ADVANCES IN RESEARCH ON THE FRAGILE X SYNDROME

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Fragile X syndrome is a neurodevelopmental disorder that results from a single gene mutation on the X chromosome. The purpose of this review is to summarize key advances made in understanding the fragile X *premutation* gene seen in carriers and the *full mutation* gene seen in persons with the syndrome. DNA testing has replaced cytogenetic testing as the primary method for identification of fragile X, although the efficacy of protein level screening is being explored. The premutation is associated with no effects, although there is evidence of physical effects—primarily premature menopause and mild outward features of the fragile X syndrome–among premutation carriers. There is much controversy regarding premutation effects on psychological development. The few experimental studies carried out to date do not suggest noticeable or significant effects. One challenge in addressing this controversy is the sometimes ambiguous differentiation between premutation and full mutation genes. There is a well-established yet highly variable phenotype of the full mutation. Research from this decade has helped to address specific aspects of this phenotype, including the early course of its development in males, the influence of home and family environments, the nature of social difficulties and autistic features seen in boys and girls with fragile X, and the potential role of hyperarousal or hyper-reactivity. Studies in these areas, and on the role of FMR protein, will contribute towards ongoing advances in our understanding of fragile X syndrome and its mechanisms. The variability in physical, social, and cognitive features, as described in this review, is one that prohibits clear-cut screening guidelines designed to avoid high rates of both false positives and false negatives. Results from recent studies indicate the need to consider behavioral features in selecting candidates for fragile X screening. © 2000 Wiley-Liss, Inc.
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During the 1990s, advances in understanding the fragile X syndrome increased at a far greater rate–and to a far greater depth–than was possible during the two previous decades. The advances made during the last decade provided answers to important questions, and gave rise to research efforts not previously available. In view of these recent advances, reviews prepared prior to 1991 include some information that is at best incomplete, and in many cases inaccurate, based on current knowledge. In this review, the brief yet rich history of the study of fragile X is summarized, as are the advances achieved in this decade. A thorough review of the advances in molecular genetics of fragile X is beyond the scope of this review, although key factors pertaining to differentiation of mutation types are addressed. The advances to be addressed pertain to the differentiation and classification of premutation versus full mutation genes, prevalence figures of premutations and full mutations, premutation versus full mutation phenotypes, and further specification of the psychological characteristics of the fragile X syndrome. A common theme to be presented throughout this review is that of further clarification of information reported during or prior to the early 1990s.

HISTORY

Fragile X is considered the most common known hereditary cause of mental retardation, affecting both males and females in an X-linked manner. Its characteristic phenotype includes physical, cognitive, and psychosocial features. Physical features often include a long face, elongated and/or protruding ears, strabismus, flat feet, a high arched palate, hyperextensible joints, and, in males, macroorchidism [Hagerman, 1996b; Hagerman, 1999]. The behavioral and cognitive features, described below in more detail, include mental retardation, borderline intellectual ability, or learning disability; autistic features such as poor eye contact and stereotypies, primarily hand–flapping and/or hand–biting, perseverative speech and perseverative behavior; cluttered speech, hyperarousal or hyperstimulation to the environment, impulsivity, and social anxiety.

The natural history of fragile X began long before its documentation, the latter of which began in 1943. At that time, Martin and Bell first described a family study of mental retardation that appeared to be inherited, X-linked, and more deleterious in males than in females. It was not until nearly 40 years later that members of this family were re-evaluated using cytogenetic techniques and diagnosed with fragile X syndrome. The role of cytogenetic testing as a diagnostic tool was initiated by Lubs' observation [Lubs, 1969] of a characteristic fragile site which he observed in cultured cells obtained from affected individuals, at the lower end of the X chromosome. His observation gave rise to the syndrome's name several years later. Despite Lubs' initial observation, cytogenetic testing (and pedigree analysis) were not regularly used for screening and identification of fragile X until soon after Sutherland [Sutherland, 1977] and colleagues specified the need for a folate-deficient media for adequate expression of this fragile site. The increase in

cytogenetic testing facilitated identification and study of fragile X syndrome. However, there were inadequacies with cytogenetic testing, primarily a high rate of false negative findings in women known to be carriers of fragile X, and these inadequacies were then not fully understood.

