American Journal of Medical Genetics 95:150–156 (2000)

Cognitive and Behavioral Profile of Fragile X Boys: Correlations to Molecular Data

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Fragile X syndrome (FXS) is the most common form of inherited mental retardation after Down syndrome. The expansion of a CGG repeat, located in the 5′-untranslated region (5′-UTR) of the FMR1 (fragile X mental retardation) gene, leads to the hypermethylation of the repeat and the upstream CpG island. Methylation is associated with transcriptional silencing of the FMR1 gene. The lack of FMR1 protein is believed to be responsible for the typical physical and mental characteristics of the syndrome. To analyze the specific phenotype of that syndrome as well as possible associations between the phenotype and the genotype, we examined a group of 49 fragile X boys and a control group of 16 patients with tuberous sclerosis. To determine the cognitive and behavioral phenotype, the Kaufman Assessment Battery for Children (K-ABC), the Child Behavior Checklist (4/18), and a structured psychiatric interview (Kinder DIPS) were used. The genotype was analyzed by the Southern blot method. The phenotype of boys with FXS is characterized by a specific cognitive profile with strengths in acquired knowledge and in simultaneous processing. The psychiatric comorbidity is high and ADHD (attention deficit hyperactivity disorder), oppositional defiant disorder, enuresis, and encopresis predominate. In a group of 24 fragile X boys, no significant correlations between the specific aspects of the phenotype and the genotype were found. Am. J. Med. Genet. 95:150–156, 2000.

KEY WORDS: fragile X syndrome; methylation; mosaicism; phenotype; cognitive profile; psychopathology

INTRODUCTION

Although the molecular genetic basis of fragile X syndrome (FXS) has been determined [Oberle et al., 1991; Verkerk et al., 1991], the relationship between the genotype and the specific phenotype remains unexplained. The main aims of the present study were to answer the following questions: Can a specific cognitive and behavioral phenotype of FXS be identified? Are there associations between behavioral and cognitive symptoms and the genotype?

To answer these questions, a group of FXS patients ascertained by molecular genetic methods as well as a specific control group (tuberous sclerosis; TSC) were analyzed with a detailed diagnostic program.

On the Phenotype and Genotype of FXS

FXS is one of the most common genetic causes of mental retardation and is associated with the fragile site Xq27.3. The molecular mechanism of the syndrome, with a prevalence of 1:4,000 [Turner et al., 1996], is based on an expansion of a CGG repeat located in the 5′-UTR (untranslated region) of the FMR1 gene (fragile X mental retardation gene 1) [Verkerk et al., 1991]. The function of the conserved CGG repeat sequence in the promoter has not yet been determined [Deelen et al., 1994]. An amplification of the repeat leads to its hypermethylation and of the upstream CpG region. Transcriptional silencing of the FMR1 gene is believed to be responsible for the typical physical characteristics, such as large ears, a narrow face, and macroorchidism, which are often not fully developed before puberty. As females carry two X-chromosomes, the production of FMR1-protein is maintained by the nonmutated X-chromosome. Hence, in females a marked reduction and wider spectrum of clinical symptoms, somatic signs, and cognitive disabilities are observed [Hagerman, 1996a]. In addition to mental retardation, behavioral symptoms such as ADHD (attention deficit hyperactivity disorder), autistic behavior, and developmental delays in speech and motor behavior predominate [Hagerman, 1996a]. Most boys with a full muta-

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Grant sponsor: Center of Molecular Medicine, University of Cologne, Germany; Grant numbers: TV 13/22.

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Received 15 February 2000; Accepted 27 June 2000

tion have an IQ at the level of mild to severe mental retardation [Borghgraef et al., 1987; Dykens et al., 1987; Hodapp et al., 1990; Benetto and Pennington, 1996; Merenstein et al., 1996]. Strengths in their cognitive profile include a relatively large vocabulary and identification of visual stimuli, as well as simultaneous information processing [Miezejeski et al., 1986; Veenema et al., 1987; Kemper et al., 1988; Hodapp et al., 1992; Wright-Talamante et al., 1996]. Weaknesses become apparent in sequential information processing and flexible problem-solving [Dykens et al., 1987; Kemper et al., 1988; Hodapp et al., 1992], in abstract thinking, and more complex linguistic skills [Franke et al., 1996]. In addition, visual-motoric skills and short-term memory are rudimentary [Maes et al., 1994; Franke et al., 1996].

