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Age-related functional brain changes in *FMR1* premutation carriers

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ABSTRACT

The *FMR1* premutation confers a 40–60% risk for males of developing a neurodegenerative disease called the Fragile X-associated Tremor Ataxia Syndrome (FXTAS). FXTAS is a late-onset disease that primarily involves progressive symptoms of tremor and ataxia, as well as cognitive decline that can develop into dementia in some patients. At present, it is not clear whether changes to brain function are detectable in motor regions prior to the onset of frank symptomatology. The present study therefore aimed to utilize an fMRI motor task for the first time in an asymptomatic premutation population.

Premutation carriers without a diagnosis of FXTAS ($n = 17$) and a group of healthy male controls ($n = 17$), with an age range of 24–68 years old, were recruited for this cross-sectional study. This study utilized neuroimaging, molecular and clinical measurements, employing an fMRI finger-tapping task with a block design consisting of sequential finger-tapping, random finger-tapping and rest conditions. The imaging analysis contrasted the sequential and random conditions to investigate activation changes in response to a change in task demand. Additionally, measurements were obtained of participant tremor, co-ordination and balance using the CATSYS-2000 system and measures of *FMR1* mRNA were quantified from peripheral blood samples using quantitative real-time PCR methodology.

Premutation carriers demonstrated significantly less cerebellar activation than controls during sequential versus random finger tapping ($FWE_{corr} < 0.001$). In addition, there was a significant age by group interaction in the hippocampus, inferior parietal cortex and temporal cortex originating from a more negative relationship between brain activation and age in the carrier group compared to the controls ($FWE_{corr} < 0.001$).

Here, we present for the first time functional imaging-based evidence for early movement-related neurodegeneration in Fragile X premutation carriers. These changes pre-exist the diagnosis of FXTAS and are greatest in older carriers suggesting that they may be indicative of FXTAS vulnerability.

1. Introduction

It is estimated that approximately 1 in 250–810 males in the general population carry the *FMR1* premutation; a genetic status conferred by an expansion of the 5′ untranslated CGG repeat region of the *FMR1* gene from < 55 repeats to between 55 and 200 repeats (Rifé et al., 2003; Sorensen et al., 2013). Up to 45 repeats is considered to be within the normal range and 45–55 repeats is considered to be an intermediate expansion. CGG repeat regions exceeding 200 usually become methylated, and as such prevents transcriptional regulation, silencing the *FMR1* gene. This is known as a full mutation, causing Fragile X Syndrome, which is functionally and pathologically separate from disorders linked to the Fragile X premutation (Tassone and Berry-Kravis, 2010).

Until relatively recently it was believed that carriers of the *FMR1* repeat expansion were phenotypically unaffected. However, research carried out over the last two decades has revealed that carriers of the *FMR1* premutation are at risk of developing a late onset neurological condition called Fragile X-associated Tremor Ataxia Syndrome (FXTAS), which is characterised by progressive core symptoms of tremor, ataxia and cognitive decline. Males have a 40–60% chance of developing FXTAS, whereas females are at lower risk, with a 8–16% chance of developing the disease (Jacquemont et al., 2004; Hall et al., 2005). The clinical presentation of FXTAS usually begins with subtle cognitive decline, especially in executive function and working memory, which develops into full dementia in about 40% of male patients (Bourgeois et al., 2007). Motor symptoms usually include tremor, ataxia and inability for tandem gait. Parkinsonism can also occur in some individuals with FXTAS, as well as peripheral neuropathy,

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autonomic dysfunction and endocrine dysfunction (Loesch et al., 2005; Berry-Kravis et al., 2007; Wang et al., 2013). In addition to these neurological symptoms, a diagnosis of FXTAS requires one or more radiological findings (Hall et al., 2016), including global brain atrophy, increased T2 signal intensity at the middle cerebellar peduncles (known as the MCP sign) and thinning of the corpus callosum (Filley et al., 2015; Brunberg et al., 2002). Evidence suggests that the pathology of FXTAS may be driven primarily by a two to eight fold increase in *FMR1* mRNA (Li and Jin, 2012; Garcia-Arocena and Hagerman, 2010). Intranuclear inclusions in multiple brain cell types are a pathological hallmark of FXTAS, and in both FXTAS models and patient brain slices these inclusions have been shown to contain *FMR1* mRNA. It is theorised that excess of *FMR1* mRNA causes sequestration of RNA binding proteins, forming aggregates and causing cell-wide disruption, resulting in oxidative stress and eventual apoptosis (Tassone et al., 2000; Hagerman and Hagerman, 2013).

