Carriers of the \( FMR1 \) premutation allele (fXPCs) have a trinucleotide expansion (55—200 CGG repeats) and are at increased risk of developing a late-onset neurodegenerative motor disorder, FXTAS (Fragile X-associated Tremor/Ataxia Syndrome). Core features of FXTAS include intention tremor, gait ataxia, and parkinsonism (Hagerman et al., 2001). Other common features include nonmotor symptoms such as peripheral neuropathy (Berr-Kravis et al., 2007), autonomic dysfunction (Coffey et al., 2008; Jacquemont et al., 2003), and psychiatric symptoms (Bourgeois et al., 2007). Significantly, the disorder is also associated with cognitive decline (Sevin et al., 2009), even in fXPCs without motor symptoms (Cornish et al., 2009). An estimated 1 in 260 to 813 males and 1 in 113 to 259 females are fXPCs (Hagerman, 2001), and nearly 40% of male and 8% of female fXPCs develop FXTAS (Jacquemont et al., 2004). FXTAS predominantly affects individuals over age 50, and risk increases with age (Hagerman et al., 2001; Jacquemont et al., 2003). Thus, the prevalence and age-dependent nature of FXTAS highlight the importance of early identification of individuals at greatest risk for developing the disorder.

The molecular etiology and clinical phenotype in fXPCs are distinct from those in carriers of full mutation alleles (>200 CGG repeats), in whom gene silencing leads to Fragile X Syndrome (FXS). Increased CGG repeat length in fXPCs is associated with increased \( FMR1 \) mRNA yet reduced \( FMR1 \) protein (FMRP) levels (Kennessy, Zhang, Hagedorn, & Warren, 2001; Tassone et al., 2000), a dissociation due to translational inefficiency of premutation mRNA (Primerano et al., 2002). The FXTAS phenotype is thought to be due to a toxic gain of function of excess \( FMR1 \) mRNA positively associated with CGG repeat length (Hagerman & Hagerman, 2004). This is supported by findings that CGG repeat length predicts motor onset of FXTAS (Tassone et al., 2007), level of motor impairment (Leehey et al., 2008), and
Cerebellar Volume in FXTAS

Decreased cerebellar volume has been observed in male and female fXPCs with FXTAS (Adams et al., 2007; Cohen et al., 2006), in male fXPCs irrespective of FXTAS status (Loesch, Litewka et al., 2005; Moore et al., 2004), and in male fXPCs asymptomatic for FXTAS (Battistella et al., 2013). Cerebellar vermis is similarly reduced in size in individuals with FXS, and this reduction is correlated with certain measures of cognitive impairment in females (Mostofsky et al., 1998; Reiss, Freund, Tseng, & Joshi, 1991). While it is unclear how much these findings may hold true across gender, it suggests that reduced cerebellar volume is a common feature across the FMR1 spectrum, and that volume reductions in fXPCs may be linked to cognitive impairment.

Cerebellar anatomy and function is linked to executive and other cognitive functions, as described in several recent reviews (O’Halloran, Kinsella, & Storey, 2012; Koziol, Budding, & Chidekel, 2012; Stoodley & Schmahmann, 2010). Patients with cerebellar lesions exhibit impairments in executive function tasks such as sequencing, set-shifting, and verbal fluency, functional activation of the cerebellum is also observed in tasks involving switching, planning, and verbal fluency (see O’Halloran et al., 2012 for review). The cerebellum is anatomically and functionally segregated, with damage to vermis lobules VI and VII impacting cognitive functions, and damage to the vermis associated with neuropsychiatric disorders (Stoodley & Schmahmann, 2010). The involvement of the cerebellum with cognitive functions, and not just motor functions, is consistent with Ito’s (1993) proposal that the cerebellum performs similar operations on information, regardless of its source. Motor functions, such as prosaccade amplitude, have been linked to volume of the vermis, indicating that vermis volume and functions attributed to the vermis can be correlated (Ettinger et al., 2005).

Cerebellar volume may relate to cognitive function in fXPCs. Reduced vermis size has been observed in several neuropsychiatric disorders associated with executive function impairments, including attention-deficit hyperactivity disorder (ADHD), autism, and schizophrenia (see O’Halloran et al., 2012 for review), although few studies examine distinct subregions of the vermis. Thus, given evidence of decreased cerebellar volume and impaired inhibitory control in fXPCs, examination of the relationship between the two could aid our understanding of anatomical and functional changes as fXPCs age and risk for developing FXTAS increases.

Executive Function in FXTAS

Executive dysfunction is associated with FXTAS, particularly impairments in inhibitory control (Grigsby et al., 2008, 2007), which refers to the ability to suppress actions or thoughts that are inappropriate or irrelevant in a given context. Inhibitory control is conceptualized as comprising several domains: (1) interference control, suppression of irrelevant stimuli; (2) cognitive inhibition, suppression of irrelevant thoughts or cognitive processes; (3) behavioral inhibition, suppression of a prepotent response; and (4) oculomotor inhibition, suppression of reflexive saccades (Nigg, 2000). Individuals with FXTAS are observed to have impaired inhibitory control in several of these domains, including interference control (Stroop Color-Word Test; Brega et al., 2008), cognitive inhibition (Behavioral Discontrol Scale (BDS), Controlled Oral Word Association Task; Brega et al., 2008; Grigsby et al., 2006, 2007, 2008), and behavioral inhibition in fXPCs asymptomatic for FXTAS (Hayling sentence completion; Hunter, Sherman, Grigsby, Kogan, & Cornish, 2012).

Genetic variability is associated with executive function. Increased CGG repeat length is associated with poorer performance in some of these tasks (Grigsby et al., 2006, 2007), and modulates the effect of age on a behavioral inhibition task in fXPCs asymptomatic for FXTAS (Hayling sentence completion; Cornish, Hocking, & Moss, 2011; Hunter et al., 2012). Additionally, increased mRNA is associated with poorer BDS scores (Grigsby et al., 2007).

