Published in final edited form as:

Dev Disabil Res Rev. 2009 ; 15(4): 343–352. doi:10.1002/ddrr.77.

Gene, Brain, and Behavior Relationships in Fragile X Syndrome: Evidence from Neuroimaging Studies

Amy A. Lightbody* and Allan L. Reiss

Center for Interdisciplinary Brain Sciences Research, Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, California

Abstract

Fragile X syndrome (FraX) remains the most common inherited cause of intellectual disability and provides a valuable model for studying gene-brain-behavior relationships. Over the past 15 years, structural and functional magnetic resonance imaging studies have emerged with the goal of better understanding the neural pathways contributing to the cognitive and behavioral outcomes seen in individuals with FraX. Specifically, structural MRI studies have established and begun to refine the specific topography of neuroanatomical variation associated with FraX. In addition, functional neuroimaging studies have begun to elucidate the neural underpinnings of many of the unique characteristics of FraX including difficulties with eye gaze, executive functioning, and behavioral inhibition. This review highlights studies with a focus on the relevant gene-brain-behavior connections observed in FraX. The relationship of brain regions and activation patterns to FMRP are discussed as well as the clinical cognitive and behavioral correlates of these neuroimaging findings.

Keywords

fragile X syndrome; MRI; FMR1; behavior and intellectual disability

Fragile X syndrome (FraX) remains the most common inherited cause of intellectual disability, occurring in ~1 in 4,000 males and 1 in 8,000 females [Crawford et al., 2001]. The disorder is caused by a mutation of the *FMR1* gene on the long arm of the X chromosome (locus Xq27.3) which includes a trinucleotide CGG repeat expansion [Verkerk et al., 1991]. Typically developing individuals have ~30 copies of this trinucleotide repeat. For individuals who carry a premutation form of *FMR1*, the repeat length ranges from 50 to 200 and expands to over 200 copies in individuals affected with the full mutation. When the repeat length exceeds 200, hypermethylation of the promoter region of the gene usually occurs, thus limiting the production of the *FMR1* protein product (“FMRP”). In this article, the term “FraX” refers to these individuals with the full mutation and reduced FMRP levels. FMRP is known to regulate the production of a number of important brain proteins and thus, reduced levels of FMRP impacts brain development and function. The presence of the

FMR1 full mutation has the potential to affect many aspects of an individual's life including cognition, adaptive behavior, social abilities, and motor skills. Males tend to be more affected than females due to females having a second X chromosome which produces some protein.

Physical features of FraX are variable and should never be considered “diagnostic.” These features can include a long, narrow face, prominent ears, flat feet, hypotonia, and macro-orchidism. Around puberty, these features may become more apparent. However, these features are often mild and not easily distinguished from the range in the normal population, thus a confirmatory diagnosis must be made using DNA testing [Garber et al., 2008].

Cognitively, most males with FraX fall into the moderate to severe range of intellectual disability whereas females may range from moderate intellectual disability to normal IQ. However, some females with FraX who have overall normal cognitive function may still manifest clinically relevant learning difficulties. When considered as a group, individuals with FraX are at increased risk for manifesting a specific profile of cognitive weaknesses including qualitative communication problems and difficulties with math, visual-spatial processing, executive function, and some aspects of memory [Bennetto et al., 2001; Kirk et al., 2005; Roberts et al., 2005; Lightbody et al., 2006; Mazzocco et al., 2006a,b; Sullivan et al., 2007; Hooper et al., 2008; Murphy and Mazzocco, 2008; Murphy, 2009]. As children with FraX age, a slowed rate of development is often noticed compared to peers. This slowing in development can manifest as a decline in IQ over time (but does not represent a loss of skills or degenerative process). To be specific, while still gaining skills, individuals with FraX may do so at a slower rate than their typically developing peers. Thus, their trajectory of development becomes increasingly divergent from what is typical for a particular age [Hall et al., 2008a]. Hallmark behavioral symptoms in FraX consist of gaze aversion, hyperactivity, inattention, impulsivity, social difficulties including some behaviors similar to those observed in idiopathic autism, anxiety and hyperarousal, and sometimes depression in females [Mazzocco et al., 1994, 1998; Baumgardner et al., 1995; Munir et al. 2000b; Keysor and Mazzocco, 2002; Lesniak-Karpiak et al., 2003; Farzin et al., 2006; Hall et al., 2006, 2008b; Hatton et al., 2006; Hessler et al., 2006; Sullivan et al., 2006; Murphy et al., 2007; Hooper et al., 2008].

Research on the impact of FMRP on cognitive and behavioral symptoms in FraX has produced more variable results. Several studies suggest that FMRP accounts for a large percentage of the variance in cognitive (e.g., IQ, executive function) scores, while others indicate a smaller relationship or no relationship [Tassone et al., 1999; Bennetto et al., 2001; Loesch et al., 2003a,b; Skinner et al., 2005; Lightbody et al., 2006; Hall et al., 2008a]. Findings on the impact of FMRP within the behavioral domain are also variable [Hessler et al., 2001; Glaser et al., 2003; Hall et al., 2006; Hatton et al., 2006; Loesch et al., 2007; Harris et al., 2008]. For example, several studies have attempted to determine the relationship between FMRP and behavioral features of FraX such as autistic symptoms. The results of these studies are inconsistent. One such study found a negative association between scores on one measure of autism and FMRP levels [Hatton et al., 2006]. Another study found no relationship using current “gold standard” assessment tools for autism [Harris et al., 2008], while a third study determined that statistically significant relationships

between autistic symptoms and FMRP no longer existed after controlling for IQ in individuals with FraX [Loesch et al., 2007]. Neuroimaging studies have been more consistent in finding a relationship between FMRP and brain region size differences and functional activation patterns. It is likely that the variance in findings noted above is due, in part, to attempts to relate a specific biological marker (FMRP) to more loosely defined cognitive and behavioral constructs. Further, because of the inaccessibility of brain tissue for research, most studies using FMRP as a bio-marker measure this protein in cells taken from blood samples. Thus, relating protein expression in blood cells to brain function and behavior is likely to be quite challenging and thus less conclusive. Regardless, the importance of understanding the gene-brain-behavior relationships in FraX remains clear.

Over the past 15 years, structural and functional magnetic resonance imaging studies have emerged with the goal of better understanding the neural pathways contributing to the cognitive and behavioral outcomes seen in individuals with FraX. Following is a review of these studies with a focus on the relevant gene-brain-behavior connections observed in FraX. Specifically, the relationship of brain regions and activation patterns to FMRP are discussed as well as the clinical cognitive and behavioral correlates of these neuroimaging findings.

STRUCTURAL STUDIES

Caudate Nucleus

One of the most striking findings to date in FraX is a significantly enlarged caudate nucleus. Located within the basal ganglia, the caudate is thought to play a role in movement as well as learning and complex behaviors and is likely involved in the repetitive behaviors often seen in FraX. The caudate is also viewed as a filter of information particularly to the frontal lobes which may play a role in regulating specific executive functions known to be deficient in FraX such as attention shifting [Masterman and Cummings, 1997;

caudate volumes were negatively correlated with FMRP levels

Ring and Serra-Mestres, 2002]. From a very early age, children with FraX demonstrate abnormalities in the development of the caudate nucleus. Hazlett et al. [2009] examined 52 boys with FraX ages 18–42 months in comparison to boys with idiopathic developmental delay (DD), autism (AUT), and typical development (TD). Results indicated a significant bilateral enlargement of the caudate in contrast to all control groups (DD = 45%, AUT = 26%, TD = 34%). Interestingly, children with FraX who also met study criteria for autism, demonstrated the same significant caudate enlargement seen in those children with FraX who did not meet criteria for autism. Both groups of children with FraX showed significantly larger caudate volumes than did the autism group, further suggesting this as a FraX specific finding. Utilizing voxel-based morphometry methods to examine regional brain differences in this same group of young boys, Hoeft et al. [2008] also indicated the enlarged caudate volumes in the group with FraX as compared to DD and TD children.