The common inheritance pattern observed during the 1970s and 1980s, among families of probands, involved identification of an affected male born to a woman who was likely to have negative cytogenetic testing and no outward signs of fragile X syndrome. Based on the understanding of X-linked inheritance, the mother was determined to be the carrier of fragile X despite negative cytogenetic results, and was thus referred to as an "obligate carrier." This obligate carrier may have in turn received fragile X from her mother or her father; yet affected status was observed only in offspring of females. This "Sherman paradox" was described [Opitz, 1986] but not understood until 1991, at which time the mutation responsible for the fragile X syndrome was identified [Fu et al., 1991; Verkerk et al., 1991; Yu et al., 1991] in the Fragile X Mental Retardation (FMR1) gene.

This mutation was the first "triplet repeat" expansion to be identified in the field of human genetics research, and thus it was a scientific breakthrough at many levels. DNA testing soon became the method of choice for diagnosis and screening, for both the affected and "full mutation" gene and the carrier status referred to as the "premutation" gene. Differentiation of the "premutation" from the "full mutation" provided an explanation for the occurrence of the Sherman paradox. Despite these breakthroughs in understanding the biological mechanism underlying fragile X, these classifications are not always so clear.

CLASSIFICATION OF THE PREMUTATION AND FULL MUTATION

In the vast majority of cases, fragile X syndrome is associated with the mutation first described at the DNA level in 1991 [Verkerk et al., 1991]. Children with the fragile X phenotype who lack an FMR1 mutation may have an FMR1 deletion [Wohrle et al., 1992; Gu et al., 1994; Trottier et al., 1994; Hirst et al., 1995; Parvari et al., 1999] or deletion and full mutation mosaicism [Petek et al., 1999]. Others may have a mutation at the FMR2 gene [Chakrabarti et al., 1996], which is far more rare than the FMRI mutation. The FMR1 and FMR2 phe-

notypes are not, however, identical [Knight et al., 1996; Abrams et al., 1997].

The FMR1 gene is comprised (in part) of cytosine, guanine, and guanine (CGG) sequences, which are repeated, a variable number of times (from approximately 6 to 54), in the general population [Fu et al., 1991]. A primary characteristic of these normal genes is that the number of these "triplet repeats" is *stable* from generation to generation. In contrast, individuals with a mutation at the FMR1 gene may often have an unstable number of repeats; the number of repeats increase

The classification of these three forms of the FMR1 gene—normal, premutation, and full mutation—are often oversimplified as based on expansion size alone. It is important to note that the stability of repeat size from carrier to offspring and the corresponding hypermethylation status, in addition to expansion size, are among the known criteria used in classifying (and differentiating) normal, premutation, and full mutation FMR1 genes.

through subsequent generations, when the mutation is transmitted through a female.

The mechanism underlying this gene size instability is unclear, with respect both to processes involved in the transition from normal stable genes to unstable premutation genes [Patsalis et al., 1999b], and those operating in the expansion from premutation to full mutation. In addition to the role of CGG expansion size on gene stability, there appears to be an associated role of the AGG (adenine, guanine, guanine) repeats

normally interspersed among the CGG sequence that are *sometimes* absent in persons with a premutation [Zhang et al., 1995]. Stability has been associated to specific haplotypes, i.e., specific arrangements of these CGG and AGG sequences, although more recent work with different ethnic groups (Caucasian and Black populations) does not support this notion [Crawford et al., 1999b]. Although the likelihood of expansion from premutation to full mutation increases with size of the premutation, there are case reports of relatively small premutations expanding to full mutations in one generation [Nolin et al., 1996]. Hypotheses concerning the timing of full mutation expansions suggest that this process may occur during germ cell proliferation [Malter et al., 1997], or during early transitional phases of embryogenesis [Reyniers et al., 1993; Wohrle et al., 1993]. To date, the available evidence has not permitted firm establishment of a precise stage or mechanism by which this expansion occurs.