Executive Function in fXPCs Asymptomatic for FXTAS

There are clinical signs of impaired inhibitory control even in fXPCs asymptomatic for FXTAS. In fXPCs, there is higher prevalence of ADHD diagnoses in women (Hunter, Rohr, & Sherman, 2010) and boys (Farzin et al., 2006), and increased ADHD symptoms in men (Dorn, Mazzocco, & Hagerman, 1994) and boys (Aziz et al., 2003). Furthermore, self-reported inattention and impulsivity in female fXPCs is positively associated with CGG repeat length (Hunter et al., 2008a). Indicative of impaired cognitive inhibition, fXPCs report higher levels of obsessive-compulsive symptoms, which are positively associated with mRNA levels (Hessl et al., 2005). There is also evidence in fXPCs of dysfunctional ability to inhibit inappropriate behaviors. For example, female fXPCs exhibit increased smoking (Hunter et al., 2010), while male fXPCs exhibit increased alcohol abuse and dependence, and are more likely than controls to endorse physical and verbal abusive behaviors (Dorn et al., 1994; Kogan, Turk, Hagerman, & Cornish, 2008).

Neuropsychological tests of executive function in fXPCs asymptomatic for FXTAS have yielded mixed results. Principal components analysis of several executive function tests revealed no differences between fXPCs and controls (Allen et al., 2011; Hunter et al., 2008b). Hunter et al. (2012), by combining samples from several preceding studies, found that fXPCs performed worse than controls on the Stroop, BDS, and Hayling Sentence Completion Part B. These discrepant findings may be due in part to variability in both age and CGG repeat length across samples. This possibility is supported by the findings that in fXPCs with >100
CGG repeats and asymptomatic for FXTAS, there is an association between age and poorer task performance on response inhibition (Hayling Sentence Completion Part B), executive working memory (letter-number sequencing) and visuospatial working memory (Cornish et al., 2011; Hocking, Kogan, & Cornish, 2012). Thus, there is only limited evidence from neuropsychological tests that fXPCs asymptomatic for FXTAS exhibit impaired inhibitory control.

**Eye Movements and Inhibitory Control**

Eye movement measures of inhibitory control have several advantages relative to traditional neuropsychological tests. First, fXPCs are at risk for developing a neurodegenerative motor disorder, so an inhibitory control task with a prominent motor component is ideally suited for use as a possible biomarker. Second, eye movement measures from different paradigms can be used to distinguish cognitive and motor characteristics. For example, the prosaccade task assesses motor function, while the antisaccade task requires both inhibitory control and motor function. Subtracting saccade latency in the prosaccade task from latency in the antisaccade task provides a measure of inhibitory control that is independent of motor demand. Third, tasks that rely on reaction time (RT) measures can only measure the focus of attention at a single moment in time (one RT per trial), while eye movements can be recorded continuously. These additional data points allow for improved delineation of the time course of attention shifts. Fourth, other inhibitory tasks such as the Stroop use facilitation and interference effects to infer attentional engagement, while eye movements are a more direct measure of attention engagement: unless instructed otherwise, the foveated location is usually the attended location. Finally, slowed or enhanced psychomotor speed in one group relative to another, such as in female fXPCs but not male fXPCs (Goodrich-Hunsaker et al., 2011c; Wong et al., 2012), can affect performance and complicate interpretations of differences or lack thereof in RT. It is important that eye movement measures relate to traditional neuropsychological measures in patient populations with inhibitory control impairments. For example, in patients with schizophrenia, antisaccade errors correlate with perseverative errors on the Wisconsin Card Sorting Task, which reflect a failure of inhibition (Crawford, Haeger, Kennard, Reveley, & Henderson, 1995; Levy et al., 1998).

If dysfunctional inhibitory control processes underlie the FXTAS phenotype, impaired oculomotor control might be present in the absence of FXTAS symptoms. Indeed, there is evidence that executive function impairments could precede FXTAS symptoms, because fXPCs without FXTAS demonstrate impairments on tests of executive function (Moore et al., 2004), even in fXPCs without motor symptoms (Cornish et al., 2009), and risk for cognitive decline in fXPCs increases with CGG repeat length (Sevin et al., 2009). However, due to a paucity of evidence in fXPCs, it is unknown whether oculomotor abnormalities are prevalent in FXTAS or whether they precede the onset of other FXTAS motor signs. In neurodegenerative disorders other than FXTAS, eye movements can provide valuable markers of disease severity or progression (see Anderson and MacAskill (2013) for a review).

**Purpose**

The purpose of this study was to examine whether eye movements are sensitive to impairments in fXPCs, and whether these impairments relate to reductions in cerebellar vermis size. Our first aim was to determine whether fXPCs asymptomatic for FXTAS exhibit impaired inhibitory control in the oculomotor domain. Oculomotor control to suppress a reflexive saccade is required in fixation and antisaccade tasks, but not in prosaccade and smooth pursuit tasks. Given our hypothesis that fXPCs exhibit impaired inhibitory control, we anticipated they would exhibit a selective impairment in tasks requiring oculomotor control. Our second aim was to examine the relationship between cerebellar vermis volume, executive function, and eye movement function. While several studies report reduced cerebellar vermis volume or impaired executive function in fXPCs, the relationship between the two in fXPCs is unclear. Examination of both structure and function in fXPCs will help inform a neurodevelopmental understanding of the fXPC phenotype.

**Method**

**Participants**

Participants were 43 men aged 18 to 48, including 22 healthy controls (HCs) and 21 fragile X premutation carriers (fXPCs; Table 1). FXPCs had at least one family member with FXS. All participants had normal or corrected-to-normal vision.