Several studies have also indicated the caudate finding in a wider age range of individuals with FraX [Reiss et al., 1995; Eliez et al., 2001; Lee et al., 2007; Gothelf et al., 2008]. Most recently, Gothelf et al. [2008] investigated 84 children and adolescents 1–22 years of age

with FraX (45 males, 39 females) compared to a group of similarly aged typically developing individuals. Results included a 23.4% increase in caudate volume for males with FraX and an 8.8% increase for females suggesting a gene-dose effect as would be expected in a semi-dominant X-linked disorder. An earlier study also demonstrated an overall increase in caudate volumes with larger increases seen for males (28%) than females (13%) in a group of 37 children (4–19 years) with FraX compared to their typically developing peers [Eliez et al., 2001]. However, Lee et al. [2007] did not find these same gender differences in a sample of 36 (18 males, 18 females) children with FraX ages 12–16 years despite an overall 10% enlargement of the caudate compared to typically developing children. Group size, age of the participants, and MRI analysis methods all may have influenced this difference in findings. Surface-based analyses of caudate morphology primarily indicated size differences to be localized in the head of the caudate [Gothelf et al., 2008].

Relationship to FMRP—A relationship between caudate volume and FMRP has been noted. Hoeft et al. [2008] demonstrated this relationship in very young children with FraX, indicating a negative correlation between caudate volume and FMRP levels. Similar to the relationship between caudate volumes and FMRP seen in young boys with FraX, caudate volumes were negatively correlated with FMRP levels in older children as well [Gothelf et al., 2008], thus suggesting that FMRP plays a significant role in the development of abnormal enlargement of the caudate seen in FraX.

Relationship to cognitive and behavioral domains—In support of previous research indicating a relationship between caudate volume and IQ [Reiss et al., 1995], Gothelf et al. [2008] demonstrated a negative correlation between caudate volume and IQ with caudate, superior temporal gyrus, and posterior vermis combined volumes accounting for 31% of the variance in IQ amongst the FraX group [Gothelf et al., 2008]. When considering behavioral correlates, this study found a positive correlation between caudate volumes and aberrant behavior as measured by the Aberrant Behavior Checklist and the Stereotypy subscale of the Autism Behavior Checklist, suggesting that the increased size of the caudate is impacting not only cognition, but also some of the specific behavioral deficits associated with FraX.

Cerebellar Vermis

In contrast to the enlarged caudate found in FraX, a consistent finding of decreased size of the cerebellar vermis has been demonstrated, particularly in the posterior segment of this midline cerebellar region [Reiss et al., 1991a,b; Guerreiro et al., 1998; Mostofsky et al., 1998; Gothelf et al., 2008; Hoeft et al., 2008]. The cerebellum is important in visual-spatial processing, learning, executive function, and language [Stoodley and Schmahmann, 2009]. Early neuroimaging results indicated a smaller posterior cerebellar vermis in both males and females with FraX as compared to typically developing individuals and those with developmental delay [Reiss et al., 1991a,b; Guerreiro et al., 1998]. More recent studies confirm these findings in larger sample sizes and wider age ranges [Mostofsky et al., 1998; Gothelf et al., 2008; Hoeft et al., 2008]. Mostofsky et al. examined 28 males with FraX (1–43 years) and 37 females with FraX (4–28 years) compared to typically developing and developmentally delayed control groups. Posterior vermis size was decreased for both males

and females with FraX compared to the control groups with no significant difference in size between the two control groups suggesting this as a FraX specific effect. Further, females with FraX demonstrated an intermediate effect and showed a correlation between gene dosage (as measured by *FMRI* activation ratio) and posterior vermis size which also suggests specific gene effects. A recent study of 51 young boys with FraX (1–3 years), 32 boys with typical development, and 18 boys with idiopathic developmental delay confirmed that a smaller posterior vermis occurs early in development for the FraX individual. In a pattern classification analysis this region was identified as one which helps to distinguish FraX from other groups further indicating that the posterior vermis may be a hallmark of brain anatomy for FraX [Hoeft et al., 2008].

Relationship to FMRP—A positive relationship between posterior vermis size and FMRP levels was shown in a group of children and adolescents with FraX [Gothelf et al., 2008]. Hoeft et al. [2008] did not find this same association in early childhood perhaps due to a more restricted range of FMRP values in this younger sample of only males.

Relationship to cognitive and behavioral domains—Associations between posterior cerebellar size and cognitive profiles have been reported. In the Mostofsky et al. [1998] study described above, posterior cerebellar vermis size was a significant predictor of IQ in their female sample, even after accounting for the significant influence of parental intelligence on child IQ. Posterior vermis size also predicted performance on tasks of visual-spatial ability (Block Design sub-test of the Wechsler scales and Rey-Osterreith Complex Figure) and executive function (Wisconsin Card Sorting Test), areas of increased difficulty for females with FraX. A more recent study confirmed a positive correlation of posterior vermis size with IQ in a group of 83 male and female children and adolescents with FraX. This same association was not found in control subjects [Gothelf et al., 2008]. Taken together, these findings indicate that the abnormalities seen in the posterior vermis for individuals with FraX contribute to some of the cognitive deficits exhibited in FraX.

In an initial look at autistic behaviors in 30 girls ages 6–16 years with FraX, Mazzocco et al. [1997] found a negative correlation between cerebellar vermis size (Lobules VI–VII) and autistic behaviors, particularly the communication deficits and restricted and stereotyped behaviors frequently associated with both autism and FraX. Thus, higher levels of such behaviors were associated with a smaller posterior vermis. More recently, Kaufmann et al. [2003] continued this investigation into the relationship of cerebellar vermis size to behaviors of autism in young boys (3–9 years) with idiopathic autism, Down syndrome, and FraX. In this case, the FraX group was divided based on whether they met study criteria for autism and then compared both to typically developing boys and to each other. Both FraX groups demonstrated decreased size of the vermis compared to controls, however this difference was only significant for the FraX group who did not meet criteria for autism. Those children who did meet study criteria for autism actually demonstrated a relative enlargement of Lobules VI–VII in comparison to the FraX group not meeting autism criteria. This relationship was also noted in the idiopathic autism group suggesting a possible link between the vermis and these behaviors. Study group sizes and methods for diagnosing autism may have influenced these findings which warrant further study to

determine whether there is a significant relationship between cerebellar vermis size and behaviors of autism in children with FraX.