On the Phenotype and Genotype of TSC

In one-third of patients, TSC is inherited in an autosomal dominant fashion; in approximately two-thirds of cases the condition is due to a "de novo" mutation [Harrison and Bolton, 1997]. Genetic studies have identified two loci that give rise to TSC, one located on chromosome 9 (9q34), referred to as TSC1, and the other on chromosome 16 (16p13), referred to as TSC2 [Nellist et al., 1993; Povey et al., 1994]. The estimated prevalence at birth is 1:8,000 to 1:12,000 [Sampson et al., 1989; Nakauchi, 1990], although accurate estimates are difficult to obtain because of the marked variability of disease expression [Harrison and Bolton, 1997]. In classic cases, life expectancy is between 20 and 30 years.

TSC can affect most parts of the body, including characteristic skin changes [Harrison and Bolton, 1997]. The number and position of brain lesions influence not only the severity of seizures and learning difficulties, but also the severity of behavioral problems such as autism [Hunt and Dennis, 1987; Curatolo et al., 1991; Hunt, 1993; Hunt and Shepherd, 1993; Gillberg et al., 1994] and sleep problems [Curatolo et al., 1991]. Intelligence varies from normal (about one-third of cases) to more serious mental retardation (about two-thirds of patients). Hyperactivity is a characteristic behavioral feature of TSC patients, with both normal and reduced intelligence [Hunt and Dennis, 1987; Curatolo et al., 1991; Hunt, 1993; Hunt and Shepherd, 1993].

The advantage of the present study is that a relatively large group of boys with FXS and a well-selected control group were analyzed with established and normed questionnaires and tests. Thereby, a differentiated analysis of genotype and phenotype was possible.

MATERIALS AND METHODS

Subjects

A group of 49 boys with FXS, ages 5/7–16/10 years (mean = 8/6) and 16 boys with TSC, ages 5/0–17/7 years (mean = 9/5), were recruited through parental self-help groups and institutes of genetics. Only boys who were able to understand the instructions of the intelligence tests were included in the study. Severely affected children with TSC were thus excluded. Following informed consent, tests and interviews were conducted in one visit.

Intelligence

Initially, it was planned to test children up to 12/5 years with the Kaufman Assessment Battery for Children (K-ABC) and older boys with traditional Wechsler tests (HAWIK-R and HAWIE). Due to the severity of mental retardation, it became preferable to administer the K-ABC in all children and adolescents up to the age of 17/7 years. Although norms exist only for the age group up to 12/5 years, it is permissible to calculate the cognitive achievements of older children with mental retardation together with the reference group comprised of 12/5-year-olds [Maluk, 1994; Maluk and Melchers, 1998]. Because of the low cognitive levels of the boys with FXS, a second analysis of the K-ABC data was undertaken. Based on the individual test values, the developmental age of each child was calculated. The norms of the developmental age were then used to recalculate the K-ABC scales [Reynolds and Clark, 1985].

The K-ABC consists of three scales with a maximum of 16 subscales [Kaufman and Kaufman, 1983; Melchers and Preuβ, 1991, 1992]. The "Simultaneous Processing" and the "Sequential Processing" scales allow calculation of the "Mental Processing Composite" scale, which represents the general IQ. The "Achievement" scale, on the other hand, measures acquired knowledge.

Psychopathology

Clinically relevant psychopathology was assessed by a structured psychiatric interview (Kinder-DIPS) [Schneider et al., 1995] and by the parental Child Behavior Checklist questionnaire (CBCL 4/18) [Achenbach, 1991].

The Kinder-DIPS [Schneider et al., 1995] is the best known German-language structured interview that codes for categorical psychiatric disorders (1. axis) according to both ICD-10 [WHO, 1993] and DSM-IV [APA, 1994] criteria. Multiple diagnoses are possible. The entire parent version of the interview was used.

