predictive models to ascertain risk for FXTAS.

Among PM males with and without FXTAS, larger CGG repeat expansions have been associated with increased risk for cognitive impairment (Sevin et al., 2009), and impairments on measures of general intelligence (Cohen et al., 2006; Hessl et al., 2005; Sevin et al., 2009), response inhibition (Cornish et al., 2011), working memory (Cornish et al., 2009; Kogan & Cornish, 2010), visuospatial function (Hocking et al., 2012) and verbal fluency (Grigsby et al., 2006). Although there appears to be stronger evidence to suggest that CGG repeat length may be more important in terms of predicting cognitive performance in the PM compared to other FMR1 molecular measures, it should be noted that of the studies mentioned above only two included quantification of mRNA (Cohen et al., 2006; Hessl et al., 2005), and only one included FMRP levels (Hessl et al., 2005). Moreover, in both of these studies, general intelligence was the only cognitive domain investigated for possible relationships with FMR1 molecular measures. As increasing CGG repeat length is associated with both increasing mRNA and decreasing FMRP (Kenneson et al.,
Chapter 4. Cohort characteristics: Cognitive

2001; Tassone, Hagerman, Taylor, Gane, et al., 2000) it is possible that if measured, these variables would be associated with other aspects of cognitive performance. This is supported by studies suggesting an inverse relationship between FMR1 mRNA and functional connectivity within prefrontal (Hashimoto, Backer, et al., 2011), fronto-parietal (Yang, Chan, et al., 2013) and cerebellar regions (Wang, Hessl, Schneider, et al., 2013), which are known to subserve the regulation of executive functions and working memory (Alexander & Crutcher, 1990).

It is well established that the prevalence and severity of symptoms associated with FXTAS increase with age (Jacquemont et al., 2004). However, the risk and protective factors for FXTAS remain largely unknown. In the absence of longitudinal follow-up of cohorts, cross-sectional examinations of neurobehavioural and radiological features in terms of their progression with age may give insight into possible trajectories of clinical involvement in the PM across the lifespan. Neurobehavioural and radiological features that remain stable with age may reflect developmental profiles associated with the PM, while those that progress more rapidly than would be expected with increasing age may reflect a degenerative process that may culminate in FXTAS. Indeed, previous studies suggesting stronger associations between increasing age and both decrements in inhibitory control (Cornish et al., 2008) and decreased structural connectivity within the brain (Battistella et al., 2013; Hashimoto, Srivastava, et al., 2011; Wang, Hessl, Schneider, et al., 2013; Wang et al., 2012) suggest that the examination of performance among PM males with increasing age may have utility in delineating clinical trajectories associated with the PM. However, possible differences in the relationships between increasing age and cognitive performance among PM males compared to controls
have yet to be examined using a comprehensive neuropsychological evaluation targeting a broad range of cognitive domains. The examination of age-related cognitive changes in PM males, regardless of FXTAS diagnosis, circumvents issues associated with the possible classification of PM males as being ‘asymptomatic’ despite showing subclinical signs that may be early indicators of FXTAS. Further, this approach avoids drawing comparisons between PM males with and without FXTAS on clinical and radiological signs that form part of FXTAS diagnostic criteria and have therefore informed their group membership. For example, comparisons between PM carriers with and without FXTAS examining impairments in executive function, memory, or cortical/cerebellar volume loss may not be meaningful, as the two PM groups are in part defined based on the presence or absence of these features.

The aims of this chapter are to (i) comprehensively characterise cognitive features among PM males and controls across a broad range of cognitive domains; (ii) investigate whether the relationship between increasing age and cognitive performance differs between PM males and controls; and (iii) explore whether cognitive features among PM males are associated with *FMR1* molecular measures (CGG repeat length, mRNA). It was hypothesised that: (1) PM males would perform worse than controls on cognitive domains of executive function, attention and working memory, information processing speed, and fine motor function; (2) performance on these domains would show a stronger negative relationship with increasing age among PM males compared to controls; and that (3) cognitive domain scores among PM males would be negatively associated with increasing CGG repeat length and *FMR1* mRNA.
4.2 Assessment and method

Participant recruitment and assessment measures were described in detail in Chapter 2 and as such are not repeated in full here. Briefly, 22 PM males (age range 26–80) and 24 age and education matched controls (age range 26–77) completed a comprehensive neuropsychological assessment. Domain scores were derived for measures of attention and working memory, information processing speed, executive function, verbal memory, visuospatial function, language and fine motor function (see Chapter 2 for details). CGG repeat length and $FMR1$ mRNA levels were quantified from blood, as described in Chapter 2.

4.3 Statistical analyses

4.3.1 Assessment of cognitive features among PM males and controls

Multiple linear regression was used to explore age-related changes in cognitive features among PM males and controls. Independent variables for each model included age, group (PM vs controls), and their interaction (age x group). Premorbid IQ and education were included as covariates in all models. Fine motor function was included as a covariate in additional models for visuospatial and information processing domain scores, as all tests included in these domains involved manual manipulation of materials. Assumptions of multiple linear regression (linearity, independence and normality of residuals, homoscedasticity) were adequately met for all models.
4.3.2 Associations between FMR1 molecular measures and cognitive features

Associations between FMR1 molecular measures (CGG repeat length, mRNA levels) and cognitive domain scores among PM males were explored using partial Pearson correlations controlling for age and premorbid IQ.

An adjusted $p$ value of .007 was used for all analyses to account for multiple comparisons across the seven cognitive domain scores. Results with $p$ values <.05 are also discussed as possible trend-level associations, although it is acknowledged that these cannot be interpreted as statistically significant.

4.4 Results

4.4.1 Participant characteristics

Participant characteristics were described in Chapter 3 and are therefore not repeated here. In brief, the combined PM group were well matched to controls with no significant differences between these groups for variables of age, education, premorbid IQ, current IQ, primary language spoken at home, ethnicity, relationship status and total household income (see Chapter 3).

4.4.2 Associations between cognitive features, carrier status and age

Mean cognitive domain scores are presented in Table 4.1.
Table 4.1

Descriptive statistics for cognitive domain scores

<table>
<thead>
<tr>
<th></th>
<th>HC (n=24)</th>
<th>PM (n=22)</th>
<th>FX+ (n=7)</th>
<th>FX- (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fine motor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.23 (0.97)</td>
<td>-0.25 (0.95)</td>
<td>-1.22 (0.66)</td>
<td>0.21 (0.68)</td>
</tr>
<tr>
<td>Range</td>
<td>-1.58–2.21</td>
<td>-2.21–1.58</td>
<td>-2.21–0.19</td>
<td>-0.97–1.58</td>
</tr>
<tr>
<td><strong>Information processing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.33 (0.88)</td>
<td>-0.36 (0.98)</td>
<td>-1.24 (0.63)</td>
<td>0.05 (0.84)</td>
</tr>
<tr>
<td>Range</td>
<td>-1.58–2.21</td>
<td>-2.21–1.58</td>
<td>-2.21–0.42</td>
<td>-1.28–1.58</td>
</tr>
<tr>
<td><strong>Attention and working memory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.22 (1.11)</td>
<td>-0.24 (0.82)</td>
<td>-0.59 (0.83)</td>
<td>-0.08 (0.79)</td>
</tr>
<tr>
<td>Range</td>
<td>-1.46–2.02</td>
<td>-2.23–1.96</td>
<td>-2.23–0.39</td>
<td>-0.89–1.96</td>
</tr>
<tr>
<td><strong>Executive function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.28 (0.93)</td>
<td>-0.31 (0.96)</td>
<td>-1.17 (0.97)</td>
<td>0.1 (0.65)</td>
</tr>
<tr>
<td>Range</td>
<td>-1.42–2.21</td>
<td>-2.21–1.28</td>
<td>-2.21–0.81</td>
<td>-0.89–1.28</td>
</tr>
<tr>
<td><strong>Memory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.27 (0.91)</td>
<td>-0.29 (0.99)</td>
<td>-0.58 (1.09)</td>
<td>-0.16 (0.95)</td>
</tr>
<tr>
<td>Range</td>
<td>-1.42–2.21</td>
<td>-2.21–1.58</td>
<td>-2.21–1.28</td>
<td>-1.81–1.58</td>
</tr>
<tr>
<td><strong>Visuospatial</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.21 (0.88)</td>
<td>-0.23 (1.1)</td>
<td>-1.3 (1.18)</td>
<td>0.27 (0.61)</td>
</tr>
<tr>
<td>Range</td>
<td>-1.36–1.83</td>
<td>-2.38–1.77</td>
<td>-2.38–0.75</td>
<td>-0.55–1.77</td>
</tr>
<tr>
<td><strong>Language</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.14 (0.93)</td>
<td>-0.15 (1.03)</td>
<td>-0.97 (1.1)</td>
<td>0.23 (0.76)</td>
</tr>
<tr>
<td>Range</td>
<td>-1.42–2.21</td>
<td>-2.21–1.42</td>
<td>-2.21–0.89</td>
<td>-1.02–1.42</td>
</tr>
</tbody>
</table>
Multiple linear regression models exploring the main effects of group (PM vs HC), age, and the interaction between these variables on cognitive domain scores are summarised in Table 4.2. Education and premorbid IQ were included as covariates in all models.

Fine motor function

The regression model including premorbid IQ, education, age, group and the interaction of age × group as predictors of fine motor function was significant ($F_{(5, 40)}=21.825, p<.001$), explaining 69.8% of variance in the domain score. PM carrier status was a significant predictor of fine motor function (B=.528, $p=.003$), indicating poorer performance among PM males compared to controls. Among PM males, increasing age was associated with poorer fine motor function (B=-.490, $p<.001$), but the interaction term of age × group was not significant (B=-.073, $p=.667$). This indicates that while scores were significantly lower among PM males and showed a negative association with age, the relationship between cognitive performance and age was comparable to that found in controls (Figure 4.1).

Information processing speed

The regression model predicting information processing speed and including premorbid IQ, education, age, group and the interaction of age × group as predictors was significant ($F_{(5, 40)}=11.071, p<.001$), explaining 52.8% of the domain score variance. A main effect of PM carrier status was observed (B=.691, $p=.002$), indicating that PM males achieved significantly lower scores on this domain compared to controls. Age was also a significant predictor of performance among
PM males (B=-.365, \(p=.002\)), suggesting poorer performance with increasing age. The interaction between these terms (age \(\times\) group) was not significant, indicating that the relationship between information processing speed and age did not differ between PM males and controls (Figure 4.1).

To determine whether the group effect on information processing speed was due to deficits in fine motor function, fine motor function domain scores were added into a subsequent model predicting information processing speed. Fine motor function did not make a significant contribution to the model (B=.224, \(p=.263\)), and the inclusion of fine motor function did not significantly change the variance explained by the overall model (\(R^2\) change=.013, \(p=.263\)). Therefore the significant main effect of group in the initial model is unlikely to be due to group differences in fine motor function.
Figure 4.1. Changes in domain scores for and information processing speed (left) and fine motor function (right) with increasing age among PM males and controls. PM males attained significantly lower domain scores for fine motor function and information processing speed compared to controls. However, the relationships between performance on these domains and age were comparable between groups.
Attention and working memory

The regression model including premorbid IQ, education, age, group and the interaction of age \times group as predictors was significant ($F_{(5, 40)}=7.052, p<.001$), explaining 40.2% of the variance in attention and working memory domain scores. No significant main effect emerged for group, indicating comparable performance on the attention and working memory domain between PM males and controls. Increasing age was not a significant predictor among PM males (B=.033, $p=.793$). However, the interaction term for age \times group approached the adjusted level for significance (B=-.403, $p=.015$), indicating a possible difference in the relationship between age and attention/working memory function between groups. Contrary to the finding in PM males, increasing age was a significant predictor of poorer performance in attention and working memory domain scores in the control group (B=-.370, $p=.004$) (Figure 4.2).
Figure 4.2. Changes in attention/working memory domain score with increasing age in PM males and controls. Although there was no difference in domain scores overall between PM males and controls, only controls demonstrated a negative relationship between performance on this domain and age.

Executive function

The regression model including premorbid IQ, education, age, group and the interaction of age × group as predictors of executive function was significant ($F_{(5,40)}=9.738$, $p<.001$) and explained 49.3% of the variance in executive function domain scores. The main effect of group was not significant ($B=.427$, $p=.056$), indicating comparable performance on the executive function domain between PM males and controls. Increasing age was associated with poorer performance among
PM males (B=-.244, p=.044), although this did not meet the adjusted significance threshold. The interaction term of age × group was not significant (B=.035, p=.809), indicating that the relationship between age and performance on this domain was comparable to that found in controls.

**Verbal memory**

The regression model for verbal memory including premorbid IQ, education, age, group and the interaction of age × group was significant ($F_{(5, 40)}=11.233, p<.001$), explaining 53.2% of the variance in domain scores. PM carrier status was not a significant predictor (B=.336, p=.115). Increasing age was not significantly associated with verbal memory scores among PM males (B=-.148, p=.196) and the interaction term for age × group was not significant (B=-.273, p=.058), indicating that the relationship between age and performance on this domain was comparable to that found in controls.

**Visuospatial function**

The regression model including premorbid IQ, education, age, group and the interaction of age × group was significant ($F_{(5, 40)}=8.719, p<.001$), explaining 46.2% of the variance in visuospatial function domain scores. The main effect of group was not significant (B=.347, p=.129), indicating comparable performance on the visuospatial function domain between PM males and controls. Increasing age was associated with poorer performance among PM males (B=-.307, p=.015), although this did not meet the adjusted significance threshold. The interaction term of age ×
group was not significant (B=-.124, p=.413), indicating a similar relationship between age and performance on this domain was for PM males and controls.

Fine motor function was included in a subsequent model predicting visuospatial function to control for the potential impact of fine motor dysfunction on performance. When included simultaneously, neither age (B=-.177, p=.458) nor fine motor function (B=.388, p=.071) were significant predictors of visuospatial domain scores among PM males. Similarly, the main effect for group (B=.143, p=.562) and the interaction between group and age (B=-.096, p=.518) were not significant.

Language

The regression analysis for language domain scores which included premorbid IQ, education, age, group and the interaction of age × group as predictors generated a significant model ($F_{(5, 40)}=10.504, p<.001$), explaining 51.4% of the variance in domain scores. PM carrier status was not a significant predictor of language domain scores (B=.159, p=.451). Increasing age was associated with poorer performance among PM males (B=-.280, p=.017), although this did not meet the adjusted significance threshold. The interaction term of age × group was not significant (B=-.076, p=.592), indicating that the relationship between age and performance on this domain was similar to that found in controls.
### Table 4.2

**Summary of multiple linear regression models exploring the effect of PM status and age on cognitive domain scores**

<table>
<thead>
<tr>
<th>Information processing</th>
<th>Attention and working memory</th>
<th>Memory</th>
<th>Visuospatial</th>
<th>Language</th>
<th>Executive function</th>
<th>Fine motor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B</strong> (S.E.)</td>
<td><strong>t</strong></td>
<td><strong>B</strong> (S.E.)</td>
<td><strong>t</strong></td>
<td><strong>B</strong> (S.E.)</td>
<td><strong>t</strong></td>
<td><strong>B</strong> (S.E.)</td>
</tr>
<tr>
<td>Constant</td>
<td>-.293</td>
<td>-.164</td>
<td>-.901</td>
<td>-4.40***</td>
<td>-8.05</td>
<td>-4.44</td>
</tr>
<tr>
<td>Premorbid IQ</td>
<td>.02</td>
<td>1.27</td>
<td>.09</td>
<td>4.45***</td>
<td>.07</td>
<td>4.10***</td>
</tr>
<tr>
<td>Education</td>
<td>.01</td>
<td>.34</td>
<td>-.06</td>
<td>-1.29</td>
<td>.00</td>
<td>.06</td>
</tr>
<tr>
<td>Age</td>
<td>-.37</td>
<td>-3.30**</td>
<td>.03</td>
<td>.26</td>
<td>-.15</td>
<td>-1.32</td>
</tr>
<tr>
<td>Group</td>
<td>.69</td>
<td>3.36**</td>
<td>.26</td>
<td>1.08</td>
<td>.336</td>
<td>1.61</td>
</tr>
<tr>
<td>Age × group</td>
<td>-.06</td>
<td>-.41</td>
<td>-.40</td>
<td>-2.55*</td>
<td>-.27</td>
<td>-1.95</td>
</tr>
</tbody>
</table>

*p<.05; **p<.01; ***p<.001
4.4.3 Associations between cognitive domains and FMR1 molecular measures

Possible associations between FMR1 molecular measures and cognitive domains among PM males were explored using partial Pearson correlations, controlling for age and premorbid IQ. Education was not included as a covariate as it was not found to be a predictor of any cognitive domain score when included in linear regression models (see Table 4.2). No correlations between cognitive and FMR1 molecular measures met the adjusted significance threshold of \( p < .007 \). Exploration of trend-level associations revealed a possible negative association between visuospatial function and both FMR1 mRNA level \((r = -0.517, p = .020)\) and CGG repeat length \((r = -0.494, p = .027)\). These correlations remained at the trend level when fine motor function was included as an additional covariate \((FMR1 \text{ mRNA: } r = -0.485, p = .035; \text{ CGG: } r = -0.465, p = .045)\). CGG repeat length and FMR1 mRNA levels were not associated with domain scores for information processing speed \((\text{CGG: } r = -0.295, p = .206; FMR1 \text{ mRNA: } r = -0.336, p = .148)\), fine motor function \((\text{CGG: } r = -0.202, p = .392; FMR1 \text{ mRNA: } r = -0.228, p = .334)\), attention and working memory \((\text{CGG: } r = -0.408, p = .074; FMR1 \text{ mRNA: } r = 0.070, p = .768)\), executive function \((\text{CGG: } r = -0.327, p = .159; FMR1 \text{ mRNA: } r = -0.269, p = .252)\), verbal memory \((\text{CGG: } r = 0.037, p = .878; FMR1 \text{ mRNA: } r = 0.133, p = .577)\) or language function \((\text{CGG: } r = -0.164, p = .490; FMR1 \text{ mRNA: } r = -0.090, p = .707)\).

4.5 Discussion

This chapter provided a comprehensive examination of cognitive features among this newly established cohort of PM males and controls. Possible differences in the associations between increasing age and cognitive performance between groups were
also explored. The findings indicated poorer performance on measures of fine motor function and information processing speed in males with PM compared to controls, providing only partial support for the hypothesis that PM males would perform worse on a range of cognitive domains as previously described (Brega et al., 2008; Cornish et al., 2009; Cornish et al., 2008; Grigsby et al., 2008; Grigsby et al., 2007; Hashimoto, Javan, et al., 2011; Schneider et al., 2011; Wang, Hessl, Schneider, et al., 2013; Yang, Chan, et al., 2013; Yang, Simon, et al., 2013). Contrary to the hypothesis that the relationships between increasing age and poorer performance on these domains would be stronger in the PM group compared to controls, the associations between performance on these cognitive domains and age were comparable between groups. In males with the PM, the hypothesis of associations between poorer performance on cognitive domains, and increasing CGG repeat length and \textit{FMR1} mRNA levels was not supported. Together, these findings suggest generally poorer performance on measures of fine motor function and information processing speed in males with the PM, although the lack of associations with age and \textit{FMR1} molecular measures are inconsistent with previous studies (Cornish et al., 2011; Cornish et al., 2009; Cornish et al., 2008; Grigsby et al., 2006; Kogan & Cornish, 2010).

The current finding of impairments in fine motor and information processing speed in the combined PM group are suggestive of greater cognitive and motor slowing among PM males compared to controls. Impairments in information processing speed and fine motor function have previously been described as being characteristic of FXTAS (Brega et al., 2008; Grigsby et al., 2008; Grigsby et al., 2007; Schneider
et al., 2011; Wang, Hessl, Schneider, et al., 2013; Yang, Chan, et al., 2013); however, the relationships between performance on these cognitive domains and increasing age among PM males in this cohort was comparable to those found in controls. This suggests an overall lower level of performance on these domains compared to matched controls that did not appear to be more marked in participants of more advanced age. The lack of a significant interaction with age may suggest a neurodevelopmental, as opposed to neurodegenerative trajectory, associated with the PM. However, this explanation would be at odds with previous studies demonstrating comparable performance on measures of information processing speed and fine motor function among asymptomatic PM males and controls (Brega et al., 2008; Grigsby et al., 2008). It is possible that the small sample size, particularly for older PM males with FXTAS, resulted in insufficient power to detect a significant difference in the relationships between age and cognitive function between groups. Nevertheless, features of cognitive and motor slowing, which may be observed in other neurodegenerative disorders including Huntington’s Disease (Bamford, Caine, Kido, Cox, & Shoulson, 1995), are consistent with dysfunction within fronto-subcortical circuits underlying cognitive and motor function (Knopman & Selnes, 2003). Although FXTAS is frequently referred to as a fronto-subcortical syndrome based on the pattern of neuropsychiatric features (Bacalman et al., 2006; Brega et al., 2008), the relationships between volume loss in specific subcortical structures and cognitive features have yet to be explored. These relationships will be the focus of more targeted analyses, presented in Chapter 6.

Examination of attention and working memory performance among PM males and controls revealed no significant difference in domain scores between groups. This is
in contrast to previous reports suggesting poorer working memory performance in PM males with and without FXTAS (Cornish et al., 2009; Kogan & Cornish, 2010). Interestingly, the results did suggest that if examined in a larger sample, PM males may demonstrate a different relationship between attention and working memory performance and age compared to controls. Although the significance of the age × group interaction term did not withstand correction for multiple comparisons, scores within the control group tended to decrease with increasing age, while no significant effects of increasing age were observed within the PM group. It is possible that the lack of a significant association between performance on this domain and age among PM males may be due to poorer performance among younger carriers in the cohort. This is illustrated in Figure 4.2, showing that younger PM males tended to have lower scores compared to controls around the same age. Although this interpretation is speculative, poorer performance among younger PM males would be consistent with studies suggesting decrements in working memory among PM males without FXTAS (Cornish et al., 2009; Kogan & Cornish, 2010) and alterations in structural and functional connectivity within the prefrontal cortex (Hashimoto, Backer, et al., 2011; Hippolyte et al., 2014). A more in-depth examination of the trajectories associated with possible decrements in attention and working memory would require longitudinal studies in a larger cohort of PM males.

In regard to language function, there was no significant difference between PM males and controls, consistent with a previous study including measures of this domain (Grigsby et al., 2008). However, males with the PM in this cohort also did not demonstrate significant impairments on measures of executive function, verbal memory, or visuospatial function, contrary to previous studies (Brega et al., 2008;
Chapter 4. Cohort characteristics: Cognitive

Cornish et al., 2011; Cornish et al., 2009; Cornish et al., 2008; Grigsby et al., 2008; Grigsby et al., 2007; Hippolyte et al., 2014; Hocking et al., 2012; Kogan & Cornish, 2010; Wong et al., 2012). The current findings are more in line with previous studies that did not detect deficits in executive function (Allen et al., 2011; Juncos et al., 2011) among PM males relative to controls. Possible explanations for the discrepancies between studies regarding cognitive domains affected in the PM may include differences in the selection of cognitive tests. In the current study, scores on individual tests of executive function were combined into an overall domain score, whereas in previous studies individual scores of tests tapping more specific aspects of executive function, such as verbal fluency and inhibitory control, have been used (Cornish et al., 2011; Cornish et al., 2008; Grigsby et al., 2006; Grigsby et al., 2007). It is possible that the domain score is not sensitive to specific deficits, such as inhibitory control, which have previously been described among PM males with and without FXTAS (Cornish et al., 2011; Cornish et al., 2008). Another possibility for the relatively milder cognitive involvement in this cohort is that scores of all PM males were collated, as opposed to being presented as subgroups according to FXTAS diagnosis. As discussed in the introduction to this chapter, deficits in executive function and verbal memory are included in the diagnostic criteria for FXTAS, and as such, comparisons of PM males with and without FXTAS on these domains may be redundant. As the overall PM group included individuals presenting with varying degrees of cognitive involvement, the combination of individual scores ranging across both upper and lower tails of the distribution unsurprisingly normalised to a mean score comparable to that of controls.
Among PM males, there was trend-level evidence to suggest that poorer performance on the visuospatial domain was associated with both increasing \textit{FMR1} mRNA and CGG repeat length. Although the finding did not withstand correction for multiple comparisons, this is consistent with previous studies suggesting CGG-related decrements in specific tasks tapping dorsal stream visuospatial processing among PM males without FXTAS (Hocking et al., 2012). It is important to note that although this association was observed, performance on the visuospatial domain was not significantly impaired among PM males compared to controls. Therefore the clinical significance of \textit{FMR1}-related changes in visuospatial function should be explored in future studies. Performance on the remaining cognitive domains was not associated with \textit{FMR1} molecular measures in this cohort. Again, this may reflect the lack of specificity of the cognitive domain scores used in the current study as opposed to more specific aspects of cognitive function which have been shown to possess a negative relationship with CGG repeat length, including response inhibition (Cornish et al., 2011), working memory (Cornish et al., 2009; Kogan & Cornish, 2010) and verbal fluency (Grigsby et al., 2006).

