Neuroanatomy of fragile X syndrome: The temporal lobe

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Article abstract—Fragile X syndrome, an X-linked genetic disorder caused by a mutation in the FMR-1 gene, is associated with a particular profile of abnormalities of behavior, learning, language, and memory, suggesting temporal lobe dysfunction. We undertook a quantitative neuroimaging study investigating the neuroanatomy of the temporal lobe in individuals with the fragile X mutation. The temporal lobe neuroanatomy of 15 young fragile X subjects was quantified and compared with that of 26 age- and IQ-matched control subjects. Analyses showed the right and left hippocampal volumes to be significantly larger in the fragile X group compared with the control group. Subjects with the fragile X mutation showed an age-related increase in volume of the hippocampus and an age-related decrease in volume of the superior temporal gyrus. Along with the findings of previous imaging studies of fragile X subjects, the results of the present investigation are consistent with studies showing a nonrandom distribution of expression of the FMR-1 gene in the developing brain, with increased expression in the cerebellum, hippocampus, and specific cortical regions. The results also suggest involvement of temporal lobe regions in the behavioral and cognitive abnormalities associated with fragile X syndrome.

Fragile X syndrome is a common genetic disorder caused by a mutation in a specific gene located on the X chromosome. Current estimates indicate that the fragile X mutation is the most common known heritable cause and at least the second most common specific genetic etiology for developmental and neurobehavioral dysfunction in the general population.1 The most recent advances in uncovering the molecular basis of fragile X syndrome have shown that there are at least two distinct categories of abnormal DNA at the fragile X locus. The fragile X premutation consists of a 150- to 600-base pair (bp) insertion or amplification (ie, additional material) within the fragile X mental retardation 1 (FMR-1) gene.2 This additional DNA primarily consists of a trinucleotide repeat of cytosine-guanine-guanine (CGG). Males and females with the premutation do not suffer the most deleterious features of the clinical syndrome, such as overt mental retardation.3,4 In the case of the fragile X full mutation, an amplification of 600 to 3,000 bp or more is typically present. The full mutation in males and females is associated with hypermethylation of the promoter region of the FMR-1 gene, a marker for gene inactivation.2 The full mutation is associated with the physical, developmental, and neurobehavioral manifestations constituting fragile X syndrome.

Although fragile X syndrome has a broad range of phenotypic features, effects on the CNS are the most important in terms of the affected individual's daily functioning. Nearly all males and approximately 50% of females with the full mutation have cognitive function in the mild to severe range of mental retardation. Descriptions of the cognitive, developmental, communication, and behavioral components of the syndrome2,9 also suggest that there is enough consistency in the quality of dysfunction within these functional domains to characterize a fragile X neurobehavioral phenotype.

In males, the behavioral component of the fragile X neurobehavioral phenotype consists of social deficits with peers, abnormalities in language and communication, unusual responses to sensory stimuli, stereotypic behavior, and hyperactivity.6,7,8,11 Some investigators6,12 have conceptualized the behavioral abnormalities occurring in males with the fragile X full mutation as having features overlapping with the behavioral syndrome of autism. Cognitive dysfunction in fragile X males includes deficits in short-term memory, visual-spatial abilities, visual-motor coordination, processing of sequential information, and attention.13-16 In addition, cross-sectional and longitudinal studies in males with...
Fragile X indicate the possibility of progressive decline in IQ, most apparent at the transition between prepuberty and puberty, suggesting that there is an ongoing degenerative component to fragile X syndrome.

Although less information is available about fragile X females, evidence suggests that females heterozygous for the full mutation demonstrate behavioral abnormalities that are similar in quality to, but less severe than, those seen in males with this condition. Social disability, anxiety, depression, and stereotypic behavior appear to be particularly important components of the female phenotype. The cognitive profile of relative strengths and weaknesses in fragile X females, including females with IQs in the normal range, resembles that in fragile X males.

As is the case for other diseases affecting the CNS, progress in understanding neurobiologic dysfunction in fragile X syndrome has been partially hindered by the inaccessibility of the living brain. However, with the recent development of technologies that allow for visualization of the CNS, the opportunity now exists to safely acquire direct and extensive information about brain structure and function in individuals with fragile X syndrome.

