Fragile-X: Neuropsychological Test Performance, CGG Triplet Repeat Lengths, and Hippocampal Volumes

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Abstract

We compared cognitive performance and hippocampal volumes using magnetic resonance imaging (MRI) in adult fragile-X [fra(X)] males and females with either premutation (pM) or full mutation (fM) (n = 10 in all groups). Cognitive performance of fM males in the Wechsler Adult Intelligence Scale—Revised was worse than that of pM males, and the deficits in fM females were qualitatively similar, but less severe. In a visual memory test, both fM groups were impaired. In a list learning test, fM males were impaired in the learning phase and in delayed recognition. In a logical memory test, fM males and females were not significantly different from pM subjects. Hippocampal volumes normalized for intracranial or brain area did not significantly differ between fM and pM groups. However, positive correlations between left normalized hippocampal volumes and performance in many delayed memory tests observed in pM subjects were absent in fM subjects. Furthermore, in > 50% of the fM subjects, nonspecific changes, such as enlargement of ventricles and perivascular spaces, focal hyperintensities in temporal pole white matter, and/or subjectively assessed atypical appearance of hippocampal morphology, were observed in MRI. The data suggest minor abnormalities in temporal lobe structures in adult fra(X) subjects with fM. (J. Clin. Invest. 1997. 100:331–338.) Key words: fragile-X syndrome pathology • temporal lobe pathology • cognition • neuropsychology

Introduction

Fragile-X [fra(X)]† syndrome is the most common form of inherited mental retardation, with an estimated incidence of 1 in 1.250 males and 1 in 2,000 females (1, 2). The syndrome is associated with an elongation of a small DNA fragment, containing a repeat of the trinucleotide CGG, located in the 5’ untranslated promoter region of the fragile-X mental retardation 1 (FMR1) gene in the X-chromosome. In normal individuals, the repetitive (CGG)n sequence is stable with an average number of 5–35 repeats per individual. In fra(X) premutation (pM), the CGG triplet repeat number is somewhere between 60 and 200 repeats, and the instability is emphasized as the number of repeats is increased. When 200 or more CGG repeats are present, the expanded repeat sequence and an adjacent CpG island are usually hypermethylated, a phenomenon associated with transcriptional silencing of the gene. This is commonly referred to as the FMR1 full mutation (fM) (3–6). In pM, where there is no hypermethylation within the promoter region, there is normal or almost normal phenotype, whereas fM is characterized by mental retardation in most males and in ~55% of females (3, 7–9).

The function of the FMR1 gene is still unknown. Methylation of the CpG island is considered a marker for inactivation of the FMR1 gene, which may lead to the fra(X) phenotype (4, 6). Correspondingly, the fM is usually associated with a lack of FMR1 protein (FMRP) expression, whereas pM carriers might have normal FMRP expression. In neurons, the protein synthesis units of the cell, polyribosomes, are located near the synapse. Since FMRP binds to RNA and ribosomes, it has been suggested that FMRP might be involved in the control of translation of certain proteins near the synapse, which are vital for synaptic maturation and plasticity (10–13). In agreement with this, immature synapse morphology in fra(X) has been reported (12).

In normal individuals, the FMRP is most abundant in neuronal cells, particularly those in the cerebellum and Purkinje cells, although it is diffusely present throughout the gray matter (14–16). During fetal development, the highest levels of FMR1 transcription occur in the cholinergic neurons of the nucleus basalis magnocellularis (nBM) and in the pyramidal neurons of the hippocampus (15). According to animal and human studies, both the hippocampus and nBM are considered to be components of those neuroanatomical pathways involved in learning, memory, attention, processing of polysensory information, and regulation of affect (17–20). The nBM is also the major source of the cholinergic innervation of cortical and limbic structures (21). The cognitive dysfunctions in fM males consist of deficits in visual short-term memory, visual-spatial abilities, visual-motor coordination, processing of sequential information, and executive function deficits in attention (5, 7–9, 22). Although there is less information about fM females, they appear to suffer cognitive abnormalities which are similar in quality, but less severe than those seen in fM males (23–27). Accordingly, there is a possibility that the absence of FMRP in the nBM region and/or hippocampus could be involved in
the neuropsychological difficulties experienced by fra(X) individuals.

