MENTAL RETARDATION AND DEVELOPMENTAL DISABILITIES
RESEARCH REVIEWS *10: 17–24 (2004)*

THE NEUROANATOMY AND NEUROENDOCRINOLOGY OF FRAGILE X SYNDROME

David Hessl,[1,2] Susan M. Rivera,[1,3] and Allan L. Reiss[4*]

[1]M.I.N.D. Institute, University of California, Davis, Sacramento, California

[2]Department of Psychiatry and Behavioral Sciences, University of California, Davis, Sacramento, California

[3]Department of Psychology, University of California, Davis, Davis, California

[4]Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, California

Fragile X syndrome (FXS), caused by a single gene mutation on the X chromosome, offers a unique opportunity for investigation of gene–brain–behavior relationships. Recent advances in molecular genetics, human brain imaging, and behavioral studies have started to unravel the complex pathways leading to the cognitive, psychiatric, and physical features that are unique to this syndrome. In this article, we summarize studies focused on the neuroanatomy and neuroendocrinology of FXS. A review of structural imaging studies of individuals with the full mutation shows that several brain regions are enlarged, including the hippocampus, amygdala, caudate nucleus, and thalamus, even after controlling for overall brain volume. These regions mediate several cognitive and behavioral functions known to be aberrant in FXS such as memory and learning, information and sensory processing, and social and emotional behavior. Two regions, the cerebellar vermis, important for a variety of cognitive tasks and regulation of motor behavior, and the superior temporal gyrus, involved in processing complex auditory stimuli, are reported to be reduced in size relative to controls. Functional imaging, typically limited to females, has emphasized that individuals with FXS do not adequately recruit brain regions that are normally utilized by unaffected individuals to carry out various cognitive tasks, such as arithmetic processing or visual memory tasks. Finally, we review a number of neuroendocrine studies implicating hypothalamic dysfunction in FXS, including abnormal activation of the hypothalamic–pituitary–adrenal (HPA) axis. These studies may help to explain the abnormal stress responses, sleep abnormalities, and physical growth patterns commonly seen in affected individuals. In the future, innovative longitudinal studies to investigate development of neurobiologic and behavioral features over time, and ultimately empirical testing of pharmacological, behavioral, and even molecular genetic interventions using MRI are likely to yield significant positive changes in the lives of persons with FXS, as well as increase our understanding of the development of psychiatric and learning problems in the general population.
MRDD Research Reviews 2004;10:17–24.

Key Words: fragile X syndrome; *FMR1* protein; MRI; fMRI; endocrine; cortisol

NEUROPATHOLOGICAL STUDIES

It has long been suggested that the decrease or absence of *FMR1* protein production associated with fragile X syndrome (FXS) may lead to brain abnormalities in affected individuals [Devys et al., 1993; Hinton et al., 1991; Rudelli et al., 1985; Tamanini et al., 1997; Wisniewski et al., 1991]. Postmortem microscopic examinations of brain tissue from patients with fragile X have revealed that although the number of neurons falls within the normal range, abnormalities in neuronal dendritic spines exist. These abnormalities are characterized by unusually long and thin, tortuous spines, with very few of the stubby, mushroom-shaped spines found in unaffected controls, and a higher density of spines along dendrites, suggesting a possible failure of synapse elimination [Hinton et al., 1991; Irwin et al., 2000a].

Brain Structure

Structural magnetic resonance imaging (MRI) studies in FXS have further explored the effects of the *FMR1* full mutation on neuroanatomy. The first brain abnormality reported in an MRI study was that of hypoplasia of the cerebellar vermis—the connecting tissue between the right and left hemispheres of the cerebellum [Reiss et al., 1988]. Vermis hypoplasia, particularly in posterior lobules VI and VII, has been confirmed in several subsequent studies [Mostofsky et al., 1998; Reiss et al., 1991a; Reiss et al., 1991b]. The vermis, which is anatomically connected to limbic structures, including the hippocampus and amygdala, has been implicated in the execution and regulation of motor behavior [Rosenthal et al., 1988], visual saccades [Hayakawa et al., 2002], auditory processing [Huang and Burkard 1986], and some aspects of language [Moretti et al., 2002, Schmitt et al., 2001]. Abnormalities of the cerebellar vermis may therefore be linked to some of the behavioral anomalies associated with FXS, including hyperactivity and repetitive movements, tactile defensiveness, attention deficits, and language dysfunction.

*Correspondence to: Allan L. Reiss, M.D., Division of Child and Adolescent Psychiatry, Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, 401 Quarry Road, Stanford, CA 94305-5719.
E-mail: areiss1@stanford.edu
Received 11 April 2003; Accepted 28 May 2003
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/mrdd.20004

Structural MRI studies also have shown that the fourth ventricle is enlarged in individuals with FXS [Mostofsky et al., 1998; Reiss et al., 1991a; Reiss et al., 1991b; Reiss et al., 1988]. Increased lateral ventricular volume also has been noted in males with FXS [Reiss et al., 1995] and it was larger in both affected males and affected females in a study of children and adolescents with FXS [Eliez et al., 2001].

Abnormalities of the temporal lobe also have been noted in FXS. Reiss and colleagues [Reiss et al., 1994] studied a sample of 15 young males and females with FXS and 26 intelligence quotient-(IQ) matched controls and reported a volumetric decrease with age of the superior temporal gyrus, an area important for processing complex auditory stimuli, including speech. In contrast to this age-related decrease, this study reported an age-related increase in both left and right hippocampal volume, a medial temporal structure important for learning, memory, and processing visuospatial information—a cognitive area known to be particularly problematic in persons with FXS [Freund and Reiss 1991; Mazzocco et al., 1993]. Similarly, Kates and colleagues [Kates et al., 1997] reported increased hippocampal volumes in children with FXS as compared to age- and gender-matched controls. Jäkälä and colleagues [Jäkälä et al., 1997] found no significant changes in normalized hippocampal volumes between full mutation and premutation groups (n = 20 in each group) but did see atypical hippocampal morphology in MRI.