This paper describes the results of an ongoing project focused on describing the neuroanatomic variations associated with fragile X syndrome. The overall goal of this project is to contribute to the elucidation of the neurodevelopmental pathway through which the fragile X mutation produces neurobehavioral dysfunction in affected individuals. The findings presented are derived from quantitative analysis of temporal lobe anatomy in individuals with the fragile X full mutation and age-matched control subjects as determined from MRIs. Animal and human studies support the involvement of temporal lobe regions in learning, memory, language, attention, processing of polymodal sensory information, and social behavior. Because these are all domains of function that are often abnormal in individuals with fragile X syndrome, we chose the temporal lobe as the focus for this study.

**Methods. Subjects.** The fragile X group comprised six males and nine females with the fragile X full mutation. The genetic status of all subjects was verified with direct DNA analysis. Thirteen fragile X subjects were noted to have the full (methylated) mutation as shown by DNA analysis. Two fragile X subjects (one male and one female) had a mosaic pattern on the DNA test, with both the premutation and the full mutation forms of the FMR-1 gene noted to be present. Ages ranged from 6 to 27 years; the mean (+SD) was 12.9 ± 6.3 years. Subjects with fragile X were recruited from families living in the mid-Atlantic region in which one or more relatives had been identified as having the fragile X full mutation. Five of six males and three of nine females with fragile X were probands, i.e., they were initially tested for this condition by a geneticist or pediatrician due to the presence of developmental disability. The remainder of the fragile X subjects were tested because the condition was present in a first- or second-degree relative.

The control group consisted of 20 males and six females with ages ranging from 3 to 24 years (mean, 10.3 ± 4.9 years). Three members of the control group were normal controls recruited for this study; two control subjects had already been scheduled to undergo MRI for evaluation of medical complaints—headaches in one and possible seizures in the other; the remaining 21 control subjects had already been scheduled to undergo MRI as a component of their medical workup for a neurobehavioral or developmental disorder. For all but the three normal controls, permission to add an additional (research) scan to the control subject’s clinical protocol was obtained from the parent or guardian. Eleven of the 26 controls underwent karyotype analysis and were found to have no evidence of the fragile X chromosome. Of the 15 control subjects who did not receive genetic testing, eight were males with an IQ in the normal range (≥85) and a psychiatric diagnosis such as attention deficit hyperactivity disorder (ADHD), depression, specific developmental disorder, or oppositional defiant disorder; three were males without evidence of developmental or psychiatric disorder; one was a female without evidence of developmental or psychiatric disorder; one was a female with an IQ of 122 and a major depressive disorder; and two were being evaluated for developmental disability—a 7-year-old girl with mental retardation and ADHD, and a 3-year-old boy with mental retardation and an autistic disorder. The latter two developmentally disabled subjects did not undergo genetic testing—the girl because her family moved abroad shortly after the MRI was completed, and the boy because he received a karyotype without culture conditions designed to elicit the fragile X chromosome. (Detailed demographic information and associated medical and psychiatric diagnoses in the fragile X and control groups are available in a table filed with the National Auxiliary Publications Service; see Note at end of article.)

**Neurobehavioral evaluation.** Fourteen fragile X subjects were evaluated with the Stanford-Binet Intelligence Scale, 4th edition. One fragile X subject was evaluated with the Wechsler Intelligence Scale for Children—Revised (WISC-R). The average IQ was 86 ± 28 in the fragile X group (range, 36 to 126). Cognitive data were available for the 22 control subjects who had undergone standard IQ batteries, such as the Stanford-Binet Intelligence Scale or the WISC-R. The average IQ among these 22 control subjects was 72 ± 31 (range, 20 to 123). The remaining four control subjects were all functioning normally vocationally or educationally and had no evidence of cognitive or behavioral abnormalities at the time they underwent imaging.

As a component of their research evaluation, subjects in the fragile X group also underwent a developmental and behavioral evaluation. For diagnostic assessment, the Diagnostic Interview for Children and Adolescents—Revised (DICA-R) and the Neuropsychiatric Developmental Interview were administered to the parents. These instruments are structured diagnostic interviews designed to detect past or present DSM-III-R diagnoses, including pervasive developmental disorders (PDDs). Parents of subjects in the fragile X group were also administered the Aberrant Behavior Checklist and the Vineland Adaptive Behavior Scales. Diagnosis for subjects in the control group were obtained from review of the medical records.