Recent developments in imaging techniques have provided noninvasive methods with which to visualize the brain. These may be of help in understanding how neurobiological factors contribute to the fra(X) syndrome. Magnetic resonance imaging (MRI) is the most accurate imaging method available. It provides excellent gray/white matter contrast, which when combined with the possibility to make multiple observations in many planes, permits detection of changes in small, irregularly shaped structures such as the hippocampus (28–30). Previously, the right and left hippocampal volumes were reported to be significantly larger in a group of young males and females (aged 6–27 yr) with fra(X) when compared with a group of age- and IQ-matched controls (31). These young fra(X) subjects also revealed an age-related increase in the volume of the hippocampus and an age-related decrease in the volume of the superior temporal gyrus. Therefore, there is evidence to implicate temporal lobe regions in the behavioral and cognitive abnormalities associated with fra(X) syndrome.

The aim of this study was to compare the neuropsychological deficits and hippocampal volumes in MRI in adult fra(X) subjects with either fM or pM. Our hypotheses were that (a) adult fM males would have the most pronounced hippocampal volume changes and that these would correlate with the neuropsychological deficits; and that (b) the hippocampal volume changes and neuropsychological deficits would correlate to the CGG triplet repeat length in the FMR1 gene.

Methods

Patients. A representative sample of males and females with either fra(X) fM or pM (n = 10 in all four study groups) were randomly selected from an east Finland population representing different pedigrees (32). The study was approved by the local Ethics Committee, and informed consent for participation in the study was obtained from each subject and a near relative. None of the subjects was institutionalized. All the subjects underwent an extensive evaluation: medical history; clinical, general, and neurological examination; Mini-Mental Status examination (MMS); activities of daily living assessed by the scale of Blessed (range 0–28, 0 representing unimpaired performance); Hamilton Psychiatric Rating Scale for Depression (range 0–64, 0 representing no depression); a neuropsychological test battery including subtests from Wechsler Adult Intelligence Scale—Revised (WAIS-R); and tests of logical and visual memory as well as a list learning task. Finally, hippocampal volumes in MRI were measured. Three fM males failed to complete the neuropsychological test battery and failed to stay motionless in the imager due to hyperactivity or severe dyskinesia. In addition, one pM male, one pM female, and one fM female failed to stay motionless in the imager. None of these three subjects differed neuropsychologically from the others in their respective groups (data not shown). 10/10 fM males and 3/10 fM females had some features of fra(X)-phenotype including prominent ears or jaw, long face, loose joints, macroorchidism, skin abnormalities, or minor limb abnormalities, whereas none of the pM subjects had any of these features. In clinical neurological examination, 10/10 fM males and 2/10 fM females had minor abnormalities in either motor coordination, visuomotor skills, diadochokinesia, or visuospatial orientation. One 16-yr-old fM male had had several partial secondarily generalized epileptic seizures. However, the patient had been seizure free for 5 yr, and without antiepileptic medication (carbamazepine) for 1 yr.

Analysis of the FMR1 CGG-repeat length. DNA was prepared from venous blood of the subjects by standard phenol-chloroform procedure. The PCR-based nonradioactive analysis of CGG-repeat region of FMR1 gene was carried out as described by Brown et al. (33) with some modifications. The amplification reactions were performed using a 5′ primer (5′-GAC GGA GGC GCC GCT GCC AGG-3′) and a 3′ primer (5′-GTG GGC TGG GCG CGC TCG AGG-3′). The reaction mixture was in a final volume of 10 ml containing 5 pmol of each primer, 200 mM each of dATP, dCTP, dTTP, and 7-deaza dGTP, 10% DMSO, 0.75 mM MgCl2, 0.25 U AmpliTaq DNA polymerase (Perkin Elmer), 1x PCR Buffer II (Perkin Elmer), and 50–100 ng of genomic DNA. The amplification conditions were: the initial denaturation at 94°C for 2 min followed by 30 cycles with denaturation at 94°C for 1 min, annealing at 62°C for 1 min, and elongation at 72°C for 2 min. The PCR products were detected by (CGG)5-chemiluminescence probe hybridization after denaturing gel electrophoresis (6% polyacrylamide, 8.3 M urea) and electroblotting. Probe, chemiluminescent spray, and hybridizing reagents were supplied as a kit (Quick-Light, FMC). Fragment sizes in CGG-repeats were determined by migration relative to previously measured CGG-repeats with lengths from 16 to 70 repeats. In cases where no fragments were seen in a sample of a male or only one fragment was seen in a sample of a female, Southern hybridization with probe StB 12.33 was carried out as previously described in Mannermaa et al. (34). The subject was considered as a being a carrier (pM) with repeats ranging from 60 to 200. The Southern hybridization procedure to detect the FMR1 mutations usually shows a series of bands, or a smear, within the full mutation size range when an affected subject is studied. This is probably due to the mitotic fragility of the large triplet repeat. Therefore, the exact measuring of the mutation size is difficult, and for fM > 200 repeats and methylation were used as criteria. In statistical analyses, the value of 200 repeats was used for fM subjects.