The CBCL 4/18 is the best-established parental questionnaire to assess children's general behavior [Achenbach, 1991]. The problem scores consist of eight specific syndrome scores and three composite scores: "Internalizing Behavior," "Externalizing Behavior," and "Total Score." The cutoffs for the clinical and borderline ranges were chosen (T-values 67 and 60, respectively) using the recently calculated German norms [Arbeitsgruppe Deutsche Child Behavior Checklist, 1998].

Genetic Analyses

Determination of the repeat amplification. DNA was extracted from peripheral blood obtained by venipuncture. The genomic DNA was then cleaved with *EcoR*I, *Eag*I, or *Pst*I, and the DNA fragments were separated by electrophoresis on 0.8% agarose gels, Southern blotted onto Boehringer nylon-plus membranes, and hybridized to the labeled *Xho*I-*Pst*I fragment of the pE5.1 plasmid [Verkerk et al., 1991]. The lengths of the amplified repeats were calculated

from band positions on the autoradiograms relative to standard size markers.

Methylation in the repeat. To determine the methylation status of the 5′-UTR of the FMR1 gene the methylation sensitive enzyme *Eag*I was used.

Statistics

Statistics were calculated with SPSS for Windows ver. 7.0. Descriptive statistics ("frequencies," "descriptives") were calculated as well as univariate statistics (*t*-tests, Mann-Whitney test, correlations). An alpha-adjustment according to Bonferroni was not employed. Due to the size of the samples (FXS = 49, TSC = 16), only a few statistically relevant differences would remain significant. Also, the main emphasis was placed on descriptive and not on inferential statistics.

RESULTS

Intelligence

Out of a total of 49, 43 boys were tested with the K-ABC and two older boys with Wechsler tests (HAWIK-R). Four children could not be tested as they were not able to concentrate on the test situation. In one child, only the language-free subtests of the K-ABC could be administered. Therefore, the K-ABC could be evaluated completely for 42 children and the "Simultaneous Processing" scale only for one boy. Five boys with FXS were older than 12/5. For them the norms of the 12/5-year-olds were used.

The average cognitive level of children with FXS was equivalent to moderately severe mental retardation, with a mean of 46.6 for the "Mental Processing Composite" scale. The values range from mild mental retardation (highest value, 67) to moderately severe mental retardation (lowest value, 40). The average values for the different scales of the K-ABC are shown in Figure 1. There were no statistically significant differences between the "Sequential Processing" (mean = 46.1) and the "Simultaneous Processing" (mean = 46.6) scales. With a mean of 48.8, the "Achievement" scale, which measures acquired knowledge, was slightly, although significantly, higher than the "Mental Processing Composite" scale, with 46.6 ($P < 0.05$). The differences between the "Sequential Processing" and the "Achievement" scale were also significant ($P < 0.05$).

K-ABC could be used for all children with TSC, but since nontestability was an exclusion criteria in the TSC group, they were not representative of the entire TSC group, but of a higher-functioning subgroup. Two boys were older than 12/5. For them the norms of the 12/5-year-old children were used.

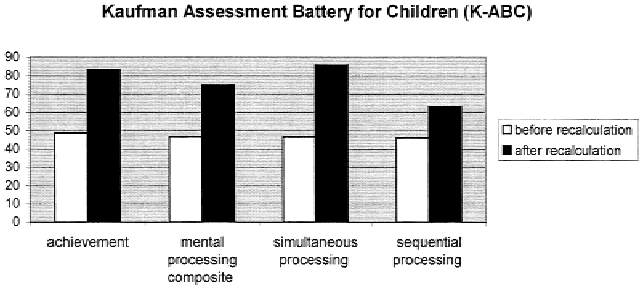

Fig. 1. Average values for the different scales of the "Kaufman Assessment Battery for Children" (K-ABC) for boys with FXS (before and after the recalculation according to the developmental age).

The boys with TSC had a general intelligence ("Mental Processing Composite" scale) at the level of mild mental retardation (mean = 59.9; minimum 40 to maximum 107). The differences between the "Simultaneous Processing" (mean = 60.6) and the "Sequential Processing" (mean = 58.9) scales, as well as between "Mental Processing Composite" scale (mean = 59.9) and the "Achievement" scale (mean = 60.6), were not significant.

