NeuroImage 65 (2013) 288–298

Contents lists available at SciVerse ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Influence of the fragile X mental retardation (*FMR1*) gene on the brain and working memory in men with normal *FMR1* alleles

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ARTICLE INFO

Article history:
Accepted 30 September 2012
Available online 12 October 2012

Keywords:
FMR1
Diffusion tensor imaging
Working memory
Cognition
White matter
Tractography

ABSTRACT

The fragile X mental retardation 1 (*FMR1*) gene plays an important role in the development and maintenance of neuronal circuits that are essential for cognitive functioning. We explored the functional linkage(s) among lymphocytic *FMR1* gene expression, brain structure, and working memory in healthy adult males. We acquired T1-weighted and diffusion tensor imaging from 37 males (18–80 years, mean ± SD = 40.7 ± 17.3 years) with normal *FMR1* alleles and performed genetic and working memory assessments. Brain measurements were obtained from fiber tracts important for working memory (i.e. the arcuate fasciculus, anterior cingulum bundle, inferior longitudinal fasciculus, and the genu and anterior body of the corpus callosum), individual voxels, and whole brain. Both *FMR1* mRNA and protein (FMRP) levels exhibited significant associations with brain measurements, with FMRP correlating positively with gray matter volume and white matter structural organization, and *FMR1* mRNA negatively with white matter structural organization. The correlation was widespread, impacting rostral white matter and 2 working-memory fiber tracts for FMRP, and all cerebral white matter areas except the fornix and cerebellar peduncles and all 4 fiber tracts for *FMR1* mRNA. In addition, the levels of *FMR1* mRNA as well as the fiber tracts demonstrated a significant correlation with working memory performance. While *FMR1* mRNA exhibited a negative correlation with working memory, fiber tract structural organization showed a positive correlation. These findings suggest that the *FMR1* gene is a genetic factor common for both working memory and brain structure, and has implications for our understanding of the transmission of intelligence and brain structure.

Introduction

The fragile X mental retardation 1 (*FMR1*) gene encodes for the fragile X mental retardation protein (FMRP), a RNA-binding protein that regulates protein synthesis for activity-dependent synaptic development and plasticity (Bassell and Warren, 2008). Both *FMR1* mRNA and FMRP have been localized in the cell body, axons, and dendrites (Bassell and Warren, 2008; Devys et al., 1993). A newly published study (Darnell et al., 2011) reported that FMRP regulates the translation of approximately one-third of the proteins in the pre- and post-synaptic proteomes, underscoring the critical role of *FMR1* in the development and maintenance of neuronal circuits.

In accordance with the importance of *FMR1* expression in brain development and function, abnormal levels of *FMR1* protein and mRNA have been found to lead to two independent brain disorders: fragile X syndrome and fragile X-associated tremor/ataxia syndrome (FXTAS) (Oostra and Willemsen, 2009). The dynamics of *FMR1* gene expression originate from expansion of the polymorphic CGG repeat in the 5′ untranslated region of the gene, which normally ranges from 5 to 44 triplets (Verkerk et al., 1991). When CGG repeats expand beyond 200 triplets (termed the full mutation range), the promoter and CGG-repeat regions of the gene generally become hypermethylated, with consequent transcriptional and translational silencing (Pieretti et al., 1991); loss of FMRP expression results in fragile X syndrome (Devys et al., 1993), the most frequent single-gene caused intellectual disability (Crawford et al., 1999). Premutation alleles with CGG lengths between 55 and 200 triplets (Pieretti et al., 1991) produce slightly reduced protein, but significantly elevated *FMR1* mRNA (Tassone et al., 2000). Transcriptional upregulation of the expanded CGG-repeat alleles is known to be toxic to both neuronal and non-neuronal cells (Garcia-Arocena and Hagerman, 2010), and has also been demonstrated to cause altered neuronal connectivity in cultured neonatal mouse neurons, and altered neuronal differentiation and migration in embryonic mice (Chen et al.,

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http://dx.doi.org/10.1016/j.neuroimage.2012.09.075

2010; Cunningham et al., 2011). Elevated levels of the expanded CGG-repeat *FMR1* mRNA could thus have an impact on child development (i.e., could be the pathogenic basis for the neurodevelopmental phenotypes among carriers of premutation alleles) (Chonchaiya et al., 2012; Farzin et al., 2006), as well as the clear RNA toxicity associated with aging (Hagerman and Hagerman, 2004). Boys with *FMR1* premutation alleles are reported to have a higher risk of developing autism spectrum disorders and attention-deficit/hyperactivity disorders (Chonchaiya et al., 2012; Farzin et al., 2006), whereas older (adult) carriers of premutation alleles may develop FXTAS, typically after 50 years of age (Hagerman and Hagerman, 2004). Among the range of noted cognitive impairments, working memory has emerged as a consistent impairment associated with both the fragile X full mutation (Lanfranchi et al., 2009) and the premutation (Cornish et al., 2009; Hashimoto et al., 2011a).