Participants were recruited through the NeuroTherapeutics Research Institute (NTRI) at the Medical Investigation of Neurodevelopmental Disorders (MIND) Institute at the University of California, Davis Medical Center, and from the community through recruitment advertisements. FXPCs were recruited from known FXS pedigrees, and HCs were recruited from pedigrees or the community. Exclusion criteria were: acute medical condition such as renal, liver, or cardiac or other disease that may be associated with brain atrophy or dysfunction; current or past history of major DSM-IV Axis I psychiatric disorder; history of head trauma, toxic encephalopathy, encephalitis, or bacterial meningitis; history of

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alcoholism or drug problem and use of medication that affects cerebral blood flow (e.g., current beta blockers). This study was approved by the Institutional Review Board at the University of California, Davis Medical Center and conformed to institutional and federal guidelines for the protection of human participants. All participants provided written informed consent prior to participation.

Procedure

We conducted this experiment as part of a larger study (see Wong et al., 2012; other manuscripts in preparation). The study visit involved administration of neuropsychological tests, a blood draw, and a battery of cognitive tests. All FXPCs were evaluated by a physician and determined to be asymptomatic for FXTAS. All control participants completed the Tremor Disability Rating Scale (Jacquemont et al., 2004). One control participant reported difficulty or disability performing any of the 31 common actions (i.e., “using eyepieces” and “threading a needle”). Because this participant’s performance was not extreme (black triangle in figures), he was included in all analyses as a HC.

Molecular assays. Molecular data were FMR1 CGG repeat length and mRNA expression level. Genomic DNA was isolated from peripheral blood leukocytes using standard methods (Puregene Kit; Gentra Inc., Valencia, CA). Repeat length was determined using Southern blot analysis and PCR amplification of genomic DNA as described previously (Tassone, Pan, Amiri, Taylor, & Hagerman, 2008). All quantifications of FMR1 mRNA were performed using a 7900 Sequence detector (PE Biosystems).

Psychological assessment. Full scale IQ (FSIQ) was measured using either the Wechsler Adult Intelligence Scale, third edition (WAIS-III) (Wechsler, 1997) or the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999). Due to time constraints during testing, FSIQ data were not available from 3 HC.

ADHD assessment. ADHD diagnoses are more prevalent, and symptoms more elevated, in FXPCs relative to controls (Hunter et al., 2010; Farzin et al., 2006; Dorn et al., 1994; Aziz et al., 2003). Adults with ADHD have been found to produce longer saccade latencies and increased anticipatory saccades (Carr, Nigg, & Henderson, 2006). Therefore, we measured ADHD status as a potential confound of oculomotor performance, which requires both attention and inhibitory control. ADHD status was measured using the 66-item Conners’ Adult ADHD Rating Scale (CAARS) (Conners, Erhardt, & Sparrow, 1999). Participants completed a self-report, and an observer report was completed by a spouse, partner, family member, or close friend. Scores were adjusted according to established age and sex norms. Due to time constraints during testing and inability to collect observer reports during testing, ADHD data were not available from all participants.

Behavioral tasks. All experiments were presented via E-Prime 2.0.8.90 (http://www.pstnet.com) on a Tobii T120 monitor (http://www.tobii.com), and gaze data were collected via Tobii Studio 2.3.2.0 eye-tracking software at a rate of 120 Hz. Participants were seated 60 cm from the eye-tracking monitor in a chin rest to maintain head position. Participants were calibrated using a 5-point system prior to the eye-tracking. Participants were observed during task performance to ensure appropriate task performance and gaze data quality, and were recalibrated between tasks as necessary. Each task began only after the participant successfully completed practice trials to demonstrate understanding of task instructions. In each trial, participants fixated on a centrally presented cross, and the trial began after the eye-tracker determined that the participant’s gaze was within 1° visual angle (VA) of the center for 150 ms.

Fixation. Participants maintained gaze position when the cross disappeared or a peripheral stimulus appeared. In the gap condition, the cross disappeared for a variable duration (200, 400, 600, or 800 ms) before the stimulus appeared. In the no-gap condition, the cross remained present during stimulus presentation. The stimulus was a black circle subtending 1.25° VA, positioned randomly 4.5–4.6° VA above, below, to the left, or to the right of fixation, appearing for 1,000 ms. A variable intertrial interval (250 or 500 ms) was included to jitter the timing of the trials. Participants completed 80 trials, separated into four blocks. The dependent measure was maximum gaze deviation from center.

Smooth pursuit. Participants maintained gaze during target presentation on a square moving at constant velocity. When the square appeared, the fixation cross disappeared. The square subtended 0.5° VA in either direction, and appeared randomly above, below, to the left, or to the right of fixation for 500 ms, then moved across the screen for 2,000 ms at a constant speed of 12° VA/sec. A variable intertrial interval (250 or 500 ms) was included to jitter the timing of the trials. Participants completed 64 trials, separated into four blocks.

Because pursuit initiation is distinct from pursuit maintenance, and to reduce the potentially confounding effect of preparing to stop pursuit, the time window for analysis began 250 ms after target movement onset and ended 250 ms before target movement offset. Thus, the duration of the analyzed tracking window was 1,500 ms. Dependent saccade measures were: number of anticipatory (in the same direction of target movement but increased position error), back-up (in the opposite direction of target movement), catch-up (in the same direction of target movement with decreased position error), and total saccades (sum of the three saccade types). Additional dependent measures were: closed loop pursuit gain (CLPG), calculated as the ratio of eye velocity to target velocity; and root mean square error (RMSE), calculated by squaring the distance between gaze and target position at each time point, then obtaining the average of the square root of these values.