When the number of triplet repeats is *unstable* and below approximately 200 the mutation is classified as a *premutation*. Unstable alleles are typically larger than approximately 50 repeats, but exceptions have been reported. Once the number of repeats reaches or exceeds 200 and methylation occurs, the mutation is classified as a *full mutation*. The classification of these three forms of the FMR1 gene—normal, premutation, and full mutation—are often oversimplified as based on expansion size alone. It is important to note that the stability of repeat size from carrier to offspring and the corresponding hypermethylation status, in addition to expansion size, are among the known criteria used in classifying (and differentiating) normal, premutation, and full mutation FMR1 genes.

It is also important to recognize the *lack* of unambiguous, consistent guidelines for classifying alleles that fall *between* the general categories of normal and premutation, or premutation and full mutation. The three criteria described above are helpful in discerning which classification is appropriate for an ambiguous gene. The distributions for "normal" and "premutation" allele sizes overlap, which may also be true for the distributions of premutation and full mutation genes. *Intermediate alleles,* also referred to as "*gray zone*" alleles, may be large normal or small premutation genes. Genes with close to 200 repeat expansions may be classified as premutations or full mutations, depending on stability and methylation status. To add to this complexity,

heterogeneity in allele size can occur, such as when both premutations and full mutation genes are found in an individual; alternatively, an individual may have both methylated and unmethylated genes in the full mutation range. The former "mosaic" pattern is not uncommon in persons with the full mutation, and both forms of mosaicism may or may not be associated with a less deleterious full mutation phenotype, depending on the degree to which the full mutation is expressed [Hagerman et al., 1994a; Wohrle et al., 1998].

The degree to which a full mutation affects development is linked with a decrease in the protein typically produced in the presence of a normal FMR1 gene. This decrease in protein results when the methylated full mutation interferes with the normal functioning of the gene and thus with its transcription [Feng et al., 1995]. FMR protein appears to be involved with mRNA processing, or with its transport or translation [Zhong et al., 1999a]. Its influence on normal brain development appears to be in synaptic pruning [Weiler and Greenough, 1999], which may account for the observation of immature dendritic spines in males with fragile X syndrome [Hinton et al., 1991]. However, the precise role of FMR protein is not yet fully understood. [Ashley et al., 1993; Brown et al., 1998; Kaufmann and Reiss, 1999; Pimentel, 1999]. Expression of FMR protein is (1) indistinguishable across carriers of the normal or premutation alleles [Feng et al., 1995]; (2) significantly decreased and abnormal, but not absent [Kaufmann et al., 1999], in males with the full mutation, relative to levels in the normal population; and (3) present at variable levels, including normal expression patterns in many cases, across females with the full mutation. This general pattern is consistent with the reports of no cognitive or behavioral effects associated with the premutation, moderate cognitive and behavioral effects among males with the full mutation, and a highly variable psychological phenotype among females with the full mutation (as described below). This pattern is also consistent with the reported association between FMR protein expression and intellectual functioning [Tassone et al., 1999b]. However, this genotype-phenotype association is not simply linear; it may be complicated by heterogeneity of full mutation and therefore protein expression across tissues, which has been reported in some cases [Taylor et al., 1999] but not in others [Tassone et al., 1999a].

Prior to the availability of DNA testing, it was assumed that "transmitting males" were carriers of the premutation who passed the mutation on to all of their daughters and none of their sons, in a classic X-linked manner. More recently, there have been several reports of "unaffected" or transmitting males demonstrating decreased expression of FMR1 protein and a mosaic pattern including full mutation alleles. What is unique about these case reports, relative to males affected by the FMR1 fM, is presence of unmethylated full mutation genes [Rousseau et al., 1994b; Wohrle et al., 1998] and greater expression of FMR1 protein, which Wohrle and colleagues interpreted as evidence for somatic instability of large FMR1 mutations. These reports are an exception to the notion that full mutations are hypermethylated, and represent the challenges in defining and differentiating premutation and full mutation alleles.