**Neuroimaging.** MRIs were acquired with a General Electric 1.5-tesla Sigma scanner and archived on nine-track tape. The research imaging protocol used for the present study consisted of a series of four acquisitions: (1) an axial T1-weighted scout (TR = 400, TE = 20, 5-mm slice thickness, 2.5-mm gap) through the posterior fossa enabling more precise midline orientation of a midsagittal image, (2) a sagittal T1-weighted scan (TR = 600, TE = 20, 3- to 5-mm slice thickness, contiguous images), (3) a coronal T1-weighted scan (TR = 600 to 700, TE = 20, 3-mm slice thickness, contiguous images through the frontal and temporal regions), and (4) an axial T1-weighted scan (TR = 600 to 700, TE = 20, 3-mm slice...
All scans were clinically evaluated by a neuroradiologist. Nonspecific clinical findings were described for four subjects: a 17-year-old fragile X boy with a 3-mm focal white matter hyperintensity, a 13-year-old fragile X girl with mild ventriculomegaly, a 16-year-old male control with asymmetry of the cerebellar hemispheres, and a 10-year-old female control with diffusely increased white matter signal.

Images were imported into a modified version of the program NIH Image (National Institute of Mental Health) for processing and analysis. Neuroanatomic variables of interest for this study included the volumes of the right and left temporal lobe, amygdala, hippocampus, superior temporal gyrus, and temporal horn of the lateral ventricles, as well as a representative brain volume. The anatomy of these regions is illustrated in figure 1. Volume measurements of the cerebellar hemispheres, fourth ventricle,pons, and midbrain, and area measures of cerebellar vermal lobules I to V, VI to VIII, and IX and X, were already available from 12 fragile X subjects and 22 control subjects as a result of previous investigations.

Predetermined procedures related to image processing were employed to facilitate the identification and specification of each region of interest (ROI) with respect to surrounding tissue. For example, each image containing an ROI was magnified \( \times 2 \) on screen using bilinear interpolation. The image was then filtered to enhance contrast, sharpen edges, and smooth distorting artifact. Tissue description, based either on thresholding (representative brain volume and temporal lobe and superior temporal gyral volumes) or on operator-drawn boundaries (amygdala, hippocampus, amygdala-hippocampal slice, temporal horn, midsagittal regions), depending on the complexity and borders of the ROI, was used. Previous descriptions\(^{35,36}\) of specific neuroanatomic definitions of ROIs, as well as interrater reliabilities, were reported for midsagittal and posterior fossa measures. The mean interrater reliability for these regions, as determined with the intraclass correlation coefficient, was 0.94. Interrater agreement for temporal lobe ROIs was determined from 12 complete and six additional partial (amygdala, hippocampus, and temporal horn only) sets of measurements obtained independently by two raters (A.L.R. and J.L.). The interrater reliabilities for this study were as follows: brain volume, 0.93; temporal lobe volume, 0.94; superior temporal gyrus volume, 0.97; amygdala volume, 0.85; hippocampal volume, 0.95; and temporal horn volume, 0.68. Operational definitions for the ROIs measured in this study were partially adapted from studies of temporal lobe anatomy in other populations\(^{37,38}\) (table 1).

Data analysis. Due to the difference in gender composition between the fragile X and control groups, a two-factor (group [fragile X versus control] \( \times \) gender) analysis of variance (ANOVA) was used to determine between-group differences. To explore possible between-group effects related to the diagnosis of pervasive developmental disorder (PDD), a second two-factor (group [fragile X versus control] \( \times \) PDD diagnosis [present versus absent]) ANOVA was used as well. Within-group associations between temporal lobe variables and age, measures of posterior fossa anatomy, IQ, and behavioral-developmental function were assessed in fragile X subjects with simple or multiple regression.