Prosaccade. Participants looked at the stimulus that appeared in the periphery. They were instructed that if the target appeared a certain distance to the left, for example, they were to look the same distance to the left. In the gap condition, the cross disappeared for 200 ms before the stimulus appeared, while in the no-gap condition, the cross remained present during stimulus presentation. The stimulus was a black circle filled with a 3 × 3 checkerboard pattern and subtending 1.25° VA, positioned randomly 4.5–4.6° VA above, below, to the left, or to the right of fixation, appearing for 1,000 ms. A variable intertrial interval (200, 400, or 600 ms) was included to jitter the timing of the trials. Participants completed 72 trials, separated into three blocks. Several dependent measures were collected: (1) latency, (2) speed, and (3) magnitude of initial saccade; and (4) number of anticipatory saccades, defined as saccades during gap presentation.

Antisaccade. The task parameters were identical to those in the Prosaccade task, except participants were instructed to look in the direction opposite the stimulus that appeared in the
periphery. They were instructed that if the target appeared a certain distance to the left, for example, they were to look to the same distance to the right. The dependent measures were identical to those in the Prosaccade task. An additional dependent measure was percentage of trials with directional errors. These were trials in which the initial saccade was not in the same direction as the target.

**Structural MRI.** Magnetic resonance images (MRI) of the participants were collected. Mid sagittal slices were used to calculate cerebellar vermis volume. Two independent raters (LMW and MZ) manually traced four subregions: 1) lobules I-V, 2) lobules VI-VII, 3) lobule VIII, and 4) lobules IX-X. Interrater reliability was 95.0% or greater for overall vermis area and each of these four subregions.

**MRI acquisition.** MR images were obtained using a Siemens Trio 3T scanner at the University of California, Davis Imaging Research Center. One of the two 3D T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) MRI sequences were acquired for each participant. One sequence consisted of 208 contiguous sagittal slices with TR (repetition time) = 1,900 ms; TE (echo time) = 2.26 ms; in-plane resolution = 0.47 × 0.47 mm; slice thickness = 0.95 mm; flip angle = 9°; field of view of 207; and an acquisition matrix of 512 × 512. The second sequence consisted of 192 contiguous sagittal slices with TR = 2170 ms; TE = 4.86 ms; in-plane resolution = 1 × 1 mm; slice thickness = 1 mm; flip angle = 7°; field of view of 192; and an acquisition matrix of 256 × 256.

**MRI preprocessing.** Using the Automatic Registration Toolbox (ART) acpdetect module (http://www.nitrc.org/projects/art/), the original acquired images were put into basic alignment so that the anterior commissure (AC) and posterior commissure (PC) were along a horizontal plane. This initial alignment, often referred to as AC-PC alignment, is a rigid-body (i.e., three-translation and three-rotation) transformation.

Using Advanced Normalization Tools (ANTS; http://www.picsl.upenn.edu/ANTS/), the images were then corrected for signal intensity inhomogeneity caused by nonuniformities in the radio frequency (RF) receiver coils implementing the N4 bias field algorithm (Sled, Zijdenbos, & Evans, 1998). The images were also normalized to have an intensity range between 0 and 100 with linear scaling by using histogram matching to the template image.

Finally, brain extraction and three-tissue segmentation were achieved using a new method, Atropos, that is a part of ANTs. Simply, the process involves taking a template brain—we used the MNI ICBM152 2009c Nonlinear Symmetric template with linear scaling by using histogram matching to the template—of the brain—we used the MNI ICBM152 2009c Nonlinear Symmetric template with linear scaling by using histogram matching to the template—and aligning it to a participant image to provide a rough template-based brain extraction. We used the symmetric diffeomorphic transformation model (SyN) approach (Avants, Epstein, Grossman, & Gee, 2008), because of its ability to capture large deformations (e.g., when brain lesions are present) while maintaining topology. Individual participant brain masks were generated by applying the nonlinear warp to the template brain mask. Spatial prior probability maps of the three tissues were also generated by applying the nonlinear warp to the template priors. Then, Atropos was used to acquire three-tissue classification by implementing the spatial prior probability maps for each participant. Gray matter, white matter, and CSF masks were generated for each participant. By combining gray matter and white matter volumes, total brain volumes were acquired for each participant.

**Statistical Analyses**

**Behavioral tasks.** For all tasks, gaze position at each time point was determined by averaging gaze position from both eyes. For the fixation and saccade tasks, saccades were identified as eye movements with velocity exceeding 100° VA/sec with at least 16 ms duration. In the Pursuit task, due to a low number of high-velocity eye movements, saccades were identified as eye movements with velocity exceeding 50° VA/sec with at least 16 ms duration. For each participant and gap duration, outlier trials were identified as having a dependent measure greater than three times the interquartile range (IQR) or less than three times the IQR, and were excluded from analyses. The dependent measure for outlier determination was gaze deviation in the Fixation task, saccade latency in the prosaccade and antisaccade tasks, and total number of saccades in the Pursuit task.

Similarly, for each group outlier participants were identified and excluded from analyses. Linear regression models with gap duration (except in Pursuit), group, age, and group × age interaction as predictors were assessed for each dependent variable for each task. If the main effect of age was significant, the effect of age was then examined within each group. In fXPCs, Pearson’s correlations between CGG repeat length and mRNA were also assessed.

**Structural MRI.** Area for each region was calculated as the average value between raters. Area was normalized by dividing by total brain volume, and normalized area was used for all subsequent statistical tests. T tests were used to identify differences in area between fXPCs and HCs. Pearson’s correlations were used to identify relationships between normalized vermis area and behavioral performance measures (both groups) and molecular variables (CGG and mRNA; fXPCs only).

