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Neuroanatomy in Fragile X Females: The Posterior Fossa

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Summary

The relative homogeneity of the neuropsychiatric phenotype in individuals with fragile (fra) X syndrome suggests that there are consistent central nervous system (CNS) abnormalities underlying the observed cognitive and behavioral abnormalities. In this study, the neuroanatomy of the posterior fossa and other selected CNS regions in 12 young fra X females were compared with those of a group of 12 age-, sex-, and IQ-matched females without evidence of the fra X syndrome. Fra X females were shown to have decreased size of the posterior cerebellar vermis and increased size of the fourth ventricle, findings that are identical to those previously reported for fra X males. When compared with fra X male and nonfra X control groups, the distribution of the posterior-vermis and fourth-ventricle variables for the fra X female group was intermediate. These results support the hypothesis that the fra X genetic abnormality leads to hypoplasia of the posterior cerebellar vermis, a neuroanatomical variation of potential importance to both developmental and neuropsychiatric syndromes.

Introduction

Fragile X (fra X) syndrome is the most common heritable cause of developmental disability currently known (Webb 1989). Nearly all males with high fragile-site expression in the karyotype appear to suffer serious cognitive and behavioral consequences. In contrast to most X-linked genetic conditions, the fra X genetic abnormality also produces identifiable cognitive or behavioral disability in at least one-third of female heterozygotes.

It has been suggested that there is enough consistency in the cognitive and behavioral deficits observed in individuals with fra X syndrome to define a "neuropsychiatric phenotype" (Reiss and Freund 1991). In males, the behavioral component of this phenotype consists of social avoidance, qualitative abnormalities in communication, unusual responses to sensory stimuli, and stereotypic behavior (Reiss and Freund 1990*b*). These features have sometimes been conceptualized as being consistent with the behavioral syndrome of autism (Brown et al. 1986; Hagerman et al. 1986; Cohen et al. 1988; Reiss and Freund 1990*b*). Cognitive dysfunction seen in fra X males includes deficits in visual short-term memory, visual/spatial abilities, and processing of sequential information (Theobald et al. 1987; Kemper et al. 1988; Freund and Reiss 1991).

Although there is less information available about fra X females, some evidence suggests that female heterozygotes demonstrate behavioral abnormalities which are similar in quality but lesser in severity than those seen in males with this condition (Hagerman and Smith 1983; Hagerman et al. 1986; Miezajeski et al. 1986; Borghgraef et al. 1990; Simon et al. 1990). Social disability appears to be a particularly important component of the female phenotype (Reiss et al. 1988; Borghgraef et al. 1990; Reiss and Freund 1991). The cognitive profile of relative strengths and weaknesses observed in fra X females also resembles that described for fra X males (Theobald et al. 1987; Prouty et al. 1988; Freund and Reiss 1991).

The relative homogeneity of the neuropsychiatric phenotype in individuals with fra X syndrome suggests that there are consistent central nervous system (CNS) abnormalities underlying the observed cognitive and

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behavioral abnormalities. However, there are relatively few studies which have looked for neuroanatomical abnormalities associated with this condition. Results from some studies utilizing computed tomographic (CT) analysis and neuropathological examination in a small number of individuals with fra X syndrome have demonstrated nonspecific findings such as ventricular enlargement and subtle abnormalities of cellular morphology and cytoarchitecture of the cortex (Dunn et al. 1962; Rudelli et al. 1985; Wisniewski et al. 1985; Veenema et al. 1987). However, one study utilizing CT reported that a 10-year-old male with fra X had "vermis atrophy" (Musumeci et al. 1988). Although further details were not given in that report, it is probable that the atrophy (or hypoplasia) of the cerebellar vermis was quite significant in this patient. Variations in anatomy of the cerebellar vermis are quite difficult to detect on axial CT images, as this method of brain imaging, unlike magnetic resonance (MR) imaging, does not provide for true imaging in the sagittal plane, a procedure which is necessary for accurate assessment of vermis morphology (Curatolo and Cotroneo 1982).

In a recent study utilizing MR imaging, brain regions in the posterior fossa of 14 fra X males were compared with those of age- and sex-matched groups of fra X–negative, developmentally disabled subjects and individuals with normal IQ (Reiss et al. 1991). The fra X group was found to have significantly decreased size of the posterior vermis and increased size of the fourth ventricle, compared with both control groups. These findings are similar to those reported for a subgroup of autistic subjects by other investigators (Courchesne et al. 1988; Murakami et al. 1989).