Neuropsychological tests. Cognitive performance was assessed by using the WAIS-R verbal and performance IQ scores (35, 36). The scores from individual subtests of WAIS-R (Information, Digit Span, Vocabulary, Arithmetic, Similarities, Picture Completion, Picture Arrangement, Block Design, Object Assembly, and Digit Symbol) were used for the assessment of different cognitive domains. To shorten the test session, the Comprehension subtest was not done. Memory was assessed by using three tests: the Logical Memory Test and the Russell’s adaptation of the Visual Reproduction Test from the Wechsler’s Memory Scale (37), and a ten-item version of the Buschke Selective Reminding Test (a list learning test) (38). In the Logical Memory Test, the scores were the number of details of a story spontaneously recalled immediately and after the delay. In the Visual Reproduction Test, the scores were the total number of details of geometrical figures reproduced immediately and after the delay. In the Buschke Selective Reminding Test, the scores recorded were the total recall in six trials and in delayed recall, spontaneous recall, and recognition (correct responses and false positives). For all the memory tests, the delayed recall was assessed after about an hour of unrelaxed testing.

MRI imaging technique. The subjects were scanned with a 1.5 T Magnetom (SP4000; Siemens Magnetom, Erlangen, Germany) using a standard head coil and a tilted coronal 3D gradient echo sequence (magnetization prepared rapid gradient echo: time of repetition 10 ms, time of echo 4 ms, time of inversion 250 ms, flip angle 12°, field of vision 250 mm, matrix 256 × 192, 1 acquisition) resulting in contiguous T1-weighted partitions with slice thickness of 2.0 mm oriented perpendicular to the long axis of the hippocampus. The boundaries of the hippocampus were outlined by a trackball driven cursor, and the number of voxels within the region was calculated by using an in-house developed program for standard work console. The outlining of the boundaries always proceeded from anterior to posterior in a sequential fashion. The volumetric measurements were performed by a single rater (M. Laakso) blinded to the clinical data and diagnostic category of the study subjects. Standard anatomical atlases of the human brain (39, 40), as well as several previous articles (28, 29, 41), were used as guidelines to determine the boundaries of the hippocampus in oblique coronal MRI sections. The hippocampus is considered to include the dentate gyrus, the hippocampus proper, and the subicular...
complex. The rostral end of the hippocampus where it first appears below the amygdala was the anatomical starting point. The caudal end of the hippocampus was taken as the section in which the crura of fornices depart from the hippocampal tail. To exclude the effect of individual brain and/or head size, the volumes were normalized for (a) brain area (BA), which refers to the area of the brain measured at the level of the anterior commissure with the ventricular spaces excluded, and for (b) coronal intracranial area (CIA) measured at the level of the anterior commissure. The intrarater and interrater agreements and reproducibility of this method have been reported previously (30, 42, 43). The normalized values were used in the statistical analyses. Normalized hippocampal volumes of fra(X) subjects were also compared to those of age- and sex-matched healthy controls (n = 10 in all control groups) derived from normal control material of Kuopio University Hospital (43). Finally, all scans were also evaluated by an experienced neuroradiologist (K. Partanen) who was blinded to the diagnostic category of the study subjects.

Statistical analyses. The data were analyzed by using Statistical Package for Social Sciences/Personal Computer (SPSS/PC+; SPSS Inc., Chicago, IL) V3.1 software. fM males and females were younger than their pM controls. Therefore, in comparisons of the means over the study groups, we used ANOVA adjusted for age. Normalized hippocampal volumes and neuropsychological tests were calculated by Pearson’s correlation test, and correlations between CGG triplet repeat lengths and MRI data as well as neuropsychological tests were calculated by the partial correlation test, controlling for sex. In fM subjects, a CGG triplet repeat value of 200 was used in statistical analysis. The data are expressed as means±SD. Values with P < 0.05 were considered significant.

Results

The age, MMS, and Blessed and Hamilton scale scores of the study subjects are shown in Table I.

Cognitive performance in WAIS-R (Table II). fM males were inferior to pM males in all the subtests of WAIS-R, and had lower verbal and performance IQs than pM males. IM females were inferior to pM females in almost all the WAIS-R subtests, and the verbal and performance IQs of IM females were lower than those of pM females. When compared with fM females, IM males were inferior in all the subtests of the verbal part, and in almost all the subtests of the performance part of WAIS-R. The verbal and performance IQs of fM males were lower than those of IM females.