**Results**

**Study Sample**

A total of 22 HCs and 21 fXPCs performed the tasks (see Table 1). Of these, some participants were excluded due to technical difficulties during data collection (two fXPCs in Fixation and one HC in the remaining three tasks). Thus, usable data were obtained from 22 HCs and 19 fXPCs for the Fixation task, and from 21 HCs and 21 fXPCs for the remaining three tasks. One participant was ineligible for MRI.

The mean age (± SD) was 30.14 ± 6.44 for HCs and 32.17 ± 7.74 for fXPCs, which did not differ significantly (t = −0.93, p = .36). The mean CGG repeat length was 29.59 ± 5.30 for HCs and 32.17 ± 24.63 for fXPCs, which differed significantly (t = −12.26, p < .001). One participant expressed two variants of the premutation allele (120 and 156). Because his performance was not extreme in any task, he was included in all analyses. To assess the effect of CGG repeat length on performance, separate correlations were tested using his mean (138) or higher (156) CGG value. The mean mRNA value was 1.43 ± 0.23 (range: 1.10–1.76) for HCs and 3.05 ± 1.34 (range: 1.85–7.81) for fXPCs, which differed significantly (t = −5.08, p < .001).
Mean FSIQ was 118.6 ± 13.27 for HCs, and 116.7 ± 13.68 for fXPCs, which did not differ between groups (t = 0.45, p = .66). ADHD self-report data were available from 20 HCs and 18 fXPCs, and observer-report data were available from 19 HCs and 15 fXPCs. None of the ADHD subscale scores differed between groups (all ps > .33). No participants met ADHD criteria on both the self- and observer report, though one HC and four fXPCs met ADHD criteria on the Total Symptoms subscale of the self-report.

**Outlier Identification**

Because several participants met outlier criteria, they were excluded from task-specific analyses. Four participants were excluded from the Fixation (one HC, one fXPC), Prosaccade (one fXPC), Antisaccade (one HC), and Pursuit (one HC) tasks. The outlier in the Prosaccade task was also an outlier in the Fixation task.

Thus, analyses included 21 HCs and 18 fXPCs in the Fixation task, 21 HCs and 20 fXPCs in the Prosaccade task, 20 HCs and 21 fXPCs in the Antisaccade task, and 20 HCs and 21 fXPCs in the Pursuit task.

The number of trials excluded for meeting outlier criteria did not differ between groups for any task (all ps > .37). The mean number of trials excluded was low in the Fixation (HCs: 0.05 ± 0.22, fXPCs: 0.17 ± 0.51), Pursuit (HCs: 0.10 ± 0.31, fXPCs: 0.19 ± 0.51), Prosaccade (HCs: 0.52 ± 0.98, fXPCs: 0.35 ± 0.93), and Antisaccade (HCs: 0.85 ± 0.81, fXPCs: 1.00 ± 1.55) tasks.

**Oculomotor Performance**

Table 2 shows the results from regression models. Table 3 shows the within-group associations between oculomotor performance and age (both groups), and CGG repeat length (fXPCs only).

**Fixation.** Fixation requires inhibitory control to inhibit reflexive eye movements toward distractors. As seen in Figure 1, although the within-group associations with age were not significant, the group × age interaction was significant, F(1, 190) = 8.97, p = .003, such that gaze deviation increased with age for HCs (r = .14, p = .56), but decreased with age in fXPCs (r = −.33, p = .18). The main effects of group and age were not significant.

**Smooth pursuit.** Smooth pursuit requires eye movements designed to match eye position with anticipated position of a moving target. As seen in Figure 2, there were no effects of group or age on anticipatory, back-up, catch-up, or total saccades (all ps > .40). Nor were there effects of group or age on CLPG or RMSE (all ps > .13).

**Prosaccade.** Prosaccades are reflexive eye movements generated toward a target. As seen in Figure 3, there were no effects of group, age, or group × age interaction for any of the four measures. Within-group comparisons revealed that anticipatory responses increased with age in HCs (r = .43, p = .05) but not in fXPCs (r = .20, p = .40), but the interaction between group and age was not significant, F(1, 77) = 1.90, p = .17.

**Antisaccade.** Antisaccades require inhibitory control to inhibit reflexive eye movements toward a target, as well as movement generation in the opposite direction. As seen in Figure 4, fXPCs produced longer latencies than HCs to initiate saccades, F(1, 77) = 13.23, p < .001. Number of anticipatory saccades increased with age, F(1, 77) = 7.01, p = .01 across groups. This was driven by an association with age in HCs (r = .46, p = .04), but not in fXPCs (r = .43, p = .92). There was a group × age interaction for errors, F(1, 77) = 14.80, p < .001. Error increased with age in HCs (r = .58, p = .007), but not in fXPCs (r = −.19, p = .40).

**Inhibitory cost.** Inhibition cost was calculated as the difference in mean latency between antisaccades and prosaccades. The effect of group was significant, F(1, 77) = 9.42, p = .03, such that fXPCs exhibited greater cost. The group × age interaction was significant, F(1, 75) = 5.25, p = .02, such that there was a trend for cost to increase with age in HCs (r = .29, p = .06) but not in fXPCs (r = −.23, p = .15).

**Associations Between Oculomotor and Molecular Measures**

**Midsagittal Cerebellar Vermis Area**

MRI data were available from 18 HCs and 19 fXPCs. There were no group differences in cerebellar vermis area for any of the four subregions or for total area (all ps > .08). In HCs, antisaccade speed correlated positively with area of lobules I-V (r = .56, p = .02) and lobules IX-X (r = .52, p = .04). In fXPCs, area of lobules I-V correlated positively with RMSE (r = .46, p = .05), and area of lobule VIII correlated negatively with number of anticipatory saccades (r = −.54, p = .02). Also in fXPCs, area of lobules VI-VII correlated positively with inhibitory cost (r = .52, p = .03). These latter findings indicate that in fXPCs, increased area in lobules VI-VII is associated with greater cost to inhibit a reflexive response and an alternative response.