In the present study, the neuroanatomy of the posterior fossa and other selected CNS regions in 12 young fra X females is compared with that in a group of 12 age-, sex-, and IQ-matched females without evidence of the fra X syndrome. On the basis of the hypothesis that vermal hypoplasia and fourth-ventricular enlargement are CNS features of the fra X syndrome, it was predicted that females with fra X syndrome would demonstrate neuroanatomical variations similar to those previously reported for fra X males. It was further predicted that the range of posterior fossa abnormalities occurring in the fra X female group would be intermediate between those in fra X male and those in non–fra X control groups. The association of neuroanatomical variations of the posterior fossa with components of the neuropsychiatric phenotype in fra X subjects is also explored and discussed.

Methods

Age, IQ, number of fra X and of total cells counted in the karyotype, and neuroanatomical data from all fra X and control subjects are shown in table 1. The fra X group consisted of 12 female outpatients ranging in age from 6 to 27 years, with a mean of 14.2 years. Standard fra X karyotyping methods revealed that 11 subjects in this group had clear cytogenetic evidence of the fra X chromosome. Percent fragility ranged from 4.0% to 50%. One subject (S9) had only one fra X chromosome detected in 170 total cells analyzed in two studies. However, family history and DNA studies indicated a high probability ($P > .98$) that she had received the fra X chromosome from her mother. Four subjects had repeat karyotypes as a function of a separate, ongoing research protocol. All fra X subjects had one or more first- or second-degree relatives with cytogenetically confirmed fra X syndrome.

IQ levels for all fra X subjects were determined either with the Stanford-Binet Intelligence Scale (Thorndike et al. 1986), fourth edition (10 subjects), or with the Wechsler Adult Intelligence Scale—Revised (two subjects) (Wechsler 1981). Eight fra X subjects had overall IQ levels in the normal range of intelligence, three tested in the mildly retarded range, and one subject had an IQ in the severe-to-profound range of mental retardation.

The control group consisted of 12 female subjects. Ethical concerns pertaining to imaging of "normal" subjects and funding limitations prevented recruiting our control group entirely from nonclinical populations. Therefore, the subjects in this group were drawn from various sources: (1) one subject (S16) was participating as a developmentally disabled control in an ongoing fra X research study; (2) two subjects (S13 and S21) were inpatients hospitalized on a short-stay child psychiatry inpatient unit who were already scheduled for an MR study as a component of their neuropsychiatric evaluation; (3) seven subjects were outpatients, also scheduled to received an MR study for a variety of neuropsychiatric and neurological problems including developmental disability (S15, S19, and S23), headaches (S22), seizures (S17 and S20), and learning disability (S14); (4) two subjects (S18 and S24) were normal volunteers. The age range of the control group was from 5 to 27 years, with a mean of 12.9 years.

IQ scores were available for eight control subjects and were obtained from records of most recent cognitive testing. IQ scores were not available for four sub-

Table 1

fra X Cells, IQ, and Neuroanatomical Variables for Fra X and Control Subjects

Group and Subject (age in years)	No. of fra x Cells/Total No. of Cells	IQ	Fourth-Ventricular Volume (cm^3)	Vermis (total) (cm^2)	Posterior Vermis (cm^2)	Anterior Vermis (cm^2)	Lobules VI and VII (cm^2)	PV/IC Ratio
Fragile x:								
S1 (6)	14/100	68	1.929	11.25	6.60	4.64	2.83	.0410
S2 (7)	3/70, 21/100[a]	92	2.082	10.84	5.91	4.93	2.71	.0383
S3 (9)	15/30	101	2.352	11.16	5.78	5.38	2.54	.0363
S4 (11)	6/25	82	1.332	9.14	5.02	4.12	2.50	.0330
S5 (11)	17/50, 18/100[a]	97	1.965	11.52	6.92	4.60	3.22	.0416
S6 (12)	4-10/100[b]	89	3.588	9.36	5.04	4.32	2.29	.0330
S7 (12)	6/25	126	2.253	11.38	6.21	5.17	3.01	.0389
S8 (13)	10/100, 8/100[a]	63	2.682	9.57	5.30	4.27	2.03	.0317
S9 (15)	1/75, 0/100[a]	95	1.890	10.01	6.50	3.51	2.59	.0427
S10 (20)	25/100	38	2.583	9.67	6.04	3.63	2.85	.0383
S11 (25)	6/150	68	2.010	11.67	6.42	5.25	3.68	.0434
S12 (27)	7/163	85	2.373	11.13	7.01	4.12	3.88	.0446
Control:								
S13 (5)	. . .[c]	122	1.746	9.54	5.31	4.23	2.26	.0354
S14 (7)	0/100	87	. . .[d]	11.72	6.50	5.22	3.36	.0453
S15 (7)	. . .[c]	55	2.196	. . .[e]	. . .[e]	. . .[e]	. . .[e]	. . .[e]
S16 (9)	0/100	55	1.425	11.62	7.05	4.57	3.40	.0457
S17 (10)	. . .[c]	. . .	2.226	11.10	7.07	4.03	3.17	.0450
S18 (11)	0/100	. . .	1.197	13.34	7.94	5.40	4.37	.0504
S19 (12)	. . .[f]	44	. . .[d]	11.26	6.89	4.37	3.00	.0455
S20 (13)	. . .[c]	92	1.710	10.93	6.68	4.25	3.50	.0484
S21 (16)	0/100	70	1.992	9.98	5.73	4.25	2.81	.0376
S22 (17)	. . .[c]	. . .	2.403	12.18	6.61	5.57	3.44	.0378
S23 (21)	0/100	20	1.278	11.10	6.67	4.33	3.41	.0419
S24 (27)	. . .[c]	. . .	1.827	10.15	5.88	4.26	2.90	.0392