Memory tests (Table III). In the Logical Memory Test, fM males tended to recall less than pM males, and recalled signifi-
Table III. Memory Test Performance in fra(X) Subjects

<table>
<thead>
<tr>
<th>Test</th>
<th>Possible range</th>
<th>fM male</th>
<th>pM male</th>
<th>fM female</th>
<th>pM female</th>
<th>ANOVA (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Logical memory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate recall</td>
<td>0–21</td>
<td>5.7±5.3*</td>
<td>9.9±4.0</td>
<td>10.4±4.6</td>
<td>12.9±2.7</td>
<td>5.6†</td>
</tr>
<tr>
<td>Delayed recall</td>
<td>0–21</td>
<td>3.7±5.0*</td>
<td>8.8±4.5</td>
<td>8.7±5.7</td>
<td>10.9±2.7</td>
<td>5.2†</td>
</tr>
<tr>
<td>Visual memory</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate recall</td>
<td>0–21</td>
<td>3.6±2.6*</td>
<td>12.7±3.7</td>
<td>7.9±3.7*</td>
<td>11.6±2.6</td>
<td>17.8†</td>
</tr>
<tr>
<td>Delayed recall</td>
<td>0–21</td>
<td>3.0±2.8*</td>
<td>11.9±4.2</td>
<td>6.1±4.1*</td>
<td>9.6±4.3</td>
<td>11.6†</td>
</tr>
<tr>
<td>List learning test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td>0–60</td>
<td>32.3±17.0*</td>
<td>44.8±6.3</td>
<td>44.8±8.9</td>
<td>51.7±3.7</td>
<td>7.5†</td>
</tr>
<tr>
<td>Long-term memory</td>
<td>0–60</td>
<td>26.9±21.1*</td>
<td>39.0±10.0</td>
<td>41.6±11.0</td>
<td>49.3±6.3</td>
<td>6.7†</td>
</tr>
<tr>
<td>Delayed recall</td>
<td>0–10</td>
<td>6.3±3.6</td>
<td>7.7±1.5</td>
<td>8.6±1.8</td>
<td>9.1±1.1</td>
<td>4.2†</td>
</tr>
<tr>
<td>Recognition: correct responses</td>
<td>0–10</td>
<td>10.0±0.0</td>
<td>9.5±1.0</td>
<td>9.9±0.3</td>
<td>10.0±0.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Recognition: false positives</td>
<td>0–10</td>
<td>4.7±4.7*</td>
<td>0.6±1.0</td>
<td>0.6±1.9</td>
<td>0.1±0.3</td>
<td>6.0†</td>
</tr>
</tbody>
</table>

ANOVA [F(3,36) for all the data except for immediate recall in Logical Memory F(3,35)] with age as a covariate was used for between-group comparisons. Overall group effects: †P < 0.05. fM males or females vs. their pM control groups, respectively: *P < 0.05. fM males vs. fM females: ‡P < 0.05.

MRI data (Table IV) and raw volumes. fM males tended to have larger BAs and CIAs than pM males, and significantly larger BAs and CIAs than fM females. fM females had larger BAs than pM females, whereas their CIAs did not differ. In general, males (fM and pM) had significantly larger CIAs (P < 0.02), and tended to have larger BAs (P = 0.07) than females (fM and pM). After controlling for group, age correlated negatively with BAs (r = −0.76, P < 0.001), and tended to correlate negatively with CIAs (r = −0.21, P > 0.1). BAs and CIAs of pM and IM males and females did not differ significantly from those of age- and sex-matched controls.

The right and left hippocampal raw volumes of fM males tended to be larger than those of pM males, and were significantly larger than those of fM females. The hippocampal raw volumes of fM and pM females did not differ. In general, males (pM and fM) had significantly larger right hippocampal volumes of fM and pM females did not differ significantly in their performance in this test. In the Visual Reproduction Test, both fM males and females reproduced significantly fewer figure details than pM males and females, respectively, both immediately after figure presentation and after a delay of 1 h. Furthermore, fM males reproduced significantly fewer figure details than fM females immediately after figure presentation. In the Selective Reminding list learning test, fM males learned fewer words than pM males and fM females did not differ significantly in their performance in this test. In the Visual Reproduction Test, both fM males and females reproduced significantly fewer figure details than pM males and females, respectively, both immediately after figure presentation and after a delay of 1 h. Furthermore, fM males reproduced significantly fewer figure details than fM females immediately after figure presentation. In the Selective Reminding list learning test, fM males learned fewer words than pM males and fM females did not differ significantly in their performance in this test. In the Visual Reproduction Test, both fM males and females reproduced significantly fewer figure details than pM males and females, respectively, both immediately after figure presentation and after a delay of 1 h. Furthermore, fM males reproduced significantly fewer figure details than fM females immediately after figure presentation. In the Selective Reminding list learning test, fM males learned fewer words than pM males and fM females did not differ significantly in their performance in this test.