For different subtests of the "Mental Processing Composite" scale, there is a striking strength in the subtest "Gestalt Closure" for the boys with both FXS and TSC. In the group of FXS boys, the result of this subtest differs significantly (*t*-tests; $P < 0.001$) from the results of all the other subtests. For the TSC group the differences to the other subtests are also significant (Wilcoxon test: for "Hand Movements," "Number Recall," "Photo Series," and "Triangles" = $P < 0.01$; for "Word Order," "Matrix Analogies," and "Spatial Memory" = $P < 0.05$).

In total, both groups showed a relatively homogeneous cognitive profile: the FXS boys in the range of moderately severe, the TSC children in the range of mild mental retardation. They differed statistically regarding the "Sequential Processing" scale ($P < 0.001$), the "Mental Processing Composite" scale ($P < 0.05$), as well as the "Achievement" scale ($P < 0.05$).

Because of the low cognitive levels of the FXS boys, the K-ABC, which was not primarily designed for mentally retarded children, reaches a "floor effect" and cannot differentiate sufficiently between the scales. Thus, individual patients could very well have shown marked differences in their cognitive profiles had the K-ABC included simpler tasks. Therefore, a second analysis of the K-ABC data was undertaken. Based on the individual test values, the developmental age of each child was calculated. The norms of the developmental age were then used to recalculate the K-ABC scales [Reynolds and Clark, 1985].

The average age of the FXS children was 8/9 years, their average developmental age 4/8 years, so that the average difference between both was 4/1 years. The values for the scales based on the developmental age documented marked statistical differences.

Based on the results of this recalculation (see Fig. 1), the "Simultaneous Processing" scale (mean = 85.7) was significantly higher than the "Sequential Processing" scale (mean = 63.3; $P < 0.001$). The differences between the "Achievement" scale (mean = 83.2) and the "Sequential Processing" scale (mean = 63.3; $P < 0.001$), as well as between general intelligence ("Mental Processing Composite" scale; mean = 74.9) and acquired knowledge ("Achievement" scale, mean = 83.2) were also statistically significant ($P < 0.01$).

The average age of the TSC children was 9/11 years, their developmental age 5/11 years, with an average difference of 4 years. The differences calculated with the Wilcoxon Test between the "Simultaneous Processing" scale (mean = 89.9) and the "Sequential Process-

ing" scale (mean = 76.9; $P < 0.01$), as well as between the "Achievement" scale (mean = 94.5) and the "Mental Processing" scale (mean = 84.2; $P < 0.05$) were statistically significant.

Psychopathology

Both the FXS and the TSC groups showed a high degree of psychiatric comorbidity regarding DSM-IV and ICD-10 diagnoses, which were gained through a structured child psychiatric interview with the parents (Kinder-DIPS) (Table I). In only 18.4% of the boys with FXS and 25% of the children with TSC could no definite psychiatric diagnosis be reached.

The most common DSM-IV diagnosis among the FXS boys was ADHD (n = 36; 74%), followed by oppositional defiant disorder (n = 14; 29%), functional enuresis (n = 13; 27%), functional encopresis (n = 10; 20%), separation anxiety disorder (n = 5; 10%), and one child (2%) with an obsessive-compulsive disorder (Fig. 2).

The psychiatric comorbidity among the TSC children was lower, the types of disorders similar to those of the FXS boys. ADHD was the most common diagnosis (n = 7; 44%), again followed by oppositional defiant disorder (n = 4; 25%), separation anxiety disorder (n = 3; 19%), functional enuresis (n = 2; 13%), and functional encopresis (n = 1; 6%).

There were significant differences regarding ADHD between the two groups ($\chi^2 = 4.76$; $P < 0.05$). There were no significant differences for the other diagnoses.

Regarding the results of the CBCL, more boys with FXS revealed relevant behavioral problems than boys with TSC. Compared to the normative population, the rate of children with "Total Problems" (89.8%) was increased by a factor of 6 (Table II). On the syndrome scales, again "Attention Problems," "Social Problems," and "Thought Problems" predominated. Boys with TSC exhibited a similar profile at a lower level.