[a] Karyotyping repeated 2 years after initial evaluation.
[b] Karyotyping interpreted as having four "definite" and six additional "questionable."
[c] Karyotyping not performed.
[d] Axial images suboptimal for morphometric analysis, because of motion artifact.
[e] Midsagittal image suboptimal for morphometric analysis, because of lateral head rotation.
[f] Subject had congenital syphilis as an infant.

jects in this group. One subject for whom IQ testing was not available was a 27-year-old adult female (S24) with a graduate degree. The three other control subjects (S17, S18, and S22) for whom IQ scores were not available ranged in age from 11 to 17 years and were attending regular classroom settings. All were described by their parents as functioning at or above grade level. Therefore, eight control subjects were considered to be of normal intelligence, one tested in the mildly retarded range of intelligence, one tested in the moderately retarded range, and two tested in the severe-to-profound range of mental retardation.

Five of the subjects (S14, S16, S18, S21, and S23) in the control group with evidence of developmental disability had been tested and found to be negative for the fra X chromosome. One of the mentally retarded control subjects (S19) had a history of congenital syphilis infection treated in infancy. One other control subject with developmental disability (S15) had moved out of the country and was not available for chromosome testing. However, review of this subject's medical records indicated that she had neither family history of X-linked mental retardation nor physical stigmata of fra X syndrome.

There was no history of exposure to potential cerebellar-toxic agents or events in any subject in either group. There was also no clinical evidence of cerebellar disease in any research subject.

After appropriate consent was obtained, MR images were obtained with a scanner operating at a 1.5-tesla magnetic field. The head was aligned with laser cross hairs centered with reference to the nasion and

midsagittal plane. T_1 weighted images, 5 mm thick with a 1.0–2.5-mm gap between slices, were obtained in the sagittal plane with a TR of 600 ms, TE of 20 ms, two excitations, 22–24-cm field of view, and a 256 × 256 matrix. Images were obtained in the axial plane by these same parameters, except that axial images were contiguous, 3 mm in thickness, and extended from the foramen magnum superiorly.

Area and volume measurements were performed on an Apple Macintosh II Image Analysis Workstation utilizing the program IMAGE (version 1.28) (Rasband 1990). MR images with all identification marks deleted were acquired for each subject as 8-bit gray-scale TIFF files utilizing a video-digitization process. Operational definitions of regions of interest (ROIs) were specified utilizing guidelines determined by an experienced neuroradiologist and with reference to standard neuroanatomical landmarks (Courchesne et al. 1989; Schnitzlein and Murtagh 1990; Reiss et al. 1991; Aylward and Reiss, in press). Scans from subjects in the control group were clinically evaluated by a neuroradiologist and read as normal, except for those of one 5-year-old girl (S13) with major depression and normal IQ who was judged to have mild dilatation of the fourth ventricle.

