

# Brain anatomy, gender and IQ in children and adolescents with fragile X syndrome

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## Summary

This study utilized MRI data to describe neuroanatomical morphology in children and adolescents with fragile X syndrome, the most common inherited cause of developmental disability. The syndrome provides a model for understanding how specific genetic factors can influence both neuroanatomy and cognitive capacity. Thirty-seven children and adolescents with fragile X syndrome received an MRI scan and cognitive testing. Scanning procedures and analytical strategies were identical to those reported in an earlier study of 85 typically developing children, permitting a comparison with a previously published template of normal brain development. Regression analyses indicated that there was a normative age-related decrease in grey matter and an increase in white matter. However, caudate and

ventricular CSF volumes were significantly enlarged, and caudate volumes decreased with age. Rates of reduction of cortical grey matter were different for males and females. IQ scores were not significantly correlated with volumes of cortical and subcortical grey matter, and these relationships were statistically different from the correlational patterns observed in typically developing children. Children with fragile X syndrome exhibited several typical neurodevelopmental patterns. Aberrations in volumes of subcortical nuclei, gender differences in rates of cortical grey matter reduction and an absence of correlation between grey matter and cognitive performance provided indices of the deleterious effects of the fragile X mutation on the brain's structural organization.

**Keywords:** fragile X syndrome, MRI, brain development, FMR1

**Abbreviation:** *FMR1* = fragile X mental retardation gene

## Introduction

Normal brain development consists of a complex series of progressive and regressive events including stages of cellular proliferation, neuropil growth, myelination, programmed cell death and synaptic elimination. It is remarkable that this complex array of developmental processes culminates in a functionally competent person most of the time. In one of every 2000–6000 live births (Gustavson *et al.*, 1986; de Vries *et al.*, 1997), a specific single gene mutation alters the course of brain development resulting in the fragile X syndrome, the most common inherited cause of mental retardation.

The genetic mutation associated with fragile X intersects with developmental pathways that influence physical development, cognitive ability and behaviour. Physical manifestations include a long and narrow face, large dysmorphic ears and a prominent jaw (Loesch and Hay, 1988; Meryash *et al.*, 1984; Davids *et al.*, 1990). These features, as well as

macroorchidism, have been observed consistently among affected males (Lachiewicz and Dawson, 1994). However, physical characteristics are highly variable, particularly among prepubertal children and females, and are insufficient for diagnosing the presence of fragile X syndrome.

Consistency among investigations of the syndrome's cognitive and behavioural features suggests predisposition for a particular neurobehavioural profile in this condition (Turk, 1992; Freund *et al.*, 1993; Einfeld *et al.*, 1994; Warren and Ashley, 1995). The phenotype is different for males and females, potentiated by the fact that the syndrome is X-linked. Females, heterozygous for the fragile X full mutation, typically have mild mental retardation or normal cognitive functioning accompanied by learning difficulties (Riddle *et al.*, 1998). Behaviourally, affected females often exhibit attention deficit, anxiety and difficulties with socialization

(Freund *et al.*, 1993). Males with the full mutation, reliant upon a single X chromosome in each cell (i.e. hemizygous), usually function in the moderate to severely mentally retarded range of intelligence, and their IQ scores may decline during middle childhood (Hodapp *et al.*, 1990). Specific areas of cognitive deficit for males include visuospatial abilities, visual-motor coordination and short-term memory (Kemper *et al.*, 1988; Crowe and Hay, 1990; Freund *et al.*, 1993). Behaviourally, males with fragile X syndrome often exhibit hyperactivity, autistic features, difficulties with peer interaction, abnormal social communication, gaze avoidance and motor stereotypies (Lachiewicz *et al.*, 1994; Baumgardner *et al.*, 1995; Turk and Cornish, 1998). Variability in phenotypical observations and shared characteristics with other disorders, such as autism (Feinstein and Reiss, 1998), preclude accurate identification of the syndrome based solely upon cognitive and behavioural features.

