Preliminary evidence of an effect of cerebellar volume on postural sway in FMR1 premutation males

R. C. Birch†, D. R. Hocking†, K. M. Cornish‡, J. C. Menant†‡, N. Georgiou-Karistianis§, D. E. Godler†, W. Wen‡, A. Hackett§§, M. C. Rogers§§ and J. N. Trollor†

Recent evidence suggests that early changes in postural control may be discernible among females with premutation expansions (55–200 CGG repeats) of the fragile X mental retardation 1 (FMR1) gene at risk of developing fragile X-associated tremor ataxia syndrome (FXTAS). Cerebellar dysfunction is well described in males and females with FXTAS, yet the interrelationships between cerebellar volume, CGG repeat length and postural control remain unknown. This study examined postural sway during standing in a cohort of 22 males with the FMR1 premutation (ages 26–80) and 24 matched controls (ages 26–77). The influence of cerebellar volume, CGG repeat length and FMR1 mRNA levels on postural sway was explored using multiple linear regression. The results provide preliminary evidence that increasing CGG repeat length and decreasing cerebellar volume were associated with greater postural sway among premutation males. The relationship between CGG repeat length and postural sway was mediated by a negative association between CGG repeat size and cerebellar volume. While FMR1 mRNA levels were significantly elevated in the premutation group and correlated with CGG repeat length, FMR1 mRNA levels were not significantly associated with postural sway scores. These findings show for the first time that greater postural sway among males with the FMR1 premutation may reflect CGG repeat-mediated disruption in vulnerable cerebellar circuits implicated in postural control. However, longitudinal studies in larger samples are required to confirm whether the relationships between cerebellar volume, CGG repeat length and postural sway indicate greater risk for neurological decline.

Keywords: Cerebellum, fragile X mental retardation 1 (FMR1) gene, FMR1 premutation, fragile X-associated tremor ataxia syndrome (FXTAS), postural balance

Received 4 November 2014, revised 5 January 2015 and 27 January 2015, accepted for publication 28 January 2015

Approximately 1 in 209 females and 1 in 430 males in the general population (Tassone et al. 2012) carry premutation (PM) expansions (55–200 CGG repeats) in the 5′ untranslated region of the fragile X mental retardation 1 (FMR1) gene (Tassone et al. 2012). Premutation expansions are associated with increased transcription and elevated levels of FMR1 messenger RNA (mRNA) (Tassone et al. 2000), and confer risk for the development of fragile X-associated tremor ataxia syndrome (FXTAS) (Hagerman et al. 2001), a neurodegenerative disorder affecting up to 45.5% of PM males and 16.5% of PM females over 50 years of age (Jacquemont et al. 2004; Rodriguez-Revenga et al. 2009). Core clinical and radiological features include intention tremor, gait ataxia, parkinsonism, cognitive dysfunction, diffuse white matter pathology and volume loss throughout the cerebrum and cerebellum (Cohen et al. 2006; Hagerman et al. 2001; Jacquemont et al. 2003). Characterization of these features in their earliest stages is crucial for the development of targeted interventions aimed at prolonging onset of symptoms, thereby increasing functional capacity and quality of life.

Evidence of impaired vestibular control of balance in asymptomatic PM males (O’Keefe et al. 2012), and age- and CGG-dependent changes in attentional demands of postural control in PM females (Kraan et al. 2013) suggest that postural sway measures during stance (maximal anterior-posterior and medio-lateral displacements) may be sensitive to the earliest PM-related effects on motor control. In PM males with and without FXTAS, CGG repeat length has also been negatively correlated with cerebellar volume and structural connectivity within the middle cerebellar peduncles (MCP) and cerebellum (Adams et al. 2007; Battistella et al. 2013; Cohen et al. 2006; Moore et al. 2004), areas that comprise...
the fronto-cerebellar tracts underlying balance control (Ouchi et al. 1999). Furthermore, in PM males with FXTAS, cerebellar volume and white matter integrity in the superior cerebellar peduncles have been negatively correlated with clinical FXTAS staging and FXTAS rating scale scores, respectively (Adams et al. 2007; Hashimoto et al. 2011a; Wang et al. 2013). Cerebellar changes have also been described among asymptomatic PM males, including grey matter volume loss in lobules VII of the vermis, lobule III (Hashimoto et al. 2011a) 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 et al. 2011b). 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.