**Results.** Between-group differences. There were no significant differences between the two groups in age \( (F = 2.30, p = 0.14) \) or IQ \( (F = 0.359, p = 0.55) \). Between-group differences in neuroanatomy are shown in table 2. The first two-factor ANOVA (group \( \times \) gender) showed the right \( (F = 10.32, p < 0.003) \) and left \( (F = 7.73, p = 0.009) \) hippocampal volumes to be significantly larger in the fragile X group. Right temporal horn volume was larger in fragile X subjects as well; however, this difference only approached significance \( (F = 4.03, p = 0.052) \). There were no significant gender or group \( \times \) gender effects for any neuroanatomic variable.

Four fragile X subjects and six control subjects met DSM-III-R criteria for PDD (autistic disorder or PDD, not otherwise specified\(^{39}\)). The second two-factor ANOVA (group \( \times \) PDD diagnosis) showed no significant PDD or group \( \times \) PDD effects for any neuroanatomic variable. However, there was a statistical trend suggesting that the volume of the left amygdala was smaller in subjects with a diagnosis of PDD compared with subjects without this diagnosis \( (F = 3.4, p = 0.073) \). Visual inspection of the data indicated that one control subject with a very small left amygdala volume accounted for this finding. This subject’s left amygdala
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Table 1. Operational definitions for regions of interest measured in the temporal lobe

| Brain: This measure encompasses all the brain matter that is not included within the temporal lobes. It is defined by its cortical surface and the boundaries that separate it from the temporal lobes. Its measure begins when the temporal lobes come into view and ends upon the appearance of the internal auditory canals. |
| Temporal lobe: In the anterior region of the brain, the temporal lobe is defined as the cortical surface around the lateral, medial, and inferior borders; superiorly, they are defined by the sylvian fissure and a line connecting the furthest extent of the inferior ramus of the Sylvian fissure to the choroid fissure. The temporal lobes are measured from the temporal pole until the slice at which the internal auditory canal is first seen. |
| Superior temporal gyrus: Boundaries are defined by the cortical surface of this structure and a line connecting the deepest invagination of the superior temporal sulcus to the furthest extent of the inferior ramus of the Sylvian fissure. The superior temporal gyrus is measured for six consecutive slices on each side beginning with the slice in which the amygdala first appears. |
| Amygdala: This ovoid, gray matter region is measured in only two slices. Therefore, the most representative slices in each temporal lobe are selected. The two slices chosen contain the maximum amygdala size and clarity of the gray matter borders. The two amygdala slices immediately precede the slice designated as the combined amygdala-hippocampus (A-H) slice (see below). The boundaries of the amygdala include the temporal horn or white matter inferiorly, white matter laterally and superiorly, and cortical surface medially. Portions of the uncus are included in the medial amygdala measurement. |
| A-H slice: This slice defines the transition between the waning amygdala and the emergent hippocampus. In this slice, both structures are evident and largely inseparable through manual or automated attempts at boundary detection. Therefore, both amygdala and hippocampus are measured as a single unit in this slice. Amygdala measurement immediately precedes this slice and hippocampus measurement immediately follows. |
| Hippocampus: The hippocampus is an ovoid, gray matter region emerging inferior to the amygdala. Measurement of the hippocampus begins in the slice immediately following the A-H slice and continues up to the slice in which the internal auditory canal is seen. The hippocampus is partially bordered laterally and superiorly by the temporal horn of the lateral ventricle and is bordered inferiorly by white matter and medially by cortical surface. The fimbria is included in the hippocampal measurement in slices in which it is visible. |
| Temporal horn of the lateral ventricle: This fluid-containing structure is measured whenever discernible up to the internal auditory canal. |