The cognitive and behavioural phenotype in fragile X results from a known genetic aetiology. Early investigations of fragile X syndrome noted that the phenotype co-segregated with a morphological abnormality of the X chromosome (Lubs, 1969). Karyotyping of cells revealed a 'fragile' site that appeared as a constriction on the distal long arm (Lubs, 1969). In 1991, the most common mutation responsible for the syndrome was identified (Rousseau *et al.*, 1991; Verkerk *et al.*, 1991) and described as an expanded number of CGG triplet repeats occurring within the initial (5') untranslated portion of the fragile X mental retardation gene (*FMR1*) (Kremer *et al.*, 1991). When >200 CGG repeats are present, hypermethylation of the promoter region of *FMR1* is probable (Oberle *et al.*, 1991), inhibiting the transcription and translation of *FMR1*, and hence resulting in a 'transcriptional silencing' of the gene.

Diminished or absent production of the FMR1 protein may lead to aberrant brain development and function (Devys *et al.*, 1993; Tamanini *et al.*, 1997), although few studies have investigated the mutation's neuroanatomical effects directly. Autopsy studies have indicated abnormalities in the dendritic arborization of the cerebral cortex among affected males (Rudelli *et al.*, 1985; Hinton *et al.*, 1991; Wisniewski *et al.*, 1991). Studies investigating *FMR1* mRNA during mammalian development have pointed to neuronal localization and particularly high gene expression in the hippocampus, cerebellum (Purkinje cells) and nucleus basalis (Devys *et al.*, 1993; Tamanini *et al.*, 1997).

MRI studies have localized the neuroanatomical effects of the *FMR1* full mutation further. Structural MRI studies of the posterior fossa show that the cerebellar vermis is decreased in size (particularly lobules VI and VII) and that the fourth ventricle is enlarged (Mostofsky *et al.*, 1998). Moreover, decreased vermis size is associated with lower verbal and performance IQ scores (Mostofsky *et al.*, 1998) and with increased stereotypic behaviour (Mazzocco *et al.*, 1998). Some investigators have shown increased volumes of the hippocampus, a structure known for its role in learning and memory (Reiss *et al.*, 1994; Kates *et al.*, 1997), although

other investigators (Jakala *et al.*, 1997) have found no differences in hippocampal volumes. Volumetric aberrations have been detected in the caudate nucleus (Reiss *et al.*, 1995). Increased lateral ventricular volumes have been observed among males with the full mutation, and enlargement of the thalamus has been noted among females (Reiss *et al.*, 1995).

In the current study, we sought to investigate further the consequences of the *FMR1* full mutation on brain structure and development in a sample of children and adolescents of varying ages. Our hypotheses were 2-fold: first, we expected that children with fragile X syndrome would differ in patterns of brain development from a comparison sample of typically developing children. Because the mutated FMR1 protein is expressed primarily in the neurones constituting grey matter (Weiler and Greenough, 1999), we anticipated that structural aberrations would be manifested primarily in the cortical and subcortical (i.e. caudate, putamen and thalamus) grey matter compartments. Secondly, we hypothesized that males and females in our sample would show different structural abnormalities that would be concordant with the gender differences in genetic status. Because fragile X syndrome is an X-linked disorder, we expected more evidence of neuroanatomical anomalies in males than in females, whose genetic status (i.e. having two X chromosomes on which to rely) places them in an 'intermediate' position for experiencing the mutation's impact.