PREVALENCE OF FMR1 MUTATIONS

Fragile X prevalence figures vary according to whether rates pertain to the premutation, the full mutation, or affected status (i.e., the fragile X syndrome). With respect to the syndrome, typical prevalence rates indicate that the majority of males and approximately 50% of females [Rousseau et al., 1994a] with the full mutation are "affected" by mental retardation. The rate for females is higher than previously reported figures of 30%, because rates cited prior to 1991 often included the "obligate carriers" later identified as carriers of the premutation. The 50% of females who do not have mental retardation may nevertheless be "affected," and may have borderline or below average cognitive functioning, learning disability, and/or psychosocial difficulties.

Fragile X Full Mutation: Prevalence

In the general population, approximately 1/4000 males and 1/8000 females [Turner et al., 1996; Crawford et al., 1999a] has the fragile X syndrome, according to studies conducted throughout the world. When statistical confidence intervals are considered, the prevalence rates are quite consistent across studies carried out worldwide. Similarly, when statistical confidence intervals are considered, the rates of fragile X syndrome among individuals with mental retardation are also comparable across studies carried out in Australia and Great Britain (4.3%, [Turner et al., 1996]), Great Britain alone (2.5%, [Wang et al., 1993]; 0.5%, [Murray et al., 1996]), Brazil

(2%, [Haddad et al., 1999]), Chile (5%, [Aspillaga et al., 1998]), China (2.8%, [Zhong et al., 1999b]), Cyprus and Greece (0.9%, [Patsalis et al., 1999a]), Finland (5.4%, [Von Koskull et al., 1994]), Holland (4.2%, [Van den Ouweland et al., 1994]), Indonesia (2.4%, [Faradz et al., 1999]), Japan (2.1%, [Hofstee et al., 1994]), Mexico (4.1%, [Kaplan et al., 1994]); Puerto Rico (3%, [Toro-Sola, 1998]), and the United States (1.1%, [Hagerman et al., 1994b]; 0.6%, [Mazzocco et al., 1998b]). Lower frequency rates of full mutation have been reported for Blacks versus Caucasians, yet more recent evidence indicates that this observation may result from ascertainment bias rather than a true difference in prevalence rates. Goldman and colleagues [1998] reported that 6.1% of 148 unrelated institutionalized black males in South Africa who were screened for fragile X had the full mutation, a rate comparable to those reported above. Crawford and colleagues [1999a] carried out the first prevalence study across nonclinically referred Black and Caucasian children, and found comparable prevalence for fragile X in both groups. Access to suitable health care resources may explain the initially observed differences in frequencies [Sherman, personal communication, 1999].

Fragile X Premutation: Prevalence

Rates of the premutation may vary across studies depending on the repeat threshold used to differentiate normal and premutation alleles. These rates have been quite comparable across studies. For example, Rousseau and colleagues [1995] reported 1/259 females and 1/379 males to have a premutation > 54 repeats, a rate similar to the estimated 1/317 premutation carriers in Crawford et al.'s [1999]control population, and rates in a study of consecutive male births [Holden et al., 1995]. It has been hypothesized that premutation alleles may be more frequent among females; predictions regarding the degree to which this is the case vary, depending on models based on pre-zygotic versus post-zygotic expansion [Ashley and Sherman, 1995; Morris et al., 1995]. However, the likelihood of full mutation expansion is comparable for male and female offspring [Ashley-Koch et al., 1998].