Hippocampus

Inconsistent findings have been reported on potential abnormalities of the hippocampus in FraX, a structure important in learning and memory and one in which FMRP mRNA has been noted to have particularly high levels during human fetal development [Abitbol et al., 1993]. Initial reports on 15 male and female individuals with FraX indicated age-related increases in bilateral hippocampal volume [Reiss et al., 1994]. Similarly, Kates et al. [1997] found a ~10% increase in hippocampal volumes for 10 children (3–12 years) with FraX compared to typically developing children. In contrast, a study looking at adults with FraX did not indicate these increases in the size of the hippocampus [Jakala et al., 1997]. Further, in an examination of 52 young boys with FraX, voxel-based morphometry analysis methods demonstrated smaller hippocampal volumes for the FraX group compared to the developmentally delayed and typically developing groups [Hoeft et al., 2008]. However, using volumetric methodology on this same sample of children, results indicated a 26% enlargement of the hippocampus in the FraX group compared to the developmentally delayed group, but no differences compared to the typically developing children or those with autism [Hazlett et al., 2009]. Given the small sample sizes in the earlier studies, the wide age ranges, and the variety in analysis methods, these inconclusive results certainly warrant further investigation and longitudinal analysis to assess potentially important age-related changes in the hippocampus, particularly in light of related functional imaging findings discussed later.

Amygdala

The amygdala plays an important role in social behavior and emotion processing and shows significant enlargement in children with idiopathic autism [Sparks et al., 2002; Schumann et al., 2004; Amaral et al., 2008]. Given that children with FraX often demonstrate behaviors similar to those seen in individuals with autism, abnormalities in the amygdala may prove relevant for further understanding of the gene-brain-behavior pathways for FraX and autism. In connection with this idea, Hazlett et al. [2009] investigated this relationship in their study of brain development in FraX. Overall, the young boys with FraX (18–42 months) demonstrated smaller amygdala volumes than the control group by 8%. When the FraX group was split into those who met study criteria for autism and those who did not and were then compared to the group of children with idiopathic autism and controls, the finding of a smaller amygdala in FraX remained regardless of autism status. Similar to the impact of the enlarged caudate, the reduction in the size of the amygdala appears to be FraX-specific and not related to behaviors of autism. Indeed, Hazlett et al. [2009] did not identify any clinical correlation between amygdala volumes and measures of autism.

Consistent with the amygdala findings demonstrated in very young children with FraX, reports of significantly reduced amygdala volumes have been shown in older children as well. Kates et al. [1997] found amygdala volumes to be ~10% smaller for a group of children with FraX compared to typically developing children. A more recent investigation of children and adolescents also reported a significant reduction in amygdala volumes

compared to typically developing and developmentally delayed groups [Gothelf et al., 2008]. Amygdala size was also an important predictor of group membership in this study.

Fusiform Gyrus and Insula

As discussed below, functional MRI studies of face and emotion processing, including investigations into gaze aversion, have indicated unique activation patterns in both the fusiform gyrus and insula for individuals with FraX. These regions also represent two additional areas where volume differences exist at an early age in FraX [Hoeft et al., 2008]. The fusiform gyrus is important in face processing while the insula plays a role in emotion processing and regulation, both areas of potential difficulty for individuals with FraX.

In a large sample of young males with FraX ages 1–3 years, voxel-based investigations found enlarged fusiform gyrus and significantly-reduced insula volumes in comparison to developmentally delayed and typically developing age and gender matched children. Further, these regions were identified as distinct for FraX in a pattern classification analysis which predicted group membership with greater than 90% accuracy [Hoeft et al., 2008].

FUNCTIONAL STUDIES

Functional neuroimaging studies in FraX have begun to emerge in the past decade. Due to the challenges of holding still and maintaining attention for extended periods of time during an MRI scan, most early fMRI studies in FraX compared relatively higher functioning females to typically developing individuals. With advancements in behavioral training and preparation for the MRI scan, successful studies of males with FraX in comparison to IQ-matched individuals, have started to emerge in the past few years. This is important for further understanding the relative impact of the *FMRI* gene and for identification of differences that may exist between males and females with FraX dependent on their behavioral and cognitive distinctions. Understanding these distinctions will help facilitate the design of potential cognitive training programs intended to improve selected brain functions in individuals with FraX.

Face and Emotion Processing

Gaze aversion remains one of the hallmark behavioral symptoms of FraX. Behavioral studies have investigated the underlying causes for this avoidance of eye contact [Hall et al., 2006; Hessel et al., 2006; Murphy et al., 2007] and several neuroimaging studies look to elucidate the neural underpinnings and behavioral correlates of this common feature [Garrett et al., 2004; Dalton et al., 2008; Holsen et al., 2008; Watson et al., 2008]. An initial study of face and gaze processing [Garrett et al., 2004] examined this function in 10–22 year old females with FraX ($N = 11$). Using an fMRI task where participants viewed photographs of forward-facing or angled faces with either direct or averted gaze, this study sought to determine how the fusiform gyrus (FG) and superior temporal sulcus (STS), areas of the brain known to activate in response to face and gaze stimuli, responded to such images in persons with FraX when compared to age-matched typically developing girls. Girls with FraX had more difficulty determining the direction of gaze than did the control group which may have been related, in part, to IQ. In response to direct gaze versus averted gaze, the

FraX group demonstrated more activation than the control group in areas such as the right insula and the cerebellum. In contrast, the control group showed more activation than the FraX group in response to direct gaze in the left posterior insula, the STS, lingual gyrus, and cerebellum. The authors suggest the idea that increased insula activation in FraX could be related to anxiety or emotional responses evoked by faces.

Region of interest findings from this study indicated that while typically developing participants demonstrated greater FG activation for forward faces than for angled faces, FraX participants did not show a difference in activation based on the direction of the face. Further, the control group showed significantly greater activation in the right hemisphere for the FG than the left; whereas, the FraX group did not demonstrate hemispheric differences. For the STS, the typically developing subjects activated this brain region, particularly the right hemisphere, significantly more than did the girls with FraX for all task stimuli. The authors concluded that the control group likely demonstrated a more specialized activation of the FG in response to forward faces because forward faces may be more socially significant. In contrast, the FraX group may not show this specialization because they have a propensity toward looking at away facing people in social situations. Thus, while the girls with FraX are processing facial stimuli in a relatively appropriate manner, they have not developed a preference for the more socially relevant forward faces in terms of brain activation. The FraX participants also had decreased activation in the STS which is consistent with previous reports of anatomical abnormalities in this region [Reiss et al., 1994]. As Garrett et al. [2004] note, whether individuals with FraX fail to develop normal gaze processing abilities due to their behavioral avoidance of eye gaze or the eye gaze avoidance results from the anatomical disruptions in this or other parts of the brain remains an area for further investigation and longitudinal studies of young children with FraX.

A follow-up study [Watson et al., 2008] examined how boys with FraX responded to the same facial stimuli utilized in the Garret et al. [2004] study. In this study, 13 adolescent boys with FraX were compared to age and gender matched typically developing ($N = 13$) and developmentally delayed ($N = 10$) participants to determine whether FraX showed unique patterns of brain activation in response to facial eye gaze. When comparing brain activation for direct gaze versus averted gaze, the TD and DD groups demonstrated greater activation in the right midfrontal gyrus and cingulate cortex in response to the direct gaze while the FraX group showed greater activation in these areas for the averted gaze. In contrast, the FraX group responded more in the left insula to the direct gaze, an area related to arousal. The study further examined the degree to which brain regions showed decreased (adaptation) or increased (sensitization) activation when direct gaze stimuli were repeated. Consistent with the authors' hypotheses, the FraX group had significant sensitization in the left amygdala compared to the two control groups. Thus, brain regions related to emotion perception and arousal showed greater sustained activation in the FraX subjects in response to direct gaze. This pattern did not exist for the TD and DD groups despite similar task performance between the DD and FraX participants.