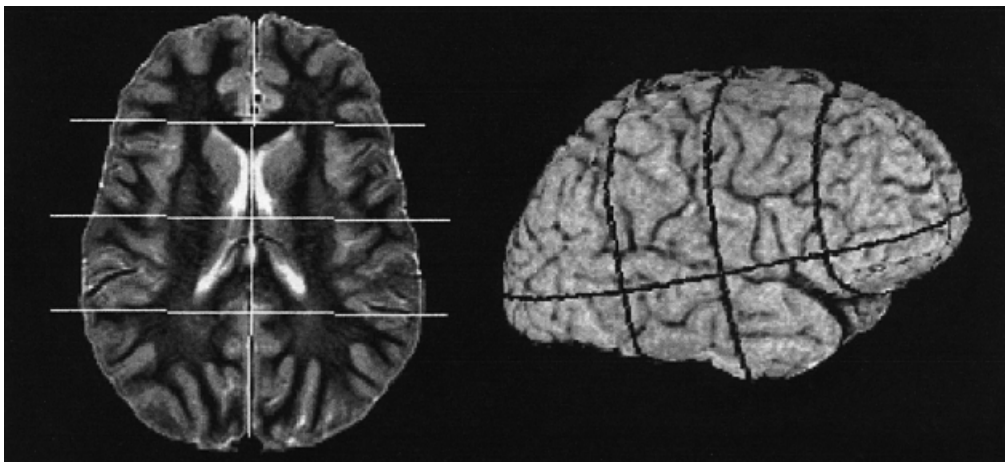
## Methods

### Subjects

Children and adolescents with the fragile X mutation were recruited from families identified by standard molecular testing. Thirty-seven children with DNA-confirmed (Rousseau *et al.*, 1991) fragile X syndrome participated in the procedures. The sample ( $n = 37$ ) consisted of 27 girls and 10 boys ranging in age from 4 to 19 years (mean  $10.2 \pm 3.8$  years). The predominance of girls in our sample is attributable to our laboratory's previous emphasis in recruiting females with genetic conditions. A previous report on molecular variables in fragile X syndrome utilized our sample as part of a larger sample ( $n = 51$ ) composed of both children and adults (Reiss *et al.*, 1995). Written informed consent was obtained from children and adolescents who understood the procedure and from the parents of all others. Approval for the research was given by The Joint Commission of Clinical Investigation of Johns Hopkins University School of Medicine.

### Image acquisition and analysis

We replicated the methodology, imaging, processing and analytical procedures utilized in our laboratory's previous investigation (Reiss *et al.*, 1996; Fig. 1) of 85 typically developing children. MRIs of each subject's brain were



**Fig. 1** The brain parcellation scheme used for subhemispheric regional volumes. Images show the location of five planes projected through the brain in a transaxial slice and three-dimensional surface rendering. See Methods for details.

acquired on a GE-Signa 1.5 T scanner (GE Imaging Systems, Milwaukee, Wisc., USA). Images were derived from an axial spin echo SD/T<sub>2</sub>-weighted scan [TR (repetition time) = 3000 and TE (echo time) = 30/100] acquired parallel to the AC/PC (anterior/posterior commissure) plane. These images were 5 mm thick and contiguous. Measurement of grey matter, white matter and CSF compartments were made after creating composite images from paired T<sub>2</sub>- and proton-weighted images. Composite images were produced by adding and subtracting, respectively, the early and late echo (paired) images. Tissue classification was determined in each slice using histogram-based segmentation algorithms which automatically establish one or more statistically optimal thresholds for separating tissue types (Cho *et al.*, 1989; Otsu, 1979). Independent assessors measured brain volumes and were blind to the subjects' identities and diagnoses.

### **Cognitive assessment**

Standardized cognitive testing was administered within 3 months of the MRI scanning procedures. Children under 6 years of age received the Stanford-Binet 4th edition (Thorndike *et al.*, 1986), whereas children who were 6 years of age or older received the WISC-R (Wechsler Intelligence Scale for Children—Revised) (Wechsler, 1981).

### **Comparison sample**

A sample from a previously published investigation of typical brain development (Reiss *et al.*, 1996) comprising 64 females (mean age  $10.6 \pm 2.9$  years) and 21 males (mean age  $10.7 \pm 2.8$  years) provided a template for comparison. Inclusion criteria for this sample were normal IQ and absence of neurological and psychiatric disorders. The comparison sample was equivalent to the fragile X sample in terms

of age range and gender composition. Cognitive testing instruments, image acquisition, pulse sequences, image processing procedures and statistical analyses were identical for both studies.

### **Statistical analyses**

Hypothesis testing was conducted using an alpha of 0.05 (two-tailed) as the threshold for statistical significance. Gender comparisons of mean structural volumes in the regions of interest were conducted using ANCOVA (analysis of covariance), covarying for the gender difference in overall brain volume. Developmental and age-related patterns of neurodevelopment were examined using linear regression. The predictive roles of gender and age on structural volumes in the regions of interest were tested hierarchically with the following predictors: total brain volume, gender, age and a gender by age interaction term. When homogeneity of variances was not evident, the regression effects were re-tested using non-parametric permutation testing and bootstrapping procedures. Relationships between IQ scores and total brain, cortical grey and subcortical grey matter volumes were quantified using linear regression procedures. Analysis of brain asymmetry (left versus right hemisphere) among children with fragile X syndrome was conducted using repeated-measures ANOVA (analysis of variance), with hemispheric volumes as within-subjects factors and gender as a between-subjects factor.