Table IV. Hippocampal Volumes in MRI in fra(X) Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>fM Male</th>
<th>pM male</th>
<th>fM female</th>
<th>pM female</th>
<th>ANOVA (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA (mm³)</td>
<td>9806±546*</td>
<td>8734±1055</td>
<td>8697±821†</td>
<td>8532±1000</td>
<td>2.69</td>
</tr>
<tr>
<td>Controls</td>
<td>9433±941</td>
<td>9129±751</td>
<td>8956±863</td>
<td>8670±754</td>
<td></td>
</tr>
<tr>
<td>CIA (mm³)</td>
<td>13998±549*</td>
<td>13541±784</td>
<td>13021±732</td>
<td>13116±1022</td>
<td>2.15</td>
</tr>
<tr>
<td>Controls</td>
<td>13250±716</td>
<td>13081±729</td>
<td>12463±757</td>
<td>12412±723</td>
<td></td>
</tr>
<tr>
<td>HDX (mm³)</td>
<td>3736±281*</td>
<td>3298±499</td>
<td>3133±378</td>
<td>3179±450</td>
<td>2.46</td>
</tr>
<tr>
<td>Controls</td>
<td>3690±638</td>
<td>3855±4600§</td>
<td>3451±618</td>
<td>3503±614</td>
<td></td>
</tr>
<tr>
<td>HSIN (mm³)</td>
<td>3668±325*</td>
<td>3085±475</td>
<td>2949±461</td>
<td>3041±557</td>
<td>3.19†</td>
</tr>
<tr>
<td>Controls</td>
<td>3478±466</td>
<td>3609±4500§</td>
<td>3185±448</td>
<td>3157±443</td>
<td></td>
</tr>
<tr>
<td>HDX:BA</td>
<td>0.381±0.027</td>
<td>0.380±0.051</td>
<td>0.361±0.037</td>
<td>0.374±0.042</td>
<td>0.62</td>
</tr>
<tr>
<td>Controls</td>
<td>0.398±0.077</td>
<td>0.424±0.046</td>
<td>0.382±0.064</td>
<td>0.442±0.050</td>
<td></td>
</tr>
<tr>
<td>HDX:CIA</td>
<td>0.267±0.020</td>
<td>0.244±0.035</td>
<td>0.241±0.031</td>
<td>0.242±0.028</td>
<td>0.77</td>
</tr>
<tr>
<td>Controls</td>
<td>0.278±0.043</td>
<td>0.277±0.027</td>
<td>0.274±0.038</td>
<td>0.293±0.041</td>
<td></td>
</tr>
<tr>
<td>HSIN:BA</td>
<td>0.376±0.043</td>
<td>0.353±0.033</td>
<td>0.339±0.036</td>
<td>0.356±0.043</td>
<td>1.07</td>
</tr>
<tr>
<td>Controls</td>
<td>0.368±0.054</td>
<td>0.413±0.065§</td>
<td>0.352±0.043</td>
<td>0.400±0.044</td>
<td></td>
</tr>
<tr>
<td>HSIN:CIA</td>
<td>0.263±0.026</td>
<td>0.228±0.029</td>
<td>0.228±0.039</td>
<td>0.232±0.038</td>
<td>1.42</td>
</tr>
<tr>
<td>Controls</td>
<td>0.263±0.034</td>
<td>0.269±0.036§</td>
<td>0.250±0.020</td>
<td>0.266±0.032</td>
<td></td>
</tr>
</tbody>
</table>

Controls are age- and sex-matched healthy control subjects. ANOVA [F(3,34) for all the data] with age as a covariate was used for between fM and pM group comparisons, overall group effects: †P < 0.05. fM males or females vs. their pM control groups, respectively: *P < 0.05. fM males vs. fM females: ‡P < 0.05. Duncan’s post-hoc analysis vs. age- and sex-matched healthy controls: †P < 0.05. HDX, right hippocampus; HSIN, left hippocampus.

334 Jakälä et al.
Normalized hippocampal volumes. Right and left hippocampal raw volumes of pM males, fM males, and pM females, or fM males and females.

