Cognitive and Visual Processing Skills and Their Relationship to Mutation Size in Full and Premutation Female Fragile X Carriers

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ABSTRACT: Background: The fragile X gene contains an unstable trinucleotide (CGG) repeat that expands as it is passed from female carriers to the affected offspring. Obligate female carriers may have a premutation or full mutation genotype. Methods: In this study, fragile X premutation and full mutation female carriers were compared on three tasks of visual processing and cognitive skills. Results: In each case, there were significant differences between premutation and full mutation carriers on a number of the subtests or the full test scores. Specifically, full mutation female carriers performed more poorly in visual-motor processing and analysis-synthesis on the Woodcock-Johnson Psycho-Educational Battery–Revised, The Developmental Test of Visual Motor Integration, and on five of the seven subtests of the Test of Visual-Perceptual Skills. Regression analyses revealed significant negative correlations between mutation size and cognitive ability. Conclusions: These findings have implications in educational planning decisions for full mutation carriers who may present with specific cognitive deficits. (Optom Vis Sci 2000;77:592–599)

Key Words: fragile X syndrome, intelligence, visual processing, fragile X females

Fragile X syndrome is one of the most frequently encountered inherited forms of mental retardation. Other characteristics of fragile X syndrome include behavioral difficulties, facial dysmorphism, speech/language deficits, macroorchidism, and connective tissue abnormalities such as hyperextensible joints and mitral valve prolapse (Table 1 has a more complete list of characteristics associated with fragile X).1 The prevalence of fragile X syndrome was initially estimated at 1/1200 males and 1/2500 females.2 However, early prevalence estimates were based on karyotype analysis, which is not as accurate as current DNA-based testing. More recent population-based estimates give a prevalence of fragile X syndrome at 1/4000 to 1/5000 for males.3 The estimated prevalence of female carriers with no clinical features is at least 1/500 and recently as high as 1/259 in general populations.4

The fragile X syndrome was first reported in 1943 by Martin and Bell,5 who described a family in which this form of mental retardation appeared to be an X-linked trait. Later, the syndrome was found to be associated with a folate sensitive fragile site (constriction) on the X chromosome at band q27.3, leading to the use of the term fragile X syndrome to describe the syndrome.6 Initially, the diagnosis of fragile X syndrome was made on the basis of clinical features along with the observation of the fragile site on the X chromosome seen in cultured cells.

In 1991, the fragile X gene (FMR-1, fragile X mental retardation-1) was isolated and found to contain a trinucleotide (CGG) repeat in the promoter region that is mutated in fragile X affected individuals.7 New molecular analysis techniques resulted in improved diagnosis and revealed a unique genetic mechanism for transmission of the syndrome. Normal individuals carry approximately 6 to 52 repeat units, and CGG repeat number remains constant over generations. Individuals with a fragile X chromosome have a greater number of CGG repeats, and the CGG repeat number is unstable and tends to increase as the gene is passed from generation to generation.8–10 These expanded forms of the FMR-1 gene present on the fragile X chromosome can be divided into two groups based on the phenotypic effects: the premutation (about 50 to 200 CGG repeats) and the full mutation (>200 CGG repeats).4 The premutation generally has little affect on FMR-1 gene expression,11 and because the CGG repeat is transcribed but not translated, individuals with the premutation do not clinically manifest...
Physical features

- Large prominent ears
- Long narrow face
- Large head in relation to body
- High arched palate
- Flat feet
- Heart murmur or click
- Narrow palpebral fissures
- Strabismus
- Epicantal folds
- Macroorchidism
- Double-jointed thumb
- Single palmar crease
- Hyperextensible finger joints
- Seizures
- Premature ovarian failure
- Nystagmus
- Behavioral features
- Attentional problems
- Emotional difficulties
- Premature ovarian failure
- Perseveration: speech and behavior
- Hyperactivity
- Autism or autistic-like features (poor eye contact, hand flapping, hand biting, and tactile defensiveness)