Another medial temporal lobe structure, the amygdala, was first hypothesized to be affected in FXS after a study of monozygotic twin girls with the full mutation who were discordant for mental retardation [Mazzocco et al., 1995; Reiss et al., 1995]. Both twins had similar CGG expansions, activation ratios, and neonatal course without brain trauma. Twin A, however, had a full-scale IQ of 105, whereas twin B had a full-scale IQ of 47. Structural MRI analyses showed that the amygdala in twin B was 35% larger than that in twin A. In addition, whereas their overall brain size was similar, twin A also had enlarged lateral and fourth ventricles, enlarged caudate and thalamus, and a smaller posterior cerebellar vermis than twin B [Reiss et al., 1995]. Another source of information about amygdala dysfunction in FXS has come from *FMR1* knockout mouse studies. Paradee and colleagues [Paradee et al., 1999] demonstrated an abnormal conditioned fear response (less freezing behavior during contextual and cued conditions) in the knockout mouse compared to controls. The amygdala, a complex structure consisting of approximately 10 distinct nuclei, is thought to mediate both conscious and unconscious emotion processing.

Reiss and colleagues [1995] also reported larger caudate nuclei in young male and female patients with FXS, as compared to controls, and replicated this finding in a subsequent study [Eliez et al., 2001.) The caudate nucleus (which, along with the putamen, forms the structure known as the basal ganglia) has many cortical connections, the most numerous being those to the frontal lobes. The function of the caudate nucleus is to regulate, organize, and filter information. Thus, the many connections between the caudate and the frontal lobes play a large role in determining behavior. Some of the frontal–subcortical circuits that involve the caudate include those important for shifting attention, motor planning, and executive functions, all of which constitute deficits in FXS [Abrams and Reiss 1995, Mazzocco et al., 1993]. In terms of other subcortical structural abnormalities, two studies have found significantly increased thalamic volume in FXS as compared with controls, but only in girls [Reiss et al., 1995; Eliez et al., 2001.)

Thus, the many connections between the caudate and the frontal lobes play a large role in determining behavior. Some of the frontal–subcortical circuits that involve the caudate include those important for shifting attention, motor planning and executive functions, all of which constitute deficits in FXS.

Although FMRP is primarily expressed in the cell body, the dysmorphology of dendrites and synapse formation in FXS could affect the development and targeting of axons linking brain regions. Barnea-Goraly and colleagues [2003] examined the structure of white matter tracts in a group of 10 females with FXS and 10 age-matched, healthy comparison subjects using diffusion tensor imaging (DTI), a recently developed MRI technique that allows investigation of brain pathways in vivo. Relative to controls, females with FXS had decreased white matter tract connectivity in frontostriatal pathways and parietal sensory-motor tracts. Given that these pathways are thought to mediate sensory processing, executive function, regulation of affect, and motor programming, these new DTI data may provide a neuroanatomical basis for disruption of these functions in FXS.

Correlations among MRI, Cognitive/Behavioral Profiles, and Molecular Variables

If it is the case that the aforementioned differences in neuroanatomy between FXS and comparison subjects reflect the effect of absence of the *FMR1* protein on brain function and development, an association among brain-based measurements, protein expression, and cognitive/behavioral profiles should be evident. A number of studies have now examined correlations between cognitive/behavioral testing results and MRI findings. These studies provide a more direct insight into brain–behavior relationships in FXS. Whereas some early studies found no correlations between temporal lobe and posterior fossa structure measures and cognitive/behavioral measures [Reiss et al., 1994, Reiss et al., 1991a,1991b], more recent studies have found this association. Mazzocco and colleagues [1997], in a study of 30 girls with FXS and age- and IQ-matched controls, found that the size of the posterior cerebellar vermis was negatively correlated with measures of stereotypic/restricted behavior, communication dysfunction, and autistic items on a parental interview, where higher scores represent more dysfunction. By contrast, measures of anxiety did not correlate with IQ or with the size of the posterior cerebellar vermis, suggesting that anxiety, which is part of the behavioral phenotype of girls with FXS, may have a different neuropathological mechanism that is unrelated to morphology of the posterior cerebellar vermis.

Similarly, Mostofsky and colleagues [1998] studied 32 males and 37 females with FXS, along with age-matched typically developing controls as

Table 1. Neuroanatomic Abnormalities in Fragile X Patients

Brain Structure	Abnormality	Reference
Cerebellar vermis	Decreased	Mostofsky et al. [1998], Reiss et al. [1988, 1991a,b]
Fourth ventricle	Enlarged	Mostofsky et al. [1998], Reiss et al. [1988, 1991a,b]
Lateral ventricles	Enlarged	Eliez et al. [2001], Reiss et al. [1995]
Superior temporal gyrus	Decreased	Reiss et al. [1994]
Hippocampus	Enlarged	Kates et al. [1997], Reiss et al. [1994]
Amygdala	Enlarged	Mazzocco et al. [1995]
Caudate nucleus	Enlarged	Eliez et al. [2001], Reiss et al. [1995]
Thalamus	Enlarged (in females)	Eliez et al. [2001], Reiss et al. [1995]

well as controls with developmental disabilities (for the males). In females with FXS, hierarchical/stepwise regression was used to determine if size of the posterior vermis predicts cognitive performance. After statistically removing the effect of mean parental IQ (which is a strong predictor of a child's cognitive ability), they found that posterior vermis size predicted 10%–23% of the variance of performance on full-scale, performance and verbal IQ, block design (a test of visuospatial ability), the Rey –Osterrieth Complex Figure Test (a test of visuospatial perception/construction and memory), and the categories achieved on the Wisconsin Card Sorting Test (a test of executive function, including strategic planning and set shifting). No correlation was observed between size of the posterior vermis and age, suggesting that hypoplasia, not atrophy, is likely the primary cause of the small size of the vermis in FXS. These results confirm not only that the posterior cerebellar vermis is significantly affected by the *FMR1* mutation but also that the vermis is important for a variety of cognitive tasks, including executive function abilities and visuospatial abilities.

Correlations also have been observed between the size of both the caudate and lateral ventricular volume and IQ. Specifically, Reiss and colleagues [1995] found that both brain structures were significantly and inversely correlated with IQ in subjects with the full mutation, whereas for control subjects, larger caudate predicted higher IQs. The authors suggested that the mechanism for increases in caudate size could be different for controls than for individuals with FXS.

Caudate volume also has been examined with respect to *FMR1* protein expression. Reiss and colleagues [1995] used activation ratio (estimated proportion of cells that are expressing the *FMR1* protein over the total number of cells) as an indirect measure of variable *FMR1* protein expression. Multiple-regression analysis showed that a higher activation ratio (*FMR1* expression) predicted a lower (more normal) caudate volume in FXS subjects with the full mutation.