In addition to the general limitations (discussed in Chapter 9 to avoid unnecessary repetition), there are a number of specific limitations that should be considered. First, the use of cognitive domain scores rather than individual test scores may have resulted in a lack of sensitivity to more specific decrements in cognitive function (e.g. within the executive function domain). The examination of cognitive domains at only a single time point is a further limitation. Therefore, findings pointing to comparable trajectories in cognitive function with increasing age between PM males and controls should be interpreted with caution. Future research with larger PM
cohorts and longitudinal follow-up will be required to determine the progression of
cognitive features with increasing age among PM males compared to controls.
In conclusion, this chapter has explored age-related changes in cognitive features
among PM males with and without FXTAS. Although PM males demonstrated
poorer performance on measures of fine motor function and information processing
speed compared to controls, the relationships between performance on these
cognitive domains and increasing age did not differ between groups. Taken together,
the findings point to a stable trajectory associated with greater cognitive and motor
slowing among males with the PM, suggestive of disruption to fronto-subcortical
neural networks subserving cognitive and motor function. Future studies should
explore the possible interrelationships between information processing speed, fine
motor function and subcortical volumes in PM males with and without FXTAS.
CHAPTER 5.

COHORT CHARACTERISTICS:

MOTOR AND RADIOLOGICAL
CHAPTER 5. COHORT CHARACTERISTICS:
MOTOR AND RADIOLOGICAL

5.1 Introduction

Examination of the cognitive characteristics of the cohort in Chapter 4 revealed poorer performance on cognitive domains of information processing speed and fine motor function among PM males compared to controls. It was proposed that cognitive and motor slowing among males with the PM may reflect dysfunction of fronto-subcortical neural networks subserving cognitive and motor function. This chapter will examine gross motor and radiological features among the cohort, including their relationships with increasing age.

Motor signs among PM carriers typically manifest from the age of 50 and become increasingly severe with advancing age (Jacquemont et al., 2004). These include intention tremor (increasing in amplitude toward the end point of a movement), and cerebellar ataxia (wide-based, unsteady gait), with less pronounced signs of parkinsonism (rigidity, bradykinesia, hypomimia, resting tremor) (Jacquemont et al., 2003; Leehey et al., 2008). Although less common, dystonia, spasticity and muscle weakness may also be present (Jacquemont et al., 2003; Jacquemont et al., 2005; Zhang, Sukharev, Schneider, Olichney, Seritan et al., 2014). Retrospective reports suggest that among PM males with FXTAS, tremor occurs prior to the onset of gait ataxia (Juncos et al., 2011; Leehey, Beffy-Kravis, Min, Hall, Rice et al., 2007), with mean (SD) ages of onset at 62.6 (8.1) and 63.6 (7.3) years, respectively (Tassone et al., 2007). Severity of parkinsonism shows the strongest association with increasing
age, followed by ataxia, and tremor (Leehey et al., 2008). Among one cohort of 55 PM males with FXTAS (Leehey et al., 2007), difficulties with ADLs emerged within nine years of initial tremor, progressing to inability to undertake most ADLs within 16 years from onset. Onset of falls and dependence on a walking aid were typically within six and 15 years of initial gait disturbance, respectively.

Motor signs including impairments in vestibular control of balance among PM males (O'Keefe et al., 2012; O'Keefe et al., 2015), and decrements in finger tapping, reaction time, and proprioception among PM females (Kraan et al., 2013; Narcisa et al., 2011) may also be discernible among PM carriers who do not meet diagnostic criteria for FXTAS. Decrements in postural control and greater reductions in gait speed and step length while concurrently performing dual-tasks have also been described among asymptomatic PM females (Hocking et al., 2015; Kraan et al., 2013). Findings relating to postural sway and dual-task related changes in gait function will be reviewed in greater detail in Chapters 7 and 8 respectively.

Among PM males with FXTAS, a positive linear association between CGG repeat length and body sway with eyes closed suggests an effect of increasing CGG expansions on postural control (Allen et al., 2008). Further, decrements in postural sway among PM carriers with and without FXTAS have also been observed with increasing age and CGG repeat length (O'Keefe et al., 2015). These findings are also supported by greater dual-task related decrements in performance on measures of gait variability (Kraan, Hocking, Georgiou-Karistianis, Metcalfe, Archibald, Fielding, Bradshaw, et al., 2014) and medial-lateral sway (Kraan et al., 2013) among PM females with increasing age and CGG repeat length. Moreover, elevated FMR1
mRNA levels have been associated with greater variability in reaction time and movement time during voluntary stepping among PM females without FXTAS (Hocking et al., 2015). As larger CGG repeat lengths have been associated with younger age of onset of tremor and/or ataxia (Tassone et al., 2007), greater severity of motor symptoms (Apartis et al., 2012; Grigsby et al., 2006; Leehey et al., 2008), and decreased age at death (Greco et al., 2006), these findings are suggestive of age-related effects of increased CGG repeat length and FMR1 mRNA on neuromotor function, that may be detectable prior to the onset of FXTAS.

As noted in Chapter 1, characteristic radiological changes observed among PM males with FXTAS include cerebral and cerebellar volume loss, hyperintensities in the MCP and splenium of the corpus callosum, and increased WMH volume in the whole brain (Apartis et al., 2012; Brunberg et al., 2002; Cohen et al., 2006; Jacquemont et al., 2003; Juncos et al., 2011; Renaud et al., 2015). Decreased grey matter density within specific regions implicated in fronto-subcortical and cortico-cerebellar pathways among PM males with FXTAS have been described, including volume loss in the cingulate cortex, dorsomedial prefrontal cortex, orbito-frontal cortex, premotor cortex, thalamus, putamen, caudate, pallidum and multiple subregions of the cerebellum (Brunberg et al., 2002; Cohen et al., 2006; Hashimoto, Javan, et al., 2011; Moore, Daly, Tassone, Tysoe, Schmitz et al., 2004; Wang, Hagerman, et al., 2013). Structural and functional changes within the cerebellum and frontal lobes have also been described in PM males without FXTAS. These include grey matter volume loss in lobules I/II of the vermis, lobule III (Hashimoto, Javan, et al., 2011) and anterior lobule VI of the cerebellum (Battistella et al., 2013), decreased structural connectivity within white matter tracts of the MCP and bilateral
cerebral peduncles (Hashimoto, Srivastava, et al., 2011), and decreased activation of the prefrontal cortex while completing a working memory task (Hashimoto, Backer, et al., 2011). Moreover, among PM carriers with and without FXTAS, greater CGG repeat length has been associated with decreased grey matter density in the dorsomedial prefrontal cortex (Hashimoto, Javan, et al., 2011) and cerebellum (Adams, Adams, Nguyen, Brunberg, Tassone et al., 2007; Cohen et al., 2006; Moore et al., 2004) in addition to reduced structural connectivity in the MCP (Battistella et al., 2013) and global cortical network (Leow, Harvey, Goodrich-Hunsaker, Gadelkarim, Kumar et al., 2014). Elevated FMR1 mRNA levels have been linked to white matter integrity within the superior cerebellar peduncles (Wang, Hessl, Schneider, et al., 2013) and decreased right ventral inferior frontal cortex activity (Hashimoto, Backer, et al., 2011). As these cortical and subcortical regions are implicated in fronto-subcortical and cortico-cerebellar networks subserving behavioural, cognitive and motor control, damage to these structures may give rise to clinical signs that may be observable prior to the onset of FXTAS.

Recent studies have described the neural correlates of specific clinical features among males with the PM. For example, atrophy in the orbitofrontal cortex, cerebellar lobules VI/VII, vermis (Hashimoto, Javan, et al., 2011), thalamus, putamen and left caudate (Wang, Hagerman, et al., 2013) as well as white matter pathology in the MCP and fornix (Hashimoto, Srivastava, et al., 2011) have been associated with greater severity of FXTAS symptoms. Volume loss in the anterior cingulate cortex and left inferior frontal cortex have been linked to lower working memory performance (Hashimoto, Javan, et al., 2011), and negative associations between white matter integrity of the dorsolateral prefrontal cortex and performance
on a verbal memory encoding task (Hippolyte et al., 2014) have also been described. Reduced event-related potential P300 amplitude and prolonged latency in the frontal lobes have also been reported among carriers with FXTAS which correlate with performance on executive function tasks (Yang, Chan, et al., 2013; Yang, Simon, et al., 2013). Psychiatric symptoms may be associated with subcortical pathology, particularly in the amygdalo-hippocampal complex. Greater psychiatric symptomatology has been described among PM males who show decreased hippocampal volume (Adams et al., 2010), and reduced activation of this region while performing a memory recall task (Koldewyn et al., 2008). Reduced amygdala activation and volume in PM males have also been associated with greater psychiatric symptomatology and autism spectrum symptoms (Hashimoto, Javan, et al., 2011; Hessl et al., 2007; Hessl et al., 2011). Collectively, these findings suggest a complex interplay between molecular factors associated with the PM, structural and functional integrity of brain regions implicated in fronto-subcortical and cortico-cerebellar circuits, and impairments in associated processes among PM carriers with and without FXTAS.

Findings of structural and functional changes in brain regions implicated in fronto-subcortical and cortico-cerebellar pathways suggest that these changes may occur prior to the onset of FXTAS symptoms; however, it is not yet known whether these changes may indicate greater risk for symptom onset. In the absence of longitudinal follow-up, it is unclear whether clinical and radiological changes observed in some asymptomatic carriers represent an independent phenotype associated with the PM, or serve as early indicators of later progression to FXTAS. As highlighted in Chapter 4, cross-sectional examinations of neurobehavioural and radiological features in
terms of their progression with age may give insight into possible trajectories of clinical involvement across the lifespan in the PM. Accelerated degradation of white matter tracts with increasing age in male PM carriers (Battistella et al., 2013; Hashimoto, Srivastava, et al., 2011; Wang, Hessl, Schneider, et al., 2013; Wang et al., 2012) raises the possibility that changes in structural connectivity may represent preclinical markers of FXTAS. Conversely, early alterations in volume that do not accelerate with age may reflect neurodevelopmental effects of \textit{FMR1} PM expansions on cortical development (Battistella et al., 2013). Similarly, motor signs that progress more rapidly than would be expected with increasing age may reflect a degenerative process that may culminate in FXTAS (Kraan, Hocking, Georgiou-Karistianis, Metcalfe, Archibald, Fielding, Bradshaw, et al., 2014; Kraan et al., 2013).

The aims of this chapter are to (i) examine motor and radiological features among PM males and age-matched controls with normal \textit{FMR1} alleles; (ii) investigate the extent to which these features change with increasing age; and (iii) explore whether motor and radiological features among PM males are associated with \textit{FMR1} molecular measures (CGG repeat length, mRNA). It was hypothesised that: (1) severity of motor symptoms would be greater among PM males compared to controls; (2) PM males would exhibit greater reductions in cortical and subcortical volumes, and increased WMH load; (3) the relationship between the severity of these features and increasing age would be stronger among PM males compared to controls; and (4) among PM males, motor and radiological changes would be associated with increasing CGG repeat length and \textit{FMR1} mRNA levels.
5.2 Assessment and measures

Detailed information regarding participant recruitment and measures was provided in Chapter 2 and are therefore not repeated in full here. Briefly, participants included 22 PM males (ages 26–80) and 24 controls (ages 26–77). CGG repeat length and \textit{FMR1} mRNA were quantified from blood. Motor symptoms including tremor, ataxia and parkinsonism were assessed using the FXTAS Rating Scale (Version 1.0) (Leehey et al., 2008). Participants also underwent T1-weighted imaging and T2-weighted FLAIR imaging. The following measures were extracted from FreeSurfer v5.3.0 for cortical volumes: Cortex (total cortical grey matter); CorticalWhiteMatter (total cortical white matter); SubCortGray (total subcortical grey matter). Cerebellar volumes were also extracted from FreeSurfer v5.3.0 (LeftCerebellumWhiteMatter; LeftCerebellumCortex; RightCerebellumWhiteMatter; RightCerebellumCortex). Total cerebellar volume was calculated as the sum of grey and white matter in both the left and right hemispheres. The sum of grey and white matter was used (as opposed to each of these separately) to avoid issues with distinguishing between grey and white matter boundaries in the presence of WMHs.

Deep and periventricular WMHs were processed using methods previously published by the neuroimaging group that assisted with this study (Wen & Sachdev, 2004; see Chapter 2 for further detail). Deep WMHs were summed using the following values from the FLAIR output: right temporal, left temporal, left frontal, right frontal, left occipital, right occipital, left parietal, right parietal. Periventricular WMHs were calculated as the sum of left anterior horn, right anterior horn, left posterior horn, right posterior horn, left periventricular body, and right
periventricular body. Values for deep and periventricular WMHs were transformed into normalised scores (Blom, 1958) to minimise the effect of extreme values. Cerebellar WMH volumes could not be extracted as a result of incomplete coverage of the cerebellum during acquisition of the FLAIR sequences.

5.3 Statistical analyses

5.3.1 Age-related changes in clinical and radiological features among PM males and controls

Multiple linear regression was used to explore age-related changes in motor and radiological features among PM males and controls. Motor and imaging variables were tested separately as dependent variables. Independent variables for each model included age, group (PM vs controls), and their interaction (age × group). ICV was used as a covariate in all models predicting brain volumes and WMH load. Assumptions of multiple linear regression (linearity, independence and normality of residuals, homoscedasticity) were adequately met for all models.

5.3.2 Relationships between clinical and radiological variables with FMR1 molecular measures

Associations between FMR1 molecular measures (CGG repeat length, mRNA levels) with motor and radiological variables among PM males were explored using Partial Pearson correlations controlling for age. ICV was included as an additional covariate for correlations between molecular measures and radiological features.
An adjusted $p$ value of $p=.007$ was used for all analyses to account for testing across the seven dependent variables (one motor; six radiological). Results with $p$ values <.05 are also discussed as possible trend-level associations as these may indicate clinical significance and be worthy of further investigation, although it is acknowledged that these cannot be interpreted as statistically significant.

5.4 Results

5.4.1 Participant characteristics

Participant characteristics were described in detail in Chapter 3 and as such are not repeated here. Briefly, the combined PM group were well matched in terms of sociodemographic variables including age, IQ, and total household income. No differences were found between groups for physical health and lifestyle factors of height, weight, systolic blood pressure, BMI, cardiovascular risk, previous diagnosis of hypertension, and alcohol use. A significantly greater proportion of PM males reported a history of past or current regular smoking.

5.4.2 Associations between motor symptoms, carrier status and age

Mean FXTAS rating scale scores are presented in Table 5.1. The regression model including age, group (PM, control) and the interaction of age × group was significant ($F_{(3, 42)}=12.954, p<.001$), explaining 44.3% of the variance in FXTAS rating scale scores. In this model, carrier status was not a significant predictor of motor symptoms (B=−.112, $p=.569$; Table 5.2). However, both increasing age (B=.560, $p<.001$) and the interaction term for age × group (B=−.371, $p=.008$) were significant. Although increasing age was marginally associated with greater severity of motor
symptoms in the control group ($B=.213, p=.047$), the significant age × group interaction indicates that the relationship between increasing age and symptom severity was stronger in the PM group (Figure 5.1).

Table 5.1

*FXTAS rating scale scores*

<table>
<thead>
<tr>
<th></th>
<th>HC (n=24)</th>
<th>PM (n=22)</th>
<th>FX+ (n=7)</th>
<th>FX- (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>13.83 (7.35)</td>
<td>22.39 (23.69)</td>
<td>49.86 (25.29)</td>
<td>10.38 (7.81)</td>
</tr>
<tr>
<td>Range</td>
<td>5–30</td>
<td>2–90</td>
<td>17–90</td>
<td>2–25</td>
</tr>
</tbody>
</table>

Table 5.2

*Summary of regression model examining the effect of age and group on FXTAS rating scale score*

<table>
<thead>
<tr>
<th></th>
<th>B (S.E)</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>2.58 (.14)</td>
<td>18.29**</td>
</tr>
<tr>
<td>Age</td>
<td>.56 (.09)</td>
<td>5.90***</td>
</tr>
<tr>
<td>Group</td>
<td>-.11 (.19)</td>
<td>-.57</td>
</tr>
<tr>
<td>Age × Group</td>
<td>-.37 (.13)</td>
<td>-2.78**</td>
</tr>
</tbody>
</table>

**$p<.01$; ***$p<.001$**
Figure 5.1. Changes in FXTAS rating scale score with increasing age among PM males and controls. Although there was no significant difference in mean FXTAS rating scale scores between PM males and controls, PM males showed a stronger positive association between increasing age and greater symptom severity.

5.4.3 Associations between radiological features, carrier status and age

Mean brain volumes for cortical grey matter, cortical white matter, subcortical grey matter, and total cerebellum in addition to deep and periventricular WMH load are presented in Table 5.3. Multiple linear regression models exploring the main effects of group (PM vs HC), age, and the interaction between these variables on MRI measures are summarised in Table 5.4.
Table 5.3

*Mean brain volumes and white matter hyperintensity (WMH) load (mm³)*

<table>
<thead>
<tr>
<th></th>
<th>HC (n=24)</th>
<th>PM (n=20)</th>
<th>FX+ (n=7)</th>
<th>FX- (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical grey a</td>
<td>Mean</td>
<td>435075.47</td>
<td>447330.24</td>
<td>408123.46</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>37431.55</td>
<td>63400.34</td>
<td>44512.70</td>
</tr>
<tr>
<td>Cortical white a</td>
<td>Mean</td>
<td>479473.5</td>
<td>474627.20</td>
<td>415138.70</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>46442.09</td>
<td>85532.70</td>
<td>46850.22</td>
</tr>
<tr>
<td>Subcortical grey a</td>
<td>Mean</td>
<td>59510.08</td>
<td>58689.12</td>
<td>49591.75</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>5376.5</td>
<td>8939.95</td>
<td>6233.98</td>
</tr>
<tr>
<td>Cerebellar</td>
<td>Mean</td>
<td>127859.99</td>
<td>118604.05</td>
<td>102069.07</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>12590.87</td>
<td>22032.81</td>
<td>17530.20</td>
</tr>
<tr>
<td>Deep WMH</td>
<td>Mean</td>
<td>1638.84</td>
<td>8301.49</td>
<td>21642.43</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1492.23</td>
<td>21631.25</td>
<td>34061.94</td>
</tr>
<tr>
<td>Periventricular WMH</td>
<td>Mean</td>
<td>2024.16</td>
<td>3557.08</td>
<td>7196.95</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1538.34</td>
<td>5618.65</td>
<td>8565.10</td>
</tr>
</tbody>
</table>

a three FX+ missing

*Total cerebellar matter volumes*

The regression model including ICV, age, group and the interaction of age × group was significant ($F_{(4, 39)}=17.352, p<.001$), explaining 60.3% of the variance in total cerebellar volume. The main effect of group was significant (B=.709, $p<.001$), indicating reduced cerebellar volume among PM males compared to controls.

Among PM males, increasing age was significantly associated with decreasing total cerebellar volume (B=−.581, $p<.001$), and the interaction term between age and group approached corrected levels of significance (B=.361, $p=.008$). Although increasing age was also a significant predictor of reduced cerebellar volume in controls (B=−.220, $p=.019$), these findings provide preliminary evidence to suggest
that the negative relationship between age and cerebellar volume was stronger in the PM group (see Figure 5.2).

*Total subcortical grey matter volumes*

The regression model for subcortical grey matter volumes including ICV, age, group and the interaction of age × group as predictors was significant ($F_{(4, 36)}=24.285$, $p<.001$), explaining 70% of the variance in total volume. The main effect of group was significant ($B=.543$, $p=.005$), indicating reduced total subcortical grey matter volume among PM males compared to controls. Among PM males, increasing age was significantly associated with decreasing subcortical grey matter volume ($B=-.617$, $p<.001$), but the interaction between age and group did not withstand correction for multiple comparisons ($B=.279$, $p=.032$). Although increasing age was also a significant predictor of reduced subcortical grey matter volume in controls ($B=-.338$, $p<.001$), this provides preliminary evidence to suggest that the negative relationship between age and subcortical grey matter volume may be stronger in the PM group (Figure 5.2).
Figure 5.2. Reductions in total cerebellar (left) and subcortical grey matter volume (right) with increasing age among PM males and controls.

PM males had significantly reduced total cerebellar and subcortical grey matter volume compared to controls. The negative effect of age on cerebellar and subcortical volumes was also stronger in the PM group, indicating greater reductions in volume with increasing age compared to controls.
Cortical grey matter volume

The regression model predicting cortical grey matter volume from ICV, age, group and the interaction of age × group was significant ($F_{(4,36)}=17.012, p<.001$) and explained 61.6% of the variance. The main effect of group was not significant (B=.103, $p=.619$), indicating comparable cortical grey matter volumes between PM males and controls. Among PM males, increasing age was a significant predictor of reduced cortical grey matter volume (B=-.406, $p<.001$), but the interaction between age and group (B=.157, $p=.141$) was not significant. This suggests that the relationship between increasing age and cortical grey matter volume among PM males was comparable to that found in controls.

Cortical white matter volume

The regression model including ICV, age, group and the interaction of age × group to predict cortical white matter volume was significant ($F_{(4, 36)}=13.859, p<.001$), explaining 56.3% of the variance. PM carrier status was not a significant predictor of reduced cortical white matter volume in this model (B=.396, $p=.077$), indicating comparable volumes between PM males and controls. Although, increasing age was a significant predictor of reduced cortical white matter volume in the PM group (B=-.340, $p=.007$), the interaction term between age and group was not significant (B=.245, $p=.112$). This suggests that the relationship between increasing age and cortical white matter volume was similar among PM males and controls.
Deep white matter hyperintensity volume

The regression model including ICV, age, group and the interaction of age × group was significant ($F_{(4,39)}=8.572, p<.001$), explaining 41.3% of the variance in deep WMH volume. The main effect of group was not significant ($B=-.044, p=.849$), indicating comparable deep WMH volume between PM males and controls. Among PM males, increasing age was a significant predictor ($B=.485, p<.001$), but the interaction term between age and group was not significant ($B=-.073, p=.641$). This suggests that the relationship between increasing age and deep WMH volume among PM males was commensurate to that found in controls.