Table 2. Results from quantitative analysis of temporal lobe anatomy in fragile X and control groups

| Brain region | Fragile X (n = 15) | Control (n = 26) |
|--------------|-------------------|-----------------|
| Brain volume (cm$^3$) | 337.90 ± 43.96 | 338.14 ± 43.78 |
| Rt temporal lobe | 55.04 ± 8.54 | 56.04 ± 11.93 |
| Lt temporal lobe | 49.10 ± 7.13 | 51.24 ± 9.14 |
| Rt sup temporal gyrus | 6.17 ± 1.81 | 6.78 ± 1.58 |
| Lt sup temporal gyrus | 5.92 ± 1.44 | 6.30 ± 1.36 |
| Rt amygdala | 1.36 ± 0.19 | 1.37 ± 0.17 |
| Lt amygdala | 1.39 ± 0.16 | 1.34 ± 0.16 |
| Rt hippocampus* | 2.54 ± 0.41 | 2.02 ± 0.36 |
| Lt hippocampus† | 2.20 ± 0.46 | 1.82 ± 0.32 |
| Rt amyg/hipp‡ | 4.61 ± 0.47 | 4.24 ± 0.53 |
| Lt amyg/hipp§ | 4.25 ± 0.54 | 3.95 ± 0.46 |
| Rt temporal horn¶ | 0.37 ± 0.22 | 0.25 ± 0.08 |
| Lt temporal horn | 0.32 ± 0.14 | 0.25 ± 0.12 |

Two-factor (group × gender) ANOVA shows fragile X > control.

*p ≤ 0.003.
†p ≤ 0.009.
‡p ≤ 0.013.
§p ≤ 0.015.
¶p ≤ 0.052.

volume (0.87 cm$^3$) was more than three SDs less than the mean volume of this region for the two groups combined (1.36 ± 0.16 cm$^3$). There was no PDD diagnostic effect for left amygdala volume when the analysis was repeated without this subject (F(1, 15) = 1.5, p = 0.222).

Age effects. Separate multiple regression analyses were run for the fragile X and control groups to investigate the possibility of age-related changes in the volumes of the ROIs measured. The groups were analyzed separately to determine whether there was a pattern of variation in ROI volume among fragile X subjects that was different from that among controls and which might suggest an ongoing degenerative or dysmaturational process. These analyses were run with temporal lobe volume as the outcome variable and brain volume and age as the predictor (independent) variables. Brain volume was included to determine whether changes in temporal lobe volume were also associated with overall size of the cerebrum. Similarly, in the assessment of regions within the temporal lobe, the volume of the corresponding temporal lobe and age were used as predictor variables. Only those multiple regression analyses in which the overall F value reached a significance level of p ≤ 0.05 were examined further for correlations between variables. These analyses showed some similarities as well as several differences between the fragile X and control groups:

1. Right and left temporal lobe volumes (outcome variables) with brain volume and age (predictor variables) for fragile X and control groups. The volumes of the right and left temporal lobes were positively correlated with the brain volume measure for both the fragile X and control groups. A similar association for temporal lobe volume and age was not observed for either group. For the fragile X group, the partial correlation...
coefficient (partial $r^2$) for right temporal lobe and brain volume was 0.69 ($p = 0.0002$) while the partial $r^2$ for the left temporal lobe and brain volume was 0.36 ($p = 0.02$). For the control group, these analyses showed a partial $r^2$ of 0.63 ($p = 0.0001$) for right temporal lobe and brain volume and a partial $r^2$ of 0.45 ($p = 0.0002$) for left temporal lobe and brain volume. These analyses indicate that, for both the fragile X and control groups, the size of the temporal lobes increases with increasing brain volume.

2. Right and left superior temporal gyrus volumes (outcome variables) with corresponding temporal lobe volumes and age (predictor variables) for the fragile X group. The volumes of the right (partial $r^2$ for age = $-0.45, p = 0.009$) and left (partial $r^2$ for age = $-0.49, p = 0.005$) superior temporal gyri were negatively correlated with age in the fragile X group. The left superior temporal gyrus volume was also positively correlated with the volume of the corresponding temporal lobe in the fragile X group (partial $r^2$ for temporal lobe volume = 0.32, $p = 0.035$). A similar association was not observed for the right gyrus volume and corresponding temporal lobe (partial $r^2$ for temporal lobe volume = 0.18, $p = 0.132$) for fragile X subjects. Thus, the volumes of both superior temporal gyri decreased with age in this cross-sectional sample of children and young adults with the fragile X mutation (figure 2). However, only the volume of the left gyrus increased with the overall size of the corresponding temporal lobe in this group.