Quantitative analyses were performed independently by two raters who were blinded as to the source of the brain image being analyzed. During the process of evaluating an ROI, measurement was omitted if the rater judged that the scan was suboptimal for determination of specific neuroanatomical landmarks or borders because of (*a*) lack of complete inclusion of that region within the scan series, (*b*) artifact, or (*c*) partial volume averaging. The sagittal image most clearly showing the cerebral aqueduct and the lobular anatomy of the vermis (Courchesne et al. 1989) was chosen as the midsagittal slice from which area measurements were taken. Care was taken to distinguish the borders of the cerebellar vermis from the cerebellar tonsils or hemispheres (see fig. 1). Interrater reliabilities for the neuroanatomical measurements included in this study were analyzed with the intraclass correlation coefficient and averaged .94.

Statistical procedures utilized for data analyses included the Student's *t*-test for two-group comparisons, one-factor analyses of variance (ANOVAs) for three-group comparisons, and the Pearson product moment correlation. When ANOVA was used, the *F*-test for simple mean comparisons was utilized for variables in which a priori predictions had been made. A level of $P \leq .05$ was adopted as the criterion of significance for the between-group analyses for which a priori, directional predictions had been made. A significance criterion of $P \leq .01$ was set for the exploratory correlational analyses in order to control for spurious significance among the multiple correlations.

Results

Area measures taken from the midsagittal scan are shown in table 2. The size of the posterior cerebellar vermis—in particular, lobules VI and VII—was smaller in the fra X group compared with the control group (fig. 1). The ratio of the area of the entire posterior vermis to the intracranial area was determined in order to take overall brain size into consideration. As

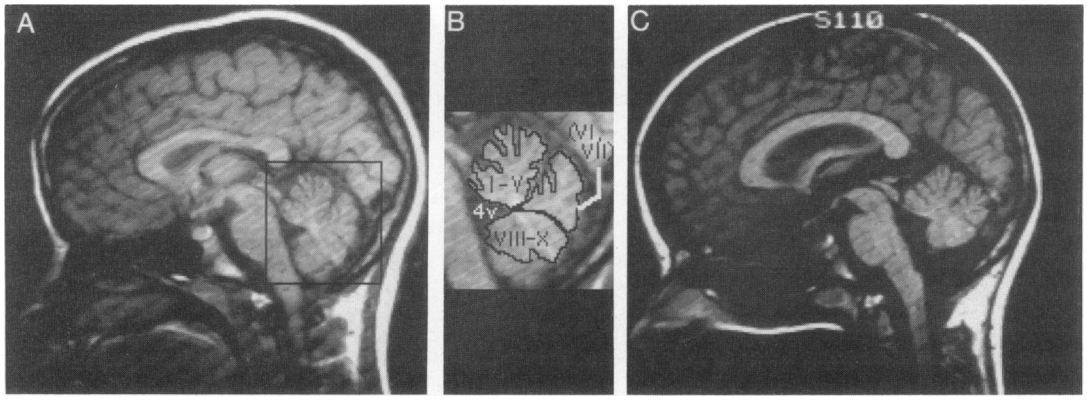

Figure 1 Representative midsagittal magnetic resonance images. *A*, Control subject showing normal anatomy. *B*, Rectangular outline at 1.5 × magnification and showing lobular anatomy of cerebellar vermis. *C*, Fra X female showing hypoplasia of posterior vermis and dilatation of fourth ventricle.

Table 2

Mean ± SD Midsagittal Area Measurements and Ratios

	Fra X (n = 12)	Control (n = 11)
Brain region:[a]		
Intracranial	157.34 ± 5.87	153.49 ± 9.42
Cortical	90.60 ± 6.68	88.79 ± 6.33
Corpus collosum	6.77 ± 1.04	6.59 ± 1.10
Vermis (total)	10.56 ± .94	11.17 ± 1.07
Anterior vermis (lobules I–V)	4.50 ± .61	4.59 ± .54
Posterior vermis (lobules VI–X)*	6.06 ± .68	6.58 ± .73
Lobules VI and VII*	2.85 ± .54	3.24 ± .52
Lobules VIII–X	3.22 ± .42	3.34 ± .36
PV/IC ratio**	.0386 ± .0043	.0429 ± .0049

[a] All data are in square centimeters.
* $P \leq .05$.
** $P \leq .025$.

in a prior study of fra X males (Reiss et al. 1991), this ratio further accentuated the decreased size of the posterior vermis in the fra X group. No differences in the size of other regions measured in the midsagittal plane (corpus collosum, cortex, and intracranial areas) were noted between the groups.