Relationship to social anxiety and autism symptoms—In a study of encoding of facial stimuli, Holson et al. [2008] examined the impact of social anxiety in 11 adolescents with FraX (5 males, 6 females) compared to an age and gender matched control group of

typically developing individuals. During the fMRI task, participants saw faces with fearful expressions and were to determine the gender of each face. Eye movements were tracked during the scan. Post MRI, participants saw the same pictures along with distracter pictures and were asked whether they had previously seen the face during the MRI scan. All participants also completed a measure of social anxiety, the Social Phobia and Anxiety Inventory (SPAI; adult and child versions). SPAI scores were converted to z-scores to maintain consistency across the two versions. As expected, individuals with FraX reported higher levels of anxiety than did the control group.

When examining the difference between groups in response to remembered faces, the control group showed more activation in both the superior frontal gyrus and medial frontal gyrus, areas associated with social cognition. Interestingly, the FraX group actually demonstrated reduced activation in these areas for both remembered and forgotten faces. While time spent looking at the eyes of a picture was not related to whether the face was remembered in either group, for the FraX group, increased time spent looking at the eye area of the faces resulted in more activation in the left angular gyrus and less activation in the posterior cingulate gyrus for those faces that were remembered. In contrast, the control group demonstrated increased activation in both the posterior cingulate gyrus and the left insula with increased eye gaze for remembered faces.

within the FraX group, those with higher levels of anxiety fail to utilize the encoding, social cognition, and memory related areas of the brain to the appropriate degree while those with lower levels of anxiety are better able to recruit these brain regions

Additional examination of the impact of anxiety on successful encoding of facial stimuli revealed SPAI scores to be negatively correlated with activation in the left inferior frontal gyrus (IFG), right medial frontal gyrus (MFG), right superior frontal gyrus (SFG), and left hippocampus in the FraX group while the typically developing individuals exhibited increased activation in connection with increased SPAI scores in the left IFG and right SFG, but no relationship in the other regions. These relationships were independent of age and IQ. The authors conclude that within the FraX group, those with higher levels of anxiety fail to utilize the encoding, social cognition, and memory related areas of the brain to the appropriate degree while those with lower levels of anxiety are better able to recruit these brain regions. The contrasting activation patterns seen in the control group reflect a possible difference in the impact of social anxiety on brain activation profiles. The authors suggest that perhaps typically developing individuals with higher levels of anxiety have heightened arousal in response to social stimuli and they, therefore, recruit encoding and social cognition regions to a greater extent in an attempt to override their anxiety. The idea that social anxiety, including time spent looking at the face and eyes, impacts multiple aspects of cognition and behavior for individuals with FraX is an interesting concept that warrants further investigation both from neuroimaging and behavioral approaches.

Finally, numerous studies have attempted to elucidate the behavioral overlap between FraX and autism [Farzin et al., 2006; Hatton et al., 2006; Loesch et al., 2007; Hall et al., 2008b; Hernandez et al., 2009]. However, while there exists symptomatic overlaps between the two disorders, perhaps the fundamental route of these symptoms is different for individuals with

FraX versus those with idiopathic autism [Reiss, 2009]. Few imaging studies have undergone attempts at identifying such a distinction at the neural level, particularly from a functional standpoint. One such study, however, does examine the face and emotion processing network for FraX as it compares to that of individuals diagnosed with an autism spectrum disorder (ASD) and typical development (TD) [Dalton et al., 2008]. Participants with FraX were assessed for characteristics of autism using the Social Communication Questionnaire (SCQ) and diagnoses of autism spectrum disorders were confirmed for the ASD group using the Autism Diagnostic Interview-Revised (ADI-R). The FraX group ($n = 9$) consisted of three males and six females, while the ASD group ($N = 14$) consisted of all males, nine of whom met criteria for autism and five of whom met criteria for Asperger's. The TD group ($N = 15$) was comprised of twelve males and three females. The FraX group was significantly older ($M = 20.7$ years) than the other two groups (ASD: $M = 15.9$; TD: $M = 16.8$ years), but did not differ significantly from the ASD group on IQ. The fMRI task used in this study required participants to view pictures of human faces and decide whether the facial expression was neutral (no emotion displayed) or emotional (happy, scared, angry). Eye movements were also tracked during the experiment and calculated based on time spent looking at the eyes, mouth, or other parts of the face that did not include the designated eye or mouth region. Accuracy of emotion judgment was similar for the FraX and AUT groups with both groups exhibiting lower accuracy scores than the TD group. Gaze fixation patterns were similar across all three groups. Brain activation maps comparing the participants with FraX to TD across all facial photographs regardless of emotional state indicated less activation in the right fusiform gyrus (FG) for the FraX group similar to that noted in the Garrett et al., [2004] study. In contrast, activation in the FG was similar across the FraX and AUT groups. Interestingly, the activation in both the left and right FG was highly correlated in both the FraX and AUT groups with the time spent looking at the eyes of the photographs. More time spent looking at the eyes resulted in more activation in the FG. Further, for the FraX group, right FG activation was negatively correlated with SCQ scores and both findings remained significant when parsing out the influence of IQ, suggesting an effect independent of cognitive level.

The FraX group in this study did demonstrate a unique pattern of significantly greater brain activation in response to facial stimuli in contrast to both the AUT and TD groups. These regions included greater activation in the left hippocampus (HIPP), right insula (INS), left postcentral gyrus (PCG), and left superior temporal gyrus (STG). The authors suggest that the increased activation in these areas could be due to reduced habituation to stimuli with an emotional basis as well as increased anxiety or fear in response to such stimuli as would be consistent with some of the social avoidance behaviors noted in FraX. When looking at the cognitive (IQ) and behavioral (SCQ) correlates within the FraX group, the authors found a negative correlation with IQ and positive correlation between SCQ score and left HIPP. While IQ and autism characteristics are highly related in FraX, the SCQ and HIPP relationship remained after controlling for IQ. Autism characteristics were not correlated with HIPP activation for the AUT group suggesting perhaps a different neural pathway for some of the specific social impairments seen in FraX [Dalton et al., 2008].

In summary, the social and gaze avoidance behaviors seen in FraX are complex and range in severity across individuals. Diagnostic symptoms of autism may capture some of these behaviors, but also may not fully explain the pathophysiology of the complex social cognition profiles shown by individuals with FraX. It is important for future studies to take this into account when examining the neural pathways of such behaviors.