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The purpose of the present study was to compare the cognitive and visual processing abilities of females with premutations and full mutations and to examine the relationship between these abilities and the number of CGG repeats in the FMR-1 gene. The first objective was to describe differences in cognitive processing using the Woodcock-Johnson Psycho-Educational Battery–Revised, a measure often used in educational settings to detect learning disabilities. It has been suggested that learning disabilities are relatively common among females with fragile X syndrome, but the identification of specific areas of deficit, including nonverbal abilities and short-term memory, has not been consistent. The second objective was to describe differences in motor and nonmotor visual information processing skills. Ocular anomalies and difficulties in visual-spatial abilities have been noted in the literature as affecting both males and females (e.g., Amin and Maino, Maes et al., Maeno et al., and Cornish et al. ). Little, however, is known about the range of visual processing abilities in higher functioning adult females with fragile X. Third, we explored the relationship between FMR-1 mutation size (number of CGG repeats) and levels of cognitive functioning and visual processing skills.
premutations were determined by polymerase chain reaction standards on Southern blots, and the number of CGG repeats in the full mutation, whereas 15 mothers carried the premutation. DNA isolated from blood samples revealed that 10 mothers carried can Americans, and 1 Asian American.

Subjects

Subjects for this study were recruited through The Fragile X Clinical/Research Group at Rush-Presbyterian St. Lukes Medical Center in Chicago, Illinois. The medical center sponsors a monthly clinic that includes medical, optometric, educational, speech/language, and advocacy services. The majority of clients are referred to the clinic by the Fragile X Association of America, a parent support and information group operating in Illinois. At the initiation of the study, the clinic had an active list of approximately 35 families. Approval for this study was obtained from the Rush-Presbyterian St. Lukes Medical Center Institutional Review Board. Of this group, 25 biological mothers aged 24 to 46 years agreed to participate in the study, and informed consent was obtained. These mothers came from middle-class urban, suburban, and rural settings. The group consisted of 22 Caucasians, 2 African Americans, and 1 Asian American.

Direct mutation analysis of the fragile X gene carried out on DNA isolated from blood samples revealed that 10 mothers carried the full mutation, whereas 15 mothers carried the premutation. Full mutations were sized via comparison to molecular weight standards on Southern blots, and the number of CGG repeats in premutations were determined by polymerase chain reaction with comparisons of product sizes to those of standards with known CGG repeat numbers. DNA from all but two study participants (both with full mutations) was analyzed by the Rush Molecular Diagnostics Laboratory. The two individuals tested elsewhere had DNA analysis via essentially the same method used at Rush. Because significant oculo-visual problems have been associated with Fragile X syndrome, each subject received a complete eye and vision evaluation before participating in this study to rule out any detrimental effects caused by the presence of significant refractive error, strabismus, amblyopia, or other similar anomaly. No subject was excluded because of ocular problems.

Statistical Analysis

Comparisons between the premutation and full mutation groups used the Student’s t-test for independent groups, except in those group comparisons with unequal variance, in which case the
nonparametric Mann-Whitney U test was used. All regression analyses used a linear model. Premutation and full mutation carriers were included in regression analysis because recent data indicates that premutation carriers with >100 repeats may show mild clinical affectedness caused by diminished translation efficiency for FMRP when long CGG repeat sequences are present in the FMR-1 mRNA. Also, our study group contained a number of patients with large premutation alleles or small full mutation alleles that might potentially produce phenotypic effects intermediate between those seen in premutation (no significant cognitive effects expected) and full mutation groups. Thus, although our carriers of smaller premutations were expected to form a grouping showing essentially normal performance, patients with larger alleles might theoretically form a continuum, at least at repeat sizes <300. Statistical calculations were performed using the GB Statistical software package (Dynamic Microsystems, Inc., Silverspring, MD). An alpha level of p < 0.05 (two-tailed) was the significance level set for rejection of the null hypothesis.