FURTHER SPECIFICATION OF THE FULL MUTATION PHENOTYPE

Variability in the Phenotype

Global descriptions of the fragile X phenotype include physical, behavioral, and cognitive features that vary between affected individuals. In general, the phe-

notype is *less* variable among males with the full mutation than among females with the full mutation, but it is nevertheless variable in males, as described further, below. The fact that females, "as a group," are less affected than males is sometimes incorrectly interpreted as indicating that affected females do not have mental retardation. The correct interpretation is that cognitive function can range from mental retardation to normal levels, in females with a fragile X full mutation. Approximately 50% of females with the full mutation have mental retardation. The remaining 50% may manifest borderline to normal intellectual functioning, learning disability, and/or psychosocial difficulties. These figures differ from earlier reports that 33% of fragile X females have mental retardation, 33% have learning disability, and 33% have normal intellectual functioning. In these earlier reports, carriers were presumed to "have" fragile X when in fact they often carried a premutation and not a full mutation. Reports based on samples of females known to have the full mutation suggest that mental retardation is more frequent than in 33% of these women. For instance, 59% of 170 females with the full mutation who were included in a multi-center study had some degree of mental retardation [Rousseau et al., 1994a]. Thus *most* males and females with the full mutation are affected. The degree to which individuals are affected varies, with different *degrees* and *ranges* of effects evident among males versus females.

The generally less deleterious effects among females are explained, in part, by the presence of two X chromosomes, only one of which is active in each cell. Correlational studies have demonstrated that an increase in the proportion of active X chromosomes containing the normal FMR1 gene (versus the fragile X full mutation) as a result of random X-inactivation is associated with a decrease in severity of fragile X symptomology [Abrams et al., 1994; Reiss et al., 1995; de Vries et al., 1996]. More recently, significant positive correlations between this activation ratio and protein levels have been described [Kaufmann et al., 1999; Tassone et al., 1999b].

The phenotype's variability in both males and females is believed to be linked, at least in part, to variable degrees of FMR protein expression. For females, X chromosome inactivation ratios appear to account for the variability, whereas in males variability in symptomology may be associated with mosaicism–including premutation/full mutation mosaicism and methylation mosaicism among full mutation alleles. Mosaicism per se does not indicate diminished severity of symptoms; the nature and degree of the mosaicism, such as the degree of unmethylated full mutation genes, is believed to affect levels of protein expression [Hagerman et al., 1994a].

Cognitive Features

The majority of males with fragile X syndrome have mental retardation, with scores in the mentally retarded range emerging by approximately age three years or earlier, and by age four years this is the case for nearly all boys with fragile X [e.g., Bailey et al., 1998a]. Yet mental retardation is neither necessary nor sufficient for describing the phenotype of every individual with fragile X syndrome, in light of the aforementioned phenotypic variability. Specificity of the cognitive phenotype can be best addressed by examining differences among

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specific domains of neuropsychological function, and these differences are more easily detected in individuals with higher level of cognitive or adaptive functioning. For this reason, much of the work on cognitive specificity is carried out with females who do not have mental retardation.

Among affected females *without* mental retardation, deficits are seen in measures of visual-spatial skills, attention, and "executive function," and math achievement scores are lower than reading achievement scores [Mazzocco et al., 1992; Kovar, 1993; Mazzocco et al., 1993]. Visual-spatial deficits do not appear to be globally represented; for instance, visual memory skills in women have been reported to fall within the average range [Mazzocco et al., 1992; Mazzocco et al., 1993]. Task-specific visual-spatial difficulties are implicated in a study of males with fragile X, for whom deficiencies were strongest on visual motor and visual construction tasks [Cornish et al., 1999]. In a preliminary study of math skills performance, there was no evident, specific pattern of errors that differentiated girls with fragile X from girls without fragile X who had comparable IQ scores [Mazzocco, 1998]. Math achievement was positively correlated with performance on all three Wechsler factor scores (verbal comprehension, perceptual/organization, and "freedom from distractibility") among girls with fragile X and girls in a peer comparison group. However, only in the peer comparison group were math achievement scores also positively correlated with performance on a "judgement of line orientation test" that involves both visual representation and "executive" skills necessary for scanning and selecting lines that match on the basis of angular position. Similar associations have been reported from a normative study of math and visual-spatial skills performance in regular kindergarten students [Mazzocco, 1997]. These findings, based on small groups of girls (26 and 25 per group, respectively), are preliminary in nature and yet suggest potential differences in the approach to, or processes that underlie, math performance in girls with fragile X relative to children without fragile X. More research on math skills performance among girls with fragile X, including longitudinal studies, is in progress [Mazzocco, 1997]. Moreover, these math performance findings are consistent with a preliminary study of brain activation using spectroscopy, that also indicates lack of uniformity in math performance among six women with fragile X [Grigsby et al., 1999].