Volume measures taken from the T_1 weighted axial scan are shown in table 3. Fourth-ventricular volume was significantly larger in the fra X group. There were no other significant differences between the two subject groups. In particular, left, right and total cerebellar volumes did not differ between the subject groups, suggesting that abnormalities of the cerebellum were confined to the midline vermis in fra X subjects.

In the previous study of fra X males (Reiss et al. 1991), separate control groups of developmentally disabled and normal IQ males were evaluated with MR imaging. The results of that study showed that the two control groups were not significantly different from one another in any of the neuroanatomical variables of interest. In order to further investigate the association between fra X and posterior fossa abnormalities, one-factor ANOVAs were performed to determine whether the female control group participating in this study differed from either of the aforementioned male control subject groups on any neuroanatomical measure of the cerebellar vermis or fourth ventricle. These analyses indicated that, for these variables, the female control group was not significantly different from the male control groups utilized in the previous study. Therefore, all non–fra X, male and female subjects analyzed in the previous and current studies were combined into one control group. The new combined control group consisted of 45 subjects (33 male and 12

Table 3

Mean ± SD Volume Measurements Taken from Axial Images

Brain Region	Fra X (n = 12)	Control (n = 10)
Medulla	4.712 ± .356	4.750 ± .596
Pons	13.837 ± 1.789	12.507 ± 2.175
Midbrain	8.882 ± 1.228	8.628 ± .942
Right cerebellar hemisphere	70.853 ± 4.898	69.177 ± 7.383
Left cerebellar hemisphere	68.529 ± 4.758	67.657 ± 7.287
Cerebellum (total)	139.381 ± 9.551	136.834 ± 14.330
Third venticle	.568 ± .171	.458 ± .175
Fourth venticle*	2.253 ± .552	1.800 ± .412

NOTE.—All data are in cubic centimeters.
* $P < .025$.

female) with an average age of 12.1 years. One-factor ANOVA failed to indicate a significant difference between the male fra X, female fra X, and combined control groups by age but did show significant differences both in the posterior vermis/intracranial (PV/IC) ratio ($F(2,68) = 14.29$, $P = .0001$) and in the fourth-ventricular volume ($F(2,60) = 10.50$, $P = .0001$). Planned comparisons demonstrated that the male fra X group had a significantly smaller PV/IC ratio than did either the female fra X ($F(1,67) = 4.81$, $P < .05$) or the combined control group ($F(1,67) = 43.70$, $P < .001$) and that the female fra X group had a significantly smaller PV/IC ratio than did the combined control group. ($F(1,67) = 11.88$, $P < .01$). Both the male and female fra X groups had significantly larger fourth-ventricular volume than did the combined control group ($F(1,60) = 19.49$, $P < .001$ and $F(1,60) = 17.46$, $P < .001$, respectively) but did not significantly differ from each other.

Figures 2 and 3 show the range of both PV/IC ratio and fourth-ventricular volume as measured in the male fra X, female fra X, and male and female control groups. For these variables, the general distribution of values for the female fra X group appears to lie intermediate between those obtained in the male fra X and those obtained in the two control groups. However, the *mean* fourth-ventricular volume for the female fra X group is substantially increased by one subject (S6) with a fourth-ventricular volume greater than 2 SD from the mean of any fra X or control group.

Correlational analyses were conducted to explore, in the fra X female group, the association between the PV/IC ratio and age ($r = .48$, $P = .11$; two-tailed test); overall IQ ($r = .08$, $P > .10$; two-tailed test), verbal reasoning ($r = .05$, $P > .10$; two-tailed test), and quantitative-reasoning ($r = .30$, $P > .10$; two-tailed test) cognitive area scores; and percent fragility in the karyotype ($r = -.21$, $P > .10$; two-tailed test).