Links between neuroanatomical abnormalities and HPA axis function in FXS have been hypothesized by several authors [Wisbeck et al., 2000; Hessl et al., 2002; Miyashiro et al., 2003; Sun et al., 2001]. (Table 1) Whereas HPA axis function and FXS are covered extensively below, in the discussion of the neuroendocrinology of FXS, it is relevant to note here that a direct neurotransmitter role for corticotropin-releasing hormone, which activates the HPA axis, has been identified in the pathogenesis of stress-related behaviors [Dunn and Berridge, 1990]. Several brain regions contain binding sites for this hormone, including the hippocampus and the amygdala—regions that have been shown, either directly or indirectly, to be anomalous in FXS [Abrams et al., 1995]. Despite many endocrine and case studies implicating hypothalamic dysfunction (described below), to date there have been no published studies that directly examine the structure or function of this brain region in individuals with FXS.

Functional Magnetic Resonance Imaging (fMRI)

Functional MRI provides a picture of the brain's dynamic activity rather than its static structure. Thus, it permits the in vivo study of the neural substrates implicated in the pathogenesis of FXS.

Although there are no published fMRI studies of FXS males, there have been a handful of published studies using female participants with the full mutation. The technique has been shown to be useful in helping to elucidate the neuropathology associated with the deficits in cognitive function that characterize the syndrome. Tamm and colleagues [2002] showed that females with FXS had a significantly different pattern of activation than comparison subjects on a cognitive interference task—the Counting Stroop. The task included interference trials (during which, for example, the word *three* might be presented on the screen two times) and neutral, control trials (during which the word *fish* was presented one, two, three, or four times on the screen). For both types of trials, subjects were instructed to press the button (1, 2, 3, or 4) that corresponded to the number of words on the screen, regardless of the word. Whereas comparison subjects showed significant activation in the inferior/middle frontal gyrus and inferior/superior parietal lobe, females with FXS showed more extensive activation in the anterior region of the prefrontal cortex and failed to show expected activation in the inferior/superior parietal lobe.

Another study [Kwon et al., 2001] examined the neural substrates of visuospatial working memory in female subjects with FXS using standard one-back and two-back tasks. During these tasks, subjects saw a circle presented in one of nine distinct visuospatial locations in a 3×3 matrix. In the one-back task, the subject was instructed to respond if the stimulus was in the same location as in the previous trial. In the two-back task, the subject was instructed to respond if the stimulus was in the same location as it was two trials back. They found that subjects with FXS performed significantly worse on the more difficult, two-back task than did age-matched control subjects. Whereas comparison subjects showed a significant increase in the inferior frontal gyrus, middle frontal gyrus, superior parietal lobule, and supramarginal gyrus on the two-back as compared to the one-back task, subjects with FXS showed no change in activation between the two. Furthermore, molecular measures correlated with brain activation on this task. That is, in subjects with FXS syndrome, significant correlations were found, during the two-back task, between FMRP expression and activation in the right inferior and bilateral middle

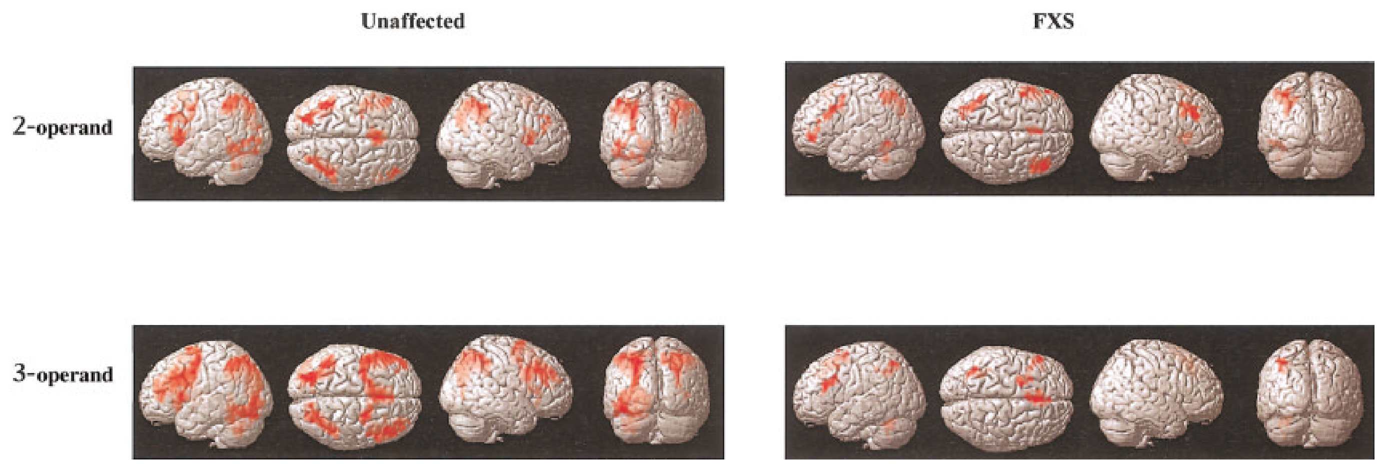

Fig. 1. Brain areas that show significantly greater activation during two- and three-operand arithmetic equations for unaffected subjects and subjects with FXS. All activations reported [Rivera et al., 2002] were significant after height ($Z > 2.33$, $P < 0.01$) and extent ($P < 0.01$) thresholding.

frontal gyri and the bilateral supramarginal gyri.

A similar finding was obtained in an fMRI study of arithmetic processing in females with the FXS full mutation [Rivera et al., 2002]. In this study, subjects with FXS exhibited less overall activation than did unaffected subjects during both two-operand (e.g., 2 + 1 = 3) and three-operand (e.g., 3 + 2 – 1 = 5) trials. Moreover, unlike the unaffected group, subjects with FXS showed no increased extent of activation in association with greater task difficulty (see Fig. 1). Between-group comparisons confirmed that, in response to increasing arithmetic complexity, unaffected subjects recruit a functional network known to be involved in arithmetic processing, but subjects with FXS do not. With respect to molecular measures, this investigation showed that with increasing levels of FMRP expression, subjects with FXS were found to activate more during the three-operand trials in areas that are involved in arithmetic processing in typically developing subjects. This result, along with the results of Kwon and colleagues [2001], provides direct evidence that decreased FMRP expression underlies deficits in cognitive performance in persons with FXS.