Progressive motor symptoms are therefore a core feature of FXTAS, with clinical signs of the condition only rarely manifesting before 50 years old. It is however possible that significant neurodegeneration is already occurring before clinical features of motor dysfunction emerge. In order to determine whether this is indeed the case we set out to investigate whether subtle differences in brain activation during motor function can be elicited using functional magnetic resonance imaging (fMRI), even in premutation carriers without a diagnosis of FXTAS. We utilized a finger tapping task which contained both sequential and random finger tapping components. Compared to planned sequential tapping, random, choice-driven tapping is known to elicit higher levels of activation in classical motor regions and rely more upon regions of higher cognitive functioning, such as the dorsolateral prefrontal cortex (Gountouna et al., 2010). To the authors' knowledge, there have been no previous neuroimaging studies which have examined these functions in premutation carriers, although other studies have investigated brain changes in carriers without FXTAS. A systematic review of neuroimaging studies in premutation carriers provides an overview of these findings (Brown and Stanfield, 2015), including a reduction in brainstem volume in carriers without FXTAS (Cohen et al., 2006), significant correlation between hippocampal volume and anxiety measures in female carriers without FXTAS (Adams et al., 2010), significant elevations in axial and radial diffusivities in male carriers without FXTAS (Hashimoto et al., 2011) and significantly lower BOLD response at the right temporoparietal junction compared to controls during a working memory task in a group of male and female asymptomatic carriers (Kim et al., 2014). Finger-tapping studies in other neurodegenerative disorders, such as Huntington Disease (Bartenstein et al., 1997; Kloppel et al., 2009), Spinocerebellar Ataxia Type 3 (Duarte et al., 2016; Cleary and Ranum, 2014) and Parkinson Disease (Lewis et al., 2011) have demonstrated impaired functional activity in motor regions and dysregulation of compensatory activity, in particular during conditions with higher demand. The finger-tapping task for this study was therefore designed to involve various levels of cognitive and motor complexity using sequential and random tapping conditions, with the intention of contrasting these two conditions during the imaging analysis to isolate regions of changing activations in response to task demands.

The primary aim of this study was therefore to determine whether, compared to controls, differences in brain activation are apparent during motor function in premutation carriers who do not have a diagnosis of FXTAS. The secondary aim was to use this cross-sectional sample to look for preliminary evidence of progressive changes to brain function in premutation carriers by comparing the relationship between age and brain function in premutation carriers and controls. It was hypothesised that differences in brain activation in motor regions, such as the premotor cortex, supplementary motor area and the cerebellum, would be seen between controls and premutation carriers without FXTAS, in addition to differences in other regional involvement of compensatory mechanisms (Lewis et al., 2011; Duarte et al., 2016; Bartenstein et al., 1997; Minkova et al., 2015; Murta et al., 2016). Finally, we also predicted that the degree of change in brain activation in carriers may show an association with levels of *FMR1* mRNA and extent of movement symptoms.

Table 1
Summary data of participant age, IQ, *FMR1* mRNA level and CATSYS-2000 performance.

Participant summary data				
	Control mean	Control SD	Carrier mean	Carrier SD
Age (years)	47.6	12.9	50.4	15.1
Verbal IQ	112.8	13.5	111.1	15.8
Non-verbal IQ	117.4	7.7	108.1	13.7
Composite IQ	116.4	9.1	110.4	14.9
FMR1 mRNA level	1.9×10^{-6}	1.9×10^{-5}	3.3×10^{-6}	3.3×10^{-5}
Tremor index	43	7.2	32.5	10.6
Co-ordination index	131.1	15.4	108.8	19.1
Balance index	171.9	15.6	173.0	13.0

2. Materials and methods

2.1. Participants and recruitment

Male premutation carriers without FXTAS and a group of age-matched healthy male controls were recruited through the Fragile X Society, UK. Invitation letters were sent to 1000 individuals of known Fragile X families across the UK via the existing database held by the Fragile X Society charitable association. Eligible participant age range was between 20 and 70 years. Potential participants falling out with an age range of 20–70 years, with a diagnosis of FXTAS or from a Fragile X family but without a genetic diagnosis of the premutation were excluded. All participants had normal or corrected-to-normal vision. All participants underwent genetic testing for *FMR1* CGG repeat length (Table 1). All participants gave fully informed written consent and were screened for MRI eligibility and safety. The study was approved by South East Scotland Research Ethics Committee, NHS Lothian.

2.2. Imaging methods

All MRI data was acquired using a 3.0 Tesla Siemens MRI scanner using an 8 channel head coil. For the acquisition of functional images the TR was 1560 ms, the TE (echo time) was 26 ms, the flip angle was 66°, the FOV (field of view) was 220 mm, slice thickness was 5 mm and slice number per volume was 26. Slice order was interleaved and bottom up in the axial orientation. Each participant also underwent a T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence, in addition to fMRI sequences, for the purpose of image preprocessing. For the MPRAGE acquisition the TR was 2300 ms, the TE was 2.98 ms, the flip angle was 9°, the FOV was 256 mm, slice thickness was 1 mm and slice number per slab was 160. Structural and functional images were acquired during the same scanning session.

The finger-tapping task designed and used for this study utilized a block design and consisted of three conditions, based on Gountouna et al., 2010. In the sequential tapping condition, participants were asked to tap their thumbs and index fingers on trigger buttons in a predetermined sequence in time with a flashing symbol. In the random tapping condition, participants were asked to tap their thumbs and fingers on the trigger buttons in time with the flashing symbol in a random order of their choosing. The final condition consisted of a flashing fixation cross, where participants were asked to rest and watch the screen (Fig. 1). The purpose of this fixation/rest condition was to allow a control condition minimising possible noise from visual stimulus. The flashing symbol appeared once every second, for a duration of 0.5 s. Each condition block had a total duration of 30 s, including a 2 second prompt screen, and was repeated 4 times during the task. This