Periventricular white matter hyperintensity volumes

The regression model to predict periventricular WMH volumes from ICV, age, group and the interaction of age × group was significant ($F_{(4,39)}=7.908, p<.001$), explaining 39.1% of the variance. PM carrier status did not make a significant contribution to the model, ($B=-.279, p=.245$), indicating comparable periventricular white matter hypensity volumes between PM males and controls. Among PM males, increasing age was a significant predictor of greater periventricular WMH load ($B=.361, p=.003$). The interaction terms between age and group was not significant ($B=.096, p=.158$), indicating a comparable relationship between increasing age and periventricular WMH load among PM males and controls.
Table 5.4

*Summary of multiple linear regression models examining the effect of age and group on brain volumes*

<table>
<thead>
<tr>
<th></th>
<th>Cortex</th>
<th>Cortical white matter</th>
<th>Subcortical grey</th>
<th>Cerebellar</th>
<th>Deep WMH</th>
<th>Periventricular WMH</th>
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<tbody>
<tr>
<td></td>
<td>B (S.E)</td>
<td>t</td>
<td>B (S.E)</td>
<td>t</td>
<td>B (S.E)</td>
<td>t</td>
</tr>
<tr>
<td>Constant</td>
<td>-.11 (.16)</td>
<td>-.71</td>
<td>-.28 (.17)</td>
<td>-1.63</td>
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<tr>
<td></td>
<td>ICV</td>
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<td>.70 (.12)</td>
<td>6.12***</td>
<td>.46 (.10)</td>
<td>4.80***</td>
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<td></td>
<td>.63 (.11)</td>
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<tr>
<td></td>
<td>Age</td>
<td>-3.66***</td>
<td>-.34 (.13)</td>
<td>-2.87**</td>
<td>-.62 (.10)</td>
<td>-6.30***</td>
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<td>-.41 (.11)</td>
<td></td>
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<tr>
<td></td>
<td>Group</td>
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<td>.50</td>
<td>1.82</td>
<td>.54 (.18)</td>
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</tr>
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<tr>
<td></td>
<td>Age × group</td>
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<td>1.11</td>
<td>.25 (.15)</td>
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<td>2.23*</td>
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<tr>
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<td></td>
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<td></td>
<td></td>
<td>.28 (.13)</td>
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</table>

*p<.05; **p<.01; ***p<.001
ICV= intracranial volume
5.4.4 Correlations with CGG and mRNA in PM males

When controlling for age, FXTAS rating scale score was not significantly correlated with CGG repeat length \( (r=-.001, p=.996) \) or \( FMR1 \) mRNA levels \( (r=-.068, p=.770) \). After controlling for age and ICV, cerebellar volume was significantly and negatively associated with CGG repeat length \( (r=-.687, p=.002) \), but not \( FMR1 \) mRNA level \( (r=-.387, p=.112) \). CGG repeat length and \( FMR1 \) mRNA levels were not associated with volumes for periventricular WMHs (CGG: \( r=-.052, p=.838 \); \( FMR1 \) mRNA: \( r=.069, p=.784 \)), deep WMHs (CGG: \( r=-.312, p=.208 \); \( FMR1 \) mRNA: \( r=-.095, p=.707 \)), cortical grey matter (CGG: \( r=-.272, p=.326 \); \( FMR1 \) mRNA: \( r=-.096, p=.734 \)), cortical white matter (CGG: \( r=-.364, p=.183 \); \( FMR1 \) mRNA: \( r=-.139, p=.621 \)) or subcortical grey matter (CGG: \( r=-.077, p=.786 \); \( FMR1 \) mRNA: \( r=.066 p=.815 \)).

5.5 Discussion

This chapter examined motor and radiological features in PM males with and without FXTAS and matched controls with normal alleles. There was no difference in the overall severity of motor symptoms between groups. Therefore the hypothesis that PM males would demonstrate greater severity of motor symptoms was not supported. However, the finding of greater age-related changes in motor symptoms in PM males supports the hypothesis that the relationship between increasing age and severity of motor symptoms is stronger in males with the PM. The hypothesis of widespread radiological changes was partially supported with PM males showing only reduced cerebellar and subcortical volumes compared to controls. Stronger relationships between increasing age and volume loss in the cerebellum and
subcortical grey matter were also observed in the PM group but these findings did not withstand correction for multiple comparisons. Therefore the hypothesis that the negative relationship between age and brain volumes would be stronger among PM males than in controls was also only partially supported. Finally, increasing CGG repeat length, but not FRM1 mRNA levels, were associated with reduced cerebellar volume among PM males providing only partial support for the hypothesis that FMR1 molecular measures would be associated with motor and radiological changes. Collectively, these findings are consistent with previous studies (Brunberg et al., 2002; Cohen et al., 2006; Hashimoto, Javan, et al., 2011; Moore et al., 2004; Wang, Hagerman, et al., 2013), indicating reduction in cerebellar and subcortical volumes alongside motor signs that appear more marked among those of advanced age.

Severity of motor symptoms, as measured by the composite FXTAS rating scale, was comparable between PM males and controls. It should be acknowledged that there were only a small number of PM males with FXTAS (n=7) included the current study. Despite this limitation, the relationship between increasing age and motor symptom severity was stronger among PM males compared to that found in controls. Overall reductions in cerebellar and subcortical grey matter volume, in addition to greater reductions in these volumes with increasing age, were also observed among PM males compared to controls. These findings are consistent with the age-related penetrance of FXTAS (Jacquemont et al., 2004), and highlight the possible involvement of subcortical brain regions in neurodegenerative manifestations of the PM. Indeed, previous studies have shown that volume loss in cerebellar lobules VI/VII, vermis (Hashimoto, Javan, et al., 2011), thalamus,
putamen and left caudate (Wang, Hagerman, et al., 2013) as well as white matter pathology in the MCP and fornix (Hashimoto, Srivastava, et al., 2011) are associated with greater severity of FXTAS symptoms. Cognitive features of FXTAS including deficits in executive function, working memory, information processing and fine motor function may also be indicative of disruption to fronto-subcortical and cortico-cerebellar neural networks (Bacalman et al., 2006; Brega et al., 2008). To date, no study has explored the interrelationships between volumes of subcortical structures and cognitive features in the PM, yet this may contribute to the understanding of mechanisms underlying cognitive impairment observable in affected carriers.

Another important finding was that among PM males, increasing CGG repeat length, but not FMR1 mRNA levels, was significantly associated with reductions in cerebellar volume. This is consistent with previous studies reporting the same negative correlation between CGG repeat length and cerebellar volume, but no significant association between cerebellar volume and FMR1 mRNA levels (Adams et al., 2007; Cohen et al., 2006; Moore et al., 2004). One potential explanation for the lack of correlation between FMR1 mRNA and cerebellar volume may be due to FMR1 mRNA levels measured in blood not being representative of those within different regions in the brain (Tassone et al., 2004). However, a recent post-mortem study of cerebellar tissue of PM males with FXTAS described increased transcript levels of FMR1 mRNA that correlated with CGG repeat length (Pretto et al., 2014). Moreover, elevated FMR1 mRNA levels in the PM have been associated with reductions in structural connectivity (as opposed to volume) within the superior cerebellar peduncle (Wang et al., 2013). The discrepancies between studies may relate to the different selection of imaging measures. Together these findings may
suggest that accelerated reduction in structural connectivity with increasing age in the PM (Hashimoto et al., 2011, Wang et al., 2013, Wang et al., 2012) may result from mRNA toxicity, while early alterations in volume that do not accelerate with age may reflect neurodevelopmental effects of FMR1 PM expansions on cortical development.

Findings of relationships between CGG repeat length and cerebellar volume in the current, and previous studies (Adams et al., 2007; Cohen et al., 2006; Moore et al., 2004) are of interest given that the core characteristics of FXTAS (intention tremor, ataxia) are clinical signs of cerebellar dysfunction (Holmes, 1939; Ilg & Timmann, 2013; Morton & Bastian, 2007). It is possible that previously observed relationships between CGG repeat length and motor signs among PM males including age of onset of tremor and/or ataxia (Tassone et al., 2007) and greater severity of motor symptoms (Apartis et al., 2012; Grigsby et al., 2006; Leehey et al., 2008) emerged due to effects of larger CGG repeats on cerebellar structure. Contrary to previous reports (Grigsby et al., 2006; Leehey et al., 2008) total FXTAS rating scale scores among PM males in the current study were not correlated with CGG repeat length. Possible explanations are that the current sample is smaller (Grigsby et al., 2006: 25 PM males with FXTAS; Leehey et al., 2008: 54 PM males), and younger than these cohorts (Grigsby et al., 2006: mean 70.0 years; Leehey et al., 2008: mean 66.9 years). These sample characteristics may have contributed to an overall milder level of motor dysfunction. Further, in contrast to the current study, in Leehey et al (2008) correlation analyses were conducted across the entire cohort, including both non-carriers and PM carriers. As FXTAS rating scale scores among PM males were significantly higher compared to controls, and these groups are defined by CGG
repeat length, the correlation coefficient observed in that study may have been artificially inflated by the inclusion of the entire cohort and could simply reflect group differences rather than a linear association.

Another possible explanation for the lack of significant association between CGG repeat length and motor symptoms in the current study is potential lack of sensitivity of the FXTAS rating scale to earlier subtle changes in motor function (Hall et al., 2014). There is evidence to suggest that alternative measures of postural control incorporating manipulation of sensory and cognitive load may provide a more sensitive measure of CGG and/or mRNA related changes in motor function among PM males and females; including decrements in body sway (Allen et al., 2008) (Kraan et al., 2013) and increased intra-individual variability of gait (Kraan, Hocking, Georgiou-Karistianis, Metcalfe, Archibald, Fielding, Bradshaw, et al., 2014) and stepping (Hocking et al., 2015). Future studies should focus on exploring whether these measures of postural control may provide more sensitive markers of cerebellar dysfunction with increasing CGG repeat length among PM males with and without FXTAS.

With regard to other radiological features (cortical grey matter, cortical white matter or WMH volumes), no significant differences were observed between PM males and controls. Again, this may be due to the small proportion of PM males with FXTAS. It is also possible that the examination of more global imaging measures (total cortical grey matter, total cortical white matter, total subcortical volume) may have washed out group differences that may have been observable within more specific regions of interest. Total volumes were selected over more specific regions of
interest for two main reasons. Firstly, this allowed for the characterisation of core radiological features associated with FXTAS in this newly established cohort. Second, given the small sample size, the use of total volumes minimised the number of multiple comparisons that would require adjustment in analyses investigating a larger number of regions.

In addition to general limitations including sample size (discussed in Chapter 9), there are a number of limitations of this study requiring consideration. As with the analyses of age-related cognitive changes presented in Chapter 4, findings pointing to different trajectories in motor and radiological features with increasing age should be interpreted with caution given the cross-sectional nature of the study design. Future research with larger PM cohorts and longitudinal follow-up will be required to determine the reliability of motor and radiological changes with increasing age among PM males compared to controls. Another limitation relates to the use of total volumes for cortical/subcortical grey and white matter as opposed to more specific regions of interest. It is reasonable to suggest that group differences and/or correlations with $FMR1$ molecular measures may have been observed if more targeted regions of interest (e.g. prefrontal cortex) were included in the analyses. Finally, WMH volumes in the cerebellum were not able to be calculated due to incomplete capture of this region on T2-FLAIR images. Therefore, the possible associations between $FMR1$ molecular measures and white matter burden in this region could not be explored.

In conclusion, this chapter has examined age-related changes in motor and radiological features among PM males with and without FXTAS and matched
controls. Despite comparable motor symptom severity across PM males and controls, the relationship between increasing age and motor symptom severity was stronger in the males with the PM. Males with the PM also exhibited significant reductions in cerebellar and total subcortical grey matter volumes, and there was preliminary evidence to suggest that the relationship between volume loss in these regions and increasing age was stronger than that found in controls. Decreasing cerebellar volume among PM males was also associated with increasing CGG repeat length. Taken together with the findings of greater cognitive and motor slowing among PM males compared to controls (Chapter 4), these findings suggest disruption to cerebellar and subcortical neural networks implicated in the regulation of cognitive and motor function in the PM. Future studies will be needed to explore the relationships between subcortical volumes and cognitive features in males with the PM. Further research is also needed to provide reliable quantitative measures of subtle changes in gait and postural control to monitor disease progression and assess the efficacy of novel interventions in future clinical trials. These future research areas are explored in Chapters 6–8.
CHAPTER 6.

DEFINING THE RELATIONSHIPS BETWEEN KEY COGNITIVE FUNCTIONS AND SUBCORTICAL STRUCTURES
CHAPTER 6. DEFINING THE RELATIONSHIPS BETWEEN KEY COGNITIVE FUNCTIONS AND SUBCORTICAL STRUCTURES

6.1 Introduction

Age-related changes in neurobehavioural and radiological features among PM males and controls were explored in Chapters 4 and 5. These analyses indicated poorer performance on measures of fine motor function and information processing among PM males compared to controls, in addition to significantly reduced total subcortical grey matter and cerebellar volumes. Although increasing age was associated with reductions in subcortical grey matter volume in both PM males and controls, there was evidence to suggest that the relationship between subcortical and cerebellar volume loss and increasing age was stronger in males with the PM. It was concluded that deficits in information processing and fine motor function among PM males might reflect a disruption to subcortical neural networks subserving cognitive and motor function. However, in Chapter 5 the interrelationships between cognitive and subcortical volumes were not explored. Moreover, the examination of total subcortical grey matter volume precluded further investigation of specific subcortical structures comprising frontal-subcortical and cortico-cerebellar networks. This chapter will address these gaps by investigating age-related changes in specific subcortical structures (thalamus, caudate, putamen and pallidum) among PM males and controls. The possible relationships between these volumes, information processing speed and fine motor function will also be examined.
It is well established that the thalamus and basal ganglia (caudate, putamen and pallidum) play a critical role in the regulation of cognitive, motor and limbic function (Alexander & Crutcher, 1990; Alexander et al., 1986; Cummings, 1993). The caudate is the major source of input to the basal ganglia, receiving projections from multiple cortical regions implicated in higher order cognitive processes including the dorsolateral prefrontal cortex, lateral orbitofrontal cortex, and frontal eye fields. Forming the striatum, the caudate and putamen are also involved in the control of voluntary movement via primary connections between the putamen and motor cortex (Alexander et al., 1986). Information from prefrontal and motor cortices is conveyed via the caudate and putamen to the pallidum, the major site of basal ganglia output. Interconnected with the basal ganglia, the thalamus relays information from the pallidum via afferent and efferent connections with cerebrum and cerebellum (Middleton & Strick, 2000). It has been proposed that these connections are segregated into five anatomically and functionally distinct circuits mediating motor/somatosensory function (motor circuit), oculomotor control (oculomotor circuit), executive function (dorsolateral prefrontal circuit), social cognition/impulsivity (lateral orbitofrontal circuit), and volition (anterior cingulate circuit) (Alexander & Crutcher, 1990; Alexander et al., 1986).

As reviewed in Chapter 4, investigations of subcortical volume have indicated reduced volume of the thalamus among PM males with and without FXTAS compared to controls (Battistella et al., 2013; Hashimoto, Javan, et al., 2011; Moore et al., 2004; Wang, Hagerman, et al., 2013). One recent study demonstrated significant reductions in caudate, putamen and pallidum volume among PM males with FXTAS, with volume loss in the thalamus, putamen and left caudate being
associated with greater severity of motor symptoms (Wang, Hagerman, et al., 2013). These findings highlight a possible role of subcortical pathology, particularly affecting the thalamus and basal ganglia structures, in motor dysfunction associated with FXTAS. Although subcortical volumes have not been associated with \textit{FMRI} molecular measures in previous studies (Battistella et al., 2013; Hashimoto, Javan, et al., 2011; Moore et al., 2004; Wang, Hagerman, et al., 2013), these require further investigation in independent cohorts. Moreover, the relationship between volume loss in these structures and cognitive features among PM males has yet to be explored. As studies in healthy and clinical groups have pointed to associations between psychomotor slowing and fronto-subcortical circuits originating in the frontal lobes (e.g. Batista, Zivadinov, Hoogs, Bergsland, Heininen-Brown et al., 2012; Van Der Werf, Tisserand, Visser, Hofman, Vuurman et al., 2001), it is possible that volume loss in the thalamus and basal ganglia may underlie psychomotor slowing in the PM.

The aims of this chapter are to (i) explore age-related changes in specific subcortical structures (thalamus, caudate, putamen and pallidum) among PM males and matched controls; (ii) investigate whether volume loss in these structures contributes to deficits in fine motor function and information processing speed among PM males; and (iii) examine possible relationships between specific subcortical volumes with \textit{FMRI} molecular factors (CGG repeat length, mRNA levels) among PM males. It was hypothesised that: (1) PM males would exhibit volume loss in all subcortical structures compared to controls; (2) the relationship between volume loss in these structures and increasing age would be stronger among PM males; (3) among PM males, reductions in subcortical regions implicated in the motor fronto-subcortical
circuit (putamen, pallidum, thalamus) would be associated with decrements in fine
motor function; (4) among PM males, volume loss in regions receiving major
projections from the frontal lobes (caudate, pallidum, thalamus) would be associated
with decrements in information processing speed; and (5) on the basis of
relationships observed between \textit{FMR1} molecular measures and changes in cerebellar
structure (Chapter 5), increasing CGG repeat length, but not \textit{FMR1} mRNA levels,
would be associated with reductions in subcortical volumes.

\textbf{6.2 Assessment and measures}

This chapter includes a subset of the PM males and controls from the overall cohort
for whom MRI scans were available. Participants were 20 PM males (ages 26–80)
and 24 age- and education- matched controls with normal \textit{FMR1} alleles (ages 26–
77). Full details regarding participant recruitment and measures are provided in
Chapter 2 and are therefore not repeated here. Domain scores for information
processing speed and fine motor function were derived from performance on
measures outlined in Chapter 2 (Neuropsychological Assessment). Participants
underwent MRI scanning and total volumes (left + right) for thalamus, caudate,
pallidum and putamen were extracted using FSL v5.01 (Jenkinson et al., 2012). A
detailed description of MRI processing methods, including screening and quality
checking of output is presented in Chapter 2. Briefly, quality checking included
screening for statistical outliers (>1.98 SD) and visual inspection of automatic
segmentations. The following subcortical volumes were excluded from analyses as
they did not meet quality control standards: thalamus (one PM male with FXTAS);
caudate (two PM males with FXTAS); putamen (two PM males with FXTAS); and pallidum (one PM male with FXTAS).

6.3 Statistical analyses

6.3.1 Participant characteristics

Although demographic characteristics of the cohort were presented in Chapter 3, these were re-examined in the current chapter to ensure that the subgroup of PM males for whom MRI was available were representative of the larger cohort. Group differences in demographic characteristics were explored using independent t-tests, ANOVA, or non-parametric equivalents where the normality assumption was violated. Pairwise intergroup comparisons were conducted for: (a) all PM carriers vs controls (PM vs HC); (b) PM carriers with FXTAS vs controls (FX+ vs HC); (c) PM carriers without FXTAS vs controls (FX- vs HC); (d) PM carriers with FXTAS vs PM carriers without FXTAS (FX+ vs FX-).

6.3.2 Age-related changes in subcortical volumes

Assessment of normality of subcortical variables using the Shapiro-Wilk test revealed that all subcortical volumes were normally distributed. Multiple linear regression was used to explore age-related changes in subcortical volumes among PM males and controls. Each subcortical volume (thalamus, caudate, pallidum and putamen) was included as a dependent variable. Independent variables for each model included age, group (PM vs controls), and their interaction (age × group). ICV was included as a covariate in all models.
6.3.3 Relationship between subcortical volumes and cognitive domain scores in PM males and controls

Linear regression analyses were conducted to examine associations between subcortical volumes and domain scores for information processing and fine motor function. The contribution of each volume was examined in separate models. As the individual contributions of age (examined in Chapter 4) and ICV (covariate for brain volumes only) were not of interest for these analyses, each subcortical volume was adjusted for age and ICV prior to being included in the models. Premorbid IQ was not included as a covariate, as previous analyses in Chapter 4 suggested no significant association between premorbid intelligence and measures of information processing speed and fine motor function. Adjusted subcortical volumes and their interaction terms with group (group × volume) were included to determine whether the relationship between subcortical volumes and cognitive performance differed between groups (PM vs HC). As measures of information processing speed involved manual manipulation of assessment materials, and performance on this domain was significantly associated with fine motor function (partial Pearson controlling for age: $r = .397, p = .007$), additional models which included fine motor function as a potential confounder were conducted. Assumptions of multiple linear regression (linearity, independence and normality of residuals, homoscedasticity) were adequately met, and an adjusted $p$ value of .025 was used to account for multiple comparisons across the two cognitive domain scores.
6.3.4 Relationship between subcortical volumes and FMR1 molecular measures

The relationships between subcortical volumes, CGG repeat length and FMR1 mRNA were explored using partial Pearson correlations controlling for age and ICV. See Chapter 4 for correlations between FMR1 molecular measures and cognitive domain scores.

6.4 Results

6.4.1 Participant characteristics

Examination of key demographic characteristics confirmed that the pattern of group differences demonstrated in the overall cohort (Chapter 3) were comparable to those found in this subsample. When comparing all PM carriers to controls, the groups were well matched on demographic variables (Table 6.1) with no significant differences for age ($t_{(42)}=-.544$, $p=.817$), education ($t_{(42)}=-.048$, $p=.785$), premorbid IQ ($t_{(42)}=-1.168$, $p=.986$), or current IQ ($t_{(42)}=-1.758$, $p=.318$). As expected, CGG repeat length (Mann-Whitney $U=0.000$, $p<.001$) and FMR1 mRNA levels (Mann-Whitney $U=39.000$, $p<.001$) were significantly greater in the PM group compared to controls.

After dividing the PM group into those with (FX+ n=7) and without (FX- n=15) FXTAS, no significant differences were detected between these groups and controls for education ($F_{(2,41)}=.622$, $p=.542$), or premorbid IQ ($F_{(2,41)}=3.098$, $p=.056$).

Consistent with the findings in the overall cohort (Chapter 3), significant differences emerged again for age ($F_{(2,41)}=5.559$, $p=.007$), current IQ ($X^2=8.085$, $p=.018$), CGG repeat length ($X^2=32.944$, $p<.001$) and FMR1 mRNA level ($X^2=22.533$, $p<.001$).
Post-hoc comparisons showed that PM males with FXTAS were significantly older than PM males without FXTAS ($p=.006$), with significantly lower current IQ than controls ($p=.007$) and PM males without FXTAS ($p=.012$). PM carriers with and without FXTAS showed significantly greater CGG repeat length and $FMR1$ mRNA levels compared to controls ($ps<.001$); however, there were no significant differences in CGG repeat length ($p=.283$) or $FMR1$ mRNA levels ($p=.968$) when comparing PM males with and without FXTAS.
Table 6.1

Participant characteristics of subsample included in Chapter 6

<table>
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<tr>
<th></th>
<th>HC (n=24)</th>
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<th>FX+ (n=7)</th>
<th>FX- (n=13)</th>
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<td>9–18</td>
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<td><strong>CGG repeat length</strong></td>
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<td>88.05 (15.69)**a</td>
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<td>85–92</td>
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<td>Mean (SD)</td>
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<td>2.16 (1.05)**a</td>
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<td>Range</td>
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<td>.95–5.16</td>
<td>1.39–4.15</td>
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<td><strong>Premorbid full scale IQ</strong></td>
<td>Mean (SD)</td>
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<td>110.34 (4.38)</td>
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<td>Range</td>
<td>100.74–119.74</td>
<td>98.26–121.39</td>
<td>98.26–121.39</td>
</tr>
<tr>
<td><strong>Current full scale IQ</strong></td>
<td>Mean (SD)</td>
<td>113.46 (11.43)</td>
<td>106.32 (14.44)</td>
<td>93.43 (16.58)**c,d</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>86–133</td>
<td>79–127</td>
<td>79–123</td>
</tr>
</tbody>
</table>

* **p < .01; ***p < .001

a > HC; b > FX-; c < HC-; d < FX-
6.4.2 Associations between subcortical volumes, age, and carrier status

Mean volumes for total thalamus, caudate, putamen and pallidum are shown in Table 6.2. Multiple linear regression analyses examining the effect of age, group (PM, control) and the interaction term for these (age × group) on thalamus, caudate, putamen and pallidum volumes are summarised in Table 6.3.