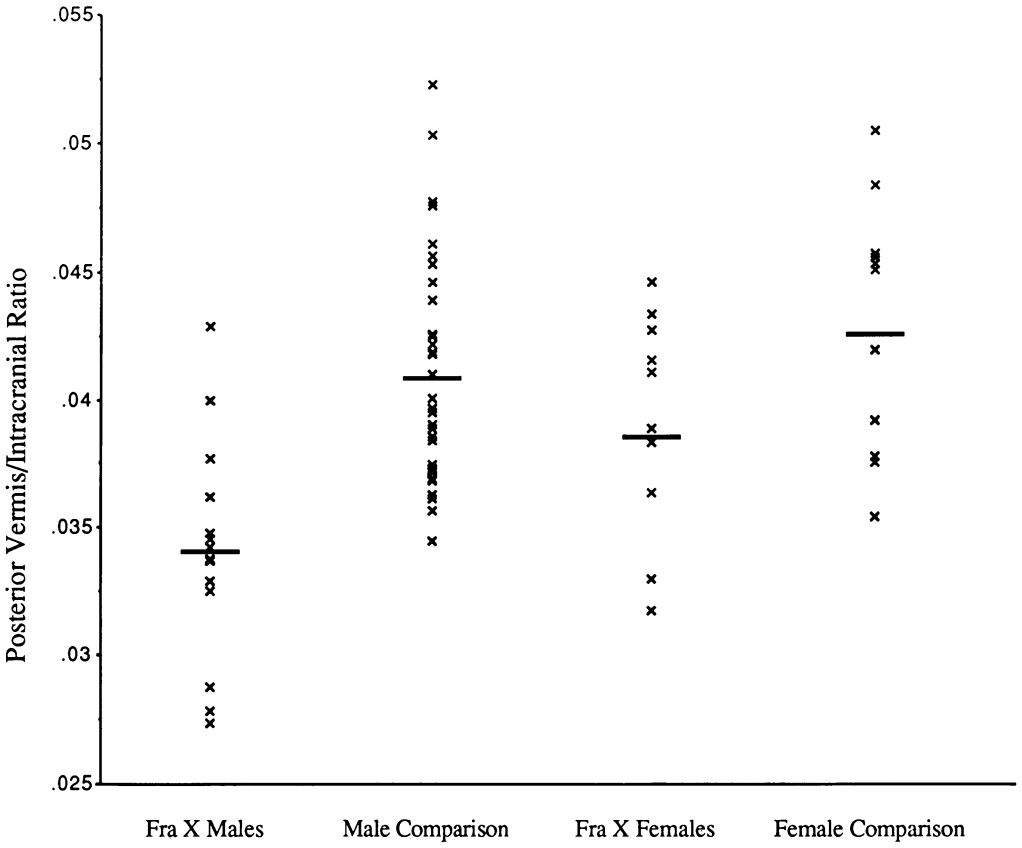

Figure 2 Scattergram showing distribution of posterior vermis area/intracranial area ratio in fragile X male, fra X female, and male and female control groups.