Neuroanatomical Findings in Male Premutation Carriers

Thus far our discussion has focused on individuals with the *FMR1* full mutation. It is important to note here that a subgroup of older males with the *FMR1* premutation (those with 55 to 200 CGG repeats, often referred to as premutation "carriers") develop a progressive neurological disorder, typically beginning after age 50, characterized by cerebellar ataxia and/or intention tremor and cognitive dysfunction [Hagerman et al., 2001, Brunberg et al., 2002; Jacquemont et al., 2003]. Structural brain MRI of these patients has revealed several abnormalities, including increased white matter signal intensity in the middle cerebellar peduncles, cerebellar cortical atrophy, and ventricular enlargement. The affected patients are reported to have *FMR1* messenger RNA (mRNA) levels 2 to 10 times higher than normal, despite mildly reduced levels of FMRP. It has been hypothesized that the elevated mRNA leads to the neuropathology, including the finding of eosinophilic intranuclear inclusions [Greco et al., 2002] and consequent clinical features. The prevalence of this newly identified syndrome is not known; however, a recent report [Macpherson et al., 2003] suggests that a significant proportion of adult males with late-onset cerebellar ataxia carry the *FMR1* premutation. (See Hagerman and Hagerman in this issue).

Neuroendocrinology

A number of studies documenting neuroendocrine dysfunction in FXS suggest that the hypothalamus may be especially affected by the decrease or absence of FMRP. Early work focused on the endocrine system to explain the physical features of the disorder such as macroorchidism (enlarged testicles). In some investigations, measures of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyroid hormone were reported to be normal [Bowen et al., 1978; Brondum Nielsen et al., 1983; Cantu et al., 1978]; however, elevated LH and FSH were documented in others [McDermott et al., 1983; Ruvalcaba et al., 1977; Turner et al., 1975]. In an evaluation of hypothalamic–pituitary–thyroid (HPT) function in 12 males with FXS, Bregman et al. [1990] reported normal levels of thyroid-stimulating hormone (TSH) but a blunted TSH response to thyrotropin-releasing hormone (TRH).

Despite a high rate of physical growth in the preadolescent period, individuals with FXS show less pubertal growth compared to normal relatives [Loesch et al., 1995]. As suggested by the investigators, this growth pattern may be an indication of premature activation of the hypothalamic–pituitary–gonadal (HPG) axis. Interestingly, growth hormone abnormalities also have been described in three separate case reports of precocious puberty in girls with FXS, each with advanced bone age and a mature response to gonadotropin-releasing hormone [Butler and Najjar 1988; Kowalczyk et al., 1996; Moore et al., 1990]. Despite the clear growth abnormalities seen in this population, to date there have been no group endocrine studies of growth hormone in FXS.

Premature ovarian failure (POF), or menopause before age 40, is found in 16%–24% of women with the fragile X premutation [Schwartz et al., 1994; Allingham-Hawkins et al., 1999; Partington et al., 1984; Vianna-Morgante et al., 1996; Cronister, 1991; Hundscheid, 2003]. In contrast, the incidence of POF in the general population of women under 40 is estimated to be 1% [Coulam et al., 1986]. The hypothesis that POF is due to hypothalamic overstimulation has been supported by several studies documenting elevated FSH in these women [Braat et al., 1999; Murray et al., 1999]. However, whether POF in the premutation is because of a hormonal abnormality, a more fundamental ovarian problem, or both is not clear.

Case studies have also supported the potential importance of hypothalamic dysfunction in FXS. For example, Fryns and colleagues [1987] described a subphenotype of FXS characterized by extreme obesity, short stature, stubby hands and feet, and hyperpigmentation similar to the features of Prader–Willi syndrome, another genetic condition associated with hypothalamic dysfunction. Interestingly, this author also described a male patient with an acquired hypothalamic lesion, macroorchidism, and facial features of FXS who was normal with regard to the *FMR1* mutation [Fryns et al., 1986].

Gould and colleagues [Gould et al., 2000] found disturbed sleep patterns and elevated day and night melatonin levels in young males with FXS in comparison to controls. Melatonin, an indoleanine derived from serotonin and regulated by the hypothalamus, is directly involved in regulating circadian rhythm and has soporific properties. It is interesting to note that these authors hypothesized that increased melatonin could be caused by malfunctioning melatonin receptors, where melatonin is overproduced to compensate for diminished receptor activation, perhaps as a consequence of the FMRP deficit. In fact, this reasoning is reminiscent of some of our own research focused on the stress hormone cortisol, also regulated by the hypothalamus. We review this work below.