Table 6.2

<table>
<thead>
<tr>
<th></th>
<th>HC (n=24)</th>
<th>PM (n=19)</th>
<th>FX+ (n=6)</th>
<th>FX- (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalamus (^a)</td>
<td>Mean</td>
<td>14993.3</td>
<td>13976.02</td>
<td>11108.14</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1731.17</td>
<td>3086.93</td>
<td>2929.85</td>
</tr>
<tr>
<td>Caudate (^b)</td>
<td>Mean</td>
<td>7359.9</td>
<td>7148.65</td>
<td>6421.18</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>988.82</td>
<td>1414.78</td>
<td>1401.59</td>
</tr>
<tr>
<td>Putamen (^b)</td>
<td>Mean</td>
<td>9861.39</td>
<td>10005.5</td>
<td>8390.38</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1534.02</td>
<td>1795.83</td>
<td>1394.26</td>
</tr>
<tr>
<td>Pallidum (^a)</td>
<td>Mean</td>
<td>3529.61</td>
<td>3414.99</td>
<td>2574.16</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>527.42</td>
<td>800.63</td>
<td>698.97</td>
</tr>
</tbody>
</table>

\(^a\) missing 1 FX+; \(^b\) missing 2 FX+

Thalamus

The regression model predicting thalamus volume including ICV, age, group and the interaction of age × group as predictors was significant \(F(4,38)=24.136, p<.001\), explaining 68.8% of the variance in total thalamus volume. The main effect of group was significant \(B=.736, p<.001\), indicating reduced thalamus volume among PM males compared to controls. In the PM group, increasing age was associated with reduced thalamus volume \(B=-.613, p<.001\), and the interaction term between age
and group was also significant ($B=.346, p=.005$). This suggests that although increasing age was significantly associated with reduced thalamus volume among controls ($B=-.267, p=.002$), the negative relationship between age and volume was stronger in males with the PM.

**Caudate**

The regression model including ICV, age, group and the interaction of age $\times$ group as predictors was significant ($F_{(4,37)}=24.934, p<.001$), explaining 70% of the variance in caudate volume. The main effect of group was significant ($B=.651, p<.001$) indicating reduced caudate volume among PM males compared to controls. Increasing age was significantly associated with decreasing caudate volume in PM males ($B=-.465, p<.001$). However, the interaction term between age and group was not significant ($B=.168, p=.187$), suggesting that the relationship between caudate volume and increasing age among PM males was comparable to that found in controls.

**Putamen**

The regression model for total putamen volume including ICV, age, group and the interaction of age $\times$ group was significant ($F_{(4,37)}=11.532, p<.001$) and explained 50.7% of the variance. There was no significant main effect of group on putamen volume, indicating comparable volumes between PM males and controls ($B=.331, p=.156$). Although increasing age was significantly associated with decreasing putamen volume in males with the PM ($B=-.544, p<.001$), the interaction term between age and group was not significant ($B=.245, p=.134$), suggesting that the
relationship between putamen volume and increasing age among PM males was comparable to that found in controls.

**Pallidum**

The regression model for pallidum volume with ICV, age, group and the interaction of age × group as predictors was significant ($F_{(4,38)}=6.951, p<.001$), explaining 36.2% of the variance. The main effect of group was not significant ($B=.418$, $p=.106$), indicating comparable pallidum volume between PM males and controls. However, the relationship between age and volume differed between groups, as indicated by the significant age × group interaction term ($B=.618$, $p<.001$). Increasing age was significantly associated with decreased pallidum volume among PM males ($B=-.598$, $p<.001$), but not for controls ($B=.019$, $p=.867$).
Table 6.3

*Summary of multiple linear regression models examining the effect of age and group on subcortical volumes*

<table>
<thead>
<tr>
<th></th>
<th>Thalamus</th>
<th></th>
<th>Caudate</th>
<th></th>
<th>Putamen</th>
<th></th>
<th>Pallidum</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (S.E)</td>
<td>t</td>
<td>B (S.E)</td>
<td>t</td>
<td>B (S.E)</td>
<td>t</td>
<td>B (S.E)</td>
<td>t</td>
</tr>
<tr>
<td>Constant</td>
<td>-.47 (.13)</td>
<td>-3.60***</td>
<td>-.45 (.14)</td>
<td>-3.31**</td>
<td>-.28 (.18)</td>
<td>-1.61</td>
<td>-.31 (.19)</td>
<td>-1.66</td>
</tr>
<tr>
<td>ICV</td>
<td>.36 (.09)</td>
<td>4.01***</td>
<td>.60 (.09)</td>
<td>6.84***</td>
<td>.38 (.11)</td>
<td>3.38**</td>
<td>.18 (.13)</td>
<td>1.40</td>
</tr>
<tr>
<td>Age</td>
<td>-.61 (.09)</td>
<td>-7.22***</td>
<td>-.47 (.10)</td>
<td>-4.79***</td>
<td>-.54 (.12)</td>
<td>-4.38***</td>
<td>-.60 (.13)</td>
<td>-4.76***</td>
</tr>
<tr>
<td>Group</td>
<td>.74 (.18)</td>
<td>4.18***</td>
<td>.65 (.18)</td>
<td>3.65***</td>
<td>.33 (.23)</td>
<td>1.45</td>
<td>.42 (.25)</td>
<td>1.66</td>
</tr>
<tr>
<td>Age × group</td>
<td>.35 (.12)</td>
<td>2.97**</td>
<td>.17 (.13)</td>
<td>1.35</td>
<td>.25 (.16)</td>
<td>1.53</td>
<td>.62 (.17)</td>
<td>3.64***</td>
</tr>
</tbody>
</table>

**p<.01; ***p<.001
6.4.3 Associations between subcortical volumes and cognitive performance

Fine motor function

Regression models exploring the relationship between adjusted subcortical volumes and fine motor function are summarised in Table 6.4.

The overall models predicting fine motor function from thalamus and pallidum volumes comprising of group, volume and group × volume as predictors were significant (thalamus: $F_{(3,39)}=5.435, p=.003$; pallidum: $F_{(3,39)}=4.102, p=.013$), and explained 24.1% and 18.1% of the variance in fine motor function, respectively. Within these models, the contribution of thalamus (B=.761, $p<.001$) and pallidum (B=.591, $p=.005$) volumes were significant, indicating that reduced thalamus and pallidum volumes were both associated with poorer fine motor function in males with the PM compared to controls. The interaction of group × volume were also significant for thalamus (B=-.980, $p=.003$) and pallidum (B=-.848, $p=.003$) volumes, suggesting that the relationships between volumetric changes in these regions and fine motor function differed between PM males and controls. Neither thalamus (B=-.219, $p=.332$) nor pallidum (B=-.245, $p=.370$) volume was significantly associated with fine motor function among the controls.

The overall models predicting fine motor function which included caudate ($F_{(3,38)}=1.424, p=.251$) and putamen ($F_{(3,38)}=.938, p=.432$) volume failed to achieve significance. Within these models, the individual contributions of subcortical volume (caudate: B=.382 $p=.099$; putamen: B=.334, $p=.176$), and their interactions with group (caudate: B=-.578, $p=.073$; putamen: B=-.230, $p=.448$), were not significant.
Table 6.4

Summary of regression models exploring the association between subcortical volumes and fine motor function

<table>
<thead>
<tr>
<th></th>
<th>B (S.E)</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalamus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>.28 (.23)</td>
<td>1.25</td>
</tr>
<tr>
<td>Thalamus</td>
<td>.76 (.21)</td>
<td>3.67***</td>
</tr>
<tr>
<td>Group</td>
<td>.04 (.30)</td>
<td>.12</td>
</tr>
<tr>
<td>Group × thalamus</td>
<td>-.98 (.304)</td>
<td>-3.22**</td>
</tr>
<tr>
<td>Caudate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>.22 (.24)</td>
<td>.92</td>
</tr>
<tr>
<td>Caudate</td>
<td>.38 (.23)</td>
<td>1.69</td>
</tr>
<tr>
<td>Group</td>
<td>.08 (.31)</td>
<td>.27</td>
</tr>
<tr>
<td>Group × caudate</td>
<td>-.58 (.31)</td>
<td>-1.85</td>
</tr>
<tr>
<td>Putamen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>.09 (.22)</td>
<td>.39</td>
</tr>
<tr>
<td>Putamen</td>
<td>.33 (.24)</td>
<td>1.38</td>
</tr>
<tr>
<td>Group</td>
<td>.12 (.29)</td>
<td>.43</td>
</tr>
<tr>
<td>Group × putamen</td>
<td>-.23 (.30)</td>
<td>-.77</td>
</tr>
<tr>
<td>Pallidum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>.02 (.20)</td>
<td>.12</td>
</tr>
<tr>
<td>Pallidum</td>
<td>.59 (.20)</td>
<td>2.98**</td>
</tr>
<tr>
<td>Group</td>
<td>.24 (.27)</td>
<td>.91</td>
</tr>
<tr>
<td>Group × pallidum</td>
<td>-.85 (.27)</td>
<td>-3.12**</td>
</tr>
</tbody>
</table>

**p<.01; ***p<.001

Information processing speed

Regression models exploring the relationship between subcortical volumes and information processing speed before and after adjusting for fine motor function are summarised in Table 6.5.

The overall models for both thalamus and pallidum volume comprising of group, volume and group × volume were significant (thalamus: \( F_{(3,39)} = 7.207, p<.001; \)
pallidum: $F_{(3,39)}=6.031, p=.002$), explaining 30.7% and 26.4% of the variance in information processing speed, respectively. Within these models, the main effects of thalamus (B=.708, $p=.001$) and pallidum (B=.477, $p=.013$) volume were significant, suggesting that reductions in these volumes were associated with poorer information processing speed in the PM group. The interaction with group for thalamus (B=-.985, $p=.002$) and pallidum (B=-.868, $p=.001$) volume were also significant predictors, indicating that the relationships between the volumes of these structures and information processing speed differed between groups. Volumes of the thalamus (B=-.276, $p=.204$) and pallidum (B=-.390, $p=.03$) were not significantly associated with information processing speed among controls at the adjusted significance threshold. After controlling for fine motor function, the individual contributions of thalamus (B=.276, $p=.153$) and pallidum (B=.152, $p=.364$) volume, and their interactions with group (thalamus: B=-.428, $p=.120$; pallidum: B=-.401, $p=.089$), were no longer significant.

The overall models that included either caudate ($F_{(3,38)}=2.577, p=.068$) or putamen ($F_{(3,38)}=2.279, p=.095$) volume were not significant when predicting information processing speed. Within these models, the individual contributions of caudate (B=.328, $p=.139$) and putamen (B=.395, $p=.096$) volume, and their interactions with group (caudate: B=-.075, $p=.805$; putamen: B=-.386, $p=.186$), were not significant. Furthermore, the individual contributions of caudate (B=.082, $p=.631$) and putamen (B=.196, $p=.303$) volume, and their interactions with group (caudate: B=.297, $p=.219$; putamen: B= -.248, $p=.284$) in their respective models, were not significant after controlling for the effect of fine motor function.
Table 6.5

Summary of regression models exploring the association between subcortical volumes and information processing speed, before\textsuperscript{a} and after\textsuperscript{b} controlling for fine motor function

<table>
<thead>
<tr>
<th></th>
<th>B (S.E)\textsuperscript{a}</th>
<th>t\textsuperscript{a}</th>
<th>B (S.E)\textsuperscript{b}</th>
<th>t\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalamus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>.04 (.22)</td>
<td>.18</td>
<td>-.12 (.18)</td>
<td>-.67</td>
</tr>
<tr>
<td>Thalamus</td>
<td>.71 (.20)</td>
<td>3.55**</td>
<td>.28 (.19)</td>
<td>1.46</td>
</tr>
<tr>
<td>Group</td>
<td>.41 (.29)</td>
<td>1.42</td>
<td>.39 (.24)</td>
<td>1.65</td>
</tr>
<tr>
<td>Group × thalamus</td>
<td>-.98 (.29)</td>
<td>-3.36**</td>
<td>-.43 (.27)</td>
<td>-1.59</td>
</tr>
<tr>
<td>Fine motor</td>
<td>-</td>
<td>-</td>
<td>.57 (.13)</td>
<td>4.51***</td>
</tr>
<tr>
<td>Caudate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-.02 (.23)</td>
<td>-.09</td>
<td>-.16 (.18)</td>
<td>-.92</td>
</tr>
<tr>
<td>Caudate</td>
<td>.33 (.22)</td>
<td>1.51</td>
<td>.08 (.17)</td>
<td>.48</td>
</tr>
<tr>
<td>Group</td>
<td>.25 (.30)</td>
<td>.83</td>
<td>.20 (.23)</td>
<td>.86</td>
</tr>
<tr>
<td>Group × caudate</td>
<td>-.08 (.30)</td>
<td>-.25</td>
<td>.30 (.24)</td>
<td>1.25</td>
</tr>
<tr>
<td>Fine motor</td>
<td>-</td>
<td>-</td>
<td>.64 (.12)</td>
<td>5.46***</td>
</tr>
<tr>
<td>Putamen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
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<td>-.54</td>
<td>-.16 (.17)</td>
<td>-.99</td>
</tr>
<tr>
<td>Putamen</td>
<td>.40 (.23)</td>
<td>1.71</td>
<td>.20 (.19)</td>
<td>1.04</td>
</tr>
<tr>
<td>Group</td>
<td>.44 (.27)</td>
<td>1.62</td>
<td>.37 (.22)</td>
<td>1.71</td>
</tr>
<tr>
<td>Group × putamen</td>
<td>-.39 (.29)</td>
<td>-1.35</td>
<td>-.25 (.23)</td>
<td>-1.09</td>
</tr>
<tr>
<td>Fine motor</td>
<td>-</td>
<td>-</td>
<td>.60 (.12)</td>
<td>4.86***</td>
</tr>
<tr>
<td>Pallidum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-.18 (.19)</td>
<td>-.96</td>
<td>-.19 (.15)</td>
<td>-1.26</td>
</tr>
<tr>
<td>Pallidum</td>
<td>.48 (.18)</td>
<td>2.60*</td>
<td>.15 (.17)</td>
<td>.92</td>
</tr>
<tr>
<td>Group</td>
<td>.58 (.25)</td>
<td>2.31**</td>
<td>.44 (.21)</td>
<td>2.15*</td>
</tr>
<tr>
<td>Group × pallidum</td>
<td>-.87 (.25)</td>
<td>-3.44**</td>
<td>-.40 (.23)</td>
<td>-1.75</td>
</tr>
<tr>
<td>Fine motor</td>
<td>-</td>
<td>-</td>
<td>.55 (.12)</td>
<td>4.55***</td>
</tr>
</tbody>
</table>

\textsuperscript{*}p<.05; \textsuperscript{***}p<.001

6.4.4 Correlations between cognitive performance, brain volumes and FMR1 molecular measures (CGG repeat length, mRNA) among PM males

Partial Pearson correlations controlling for age and ICV were conducted to explore possible associations between FMR1 molecular measures and subcortical volumes
among PM males. These analyses showed that increasing CGG repeat length was associated with reduced putamen volume ($r = -0.654, p = 0.006$), but no correlations were found with volumes of the thalamus ($r = -0.328, p = 0.198$), caudate ($r = -0.358, p = 0.176$), or pallidum ($r = -0.372, p = 0.141$). *FMR1* mRNA levels were not significantly associated with volumes of the thalamus ($r = -0.292, p = 0.255$), caudate ($r = -0.082, p = 0.762$), putamen ($r = -0.291, p = 0.275$) or pallidum ($r = -0.093, p = 0.723$). As reported in Chapter 4, there were no significant associations between *FMR1* molecular measures and fine motor function or information processing speed among PM males.

### 6.5 Discussion

This chapter examined interrelationships between volumes of specific subcortical structures (thalamus, caudate, putamen, pallidum), fine motor function and information processing speed among PM males and controls. The results showed that PM males had reduced volumes of the thalamus and caudate when compared to controls, partially supporting the hypothesis of reductions in subcortical volumes in the PM group. The hypothesis of stronger relationships between increasing age and reductions in subcortical volume in PM males was only partially supported, with stronger associations between reductions in volumes of the thalamus and pallidum with increasing age in males with the PM. Importantly, reduced thalamus and pallidum volumes were associated with poorer fine motor function among PM males providing partial support for the hypothesis that reductions in subcortical regions implicated in the motor fronto-subcortical loop (thalamus, putamen, pallidum) would be associated with decrements in fine motor function. Although the associations between volumetric reductions in the thalamus and pallidum and information
processing speed differentiated between the groups, these relationships were no longer significant in PM males and controls after controlling for fine motor function. Therefore, the hypothesis that volume loss in subcortical regions receiving major projections from the frontal lobes (caudate, pallidum and thalamus) would be associated with decrements in information processing speed among PM males was not supported. In addition, there were significant associations between reduced putamen volumes and increasing CGG repeat length in males with the PM, partially supporting the hypothesis of associations between subcortical volumes and CGG repeat length. These results extend previous findings of subcortical involvement in PM males (Battistella et al., 2013; Hashimoto, Javan, et al., 2011; Moore et al., 2004; Wang, Hagerman, et al., 2013) suggesting important relationships between volumetric changes in the thalamus and basal ganglia and decline in fine motor function.

The current finding of significantly reduced volume of the thalamus among PM males compared to controls is consistent with previous imaging studies (Battistella et al., 2013; Hashimoto, Javan, et al., 2011; Wang, Hagerman, et al., 2013). Further, PM males showed a stronger relationship between thalamus volume loss and increasing age compared to controls. This finding is in contrast to a previous study that did not show age-related changes in thalamus volume, suggestive of neurodevelopmental mechanisms (Battistella et al., 2013). This discrepancy may relate to differences in sample composition, with the Battistella et al (2013) study comprising of only PM males without FXTAS, whereas the current study included PM males both with and without FXTAS. Given that thalamus volume loss has been significantly associated with FXTAS stage (Wang, Hagerman, et al., 2013), the
inclusion of PM males with FXTAS in the current study may have resulted in greater rates of volume loss with increasing age. However, it is also possible that reductions in thalamus volume may reflect both neurodevelopmental and neurodegenerative effects of the PM at different points in the lifespan. Whether these effects occur independently, or as early and later stages of the same process, awaits future longitudinal investigations.

Another important finding was that although no significant difference in pallidum volume was observed between PM males and controls, the relationship between pallidum volume loss and increasing age was stronger in the PM group. One potential explanation is that, similar to the thalamus, volumetric loss in the pallidum may be indicative of an age-related neurodegenerative process associated with the PM. Both the pallidum and thalamus form critical parts of the fronto-striatal circuit implicated in the planning and execution of movement to facilitate goal-directed actions (Alexander & Crutcher, 1990; Alexander et al., 1986). These include the location of a target in space, the direction of limb movement, and muscle force/pattern (Alexander, Crutcher, & DeLong, 1990). In the current study, thalamus and pallidum volume were significantly associated with fine motor function among PM males, suggestive of poorer performance among those with reduced thalamus and pallidum volumes. Taken together, these findings highlight the possible role of thalamic and pallidal degeneration with increasing age in motor dysfunction seen in males with the PM. Although these regions appear to play an important role in a neurodegenerative process associated with the PM, the possible implications for the development of targeted treatments remain unknown.
In regard to the caudate, volume of this region was significantly reduced among PM males compared to controls, but the relationship between volume loss and increasing age was comparable between groups. This suggests a possible neurodevelopmental effect of PM expansions on caudate development. However, it should be noted that caudate volumes for two PM males with FXTAS were excluded due to severe atrophy resulting in poor quality segmentation. It is therefore possible that the exclusion of these two PM males with severe caudate volume loss resulted in insufficient power to detect a significant age × group interaction. To further explore this possibility, caudate volume was plotted against age with slopes calculated for both PM males and controls independently (Figure 6.1). If the rate of caudate volume loss with increasing age is comparable between groups, the slope of each group should be parallel. Alternatively, if the slopes intersect, this would suggest that the relationship between age and volume loss is different between groups. As can be seen in Figure 6.1, the slope for PM males is steeper and intersects with that of the control group, suggesting a stronger association between caudate volume loss and increasing age among PM males. Although this observation cannot be considered statistically significant, it does add to the interpretation of these findings to suggest that the lack of a significant interaction between age and group probably relates to a lack of statistical power, as opposed to any neurodevelopmental process.
Figure 6.1. Changes in caudate volume with increasing age among PM males and controls. Although the interaction term between age and group was not significant, visual inspection of the data suggests a stronger relationship between volume loss and increasing age in the PM group compared to controls.

The current findings of interrelationships between subcortical volumes, fine motor function and information processing speed suggest that reductions in caudate volume among PM males were not associated with fine motor function or information processing domain scores. This finding was unexpected given previous research in healthy populations showing positive associations between both caudate activation (Forn, Belloch, Bustamante, Garbin, Parcet-Ibars et al., 2009) and striatal white matter integrity (Hedden, Schultz, Rieckmann, Mormino, Johnson et al., 2014) and measures of information processing speed. Further, deficits in information
processing speed are often observed in clinical populations with predominant caudate involvement (e.g. Huntington’s disease; Ho, Sahakian, Brown, Barker, Hodges et al., 2003) and may be a sensitive marker of striatal dysfunction even in pre-manifest stages of disease (Harrington, Liu, Smith, Mills, Long et al., 2014; Jurgens, van de Wiel, van Es, Grimbergen, Witjes-Ané et al., 2008). It is possible that the limited measures of cognitive function included in the current analyses were not sensitive to caudate dysfunction among this cohort of PM males. Given the role of the caudate in filtering information through the dorsolateral prefrontal and oculomotor fronto-striatal circuits (Alexander & Crutcher, 1990; Alexander et al., 1986), oculomotor paradigms assessing inhibitory control may provide a more sensitive measure of caudate dysfunction in the PM. Alternatively, deficits in information processing speed may relate to dysfunction within frontal or cerebellar brain regions which are also implicated in fronto-subcortical and cortico-cerebellar networks regulating cognitive function (Alexander & Crutcher, 1990; Alexander et al., 1986; Bernard et al., 2012; Strick et al., 2009).

The investigation of possible relationships between subcortical volumes and FMR1 molecular measures in the current study indicated that although putamen volume did not differ significantly between PM males and controls, reduction in putamen volume within the PM group was associated with increasing CGG repeat length. However, neither CGG repeat length or FMR1 mRNA among PM males were associated with any other subcortical volume. Correlation coefficients between CGG repeat length and volumes of the thalamus and caudate were moderate (> .3), so it is possible that these may have been significant if tested within a larger sample. Alternatively, it is possible that the effect of increasing CGG repeat length on
subcortical volume differs between individual structures with preferential atrophy of the putamen, analogous to observations of early preferential striatal atrophy in Huntington’s disease (Kipps, Duggins, Mahant, Gomes, Ashburner et al., 2005; Tabrizi, Reilmann, Roos, Durr, Leavitt et al., 2012). If replicated by future studies, an effect of increasing CGG repeat length on putamen volume may have implications for motor signs in the PM. Together with the thalamus and pallidum, the putamen forms a central component of the motor subcortical circuit, receiving input from premotor areas (including the arcuate premotor area and supplementary motor area) and the primary motor cortex (Winn, Wilson, & Redgrave, 2010). Although putamen volume among PM males was not found to be significantly associated with fine motor function, alterations in putamen structure may indirectly affect motor function via its inhibitory action on the pallidum, which in turn regulates the action of the thalamus on cortical regions (Winn et al., 2010). This would be consistent with previous studies pointing to CGG-related disruption to the motor fronto-striatal circuit in males with the PM, including significant associations between CGG repeat length and volume loss in the supplementary motor area (Hashimoto, Javan, et al., 2011) and negative correlations between putamen volume and FXTAS stage (Wang, Hagerman, et al., 2013). However, this interpretation remains speculative, and the possible relationship between CGG repeat length and putamen volume awaits replication in larger longitudinal studies.