Significant differences between boys with FXS and TSC were found regarding the scales "Attention Problems" ($\chi^2 = 5.81$; $P < 0.05$) and the "Total Problem" score ($\chi^2 = 4.26$; $P < 0.05$).

Molecular Genetic Data and Correlations to the Phenotype

The genomic DNA of 24 fragile X boys (from a total of 49) was analyzed by the Southern blot hybridization technique to determine the repeat lengths. Data of the other 25 boys could not be analyzed, as parents and children refused to have blood samples taken. The repeat lengths were calculated from autoradiograms (data not shown). The shortest and the longest fragments are indicated in Table III. These results were then correlated to the different aspects of the cognitive and behavioral phenotypes. The results show that there are no correlations between the lengths of the CGG repeat and the specific characteristics of the phenotype. In detail, the variations in repeat length and status of mosaicism have no apparent correlations to the different aspects of the phenotype, such as intelligence (K-ABC), behavioral problems, and psychiatric diagnoses (CBCL; Kinder DIPS). In Table III the molecular data from the 24 boys with FXS and some of the cognitive and behavioral data are shown.

In all analyzed DNA samples of patients, the methylation-sensitive restriction site *Eag*I was methylated (data not shown). It is known that the methylation of the promoter sequence can silence gene expression and a lack of expression of the FMR1 protein is believed to be responsible for the phenotype of FXS. Since only minimal size blood samples were available, Western blot analyses to test for the presence of the FMR1 protein in peripheral white blood cells (PWBCs) could be performed on cell extracts from only four FXS boys and two premutation carriers. In the isolated protein extracts from these four FXS patients, no FMR1 protein was detectable. In the extracts from the premutation carriers, reduced amounts of FMR1 protein were found (data not shown).

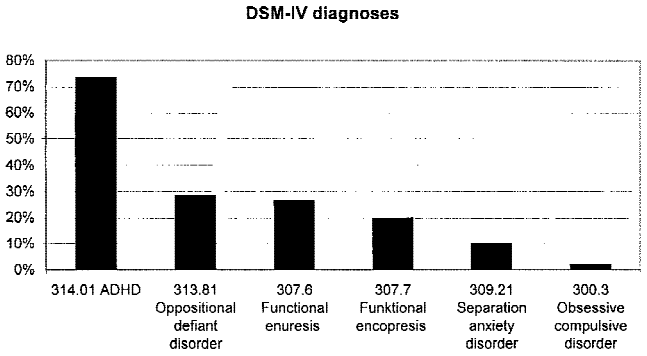

Fig. 2. DSM-IV diagnoses (FXS).

TABLE I. Number of DSM-IV or ICD-10 Diagnoses (Structured Psychiatric Interview: Kinder-DIPS)

	DSM-IV or ICD-10 diagnoses
FXS	
9 of 49 (18.4%)	No diagnosis
17 of 49 (34.7%)	One diagnosis
13 of 49 (26.5%)	Two diagnoses
4 of 49 (8.2%)	Three diagnoses
6 of 49 (12%)	Four diagnoses
TSC	
4 of 16 (25%)	No diagnosis
8 of 16 (50%)	One diagnosis
3 of 16 (19%)	Two diagnoses
1 of 16 (6%)	Three diagnoses

DISCUSSION

In order to analyze the specific phenotype of FXS and possible associations with the lengths of the CGG repeats, 49 boys with FXS and 16 boys with TSC as a control group were examined. The cognitive phenotype revealed a general intelligence corresponding to mild to moderately severe mental retardation, as previously published [Dykens et al., 1987; Borghgraef et al., 1987; Hodapp et al., 1990; Benetto and Pennington, 1996; Merenstein et al., 1996]. Significant strength regarded acquired knowledge ("Achievement" scale) compared to general intelligence ("Mental Processing Composite" scale). This difference could be due to ascertainment biases due to the recruitment procedures through self-