Here we explore for the first time the influence of cerebellar volume on changes in postural control in adult PM males with and without FXTAS. Specifically, we aimed 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.

Materials and methods

Participants

Twenty five PM males aged 26–80 and 25 age- and education-matched controls with normal FMR1 alleles aged 26–77 were recruited as part of a study exploring cognitive and neuromotor profiles associated with the FMR1 PM. Participants underwent comprehensive clinical assessments including examination of motor, cognitive and psychiatric signs in addition to brain magnetic reso-
nance imaging (MRI). Premutation males were recruited 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. Controls 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. Three PM males were excluded due to PM/full mutation (>200 CGG repeats) mosaicism, and MRI scans were missing for an additional two PM males. One control was excluded due to cognitive dysfunction resulting from a vitamin B12 deficiency.

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, Hunter New England Health, New South Wales Institute of Psychiatry and Monash University.

Clinical assessments

Assessments were completed from 2011 to 2013 in New South Wales and Victoria at Neuroscience Research Australia (NeuRA), the GOLD Service, Monash Biomedical Imaging and participants’ homes. Mea-
sures were administered as per test manuals by the same examiner for all participants. Full-scale IQ (FSIQ) was assessed using the four subtest version of the Wechsler Abbreviated Scale for Intelligence (WASI) (Wechsler 1999) and neurological symptoms were examined using the FXTAS Rating Scale (Version 1.0) (Leehey et al. 2008). Information regarding functional status included the number of falls within the past 12 months, and FXTAS stage (Bacalman et al. 2006). Stages of FXTAS are based on a 7-point scale incorporating motor symptoms (including tremor), cognitive decline, cognitive decline, and behavioral symptoms, with scores increasing in severity, walking capacity and impact of motor symptoms on activi-
ties of daily living (ADLs). Scores are assigned as follows: 0 (normal); 1 (subtle or questionable signs, i.e. subtle tremor and/or mild balance problems, but no interference with 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)). Diagnostic classifications for FXTAS in the PM group were based on clinical and radiological features according to consensus criteria (Jacquemont et al. 2003).

Postural sway was quantified using a swaymeter, affixed via a belt that is fitted at the 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 been used extensively in studies of bal-
ance and falls risk assessment in older people and clinical populations (Brooke-Wavell et al. 2001; Hinman et al. 2002; Lord & Ward 1994; Lord et al. 1991) and provides a simple way to assess postural sway during standing. It has showed good concurrent and convergent validity for the assessment of postural sway under different sensory conditions, and shows good immediate test–retest reliability (Lord et al. 1994). The 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 m away. Sway displacement was measured in the anterior-posterior and medio-lateral directions in millimetres for 30 seconds across four different conditions in a fixed order: (1) standing on the floor with eyes open, (2) standing on the floor with eyes closed, (3) standing on a foam mat (15 cm 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 & Ward 1994). Participants that were not able to complete an easier trial did not progress on to the more difficult conditions.

Structural brain MRI

Brain MRI scans were conducted using a Phillips 3T Achieva Open Bore Global Dual Scanner (Phillips Medical Systems, Best, The Netherlands) located at NeuRA, Sydney. Three-dimensional (3D) T1-weighted scans were acquired using the following parameters: TR = 6.39 milliseconds, TE = 2.9 milliseconds, flip angle = 8°, matrix size = 256 x 256, FOV = 256 x 256 x 190 and slice thickness = 1 mm with no interslice gap, yielding 1 x 1 x 1 mm3 isotropic voxels.

Molecular analyses

Asuragen® AmpliPrep™ FMR1 PCR Kit (Austin, Texas) was used to perform CGG sizing on blood DNA as previously described (Kraan et al. 2014). The PM or control status was confirmed 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 analy-
sis as described in Godier et al. (2010). Peripheral blood mononu-
clear cell (PBMC) isolation was performed on 0 to 15 mL of blood collected in EDTA-treated tubes using Ficol gradient separation, as per manufacturer’s instructions (Amersham Pharmacia Biotech, Upp-
sala, Sweden). Reverse-transcription real-time PCR was performed on RNA extracted from one million PBMCs as previously described (Loesch et al. 2011). The relative standard curve method was used
to quantify FMR1-5′, FMR1-3′ mRNA standardized to three internal control genes (GUS, EIF4A2 and SDHA) using the ViIA™ 7 Real-Time PCR System (Life technologies, Foster City, CA, USA). Reverse-transcription for each RNA sample was performed in two separate cDNA reactions, with each cDNA analysed in two separate RT-PCR reactions. The mean of the four outputs was used as a summary measure for FMR1 mRNA expression for each participant.