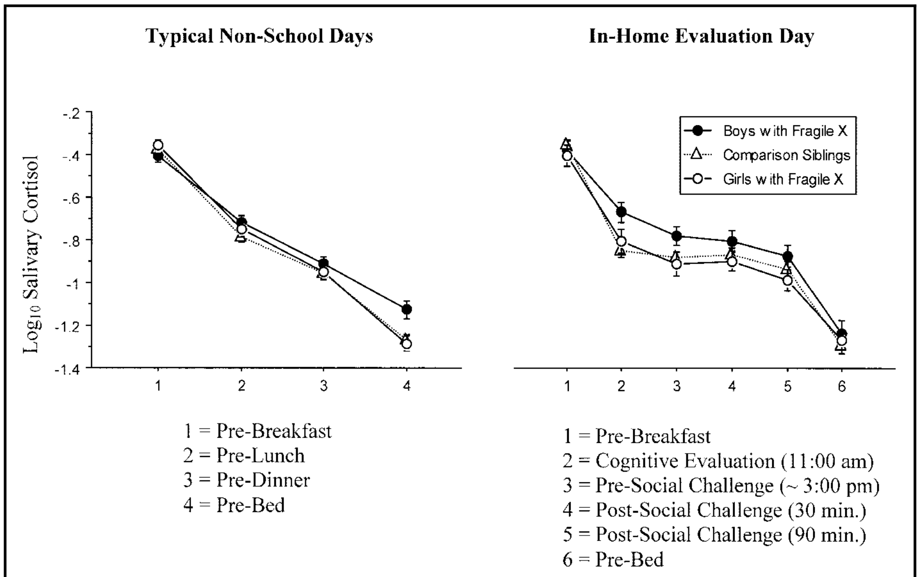

Fig. 2. Mean salivary cortisol levels ($\log_{10}$ transformed) of 39 females and 70 males with the fragile X full mutation and their unaffected siblings (58 females and 51 males) on 2 typical nonschool days (collapsed across days) and an evaluation day. On typical days, a significant main effect of diagnosis (t = -2.52, P = 0.013) and a diagnosis by gender interaction (t = -2.76, P = 0.007) showed that, in comparison to their siblings, children with fragile X, especially males, had higher levels of salivary cortisol. On the evaluation day, a significant sample time by diagnosis interaction (t = 3.01, P = 0.003) showed that children with fragile X demonstrated increased cortisol reactivity to the experience of the cognitive evaluation and meeting unfamiliar experimenters. Error bars show standard errors.

Given the numerous endocrine findings, neuroanatomical abnormalities in limbic areas, and the now well-described behavioral features of social anxiety and avoidance, we have focused our neuroendocrine research on the HPA axis, the primary biological stress response system in humans. The HPA axis is among the most intensively studied and best-described components of the neuroendocrine system. Regulation of the HPA axis is complex and involves feedback mechanisms occurring at the level of the hypothalamus, pituitary, hippocampus, amygdala, and frontal cortex. The HPA axis reacts to stress by causing the hypothalamus to secrete corticotropin-releasing hormone (CRH), which stimulates the pituitary to secrete ACTH, which then stimulates the adrenal to secrete cortisol. Cortisol is found in plasma but also is reliably measured in saliva. Secretion of ACTH by the pituitary is episodic through the 24-hour cycle, and, similarly to other hormones, basal cortisol secretion by the adrenal gland shows prominent circadian variation characterized by peak levels in the morning followed by a steady decline to the nadir at night. The HPA response to stress is adaptive in that it prepares the individual for dealing with the source of the stress; however, chronic elevations or disruptions in the typical diurnal rhythm of cortisol can lead to medical problems associated with immune suppression [McEwen et al., 1997] and adverse effects on the brain that interfere with learning and memory [Sapolsky 2000]. Recent evidence has clearly implicated several limbic regions, in particular the amygdala and the hippocampus, as being intimately involved in the regulation of the HPA system [Herman and Cullinan 1997].

A pilot study of salivary cortisol in eight male and seven female children with the fragile X full mutation was conducted [Wisbeck et al., 2000]. In comparison to normative cortisol data taken from a large group of typically developing children, children with FXS had higher levels of cortisol on 2 routine days. On an experimental day during which subjects were engaged in a socially challenging task, males with FXS had cortisol elevations following the task, and they continued to have higher levels at bedtime. In an effort to replicate and extend these results, we conducted a comprehensive, in-home study of 120 families throughout the United States and Canada, including assessment of the HPA axis via measures of salivary cortisol [Hessl et al., 2002]. The families each had at least one child with the FXS full mutation (proband) and one unaffected biological sibling (sibling comparison). Two experimenters spent a full day with each family in the home, completing neuropsychological testing and behavioral assessment of each child and both parents, interviewing parents about the children and their educational and therapeutic services, observing the physical and social qualities of the home environment, and engaging the children in a structured, socially challenging series of tasks (the interview included insistence on direct eye contact, silent and oral reading, and singing). A blood sample was obtained from each child for FXS DNA testing and to measure expression of the *FMR1* protein in lymphocytes. To measure cortisol, four samples of saliva were collected on each of 2sss weekend days (estimate of basal diurnal rhythm of cortisol) and six samples were collected during the home visit (to measure cortisol response to social and cognitive challenge). Cortisol concentration was determined by radioimmunoassay.

The results of the study documented that males with FXS had elevated basal cortisol during the day and before bedtime, and they had a greater cortisol response to the diverse challenges of the home visit (meeting the examiners, un-

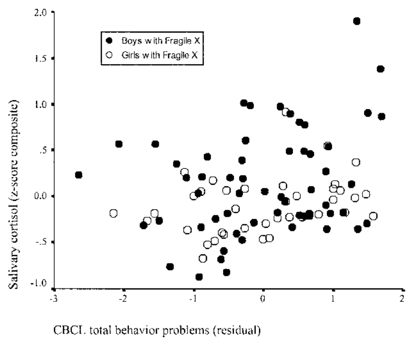

Fig. 3. Relation between salivary cortisol and CBCL total behavior problem residual scores in 58 boys and 39 girls with the fragile X full mutation. Hierarchical multiple regression analyses showed that, after removing effects of other significant factors, including IQ, *FMR1* gene protein level, and the effectiveness of educational and therapeutic services, cortisol accounted for a significant proportion of the variance (8% and 14% in boys and girls, respectively) in total behavior problems such that increased levels were associated with greater severity of problems. Cortisol levels were standardized by *z*-score transformation and then averaged to derive the composite score. Residual scores represent behavior scores after the effects of the other independent variables in the multiple regression are removed.

dergoing neuropsychological testing, and engaging in tasks designed to elicit social anxiety) in comparison to their unaffected biological siblings (Fig. 2). Despite clear manifestation of social anxiety in females with the full mutation, the girls in this study, as a group, did not demonstrate increases in cortisol relative to comparison siblings. However, in both boys *and* girls with FXS, salivary cortisol (a composite score representing overall cortisol level across samples taken during the home visit) was positively associated with severity of behavior problems (Fig. 3), predominantly withdrawn behavior, social problems, and attention problems. In contrast, no association was found between cortisol and behavior in the unaffected siblings. The association in FXS was present after accounting for several other factors shown to predict behavior in these children, including child IQ, FMRP, parental psychopathology, and the quality of the home environment. Thus, there does seem to be a unique association between HPA function and behavior in children with FXS. A critical question is whether elevation in HPA activity is the result of the *experience* of increased stress or whether cortisol elevation actually contributes to or causes anxiety and associated maladaptive behaviors in individuals with FXS. We are currently examining the relation between cortisol response during social challenge and several specific behaviors of interest during this task (i.e., quality of gaze, task avoidance, level of discomfort) that may help clarify this question. Future longitudinal studies in which cortisol and behavioral patterns can be tracked over time, as well as studies focused on other levels of the HPA axis, also will help to confirm the direction of these effects. Nevertheless, our work has provided additional evidence of hypothalamic dysregulation in FXS.