Results from the fragile X sample data were compared with data reported in a previous study of 85 typically developing children (Reiss *et al.*, 1996). Independent measures *t* tests were used to compare mean brain volumes and Fischer *r* to *z* conversions (Viana, 1980) were used to compare correlation coefficients from the two studies.

## Results

### Cerebral volume in children with fragile X syndrome and typically developing children

As shown in Table 1, cross-study comparisons indicated statistically significant differences between children with fragile X syndrome and typically developing children in two brain regions of interest. Volumes of the caudate nucleus (males:  $t = 4.58$ ,  $P < 0.001$ ; females:  $t = 3.81$ ,  $P < 0.01$ ) were larger in children with fragile X syndrome. Volumes of the thalamus were also larger in the children with fragile X syndrome, although this difference only reached statistical significance for affected females ( $t = 2.38$ ,  $P < 0.05$ ). Finally, ventricular CSF was larger in both affected females ( $t = 3.01$ ,  $P < 0.01$ ) and affected males ( $t = 2.39$ ,  $P < 0.05$ ).

### Cerebral volume in children with fragile X syndrome—gender effects

Table 1 also shows that there was a significant gender difference in total cerebral volume [ $F(1,35) = 6.5$ ,  $P = 0.016$ ; male > female]. Total cerebral volume in males was ~8% larger than in females for both the right [ $F(1,35) = 6.3$ ,  $P = 0.017$ ] and left hemispheres [ $F(1,35) = 6.5$ ,  $P = 0.015$ ]. Males also had larger volumes than females of total grey matter [ $F(1,34) = 7.5$ ,  $P = 0.01$ ], cortical grey matter [ $F(1,34) = 7.4$ ,  $P = 0.01$ , male > female] and caudate nucleus [ $F(1,34) = 6.9$ ,  $P = 0.012$ , male > female]. No gender differences were detected in the mean volumes of putamen and thalamus. Raw means of white matter volume were higher in males (mean  $468.5 \pm 57$ ) than in females (mean  $457.6 \pm 77$ ). However, after covarying for differences in overall brain size, estimated marginal means indicated that females exhibited significantly larger amounts of total white matter relative to total cerebral volume [ $F(1,34) = 6.2$ ,  $P = 0.018$ ]. No gender differences were detected in mean volumes of extraventricular and ventricular CSF.

### Change in cerebral volumes with age

Age was unrelated to total cerebral volume in males and females with fragile X syndrome ( $r = 0.10$ ,  $P = 0.55$ ). As illustrated in Table 2, there was a significant robust decrease in cortical grey matter volume with age. Age predicted 27% ( $\Delta r^2 = 0.27$ ,  $P < 0.0001$ ) of the variance in total cortical grey matter. Both males and females exhibited a significant decline in cortical grey matter. However, age-related effects were significantly different between boys and girls, with boys showing a slower rate of cortical grey matter decline ( $\Delta r^2 = 0.05$ ,  $P = 0.001$ ). Age-related regression results also indicated a robust increase in white matter over time in the fragile X sample; age explained 16% ( $\Delta r^2 = 0.16$ ,  $P < 0.0001$ ) of the variance in white matter volume. Significant age-related decreases were noted in two subcortical grey matter regions, particularly caudate grey ( $\Delta r^2 = 0.065$ ,  $P = 0.05$ ) and thalamic grey matter volumes ( $\Delta r^2 = 0.11$ ,  $P = 0.035$ ).