TABLE II. Percentages of FXS and TSC Boys With Behavioral Symptoms in the Clinical and Borderline Range: CBCL Composite and Syndrome Scales

	Normative population (clinical + borderline range)	FXS (N = 49) (clinical + borderline range)	TSC (N = 16) (clinical + borderline range)
Withdrawn	5%	38.8%	25%
Somatic complaints	5%	14.3%	25%
Anxious/depressed	5%	22.4%	31.3%
Social problems	5%	75.5%	56.3%
Thought problems	5%	55.1%	37.6%
Attention problems	5%	77.5%	43.8%
Delinquent behavior	5%	22.5%	12.6%
Aggressive behavior	5%	40.8%	25%
Internalizing score	15%	63.3%	50%
Externalizing score	15%	67.3%	43.8%
Total problem score	15%	89.8%	68.8%

help groups with highly motivated parents. On the other hand, the TSC boys (higher functioning subgroup) had no significant differences even though they were recruited similarly. In a small study of only 10 boys, Hodapp et al. [1992] also demonstrated strengths on the "Achievement" scale.

The previously described weaknesses in sequential versus simultaneous processing in boys with FXS could not be demonstrated in a traditional analysis of the K-ABC [Dykens et al., 1987; Hodapp et al., 1992], but could by a recalculation using the individual developmental age. These differences were greater among the FXS as compared to the TSC boys. By basing their analysis on developmental age, Hodapp et al. [1992] also found a general weakness in sequential processing in different groups of mentally retarded children, which were most apparent among the FXS group (10 of 10) as compared to a Down syndrome group (3 of 10) and a nonspecific mental retardation group (7 of 10). Therefore, it seems that FXS individuals show a similar, but more pronounced, type of cognitive profile than individuals with other forms of mental retardation.

The behavioral phenotype of FXS and TSC boys (higher functioning subgroup) is similar, with a high psychiatric comorbidity. In both groups, the diagnoses included ADHD, oppositional defiant disorder, functional enuresis, and functional encopresis.

ADHD was the most common diagnosis, as in other studies which have reported hyperactivity in the majority of boys with FXS [Bregman et al., 1988; Hagerman, 1996a]. In the few controlled studies with IQ-matched controls, ADHD remained a significant problem [Baumgardner et al., 1995]. Only the studies of Einfeld et al. [1991, 1994] and Borghgraef et al.

TABLE III. Associations Between Repeat Length and Cognitive and Behavioral Data

Proband	Mosaic*	5′-d(CGG)$_n$-3′-length of repeat n =	Mental processing composite (K-ABC; IQ equivalent)	CBCL** (internalizing/externalizing/total score)	Number of DSM-IV diagnoses
2.	+++	250–1400	41	0/2/2	4
3.	+++	50–1900	46	0/2/2	4
5.	++	200–700	44	0/0/0	—
1.	+	450/1000	–	1/2/2	3
6.	++	500/600	51	0/0/1	3
8.	+	450	52	2/2/2	2
10.	+++	200–800	48	2/2/2	4
11.	+++	800–1400	—	0/2/2	3
17.	+	400–800	40	2/2/2	1
13.	–	600	45	0/2/2	1
14.	+	375	45	2/2/2	2
16.	+	1400–1600	—	2/1/2	1
21.	++	650–1500	42	2/2/2	1
20.	++	300–1400	40	1/2/2	2
25.	–	1000	47	1/1/2	—
26.	–	400	44	0/0/0	—
7.	–	800/1000	—	2/2/2	2
41.	–	300	48	2/2/2	2
43.	+	1000	44	1/0/2	1
30.	–	800	41	2/2/2	4
33.	+	300–500	44	1/2/2	1
40.	+++	100–1000	43	0/0/1	1
39.	+++	100–1000	47	2/0/2	2
27.	–	600	67	2/2/2	—

*Mosaic: +++ = very high variability of repeat length; ++ = variability of repeat length; + = low variability of repeat length; – = no variability of repeat length.
**CBCL: 0 = normal range; 1 = borderline range; 2 = clinical range.