**Discussion**

This study provides the first description of basic oculomotor function in a group of fXPCs and demonstrates that eye movements are sensitive to impairments in fXPCs asymptomatic for FXTAS. We found that fXPCs exhibited impaired inhibitory control, when measured either as antisaccade latency or as latency difference between antisaccade and prosaccade conditions (inhibitory cost). Meanwhile, fXPCs did not differ in inhibitory control, measured in the Fixation task, nor in motor function, measured in the smooth Pursuit and Prosaccade tasks. Although midsagittal vermis area did not differ between groups, area of lobules VI-VII correlated positively with inhibitory cost in fXPCs.

Inhibitory control is one aspect of executive function, and the current findings are consistent with previously reported research on executive function impairment in fXPCs (Cornish et al., 2009, 2008; Grigsby et al., 2006, 2007, 2008), while extending those findings into the motor domain. Because inhibitory control impair-
ments were observed in the oculomotor domain in fXPCs asymptomatic for FXTAS, this study provides evidence that inhibitory control impairments may precede gross body motor impairments in fXPCs at risk for developing FXTAS. In this discussion, we first address functioning of the visual system and oculomotor system across the FMRI1 spectrum (premutation and full mutation carriers). Then, we describe the role of the cerebellum in eye movements, as well as the evidence for cerebellar abnormalities in fXPCs asymptomatic for FXTAS. Finally, we propose a possible link between cerebellar abnormalities and inhibitory control impairments in fXPCs.

Visual Function Across the FMRI1 Spectrum

Evidence suggests a specific impairment in magnocellular pathway and intact parvocellular pathway functioning in male (Kéri & Benedek, 2012) and female (Kéri & Benedek, 2009) fXPCs. The magnocellular pathway projects to cortical areas responsible for spatial processing (e.g., detecting spatial locations, perceiving motion, and visuomotor coordination), while the parvocellular pathway projects to cortical areas responsible for object recognition and color perception. These psychophysical studies are supported by behavioral studies reporting: (1) impaired spatial processing in men (Hocking et al., 2012; Wong et al., 2012) as well as women (Goodrich-Hunsaker et al., 2011a, 2011b) carrying the fragile X premutation, (2) impaired motion perception in female fXPCs (Kéri & Benedek, 2010) and men with FXS (Kogan et al., 2004), and (3) reduced resolution of temporal attention in infants with FXS (Farzin, Rivera, & Whitney, 2011). Visual function (motion coherence threshold and contrast sensitivity at low spatial/high temporal frequency) improved with increasing FMRP levels in healthy male volunteers (Kéri & Benedek, 2011), and temporal function (phase individuation threshold) declined with increasing CGG repeat length in infants with FXS (Farzin, Rivera et al., 2011). Together, these studies indicate that FMRI1 gene mutations modulate visual function.

Oculomotor Function Across the FMRI1 Spectrum

There has been only one other examination of oculomotor function in fXPCs, which was a case study of an 80-year-old man with FXTAS (Sulkowski & Kaufman, 2008). He complained of intermittent diplopia and was found to have “binocular diplopia, a small combatant vertical ocular misalignment, convergence insufficiency type intermittent exotropia, loss of vertical and horizontal fusional mergence amplitudes, saccadic pursuits, and hypome-
tropic saccades.” It is possible that decreased saccade magnitude and number of saccades during pursuit are associated with FXTAS progression, but we did not observe this pattern in this sample of fXPCs asymptomatic for FXTAS.

All other studies of oculomotor function have examined individuals with FXS. Ocular studies indicate that approximately 25% of children with FXS have clinically significant ocular findings, including refractive errors (primarily hyperopia and astigmatism) and strabismus (Hatton, Buckley, Lachiewicz, & Roberts, 1998; Maino, Wesson, Schlange, Cibis, & Maino, 1991). Eye tracking and pupillometry measures exhibit test–retest reliability in individuals with FXS (Farzin, Scaggs, Hervey, Berry-Kravis, & Hessl, 2011). Females with FXS, relative to controls, are slower to generate: (1) prosaccades made in the overlap condition of a gap/overlap task, (2) predictive saccades, and (3) memory-guided saccades (Lasker, Mazzocco, & Zee, 2007).

Cerebellar Role in Eye Movements

Through its connections, the cerebellum is involved with executive and motor function (Ramnani, 2006). Cerebellar lesion studies demonstrate that the cerebellum plays a role in deploying covert attention, possibly via oculomotor control mechanisms (Baier, Dieterich, Stoeter, Birklein, & Müller, 2010). Because FMRP is expressed in the cerebellum (Zangenehpour, Cornish, & Chaudhuri, 2009), Purkinje cell-specific knockouts of FMR1 can demonstrate the implications of cerebellar abnormalities in FXS, such as impaired eyeblink conditioning (Koekkoek et al., 2005). Cerebellar error correction may affect executive and motor systems similarly through the general use of temporal information to link stimulus and response. Temporal information processing in a CGG knock-in (KI) mouse model of the premutation is impaired in an age-dependent manner (Borthwell, Hunsaker, Willemsen, & Berman, 2012; Hunsaker, Goodrich-Hunsaker, Willemsen, & Berman, 2010). Thus, inaccurate temporal input to the cerebellum, or dysfunctional processing within the cerebellum, may lead to executive function and motor impairment.

Cerebellar Atypicalities in fXPCs

The cerebellum, or parts of it, is reduced in size in men and women with FXTAS (Adams et al., 2007; Cohen et al., 2006; Hashimoto, Javan et al., 2011), which is thought to be due to atrophy. Increased FXTAS severity is linked to decreased cerebellar volume (Adams et al., 2007), particularly in part of the vermis (Hashimoto, Javan et al., 2011). Cerebellar volume reduction is also linked to increased CGG repeat length (Adams et al., 2007; Cohen et al., 2006).