**Table 1** Tissue volumes in brain regions of children with fragile X syndrome and typically developing children

Region	Fragile X Volume (cm <sup>3</sup> )		Gender comparison (within fragile X)		Typically developing Volume (cm <sup>3</sup> )		Cross-study comparisons					
	Males (n = 10) mean ± SD		Female (n = 27) mean ± SD		F	P	Males (n = 21) mean ± SD		Females (n = 64) mean ± SD		Males	Females
	t value	P	t value	P	t value	P	t value	P	t value	P	t value	P
Total cerebrum*	1335.3 ± 82	1225.3 ± 127	6.5	0.016	1290.6 ± 147	1182 ± 105	0.89	n.s.	1.67	n.s.	0.89	n.s.
Right hemisphere*	666.3 ± 43	611.3 ± 64	6.3	0.017	643.9 ± 73	591.1 ± 53	0.90	n.s.	1.57	n.s.	0.90	n.s.
Left hemisphere*	669.0 ± 39.6	614.0 ± 64	6.5	0.015	646.7 ± 75	591.4 ± 53	0.88	n.s.	1.75	n.s.	0.88	n.s.
Total grey matter†	752.3 ± 54	656.3 ± 70	7.5	0.01	707.4 ± 71	637.2 ± 66	1.76	n.s.	1.24	n.s.	1.76	n.s.
Cortical grey matter†	714.1 ± 52	622.2 ± 67	7.3	0.01	674.8 ± 68	605.6 ± 64	1.62	n.s.	1.12	n.s.	1.62	n.s.
Caudate nucleus†	14.2 ± 1	12.1 ± 2	6.9	0.012	11.1 ± 2	10.7 ± 2	4.58	<0.0001	3.81	<0.01	4.58	<0.001
Putamen†	10.9 ± 2	9.6 ± 1	1.1	0.303	9.9 ± 2	9.5 ± 2	1.69	n.s.	0.29	n.s.	1.69	n.s.
Thalamus†	13.1 ± 1	12.4 ± 2	0.07	0.792	11.6 ± 2.0	11.4 ± 2	2.01	n.s.	2.38	<0.05	2.01	n.s.
White matter†	468.5 ± 57	457.6 ± 77	6.2	0.018	488.3 ± 92	443.8 ± 62	-0.62	n.s.	0.91	n.s.	-0.62	n.s.
Total CSF†	114.5 ± 26	111.4 ± 37	1.7	0.202	94.9 ± 29	101.5 ± 25	1.83	n.s.	1.49	n.s.	1.83	n.s.
Extraventricular CSF†	90.1 ± 19	93.8 ± 28	3.2	0.081	79.4 ± 24	89.6 ± 24	1.24	n.s.	0.73	n.s.	1.24	n.s.
Ventricular CSF†	24.4 ± 10	17.6 ± 12	0.08	0.776	15.5 ± 10	11.9 ± 6	2.39	<0.05	3.01	<0.01	2.39	<0.05

The left part of the table shows the comparison of fragile X male and female group volumes for neuroanatomical regions of interest. \*ANOVAs (d.f. = 1;35) were utilized to compare mean total cerebral volumes of males and females. †For designated regions of interest, ANCOVAs (d.f. = 1;34) were utilized with total cerebral volume as a co-variate to control for overall differences in male and female brain size. P values indicate the significance levels for the main effect of group (male versus female). The right part of the table presents the mean total and regional brain volumes from the previously reported sample of typically developing children (Reiss *et al.*, 1996). Independent-measures *t* statistics and their corresponding significance levels are reported. n.s. = not significant.