The findings of this study have highlighted a number of methodological considerations that may guide future studies exploring the associations between subcortical volumes and neurobehavioural features among PM males with and without FXTAS. General limitations common to multiple chapters in this thesis are
discussed in Chapter 9. More specific limitations relate to the automatic processing of subcortical volumes, the selected measures of information processing speed, and the limited number of cognitive domains included in these analyses. These limitations, along with suggestions for future studies, are discussed below.

First, the use of automatic processing of subcortical volumes precluded any manual editing of poor segmentations. Poor automatic segmentations typically occurred due to severe atrophy or the presence of WMHs; radiological signs which are frequently observed among PM males with increasing age. The exclusion of volumes that failed quality control resulted in missing volumes for caudate (n=2), putamen (n=2), thalamus (n=1), and pallidum (n=1), all obtained from three of the most clinically affected PM males with FXTAS. Manual tracing of subcortical structures may offer an alternative approach that would enable inclusion of poor segmentations, especially in those PM males with more extensive subcortical pathology.

The second limitation relates to the assessment of information processing speed, which included measures involving manual manipulation of test materials (see Chapter 2 for full details). In this chapter, poorer performance on the information processing speed domain score was associated with volume loss in both the thalamus and pallidum, but this relationship was no longer significant after controlling for the effect of fine motor function. This highlights an important issue regarding the delineation of cognitive and motor contributions to impairments on tests of psychomotor slowing among PM males, who may be exhibiting both cognitive and motor signs. More appropriate measures of information processing speed may include those that do not rely on manual dexterity (e.g. verbal adaptations, as used in
previous studies in the PM; Grigsby et al., 2007) to obtain a purer measure of
cognitive performance.

Finally, the associations between subcortical volumes and cognitive function were
examined using only two domain scores. These measures were selected for targeted
analyses as PM males demonstrated significantly poorer performance on these
domains compared to controls (results presented in Chapter 4). Future research in
larger cohorts should examine the relationships between subcortical volume loss and
a broader array of cognitive domains (e.g. inhibitory control) which may provide
sensitive measures of basal ganglia dysfunction and disease progression.

In conclusion, the findings presented in this chapter provide evidence to suggest that
greater age-related reductions in thalamus and basal ganglia volumes may contribute
to neurodegenerative manifestations of the PM. Among PM males, decrements in
fine motor function, but not information processing speed, were associated with
volume loss in the thalamus and pallidum. These findings indicate that measures of
fine motor function may be more sensitive to disruption within subcortical neural
networks in the PM than measures of information processing speed. Future studies
are required to explore the interrelationships between changes in subcortical
structures and a broader range of higher order cognitive functions among males with
the PM. This awaits future longitudinal studies in larger cohorts to determine
whether decrements in fine motor function prior to the onset of FXTAS may serve to
predict later neurological decline.
CHAPTER 7.

EXPLORING THE INTERRELATIONSHIPS BETWEEN POSTURAL SWAY, CEREBELLAR VOLUME AND $FMR1$ MOLECULAR MEASURES
CHAPTER 7. EXPLORING THE INTERRELATIONSHIPS BETWEEN POSTURAL SWAY, CEREBELLAR VOLUME, AND FMRI MOLECULAR MEASURES

7.1 Introduction

As reviewed in Chapter 3, cerebellar pathology is well described in FXTAS, including volume loss, diffuse white matter changes, spongiosis of the MCP, and variable degrees of glial and purkinje cell loss (Cohen et al., 2006; Greco et al., 2006; Greco et al., 2002; Hashimoto, Srivastava, et al., 2011; Jacquemont et al., 2003; Wang et al., 2012). A number of cerebellar changes have also been described among PM carriers without FXTAS, including grey matter volume loss in lobules I/II of the vermis, lobule III (Hashimoto, Javan, et al., 2011) and anterior lobule VI of the cerebellum (Battistella et al., 2013), and decreased structural connectivity within white matter tracts of the MCP and bilateral cerebral peduncles (Hashimoto, Srivastava, et al., 2011). These findings suggest that changes in both grey and white matter of the cerebellum may occur prior to the onset of FXTAS symptoms; however, it is not yet known whether these changes may indicate greater risk for symptom onset.

In line with previous studies, the analyses included in Chapter 5 showed that cerebellar volumes were significantly reduced among the cohort of PM males described in this thesis compared to controls. In addition, increasing CGG repeat
length was significantly associated with decreasing cerebellar volume among PM males. This was consistent with previous studies of PM males with and without FXTAS that have shown negative relationships between CGG repeat length and volume of the cerebellum (Adams et al., 2007; Cohen et al., 2006; Moore et al., 2004). Cerebellar volume and white matter integrity in the superior cerebellar peduncles in turn have shown negative associations with clinical FXTAS staging and FXTAS rating scale scores respectively (Adams et al., 2007; Hashimoto, Javan, et al., 2011; Wang, Hessl, Schneider, et al., 2013). Yet, the interrelationships between changes in postural control, cerebellar volume and \textit{FMR1} molecular measures remain unknown.

The cerebellum includes areas that comprise the fronto-cerebellar tracts underlying control of posture (Ouchi, Okada, Yoshikawa, Nobezawa, & Futatsubashi, 1999). It follows then that changes in postural control may provide a sensitive marker of cerebellar dysfunction in the PM. One study utilising computerised dynamic posturography among PM males with and without FXTAS described impairments in automatic responses to postural displacement and reduced maximal balance range without falling or stepping (O'Keefe et al., 2012). Notably, deficits on test conditions involving vestibular control of balance were reported not only among PM males with FXTAS, but also those without FXTAS (O'Keefe et al., 2012). Deficits in postural sway became more apparent with increasing age and CGG repeat length when the cohort was expanded to include a larger sample (O'Keefe et al., 2015). Among PM males, increased body sway as measured by the coordination tremor balance system (CATSYS) has also been suggested as a possible early marker of ataxia, as in some PM males changes in postural control were observed prior to symptoms being
endorsed via self-report (Allen et al., 2008; Juncos et al., 2011). Significantly, impairments in body sway with eyes closed were shown to correlate with increasing CGG repeat length in PM males (Allen et al., 2008). Similarly, there is evidence to suggest that changes in postural control may be observable among PM females without FXTAS. PM females have demonstrated greater cognitive-motor interference in postural sway (Kraan et al., 2013) and step initiation time (Hocking et al., 2015) while performing a concurrent verbal fluency task compared to matched controls. Moreover, positive associations between FMR1 molecular measures (FMR1 mRNA and/or CGG repeat length) and dual-task related decrements in postural control were described in this cohort (Hocking et al., 2015; Kraan et al., 2013). Collectively, these findings suggest that changes in postural control may be observable among PM carriers prior to the onset of FXTAS, and that this measure may be sensitive to possible dose-effects of larger PM expansions (CGG repeat length, FMR1 mRNA) on motor function.

This chapter extends upon the findings of reduced cerebellar volume among PM males described in Chapter 5 by exploring the influence of cerebellar volume on changes in postural control among PM males and controls. Specifically, the aims of this chapter are to (i) examine postural sway (maximal anterior-posterior and medio-lateral displacements) during perturbation of visual and proprioceptive input; and (ii) explore the interrelationships between postural sway, cerebellar volume, CGG repeat length and FMR1 mRNA levels. It was hypothesised that: (1) PM males would demonstrate greater postural sway compared to controls; (2) greater body sway among PM males would be associated with FMR1 molecular measures (larger CGG repeat length, elevated mRNA) and decreased cerebellar volume. The candidate’s
publication ‘Preliminary evidence of an effect of cerebellar volume on postural sway in \textit{FMRI} premutation males’ (Birch, Hocking, Cornish, Menant, Georgiou-Karistianis et al., 2015; see Appendix C) arises directly from the content of this chapter.

### 7.2 Assessment and measures

Twenty-two PM males (ages 26–80) and 24 controls with normal \textit{FMRI} alleles (ages 26–77) were included in postural sway analyses. Materials and methods are described in detail in Chapter 2. Postural sway during quiet stance was quantified using the sway meter (Lord et al., 2003) as described in Chapter 2. Briefly, sway displacement was measured in the anterior-posterior and medio-lateral directions in millimetres for 30 seconds across four different conditions: (1) standing on the floor with eyes open; (2) standing on the floor with eyes closed; (3) standing on a foam mat (15cm thick) with eyes open; and (4) standing on foam with eyes closed. 3D T1-weighted MRI scans were obtained for all participants except two PM males without FXTAS for whom MRI was contraindicated. CGG repeat length and \textit{FMRI} mRNA levels were quantified from blood.

### 7.3 Statistical analyses

As described in Chapter 2, measures of anterior-posterior and medio-lateral postural displacement under each of the sway conditions were entered into a principal components analysis, and the first principal component was extracted to provide an overall sway score. The overall sway score was then normalised using Blom’s rank-based transformation (Blom, 1958), and compared using analysis of covariance.
(ANCOVA) controlling for age as follows: (a) PM vs HC; and (b) FX+ vs FX- vs HC. For all measures of sway, a higher score denotes greater postural displacement, i.e., poorer postural control.

Details of image processing for T1-weighted scans are provided in Chapter 2. Total cerebellar volumes, which included all components of the cerebellum, were calculated as the sum of grey and white matter in both the left and right hemispheres. All analyses used cerebellum volume adjusted for ICV. Linear regression analyses were conducted separately in PM and control groups to determine associations between cerebellar volume and postural sway. In the PM group only, linear regression was also used to examine the relationship between CGG repeat length, $FMRI$ mRNA levels and postural sway. In all regression models, age and CGG repeat length were scaled (decades and per 10 CGG repeats, respectively) to enhance the interpretability of regression coefficients. Significant predictors were included in a final multiple linear regression model. Assumptions required by multiple linear regression (linearity, independence and normality of residuals, homoscedasticity) were adequately met. Where indicated, the Sobel Test (Sobel, 1982) was used to explore mediation between predictor and outcome variables. A significance threshold of $p<.05$ was used for linear regression and mediation analyses to minimise the chance of Type II error as recommended by Rothman (Rothman, 1990).
7.4 Results

7.4.1 Participant characteristics

Participant characteristics such as age, IQ, severity of motor symptoms (as measured by the FXTAS rating scale), CGG repeat length, and mRNA levels are described in Chapter 3. Therefore they are not repeated in great detail here. In brief, the combined PM group were well matched to controls in terms of demographic characteristics including age, education, current and premorbid IQ and severity of motor symptoms. PM males with FXTAS were significantly older than PM males without FXTAS, and performed worse than both PM males without FXTAS and controls on measures of current IQ and FXTAS symptoms (see Chapter 3 for further details).

7.4.2 Group differences in postural sway

Mean raw scores (mm²) for individual sway trials are presented in Table 7.1. Group comparisons were performed using the normalised overall sway factor scores. When PM males with and without FXTAS were combined, an ANCOVA controlling for age showed that PM males performed significantly worse than controls on the overall sway factor score ($F_{(1,43)}= 13.066$, $p<.001$). After classifying the PM group according to FXTAS diagnosis, a second ANCOVA controlling for age again showed that the groups differed on the overall sway factor score ($F_{(2,42)}=8.935$, $p<.001$). Post-hoc comparisons revealed that PM males with FXTAS performed significantly worse than controls on the overall sway factor ($p<.001$). No significant differences were found between PM males without FXTAS and controls ($p=.084$), or PM males with FXTAS ($p=.163$).
Table 7.1

*Mean (SD) anterior-posterior and medio-lateral postural displacement across different test conditions (mm)*

<table>
<thead>
<tr>
<th>Sway condition</th>
<th>HC (n=24)</th>
<th>PM (n=22)</th>
<th>FX+ (n=7)</th>
<th>FX- (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sway on floor, eyes open (AP)</td>
<td>14.4 (6.9)</td>
<td>24.1 (10.7)</td>
<td>31.6 (14.0)</td>
<td>20.6 (6.9)</td>
</tr>
<tr>
<td>Sway on floor, eyes open (ML)</td>
<td>11.3 (6.9)</td>
<td>15.0 (10.3)</td>
<td>19.6 (15.4)</td>
<td>12.9 (6.6)</td>
</tr>
<tr>
<td>Sway on floor, eyes closed (AP)</td>
<td>16.4 (6.1)</td>
<td>23.4 (14.0)</td>
<td>39.0 (27.8)</td>
<td>20.0 (7.3)</td>
</tr>
<tr>
<td>Sway on floor, eyes closed (ML)</td>
<td>12.0 (6.8)</td>
<td>16.5 (11.9)</td>
<td>30.3 (19.6)</td>
<td>13.6 (7.9)</td>
</tr>
<tr>
<td>Sway on foam, eyes open (AP)</td>
<td>26.3 (8.5)</td>
<td>35.8 (16.2)</td>
<td>45.2 (24.9)</td>
<td>32.6 (11.6)</td>
</tr>
<tr>
<td>Sway on foam, eyes open (ML)</td>
<td>18.6 (8.7)</td>
<td>22.4 (14.3)</td>
<td>33.2 (18.6)</td>
<td>18.8 (11.1)</td>
</tr>
<tr>
<td>Sway on foam, eyes closed (AP)</td>
<td>39.7 (22.7)</td>
<td>37.8 (16.5)</td>
<td>71.5 (12.0)</td>
<td>32.7 (9.3)</td>
</tr>
<tr>
<td>Sway on foam, eyes closed (ML)</td>
<td>25.5 (10.2)</td>
<td>33.5 (19.6)</td>
<td>56.3 (27.9)</td>
<td>30.0 (16.9)</td>
</tr>
<tr>
<td>Normalised sway factor score</td>
<td>-.4 (.9)</td>
<td>0.4 (.9)***t</td>
<td>1.2 (.8)***t</td>
<td>.1 (.8)</td>
</tr>
</tbody>
</table>

***p<.001

AP = anterior-posterior; ML = medio-lateral
a=one missing; b=two missing; c=four missing; d=five missing; e=Normalised rank transformation, includes imputed missing values; f>HHC

7.4.3 Group differences in cerebellar volumes

Differences in cerebellar volume between the combined PM group and controls were explored in Chapter 5, and as such, these comparisons are not repeated here. Briefly,
the findings indicated significantly reduced cerebellar volume among PM males compared to controls (see Chapter 5 for further detail).

7.4.4 Association between cerebellar volume and postural sway factor score

Linear regression analyses indicated that when controlling for age, smaller cerebellar volume was associated with poorer performance on the postural sway factor in the PM group (B=-.928, \(p<.001\); Table 7.2). An unexpected negative effect of age on sway (B=-.367; \(p=.020\)) can also be seen in this solution. Since the Pearson correlation coefficient between age and sway is positive (\(r=.343, p=.118\)), the negative regression coefficient for age is most likely due to multicollinearity between age and cerebellar volume, resulting from the high correlation between these two variables (\(r=-.799, p<.001\)). When controlling for age, cerebellar volume was not a significant predictor of postural sway in the control group (B=-.440, \(p=.117\)).

Table 7.2

Summary of linear regression models exploring associations between cerebellar volume (adjusted for intracranial volume) and normalised postural sway factor score

<table>
<thead>
<tr>
<th></th>
<th>HC (n=24)(^a)</th>
<th></th>
<th>PM (n=20)(^b)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (S.E)</td>
<td>(t)</td>
<td>B (S.E)</td>
<td>(t)</td>
</tr>
<tr>
<td>Constant</td>
<td>-1.19 (.76)</td>
<td>-1.57</td>
<td>2.02 (.71)</td>
<td>2.83*</td>
</tr>
<tr>
<td>Age (decades)</td>
<td>.17 (.13)</td>
<td>1.32</td>
<td>-.37 (.14)</td>
<td>-2.57*</td>
</tr>
<tr>
<td>Cerebellar volume</td>
<td>-.44 (.27)</td>
<td>-1.64</td>
<td>-.93 (.19)</td>
<td>-5.00***</td>
</tr>
</tbody>
</table>

\(^a\)\(^p<.05\); \(^b\)\(^p<.001\)

\(^a\)\(F_{(2,21)}=4.327, p=.027, \text{adjusted } R^2=.224\); \(^b\)\(F_{(2,17)}=15.261, p<.001, \text{adjusted } R^2=.600\)
7.4.5 Associations between CGG repeat length, FMR1 mRNA and postural sway in PM males

Results of the linear regression model including age and CGG repeat length showed that both increasing age (B=.277, \( p=.023 \)), and increasing CGG repeat length (B=.302, \( p=.012 \)) were significant predictors of greater postural sway among PM males (Table 7.3). FMR1 mRNA was not a significant predictor of postural sway (B=.309, \( p=.091 \)).

Table 7.3

Summary of linear regression analyses examining CGG repeat length\(^a\) and FMR1 mRNA levels\(^b\) as predictors of postural sway factor scores in PM males

<table>
<thead>
<tr>
<th></th>
<th>B (S.E)</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-3.70 (.25)</td>
<td>-2.97**</td>
</tr>
<tr>
<td>Age (decades)</td>
<td>.28 (.12)</td>
<td>2.48*</td>
</tr>
<tr>
<td>CGG repeat length (per 10 repeats)</td>
<td>.30 (.11)</td>
<td>2.79*</td>
</tr>
<tr>
<td>Constant</td>
<td>-1.32 (.76)</td>
<td>-1.73</td>
</tr>
<tr>
<td>Age (decades)</td>
<td>.20 (.12)</td>
<td>1.70</td>
</tr>
<tr>
<td>FMR1 mRNA</td>
<td>.31 (.17)</td>
<td>1.78</td>
</tr>
</tbody>
</table>

\(^a\)F\(_{(2,19)}\)=5.680, \( p=.012 \), adjusted \( R^2=.308 \); \(^b\)F\(_{(2,19)}\)=3.069, \( p=.070 \), adjusted \( R^2=.165 \)

7.4.6 Mediation analysis between cerebellar volume, CGG repeat and postural sway

Cerebellar volume was included with CGG repeat length in a final model predicting postural sway factor score in PM males. Age was also included in this model as a control variable. With the addition of cerebellar volume in this model, CGG repeat
length was no longer a significant predictor of the postural sway factor score
(B=.092, S.E=.112, p=.422). This result suggests that cerebellar volume is a potential
mediator of the effect of CGG repeat length on postural sway (see Figure 7.1). To
examine the statistical significance of such a mediation effect using the Sobel test
(Sobel, 1982), an additional regression analysis was carried out with cerebellar
volume as the dependent variable and CGG repeat length as the independent
variable, with age again included in the model as a control variable. This showed a
significant effect of CGG repeat length on cerebellar volume (B=-.292, SE=.087,
p=.004). Using this result, together with the finding of the effect of cerebellar
volume on postural sway (B=-.803, SE=.242), the Sobel test reflected that the
indirect effect of CGG repeat length on postural sway, via the mediating variable,
cerebellar volume, was statistically significant (Z=2.360, p=.018). The strength of
the indirect path, calculated as the product of the two component paths (B=.234) was
approximately two and a half times stronger than the direct path (B=.092), indicating
that cerebellar volume almost entirely mediates the influence of CGG repeat length
on postural sway in this cohort of PM males.

![Diagram](attachment:Diagram.png)

Figure 7.1. Cerebellar volume as a mediator of the effect of CGG repeat length on
postural sway. Final model: \( F_{(3,16)}=10.207, p<.001 \), adjusted \( R^2 = .592 \). Age is
included as a covariate in all models.
7.5 Discussion

The current study is the first to examine the interrelationships between cerebellar volume, CGG repeat length, \textit{FMR1} mRNA levels, and postural sway during manipulation of visual and proprioceptive input in PM males with and without FXTAS. The findings showed significantly poorer performance on postural sway measures among PM males especially among those with FXTAS, supporting the hypothesis that PM males would demonstrate greater postural sway compared to controls with normal alleles. In all PM males, reductions in cerebellar volume and greater CGG repeat size were associated with greater postural sway. Importantly, cerebellar volume significantly mediated the relationship between CGG repeat length and postural sway in PM males. As \textit{FMR1} mRNA levels were not significantly associated with postural sway, the findings provide partial support for the hypothesis that greater postural sway among PM males would be associated with larger CGG repeat length, elevated \textit{FMR1} mRNA levels and decreased cerebellar volume. Collectively, these findings extend previous studies showing postural control abnormalities in the PM (Allen et al., 2008; Hocking et al., 2015; Kraan et al., 2013; O'Keefe et al., 2012; O'Keefe et al., 2015), to indicate early effects of cerebellar changes on postural stability in PM males that may be linked to CGG repeat length.

After classifying PM carriers according to FXTAS diagnostic categories, PM males with FXTAS demonstrated greater postural displacement compared to controls. No significant difference was detected between PM males without FXTAS and controls on the overall sway factor score, which was derived by extracting the first principal
component of all sway variables. Interestingly, no significant differences in postural sway were detected between PM males with and without FXTAS on the overall sway factor score. The scores of asymptomatic PM males fell between those obtained by PM males with FXTAS and controls, suggesting that a proportion of younger PM males that do not meet diagnostic criteria for FXTAS may exhibit subtle changes in postural control which are not sufficiently severe to distinguish them from controls with normal alleles. A separate study using the same measures of postural sway in asymptomatic PM females demonstrated greater postural displacements compared to controls, but only when performing a concurrent cognitive task of excluded letter verbal fluency (Kraan et al., 2013). This suggests a differential effect of increasing cognitive load on postural sway; however, it is difficult to interpret these previous findings in the context of the current study that did not incorporate a dual-task paradigm in males with the PM. Future efforts will be required to explore whether dual-task paradigms may provide a more sensitive measure of changes in postural control in PM males. Even among individuals without FXTAS, deficiencies on measures of postural sway and balance could contribute to difficulties with everyday activities (e.g. walking, ascending stairs) and may represent an early marker for greater risk of falls (Lord et al., 1991; Lord et al., 2003). As such, early identification of abnormalities in postural control may become important not only to discern possible risk for FXTAS, but also to identify individuals who may benefit from cognitive and physical interventions targeting postural control (e.g. DiBrezzo, Shadden, Raybon, & Powers.M., 2005; Lord, Ward, & Williams, 1996; Yasuda, Kawasaki, & Higuchi, 2012) that may reduce risk of falls, increase functional capacity, and improve quality of life.
Another important finding was that poorer performance on the postural sway factor was associated with decreased cerebellar volume in PM males. This is consistent with previous evidence of structural alterations in regions involved in postural control, including the anterior cerebellum and cerebellar vermis (Battistella et al., 2013; Hashimoto, Javan, et al., 2011; Wang, Hessl, Schneider, et al., 2013).