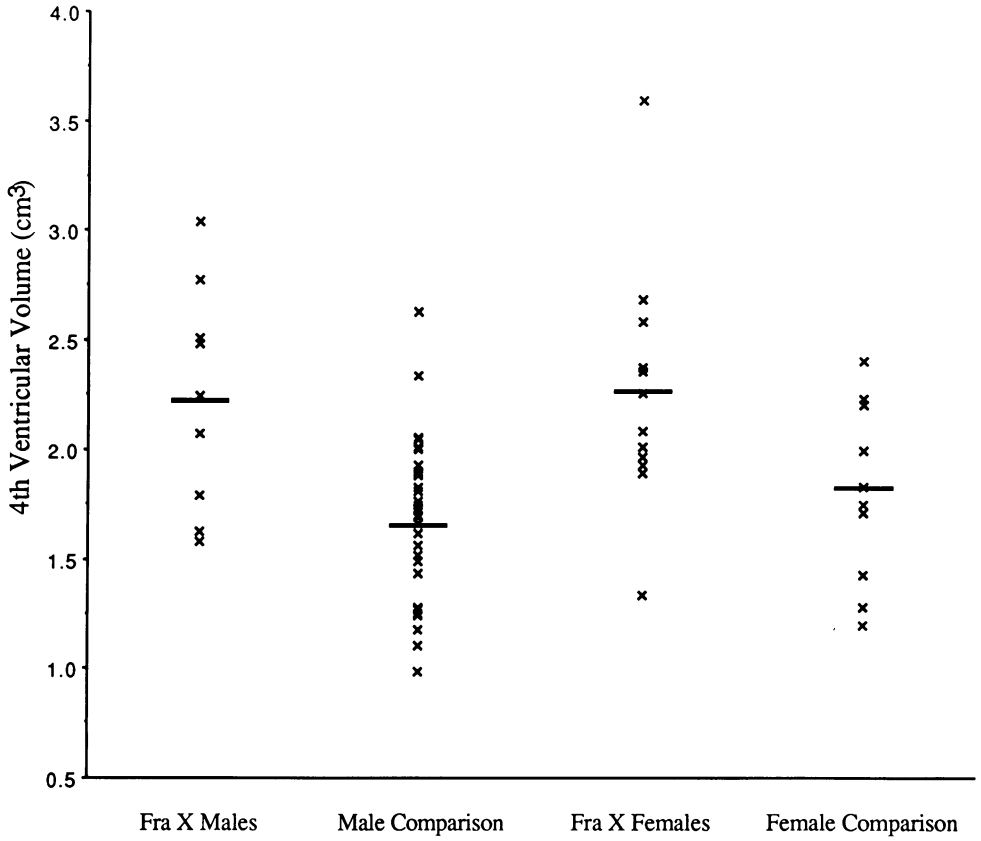

Figure 3 Scattergram showing distribution of fourth ventricular volume in fra X male, fra X female, and male and female control groups.

Examination of the association between fourth-ventricular volume and these same variables yielded no correlation coefficient greater than $\pm .25$.

Laird (1987) has suggested that higher rates of percent fragility in the karyotype or mental subnormality in a female heterozygous for fra X indicates both the presence of an "imprinted" fragile X chromosome and a more deleterious state of the fra X mutation. Therefore, as an exploratory analysis, the two female groups were compared with one another by omitting one or more subjects from the fra X group having both low fragility ($\leq 10\%$) and IQ levels within 1 SD of normal (≥ 85). Specifically, in the first analysis, the one subject (S9) in the fra X group who had very low percent fragility was omitted from the fra X group. In a second analysis, two subjects in the fra X group who showed relatively lower rates of fragility (S6 and S12) were omitted along with S9. Despite this reduction in the size of the fra X group in these analyses, significant differences ($P < .05$) between the fra X group and female control group remained for the posterior vermis area, PV/IC ratio, and fourth-ventricular-volume variables.

Discussion

In this study, a group of young fra X females were shown to have decreased size of the posterior cerebellar vermis and increased size of the fourth ventricle, compared with a group of females matched for age and IQ level. These findings are identical to those previously reported for fra X males (Reiss et al. 1991). Furthermore, in the fra X female group, with the exception of one 12-year-old female (S6) with particularly increased size of her fourth ventricle, the general distributions of both the posterior-vermis variable and the fourth-ventricle variables were intermediate between those in the fra X male group and those in the control group. Although these results do not prove a direct, causal relation between fra X and these CNS

variations, they are findings which would be predicted if vermal hypoplasia and fourth-ventricular enlargement were specifically associated with an X-linked genetic abnormality such as fra X.

One limitation of this study was that one female subject in the control group who had mild mental retardation and no known etiology for her developmental disability could not be tested for the fra X chromosome. Karyotypes were also not performed on five control-group subjects who had no evidence of developmental disability. Therefore, it is possible that one or more of these subjects could be heterozygous for fra X. However, none of these subjects had physical signs or family history suggestive of the diagnosis of fra X syndrome. Furthermore, because the prevalence of fra X in the developmentally disabled and general female populations has been estimated at $\leq 7/100$ and $<0.6/1{,}000$, respectively (reviewed in Webb 1989), the statistical likelihood that any of these subjects has fra X syndrome is quite low.

Fra X males are hemizygous for the fra X chromosome; the genetic abnormality occurs on the only X chromosome present in each somatic cell. Fra X females are heterozygous for the fra X chromosome; the genetic defect occurs on only one of the two X chromosomes present in each somatic cell. However, because of the process of random X chromosome inactivation, somatic tissue in mature female heterozygotes consists of a mosaic pattern of cell clones in which either the fragile or normal X chromosome retains nearly full capacity for genetic expression. This factor is believed to account for some of the variability in phenotypic expressivity observed in females with fra X syndrome (reviewed in Reiss and Freund 1990*a*). Accordingly, since fra X appears to be an X-linked semidominant disorder, females heterozygous for the fra X chromosome would be predicted to show an intermediate range of CNS effects which, for most females, would be less severe than those occurring in male hemizygotes.

Cognitive dysfunction is a major clinical feature of the fra X syndrome. Therefore, one might predict that a specific CNS abnormality in fra X subjects should be correlated with IQ variables. Furthermore, the likelihood of detecting such an association should be greater in female heterozygotes than in male hemizygotes. This is because individual females who are heterozygous for an X-linked condition theoretically receive varying "doses" of the genetic abnormality secondary to varying patterns of random X chromosome inactivation occurring within the CNS. Therefore, a pertinent CNS variable should be broadly distributed in a population of female heterozygotes—from normal to affected at a level equivalent to that in male hemizygotes. A gene "dose"–CNS "response" relation is thus predicted to be more readily apparent in female heterozygotes than in male hemizygotes who theoretically all receive the same "dose" of the genetic defect.