**Table 2** Influence of age and gender on regional brain volumes

Dependent variable	Regression step	Independent variable	Beta	$\Delta$ in $r^2$	$P$
Cortical grey matter	1	Total cerebral volume	0.735	0.456	<0.0001
	2	Gender	-0.444	0.097	0.0100
	3	Age	-1.262	0.271	<0.0001
	4	Gender $\times$ age	-0.784	0.050	0.0012
Cerebral white matter	1	Total cerebral volume	0.735	0.585	<0.0001
	2	Gender	0.212	0.064	0.0190
	3	Age	0.829	0.160	<0.0001
	4	Gender $\times$ age	-0.446	0.016	0.0963
Ventricular CSF	1	Total cerebral volume	0.407	0.297	0.0005
	2	Gender	0.635	0.001	0.7757
	3	Age	0.887	0.078	0.0509
	4	Gender $\times$ age	-0.655	0.035	0.1794
Extraventricular CSF	1	Total cerebral volume	2.415	0.201	0.0054
	2	Gender	0.446	0.069	0.0809
	3	Age	1.094	0.112	0.0201
	4	Gender $\times$ age	-0.820	0.054	0.0888
Caudate nucleus	1	Total cerebral volume	0.483	0.289	0.0006
	2	Gender	-0.004	0.121	0.0123
	3	Age	-0.617	0.065	0.0512
	4	Gender $\times$ age	0.383	0.012	0.3960
Lenticular nucleus	1	Total cerebral volume	0.591	0.347	0.0001
	2	Gender	0.015	0.020	0.3032
	3	Age	-0.298	0.047	0.1110
	4	Gender $\times$ age	0.071	0.001	0.8821
Thalamus	1	Total cerebral volume	0.465	0.128	0.0294
	2	Gender	-0.468	0.002	0.7928
	3	Age	-0.824	0.111	0.0351
	4	Gender $\times$ age	0.520	0.022	0.3371

The table shows regression results for seven separate hierarchical regression analyses. The standardized regression coefficients (beta weights) and the incremental changes in  $r^2$  correspond to the relationship between each predictor variable and the criterion variable, or region of interest. Beta coefficients represent the standardized weight of each criterion derived from the final step of the regression analysis.  $P$  values refer to the significance level for the contribution of each predictor to the explained variance of the criterion.

Smaller yet statistically significant developmental increases were detected in volumes of CSF: age was a significant predictor of both ventricular ( $\Delta r^2 = 0.08$ ,  $P = 0.05$ ) and extraventricular ( $\Delta r^2 = 0.12$ ,  $P = 0.02$ ) CSF volumes. Non-parametric permutation testing using the bootstrapping method confirmed all of the gender and age regression results presented in Table 2.

### Brain asymmetry in children with fragile X syndrome

For total brain tissue, volumes were equivalent in the right and left hemispheres. Patterns of hemispheric asymmetry were detected in total grey [ $F(1,35) = 7.3$ ,  $P = 0.011$ ; right > left] and cortical grey matter volumes [ $F(1,35) = 6.9$ ,  $P = 0.013$ ; right > left] but the amount of white tissue was not asymmetrical. Significant asymmetry was also noted in non-ventricular [ $F(1,35) = 24.8$ ,  $P < 0.001$ ; left > right] but not in ventricular CSF volumes. No gender  $\times$  side interactions were obtained.

### Neuroanatomy-IQ associations in children with fragile X syndrome

There was a robust, statistically significant gender difference ( $F = 23.7$ ,  $P < 0.0001$ ) in mean full scale IQ scores ( $85 \pm 18$  for females and  $54 \pm 15$  for males). For the 27 females and 10 males with fragile X syndrome, IQ scores were unrelated to volumes of cortical and subcortical grey matter. IQ scores were inversely but not significantly correlated with total cerebral volume [ $r(25) = -0.175$ ,  $P = 0.30$ ]. After statistically correcting for the 8% difference between males and females, residualized cerebral volume did not explain the significant variance in IQ scores [ $r^2(25) = 0.007$ ,  $P = 0.63$ ]. Using the Fisher  $r$  to  $z$  standardization and transformation procedure, a cross-study comparison with the correlation reported in the previous study [i.e.  $r(83) = 0.454$ ,  $P = 0.0005$  (Reiss *et al.*, 1996)] indicated a statistically significant difference between typically developing children and children with fragile X in IQ-brain volume association (Fisher  $z = 2.00$ ,  $P < 0.05$ ).

## Discussion

Utilizing MRI data, the current study provides quantitative evidence for the neuroanatomical consequences of the *FMR1* mutation in children and adolescents. Interpretations of brain alterations in children and adolescents with fragile X syndrome in this study have been facilitated by a comparison with previously generated templates of typical brain development (Reiss *et al.*, 1996). Our inferences about developmental patterns from age-related findings are certainly limited by the study's cross-sectional rather than longitudinal design (Kraemer *et al.*, 2000), as these conclusions may be confounded by the inadvertent sampling biases that occur when recruiting older versus younger affected children.