Furthermore, increasing CGG repeat length was associated with greater postural sway, consistent with previous studies suggesting effects of larger FMR1 expansions on motor function in the PM, including impairments in gait and postural control (Allen et al., 2008; Kraan, Hocking, Georgiou-Karistianis, Metcalfe, Archibald, Fielding, Bradshaw, et al., 2014; Kraan et al., 2013; O'Keefe et al., 2015), severity of motor symptoms (Leehey et al., 2008), and age of onset of motor symptoms (Tassone et al., 2007). It is therefore plausible that compromised postural control in PM males may relate to disruptions to cerebellar regions, which have been shown to play an important role in the maintenance of upright posture and regulation of postural sway (Ouchi, Okada, Yoshikawa, Futatsubashi, & Nobezawa, 2001; Ouchi et al., 1999; Sullivan, Deshmukh, Desmond, Lim, & Pfefferbaum, 2000; Sullivan et al., 2006).

Importantly, the findings of the current study demonstrate that cerebellar volume mediates the relationship between CGG repeat length and postural sway in males with the PM. These findings are in line with previous studies showing negative relationships between CGG repeat length and cerebellar volume (Adams et al., 2007; Cohen et al., 2006; Moore et al., 2004); and evidence of associations between cerebellar changes and severity of motor symptoms associated with FXTAS (Adams et al., 2007; Hashimoto, Javan, et al., 2011; Wang, Hessl, Schneider, et al., 2013).
Taken together with the findings of the current study, this raises the possibility that CGG repeat-dependent changes in cerebellar volume may inform predictive models to evaluate risk of postural control difficulties in PM males with and without FXTAS.

*FMR1* mRNA levels were not significantly associated with postural sway among PM males, despite being significantly correlated with CGG repeat length. This is consistent with previous studies describing significant negative relationships between cerebellar volume and CGG repeat length, but not *FMR1* mRNA levels (Adams et al., 2007; Cohen et al., 2006; Moore et al., 2004). While this may be due to *FMR1* mRNA levels measured in blood not being representative of those within different regions in the brain (Tassone, Hagerman, Garcia-Arocena, Khandjian, Greco et al., 2004), a recent post-mortem study of cerebellar tissue of PM males with FXTAS described increased transcript levels of *FMR1* mRNA that correlated with CGG repeat length (Pretto, Kumar, Cao, Cunningham, Durbin-Johnson et al., 2014). Moreover, elevated *FMR1* mRNA levels in the PM have been associated with reductions in structural connectivity (as opposed to volume) within the superior cerebellar peduncle (Wang, Hessl, Schneider, et al., 2013), and attentional interference in a choice-stepping reaction time test (Hocking et al., 2015), a composite measure of falls risk (Lord & Fitzpatrick, 2001). The discrepancies between studies may relate to the different selection of imaging and neuromotor measures. For example, accelerated reduction in structural connectivity with increasing age in PM males (Hashimoto, Srivastava, et al., 2011; Wang, Hessl, Schneider, et al., 2013; Wang et al., 2012) may result from mRNA toxicity, while early alterations in volume that do not accelerate with age may reflect
neurodevelopmental effects of FMR1 PM expansions on cortical development (Battistella et al., 2013). The lack of significant association between FMR1 mRNA levels and body sway among PM males may also suggest contribution from other molecular PM-specific factors that may act independently of FMR1 mRNA (Loesch et al., 2011; Pastori, Peschansky, Barbouth, Mehta, Silva et al., 2014; Todd, Oh, Krans, He, Sellier et al., 2013).

Notwithstanding the inherent limitations relating to sample size and the cross-sectional study design (see Chapter 9 for full discussion), the conclusions that can be drawn from this study are limited by the lack of parcellation of cerebellar volume. Future research could examine the integrity of specific subregions of the cerebellum in which alterations have previously been shown among PM carriers with and without FXTAS (Battistella et al., 2013; Hashimoto, Srivastava, et al., 2011; Wang, Hessl, Schneider, et al., 2013), and the relationships with performance on sensitive measures of postural control with dual-task interference. The use of measures of white matter integrity and structural connectivity would also allow for the exploration and potential separation of neurodevelopmental and neurodegenerative effects of the PM on cortical development and postural control (Battistella et al., 2013; Hashimoto, Srivastava, et al., 2011; Hippolyte et al., 2014). Further, the relationship between cerebellar integrity and other PM molecular markers including reduced FMRP (Ludwig, Espinal, Pretto, Jamal, Arque et al., 2014), abnormal expression of long non-coding RNA genes, ASFMRI/FMR4 (Loesch et al., 2011), FMR5 and FMR6 (Pastori et al., 2014), and repeat associated non-ATG translation (Todd et al., 2013) should be explored.
In conclusion, this chapter has demonstrated the novel finding that postural control deficits may be mediated by a negative relationship between CGG repeat length and cerebellar volume among PM males with and without FXTAS. Increasing CGG repeat length was associated with decreased cerebellar volume, which in turn was associated with poorer performance on measures relating to postural sway. This indicates that subtle changes in postural sway may reflect CGG repeat-mediated disruption in vulnerable cerebellar circuits implicated in postural control. These findings may inform the development of predictive models to estimate risk for the development of postural control deficits in PM males. The capacity to identify individuals at greatest risk would have significant clinical implications and would inform the development of tailored intervention strategies. Longitudinal studies in larger independent cohorts are required to determine the relationship between potential early changes in postural control and progression to more severe indicators of cerebellar dysfunction seen in FXTAS.
CHAPTER 8.

COGNITIVE-MOTOR INTERFERENCE IN GAIT

CHARACTERISTICS: RELATIONSHIPS WITH
CEREBELLAR VOLUME AND FMR1 MOLECULAR
MEASURES
CHAPTER 8. COGNITIVE-MOTOR INTERFERENCE IN GAIT CHARACTERISTICS: RELATIONSHIPS WITH CEREBELLAR VOLUME AND FMRI MOLECULAR MEASURES

8.1 Introduction

The development of measures sensitive enough to detect subtle motor dysfunction early in the disease process of FXTAS is crucial when considering potential outcome measures for use in future clinical trials. As discussed in Chapter 7, previous studies among PM males with and without FXTAS indicate measures of body sway may be sensitive to early changes in postural control among PM males without FXTAS (Aguilar et al., 2008; Allen et al., 2008; Juncos et al., 2011; Narcisa et al., 2011). The results presented in Chapter 7 extended upon these findings to suggest that the assessment of postural sway during standing may provide a sensitive measure of CGG-related cerebellar dysfunction among PM males with and without FXTAS. These findings were in line with a previous study demonstrating a positive association between postural sway with eyes closed and CGG repeat length among PM males with and without FXTAS when measured using the CATSYS computerised neuromotor testing battery (Allen et al., 2008). Despite these positive findings, existing studies of postural control among PM males are limited by lack of fine-grained assessment of gait patterns and step-to-step variability which could represent the earliest indicators for FXTAS. Gait ataxia is a core feature of FXTAS and the characterisation of these changes in their earliest stages would have important clinical implications for the identification and management of those at
greatest risk of adverse outcomes including greater risk of falls (Verghese, Ambrose, Lipton, & Wang, 2010).

One of the most sensitive approaches for investigating neuromotor function is that of dual-task related changes in gait and postural stability. Gait is a complex task requiring input from multiple sensory and cognitive systems, particularly those regulating executive and attentional control (Al-Yahya, Dawes, Smith, Dennis, Howells et al., 2011; Yogev-Seligmann, Hausdorff, & Giladi, 2008). Dual-task paradigms, which examine the effect of interference from a secondary cognitive task on a primary motor task, can provide a sensitive marker capable of detecting the earliest changes in prodromal groups including mild cognitive impairment (Maquet, Lekeu, Warzee, Gillain, Wojtasik et al., 2010), autosomal-dominant Parkinson’s Disease (Mirelman et al., 2011) and pre-manifest Huntington’s Disease (Rao, Mazzoni, Wasserman, & Marder, 2011). It has been proposed that dual-task related changes in gait, for example a decrease in speed while performing a concurrent cognitive task, arise due to the sharing of limited attentional resources between cognitive and motor tasks (Hausdorff, Yogev, Springer, Simon, & Giladi, 2005; Pashler, 1994). A recent study demonstrated that PM females show greater dual-task interference for gait domains of pace and variability when performing a concurrent digit subtraction task, especially for those PM women with lower working memory capacity (Kraan, Hocking, Georgiou-Karistianis, Metcalfe, Archibald, Fielding, Bradshaw, et al., 2014). In addition, the associations between increasing age and dual-task effects on gait variability among PM females were moderated by CGG repeat length. These findings suggest an age-related effect of larger CGG repeat expansions on gait function in PM females which may be observable prior to the
onset of FXTAS. The effect of dual-task interference in gait among males with the PM has yet to be investigated. Compared to females, PM males are at greater risk of developing FXTAS (Rodriguez-Revenga et al., 2009), and also tend to exhibit greater severity of cognitive and motor symptoms (Hagerman et al., 2004). As such the characterisation of early gait changes is a worthy area of study, as these may indicate a prodromal phase for FXTAS. The identification of prodromal features of FXTAS would allow for the earlier implementation of treatments as they become available.

The cerebellum forms an integral part of cortico-cerebellar networks regulating motor and cognitive functions including balance and locomotion (Alexander & Crutcher, 1990; Alexander et al., 1986; Morton & Bastian, 2007; Schmahmann & Sherman, 1998; Strick et al., 2009; Wu, Liu, Hallett, Zheng, & Chan, 2013). The medial, intermediate and lateral zones of the cerebellum, which are separated based on the functional organisation of connections with other brain regions, regulate specific aspects of movement control (Ilg & Timmann, 2013; Morton & Bastian, 2007). These include the maintenance of upright posture and dynamic balance control (medial zone, vermis and flocculonodular lobe), coordination and correct placement of limbs (intermediate zone), and adaptive adjustments in movement and timing to complex or changing environments (lateral zone) (Ilg & Timmann, 2013; Morton & Bastian, 2007). Due to the critical role of the cerebellum in these aspects of movement control, abnormalities in gait and postural control provide a sensitive indicator of cerebellar disruption (Ilg & Timmann, 2013; Morton & Bastian, 2007; Thach & Bastian, 2004).
As reported in Chapter 5, cerebellar volume among the cohort of PM males included in this thesis was significantly reduced compared to controls. This finding was consistent with previous studies that have indicated changes in cerebellar grey and white matter among PM males, some of which may be observed prior to the onset of FXTAS (Battistella et al., 2013; Brunberg et al., 2002; Cohen et al., 2006; Hashimoto, Javan, et al., 2011; Hashimoto, Srivastava, et al., 2011; Wang et al., 2012). Significantly, reductions in cerebellar volume have been associated with increasing CGG repeat length in this cohort (see Chapter 5) and also in previous studies (Adams et al., 2007; Cohen et al., 2006; Moore et al., 2004). Furthermore, elevated FMR1 mRNA levels have been linked to disruption to superior cerebellar peduncles, areas that serve an important role in cerebellar motor and cognitive networks (Wang, Hessl, Schneider, et al., 2013). However, the interrelationships between early gait changes, CGG repeat length, FMR1 mRNA and cerebellar structure among PM males have not yet been explored.

The aims of this chapter are to: (i) examine possible differential effects of working memory dual-tasks (serial subtraction) on spatiotemporal gait characteristics and variability in PM males and matched controls with normal FMR1 alleles; (ii) explore the relationship between dual-task gait interference and cerebellar volume; and (iii) investigate whether specific dual-task changes in gait are associated with cerebellar volume, CGG repeat length and FMR1 mRNA levels. It was hypothesised that: (1) PM males would exhibit greater interference in spatiotemporal gait characteristics when concurrently performing serial subtraction tasks; (2) decrements in dual-task performance on gait parameters would be associated with decreased cerebellar volume, increased CGG repeat length, and elevated FMR1 mRNA.
8.2 Assessment and measures

This chapter includes a subset of PM males and controls from the overall cohort who were able to complete gait assessments. Participants completing gait assessments included 20 PM males (six with FXTAS) aged 26–75 years and 24 controls aged 26–77 years. Two PM males were excluded from analyses in this chapter due to acute knee injury obscuring natural gait (one PM male without FXTAS) and inability to walk without use of a walking aid (one PM male with FXTAS). Details regarding participant recruitment and measures are presented in Chapter 2 and therefore not repeated in full here. Briefly, general intelligence was assessed using the four subtest version of Wechsler Abbreviated Scale for Intelligence (WASI) (Wechsler, 1999). Neurological symptoms including tremor, ataxia and parkinsonism were assessed using the FXTAS Rating Scale (Version 1.0) (Leehey et al., 2008). CGG repeat length and FMR1 mRNA levels were quantified from blood.

Spatiotemporal gait characteristics were assessed using the GAITRite system (CIR Systems Inc., Clifton, NJ, USA). Quantitative gait measures including gait speed (cm/s), step length (cm), step length variability, step width (cm), and step width variability were obtained under three conditions: (1) single-task, in which participants were asked to walk at their preferred pace; (2) -3s dual-task, where participants were required to count backwards by 3s while walking; and (3) -7s dual-task, where participants were asked to count backwards by 7s while walking. For the dual-task conditions, participants began counting from a starting number provided by the examiner. Intra-individual variability for gait variables were calculated as CoV= ((SD / Mean) × 100). To quantify dual-task interference as a proportion of baseline
performance, DTC was calculated using the following formula: \( \text{DTC} = \frac{(\text{dual-task score} - \text{single-task score})}{\text{single-task score}} \times 100 \). Total cerebellar volume (sum of grey and white matter of both hemispheres) was obtained by processing T1-weighted MRI scans of participants and adjusted for ICV. Brain scans were missing for two PM males without FXTAS.

### 8.3 Statistical analyses

#### 8.3.1 Participant demographics and spatiotemporal gait characteristics

Although group differences in demographic variables, cerebellar volume, and the relationships between \( \text{FMRI} \) molecular measures and cerebellar volume were presented in Chapter 3, these were re-examined in the current chapter following the exclusion of two PM males who were not able to complete the gait assessments. Normality of variables was assessed using the Shapiro-Wilk test. Group differences in demographic and gait characteristics were tested using independent \( t \)-tests, ANOVA, or non-parametric equivalents where the normality assumption was violated. Pairwise intergroup comparisons were conducted for: (a) all PM carriers vs controls (PM vs HC); (b) PM carriers with FXTAS vs controls (FX+ vs HC); (c) PM carriers without FXTAS vs controls (FX- vs HC); (d) PM carriers with FXTAS vs PM carriers without FXTAS (FX+ vs FX-). Differences in adjusted cerebellar volume were explored using multivariate analysis of covariance (MANCOVA) controlling for age. Relationships between CGG repeat length, \( \text{FMRI} \) mRNA and adjusted cerebellar volume in PM males were explored using partial Pearson correlations (controlling for age).
8.3.2 Associations between adjusted cerebellar volume and dual-task gait characteristics

Linear regression analyses were conducted to examine associations between adjusted cerebellar volume and dual-task performance on each gait parameter. Gait parameters were transformed into normalised rank scores using Blom’s formula (Blom, 1958) to minimise the influence of extreme scores and fulfil the assumptions of multiple linear regression (linearity, independence and normality of residuals, homoscedasticity). Independent variables included adjusted cerebellar volume, group (PM vs control) and an interaction term of these variables (group × cerebellar volume) to determine whether the relationship between cerebellar volume and dual-task gait performance differed between groups. Age was included as a covariate in all models.

8.3.3 Associations between FMR1 molecular measures and dual-task gait characteristics among PM males

Further linear regressions comprising age, CGG repeat length, FMR1 mRNA, and interaction terms (age × CGG repeat length; age × mRNA level) were conducted to identify relationships with gait domains within the PM group. A Bonferroni correction was used for regression coefficients ($p=.005$) to adjust for multiple testing across the five gait variables under two dual-task conditions (totalling 10 dependent variables). As this is the first study to explore the possible interrelationships between dual-task gait interference, cerebellar volume and FMR1 molecular measures among PM males, $p$ values <.05 were also reported to explore trend-level associations between variables. Although it is acknowledged that these cannot be interpreted as
statistically significant, they may be of clinical significance and worthy of further investigation in larger sample sizes.

8.4 Results

8.4.1 Participant characteristics

When comparing all PM carriers to controls, the groups were well matched on demographic and anthropometric measures with the exception of weight. Of those included in this subsample of the overall cohort, PM males were significantly heavier than controls ($t_{(42)}=2.201, p=.033$). There were no significant differences for age ($t_{(42)}=-.622, p=.512$), education (Mann-Whitney $U=232.000, p=.849$), height ($t_{(42)}=1.240, p=.222$), leg length ($t_{(42)}=1.136, p=.262$), FSIQ ($t_{(42)}=-1.612, p=.114$), or FXTAS rating scale score (Mann-Whitney $U=234.500, p=.897$) (Table 8.1). As expected, CGG repeat length (Mann-Whitney $U=.000, p<.001$) and FMR1 mRNA levels (Mann-Whitney $U=46.000, p<.001$) were significantly elevated in the PM group compared to controls. During the dual-task conditions, no significant differences were observed between PM carriers and controls in the difficulty rating or the number of correct responses for the -3s and -7s cognitive tasks (-3s difficulty: Mann-Whitney $U=163.000, p=.104$; -3s correct responses: $t_{(41)}=-1.121, p=.269$; -7s difficulty: $t_{(40)}=.611, p=.545$; -7s correct responses: $t_{(40)}=-1.883, p=.067$), indicating comparable performance between the groups.

After separating the PM group into those with (FX+ n=6) and without (FX-n=14) FXTAS, no significant differences were detected between these groups and controls for age ($X^2=5.285, p=.071$), education ($X^2=.202, p=.904$), height ($F_{(2,41)}=.903$,
Pairwise comparisons revealed that PM males without FXTAS were significantly heavier than controls ($X^2=5.501$, $p=.019$). With regard to performance on the cognitive dual-task, no significant differences in the difficulty rating or the number of correct responses for the -3s and -7s conditions emerged (-3s difficulty: Mann-Whitney $U=163.000$, $p=.104$); -3s correct responses: $F_{(2,40)}=1.242$, $p=.300$; -7s difficulty: $F_{(2,39)}=.321$, $p=.728$; -7s correct responses: $F_{(2,39)}=1.936$, $p=.158$). However, group differences emerged for FSIQ ($F_{(2,41)}=5.999$, $p=.005$), FXTAS rating scale score ($X^2=18.761$, $p<.001$), CGG repeat length ($X^2=32.295$, $p<.001$) and $FMR1$ mRNA ($X^2=21.047$, $p<.001$).

Specifically, PM carriers with FXTAS performed significantly worse than both controls and PM males without FXTAS on measures of FSIQ (vs HC $p=.005$; vs FX- $p=.014$) and FXTAS symptoms (vs HC $p<.001$; vs FX- $p<.001$). PM carriers with and without FXTAS both had significantly greater CGG repeat length and $FMR1$ mRNA levels compared to controls ($p$ values $<.001$); however, there were no significant differences in $FMR1$ CGG repeat length ($p=.274$) or $FMR1$ mRNA levels ($p=.968$) when comparing PM males with and without FXTAS.
Table 8.1

Participant characteristics of subsample included in Chapter 8

<table>
<thead>
<tr>
<th></th>
<th>HC (n=24)</th>
<th>PM (n=20)</th>
<th>FX- (n=14)</th>
<th>FX+ (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean (SD)</td>
<td>55.33 (14.60)</td>
<td>52.40 (14.68)</td>
<td>47.36 (14.48)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>26–77</td>
<td>26–75</td>
<td>26–69</td>
</tr>
<tr>
<td>Education (years)</td>
<td>Mean (SD)</td>
<td>13.50 (3.44)</td>
<td>13.40 (3.46)</td>
<td>13.64 (3.78)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>9–20</td>
<td>9–21</td>
<td>9–21</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Mean (SD)</td>
<td>173.18 (7.57)</td>
<td>175.70 (5.47)</td>
<td>176.24 (6.06)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>163.00–189.00</td>
<td>165.20–185.00</td>
<td>165.20–185.00</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Mean (SD)</td>
<td>80.35 (13.77)</td>
<td>89.35 (13.20)*</td>
<td>91.09 (13.71)*</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>57.20–118.30</td>
<td>63.50–124.80</td>
<td>76.00–124.80</td>
</tr>
<tr>
<td>Average leg length (cm)</td>
<td>Mean (SD)</td>
<td>81.15 (4.54)</td>
<td>82.66 (4.21)</td>
<td>82.37 (3.84)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>74.25–90.00</td>
<td>76.75–91.00</td>
<td>72.00–89.50</td>
</tr>
<tr>
<td>CGG repeat length</td>
<td>Mean (SD)</td>
<td>29.88 (4.09)</td>
<td>88.30</td>
<td>87.93</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>(16.34)**a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FMRI mRNA</td>
<td>Mean (SD)</td>
<td>1.08 (.25)</td>
<td>2.07 (1.00)**a</td>
<td>2.07 (1.11)**a</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>.73–1.57</td>
<td>.95–5.16</td>
<td>.95–5.16</td>
</tr>
<tr>
<td>Full scale IQ</td>
<td>Mean (SD)</td>
<td>113.46 (11.43)</td>
<td>107.30 (13.93)</td>
<td>112.36 (8.86)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>86–133</td>
<td>79–127</td>
<td>92–127</td>
</tr>
<tr>
<td>FXTAS rating scale</td>
<td>Mean (SD)</td>
<td>13.83 (7.35)</td>
<td>19.30 (20.00)</td>
<td>8.43 (6.37)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>13.64 (1.61)</td>
<td>19.30 (20.00)</td>
<td>8.43 (6.37)</td>
</tr>
<tr>
<td>-3s correct</td>
<td>Mean (SD)</td>
<td>28.71 (8.65)</td>
<td>25.68 (8.96)</td>
<td>24.36 (8.00)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>9–43</td>
<td>12–46</td>
<td>12–37</td>
</tr>
<tr>
<td>-7s correct</td>
<td>Mean (SD)</td>
<td>19.62 (7.90)</td>
<td>15.17 (7.16)</td>
<td>14.57 (7.49)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>6–37</td>
<td>4–32</td>
<td>4–32</td>
</tr>
<tr>
<td>-3s difficulty rating</td>
<td>Mean (SD)</td>
<td>2.92 (1.61)</td>
<td>3.95 (2.04)</td>
<td>3.64 (1.69)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>1–7</td>
<td>1–8</td>
<td>1–6</td>
</tr>
<tr>
<td>-7s difficulty rating</td>
<td>Mean (SD)</td>
<td>5.29 (2.33)</td>
<td>5.72 (2.16)</td>
<td>5.57 (2.07)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>2–10</td>
<td>2–9</td>
<td>2–9</td>
</tr>
</tbody>
</table>

*p<.05; ***p<.001

*a> HC; b< HC; c< FX-; d> FX-
8.4.2 Single and dual-task gait performance

Gait characteristics for single- and dual-task conditions are presented in Table 8.2. No significant differences were detected between groups for any of the spatiotemporal gait variables during the single-task (walking only) or -3s dual-task conditions. When comparing dual-task gait performance of all PM males to controls on the most difficult concurrent cognitive task (-7s), controls exhibited greater reduction in step length ($\chi^2=133.00, p=0.035$) and step width ($\chi^2=138.000, p=0.047$) while dual-tasking. Group differences emerged again for DTCs in step length ($F_{(2,39)}=4.646, p=0.015$) and step width ($F_{(2,39)}=4.440, p=0.018$) after classifying PM males according to FXTAS diagnosis. Post-hoc comparisons with Bonferroni adjustment showed significant differences in DTC between PM males with FXTAS and controls for step length ($p=0.020$) and step width ($p=0.024$). PM males with FXTAS tended to increase step length and step width while undertaking the concurrent cognitive task, while controls showed reductions in step length and step width, relative to the single task condition. No significant differences in dual-task gait performance were found between PM males without FXTAS and controls, or between PM males with and without FXTAS.