In this study, the neuroanatomical variables distinguishing fra X females from control subjects were not found to be associated with either overall IQ, cognitive-subtest scores, or percent fragility. There are several possible explanations for this finding. First, the female fra X group size may not have been large enough to allow an effect to be observed. Second, the neuroanatomical abnormalities detected in fra X subjects may not be directly caused by the genetic defect or, if secondary to the fra X genetic dysfunction, may not be etiologically related to the cognitive variables. Accordingly, abnormal size of posterior-fossa structures in fra X subjects could be a temporal marker indicating a period of brain development during which the genetic mutation is most influential. If this were the case, developmental disruption to other brain regions undergoing significant development during this period would also be expected. Third, all but one of the subjects in the fra X group had X chromosome fragility detected in the karyotype. Therefore, this subject group may not have been representative of the general fra X female population, which includes a large proportion of females who show either no or low fragility in the karyotype (Reiss et al. 1989).

Another explanation is that the clinical construct measured by IQ or cognitive-subtest scores in fra X females may not be precise enough to ascertain and specify a meaningful association with the neuroanatomical variables of interest. There is considerable evidence that both developmentally disabled and normal IQ fra X females manifest a particular profile of cognitive and neuropsychological problems including specific deficits in visual short-term memory, nonverbal reasoning, mathematics skills, and visual/spatial–visual/motor function (Kemper et al. 1986; Freund and Reiss 1991). Therefore, the pertinent neuroanatomical variables described in this study may be associated with more specific and restricted measures of cognitive, language, or neuropsychological functioning.

Neuroanatomical variables could also be related to behavioral features not measured by standard cognitive assessment—i.e., features such as abnormalities in modulation of attention, mood, or social interaction, all of which appear to be components of the

neuropsychiatric phenotype of the fra X female (Reiss and Freund 1991). As reviewed elsewhere (Courchesne et al. 1988; Reiss et al. 1991), clinical, neuroanatomical, and animal research has increasingly implicated the cerebellar vermis as an important component in functional brain systems subserving sensory and motor integration, attention, language, and modulation of agonistic behaviors. The finding that both hypoplasia of the cerebellar vermis and increased fourth-ventricular size may be a neuroanatomical feature of a subgroup of autistic children, including those with normal IQ (Courchesne et al. 1988), also suggests that neurodevelopmental abnormalities of this region are more likely to be associated with social, language, or sensory function than with general cognitive abilities. A future study will address how more specific cognitive, neuropsychological, and behavioral variables in fra X males and females are related to the neuroanatomical variations specific in the present paper.

Exploratory analyses were conducted to examine whether X chromosome imprinting (Laird 1987) may have contributed to the variability in neuroanatomical results in the female fra X group. These analyses did not alter the significance of the results when the two female groups were compared. However, only one fra X subject (S9) with normal IQ and very low fragile-site expression could be clearly characterized as having the more benign, "nonimprinted" state of the fra X mutation. Other fra X subjects showing relatively lower ($\leqslant 10\%$) fragile-site expression showed evidence of mental retardation, significant learning disabilities, or neuropsychiatric abnormalities that we have previously described in "affected" fra X females (Freund and Reiss 1991; Reiss and Freund 1991). Analysis of neuroanatomy from a larger group of fra X females with a broad range of fragile-site expression and cognitive abilities will help to clarify whether there is evidence of X chromosome imprinting effects at the level of brain structure.

Although appearing to be a consistent feature of the fra X syndrome in both males and females, posterior-fossa abnormalities are only a starting point in the search for specific neuroanatomical correlates of this important genetic cause of developmental and neuropsychiatric disability. Continued detailed study of brain structure and function in individuals with fra X syndrome is needed to provide a more coherent picture of the CNS dysfunction occurring in this genetic condition. In particular, the cognitive-behavioral phenotype observed in individuals with fra X syndrome includes abnormalities of memory, language, attention, movement, and modulation of affect (Reiss and Freund 1991). This indicates that future brain-imaging investigations should also focus on regions such as the amygdala, hippocampus, basal ganglia, planum temporale, and frontal lobe. Corresponding gross anatomical and ultrastructural investigations of the brain from neuropathological specimens should provide a critical and complementary source of information.

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