3. Right and left superior temporal gyrus volumes (outcome variables) with corresponding temporal lobe volumes and age (predictor variables) for the control group. The volumes of the right (partial $r^2$ for temporal lobe volume = 0.55, $p = 0.0001$) and left (partial $r^2$ for temporal lobe volume = 0.37, $p = 0.0007$) superior temporal gyri were positively correlated with the volumes of the corresponding temporal lobes, but not with age, in the control group. Therefore, in contrast with the fragile X group, the volumes of the superior temporal gyri in the control group were directly related to the overall size of the corresponding temporal lobe rather than to age.

4. Right and left hippocampal volumes (outcome variables) with corresponding temporal lobe volumes and age (predictor variables) for fragile X and control groups. The volumes of the right (partial $r^2$ for age = 0.41, $p = 0.014$) and left (partial $r^2$ for age = 0.41, $p = 0.013$) hippocampi were positively correlated with age but not with the volumes of the corresponding temporal lobes in the fragile X group. This indicates that hippocampal volume increased with age in the fragile X group. In control subjects, the right and left hippocampal volumes were not significantly correlated with age or with the volumes of the corresponding temporal lobes. However, the correlation between age and right hippocampal volume nearly reached significance (overall $p = 0.058$; partial $r^2$ for age = 0.15, $p = 0.064$) in the control group.

Exploratory analyses. Additional analyses to investigate associations between the temporal lobe regions measured in this study and cognitive, behavioral, developmental, and posterior fossa volumes were carried out for the fragile X group with simple regression. Because of the exploratory nature of these analyses, the significance level was set to $p \leq 0.01$ (two-tailed). The volumes of the four temporal lobe variables found to be of interest in this study (right and left hippocampi and right and left superior temporal gyri) were analyzed for associations with the Stanford-Binet Composite IQ and four Stanford-Binet area IQ scores, the Vineland Composite and three domain scores, and the five factor scores from the Aberrant Behavior Checklist (parent version). These analyses failed to reveal any significant correlations.

Further exploratory analyses were conducted to investigate the possibility of associations between the volumes of the four temporal lobe variables described above and volumes of neuroanatomic regions within the posterior fossa previously shown\textsuperscript{15} to differentiate fragile X from control subjects. The latter regions consisted of the area of the posterior cerebellar vermis (lobules VI to X) and the volume of the fourth ventricle. There were no significant correlations noted for these analyses.

**Discussion.** The principal findings of this study are that (1) compared with controls, young males and females with the fragile X full mutation have increased volume of the hippocampus bilaterally and (2) subjects with fragile X show an age-related increase in volume of the hippocampus and an age-related decrease in volume of the superior temporal gyrus.

The hippocampus has been relatively well studied compared with many other areas of the CNS. It is a component of neurofunctional pathways involved in learning, memory, attention, processing of polysensory information, and regulation of affect.\textsuperscript{25, 26, 40, 41} Accordingly, abnormal function of the hippocampus is potentially of interest with respect to understanding the mechanisms underlying brain dysfunction in fragile X.

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**Figure 2.** Regression plot showing the association between the volume of the right superior temporal gyrus and age for subjects with fragile X syndrome. A significant age-related decrease in volume of this neuroanatomic region is apparent.
syndrome because of the abnormalities of cognition, memory, attention, affect, socialization, and sensory response that are observed in individuals with this condition.\(^6\) The superior temporal gyrus is also potentially of interest with respect to understanding neurobehavioral abnormalities associated with fragile X syndrome. This cortical area contains regions that participate in the processing of complex auditory stimuli and language.\(^7\) Dysfunction of this region would be consistent with the abnormalities of language and communication that are common features of fragile X syndrome.