One of the most telling results of the study was a highly significant familial pattern in cortisol response. That is, although the children with FXS tended to have higher cortisol levels and were more cortisol-reactive than their unaffected brothers and sisters, the "family" effect indicated that the matched siblings had very similar cortisol profiles. This finding is quite consistent with studies of inheritance and genetics of HPA function, but it may also reflect social, emotional, and physical environmental factors that are shared in each proband–sibling pair. Thus, as previous studies of cognition, behavior, and physical features have emphasized, accounting for background familial and genetic variance is especially important in future studies of individuals with FXS. Prospective, longitudinal studies of individuals affected by FXS will be critical in unraveling the complex interaction among factors related to genetic influences (fragile X mutation characteristics), brain function, learning and behavior, the environment, and HPA function. Two such studies have recently commenced at Stanford, one involving follow-up of the families described above and the other following very young children with FXS throughout the preschool age range.

Because the HPA axis is the primary stress response system, it may mediate causal connections between molecular alterations and stress-related behavior in FXS. Glucocorticoid hormones regulate neuron birth, death, and dendritic arborization, which may explain brain morphological alterations found in several neuropsychiatric disorders associated with stress [Sapolsky, 2000]. Studies of both animals and humans show that abnormal corticosteroid levels can affect hippocampal morphology and volume [Bremner et al., 1995; McEwen et al., 1992]. Glucocorticoid receptors are found in many regions of the brain involved in the regulation of emotion, attention, and memory, including the hippocampus and amygdala [McEwen et al., 1986]. These brain regions are known to mediate emotional appraisal of social stimuli, memory, and learning, all of which are significantly impacted by FXS. Furthermore, as cited above, alterations in the structure or function of several brain regions, including the hippocampus, amygdala, thalamus, and frontal cortex, have been implicated in the pathogenesis of the FXS neurobehavioral phenotype. Thus, although decreased FMRP contributes to neuronal dysmorphology [Weiler and Greenough, 1999] and diminished synaptic transmission [Irwin et al., 2000b], we have hypothesized that there may be secondary HPA axis effects in brain regions that have an abundance of glucocorticoid receptors, including mesial temporal and frontal regions. We are currently testing this hypothesis with studies examining the relation between cortisol and brain structure and function in individuals with FXS.

The hypothesis that the HPA axis mediates causal connections between molecular alterations and stress-related behavior in FXS has recently gained empirical support by studies examining the molecular targets of FMRP in the brains of *FMR1* knockout mice and in human lymphocytes. For example, Miyashiro and colleagues [Miyashiro et al., 2003] recently showed that FMRP directly interacts with a number of other gene mRNAs, including the mRNA of the glucocorticoid receptor. In addition, these authors showed that decreased concentration of glucocorticoid receptors are found in the hippocampal dendrites of *FMR1* knockout as compared to wild-type mice. These studies suggest that the FMRP deficit alters GR mRNA and perhaps alters the balance of GR receptors and subsequent HPA activity. Additional support for HPA axis disruption in FXS comes from Sun and colleagues [2001], who discovered abnormal expression of a glucocorticoid-modulating protein, Annexin-1, in blood lymphocytes in a group of adult males with FXS in comparison to those with other developmental disabilities and typical controls.

If the HPA axis plays a causal or mediating role in development of the fragile X behavioral phenotype, pharmacological or environmental interventions designed to normalize HPA function might help to reduce stress-related behavior and improve mood and emotion regulation in affected individuals. For example, the glucocorticoid receptor agonist mifepristone, also known as RU486, rapidly reduces psychosis and depression in adults with psychotic major depression [Belanoff et al., 2002], a group known to have significant HPA abnormality. In addition, alterations in the home and educational environment, such as increased structure and predictability and reduced sensory stimuli and social demands, may normalize HPA responses to stress and reduce anxiety in affected individuals.

Neuroanatomy, Neuroendocrinology, and Genetics in Context

Fragile X syndrome offers a unique opportunity to study complex relations among genetic, neurobiological, and environmental systems leading to cognitive and emotional dysfunction. Since the discovery of the *FMR1* gene a little over a decade ago, there have been important breakthroughs in understanding the molecular genetics and neurobiology of FXS, many of which are reviewed in this volume. But we know that the life experience of each person with FXS is determined by more than his or her *FMR1* gene, neuroanatomy, or other biological factors. To exemplify this point, we have shown that, after accounting for *FMR1* protein expression and other background factors, the quality of the home environment (i.e., parent responsivity, availability of learning materials, cultural, recreational, or artistic enrichment, family companionship, and the quality of the physical environment) is independently associated with cognitive ability [Dyer-Friedman et al., 2002], adaptive skills [Glaser et al., 2003], and autistic behavior [Hessl et al., 2001] in children with FXS. Thus, the degree to which individuals with FXS are susceptible to both neurobiological *and* environmental factors is critical in charting the course of effective treatment studies. ■

REFERENCES

Abrams MT, Reiss AL. 1995. The neurobiology of fragile X syndrome. Ment Retard Dev Disab Res Rev 1:269–275.

Allingham-Hawkins DJ, Babul-Hirji R, Chitayat D, et al. 1999. Fragile X premutation is a significant risk factor for premature ovarian failure: The international collaborative POF in fragile X study- preliminary data. Am J Med Genet 83:322–325.

Barnea-Goraly N, Eliez S, Hedeus M, et al. 2003. White matter tract alterations in fragile X syndrome: Preliminary evidence from diffusion tensory imaging. Am J Med Genet 118B: 81–88.

Belanoff JK, Rothschild AJ, Cassidy F, et al. 2002. An open label trial of C-1073 (mifepristone) for psychotic major depression. Biol Psychiatry 52:386–392.

Bowen P, Biederman B, Swallow KA. 1978. The X-linked syndrome of macroorchidism and mental retardation: further observations. Am J Med Genet 2:409–414.

Braat DDM, Smits APT, Tomas CMG. 1999. Menstrual disorders and endocrine profiles in fragile X carriers prior to 40 years of age: a pilot study. Am J Med Genet 83:327–328.