**Statistical analyses**

Data were analysed using IBM SPSS Statistics Version 22. Normality was assessed using the Shapiro-Wilk test. Independent t-tests, analysis of variance (ANOVA) or non-parametric equivalents were used to explore group differences in demographic variables. Pairwise inter-group comparisons were conducted for: (1) all PM carriers vs. controls (PM vs. HC); (2) PM carriers with FXTAS vs. controls (FX+ vs. HC); (3) PM carriers without FXTAS vs. controls (FX− vs. HC); (4) PM carriers with FXTAS vs. PM carriers without FXTAS (FX+ vs. FX−).

Missing postural sway scores were imputed based on performance on available sway trials using the missing values analysis function in SPSS (four FX+ and one FX− did not attempt eyes closed conditions as these were introduced in a later phase of the project; two FX+ were unable to complete the foam conditions; and one FX− was unable to complete the eyes closed on foam condition). Missing data were imputed using the expectation-maximization algorithm (Dempster et al. 1977). This method estimates the most likely missing value from available data, and has been shown to provide a more valid estimate reflective of the true value than other methods (Musil et al. 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. The overall sway score was then normalized using Blom’s rank-based transformation (Blom 1958), and compared using analysis of covariance (ANCOVA) controlling for age as follows: (1) PM vs. HC and (2) FX+ vs. FX− vs. HC. For all measures of sway, a higher score denotes greater postural instability for age as follows: (1) PM vs. HC and (2) FX+ vs. FX− vs. HC.

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 et al. 2010), removal of non-brain tissue (Segonne et al. 2004), automated Talairach transformation, segmentation of subcortical structures (Fischl et al. 2002; Fischl et al. 2004), intensity normalization (Sled et al. 1998), tessellation of grey matter and white matter boundary and automated topology correction (Fischl et al. 2001; Segonne et al. 2007), and surface deformation (Fisch & Dale 2000). 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 and adjusted for intracranial volume.

Group differences in adjusted cerebellar volumes were explored using ANCOVAs controlling for age. Linear regressions were then 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, FMR1 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, and 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<0.05 was used for linear regression and mediation analyses to minimize the chance of Type II error as recommended by Rothman (1990).

**Results**

**Participant characteristics**

When comparing all PM carriers to controls, there were no significant differences for age (t₄₄ =−0.366, P =0.716), education (Mann–Whitney U =245.500, P =0.681), height (t₄₄ =0.836, P =0.408), weight (t₄₄ =1.861, P =0.069), FSIQ (t₄₄ =−1.868, P =0.068), or FXTAS rating scale score (Mann–Whitney U =255.500, P =0.852) (Table 1).