Smaller size of a neuroanatomic region is traditionally viewed as being consistent with damage to, and dysfunction of, that same region. However, more recent findings\(^8\)-\(^10\) suggest that larger-than-normal size of a specific neuroanatomic region may also be associated with neuropsychiatric conditions such as schizophrenia or affective disorder. Abnormally large size of a CNS region could result from interference with normal pruning of selected neurons or synapses, a process thought to result in more specific and functional neuronal connectivity.\(^11\) Prenatal interference with the normal biochemical, structural, spatial, or temporal aspects of neuronal migration could also lead to cellular overproliferation or misalignment and increased size of a specific region. In the case of the mammalian hippocampus, astrocytic hypertrophy of hyperplasia, thickening of dendritic fields, and abnormal increases in axonal sprouting can result from exposure to deleterious stimuli such as neurotoxins, seizures, or denervation of major afferent pathways.\(^12\)-\(^15\) Analogous hypertrophy of the inferior olivary nucleus has been demonstrated following lesions of the central tegmental tract.\(^16\) Although none of the fragile X subjects in this study had a history of a seizure disorder or exposure to the deleterious stimuli noted above, the possibility of subclinical seizures or kindling\(^17\)-\(^18\) in the mesial temporal region leading to increased hippocampal size cannot be ruled out.

The hippocampus is also a region that is rich in receptors for a variety of hormones and neuronal trophic factors, including receptors for thyroid hormone, adrenal steroids, gonadal steroids, and nerve growth factor.\(^19\)-\(^21\) These substances have been shown\(^22\)-\(^23\) to have a regulatory role in the growth, maintenance, morphology, and survival of hippocampal neurons. Accordingly, abnormalities of the cellular-neurochemical-receptor interaction might lead to structural abnormalities of the hippocampal region.

Age-related changes in the volumes of both the hippocampus and the superior temporal gyrus that were not present in the control group were observed in fragile X subjects. These findings raise the question of whether the fragile X mutation produces ongoing deleterious effects on the postnatal CNS. Recent longitudinal studies of males with fragile X syndrome\(^24\)-\(^26\) have demonstrated a plateauing in the trajectory of both intellectual skills and adaptive behavior in late childhood or early adolescence. Although these findings support imaging data suggesting that the fragile X mutation leads to ongoing detrimental effects on the postnatal CNS, a cautionary note is advised. Specifically, the age-related changes in the volumes of the hippocampus and superior temporal gyrus in the study reported here were detected in a cross-sectional group of fragile X subjects who underwent a single MRI as opposed to a group of subjects undergoing multiple scans in a longitudinal study. Therefore, the finding of age-related changes in neuroanatomy could have been influenced by ascertainment bias in that older fragile X subjects might also have had greater neuroanatomic deviation compared with younger subjects.

The absence of a statistically significant association between the neuroanatomic variables investigated here and cognitive-behavioral measures is disappointing but not surprising. The functions measured by standard cognitive or behavioral evaluations map to complex, multilocus systems of the brain rather than to specific CNS regions such as the ones measured in this study. Continued investigation in this area must utilize more focused neuropsychological assessments to increase the likelihood of detecting significant brain-behavior correlations in individuals with fragile X syndrome.

The findings of this study, as well as previous neuroimaging investigation,\(^27\)-\(^29\) suggest an association between the FMR-1 mutation and abnormalities of particular neuroanatomic regions. To date, brain regions observed to be abnormal in individuals with fragile X syndrome include the cerebellum, hippocampus, and superior temporal gyrus. Initial validation of these neuroimaging findings can be found in reports describing the pattern of FMR-1 gene expression in the mammalian brain. Hinds et al\(^30\) reported that the expression of the murine homologue of the FMR-1 gene was most concentrated in the granular layers of the hippocampus and cerebellum. The pattern of expression in the cortex of the adult mouse brain was also consistent with concentration within specific neuronal populations as opposed to comparable expression in all cortical cells. The latter finding is consistent with a pattern of degeneration that affects selected cortical regions more than others. More recently, Abitbol et al\(^31\) described the pattern of FMR-1 mRNA distribution in the human fetal brain. Although FMR-1 mRNA was distributed widely throughout the fetal brain, the highest levels were detected in cholinergic neurons of the nucleus basalis and in pyramidal neurons of the hippocampus. Given that the mammalian CNS shows regional differences in the developmental distribution of FMR-1 expression, it is not surprising that specific regions such as the hippocampus show volume alterations in the absence of changes in the more general measures, such as temporal lobe volume, used in this study.