Bregman JD, Leckman JF, Ort SI. 1990. Thyroid function in fragile-X syndrome males. Yale J Biol Med 63:293–299.

Bremner JD, Randall P, Scott TM, et al. 1995. MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. Am J Psychiatry 152: 973–981.

Brondum Nielsen K, Tommerup N, Friis B, et al. 1983. Folic acid metabolism in a patient with fragile X. Clin Genet 24:153–155.

Brunberg JA, Jacquemont S, Hagerman RJ, et al. 2002. Fragile X premutation carriers: Characteristic MR imaging findings of adult male patients with progressive cerebellar and cognitive dysfunction. Am J Neuroradiol 23:1757–1766.

Butler MG, Najjar JL. 1988. Do some patients with fragile X syndrome have precocious puberty? Am J Med Genet 31:779–781.

Cantu JM, Scaglia HE, Gonzalez-Diddi M, et al. 1978. Inherited congenital normofunctional testicular hyperplasia and mental deficiency. A corroborative study. Hum Genet 41:331–339.

Coulam, CB, Adamson SC, Annegers JF. 1986. Incidence of premature ovarian failure. Obstet Gynecol 67:604–606.

Cronister A, Schreiner R, Wittenberger M, et al. 1991. Heterozygous fragile X female: Historical, physical, cognitive, and cytogenetic features. Am J Med Genet 38:269–274.

Devys D, Lutz Y, Rouyer N, et al. 1993. The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. Nat Genet 4:335–340.

Dunn AJ, Berridge CW. 1990. Physiological and behavioral responses to corticotropin-releasing factor administration: Is CRF a mediator of anxiety or stress responses? Brain Res Brain Res Rev 15:71–100.

Dyer-Friedman J, Glaser B, Hessl D, et al. 2002. Genetic and environmental influences on the cognitive outcomes of children with fragile X syndrome. J Am Acad Child Adolesc Psychiat 41:237–244.

Eliez S, Blasey CM, Freund LS, et al. 2001. Brain anatomy, gender and IQ in children and adolescents with fragile X syndrome. Brain 124: 1610–1618.

Freund LS, Reiss AL. 1991. Cognitive profiles associated with the fra(X) syndrome in males and females. Am J Med Genet 38:542–547.

Fryns JP, Dereymaeker AM, Hoefnagels M, et al. 1986. Partial fra(X) phenotype with megalotestes in fra(X) negative patients with acquired lesions of the central nervous system (CNS). Am J Med Genet 23:213–219.

Fryns JP, Haspeslagh M, Dereymaeker AM, et al. 1987. A peculiar subphenotype in the fra(X) syndrome: Extreme obesity-short stature-stubby hands and feet-diffuse hyperpigmentation. Further evidence of disturbed hypothalamic function in the fra(X) syndrome? Clin Genet 32:388–392.

Glaser B, Hessl D, Dyer-Friedman J, et al. 2003. Biological and environmental contributions to adaptive behavior in fragile X syndrome. Am J Med Genet 117A:21–29.

Gould EL, Loesch DZ, Martin MJ, et al. 2000. Melatonin profiles and sleep characteristics in boys with fragile X syndrome: A preliminary study. Am J Med Genet 95:307–315.

Hagerman RJ, Leehey M, Heinrichs W, et al. 2001. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. Neurology 57:127–130.

Hayakawa Y, Nakajima T, Takagi M, et al. 2002. Human cerebellar activation in relation to saccadic eye movements: A functional magnetic resonance imaging study. Ophthalmologica 216:399–405.

Herman JP, Cullinan WE. 1997. Neurocircuitry of stress: Central control of the hypothalamo–pituitary–adrenocortical axis. Trends Neurosci 20:78–84.

Hessl D, Dyer-Friedman J, Glaser B, et al. 2001. The influence of environmental and genetic factors on behavior problems and autistic symptoms in boys and girls with fragile X syndrome. Pediatrics 108:E88.

Hessl D, Glaser B, Dyer-Friedman J, et al. 2002. Cortisol and behavior in fragile X syndrome. Psychoneuroendocrinology 27:855–872.

Hinton VJ, Brown WT, Wisniewski K, et al. 1991. Analysis of neocortex in three males with the fragile X syndrome. Am J Med Genet 41: 289–294.

Huang CM, Burkard R. 1986. Frequency sensitivities of auditory neurons in the cerebellum of the cat. Brain Res 371:101–108.

Hundscheid RD, Smits AP, Thomas CM, et al. 2003. Female carriers of fragile X premutations have no increased risk for additional diseases other than premature ovarian failure. Am J Med Genet:117A, 6–9.

Irwin SA, Galvez R, Greenough WT. 2000a. Dendritic spine structural anomalies in fragile-X mental retardation syndrome. Cereb Cortex 10:1038–1044.

Irwin SA, Swain RA, Christmon CA, et al. 2000b. Evidence for altered Fragile-X mental retardation protein expression in response to behavioral stimulation. Neurobiol Learn Mem 73:87–93.

Jacquemont S, Hagerman RJ, Leehey M, et al. 2003. Fragile X premutation tremor/ataxia syndrome: Molecular, clinical, and neuroimaging correlates. Am J Hum Genet 72:869–878.

Jäkälä P, Hanninen T, Ryynanen M, et al. 1997. Fragile-X: Neuropsychological test performance, CGG triplet repeat lengths, and hippocampal volumes. J Clin Invest 100:331–338.

Kates WR, Abrams MT, Kaufmann WE, et al. 1997. Reliability and validity of MRI measurement of the amygdala and hippocampus in children with fragile X syndrome. Psychiatry Res 75:31–48.

Kowalczyk CL, Schroeder E, Pratt V, et al. 1996. An association between precocious puberty and fragile X syndrome? Disabil Rehabil Adolesc Gynecol 9:199–202.

Kwon H, Menon V, Eliez S, et al. 2001. Functional neuroanatomy of visuospatial working memory in fragile X syndrome: Relation to behavioral and molecular measures. American Journal of Psychiatry 158:1040–1051.

Loesch DZ, Huggins RM, Hoang NH. 1995. Growth in stature in fragile X families: A mixed longitudinal study. Am J Med Genet 58:249–256.

Macpherson J, Waghorn A, Hammans S, et al. 2003. Observation of an excess of fragile-X premutations in a population of males referred with spinocerebellar ataxia. Hum Genet 112: 619–620.