Executive Function

Individuals with FraX have known deficits in executive function, particularly in the areas of working memory (the ability to hold and manipulate stored information in the brain), attention, and behavioral inhibition [Munir et al., 2000a,b; Kirk et al., 2005; Farzin et al., 2006; Lightbody et al., 2006; Sullivan et al., 2006, 2007; Hooper et al., 2008]. Functional MRI studies have attempted to elucidate the underlying neural mechanisms at work in these deficits and how they relate to the biological determinants of FraX such as FMRP.

the social and gaze avoidance behaviors seen in FraX are complex and range in severity across individuals. Diagnostic symptoms of autism may capture some of these behaviors, but also may not fully explain the pathophysiology of the complex social cognition profiles shown by individuals with FraX

Working memory—Kwon et al. [2001] and Menon et al. [2000] describe the relationship between FMRP, neural substrates, and behavioral outcomes in a study utilizing a visuospatial working memory task tapping the particular processes involved in working memory. Ten females with FraX (10–23 years) were compared to 15 typically developing females (8–22 years) on a visuospatial working memory fMRI task with three conditions. Participants saw the letter “O” presented on a screen in various locations. In the control condition, the subject pushed a button if the stimulus was in the center of the screen. In the “1-back” condition, the button was pushed if the stimulus location was the same as in the immediately prior trial. For the “2-back” condition, subjects responded if the stimulus was in the same position as that presented two trials back. Imaging analyses examined particular brain regions known to activate in working memory tasks including the inferior and middle frontal gyri, superior parietal lobule, and supramarginal gyrus. Behavioral data indicated significant deficits in working memory abilities for the FraX group, even when controlling for the impact of IQ. For both groups, the two-back condition was much more challenging, and significantly more so for the FraX group. Imaging results indicated that both the control group and the FraX group recruited the areas indicated for working memory when performing this task. However, a diagnosis by condition interaction was seen in that the control group exhibited increased brain activation in the two-back condition that indicated the additional effort required to perform the more difficult task. However, the females with FraX did not show this same increase in activation for the two-back condition suggesting that they were not able to recruit additional neural resources to meet the demands of the task. Further, FMRP was correlated with the brain activation seen in the two-back condition, but less so for the one-back condition. The authors hypothesized that the FraX group was recruiting the majority of their cognitive resources to accomplish the simpler one-back task and when asked to complete the two-back task, the impact of FMRP became more apparent.

Another known deficit in functioning for individuals with FraX is in the area of mathematics which often utilizes working memory capacities, particularly for mental math [Murphy, 2009]. Looking to better understand underlying mechanisms of this arithmetic deficit seen behaviorally in FraX, Rivera et al. [2002] undertook an fMRI study of 16 females with FraX (10–22 years) and 16 age and gender matched typically developing participants. In the MRI scanner, the women viewed two and three operand math equations and responded as to whether the given answer was correct. Similar to the behavioral performance outcomes seen in the N-back task described above, the FraX group performed similarly to the control group for the two operand items, but showed significantly worse performance on the three operand stimuli. This performance discrepancy occurred despite controlling for IQ differences between the groups.

The FraX and control groups both activated areas of the brain thought to be involved in mathematical computation. For the FraX group, these areas included bilateral prefrontal cortex and left angular gyrus. The control group also activated the bilateral prefrontal cortex along with parietal cortices. Again, similar to activation patterns seen with the more direct assessment of working memory, the FraX group was not able to recruit additional resources in the form of increased brain activation during the more difficult part of the task. However, the ability to recruit these resources in the three operand condition was correlated with higher level of FMRP expression. The results of these parametric study designs suggest that FMRP may be important for allowing the brain to rapidly respond to changing and more challenging aspects of the environment.

Attention and impulse control—Utilizing a version of the Stroop task adapted for the MRI scanner, Tamm et al. [2002] examined the brain activation patterns of 14 females with FraX (10–22 years) and 14 age matched typically developing females. In place of the classic Stroop color word interference task, the study employed a counting task where participants pushed a button to correspond to the number of words presented on the screen during each trial. In the control condition non-number words were presented up to four times. In the interference condition, the words for the numbers 1–4 were presented on the screen up to four times. To better understand any discrepancies in performance and brain activation that were not due to the IQ differences between the two groups, all analyses controlled for the effect of IQ. The females with FraX had more difficulty with the task and appeared to trade speed for accuracy, whereas the control group did not need that trade-off to accomplish the task. Both the FraX and control groups demonstrated activation in the inferior and middle frontal gyrus with the FraX group showing this bilaterally while the control group activation in this region was primarily in the left hemisphere. Further, the control group utilized the right inferior parietal lobe and left superior parietal lobe while the FraX group additionally recruited the left supplementary motor area. Direct group comparisons showed significantly more activation in the control group for the bilateral orbitofrontal gyrus, left insular cortex, and the left superior temporal gyrus. When accounting for varying patterns of deactivation, the left orbitofrontal gyrus emerged as the primary region where the FraX group demonstrated reduced activation compared to controls. As an area thought to be involved in executive functions and goal-directed behavior, it appears particularly relevant to the more

challenging aspects of the task for the FraX group and perhaps some of the behavioral symptoms seen in females with FraX as suggested by the authors [Tamm et al., 2002].

More recently, Hoeft et al. [2007] examined the gene-brain-behavior interplay for response inhibition in 10 adolescent males with FraX compared to 10 typically developing (TD) and 10 developmentally delayed (DD) age matched males. Using a Go/No Go fMRI task in which letters were individually presented on a screen, participants were to push a button for every letter except the letter “X.” They were to withhold their response when they saw the letter X. In the Go condition, the letter X was never seen, while in the NoGo condition, the letter X was presented half of the time. Based on previous studies and known regions underlying response inhibition, specific regions of interest included right ventrolateral prefrontal cortex (VLPFC), right caudate head, and left VLPFC.

Behavioral analyses indicate that the FraX and TD groups performed similarly with the DD group showing a trend toward lower task performance. However, brain activation looked similar for the TD and DD groups with both exhibiting greater activation than the FraX group in the ventrolateral pre-frontal cortex (VLPFC), basal ganglia, hippocampus, temporal cortex, anterior and posterior cingulate cortex, right lingual gyrus and left dorsolateral pre-frontal cortex (DLPFC). Positive correlation with task performance for the control groups was observed for the right VLPFC, right caudate, and left insula. The FraX group showed greater activation compared to both control groups in the left VLPFC, bilateral DLPFC, right cingulate, left thalamus, left intraparietal lobule, and left precuneus. Significant correlation between task performance and activation existed for the FraX group in the left VLPFC only. The authors note that while control groups were successfully recruiting the right fronto-striatal network when performing this response inhibition task, individuals with FraX demonstrated a possible compensatory strategy through increased activation in the left VLPFC.

In examining the impact of FMRP on the activation patterns shown by the FraX group, FMRP best predicted VLPFC activation in conjunction with right caudate activation. Higher levels of FMRP were associated with more typical recruitment of the fronto-striatal network, while reduced activation in the right caudate in conjunction with higher levels of FMRP resulted in more compensatory left VLPFC activation. Given the known structural abnormalities in the caudate for FraX, this interaction is particularly interesting and relevant to the understanding of this network.

The results presented above [Hoeft et al., 2007] add to a previous study looking at the same response inhibition pathways in 18 female adolescents with FraX compared to their typically developing peers [Menon et al., 2004]. Performance on the Go/NoGo task was similar across the two groups. However, the typically developing group showed significantly greater activation than the FraX group in the supplementary motor area, cingulate cortex, basal ganglia, thalamus, hippocampus, and fusiform gyrus. Further, as the authors summarize, FMRP levels correlated with areas known to be important in response inhibition including the DLPFC and VLPFC as well as the hippocampus, and basal ganglia. Activation in these areas was also related to better task performance in the typically developing group. Again, the relationship of the structural abnormalities in the caudate and the relationship to FMRP

and response inhibition skills further solidify this region as one of significant importance to understanding cognitive deficits in FraX.