The notion that a particular genetic mutation is linked to effects on specific regions or cell types in the brain or causes a particular profile of behavioral abnormalities would not be unique to the FMR-1 locus or the human brain. Several mutant mouse models have been described that are associated with abnormalities of specific brain regions and particular behavioral deficits. One example is the *fyn* mouse mutation, which results from a defect in a gene encoding one of four non-receptor tyrosine kinases.\(^32\) This mutation results in blunting of hippocampal long-term potentiation (LTP),
impaired spatial learning, and a 25% increase in neuron number in the CA3 region and dentate gyrus of the hippocampal formation. Another strain of mutant mice does not express the mu isoform of calcium-calmodulin-dependent protein kinase type II (μ-CaMKII). The μ-CaMKII mutant also shows deficient LTP in the hippocampus, impaired spatial learning, aberrant behavior—including “nervousness”—and abnormal exploratory activity. Behavioral features also described in animals with hippocampal lesions.

Although the results of this study suggest that human brain development is altered as a consequence of the absence of the FMR-1 protein, potential limitations of the study design should be noted. First, the mean age of the fragile X group was higher than that of the control group. Although this difference was not statistically significant, the difference could have contributed to the differences in neuroanatomy between the two groups. Second, the control group was not well matched with the fragile X group with respect to gender. However, it is unlikely that lack of gender matching affected the results, as statistical analysis failed to show significant gender or group by gender effects for any neuroanatomic variable. Third, the control group was a heterogeneous collection of patients with developmental disabilities, psychiatric disorders, and medical problems, as well as normal controls. A separate comparison with a more homogeneous control group (eg, subjects with Down's syndrome or tuberous sclerosis) would have been useful in confirming the specificity of the neuroanatomic findings observed in the fragile X subjects. Fourth, 15 of the control subjects were not tested for the fragile X chromosome. Of these 15 subjects, 11 were males with IQ scores in the normal range or with IQs inferred to be in the normal range. Given the abundance of data indicating that males with the fragile X full mutation nearly always have cognitive abilities in the mentally retarded range, it is unlikely that any of these 11 control males actually had the fragile X syndrome. Although it is possible that the two female control subjects with normal IQs or the two developmentally disabled control subjects who were not tested were actually carriers of the fragile X full mutation, the prevalence figures for the mutation in the general population (=1 in 1,000) and among the mentally retarded (=5 to 7%) make this possibility unlikely.

The findings of this study contribute to the emerging picture of specific neurobiologic and neurobehavioral facets of fragile X syndrome. The opportunity to study individuals with homogeneous genetic conditions permits more focused research on the effects of specific genes on neurodevelopmental pathways leading to normal and abnormal behavior in humans. Therefore, continued investigation into the neurobiology of fragile X syndrome may lead to improved understanding of general gene-brain and brain-behavior principles and to specific benefit for individuals affected by this genetic condition.

A particular need exists for future confirmation of the imaging findings reported here in a longitudinal/prospective study of children with fragile X syndrome followed with serial neuroanatomic and neurobehavioral assessments. Documentation of deleterious postnatal effects of the fragile X mutation on the human CNS might influence the decision regarding when and whether to treat with focused genetic interventions, if such treatments should become available. Also warranted are imaging studies focused on other regions of the brain of potential importance to the neurobehavioral phenotype associated with fragile X syndrome. These would include areas involved in modulating executive function, motoric behavior, and visual-perceptual skills—areas such as the basal ganglia, the thalamus, the frontal lobe, and the nondominant parietal-occipital cortex. Other areas of the brain warrant investigation by virtue of being neuroanatomically connected to regions already suspected of being abnormal in individuals with fragile X syndrome or because they have a particularly high expression of FMR-1 during development. These would include the nucleus basalis, entorhinal cortex, septal region, subiculum, fornix, mammillary body, thalamus, midbrain, and pons.

Correlation of findings obtained from structural imaging studies with data from functional imaging research and neuropathology studies would also greatly enhance our understanding of the neurobiology of fragile X syndrome.

Note. Readers can obtain a table (3 pages) detailing demographic and diagnostic information on the fragile X and control subjects from the National Auxiliary Publications Service, c/o Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, NY 10163-3513. Request document no. 05117. Remit with your order (not under separate cover), in US funds only, $4.50 for microfiche and $15.00 invoicing charge on all orders filled before payment.

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