Nonspecific findings including subjective enlargement of ventricles and perivascular spaces, atrophy relative to age, white matter and perivascular changes in temporal poles, and subjectively atypical morphology of the hippocampi, were seen more often in fM subjects than in pM subjects (Table V). In addition to these changes, a 29-yr-old pM male had an old small contusional change in the right frontobasal region. The hippocampi of the fM male who had a history of partial secondary generalized seizures appeared normal.

Correlations between normalized hippocampal volumes, neuropsychology, and CGG triplet repeat lengths. Left and right hippocampal volumes normalized for BA and CIA were also analyzed for correlations between MMS and Blessed scale scores, the WAIS-R subtest scores, and the memory test scores in each group.

In pM males, left hippocampal volumes normalized for BA correlated positively with both immediate (r = 0.73, P < 0.05) and delayed (r = 0.79, P < 0.02) recall in the Logical Memory Test, and with Digit Span Forward (r = 0.74, P < 0.05) in WAIS-R. Left hippocampal volumes normalized for CIA correlated positively with both immediate (r = 0.81, P < 0.01) and delayed (r = 0.74, P < 0.05) recall in the Visual Reproduction Test, total recall (r = 0.85, P < 0.005), long-term memory (r = 0.81, P < 0.01), and delayed spontaneous recall (r = 0.72, P < 0.05) in the Selective Reminding list learning test, MMS test scores (r = 0.80, P < 0.02), as well as Information (r = 0.68, P < 0.05), Object Assembly (r = 0.67, P < 0.05), Block Design (r = 0.78, P < 0.02), and Digit Symbol (r < 0.75, P < 0.05) in WAIS-R. Right hippocampal volumes normalized for CIA correlated positively with MMS test scores (r = 0.67, P < 0.05).

In pM females, left hippocampal volumes normalized for BA correlated positively with delayed recall in the Logical Memory Test (r = 0.63, P < 0.05), total (r = 0.66, P < 0.05) and delayed recall (r = 0.69, P < 0.05) in the Selective Reminding Test, as well as MMS test scores (r = 0.65, P < 0.05).

In fM males, left hippocampal volumes normalized for either BA or CIA correlated negatively with Similarities (r = 0.83, P < 0.05, and r = 0.84, P < 0.02, respectively) and Picture Arrangement (r = 0.86, P < 0.02, and r = 0.82, P < 0.05, respectively) subtests of WAIS-R.

In fM females, no significant correlations between normalized hippocampal volumes and any of the neuropsychological tests were found.

Table V. Nonspecific MRI Changes in fra(X) Subjects

<table>
<thead>
<tr>
<th></th>
<th>pM male</th>
<th>fM male</th>
<th>pM female</th>
<th>fM female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enlargement of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>perivascular spaces</td>
<td>1/9</td>
<td>4/7</td>
<td>0/9</td>
<td>3/9</td>
</tr>
<tr>
<td>Focal hyperintensities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in temporal pole</td>
<td>0/9</td>
<td>3/7</td>
<td>0/9</td>
<td>2/9</td>
</tr>
<tr>
<td>Slightly atypical</td>
<td></td>
<td></td>
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<td>appearing hippocampal</td>
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<td>Enlargement of</td>
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<td>Infarcts</td>
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Figure 1. Right (A) and left (B) hippocampal volumes normalized for coronal intracranial area do not significantly differ between fM and pM males, fM and pM females, or fM males and females.
analyzed for correlations between MRI data, MMS and Blessed scale scores, all the memory test scores, and verbal as well as performance IQs. The length of the CGG triplet repeat correlated positively with BA ($r = 0.38$, $P < 0.05$) and Blessed scale scores ($r = 0.50$, $P < 0.01$), and negatively with MMS test scores ($r = -0.52$, $P < 0.01$), verbal IQ ($r = -0.54$, $P < 0.005$), performance IQ ($r = -0.43$, $P < 0.02$), and immediate recall in the Visual Reproduction Test ($r = -0.47$, $P < 0.02$).