Working memory is defined as a system that temporarily holds and manipulates a limited amount of information for the accomplishment of complex cognitive tasks such as comprehension, learning, and reasoning (Baddeley, 2000). Extensive research has been conducted to understand and localize this complex cognitive function. Working memory has been recognized as a central component of human intelligence (Wechsler, 1997a). It is highly heritable (Karlsgodt et al., 2010) and is affected in a number of neurogenetic disorders in addition to fragile X syndrome, including schizophrenia, 22q deletion syndrome, and autism spectrum disorders (Greene et al., 2008; Karlsgodt et al., 2011). Functional neuroimaging studies have identified a distributed neural network that mediates the function of working memory (Rama, 2008; Zimmer, 2008). This network encompasses both modality-specific cortical areas in the temporal, parietal, and occipital lobes for encoding sensory-motor information and supramodal regions in the frontal and parietal lobes for maintaining and manipulating task information. Structural MRI studies (Burzynska et al., 2011; Charlton et al., 2010; Sepulcre et al., 2009; Takeuchi et al., 2010; Vestergaard et al., 2011; Zahr et al., 2009) have also been conducted to search for white matter tracts serving as the structural backbone of the working memory network and reported the following fiber tracts as being associated with working memory performance: the superior longitudinal fasciculus (SLF; carrying the frontoparietal pathways), inferior longitudinal fasciculus (ILF; contains the superior parietal lobule pathway in Charlton et al., 2010), corpus callosum, fornix, cingulum, thalamic radiation, and cerebellar white matter.

Only one study (Hashimoto et al., 2011a) has investigated brain-mediated *FMR1* effect on working memory. This functional MRI study reported reduced activation during a verbal working memory task in core working memory areas—right ventral inferior frontal cortex and left premotor/dorsal inferior frontal cortex for both premutation carriers with and without FXTAS compared to healthy controls. In addition, the study detected a negative effect of CGG repeat length on the activation of the right ventral inferior frontal cortex when the carriers with and without FXTAS were combined. Currently, the influence of *FMR1* expression on the brain and working memory in individuals with normal alleles remains unknown.

Accordingly, the principal-objective of the current study is to assess the functional linkage(s) among *FMR1* gene expression, the brain white and gray matter structure, and working memory performance in healthy adult males. To this end, we have acquired diffusion tensor imaging (DTI) (Basser and Pierpaoli, 1996; Mori et al., 1999) as well as T1-weighted MRI to perform an in vivo examination of the macro- and micro-structures of the gray and white matter. We found that FMRP showed a significant positive correlation with gray matter volume, and *FMR1* mRNA showed a negative correlation with brain-level white matter structural organization. Both FMRP and *FMR1* mRNA exhibited correlations with DTI tractography- and voxel-based measurements, where higher FMRP and lower *FMR1* mRNA were associated with improved white matter structural organization. In addition, *FMR1* protein and mRNA levels as well as tractography measurements correlated significantly with working memory, demonstrating the close relationships between the *FMR1* gene, the brain structure, and working memory observable in normal populations.

Materials and methods

Research participants

We recruited 37 healthy males from the local community, among which 26 were non-Hispanic Caucasian, 3 Hispanic Caucasian, 4 Asian, 3 with more than one race, and 1 of unknown ethnic background. None of the participants had neuropsychological disorders, prolonged loss of consciousness, brain infection, major psychiatric illness such as bipolar or schizophrenia, major chronic illness (cancer, liver disease, etc.), head trauma, a history of substance or alcohol abuse, or extensive T2-hyperintensive lesions or retinal damage due to diabetes or hypertension. While none of the younger males (<age 50) had diabetes or hypertension, 1 of the 13 older males (>50 years) was diagnosed as having type II diabetes and 4 had hypertension. Structured Clinical Interviews for DSM-IV (SCID-I) revealed that at the time of the study, 4 participants had a mood and/or anxiety disorder and 14 had a past mood and/or anxiety disorder (lifetime). All participants signed an informed consent and agreed to participate in the study. The local institutional review board at the University of California, Davis approved the research protocol.

Working memory assessments

Working memory performance was measured using the working memory subtests from the Wechsler Memory Scale, Third Edition (WMS) (Wechsler, 1997b), which provide standardized, age-scaled scores. The average index score is 100, with a standard deviation 15.

Molecular genetic measures

Genomic DNA was isolated from peripheral blood lymphocytes using standard methods (Qiagen, Valencia, CA). CGG repeat size was determined using real time polymerase chain reaction and Southern blot analysis performed on an Alpha Innotech FluorChem 8800 Image Detection System (San Leandro, CA) as previously described (Filipovic-Sadic et al., 2010). *FMR1* mRNA was quantified on a 7900 sequence detector (PE Biosystems) using the published method (Filipovic-Sadic et al., 2010; Tassone et al., 2000). FMRP level was measured utilizing a recently described sandwich enzyme linked immunosorbent assay for FMRP (Iwahashi et al., 2009), which quantifies the FMRP level directly rather than counting the proportion of cells with detectable staining in the commonly used immunocytochemistry method.

Neuroimaging data acquisition

We acquired DTI from 32/37 participants, the only imaging modality that allows us to examine white matter spatial organization in vivo (Basser and Pierpaoli, 1996; Mori et al., 1999). DTI measures spatial distribution of water diffusion in the brain which is constrained by brain tissues. In the white matter where axons form large fiber bundles and run in parallel, water molecules tend to diffuse more along than against fiber tracts. Based on this characteristic, the dominant diffusion direction is found by modeling the spatial distribution of water diffusion using tensors. From tensors, diffusion measurements are calculated including fractional anisotropy (FA) for measuring water-diffusion directionality and mean diffusivity (MD) for the amount of restriction to water diffusion due to the presence of brain tissues. Additional useful measurements include axial diffusivity (AD), the amount of diffusion along the dominant diffusion direction, and radial diffusivity (RD), the average diffusion perpendicular to the dominant diffusion direction. Although controversial, AD and RD have been proposed for