RESULTS

Cognitive Processing: WJ-R

Significant differences in performance were observed on two of the seven subtests on the WJ-R in mothers with full mutations compared with mothers with premutations (Table 4). Mothers with full mutations had lower scores in processing speed (Visual Matching, p < 0.03) and in abstract problem solving (Analysis-Synthesis, p < 0.05). On the overall measure of cognitive ability, differences between mothers with full mutations and premutations approached significance at p < 0.06 with lower scores in mothers with full mutations. Regression analysis revealed significant negative correlations between the number of CGG repeats and scores on processing speed (visual matching, r = -0.56, p < 0.02) and abstract problem solving (analysis-synthesis, r = -0.51, p < 0.03) and the overall cognitive level (broad cognitive ability, r = -0.60, p < 0.007) of the WJ-R (Fig. 1). No significant differences between groups were found in long-term retrieval (Memory for Names), short-term memory (Memory for Sentences), and comprehension knowledge (Picture Vocabulary). There were also no significant differences found between groups in auditory or visual closure skills (Incomplete Words and Visual Closure, respectively).

Visual Processing: TVPS and VMI

Significant performance differences in visual processing were found in mothers with premutations compared with mothers with full mutations. Mothers with full mutations had lower scores on five of the seven subtests of the TVPS: visual discrimination, visual memory, visual form constancy, visual figure ground, and visual closure (see Table 5 for means and p values). Mothers with full mutations also had lower scores on the VMI (p < 0.007). Regression analysis revealed significant negative correlations between the number of CGG repeats and scores on each of the individual TVPS subtests (Fig. 2) as well as the VMI (Fig. 3 and see Table 6 for r and p values). No significant differences in performance between groups were found on the visual spatial relations or visual sequential memory subtests of the TVPS.

DISCUSSION

Four major findings were described in this study. First, the female full mutation group performed significantly below the female premutation group in two of the seven areas of cognitive processing measured by the WJ-R. These areas involved visual-motor processing speed and analysis-synthesis skills. In addition, the difference between groups on broad cognitive ability approached significance (p < 0.06). No subjects fell below a score of 70, which is a historic indicator of mental retardation. Second, the full mutation group performed significantly below the premuta-

TABLE 3.
Descriptions of the subtests of the Test of Visual-Perceptual Skills (non-motor)

<table>
<thead>
<tr>
<th>Subtest</th>
<th>Evaluates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual Discrimination</td>
<td>Evaluates the ability to match or determine the characteristics of a form from among similar forms</td>
</tr>
<tr>
<td>Visual Memory</td>
<td>Evaluates the ability to remember for immediate recall the characteristics of a given form</td>
</tr>
<tr>
<td>Visual Sequential Memory</td>
<td>Evaluates the ability to remember for immediate recall a series of forms from among a group of similar forms</td>
</tr>
<tr>
<td>Visual Spatial Relations</td>
<td>Evaluates the ability to determine from among forms of identical configuration, the one single form that is arranged in the same direction as the stimulus</td>
</tr>
<tr>
<td>Visual Form Constancy</td>
<td>Evaluates the ability to find an individual form when it is presented in such a way that it is smaller, larger, rotated, reversed, or hidden among other forms</td>
</tr>
<tr>
<td>Visual Figure-Ground</td>
<td>Evaluates the ability to discriminate the figure to be attended to relative to the background information and to be aware of the relationship between the stimulus figure and the background</td>
</tr>
<tr>
<td>Visual Closure</td>
<td>Evaluates the ability to be aware of clues in the visual figure that help to determine what the figure would be if all details were present</td>
</tr>
</tbody>
</table>