FXPCs asymptomatic for FXTAS show a similar, though less consistent, pattern of results. Voxel-based morphometry (VBM) studies using region of interest (ROI) (Hashimoto, Javan et al., 2011) and whole-brain analyses (Battistella et al., 2013) found that asymptomatic fXPCs had gray matter reductions in anterior sub-regions of the cerebellar vermis and hemisphere. Meanwhile, other studies of fXPCs asymptomatic for FXTAS found volumetric reductions in the brainstem (Cohen et al., 2006), but in no other regions (Adams et al., 2007). An alternate technique, magnetic resonance spectroscopy, used in three men with FXTAS demonstrated atrophic cerebellum and brain stem, but relatively spared vermis (Ginestroni et al., 2007). These differential findings are likely due to variation in onset and rate of neurodegeneration across brain regions.

A major diagnostic feature of FXTAS is white matter hyperintensity on the T2-weighted magnetic resonance images of the cerebellar white matter and middle cerebellar peduncles (MCP sign) (Jacquemont et al., 2003), although nonspecific white matter

Figure 1. Fixation performance. (A) Magnitude of gaze deviation did not differ between groups. (B) Gaze deviation interacted with age, such that it increased with age in HCs, and decreased with age in fXPCs. Black triangle = HC with motor difficulty.

Figure 2. Pursuit performance. There was no effect of group (A, C, E) or age (B, D, F) on performance. Black triangle = HC with motor difficulty.
lesions are also found throughout the cerebellum and cerebral hemispheres (Brunberg et al., 2002). The MCP sign has been reported in a woman with FXTAS (Hagerman et al., 2004), indicating that females exhibit this feature, even though they possess a second X chromosome and should be less affected than males.

White matter tracts through the MCP, which connect the cerebellum to the rest of the brain, are also affected in fXPCs. In older fXPCs (>40 years old), diffusion tensor imaging (DTI) was used to show that mean diffusivity was elevated in the MCP and left cerebral peduncle (Hashimoto, Srivastava, Tassone, Hagerman, & Rivera, 2011). DTI tractography and tract-based spatial statistics (TBSS) were used to show that fXPCs with FXTAS, relative to controls, exhibited lower structural connectivity and white matter integrity, and greater age-related decline in these measures (Wang, Hessl, Hagerman, Tassone, & Rivera, 2011). These differences were observed in several white matter tracts, including the motor fiber tract through the cerebellar peduncle. Younger (<45 years old) fXPCs asymptomatic for FXTAS were also affected, because they were found to have greater age-related decline in structural connectivity in the extreme capsule. These results suggest increased neurodegeneration within the cerebellum and along relevant white matter pathways in fXPCs.

Midsagittal Cerebellar Vermis Area

We observed that midsagittal cerebellar vermis area did not differ between HCs and fXPCs. This is inconsistent with prior reports of reduced cerebellar volume in fXPCs (Adams et al., 2007; Battistella et al., 2013; Cohen et al., 2006; Loesch, Litewka et al., 2005; Moore, 2004). Several reasons may explain this discrepancy. First, previous studies examined whole cerebellar volume, often normalized to total cranial volume (TCV). The current study examined midsagittal cerebellar vermis area normalized to total brain volume, which, while reduced in size in individuals with FXS (Mostofsky et al., 1998; Reiss et al., 1991), might not be reduced in size in fXPCs. It could be the case that, in fXPCs asymptomatic for FXTAS, vermis regions other than mid-sagittal vermis area are more sensitive to group differences between fXPCs and HCs.

Second, previous studies examined fXPCs with FXTAS (Adams et al., 2007; Cohen et al., 2006) or unknown FXTAS status (Loesch, Churchyard, Brotchie, Marot, & Tassone, 2005; Moore, 2004). Our sample examined fXPCs asymptomatic for FXTAS. Thus the lack of group difference observed in our sample may reflect that cerebellar volume reductions are caused by degeneration, and that this degeneration is age-dependent and/or reflects FXTAS disease progression. It is possible that our sample, if examined when they were older, would demonstrate group differences. Alternatively, reduced cerebellar vermis size may be a feature of FXTAS disease progression (including prodromal stages), and thus may not be present in fXPCs who do not develop FXTAS.

Third, our sample size may have been too small to observe group differences with small effect size. The study that observed differences between asymptomatic fXPCs and HCs had a sample size twice as large (Battistella et al., 2013). Future studies with large sample sizes as well as behavioral measures are needed to examine the functional impact of reduced cerebellar volume in fXPCs.

Finally, our finding of group differences in function (i.e., inhibitory control) but not in structure (i.e., cerebellar vermis area), while unexpected, is informative. First, it indicates that inhibitory control impairments in fXPCs are not due to gross reductions in size in the vermis. Second, interpretations of our findings lead to several, testable hypotheses. Specifically, our finding might suggest that cerebellar vermis volume reductions in fXPCs represents a degenerative process, and that inhibitory control deteriorates at an earlier stage. Alternatively, it might also suggest that measuring the whole cerebellar vermis, as opposed to midsagittal area, is more sensitive to group differences in this population. Another interpretation might be that inhibitory control impairments are a more common feature than cerebellar atypicalities in fXPCs. Lines of questioning that arise from our results could clarify what features are common in all fXPCs and which are specific to FXTAS, and what features characterize asymptomatic fXPCs who will later develop FXTAS.
Inhibitory Control

FXTAS is often accompanied by inhibitory control impairments (Cornish et al., 2011; Grigsby et al., 2007; 2008), even in fXPCs without motor symptoms (Cornish et al., 2009). This suggests that inhibitory control impairments might precede motor impairments. The cerebellum is thought to be involved in executive functions such as inhibitory control (Bellebaum & Daum, 2007), and is known to be involved in oculomotor control. Because of this, cerebellar abnormalities in fXPCs, even in those asymptomatic for FXTAS, may mediate the link between impaired inhibitory control and impaired motor control.