[1987] found that hyperactivity was not more common in FXS than in other IQ-matched mentally retarded children. Their conclusions must be questioned due to methodological problems: inclusion of both boys and girls, assessment of ADHD by three items of an autism questionnaire and one of the DBC (Developmental Behavior Checklist); lack of exclusion of FXS in the control group [Einfeld et al., 1991, 1994]; small case numbers; and difficulties with the diagnoses of FXS in the premolecular genetic era [Borghgraef et al., 1987]. In conclusion, all studies with a high methodological standard have shown the association of FXS and ADHD for boys but not for girls [Hagerman, 1996b].

The association of FXS and enuresis, as well as encopresis, has not been reported so far. In an epidemiological study of 3,206 7-year-old children, the prevalence of enuresis was 9.8% for all children and 26.6% for the group of the handicapped and mentally retarded children [Järvelin et al., 1988]. This corresponds exactly with the 27% rate of enuresis in FXS boys. The exact prevalence of encopresis among handicapped children is not known.

Nearly all boys with FXS (89.9%) had behavioral problems in the clinical or borderline range according to parental assessment (CBCL "Total Score"). This rate is 6 times higher when compared to the normative population (15%). For the group of TSC boys, the value for the "Total Score" is increased by a factor of >4. The behavioral profile is similar, but at a lower level. In both groups "Attention Problems," "Social Problems," and "Thought Problems" predominate. The CBCL has only been used in a study of 38 girls with FXS [Lachiewicz, 1992], but not in a large group of boys with FXS, so that comparisons are not possible.

Despite methodological limitations of the instruments used, the cognitive and behavioral phenotype of FXS boys could be well delineated. Still, no associations between any of the parameters analyzed and the lengths of the CGG repeat could be demonstrated. Specifically, there were no associations between the methylation of the repeat and its lengths and the phenotype. We could not find cognitive differences between mosaic and full mutations, as other studies implied [Staley et al., 1993; Merenstein et al., 1996].

The findings that individuals with nonmethylated alleles have a normal phenotype, and those with abnormal methylation profiles are affected, suggest that the phenotype is associated with the methylation of the locus rather than with the lengths of the amplified repeats [Loesch et al., 1993; Rousseau et al., 1994; Feng et al., 1995; Steyaert et al., 1996]. In most instances, the amplification of the CGG repeat sequence is associated with methylation and the FMR1 gene is silenced. Previous reports [Hwu et al., 1993; Sandberg and Schalling, 1997; Genç et al., 2000] have shown that methylation of the FMR1 promoter region in vitro can inhibit gene expression. It can be concluded that not the repeat lengths per se but the level of FMR1 protein is probably responsible for the behavioral and cognitive phenotype of FXS. The physical and cognitive symptoms show a wide variability and do not fall into distinct classes, as one would expect. Therefore, it is conceivable that the incomplete methylation of the CG-rich region allows the expression of the FMR1 protein at a low level. The lack of FMR1 protein during embryonic development leads to the phenotype of FXS. In high-functioning males [Hagerman et al., 1994; Taylor et al., 1999], reduced levels of the FMR1 protein ameliorate the severity of the phenotype. Low levels of FMR1 protein expression are possibly due to amplified but unmethylated alleles. The function of the FMR1 protein, a cytoplasmic RNA-binding protein, is still unknown. It is widely expressed in most adult and fetal tissues and high levels are found particularly in brain and testis [Devys et al., 1993].

However, in spite of full expansion, males with a normal phenotype could be found in a few instances in which the FMR1 promoter sequence remained unmethylated [Smeets et al., 1995; Hagerman et al., 1994]. The methylation of the upstream region seems to be more important than the amplification of the repeat. Although the repeat sequence is amplified in premutation carriers, no methylation can be detected [Feng et al., 1995]. These data suggest that it is not the repeat length that is exclusively responsible for the behavioral phenotype. To initiate methylation, other factors than the amplification could be involved. The functional consequences of the interaction of proteins, such as CGGBP1 [Deissler et al., 1996, 1997], a specific CGG binding protein, have not yet been clarified.

In conclusion, further analyses are needed to study the interdependence of repeat amplification and methylation, as well as between protein expression and the somatic, cognitive, and behavioral phenotypes.

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