Our first hypothesis was that brain morphology in children with fragile X syndrome would differ from the neuroanatomical patterns observed in typically developing children. A comparison with a similar imaging study on normal brain development (Reiss *et al.*, 1996) indicates that children and adolescents with fragile X syndrome exhibit several normal neurodevelopmental patterns (Giedd *et al.*, 1999; Reiss *et al.*, 1996). These include: (i) constancy of total cerebral volume after 5 years of age; (ii) an age-related reduction of grey matter; and (iii) a complementary age-related increase of white matter. Further, our results demonstrate that children with fragile X syndrome exhibit the normal gender difference in total cerebral volume (i.e. males ~10% larger than females) that has been observed consistently in imaging research (Pfefferbaum *et al.*, 1994; Giedd *et al.*, 1996, 1999; Reiss *et al.*, 1996). Increased cortical grey matter among males is the primary contributor to this gender discrepancy in both typically developing and fragile X children. Given the purported increased neuronal density in the granule layers of the cerebral cortex among females, our findings are concordant with the hypothesized link between gender differences in brain volume and observed gender differences in cortical neuronal density (Witelson *et al.*, 1995). The similarities in volumes of cerebral white matter observed in typically developing children and our fragile X sample are consistent with observations showing that the *FMR1* protein is normally expressed only in the neuronal bodies and not in glial cells, axons or oligodendrocytes (Feng *et al.*, 1997; Tamanini *et al.*, 1997).

In contrast to the aforementioned similarities to typically developing children, several aberrant structural and developmental patterns were observed in our fragile X sample. Unique to the fragile X sample was a gender difference in the rate of reduction of cortical grey matter with age. Also, both male and female children with fragile X syndrome exhibited increased caudate grey matter volumes (i.e. 28% for males and 13% for females) when compared with their typically developing counterparts (Reiss *et al.*, 1996). Enlarged thalamic volumes, bordering on statistical significance, were also observed. Volumetric abnormalities in the caudate, as well as in the thalamus, are probably related to suppressed production of the *FMR1* protein in the

neurons of subcortical nuclei (Devys *et al.*, 1993; Tamanini *et al.*, 1997). Deficits in expression of the *FMR1* protein in neurons appear to result in abnormal increased dendritic density (Comery *et al.*, 1997; Feng *et al.*, 1997), which may reflect abnormal development of the organizational process of synapse development and stabilization and decrement in synaptic pruning (Comery *et al.*, 1997). Also unique to the fragile X sample, we observed a gross increase in lateral ventricular volumes with age, echoing a recent study (Guerreiro *et al.*, 1998) reporting ventricular and frontal lobe anomalies. Interestingly, the gender difference (male > female) in ventricular volumes consistently reported in normal populations (Giedd *et al.*, 1996; Reiss *et al.*, 1996) was not evident in our fragile X sample. Structural changes in the caudate and ventricular CSF, not evident in the normal comparison study, may reflect an unusual turnover of subcortical grey matter resulting from cytoarchitectonic disorganization, abnormal dendritic density or possibly premature neuronal death (Rudelli *et al.*, 1985; Hinton *et al.*, 1991; Comery *et al.*, 1997).

Inferences from the asymmetry analyses offer additional insights into the neurodevelopmental impact of the fragile X mutation. The leftward predominance of lateral ventricular volumes that was observed in typically developing children (Reiss *et al.*, 1996) was notably absent in our sample. Given that asymmetry is considered a hallmark of regional specialization and efficiency (Hustler *et al.*, 1998), this result possibly reflects impairment of neuroanatomical organization in children with fragile X syndrome. However, such conclusions necessitate replication with larger samples, as limited power may have precluded detection of statistically significant asymmetry for other regions. Future research with larger sample sizes will be needed for exploration of whether the fragile X mutation results in symmetry changes within lobar subregions of the brain.