TABLE 4.
Results of Cognitive Processing Tests: mean and SD for each subtest

<table>
<thead>
<tr>
<th>Subtest</th>
<th>Premutation (N = 11)</th>
<th>Full Mutation (N = 8)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory for Names</td>
<td>93.5 ± 18.9</td>
<td>87.9 ± 26.6</td>
<td>NS</td>
</tr>
<tr>
<td>Memory for Sentences</td>
<td>100.8 ± 19.0</td>
<td>97.4 ± 14.6</td>
<td>NS</td>
</tr>
<tr>
<td>Visual Matching</td>
<td>109.9 ± 12.0</td>
<td>95.0 ± 14.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Incomplete Words</td>
<td>95.3 ± 8.5</td>
<td>94.9 ± 7.5</td>
<td>NS</td>
</tr>
<tr>
<td>Visual Closure</td>
<td>108.5 ± 13.9</td>
<td>101.5 ± 9.0</td>
<td>NS</td>
</tr>
<tr>
<td>Picture Vocabulary</td>
<td>92.9 ± 11.6</td>
<td>90.6 ± 7.7</td>
<td>NS</td>
</tr>
<tr>
<td>Analysis-Synthesis</td>
<td>101.8 ± 13.8</td>
<td>89.9 ± 8.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Broad Cognitive Ability</td>
<td>98.5 ± 10.4</td>
<td>88.8 ± 10.8</td>
<td>NS</td>
</tr>
</tbody>
</table>
Data from this study also indicate that larger mutation size predicts more severe deficits in visual processing and cognitive functioning. Similar correlations have been noted in other studies that analyzed data from mixed premutation and full mutation groups. When premutation and full mutation groups were analyzed alone, such correlations have not been noted, although one of these studies reported a correlation approaching significance (p < 0.05) between mutation size and cognitive functioning in a full mutation group. Variability in the strength of correlation between repeat size and cognitive function is likely somewhat dependent on the portion of individuals analyzed with large premutations or small full mutations, which are variably methylated and produce an intermediate phenotypic effect and result in a continuum of phenotypes rather than an “all or none” effect. Recent molecular data demonstrates that even fully unmethylated large premutations are inefficiently translated despite cellular upregulation of FMR-1 mRNA levels, providing an additional molecular mechanism through which large premutations may produce a mild continuum of clinical phenotypes. Our patient set included four patients with CGG repeat numbers between 100 and 300, likely contributing to the observed correlations in the regression analysis. As is typical for large premutation and small full mutation alleles, several of these patients showed a partial methylation of their expanded allele. Indeed, future studies would benefit from measurement of activation ratios that are more sensitive and directly measure the proportion of active FMR-1 genes in a DNA sample and, conversely, indicate the amount of normally active FMR-1 genes that have been inactivated by a mutation in individual subjects. Previous research has indicated that the proportion of active normal X (inactive fragile X) chromosomes correlates with overall cognitive performance and that activation ratios in DNA correlate with cognitive abilities, particularly...
with nonverbal intelligence measures including visual spatial information processing and with executive functions. This link has not been demonstrated in all cases. Unfortunately, activation ratios were not available for all of the full mutation carriers participating in this study. Ongoing studies with larger sample sizes will be needed to further explore the relationship of activation ratios, actual FMRP levels, and mutation size to visual information processing and cognitive functioning.

The conclusion that premutation status does not seem to adversely affect overall cognitive ability, specific cognitive, or visual processes is supported by data in this study and by previous research in which subjects were also categorized by mutation status. The failure to identify specific processing deficits or patterns in nonexpressing female carriers in studies that used older diagnostic methods can be interpreted as further support of this conclusion. Overall, a preponderance of the evidence suggests no difference on cognitive measures between most female fragile X carriers who have premutations in at least small or moderate size range and noncarrier peers.

Previous studies that did not use DNA analysis in the identification of fragile X have reported conflicting findings with regard to various visual processing abilities in females. The clear dif-
ferences between full and premutation groups reported in this study, however, should help to identify future areas of study.

This study has clear implications for educators in providing appropriate programs for fragile X females with the full mutations who function in the average range of intelligence. We have demonstrated that these females have visual information processing deficits, which, if they remain unaddressed, may interfere with their ability to learn.\(^{50}\) Indeed, children who have been diagnosed with specific learning difficulties according to criteria in the Individuals with Disabilities Education Act\(^ {51}\) often demonstrate deficits in recognizing, recalling, and manipulating visual information as well as difficulties integrating visual information with motor output.\(^ {52, 53}\)

These deficits may present as educational difficulties, including problems in spelling, mathematics, handwriting, and reading performance. Individuals at risk for visually related learning problems should be referred for a comprehensive vision evaluation that would include an assessment of basic visual and visual information processing skills.\(^ {54, 55}\) The study described in this paper suggests that female carriers of the fragile X full mutation are an identifiable group at risk for visual information processing problems and should be evaluated for such problems.

REFERENCES


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