Follow-up analyses were conducted to explore whether the increased dual-task cost among those with FXTAS could be attributed to significantly lower FSIQ scores among PM males with FXTAS (see Table 8.1). When comparing FSIQ scores of only those who were able to complete the -7s dual-task condition, no significant differences emerged between groups ($F_{(2,39)}= 1.990, p=0.150$). As those PM males with FXTAS who were able to complete the -7s condition obtained comparable
FSIQ scores compared to controls (101.25 ±18.46), it is unlikely that differences in dual-task cost on this condition could be attributed to differences in FSIQ.
Table 8.2

Median (quartiles) for single-task spatiotemporal gait parameters and dual-task costs (%) when counting backwards by 3s and 7s

<table>
<thead>
<tr>
<th>Domain</th>
<th>Condition</th>
<th>HC (n=24)</th>
<th>PM (n=20)</th>
<th>FX- (n=14)</th>
<th>FX+ (n=6)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed (cm/s)</td>
<td>Single-task</td>
<td>135.58 (130.71, 149.60)</td>
<td>136.42 (128.22, 145.04)</td>
<td>139.53 (129.83, 152.17)</td>
<td>131.88 (85.77, 144.56)</td>
</tr>
<tr>
<td></td>
<td>-3s DTC</td>
<td>-6.91 (-12.88, -2.02)</td>
<td>-9.16 (-11.45, -0.86)</td>
<td>-9.44 (-12.06, -3.85)</td>
<td>-2.45 (-29.21, 4.91)</td>
</tr>
<tr>
<td></td>
<td>-7s DTC</td>
<td>-12.78 (-21.41, -6.04)</td>
<td>-7.98 (-19.08, -3.49)</td>
<td>-8.46 (-21.71, -4.48)</td>
<td>-3.10 (-14.89, 2.63)</td>
</tr>
<tr>
<td>Step length (cm)</td>
<td>Single-task</td>
<td>75.72 (72.98, 80.17)</td>
<td>73.93 (70.01, 78.39)</td>
<td>74.86 (73.46, 79.62)</td>
<td>69.50 (55.90, 77.81)</td>
</tr>
<tr>
<td></td>
<td>-3s DTC</td>
<td>-2.58 (-5.83, -0.42)</td>
<td>-1.64 (-5.04, 1.59)</td>
<td>-1.70 (-4.51, 1.88)</td>
<td>-0.35 (-12.43, 4.91)</td>
</tr>
<tr>
<td></td>
<td>-7s DTC</td>
<td>-6.12 (-8.69, -1.80)</td>
<td>-3.08 (-5.83, 2.11)*b</td>
<td>-3.41 (-6.79, -2.6)</td>
<td>1.29 (-4.46, 10.65)*b</td>
</tr>
<tr>
<td>Step length variability</td>
<td>Single-task</td>
<td>2.58 (1.85, 3.44)</td>
<td>2.47 (2.06, 4.29)</td>
<td>2.39 (1.98, 3.15)</td>
<td>3.38 (2.24, 3.38)</td>
</tr>
<tr>
<td></td>
<td>-3s DTC</td>
<td>16.48 (-6.01, 50.54)</td>
<td>32.38 (10.22, 57.33)</td>
<td>28.73 (-1.25, 56.02)</td>
<td>32.38 (11.58, 239.74)</td>
</tr>
<tr>
<td></td>
<td>-7s DTC</td>
<td>41.10 (7.09, 74.37)</td>
<td>16.82 (-12.40, 54.89)</td>
<td>11.43 (-13.12, 11.43)</td>
<td>38.71 (28.55, 126.15)</td>
</tr>
<tr>
<td>Step width</td>
<td>Single-task</td>
<td>76.57 (73.93, 80.76)</td>
<td>74.98 (71.51, 79.68)</td>
<td>76.03 (74.67, 80.85)</td>
<td>70.71 (58.05, 79.30)</td>
</tr>
<tr>
<td></td>
<td>-3s DTC</td>
<td>-2.57 (-5.74, .09)</td>
<td>-1.44 (-5.41, 1.39)</td>
<td>-1.61 (-3.79, 1.62)</td>
<td>.38 (-10.60, 4.50)</td>
</tr>
<tr>
<td></td>
<td>-7s DTC</td>
<td>-5.88 (-8.31, -1.37)</td>
<td>-3.11 (-5.97, 1.95)*b</td>
<td>-3.24 (-6.34, -5.3)</td>
<td>1.45 (-4.70, 10.07)*b</td>
</tr>
<tr>
<td>Step width variability</td>
<td>Single-task</td>
<td>2.65 (1.99, 3.26)</td>
<td>2.47 (2.15, 3.98)</td>
<td>2.33 (2.07, 4.46)</td>
<td>3.38 (2.24, 4.94)</td>
</tr>
<tr>
<td></td>
<td>-3s DTC</td>
<td>17.47 (-13.60, 41.65)</td>
<td>23.90 (-5.25, 36.17)</td>
<td>15.21 (-6.18, 36.77)</td>
<td>25.46 (17.91, 133.26)</td>
</tr>
<tr>
<td></td>
<td>-7s DTC</td>
<td>29.67 (2.69, 57.13)</td>
<td>11.68 (-10.79, 57.53)</td>
<td>4.39 (-13.52, 45.80)</td>
<td>37.73 (14.34, 139.39)</td>
</tr>
</tbody>
</table>

*p<.05

DTC = dual-task cost

*one FX+ missing for -3s and two FX+ missing for -7s; b>HC
8.4.3 Group differences in adjusted cerebellar volume

Mean cerebellar volumes are presented in Table 8.3. The combined PM group had significantly smaller adjusted cerebellar volume compared to controls after adjusting for age ($F_{(1,39)}=9.806$, $p=.003$). Group differences also emerged when the PM group were separated according to FXTAS diagnosis ($F_{(2,38)}=10.008$, $p<.001$). Post-hoc comparisons revealed that PM males with FXTAS had significantly smaller adjusted cerebellar volume compared to PM males without FXTAS ($p=.019$) and controls ($p<.001$). There was no significant difference in cerebellar volume between PM males without FXTAS and controls ($p=.598$).

Table 8.3

<table>
<thead>
<tr>
<th></th>
<th>HC (n=24)</th>
<th>PM (n=18)</th>
<th>FX- (n=12)$^c$</th>
<th>FX+ (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>127858.99</td>
<td>120602.71**a</td>
<td>128828.81</td>
<td>104150.52***a,b</td>
</tr>
<tr>
<td>SD</td>
<td>12590.89</td>
<td>22043.85</td>
<td>19453.62</td>
<td>18231.21</td>
</tr>
</tbody>
</table>

*$p<.05$; ***$p<.001$

$^a$< HC; $^b$< FX-; $^c$MRI missing for two FX-

8.4.4 Associations between CGG repeat length, FMR1 mRNA levels and cerebellar volume in PM males

Partial Pearson correlations exploring the relationships between FMR1 molecular measures and adjusted cerebellar volume among the subset of PM males included in this chapter were consistent with those reported in the overall sample (see Chapter 5). When controlling for age, increasing CGG repeat length among PM males was
significantly associated with reduced cerebellar volume \((r=-.663, p=.004)\). Adjusted cerebellar volume was not associated with \(FMRI\) mRNA level \((r=-.402, p=.110)\).

### 8.4.5 Associations between cerebellar cortex volume and dual-task gait interference

Regression models exploring the relationship between adjusted cerebellar volume and dual-task gait interference are summarised in Table 8.4. Age was included as a covariate in all models. No regression coefficients met the adjusted significance threshold of \(p=.005\), and only one trend-level association \((p<.05)\) was observed. Using the unadjusted \(p\) value, reduction in cerebellar volume was associated with increased step width variability on the -3s dual task condition in PM males \((B=-.573, p=.020)\) but not controls \((B=.069, p=.824)\). Although the interaction term between group and cerebellar volume was not significant \((B=.642, p=.073)\), the findings suggest that a stronger relationship between cerebellar volume and step width variability among PM males compared to controls may be demonstrated in a larger sample. The interaction term for cerebellar volume and group for step length variability on the -3s condition also approached the cut-off for trend level association \((B=.712, p=.051)\), with stronger associations between cerebellar volume and step length variability among PM males \((B=-.454, p=.067)\) compared to controls \((B=.258, p=.417)\).
### Table 8.4

*Summary of regression models exploring the association between cerebellar volume and dual-task gait function*

<table>
<thead>
<tr>
<th></th>
<th>3s DTC</th>
<th></th>
<th>7s DTC</th>
<th></th>
</tr>
</thead>
<tbody>
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<td></td>
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<td>B (S.E)</td>
<td><em>t</em></td>
</tr>
<tr>
<td><strong>Speed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-1.65 (.65)</td>
<td>-2.54*</td>
<td>-1.60 (.66)</td>
<td>-2.43*</td>
</tr>
<tr>
<td>Age (decades)</td>
<td>.31 (.13)</td>
<td>2.43*</td>
<td>.33 (.13)</td>
<td>2.59*</td>
</tr>
<tr>
<td>Group</td>
<td>-.11 (.33)</td>
<td>-.32</td>
<td>-.45 (.33)</td>
<td>-1.33</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>.38 (.23)</td>
<td>1.69</td>
<td>.24 (.26)</td>
<td>.92</td>
</tr>
<tr>
<td>Group × cerebellum</td>
<td>.07 (.33)</td>
<td>.21</td>
<td>.28 (.36)</td>
<td>.79</td>
</tr>
<tr>
<td><strong>Step length</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-.60 (.67)</td>
<td>-.90</td>
<td>-1.05 (.60)</td>
<td>-1.75</td>
</tr>
<tr>
<td>Age (decades)</td>
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<td>1.07</td>
<td>.26 (.12)</td>
<td>2.26*</td>
</tr>
<tr>
<td>Group</td>
<td>-.41 (.34)</td>
<td>-1.21</td>
<td>-.83 (.30)</td>
<td>-2.73**</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>.20 (.23)</td>
<td>.84</td>
<td>-.03 (.24)</td>
<td>-.12</td>
</tr>
<tr>
<td>Group × cerebellum</td>
<td>.07 (.34)</td>
<td>.20</td>
<td>.54 (.33)</td>
<td>1.65</td>
</tr>
<tr>
<td><strong>Step length variability</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Constant</td>
<td>.56 (.69)</td>
<td>.81</td>
<td>.29 (.71)</td>
<td>.41</td>
</tr>
<tr>
<td>Age (decades)</td>
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<td>-.74</td>
<td>-.09 (.14)</td>
<td>-.62</td>
</tr>
<tr>
<td>Group</td>
<td>-.18 (.35)</td>
<td>-.51</td>
<td>.28 (.36)</td>
<td>.78</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>-.45 (.24)</td>
<td>-1.89</td>
<td>.12 (.28)</td>
<td>.41</td>
</tr>
<tr>
<td>Group × cerebellum</td>
<td>.71 (.35)</td>
<td>2.02</td>
<td>.09 (.39)</td>
<td>.22</td>
</tr>
<tr>
<td><strong>Step width</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
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<td>-.90</td>
<td>-1.12 (.60)</td>
<td>-1.86</td>
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<tr>
<td>Age (decades)</td>
<td>.14 (.13)</td>
<td>1.08</td>
<td>.27 (.12)</td>
<td>2.34*</td>
</tr>
<tr>
<td>Group</td>
<td>-.41 (.34)</td>
<td>-1.20</td>
<td>-.81 (.31)</td>
<td>-2.67*</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>.19 (.24)</td>
<td>.79</td>
<td>.00 (.24)</td>
<td>.00</td>
</tr>
<tr>
<td>Group × cerebellum</td>
<td>.05 (.35)</td>
<td>.14</td>
<td>.52 (.33)</td>
<td>1.59</td>
</tr>
<tr>
<td><strong>Step width variability</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>.88 (.68)</td>
<td>1.30</td>
<td>.23 (.72)</td>
<td>.32</td>
</tr>
<tr>
<td>Age (decades)</td>
<td>-.18 (.13)</td>
<td>-1.35</td>
<td>-.07 (.14)</td>
<td>-.53</td>
</tr>
<tr>
<td>Group</td>
<td>.05 (.34)</td>
<td>.14</td>
<td>.32 (.36)</td>
<td>.88</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>-.57 (.24)</td>
<td>-2.43*</td>
<td>-.16 (.29)</td>
<td>-.54</td>
</tr>
<tr>
<td>Group × cerebellum</td>
<td>.64 (.35)</td>
<td>.64</td>
<td>.20 (.39)</td>
<td>.52</td>
</tr>
</tbody>
</table>

*p* < .05; **p** < .01

DTC = dual task cost
8.4.6 Associations between CGG repeat length, FMR1 mRNA and spatiotemporal gait characteristics

The relationships of CGG repeat length and FMR1 mRNA levels with dual-task gait interference in PM males were explored using linear regression (Tables 8.5 and 8.6 respectively). No statistically significant relationships emerged following adjustment for multiple comparisons. Exploration of trend-level associations revealed that greater CGG repeat length was associated with increased step width variability on the -3s condition (B=.439, \( p=.042 \)). Further, the interaction term between age and CGG repeat length as a predictor of step width variability on the -7s condition (B=.422, \( p=.045 \)) suggests that the relationship between increasing CGG repeat length and greater dual-task interference in step width variability may become stronger with increasing age. FMR1 mRNA was not associated with dual-task interference in any of the spatiotemporal gait parameters.
Table 8.5

Summary of regression models exploring the association between CGG repeat length and dual-task gait function among PM males

<table>
<thead>
<tr>
<th>Variable</th>
<th>-3s DTC</th>
<th></th>
<th></th>
<th>-7s DTC</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>B (S.E)</td>
<td>t</td>
<td>B (S.E)</td>
<td>t</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Speed</strong></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-.02 (.24)</td>
<td>.09</td>
<td>.18 (.19)</td>
<td>.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (decades)</td>
<td>.20 (.18)</td>
<td>1.14</td>
<td>.27 (.14)</td>
<td>2.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGG (per 10 repeats)</td>
<td>-.10 (.20)</td>
<td>-.51</td>
<td>-.21 (.15)</td>
<td>-1.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age × CGG</td>
<td>.05 (.12)</td>
<td>.39</td>
<td>.03 (.09)</td>
<td>.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Step length</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>.12 (.29)</td>
<td>.40</td>
<td>.34 (.21)</td>
<td>1.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (decades)</td>
<td>.10 (.21)</td>
<td>.51</td>
<td>.23 (.15)</td>
<td>1.48</td>
<td></td>
<td></td>
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<tr>
<td>CGG (per 10 repeats)</td>
<td>-.23 (.24)</td>
<td>-.98</td>
<td>-.25 (.17)</td>
<td>-1.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age × CGG</td>
<td>-.16 (.15)</td>
<td>-1.11</td>
<td>-.12 (.11)</td>
<td>-1.16</td>
<td></td>
<td></td>
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<tr>
<td><strong>Step length variability</strong></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>.29 (.27)</td>
<td>.85</td>
<td>.00 (.25)</td>
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<tr>
<td>Age (decades)</td>
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<td>.91</td>
<td>-.03 (.18)</td>
<td>-.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGG (per 10 repeats)</td>
<td>.30 (.22)</td>
<td>1.35</td>
<td>.27 (.20)</td>
<td>1.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age × CGG</td>
<td>.11 (.14)</td>
<td>.78</td>
<td>.25 (.12)</td>
<td>2.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Step width</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>.12 (.29)</td>
<td>.43</td>
<td>.33 (.22)</td>
<td>1.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (decades)</td>
<td>.13 (.20)</td>
<td>.62</td>
<td>.24 (.15)</td>
<td>1.54</td>
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<td></td>
</tr>
<tr>
<td>CGG (per 10 repeats)</td>
<td>-.22 (.24)</td>
<td>-.91</td>
<td>-.24 (.17)</td>
<td>-1.39</td>
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<td></td>
</tr>
<tr>
<td>Age × CGG</td>
<td>-.16 (.15)</td>
<td>-1.08</td>
<td>-.12 (.11)</td>
<td>-1.11</td>
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<td></td>
</tr>
<tr>
<td><strong>Step width variability</strong></td>
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<td></td>
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</tr>
<tr>
<td>Constant</td>
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<td>.80</td>
<td>.05 (.27)</td>
<td>.20</td>
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<td></td>
</tr>
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<td>.13 (.19)</td>
<td>.66</td>
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<tr>
<td>CGG (per 10 repeats)</td>
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<td>.42 (.22)</td>
<td>1.96</td>
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<tr>
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<td>1.19</td>
<td>.29 (.13)</td>
<td>2.20*</td>
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<td></td>
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</tbody>
</table>

*p < .05

DTC = dual task cost
Table 8.6

*Summary of regression models exploring the association between FMR1 mRNA and dual-task gait function among PM males*

<table>
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<tr>
<th></th>
<th>-3s DTC</th>
<th></th>
<th>-7s DTC</th>
<th></th>
</tr>
</thead>
<tbody>
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<td></td>
<td>B (S.E)</td>
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<td>B (S.E)</td>
<td>t</td>
</tr>
<tr>
<td>Speed</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
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<td>-.04</td>
<td>.19 (.18)</td>
<td>1.03</td>
</tr>
<tr>
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<td>1.54</td>
<td>.33 (.14)</td>
<td>2.42*</td>
</tr>
<tr>
<td>FMR1 mRNA</td>
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<td>-.01</td>
<td>-.33 (.21)</td>
<td>-1.59</td>
</tr>
<tr>
<td>Age × FMR1 mRNA</td>
<td>.17 (.21)</td>
<td>.79</td>
<td>.01 (.16)</td>
<td>.06</td>
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<td>Step length</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
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<td>.77</td>
<td>.43 (.20)</td>
<td>2.18*</td>
</tr>
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<td>.26 (.15)</td>
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<td>-.60</td>
<td>-.32 (.23)</td>
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<td>-.12 (.17)</td>
<td>-.67</td>
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<td>.46</td>
<td>-.13 (.24)</td>
<td>-.54</td>
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<td>-.03 (.18)</td>
<td>-.16</td>
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<td>.19 (.27)</td>
<td>.71</td>
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<td>.35 (.21)</td>
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<td>.76</td>
<td>.41 (.20)</td>
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<td>.64</td>
<td>.26 (.15)</td>
<td>1.78</td>
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<td>FMR1 mRNA</td>
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<td>-.65</td>
<td>-.34 (.23)</td>
<td>-1.51</td>
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<td>-.12 (.25)</td>
<td>-.50</td>
<td>-.13 (.17)</td>
<td>-.77</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Constant</td>
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<td>.22</td>
<td>-.11 (.27)</td>
<td>-.43</td>
</tr>
<tr>
<td>Age (decades)</td>
<td>.10 (.19)</td>
<td>.53</td>
<td>.09 (.19)</td>
<td>.47</td>
</tr>
<tr>
<td>FMR1 mRNA</td>
<td>.27 (.30)</td>
<td>.90</td>
<td>.37 (.30)</td>
<td>1.23</td>
</tr>
<tr>
<td>Age × FMR1 mRNA</td>
<td>-.04 (.22)</td>
<td>-.19</td>
<td>.38 (.23)</td>
<td>1.70</td>
</tr>
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</table>

*p <.05

DTC = dual task cost
8.5 Discussion

This is the first study to explore cognitive-motor interference on spatiotemporal gait parameters among PM males with and without FXTAS. The findings indicate differences in the effect of increasing cognitive load on dual-task interference between PM males with FXTAS and controls, supporting the hypothesis of greater interference in spatiotemporal gait characteristics when concurrently performing a serial subtraction task. Relationships between cerebellar volume and dual-task interference for step width variability, and the trend-level associations with age and CGG repeat length did not withstand correction for multiple comparisons. Therefore, the hypothesis that decrements in dual-task performance on gait parameters would be associated with decreased cerebellar volume, increased CGG repeat length, and elevated \textit{FMR1} mRNA was not supported. These findings suggest that dual-task gait interference may be a sensitive marker of subtle neuromotor dysfunction in PM males and may therefore show some utility for tracking progression of motor signs in FXTAS. However, longitudinal studies in larger samples are required to confirm whether the relationships between cerebellar volume, CGG repeat length and gait function indicate at-risk profiles for the later onset of FXTAS.

Although gait performance during baseline walking without interference was comparable between PM males and controls, significant differences in dual-task cost in step width and step length emerged while concurrently counting backwards by 7s. Males with FXTAS demonstrated mean increases in both step width and step length relative to single-task performance; in contrast to controls and asymptomatic PM males who demonstrated mean decreases in these parameters while dual-tasking (see
Figure 8.1). As increasing step width provides a greater base of support (Kirtley, 2006), the dual-task related increase in step width among PM males with FXTAS may be compensatory for instability associated with dividing attentional resources between cognitive and motor tasks. The finding of a dual-task related increase in step length among males with FXTAS was unexpected, given previous reports of reduced mean step length among patients with cerebellar ataxia compared to healthy controls under normal walking conditions (Morton & Bastian, 2003; Palliyath, Hallett, Thomas, & Lebiedowska, 1998; Serrao, Pierelli, Ranavolo, Draicchio, Conte et al., 2012). A possible explanation for this difference could relate to the use of mean values for step length to quantify dual-task gait performance among PM males with FXTAS. Ataxic gait is characterised by staggering steps that are irregular in length (Holmes, 1939), and as such, overall mean values for step length may be less meaningful than the step-to-step variability on this measure (Ilg & Timmann, 2013).

Consistent with the findings for the overall cohort (Chapter 3), larger CGG repeat expansions, but not \textit{FMRI} mRNA levels, were associated with decreased total cerebellar volume in PM males. This is consistent with previous studies describing significant negative relationships between cerebellar volume and CGG repeat length, but not \textit{FMRI} mRNA levels (Adams et al., 2007; Cohen et al., 2006; Moore et al., 2004). In the current study, decreased cerebellar volume among PM males showed trend-level associations with greater dual-task cost in intra-individual variability of step width when performing a concurrent serial subtractions task. This is in line with previous findings that the cerebellum plays an integral role as part of cortico-cerebellar networks implicated in the execution of concurrent cognitive and motor tasks (Wu et al., 2013). Although the findings in the current study did not reach
statistical significance at the adjusted value, the emergence of trend-level associations suggests that further research in this area may be warranted. Examination of the impact of cerebellar dysfunction on dual-task related changes in step-to-step variability among larger cohorts would be worthwhile to ascertain whether gait changes provide a sensitive marker of cerebellar dysfunction among PM males with and without FXTAS.

Figure 8.1. Mean step width (cm) across single and dual-task walking conditions. PM males with FXTAS demonstrated mean increases in step width while undertaking the concurrent cognitive task, while controls and PM males without FXTAS tended to decrease step width.

Further trend-level findings in the current study suggested that greater dual-task related increases in step width variability among PM males may represent subtle age-related changes that are more apparent with increasing CGG repeat size. Although these findings did not withstand correction for multiple comparisons, they highlight
an important focus for future research in the PM, as increased gait variability has been shown to contribute to greater risk of falls in healthy aging (Callisaya, Blizzard, Schmidt, Martin, McGinley et al., 2011; Hausdorff, Rios, & Edelberg, 2001; Nordin, Moe-Nilssen, Ramnemark, & Lundin-Olsson), and may be a marker of pre-symptomatic motor changes in individuals at risk of neurodegenerative disorders including Parkinson’s Disease (Mirelman et al., 2011) and Huntington’s Disease (Rao et al., 2011). Moreover, these findings are consistent with a previous study in female PM carriers, where the same effect of increasing age on a gait variability domain score (comprising step time variability and step width variability) were described among asymptomatic PM females with larger CGG repeat expansions (Kraan, Hocking, Georgiou-Karistianis, Metailfe, Archibald, Fielding, Bradshaw, et al., 2014). Variation in step width from step to step may reflect a compensatory response to lateral postural instability elicited by the greater cognitive load associated with dual-tasking (Brach, Studenski, Perera, VanSwearingen, & Newman, 2008; Collins & Kuo, 2013; Gabell & Nayak, 1984). These findings therefore suggest that CGG repeat length may be an important predictor of cerebellar dysfunction and age-related increases in dual-task interference in gait variability among PM males with and without FXTAS, in line with previous studies showing significant associations between increasing CGG repeat length motor dysfunction among PM males (Allen et al., 2008; Grigsby et al., 2006; Leehey et al., 2008; Tassone et al., 2007). Age-related changes in cerebellar volume and gait variability among PM carriers with larger CGG expansions may have significant implications for monitoring symptom progression, and may inform predictive models for determining risk for symptom onset. However, the relationship between markers of gait variability and later decline associated with FXTAS should be investigated.
further using multi-site prospective longitudinal designs to track the progression of changes in postural stability and gait function.