CONCLUSION

Advances in neuroimaging, including behavioral training techniques to help individuals with FraX suppress head movement during MRI scans, has resulted in an increasing number of structural imaging studies with larger samples sizes and younger age ranges. These studies have established and begun to refine the specific topography of neuroanatomical variation associated with FraX. For example, both an enlarged caudate nucleus and a reduced cerebellar vermis appear consistent across studies and age ranges. In addition, both of these regions seem to correlate with levels of the protein product of the *FMR1* gene (FMRP) as well as with some cognitive and behavioral symptoms of FraX such as IQ, aberrant behavior, and autism related features emphasizing the importance of conducting research across scientific levels of investigation (i.e., gene-brain-behavior). Evidence also suggests a reduction in amygdala and insular volumes that may be related to affective symptoms of FraX. An enlarged fusiform gyrus has been noted in very young children with FraX, which, in conjunction with amygdala and insula anatomical findings, may prove particularly helpful in interpreting findings from fMRI studies involving face and emotion processing. Results for the hippocampus have been more variable with studies of younger children suggesting enlargement in this region while adults have not shown this difference. It is possible that the hippocampus may experience age related morphological changes in FraX, perhaps in part, as a result of the neurotoxic effects of chronic stress, thus contributing to differing study outcomes. Further investigation is required to test this hypothesis.

As evidenced by the neuroanatomical “profile” occurring in FraX, the impact of the *FMR1* full mutation and the subsequently reduced FMRP levels, appear to differentially affect the development of several brain structures. The developing brain goes through waves of synapse production, maturation and pruning, both pre- and postnatally, and brain structures may undergo this process at different rates [Huttenlocher and Dabholkar, 1997]. Environmental factors likely influence this process as well given the importance of learning and experience in synaptic pruning and strengthening. Molecular biological studies of FraX demonstrate the impact of FMRP on dendritic spine and synapse maturation and pruning. The brains of individuals with FraX show long, thin dendritic spines and increased spine density typical of early development rather than the larger, shorter spines seen in healthy brain maturation [Irwin et al., 2000; Weiler and Greenough, 1999]. Certain brain structures may be more sensitive to the impact of FMRP than others and the timing of when FMRP plays a critical role in neurodevelopment may differ on a region-by-region basis. Thus, one might hypothesize that the neuroanatomical profile observed in FraX is strongly related to the neurodevelopmental period when FMRP has the greatest impact. For example, it is possible that regional enlargement seen in FraX may result from a lack of synapse maturation and pruning after birth, while smaller regional size results from an early (prenatal) insult to the brain related to deficient FMRP. As such, regional size differences seen in the FraX brain could possibly indicate a time stamp for the topographic impact of FMRP on the brain.

Functional neuroimaging studies have begun to elucidate the neural underpinnings of many of the unique characteristics of FraX including difficulties with eye gaze, executive functioning, and behavioral inhibition. Several studies demonstrate similarities in brain function between typically developing and developmentally delayed individuals, with differences shown in activation patterns for FraX. This suggests a relative impact of the *FMRI* gene on functional outcomes that is independent of cognitive level.

relevant knowledge about the impact of the *FMRI* gene on brain development combined with evidence from neuroimaging data can inform treatment research for FraX that targets disease specific causes of cognitive and behavioral outcomes

Both males and females with FraX demonstrate activation patterns that differ from typically developing individuals in response to face stimuli. In females, reduced activation or inadequate hemispheric specificity in the insula and fusiform gyrus may indicate a lack of specialization seen in typically developing individuals in response to forward versus averted faces. Males demonstrate increased amygdalar and insular activity in response to direct gaze and show a negative correlation with anxiety in certain regions such as left inferior frontal gyrus, right medial frontal gyrus, right superior frontal gyrus, and left hippocampus when processing facial stimuli. Taken together with structural differences noted in the amygdala, fusiform gyrus, and insula, further investigation into the role of these regions in face processing and the influence of anxiety levels would prove enlightening and provide valuable information for intervention design.

Despite some behavioral phenotypic similarities between FraX and idiopathic autism, functional brain activation pattern differences in response to facial stimuli begin to suggest possible underlying neural differences between these groups. This is not an unexpected result, however, given the specificity of FraX as a specific “disease” with reliable biomarkers and the heterogeneity of autism as a behaviorally defined diagnosis. However, more investigation is needed to better understand which neural pathways impact the complex social cognition profiles of individuals with FraX.

Finally, functional MRI studies have begun to explain the neural functioning behind particular cognitive deficits seen in FraX such as working memory and impulse control. Individuals with FraX appear to have difficulty recruiting additional neural resources as working memory task difficulty increases. Further, this ability to utilize the brain efficiently in solving more complex problems relates to increases in FMRP levels. During response inhibition tasks, higher levels of FMRP relate to more typical recruitment of the fronto-striatal network while these higher levels of FMRP in conjunction with reduced activation in the caudate result in more compensatory utilization of the ventrolateral prefrontal cortex. These findings suggest that FMRP may influence how the human brain adapts to changes within the environment on a “real-time” basis.

Future Directions

It remains an ongoing goal of all research groups to take the information learned from studies of gene-brain-behavior investigations in FraX and apply it to the design of new interventions that target specific areas of known dysfunction in this condition. For example,

in a study of episodic memory in females [Greicius et al., 2004], results indicated decreased activation in the hippocampus and basal forebrain for girls with FraX compared to their typically developing peers. In conjunction with the finding that high levels of *FMRI* mRNA occurs in both of these brain regions during human fetal development [Abitbol et al. 1993] and the knowledge that cholinergic pathways in the basal forebrain and hippocampus are critical to numerous important cognitive functions, these data provided motivation to design a clinical trial of a medication intended to enhance cholinergic neurotransmission in the brain and subsequently improve learning and memory [Kesler et al., 2009]. In this way, relevant knowledge about the impact of the *FMRI* gene on brain development combined with evidence from neuroimaging data can inform treatment research for FraX that targets disease specific causes of cognitive and behavioral outcomes.

REFERENCES

- Abitbol M, Menini C, Delezoide AL, et al. Nucleus basalis magnocellularis and hippocampus are the major sites of FMR-1 expression in the human fetal brain. *Nat Genet.* 1993; 4:147–153. [PubMed: 8348153]
- Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. *Trends Neurosci.* 2008; 31:137–145. [PubMed: 18258309]
- Baumgardner TL, Reiss AL, Freund LS, et al. Specification of the neurobehavioral phenotype in males with fragile X syndrome. *Pediatrics.* 1995; 95:744–752. [PubMed: 7724315]
- Bennetto L, Pennington BF, Porter D, et al. Profile of cognitive functioning in women with the fragile X mutation. *Neuropsychology.* 2001; 15:290–299. [PubMed: 11324870]
- Crawford DC, Acuna JM, Sherman SL. *FMRI* and the fragile X syndrome: human genome epidemiology review. *Genet Med.* 2001; 3:359–371. [PubMed: 11545690]
- Dalton KM, Holsen L, Abbeduto L, et al. Brain function and gaze fixation during facial-emotion processing in fragile X and autism. *Autism Res.* 2008; 1:231–239. [PubMed: 19360673]
- Eliez S, Blasey CM, Freund LS, et al. Brain anatomy, gender and IQ in children and adolescents with fragile X syndrome. *Brain.* 2001; 124:1610–1618. Part 8. [PubMed: 11459752]
- Farzin F, Perry H, Hessel D, et al. Autism spectrum disorders and attention-deficit/ hyperactivity disorder in boys with the fragile X premutation. *J Dev Behav Pediatr.* 2006; 27(2 Suppl):S137–S144. [PubMed: 16685180]
- Garber KB, Visootsak J, Warren ST. Fragile X syndrome. *Eur J Hum Genet.* 2008; 16:666–672. [PubMed: 18398441]
- Garrett AS, Menon V, MacKenzie K, et al. Here's looking at you, kid: neural systems underlying face and gaze processing in fragile X syndrome. *Arch Gen Psychiatry.* 2004; 61:281–288. [PubMed: 14993116]
- Glaser B, Hessel D, Dyer-Friedman J, et al. Biological and environmental contributions to adaptive behavior in fragile X syndrome. *Am J Med Genet A.* 2003; 117A:21–29. [PubMed: 12548736]
- Gothelf D, Furfaro JA, Hoeft F, et al. Neuroanatomy of fragile X syndrome is associated with aberrant behavior and the fragile X mental retardation protein (FMRP). *Ann Neurol.* 2008; 63:40–51. [PubMed: 17932962]
- Greicius MD, Boyett-Anderson JM, Menon V, et al. Reduced basal forebrain and hippocampal activation during memory encoding in girls with fragile X syndrome. *Neuroreport.* 2004; 15:1579–1583. [PubMed: 15232287]
- Guerreiro MM, Camargo EE, Kato M, et al. Fragile X syndrome. Clinical, electro-encephalographic and neuroimaging characteristics. *Arq Neuropsiquiatr.* 1998; 56:18–23.
- Hall S, DeBernardis M, Reiss A. Social escape behaviors in children with fragile X syndrome. *J Autism Dev Disord.* 2006; 36:935–947. [PubMed: 16897394]
- Hall SS, Burns DD, Lightbody AA, et al. Longitudinal changes in intellectual development in children with fragile X syndrome. *J Abnorm Child Psychol.* 2008a; 36:927–939. [PubMed: 18347972]