Discussion

In line with previous studies (5, 7–9, 22, 24, 26), subjects with fra(X) fM had extensive and major cognitive deficits in WAIS-R when compared with pM subjects. fM males performed worse than pM males in all the subtests, and the deficits of fM females were qualitatively similar to those of fM males, although they were less severe. In the visual memory test, the abstract material to be remembered has to be drawn by the subject (37). Therefore, it is possible that the deficits in both immediate and delayed recall by both fM groups may relate more to deficits in visuoconstructive and/or visuospatial abilities (as indicated also by impaired performance by both fM groups in Block Design and Object Assembly in WAIS-R as compared to their pM counterparts), than to deficits in visual memory per se. However, in an earlier study (9), weaknesses in both fM males and females were found in short-term memory recall of visually presented abstract stimuli, whereas a consistent strength was observed for short-term memory recall of visually presented meaningful stimuli. Neither fM males nor fM females performed significantly worse than pM subjects in a logical memory test. In a list learning test, fM males were impaired in the learning phase and in delayed recognition, but not in delayed spontaneous recall. The lack of significant deficits in all the verbal memory measures in fM subjects when compared with pM subjects is partly in agreement with previous studies (5, 7–9), and suggests that fM subjects may have relative strengths in the domains of verbal memory or verbal functions when compared with their deficits in other cognitive functions. However, the small sample size in this study could also partially account for the failure to reach statistically significant differences in all the memory test variables between fM and pM groups.

The hippocampus is considered to belong to those neuropsychological networks involved in learning, memory, attention, processing of polysensory information, and regulation of affect (17–20). These are all the domains of function which are often abnormal in subjects with fra(X) syndrome. Thus, hippocampal dysfunction and/or pathology could well account for some of the cognitive deficits observed in fra(X). Previously, the right and left raw hippocampal volumes were reported to be significantly larger in a group of young fra(X) subjects when compared with controls (31). In this study in adult fra(X) subjects, we found no statistically significant differences in hippocampal volumes normalized for CIA or BA in fM subjects when compared with pM subjects. Furthermore, the normalized hippocampal volumes of pM subjects tended to be smaller, not larger, than those of their age- and sex-matched control subjects.

There are several methodological differences between the earlier study in young fra(X) subjects (31) and this study in adult fra(X) subjects which may explain the conflicting results in hippocampal volumes between the studies. (a) The fra(X) group in the study of Reiss et al. (31) consisted of young (mean age 12.9±6.3 yr) subjects, whereas we compared adult subjects (the mean age in years being 29 in fM males, 52 in pM males, 34 in fM females, and 48 in pM females). (b) Reiss et al. (31) combined fra(X) males and females into one group (six males and nine females), whereas in the present study fM males and fM females were kept as separate groups in the analyses. (c) Two of the fra(X) subjects in the study by Reiss et al. (31) were mosaics and 13 had fM, whereas in the present study no mosaics were included. (d) The control group of Reiss et al. (31) consisted of subjects with different psychiatric diagnoses such as attention deficit hyperactivity disorder, depression, specific developmental disorder, oppositional defiant disorder, and autistic disorder, whereas we compared fM and pM groups between each other and vs. age- and sex-matched normal healthy control subjects. (e) In this study, we used hippocampal volumes normalized for either BA or CIA (see Methods), whereas Reiss et al. (31) used hippocampal raw volumes, although they had also ascertained that there were no differences in so-called “brain” values encompassing all the brain matter which was not included within the temporal lobes. It is also relevant to note that in this study, males (pM and fM) in general had larger CIAs, BAs, and hippocampal raw volumes than females (pM and fM). Therefore, males and females (fM males and fM females and pM males and pM females) were not combined in the analysis. Furthermore, age-adjusted hippocampal raw volumes correlated significantly with BAs and CIAs. This indicates that the larger the BAs or CIAs of the individual adult fra(X) subject, the larger one would expect the raw hippocampal volumes to be. However, after the hippocampal raw volumes were normalized for brain or head size, there were no differences between adult pM and fM subjects at the group level.

In the earlier study, an age-related increase in the volume of the hippocampus and an age-related decrease in the volume of the superior temporal gyrus was seen in young fra(X) subjects (31). In contrast, in this study, age correlated negatively with hippocampal raw volumes and brain areas, i.e., the older the subjects, the smaller the hippocampal raw volumes and BAs. Thus, the increase in hippocampal raw volumes in young fra(X) subjects in the previous study could have been related to a growth and maturation process (31).

Although we found no significant changes in normalized hippocampal volumes between fM and pM groups, nonspecific changes were detected in the MRI investigation in > 50% of fM subjects. These changes included enlargement of ventricular and perivascular spaces, focal hyperintensities in temporal pole white matter, and/or somewhat atypical hippocampal morphology, indicative of structural abnormalities in temporal lobe structures in fM subjects.

Previously, the size of the hippocampus was also assessed in other genetic disorders using MRI. In Down’s syndrome, MRI studies have reported hippocampal atrophy, this being more severe in demented patients. The same studies also reported an unexplained enlargement of the parahippocampal gyrus in Down’s syndrome (44, 45). In Turner’s syndrome, the size of the hippocampus has also been reported to be diminished (46).