**Table 1: Sample characteristics**

<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>Age (years)</td>
<td>Mean (SD)</td>
<td>55.3 (14.6)</td>
<td>53.7 (15.2)</td>
<td>66.4 (8.1)* †</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>26–77</td>
<td>26–80</td>
<td>56–80</td>
</tr>
<tr>
<td>Education (years)</td>
<td>Mean (SD)</td>
<td>13.5 (3.4)</td>
<td>13.2 (3.4)</td>
<td>12.3 (2.9)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>9–20</td>
<td>9–21</td>
<td>9–18</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Mean (SD)</td>
<td>173.2 (7.6)</td>
<td>174.9 (5.9)</td>
<td>173.5 (4.9)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>163–189</td>
<td>165.2–185.0</td>
<td>1675–180</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Mean (SD)</td>
<td>80.3 (13.8)</td>
<td>87.9 (13.7)</td>
<td>84.9 (11.0)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>57.2–118.3</td>
<td>63.5–124.8</td>
<td>63.5–97.8</td>
</tr>
<tr>
<td>CGG repeat length</td>
<td>Mean (SD)</td>
<td>29.9 (4.1)</td>
<td>88.0 (15.7)* †</td>
<td>89.6 (3.2)* †</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>20–44</td>
<td>72–134</td>
<td>85–92</td>
</tr>
<tr>
<td>FMR1 mRNA</td>
<td>Mean (SD)</td>
<td>1.1 (0.3)</td>
<td>2.2 (1.0)* †</td>
<td>2.3 (1.1)* †</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.7–1.6</td>
<td>1.0–5.2</td>
<td>1.4–4.2</td>
</tr>
<tr>
<td>FSIQ</td>
<td>Mean (SD)</td>
<td>113.5 (11.4)</td>
<td>106.3 (14.4)</td>
<td>93.4 (16.6)</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>86–133</td>
<td>79–127</td>
<td>79–123</td>
</tr>
<tr>
<td>FXTAS rating scale</td>
<td>Mean (SD)</td>
<td>13.8 (7.3)</td>
<td>22.3 (24.2)</td>
<td>49.9 (25.3)* †</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>5–50</td>
<td>2–90</td>
<td>17–90</td>
</tr>
</tbody>
</table>

HC, healthy controls; PM, all premutation carriers; FX+, PM carriers with FXTAS; FX−, PM carriers without FXTAS.

†>HC
‡>FX−
§>HC
¶<FX−

*P <0.05, **P <0.01, ***P <0.001.
Significantly higher FMR1 mRNA levels were found in the PM group compared to controls (Mann–Whitney U = 50.000, P < 0.001), and among PM males these levels were significantly correlated with CGG repeat length (Spearman’s rho = 0.595, P = 0.004). 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 (χ² = 0.949, P = 0.622), height (F(2,4) = 0.561, P = 0.575) or weight (χ² = 4.252, P = 0.119). However, group differences emerged for age (F(2,4) = 4.492, P = 0.017), FMR1 mRNA (χ² = 23.247, P < 0.001), FSIQ (χ² = 8.074, P = 0.018) and FXTAS rating scale score (χ² = 17.761, P < 0.001). Specifically, PM males with FXTAS were significantly older than PM males without FXTAS (P = 0.015). Premutation males with FXTAS also performed significantly worse than both controls and PM males without FXTAS on the measure of FSIQ (vs. HC P = 0.005; vs. FX– P = 0.014) and the FXTAS rating scale (vs. HC P < 0.001; vs. FX– P < 0.001). Premutation males with and without FXTAS both had significantly elevated FMR1 mRNA levels compared with controls (P values < 0.001), however, there were no significant differences in FMR1 CGG repeat length (P = 0.162) or FMR1 mRNA levels (P = 0.731) when comparing PM males with and without FXTAS.

In terms of functional status, 5 PM males (4 with FXTAS) reported having at least one fall within the previous 12 months (range in PM group = 0–4). Stages of FXTAS in the PM group also ranged from 0 to 4 (Bacalman et al. 2006). Fourteen PM males without FXTAS were classified at stage 0 (normal). One PM male without FXTAS and 2 PM males with FXTAS showed mild tremor or balance problems but no interference with ADLs (stage 1). Two PM males with FXTAS showed 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), however, were able to stand on floor unassisted for the duration of the postural sway trial.

**Group differences in postural sway**

Mean raw scores (mm²) for individual sway trials are presented in Table 2. Group comparisons were performed using the normalized 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 (F₁,₄₃ = 13.066, P < 0.001). 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 (F₁,₄₃ = 8.935, P < 0.001). Post-hoc comparisons showed that PM males with FXTAS performed significantly worse than controls on the overall sway factor (P < 0.001). No significant differences were found between PM males without FXTAS and controls (P = 0.084), or PM males with FXTAS (P = 0.163).

**Group differences in cerebellar volumes**

Controlling for age, the combined PM group had significantly smaller adjusted cerebellar volumes compared with controls (F₁,₄₁ = 11.886, P = 0.001) (Table 3). Group differences also emerged when the PM group were separated according to FXTAS diagnosis (F₁,₄₃ = 11.943, P < 0.001). Post-hoc comparisons showed that PM males with FXTAS had significantly smaller cerebellar volumes compared with PM males without FXTAS (P = 0.011) and controls (P < 0.001). There was no significant difference in cerebellar volume between PM males without FXTAS and controls (P = 0.427).