Children with fragile X may also differ from typically developing children in the relationship between neuroanatomical structures and IQ. Previous research has shown that total cerebral volume explains up to 20% of the variance in IQ scores among normal children and adults and has specifically pointed to prefrontal and subcortical grey matter as the regions most responsible for this association (Andreassen *et al.*, 1993; Reiss *et al.*, 1996). In our sample, IQ was not related to total brain, cortical grey or subcortical grey matter volumes. Inferences from these null findings are limited because of a difference in statistical power between the current and previous studies. However, it is possible that these findings reflect a disruption of normal cortical grey matter development among affected males, and the aberrations in a substantial portion of subcortical grey matter, the caudate nucleus. Individuals with fragile X syndrome have enlarged grey matter structures, coupled with mental retardation; thus the relationship between larger brain volume and cognitive functioning, demonstrated in typically developing children, may not extend to this special population.

Our second hypothesis anticipated that affected females would resemble their typically developing counterparts more than affected males, placing females in an 'intermediate' status regarding the neuroanatomical consequences of the syndrome. The more pronounced aberrations in caudate and the lag in normal cortical grey matter decline among males supports the hypothesis that the genetic and phenotypical differences in males and females with fragile X syndrome are indeed reflected in neuroanatomy. Because *FMR1* normally is expressed in neurones, both the slowdown in the rate of cortical grey matter reduction and the more pronounced volumetric aberration in the caudate among affected males are consistent with previous research showing abnormal dendritic morphology in fragile X males. Absence of the expected cortical grey matter decrease suggests that males with fragile X syndrome experience decreased pruning of synapses (Hinton *et al.*, 1991; Wisniewski *et al.*, 1991; Weiler and Greenough, 1999). The resultant excess in number and length of dendrites, resembling the immature spinal dendrites observed in early development, has already been shown in fragile X research (Hinton *et al.*, 1991; Comery *et al.*, 1997; Weiler and Greenough, 1999). As Weiler and Greenough recently purported, the FMR1 protein might be necessary for normal synaptic pruning and maturation (Weiler and Greenough, 1999). Conversely, deficits in the FMR1 protein could result in excessive synaptic and dendritic density leading to increased volume and decreased plasticity of grey matter. Weiler's hypothesis is concordant with our observation of abnormal cortical grey matter decline as well as with the excessive brain volumes of the caudate nucleus among males with fragile X.

The gender difference in caudate volume observed within our fragile X sample contrasted with the pattern observed among typically developing children; affected males had significantly larger caudate volumes than affected females, whereas previous research with healthy children has demonstrated either no gender differences (Reiss *et al.*, 1996) or larger caudate volumes in females (Giedd *et al.*, 1996). Females with fragile X syndrome, as well as males, had enlarged caudate volumes compared with their typically developing counterparts (see Table 1); that the difference was more pronounced in males supports our hypothesis that females represent an intermediate status regarding the impact of the *FMR1* gene mutation on brain development.

The fragile X mutation clearly affects the cognitive development of females to a lesser extent than males. While the current study did not identify the brain structures that directly mediate gender differences in cognitive functioning, it is intriguing to consider whether the gender discrepancy in cortical grey matter decline and in caudate volumes underlie the observed IQ differences. Males and females with fragile X syndrome follow different developmental trajectories of cognitive decline that may relate to the observed gender difference in cortical grey matter development: IQ in males declines more rapidly during childhood, than in females, which tends to remain more stable with age (Wright-

Talamante *et al.*, 1996; Fisch *et al.*, 1999). In the future, functional imaging studies will be needed to specify the relationship between structure, function and cognitive performance in fragile X syndrome.

More definitive conclusions regarding the association between molecular changes and structural anomalies (e.g. subcortical nuclei) await the advances of future research. Future investigations should utilize sample sizes that increase statistical power and allow for separate analyses of males and females. A comprehensive model of the developmental impact of the fragile X mutation will require research on four levels: (i) intracellular changes relating to protein expression and function; (ii) changes in individual cell functioning and morphology; (iii) brain tissue development and cytology of selected subregions; and (iv) the effects of tissue organization on brain volume and function. Recent efforts have advanced our knowledge of the first (Comery *et al.*, 1997; Feng *et al.*, 1997; Tamanini *et al.*, 1997) and fourth levels (Guerreiro *et al.*, 1998; Mostofsky *et al.*, 1998), whereas the mediating pathways between these stages have yet to be elucidated.

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*Received October 16, 2000. Revised March 27, 2001.*

*Accepted April 5, 2001*