- Hall SS, Lightbody AA, Reiss AL. Compulsive, self-injurious, and autistic behavior in children and adolescents with fragile X syndrome. *Am J Ment Retard.* 2008b; 113:44–53. [PubMed: 18173299]
- Harris SW, Hessel D, Goodlin-Jones B, et al. Autism profiles of males with fragile X syndrome. *Am J Ment Retard.* 2008; 113:427–438. [PubMed: 19127654]
- Hatton DD, Sideris J, Skinner M, et al. Autistic behavior in children with fragile X syndrome: prevalence, stability, and the impact of FMRP. *Am J Med Genet A.* 2006; 140A:1804–1813. [PubMed: 16700053]
- Hazlett HC, Poe MD, Lightbody AA, et al. Teasing apart the heterogeneity of autism: same behavior, different brains in toddlers with fragile X syndrome and autism. *J Neurodev Disord.* 2009; 1:81–90. [PubMed: 20700390]
- Hernandez RN, Feinberg RL, Vaurio R, et al. Autism spectrum disorder in fragile X syndrome: a longitudinal evaluation. *Am J Med Genet A.* 2009; 149A:1125–1137. [PubMed: 19441123]
- Hessel D, Dyer-Friedman J, Glaser B, et al. The influence of environmental and genetic factors on behavior problems and autistic symptoms in boys and girls with fragile X syndrome. *Pediatrics.* 2001; 108:E88. [PubMed: 11694672]
- Hessel D, Glaser B, Dyer-Friedman J, et al. Social behavior and cortisol reactivity in children with fragile X syndrome. *J Child Psychol Psychiatry.* 2006; 47:602–610. [PubMed: 16712637]
- Hoeft F, Hernandez A, Parthasarathy S, et al. Fronto-striatal dysfunction and potential compensatory mechanisms in male adolescents with fragile X syndrome. *Hum Brain Mapp.* 2007; 28:543–554. [PubMed: 17437282]
- Hoeft F, Lightbody AA, Hazlett HC, et al. Morphometric spatial patterns differentiating boys with fragile X syndrome, typically developing boys, and developmentally delayed boys aged 1 to 3 years. *Arch Gen Psychiatry.* 2008; 65:1087–1097. [PubMed: 18762595]
- Holsen LM, Dalton KM, Johnstone T, et al. Prefrontal social cognition network dysfunction underlying face encoding and social anxiety in fragile X syndrome. *Neuroimage.* 2008; 43:592–604. [PubMed: 18778781]
- Hooper SR, Hatton D, Sideris J, et al. Executive functions in young males with fragile X syndrome in comparison to mental age-matched controls: baseline findings from a longitudinal study. *Neuropsychology.* 2008; 22:36–47. [PubMed: 18211154]
- Huttenlocher PR, Dabholkar AS. Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neurol.* 1997; 387:167–178. [PubMed: 9336221]
- Irwin SA, Galvez R, Greenough WT. Dendritic spine structural anomalies in fragile-X mental retardation syndrome. *Cereb Cortex.* 2000; 10:1038–1044. [PubMed: 11007554]
- Jakala P, Hanninen T, Ryynanen M, et al. Fragile-X: neuropsychological test performance. CGG triplet repeat lengths, and hippocampal volumes. *J Clin Invest.* 1997; 100:331–338.
- Kates WR, Abrams MT, Kaufmann WE, et al. Reliability and validity of MRI measurement of the amygdala and hippocampus in children with fragile X syndrome. *Psychiatry Res.* 1997; 75:31–48. [PubMed: 9287372]
- Kaufmann WE, Cooper KL, Mostofsky SH, et al. Specificity of cerebellar vermal abnormalities in autism: a quantitative magnetic resonance imaging study. *J Child Neurol.* 2003; 18:463–470. [PubMed: 12940651]
- Kesler SR, Lightbody AA, Reiss AL. Cholinergic dysfunction in fragile X syndrome and potential intervention: a preliminary 1H MRS study. *Am J Med Genet A.* 2009; 149A:403–407. [PubMed: 19215057]
- Keysor CS, Mazzocco MM. A developmental approach to understanding fragile X syndrome in females. *Microsc Res Tech.* 2002; 57:179–186. [PubMed: 12112455]
- Kirk JW, Mazzocco MM, Kover ST. Assessing executive dysfunction in girls with fragile X or Turner syndrome using the Contingency Naming Test (CNT). *Dev Neuropsychol.* 2005; 28:755–777. [PubMed: 16266248]
- Kwon H, Menon V, Eliez S, et al. Functional neuroanatomy of visuospatial working memory in fragile X syndrome: relation to behavioral and molecular measures. *Am J Psychiatry.* 2001; 158:1040–1051. [PubMed: 11431225]
- Lee AD, Leow AD, Lu A, et al. 3D pattern of brain abnormalities in fragile X syndrome visualized using tensor-based morphometry. *Neuroimage.* 2007; 34:924–938. [PubMed: 17161622]