### Table 2: Mean (SD) anterior-posterior and medio-lateral postural displacement across different test conditions (mm)

<table>
<thead>
<tr>
<th>Sway condition</th>
<th>HC</th>
<th>PM</th>
<th>FX+</th>
<th>FX–</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>Normalized sway factor score</td>
<td>-0.4 (0.9)</td>
<td>0.4 (0.9)*** , †</td>
<td>1.2 (0.8)*** , †</td>
<td>0.1 (0.8)</td>
</tr>
</tbody>
</table>

HC, healthy controls; PM, all premutation carriers; FX+, PM carriers with FXTAS; FX–, PM carriers without FXTAS; AP, anterior-posterior; ML, medio-lateral.
† > HC
† Normalized rank transformation, includes imputed missing values.
*** P < 0.001.
Postural sway in males with the FMR1 premutation

Table 3: Mean cerebellar volumes (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>Mean</td>
<td>127859.0</td>
<td>118604.0**</td>
<td>102069.1***</td>
<td>1275075</td>
</tr>
<tr>
<td>SD</td>
<td>12590.9</td>
<td>22032.8</td>
<td>17530.2</td>
<td>19225.1</td>
</tr>
</tbody>
</table>

HC, healthy controls; PM, all premutation carriers; FX+, PM carriers with FXTAS; FX−, PM carriers without FXTAS.

†<HC
†<FX−
†MRI missing for 2 FX−.
* P < 0.05. ** P < 0.01. *** P < 0.001.

Table 4: Summary of linear regression models exploring associations between cerebellar volume (adjusted for intracranial volume) and normalized postural sway factor score

<table>
<thead>
<tr>
<th></th>
<th>HC (n = 24)†</th>
<th></th>
<th>PM (n = 20)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (SE)</td>
<td>t</td>
<td>B (SE)</td>
</tr>
<tr>
<td>Constant</td>
<td>−1.195 (0.760)</td>
<td>−1.572</td>
<td>2.018 (0.714)</td>
</tr>
<tr>
<td>Age (decades)</td>
<td>0.167 (0.127)</td>
<td>1.315</td>
<td>−0.367 (0.143)</td>
</tr>
<tr>
<td>Adjusted cerebellar volume</td>
<td>−0.440 (0.269)</td>
<td>−1.635</td>
<td>−0.928 (0.186)</td>
</tr>
</tbody>
</table>

HC, healthy controls; PM, all premutation carriers.
†F₂,2₁ = 4.327, P = 0.027, adjusted R² = 0.224.
‡F₂,1₇ = 15.261, P < 0.001, adjusted R² = 0.600.
* P < 0.05. ** P < 0.01. *** P < 0.001.

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 = −0.928, P < 0.001) (Table 4). An unexpected negative effect of age on sway (B = −0.367; P = 0.020) can also be seen in this solution. As the Pearson correlation coefficient between age and sway is positive (r = 0.343), 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 = −0.799). When controlling for age, cerebellar volume was not a significant predictor of postural sway in the control group (B = −0.440, P = 0.117).

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 = 0.277, P = 0.023), and increasing CGG repeat length (B = 0.302, P = 0.012) were significant predictors of greater postural sway among PM males (Table 5). Postural sway was not significantly associated with FMR1 mRNA level (B = 0.309, P = 0.091).

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 (B = 0.092, SE = 0.112, P = 0.422). This result suggests that cerebellar volume is a potential mediator of the effect of CGG repeat length on postural sway (see Fig. 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 = −0.292, SE = 0.087, P = 0.004). Using this result, together with the finding of the effect of cerebellar volume on postural sway (B = −0.803, SE = 0.242), the Sobel test showed that the indirect effect of CGG repeat length on postural sway, via
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Figure 1: Cerebellar volume as a mediator of the effect of CGG repeat length on postural sway. Final model: $F_{2,16} = 10.207$, $P < 0.001$, adjusted $R^2 = 0.592$. Age is included as a covariate in all models.