Our results are consistent with this mechanism. We found that fXPCs have longer saccade latency in the antisaccade task, which indicates they have impaired inhibitory control. Inhibitory cost, calculated as the difference in RT in between the antisaccade and prosaccade tasks, was greater in fXPCs than HCs, and tended to increase with age in HCs, but not in fXPCs. This suggests that inhibitory control typically declines with age (e.g., inhibitory cost increases with age in HCs), and that fXPCs exhibit inhibitory control impairments earlier in life relative to HCs (e.g., inhibitory cost is already elevated). Because ADHD symptoms did not differ between groups, it is unlikely that group differences in inhibitory cost are related to increased ADHD symptoms and prevalence in fXPCs.

We observed that in fXPCs, inhibitory cost correlated positively with CGG repeat length. This finding was significant when the larger repeat length (156) was used, but not when the mean repeat length (138) was used, for the individual who expressed two variants of the \textit{FMR1} allele. This suggests that the presence of one longer allele might actually be more detrimental than the presence of two shorter alleles.

Inhibitory cost correlated positively with area of cerebellar vermis lobules VI-VII in fXPCs. Because we found that fXPCs have increased inhibitory cost, and other studies report that fXPCs have decreased cerebellar vermis volume, this correlation may at first seem counterintuitive. We interpret these results to suggest that lobules VI-VII are particularly important for orienting of spatial attention, but that this region is not reduced in size in fXPCs relative to HCs (Adams et al., 2007; Battistella et al., 2013; Cohen et al., 2006; Loesch, Litewka et al., 2005; Moore, 2004). This may be because other regions of the cerebellum, such as the lateral hemispheres, are more sensitive to group differences than this specific region. Alternatively, this region may be sensitive to group differences, but measuring the midsagittal area is insufficient to detect these differences.

Figure 4. Antisaccade performance. (A–B) fXPCs had longer saccade latency than HCs, and latency did not change with age for either group. (C–D) Saccade magnitude did not differ between groups or with age. (E–F) Number of anticipatory saccades did not differ between groups, and increased with age in HCs. (G–H) Percentage of trials with directional errors did not differ between groups, and increased with age in HCs. (I–J) Inhibitory cost was greater in fXPCs than HCs, and increased with age in HCs. Black triangle = HC with motor difficulty. † \( p = 0.06. \) * \( p < 0.05. \) ** \( p < 0.06. \)
Limitations

Although this study examined the effect of age on oculomotor performance, this study was cross-sectional in design, so we cannot make conclusions about how oculomotor control develops across adulthood. Our age range was limited to 18–48 years old. Because FXTAS risk is age-dependent (Jacquemont et al., 2004), and we found that some oculomotor functions are age-dependent, the participants in our sample may have been too young to exhibit impairments in these tasks. This may partially explain why we did not observe group differences in the Fixation task, which requires inhibitory control but may be easier than the Antisaccade task.

Due to our relatively small sample size, we could not compare groups of older and younger FXPCs. Sample size may have also affected our ability to detect an association between CGG and oculomotor performance. Because CGG has a nonlinear effect on some measures (Roberts et al., 2009; Seltzer et al., 2012), a larger sample would have allowed comparison of FXPCs in the upper versus lower premutation range.

Women were not included in this study because they do not develop FXTAS at rates as high as in their male counterparts (Jacquemont et al., 2004). Females may also have a different population distribution of CGG expansions than males, with more frequent high-repeat alleles (Hunter et al., 2008b). This increased variability in CGG repeat length makes observations of associations between performance and CGG repeat length more likely in women than men.

Conclusion

Because not all carriers of the fragile X premutation develop FXTAS, it is important to better specify the characteristics of FXTAS, including risk factors, early onset symptoms, and pattern of disease progression. In this study we examined whether inhibitory control impairments are present in FXPCs asymptomatic for FXTAS, and we found that they are present in the oculomotor domain. This demonstrates that the eye movement system is sensitive to impaired inhibitory control in FXPCs who do not exhibit clinical or gross body motor impairment. Thus, eye movements may be useful in assessing FXTAS risk or disease progression.

References


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Tassone, F., Pan, R., Amiri, K., Taylor, A. K., & Hagerman, P. J. (2008). A rapid polymerase chain reaction-based screening method for identifi-
cation of all expanded alleles of the fragile X (FMR1) gene in newborn and high-risk populations. The Journal of Molecular Diagnostics, 10, 43–49. doi:10.2353/jmoldx.2008.070073


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Correction to Wong et al. (2014)

In the article “Eye Movements Reveal Impaired Inhibitory Control in Adult Male Fragile X Premutation Carriers Asymptomatic for FXTAS” by Ling M. Wong, Naomi J. Goodrich-Hunsaker, Yingratana McLennan, Flora Tassone, Melody Zhang, Susan M. Rivera, and Tony J. Simon (Neuropsychology, Advance online publication. April 28, 2014. doi: 10.1037/neu0000066), in the Method section under the Participants subsection, the last two exclusion criteria for participants are distinct and should have been listed as two entities, separated by a semicolon: “history of alcoholism or drug problem; and use of medication that affects cerebral blood flow (e.g., current beta blockers).” Likewise, the authors wish to clarify that the dependent measure for the fixation behavioral task, in the Procedure subsection, was maximum gaze deviation from center during target presentation. Lastly, the last sentence in the Antisaccade task procedure section should read: “These were trials in which the initial saccade was in the same direction as the target.”

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