- Lesniak-Karpiak K, Mazzocco MM, Ross JL. Behavioral assessment of social anxiety in females with Turner or fragile X syndrome. *J Autism Dev Disord*. 2003; 33:55–67. [PubMed: 12708580]
- Lightbody AA, Hall SS, Reiss AL. Chronological age, but not FMRP levels, predicts neuropsychological performance in girls with fragile X syndrome. *Am J Med Genet B Neuropsychiatr Genet*. 2006; 141B:468–472. [PubMed: 16741913]
- Loesch DZ, Bui QM, Grigsby J, et al. Effect of the fragile X status categories and the fragile X mental retardation protein levels on executive functioning in males and females with fragile X. *Neuropsychology*. 2003a; 17:646–657. [PubMed: 14599277]
- Loesch DZ, Huggins RM, Bui QM, et al. Effect of fragile X status categories and FMRP deficits on cognitive profiles estimated by robust pedigree analysis. *Am J Med Genet A*. 2003b; 122A:13–23. [PubMed: 12949966]
- Loesch DZ, Bui QM, Dissanayake C, et al. Molecular and cognitive predictors of the continuum of autistic behaviours in fragile X. *Neurosci Biobehav Rev*. 2007; 31:315–326. [PubMed: 17097142]
- Masterman DL, Cummings JL. Frontal-subcortical circuits: the anatomic basis of executive, social and motivated behaviors. *J Psychopharmacol*. 1997; 11:107–114. [PubMed: 9208374]
- Mazzocco MM, Pennington BF, Hagerman RJ. Social cognition skills among females with fragile X. *J Autism Dev Disord*. 1994; 24:473–485. [PubMed: 7961331]
- Mazzocco MM, Kates WR, Baumgardner TL, et al. Autistic behaviors among girls with fragile X syndrome. *J Autism Dev Disord*. 1997; 27:415–435. [PubMed: 9261667]
- Mazzocco MM, Baumgardner T, Freund LS, et al. Social functioning among girls with fragile X or Turner syndrome and their sisters. *J Autism Dev Disord*. 1998; 28:509–517. [PubMed: 9932237]
- Mazzocco MM, Singh Bhatia N, Lesniak-Karpiak K. Visuospatial skills and their association with math performance in girls with fragile X or Turner syndrome. *Child Neuropsychol*. 2006a; 12:87–110. [PubMed: 16754531]
- Mazzocco MM, Thompson L, Sudhalter V, et al. Language use in females with fragile X or Turner syndrome during brief initial social interactions. *J Dev Behav Pediatr*. 2006b; 27:319–328. [PubMed: 16906008]
- Menon V, Kwon H, Eliez S, et al. Functional brain activation during cognition is related to *FMR1* gene expression. *Brain Res*. 2000; 877:367–370. [PubMed: 10986352]
- Menon V, Leroux J, White CD, et al. Frontostriatal deficits in fragile X syndrome: relation to *FMR1* gene expression. *Proc Natl Acad Sci USA*. 2004; 101:3615–3620. [PubMed: 14993603]
- Mostofsky SH, Mazzocco MM, Aakalu G, et al. Decreased cerebellar posterior vermis size in fragile X syndrome: correlation with neurocognitive performance. *Neurology*. 1998; 50:121–130. [PubMed: 9443468]
- Munir F, Cornish KM, Wilding J. Nature of the working memory deficit in fragile-X syndrome. *Brain Cogn*. 2000a; 44:387–401. [PubMed: 11104532]
- Munir F, Cornish KM, Wilding J. A neuropsychological profile of attention deficits in young males with fragile X syndrome. *Neuropsychologia*. 2000b; 38:1261–1270. [PubMed: 10865102]
- Murphy MM. A review of mathematical learning disabilities in children with fragile X syndrome. *Dev Disabil Res Rev*. 2009; 15:21–27. [PubMed: 19213014]
- Murphy MM, Mazzocco MM. Rote numeric skills may mask underlying mathematical disabilities in girls with fragile x syndrome. *Dev Neuropsychol*. 2008; 33:345–364. [PubMed: 18473203]
- Murphy MM, Abbeduto L, Schroeder S, et al. Contribution of social and information-processing factors to eye-gaze avoidance in fragile X syndrome. *Am J Ment Retard*. 2007; 112:349–360. [PubMed: 17676959]
- Reiss AL. Childhood developmental disorders: an academic and clinical convergence point for psychiatry, neurology, psychology and pediatrics. *J Child Psychol Psychiatry*. 2009; 50:87–98. [PubMed: 19220592]
- Reiss AL, Aylward E, Freund LS, et al. Neuroanatomy of fragile X syndrome: the posterior fossa. *Ann Neurol*. 1991a; 29:26–32. [PubMed: 1996876]
- Reiss AL, Freund L, Tseng JE, et al. Neuroanatomy in fragile X females: the posterior fossa. *Am J Hum Genet*. 1991b; 49:279–288. [PubMed: 1867191]

- Reiss AL, Lee J, Freund L. Neuroanatomy of fragile X syndrome: the temporal lobe. *Neurology*. 1994; 44:1317–1324. [PubMed: 8035938]
- Reiss AL, Abrams MT, Greenlaw R, et al. Neurodevelopmental effects of the FMR-1 full mutation in humans. *Nat Med*. 1995; 1:159–167. [PubMed: 7585014]
- Ring HA, Serra-Mestres J. Neuropsychiatry of the basal ganglia. *J Neurol Neurosurg Psychiatry*. 2002; 72:12–21. [PubMed: 11784818]
- Rivera SM, Menon V, White CD, et al. Functional brain activation during arithmetic processing in females with fragile X Syndrome is related to *FMR1* protein expression. *Hum Brain Mapp*. 2002; 16:206–218. [PubMed: 12112763]
- Roberts JE, Schaaf JM, Skinner M, et al. Academic skills of boys with fragile X syndrome: profiles and predictors. *Am J Ment Retard*. 2005; 110:107–120. [PubMed: 15762821]
- Schumann CM, Hamstra J, Goodlin-Jones BL, et al. The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. *J Neurosci*. 2004; 24:6392–6401. [PubMed: 15254095]
- Skinner M, Hooper S, Hatton DD, et al. Mapping nonverbal IQ in young boys with fragile X syndrome. *Am J Med Genet A*. 2005; 132A:25–32. [PubMed: 15551333]
- Sparks BF, Friedman SD, Shaw DW, et al. Brain structural abnormalities in young children with autism spectrum disorder. *Neurology*. 2002; 59:184–192. [PubMed: 12136055]
- Stoodley CJ, Schmahmann JD. Functional topography in the human cerebellum: a meta-analysis of neuroimaging studies. *Neuroimage*. 2009; 44:489–501. [PubMed: 18835452]
- Sullivan K, Hatton D, Hammer J, et al. ADHD symptoms in children with FXS. *Am J Med Genet A*. 2006; 140:2275–2288. [PubMed: 17022076]
- Sullivan K, Hatton DD, Hammer J, et al. Sustained attention and response inhibition in boys with fragile X syndrome: measures of continuous performance. *Am J Med Genet B Neuropsychiatr Genet*. 2007; 144B:517–532. [PubMed: 17427192]
- Tamm L, Menon V, Johnston CK, et al. fMRI study of cognitive interference processing in females with fragile X syndrome. *J Cogn Neurosci*. 2002; 14:160–171. [PubMed: 11970783]
- Tassone F, Hagerman RJ, Ikle DN, et al. FMRP expression as a potential prognostic indicator in fragile X syndrome. *Am J Med Genet*. 1999; 84:250–261. [PubMed: 10331602]
- Verkerk AJ, Pieretti M, Sutcliffe JS, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell*. 1991; 65:905–914. [PubMed: 1710175]
- Watson C, Hoefl F, Garrett AS, et al. Aberrant brain activation during gaze processing in boys with fragile X syndrome. *Arch Gen Psychiatry*. 2008; 65:1315–1323. [PubMed: 18981343]
- Weiler LJ, Greenough WT. Synaptic synthesis of the fragile X protein: possible involvement in synapse maturation and elimination. *Am J Med Genet*. 1999; 83:248–252. [PubMed: 10208156]