Discussion

This study is the first to examine the interrelationships between cerebellar volume, CGG repeat length, FMR1 mRNA levels and postural sway during manipulation of visual and proprioceptive demands in PM males with and without FXTAS. The findings showed significantly poorer performance on postural sway measures among PM males with FXTAS compared with 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. Collectively, these findings extend previous studies showing postural control abnormalities in PM males without FXTAS. The findings showed significantly poorer visual and proprioceptive demands in PM males with and 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 for 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 that may reduce risk of falls, increase functional capacity and improve quality of life (e.g., Dibrezzo et al. 2005; Lord et al. 1996; Yasuda et al. 2012).

Another important finding of this study 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 in males with the PM (Battistella et al. 2013; Hashimoto et al. 2011a; Wang 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 PM carriers, including impairments in gait and postural control (Allen et al. 2008; Kraan et al. 2013, 2014; severity of motor symptoms (Leehey et al. 2008), and age of onset of motor symptoms (Tassone et al. 2007). Collectively these findings suggest 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 et al. 1999; Ouchi et al. 2001; Sullivan et al. 2000; Sullivan et al. 2006). We have also provided preliminary evidence to suggest 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 et al. 2011a; Wang et al. 2013). Taken together with the findings of this 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. However, this requires investigation in longitudinal studies with larger samples.

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 (Leehey et al. 2008; Tassone 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 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), and attentional interference in a choice-stepping reaction time test (Hocking et al. in press), 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 et al. 2011a,b; Wang et al. 2012; Wang et al. 2013) 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 et al. 2014; Todd et al. 2013).

The conclusions that can be drawn from this study are limited by the small sample size, the cross-sectional design and 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 et al. 2011b; Wang et al. 2013), and the relationships with performance on sensitive measures of postural control (e.g. with dual-task interference). The relationship between cerebellar integrity and other PM molecular markers including reduced FMR1 protein (FMRP) (Ludwig et al. 2014), abnormal expression of long non-coding RNA genes, ASFMR1/FMR4 (Loesch et al. 2011), FMR5 and FMR6 (Pastori et al. 2014), and repeat associated non-ATG translation (Todd et al. 2013) should also be explored. Further, although PM males were classified according to FXTAS diagnostic criteria, the penetrance is age-related (Jacquemont et al. 2004), and it is not possible to determine which of the younger carriers without FXTAS will go on to develop symptoms with time. Longitudinal studies are required to determine whether changes in postural control are related to later decline associated with FXTAS. In addition to longitudinal study designs, the use of measures of white matter integrity and structural connectivity will 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; Hippolyte et al. 2011b; Hippolyte et al. 2014).

In conclusion, we report for the first time 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. These preliminary findings showed that increasing CGG repeat length was associated with decreased cerebellar volume, which in turn was associated with poorer performance on measures relating to postural sway. We propose that 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.

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Acknowledgments

We thank all of the families who participated in our research. This work was supported by The Australian Research Council (Discovery Project Grant DP110103346, awarded to Chief Investigators K.C., J.T., N.G.K. and W.W.); The New South Wales Institute of Psychiatry (Training Fellowship in Psychiatric Research awarded to R.B.); Monash Research Fellowship to D.H.; the Dementia Collaborative Research Centre – Assessment and Better Care, University of New South Wales as part of the Australian Government’s Dementia Initiative; and the Genetics of Learning Disability Service. Salary for the molecular component in part was supported by an NHMRC project grant (No 104299 to D.G.). We thank the Genetics of Learning Disability Service, Dr Alison Archibald and the Victorian Clinical Genetics Service, Dr Jonathan Cohen and the Fragile X Alliance Inc., and the Fragile X Association of Australia for assistance with recruitment of participants. We are also grateful to Professor Sylvia Metcalfe for her involvement in conceptualization of the study; Professor Stephen Lord, the Falls and Balance Research Group (Neuroscience Research Australia) and Tania Zareen (The University of New South Wales) for assisting with clinical assessments; the Neuroimaging Laboratory (Centre for Healthy Brain Ageing, The University of New South Wales) for processing brain MRI scans; Ern Turbitt (Murdock Children’s Research Institute) for assisting with FMR1 analyses; Jonathan Whitty and Healthscope for providing support for CGG repeat sizing; and Dr John Crawford (Centre for Healthy Brain Ageing, The University of New South Wales) for assistance with statistical analyses.