While the current study presented the first evidence of a subtle cognitive-motor interference effect on gait variability in male PM carriers, the small sample size and cross-sectional design limit the conclusions that can be drawn from the study. Although the contributions of cerebellar volume to models examining dual-task gait interference did not meet adjusted cut-offs for statistical significance, the findings have highlighted specific aspects of gait function that may be linked to cerebellar dysfunction and warrant further exploration among PM males. Future studies utilising multi-site longitudinal designs will be critical to establishing the reliability of the findings in this study. Another limitation relates to the imaging techniques that focused on total cerebellar volume and did not examine white matter integrity. Future research could also examine the integrity of specific subregions of the cerebellum in which alterations have previously been shown among PM carriers with and without FXTAS (Battistella et al., 2013; Hashimoto, Srivastava, et al., 2011), and the relationships with changes in gait variability under dual-task conditions. Future investigations incorporating measures of white matter integrity would also be worthwhile when disentangling possible developmental versus degenerative mechanisms among PM males (Battistella et al., 2013; Hashimoto, Srivastava, et al., 2011; Hippolyte et al., 2014; Wang, Hessl, Schneider, et al., 2013). The possible effects of reductions in FMRP, which has also been shown to alter genotype-phenotype relationships in FMR1 PM carriers (Hessl et al., 2011), in addition to other molecular markers related to the PM (Loesch et al., 2011; Pastori et al., 2014; Todd et al., 2013), await exploration in larger studies.
In conclusion, the findings presented in this chapter provide the first evidence of cognitive-motor interference effects on gait function among PM males. PM males with FXTAS demonstrated dual-task related increases in step length and step width which may reflect a compensatory strategy for compromised postural stability when cognitive resources are diverted to a secondary task. Although not withstanding corrections for multiple comparisons, the current findings suggest relationships between decreased cerebellar volume and dual-task interference in step width variability; a marker previously associated with increased risk of falls (Callisaya et al., 2011; Hausdorff et al., 2001; Nordin et al.). There were also trend-level associations indicating that dual-task associated decrements in step width variability became more apparent with increasing age and CGG repeat length in PM males. This suggests that age-related changes in step width variability among PM males with larger CGG repeat sizes may represent a possible early marker of cerebellar dysfunction and neuromotor dysfunction. If confirmed in future studies with larger PM cohorts, these findings have significant implications for the development of predictive models to ascertain risk and for guiding future research exploring the use of quantitative gait measures as sensitive endpoints in future clinical trials. Longitudinal studies are required to confirm the extent to which changes in dual-task related gait function in males with the PM represent the earliest indicators of neurological decline associated with FXTAS.
CHAPTER 9.

SUMMARY AND CONCLUSIONS
CHAPTER 9. SUMMARY AND CONCLUSIONS

This thesis explored neurobehavioural and radiological features of a newly established cohort of adult male FMR1 PM carriers in Australia. In addition to detailed characterisation of the cohort, the thesis explored interrelationships between specific neurobehavioural, radiological and molecular measures among PM males, and investigated the capacity of specific neurobehavioural measures to identify symptoms associated with FXTAS. This final chapter integrates the main findings of the thesis and summarises the theoretical and clinical implications arising from this work. The limitations and avenues for future research are also discussed.

9.1 Effects of the FMR1 PM from a dimensional perspective

Throughout this thesis, the possible interrelationships between neurobehavioural, radiological and molecular measures were explored in the overall PM cohort as opposed to within subgroups based on FXTAS +/- diagnostic categories. This allowed for a more dimensional consideration of clinical features in the PM along a continuum of involvement than what could have been achieved using discrete categories to denote the presence or absence of disorder. In the field of psychiatry it is increasingly recognised that while categorical classification systems for diagnosis have their advantages in clinical and research settings, for example, in determining whether symptoms are sufficiently severe to warrant treatment; dimensional approaches may be more useful when examining dynamic relationships between clinical symptoms and biological or psychosocial factors (Goldberg, 2000). In the current study, the use of a dimensional approach facilitated the identification of a number of associations between neurobehavioural, radiological and molecular
measures that may be clinically important for PM males who fall both above and below the categorical diagnostic thresholds for FXTAS.

9.2 The \textit{FMR1} PM and vulnerability to physical and mental health conditions

An important contribution to the literature arising from this thesis is support for the notion that PM males may demonstrate vulnerability to a wide range of physical and mental health conditions that extend beyond those said to be characteristic of FXTAS (e.g. Hamlin et al., 2012; Juncos et al., 2011). In the current study, a number of clinical features including hearing loss, history of psychiatric disorder and psychomotor slowing were overrepresented among PM males compared to controls. Elevated rates of mood and anxiety disorders and psychomotor slowing among PM males suggest dysfunction of fronto-subcortical neural networks, as seen in other neurodegenerative disorders including Huntington’s disease (Bacalman et al., 2006; Birch et al., 2014; Knopman & Selnes, 2003). However, deficits in information processing speed were not associated with thalamus or basal ganglia volumes after controlling for the effect of fine motor function, and the lack of clinically significant current psychiatric symptomatology precluded the investigation between psychiatric symptoms and measures of subcortical volumes. Moreover, no robust associations between neuropsychiatric features and \textit{FMR1} molecular measures in the PM group emerged. Therefore the mechanisms underlying neuropsychiatric involvement in this cohort remain unclear. Further investigation in this area is warranted as this would contribute to a greater understanding of neuropsychiatric involvement in the PM and may have important implications for the development of targeted treatments.
In terms of clinical implications, the findings of this thesis highlight the importance of proactively screening for and managing a range of physical and mental health concerns that may impact on functional abilities and quality of life. Although preliminary, results of analyses exploring age-related changes in cognitive function may also have clinical implications for health practitioners considering differential diagnosis and assessment of atypical presentations. The current findings suggest that PM males in this cohort demonstrated deficits in specific aspects of cognitive function; however, the relationship between cognitive performance and age was comparable to that found in controls. Although these findings would require replication in larger cohorts, it is possible that patients who experience a more rapid decline in cognitive function with increasing age may have an additional comorbid condition (e.g. Alzheimer’s Disease, Dementia with Lewy Bodies) requiring investigation and appropriate management.

9.3 Linking neuropsychiatric features with radiological and molecular signatures

Further studies are needed to explore the interrelationships between cognitive and psychiatric features as findings may lead to a better understanding of neuropsychiatric manifestations in PM males. This work could be informed by recent evidence in PM females without FXTAS describing a number of interrelationships between reaction time on a symbolic sequence learning task known to activate the cerebellum (Bo, Peltier, Noll, & Seidler, 2011), and measures of executive function, visuospatial function, depression, anxiety and ADHD symptomatology (Kraan, Hocking, Bradshaw, Georgiou-Karistianis, Metcalfe et al., 2014). The findings of
Kraan et al. (Kraan, Hocking, Bradshaw, et al., 2014) suggest that cortico-cerebellar involvement may underlie cognitive and affective dysfunction in some females with the PM. Further research is needed to determine whether a subset of PM males demonstrate a similar cognitive-affective profile indicative of cortico-cerebellar dysfunction that may be observable prior to the onset of FXTAS (Kraan, Hocking, Bradshaw, et al., 2014).

Future studies should also explore the associations between neuropsychiatric features and radiological and molecular measures that were not included in the current study. This may include specific MRI techniques such as diffusion tensor imaging and functional MRI, to examine the integrity of tracts and functional connections within frontal and limbic brain systems including the hippocampal-prefrontal cortex pathway, which is implicated in a range of psychiatric disorders (Godsil et al., 2013). Efforts could also be made to further explore the effects of microstructural white matter integrity within fronto-cerebellar networks and cognitive performance. This would expand upon existing evidence which suggests that metabolic and white matter changes in the MCP and genu of the corpus callosum in PM carriers with and without FXTAS contribute to performance on measures of executive function and information processing speed (Filley, Brown, Onderko, Ray, Bennett et al., 2014). Examination of the associations between neuropsychiatric features and a broader range of PM-specific molecular markers than those included in the current study may also enhance the understanding of mechanisms underlying cognitive and psychiatric involvement in the PM. Levels of FMRP have been shown to play an important role in limbic function in asymptomatic PM males (Hessl et al., 2011) and should be further explored in longitudinal studies that include PM males with FXTAS. It
should be noted that quantification of FMRP levels was included in the protocol for the current study; however these were not suitable for inclusion in the thesis due to technical issues (i.e., failure of the reagent during laboratory processing).

9.4 Insights into the impact of the FMR1 PM on postural control

A second contribution of this thesis is the finding that measures of body sway and gait variability may be sensitive to CGG-related disruption within cerebellar networks underlying postural control. These findings raise the possibility that CGG repeat-dependent changes in cerebellar volume may inform predictive models to evaluate risk of postural control difficulties in PM males with and without FXTAS. The capacity to identify those at greatest risk would afford opportunities for early intervention and proactive management of postural instability, which in turn may prevent negative outcomes such as loss of functional independence and falls. The findings from the current study also suggest that measures of postural control may have utility as sensitive end-points in future clinical trials as treatments for FXTAS become available.

9.5 Utility of a new clinical staging approach in the PM

An overarching aim of this thesis was to identify neurobehavioural measures sensitive to prodromal features and progression of FXTAS and thereby suitable for inclusion in a new clinical staging model. However, it was not feasible to draw conclusions to allow fulfilment of this aim based on this small cross-sectional sample. Rather, the findings of this thesis have highlighted that measures of postural control incorporating the manipulation of cognitive and sensory input may be
sensitive to cerebellar dysfunction in PM males with and without FXTAS and are worthy of future large-scale longitudinal study. The development of a clinical rating scale incorporating preclinical features that may progress to FXTAS would allow for the provision of interventions that may slow, or even prevent progression to more severe stages of disease. Longitudinal studies with larger PM cohorts are required to determine whether early changes in postural stability may serve as a sensitive marker of cerebellar dysfunction predictive of more severe neurological decline associated with FXTAS.

9.6 General limitations

Whilst specific methodological issues were raised within relevant chapters, a number of broader limitations of this study must be acknowledged. The study included a small sample of 22 PM males, seven of whom met diagnostic criteria for FXTAS. This may have resulted in the study being underpowered to detect small group effects, particularly after dividing PM males according to FXTAS +/- diagnostic categories. While it is possible that findings from this restricted cohort may not be generalisable to the wider PM population, the recruitment of participants through diverse sources, irrespective of symptom presentation, is likely to have resulted in a more representative sample than cohorts recruited through contact with clinical services alone. However, there is still a risk of ascertainment bias that may influence the findings. Only PM males who were able to travel to and attend the clinical assessments were included in the study, and the screening out of those who were unable to travel to the study site due to advanced FXTAS may have resulted in a sample with milder clinical involvement. Similarly, males with the PM may
experience higher rates of social phobia and abnormalities in social cognition (Bourgeois et al., 2011) and those experiencing more severe symptoms may have been less inclined to make contact with the research team and take part in the study (Hunter et al., 2009). The risk of sampling bias also applies to the recruitment of healthy controls, as individuals who volunteer and meet strict inclusion criteria for research studies may experience better general health relative to the general population. Future studies with larger samples are required to determine the reliability of the findings arising from this thesis.

A further limitation of the study was that assessments were completed at a single time-point. Without longitudinal follow-up of the cohort it is not possible to determine whether early neuropsychiatric, motor, and radiological changes may indicate greater risk for FXTAS in later years. Longitudinal studies that follow PM males across asymptomatic and symptomatic phases will be vital in determining specific risk and protective factors that may contribute to the likelihood of developing FXTAS.

9.7 Future priorities in FMR1 PM research

Despite its limitations, this thesis has highlighted key areas of consideration for future research. Longitudinal studies should explore the utility of a range of neurobehavioural markers that may be sensitive to the onset and progression of neurological symptoms in the PM. In addition to postural control measures as suggested by the current study, alternative measures sensitive to cerebellar dysfunction (e.g. oculomotor control, implicit sequence leaning) should also be
explored. Abnormalities in oculomotor function have been described in asymptomatic PM males and females and may provide a sensitive marker of cerebellar dysfunction prior to the onset of FXTAS (Shelton, Cornish, Kraan, Georgiou-Karistianis, Metcalfe et al., 2014; Shelton, Cornish, Godler, Clough, Kraan et al., 2015; Wong, Goodrich-Hunsaker, McLennan, Tassone, Zhang et al., 2014). A single case report (Sulkowski & Kaufman, 2008) has also suggested that oculomotor abnormalities may be observed in FXTAS, but this awaits further investigation in larger studies. Deficits in implicit sequence learning have also been described in PM females without FXTAS (Kraan, Hocking, Bradshaw, et al., 2014), while impairments in motor sequence learning have been observed in asymptomatic PM males (Grigsby et al., 2008). Collectively, these findings highlight the possible utility of measures of cerebellar dysfunction for tracking symptom progression from asymptomatic through to symptomatic stages. The incorporation of a range of neurobehavioural measures in future longitudinal study protocols will also be crucial to determine whether these markers could be used as end-points in clinical trials as treatments for FXTAS become available.

Future investigations could also focus on the role of subcortical pathology in clinical manifestations in the PM. Subcortical volume loss does not feature in the diagnostic criteria for FXTAS and has been relatively understudied compared to other radiological measures. Yet in the current study, volume loss in subcortical structures was more pronounced among PM males than measures of cortical volume loss and WMH load. This suggests that subcortical volume loss may have a more important role in neurodegenerative manifestations of the PM than has previously been recognised. Moreover, disruption within subcortical circuits involving the thalamus...
and basal ganglia may extend beyond the impairments in fine motor function observed in this study. As basal ganglia circuitry is involved in the regulation of motor and non-motor systems, disruption within these regions may underlie motor and neuropsychiatric signs including parkinsonism, resting tremor, and obsessive-compulsive symptoms (DeLong & Wichmann, 2007; Ring & Serra-Mestres, 2002). Given the increasing evidence of associations between subcortical volume loss and clinical features of FXTAS, further studies exploring the relationships between subcortical volume loss and clinical signs are warranted. In future studies, the implementation of manual tracing techniques (as opposed to automatic MRI processing) may be appropriate, particularly in samples of PM males with more extensive subcortical pathology.

9.8 Conclusion

The understanding of the core characteristics and underlying pathophysiology of FXTAS has progressed rapidly since its initial identification in 2001 (Hagerman et al., 2001). Previously thought to be clinically unaffected and merely ‘normal transmitting males’, it is now well recognised that males with the PM may exhibit a range of neurobehavioural and radiological changes including but not necessarily exclusive to those attributed to FXTAS. Notwithstanding these advances, a systematic review published by the author earlier in the candidacy identified a need for more comprehensive and controlled studies exploring neuropsychiatric, motor and radiological changes in independent PM cohorts. Moreover, the interrelationships between these features across neuropsychiatric, motor, radiological and genetic levels remain underexplored.
The findings arising from this thesis indicate that changes in motor postural control and volume loss in subcortical brain regions (especially the cerebellum) were the most prominent features consistent with a possible neurodegenerative trajectory associated with FXTAS among this cohort. Interestingly, a number of associations between increasing CGG repeat length and specific features of this trajectory (cerebellar volume and postural control) suggest that the length of gene expansion may contribute to the onset and/or progression of symptoms. In addition to a neurodegenerative trajectory consistent with FXTAS, the findings of this thesis also suggest that neurodegenerative changes may either exacerbate, or be superimposed onto a more diffuse vulnerability to fronto-subcortical dysfunction. This was evidenced by increased rates of psychiatric disorder and psychomotor slowing that appeared to be observable earlier in the lifespan. Longitudinal studies in larger cohorts are required to determine whether these neuropsychiatric, motor and radiological features provide sensitive measures of preclinical changes and symptom progression among PM males with and without FXTAS. If validated by longitudinal studies, these measures may be suitable for use in both clinical and research settings, including monitoring symptom progression and response to treatment. Such measures could also be incorporated into a more sophisticated clinical staging model for FXTAS across asymptomatic, prodromal, and symptomatic stages, allowing for the dynamic assessment of symptom severity and change over time.

Completion of this thesis has afforded the author invaluable insight into the experiences of PM males and their families. Interactions with participants and their families throughout the course of the study have highlighted the need for future work
to focus not only on the expansion and follow-up of the cohort but also the
translation of knowledge generated from the study of the cohort into practice.
Despite the varied clinical presentations among PM males in the current study,
concerns regarding the lack of awareness of fragile X-associated disorders in the
medical community were unanimously raised. Those with FXTAS typically
described convoluted pathways through care and all PM males described a lack of
consumer resources regarding their risk of developing various physical and mental
health conditions. These concerns were in line with findings in the United States
suggesting that FXTAS is largely underdiagnosed and misdiagnosis is common,
possibly due to heterogeneity in clinical symptoms and the resemblance of features
to a constellation of issues commonly seen in ageing populations (Leehey, Berry-
Kravis, Goetz, & Hagerman, 2010; Leehey, 2009; Leehey, Berry-Kravis,
Jacquemont, Zhang, Hagerman et al., 2004). The development of educational
resources for both health practitioners and consumers should be considered a priority
to increase awareness and understanding of FXTAS and other fragile X-associated
disorders. This would facilitate access to appropriate information for PM males and
their families regarding their health risks, and improve the capacity of health
practitioners to provide optimal levels of care.
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APPENDIX A.

Understanding the neuropsychiatric phenotype of fragile X-associated tremor ataxia syndrome: a systematic review

(Birch et al., 2014, Neuropsychology Review.

doi:10.1007/s11065-014-9262-9)
APPENDIX B.

Summary of socio-demographic and medical history data
APPENDIX B: Summary of socio-demographic and medical history data²

B.1 Socio-demographic characteristics

1. Date of birth
2. Country of birth
3. First language spoken
4. Primary language spoken at home
5. Race/ethnicity
   a. White/Caucasian
   b. Aboriginal
   c. Asian
   d. African
   e. Pacific Islander
   f. Torres Strait Islander
   g. Hispanic
   h. Other
6. Current living arrangements
   a. Home owner
   b. Renting
   c. Living with family/friends

² Full versions of questionnaires are available upon request from the author.
7. Relationship status
   a. Single
   b. In a relationship
   c. Living with partner
   d. Married
   e. Divorced or separated
   f. Widowed

8. Highest qualification
   a. Primary school
   b. Some high school
   c. Completed high school
   d. Trade or technical (Certificate Level)
   e. Advanced Diploma
   f. Bachelor Degree
   g. Graduate Diploma or Certificate

9. Total years formal education

10. Current work status
    a. Retired
    b. Not working
    c. Full time homemaker
    d. Full time work
    e. Part time work
    f. Casual work

11. Main occupation
12. Most senior occupation held throughout employment history

13. Details of financial benefits/pensions

14. Number of people in household

15. Family money situation
   a. Spending more money than we get
   b. Just enough money to get through to the next pay day
   c. Some money left over each week but it gets spent
   d. Can save a bit every now and again
   e. Can save a lot
   f. Prefer not to answer

16. Total household gross annual income
   a. <18,999
   b. $18,200–$33,799
   c. $33,800–$41,599
   d. $52,000–$72,799
   e. $88,300–$129,999
   f. ≥130,000
   g. Don’t know
   h. Prefer not to answer
B.2 Medical history

1. Details of previous *FMR1* testing (year, location, reason for testing)

2. History of stroke
   a. No
   b. Not sure
   c. Yes- only one
   d. Yes- more than one

3. History of transient ischemic attack
   a. No
   b. Not sure
   c. Yes, only one
   d. Yes, 2–5
   e. Yes, 6 or more

4. History of heart attack
   a. No
   b. Yes but not admitted to hospital
   c. Yes and admitted to hospital
   d. If yes, number of heart attacks

5. Immediate family history (if yes, who?)
   a. Stroke
   b. Transient ischemic attack
   c. Ischemic heart disease
   d. Peripheral vascular disease
6. Other diagnosed heart conditions
   a. Angina
   b. High blood pressure
   c. Low blood pressure
   d. Orthostatic hypotension
   e. High cholesterol
   f. Circulation problems
   g. Other

7. Previously diagnosed diabetes
   a. No
   b. Yes
   c. If yes, age of diagnosis and treatment:
      i. No treatment
      ii. Diet
      iii. Pills/tablets
      iv. insulin

8. Previous head injuries requiring visit to a doctor or hospital

9. Falls resulting in broken bone

10. Falls within last 12 months

11. Previous diagnosis of thyroid disorder
    a. No
    b. Yes- underactive
    c. Yes- overactive
12. Previous diagnosis of autoimmune disorder
   a. Lupus
      a. Rheumatoid arthritis
      b. Sjorgen’s syndrome/disease
      c. Optic neuritis
      d. other

13. Previous diagnosis of epilepsy
   a. No
   b. Yes, but not on treatment
   c. Yes, on treatment
   d. If yes, age at last seizure

14. History of meningitis or brain fever

15. History of the following conditions
   a. Osteoporosis
   b. Autism
   c. Fibromyalgia
   d. ADHD
   e. Vocal or motor tic disorder or Tourette’s syndrome
   f. Hyper-extensible joints
   g. Hypotonia
   h. Flat feet
16. Hearing problems
   a. No
   b. Yes:
      c. If yes, degree of hearing loss
         i. Mild to moderate
         ii. Severe to profound
   d. If yes, has a hearing aid been recommended and is one used

17. Alcohol consumption- frequency
   a. Never
   b. Once a month or less
   c. 2-4 times per month
   d. 2-3 times per week
   e. 4 or more times per week

18. Alcohol consumption- standard drinks on a typical drinking day
   a. 1 or 2
   b. 3 or 4
   c. 5 or 6
   d. 7–9
   e. ≥10

19. Average number of drinks per week
20. Smoking status
   a. Non-smoker
   b. Occasional
   c. Ex-smoker
      i. Age started smoking
      ii. Age stopped smoking
      iii. Total years smoking
      iv. Average number of cigarettes per day
   d. Smoker
      i. Age when started smoking
      ii. Total years smoking
      iii. Average number of cigarettes per day

21. History of illicit drug use
   a. No
   b. Yes
   c. If yes, name of drug, frequency, and quantity consumed per episode

22. History of prescription substance misuse
   a. No
   b. Yes
   c. If yes, name of medication, frequency, and quantity consumed per episode
23. Previous consultation with health profession about mental health concerns
   a. No
   b. If yes, diagnosis given (if any)

24. Details of previous operations

25. Number of times put under general anaesthesia

26. Details of any other serious illnesses

27. Frequency of vigorous physical activity during lifetime
   a. Ages 18–24
   b. Ages 18–25
   c. Ages 45–64
   d. Ages 65+

28. List of all current medications
   a. Name of medication
   b. Dose
   c. Frequency
   d. Duration
APPENDIX C.

Preliminary evidence of an effect of cerebellar volume on postural sway in \textit{FMR1} premutation males

(Birch et al., 2015, Genes, Brain and Behavior, 14(3), 251-9.

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