Mazzocco MM, Freund L, Baumgardner TL, et al. 1995. The neurobehavioral and neuroanatomical effects of the *FMR1* full mutation: Monozygotic tiwns discordant for fragile X syndrome. Neuropsychology 9:470–480.

Mazzocco MM, Kates WR, Baumgardner TL, et al. 1997. Autistic behaviors among girls with fragile X syndrome. J Autism Dev Disord 27:415–435.

Mazzocco MM, Pennington BF, Hagerman RJ. 1993. The neurocognitive phenotype of female carriers of fragile X: additional evidence for specificity. J Dev Behav Pediatr 14:328–335.

McDermott A, Walters R, Howell RT, et al. 1983. Fragile X chromosome: clinical and cytogenetic studies on cases from seven families. Journal of Medical Genetics 20:169–178.

McEwen BS, Biron CA, Brunson KW, et al. 1997. The role of adrenocorticoids as modulators of immune function in health and disease: Neural, endocrine and immune interactions. Brain Res Brain Res Rev 23:79–133.

McEwen BS, De Kloet ER, Rostene W. 1986. Adrenal steroid receptors and actions in the nervous system. Physiol Rev 66:1121–1188.

McEwen BS, Gould EA, Sakai RR. 1992. The vulnerability of the hippocampus to protective and destructive effects of glucocorticoids in relation to stress. Br J Psychiat Suppl 15: 18–23.

Miyashiro KY, Beckel-Mitchener A, Purk TP, et al. 2003. RNA Cargoes associating with FMRP reveal deficits in cellular functioning in *FMR1* null mice. Neuron 37:417–431.

Moore PS, Chudley AE, Winter JS. 1990. True precocious puberty in a girl with the fragile X syndrome. Am J Med Genet 37:265–267.

Moretti R, Bava A, Torre P, et al. 2002. Reading errors in patients with cerebellar vermis lesions. J Neurol 249:461–468.

Mostofsky SH, Mazzocco MM, Aakalu G, et al. 1998. Decreased cerebellar posterior vermis size in fragile X syndrome: Correlation with neurocognitive performance. Neurology 50: 121–130.

Murray A, Webb J, MacSwiney F, et al. 1999. Serum concentrations of follicle stimulating hormone may predict premature ovarian failure in FRAXA premutation women. Hum Reprod 14:1217–1218.

Paradee W, Melikian HE, Rasmussen DL, et al. 1999. Fragile X mouse: Strain effects of knockout phenotype and evidence suggesting deficient amygdala function. Neuroscience 94:185–192.

Partington MW. 1984. The fragile X syndrome II: Preliminary data on growth and development in males. Am J Med Genet 17:175–194.

Reiss AL, Abrams MT, Greenlaw R, et al. 1995. Neurodevelopmental effects of the FMR-1 full mutation in humans. Nat Med 1:159–167.

Reiss AL, Aylward E, Freund LS, et al. 1991a. Neuroanatomy of fragile X syndrome: The posterior fossa. Ann Neurol 29:26–32.

Reiss AL, Freund L, Tseng JE, et al. 1991b. Neuroanatomy in fragile X females: the posterior fossa. Am J Hum Genet 49:279–288.

Reiss AL, Lee J, Freund L. 1994. Neuroanatomy of fragile X syndrome: The temporal lobe. Neurology 44:1317–1324.

Reiss AL, Patel S, Kumar AJ, et al. 1988. Preliminary communication: neuroanatomical variations of the posterior fossa in men with the fragile X (Martin-Bell) syndrome. Am J Med Genet 31:407–414.

Rivera SM, Menon V, White CD, et al. 2002. Functional brain activation during arithmetic processing in females with fragile X Syndrome is related to *FMR1* protein expression. Hum Brain Mapp 16:206–218.

Rosenthal G, Gilman S, Koeppe RA, et al. 1988. Motor dysfunction in olivopontocerebellar atrophy is related to cerebral metabolic rate studied with positron emission tomography. Ann Neurol 24:414–419.

Rudelli RD, Brown WT, Wisniewski K, et al. 1985. Adult fragile X syndrome: Clinico-neuropathologic findings. Acta Neuropathol 67:289–295.

Ruvalcaba RH, Myhre SA, Roosen-Runge EC, et al. 1977. X-linked mental deficiency megalotestes syndrome. J Am Med Assoc 238:1646–1650.

Sapolsky RM. 2000. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiat 57:925–935.

Schmitt JE, Eliez S, Warsofsky IS, et al. 2001. Enlarged cerebellar vermis in Williams syndrome. J Psychiatr Res 35:225–229.

Schwartz CE, Dean J, Howard Peebles PN, et al. 1994. Obstetrical and gynecological complications in fragile X carriers: A multicenter study. Am J Med Genet 51:400–402.

Sun H-T, Cohen S, Kaufmann WE. 2001. Annexin-1 is abnormally expressed in Fragile X syndrome: A two-dimensional electrophoresis study in lymphocytes. Am J Med Genet 103:81–90.

Tamanini F, Willemsen R, van Unen L, et al. 1997. Differential expression of *FMR1*, FXR1 and FXR2 proteins in human brain and testis. Hum Mol Genet 6:1315–1322.

Tamm L, Menon V, Johnston CK, et al. 2002. fMRI study of cognitive interference processing in females with fragile X syndrome. J Cogn Neurosci 14:160–171.

Turner G, Eastman C, Casey J, et al. 1975. X-linked mental retardation associated with macro-orchidism. J Med Genet 12:367–371.

Vianna-Morgante AM, Mingroni-Netto RC, Barbosa AC, et al. 1996. FRAXF in a patient with chromosome 8 duplication. J Med Genet 33:611–614.

Weiler IJ, Greenough WT. 1999. Synaptic synthesis of the Fragile X protein: possible involvement in synapse maturation and elimination. Am J Med Genet 83:248–252.

Wisbeck JM, Huffman LC, Freund L, et al. 2000. Cortisol and social stressors in children with fragile X: A pilot study. J Dev Behav Pediatr 21:278–282.

Wisniewski KE, Segan SM, Miezejeski CM, et al. 1991. The Fra(X) syndrome: Neurological, electrophysiological, and neuropathological abnormalities. Am J Med Genet 38:476–480.