The delayed memory test performance is traditionally viewed as a “measure of hippocampal function.” Previously, in both Alzheimer’s disease and temporal lobe epilepsy, associations between delayed recall performance in memory tests and
hippocampal volumes detected by MRI have been noted (42, 43, 47). For example, in Alzheimer patients, the volumes of the left hippocampus correlated significantly with immediate and delayed verbal memory; the smaller the volume, the more impaired the performance (42, 43). Furthermore, both animal and human studies have demonstrated that damage to the hippocampus and adjacent cortical areas causes profound learning and memory deficits, especially in delayed recall of information (17, 18). In this study, there were positive correlations between the left normalized hippocampal volumes and performance in many delayed verbal and visual memory tests and WAIS-R subtests in pM males and females, indicating that the performance was better in those with larger left normalized hippocampal volumes. It is tempting to speculate that the absence of these correlations in fM males and females could be a reflection of abnormalities in the function of hippocampal circuitries in fM subjects.

Our hypothesis was that the neuropsychological deficits and hippocampal volumes would correlate with the lengths of the CGG triplet repeats. Indeed, the length of the CGG triplet repeat correlated positively with brain area (see Methods) and Blessed scale scores, and negatively with MMS test scores, verbal and performance IQs, and immediate visual memory. These results suggest that those fra(X) patients with longer CGG triplet repeat lengths have larger brain areas, lower IQs, and poorer cognitive performance as measured by the MMS test, Blessed scale, or visual memory tests. However, we could not find any associations between normalized or raw hippocampal volumes and CGG triplet repeat lengths.

It should also be noted that the memory deficits associated with fra(X) syndrome may not be due to hippocampal dysfunction and/or pathology alone, but could be related to pathology or abnormal function of other brain area(s) known to be important for learning and memory, e.g., diencephalic regions or frontal lobe structures, which were not measured in the present study. Furthermore, high levels of FMR1 transcription occur in the cholinergic neurons of the nBM (15). In Alzheimer’s disease, the cholinergic cells of the basal forebrain, including nBM, degenerate and this cholinergic deafferentation correlates with clinical severity of the dementia (48). Therefore, the absence of FMRP in the nBM region and cholinergic dysfunction could also lead to the neuropsychological difficulties encountered by fra(X) patients. The posterior cerebellar vermis was reported previously to be hypoplastic in both fra(X) males and females when compared with controls, and this was suggested to correlate with deficits in the processing or modulation of sensory stimuli, motor behavior, and language functions in fra(X) subjects (49, 50). The expression of the murine homologue of the FMR1 gene is most highly concentrated in the granular layers of the hippocampus and cerebellum, and expression of an FMR1/beta-galactosidase fusion gene in transgenic mice is most abundant in the hippocampus and granule cell layer of the cerebellum (16). Therefore, abnormal function of the hippocampus, nBM, or cerebellum could produce separate, perhaps dissociable, contributions to the neurobehavioral and neuropsychological disturbances experienced by individuals with fra(X) syndrome. Finally, subjects with fra(X) were reported to have an increased volume of the caudate nucleus, and, in males, also the lateral ventricle (51). Both caudate and lateral ventricular volumes correlated with IQ, and caudate volume correlated with methylation status of the FMR1 gene. Thus, caudate dysfunction could also contribute to the neuropsychological deficits seen in fra(X) syndrome.

In conclusion, adult fra(X) fM subjects did not have significantly larger hippocampal volumes normalized for either BA or CIA than adult fra(X) pM subjects. However, the absence of volumetric changes in MRI does not exclude the possibility that adult fM subjects may have neuropsychological changes affecting the hippocampus. Indeed, the nonspecific MRI changes observed in fM subjects suggest that these subjects may have minor structural abnormalities in their hippocampi. In line with this, the positive correlations between left normalized hippocampal volumes and performance in delayed memory tests observed in pM subjects were absent in fM subjects. Recent developments in the functional imaging techniques (e.g., T2 relaxation times in MRI, MR spectroscopy, and functional MRI) may help to reveal abnormalities in hippocampal function in adult fra(X) subjects with fM. Furthermore, neuropsychological investigations of the temporal lobe structures and characterization of FMRP expression in hippocampus and other areas of the brain in adult fra(X) subjects will help to clarify the neurobiology of this tragic disorder.

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tein binding to translation complexes. The 5th International Fragile X Conference, Portland, OR.