In contrast to math skills performance, verbal skills are relatively spared in females with fragile X, although girls with fragile X do have lower verbal skills relative to their unaffected sisters [Mazzocco et al., 1998a]. Women with fragile X show linguistic deficits at the level of pragmatic reasoning in discourse [Simon and Keenan, 1996; Simon and Keenan, 1998]. The deficits in discourse appear related to making inferences and successful set shifting, such as in difficulty generating or selecting joke responses for story endings that require a shift in how the preceding story information is interpreted. However, these discourse difficulties do not appear related to deficits in making inferences per se, but rather to the memory demands required when making contextually-based inferences, and to inhibiting more automatic interpretations. These findings are consistent with anecdotal reports of literal speech

among individuals with fragile X, and point to a cognitive rather than social contribution to linguistic deficits independent of basic semantic reasoning ability.

The combination of verbal strengths and math weaknesses led some researchers to speculate that the nonverbal learning disability (NLD) proposed by Rourke [e.g., Rourke, 1989; 1993] is a useful construct for understanding the fragile X syndrome phenotype. A thorough discussion of this notion is beyond the scope of this review, but it is important to note that this notion has been challenged [Miezejeski and Hinton, 1992; Kovar, 1993]). Empirical findings inconsistent with the NLD construct include strong performance in areas reported to be deficit in NLD, including nonverbal short term and long term memory for designs [Kovar, 1993; Mazzocco et al., 1993] and difficulty with auditory attention [Mazzocco et al., 1993] a proposed asset in the NLD model. Consistent with the NLD model are the frequent reports of social difficulties in both males and females with fragile X, as discussed below.

Social Behavioral Features

The social deficits that comprise a component of the full mutation phenotype range from autistic features to social anxiety and pragmatic language deficits. The social difficulties do not appear to be related to difficulty in perspective taking, as both girls [Kovar, 1993] and women [Mazzocco et al., 1993] demonstrate the ability to consider another person's perspective in story telling and story interpretation. Women affected by fragile X do not show deficits in emotion perception tasks that involve matching illustrations of persons' affect, and their degree of psychosocial difficulties indicated by personality inventory ratings are not correlated with cognitive or neuropsychological functioning [Sobesky et al., 1994]. The range of social behavioral difficulties is considered in more detail, below.

Autism

An association between autism and fragile X has been described, although a diagnosis of fragile X does not implicate autism, or vice versa. The incidence of autism in boys and girls with fragile X is higher than that reported in the general population, yet there are still some (albeit a minority of) children (e.g., 7% to 25% of boys) with fragile X who have autism [Baumgardner et al., 1995; Bailey et al., 1998b]. The frequency rates appear to diminish with age; however, this finding is drawn from cross-sectional studies and comparisons across studies using different diagnostic techniques. It remains to be seen how autistic features vary across the life span in children with fragile X, and this question is a topic of ongoing research [Bailey et al., 1998b].

The behavioral profiles observed among children who have both fragile X and autism include important differentiating features relative to children with autism who do not have fragile X syndrome. Children with both fragile X and autism show social interaction patterns suggestive of social aversion and discomfort, primarily with persons who are not primary caregivers, rather than a lack of interest in the social environment [Cohen, 1995]. Their social skills delay is less

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severe than that seen in autistic children who do not have fragile X [Bailey et al., 1999]. The social discomfort or avoidance is described as diminishing over time during a session with an initially unfamiliar person, on the basis of clinical observations [Bailey et al., 1998a]. Qualitative differences in temperament emerge between autistic boys with or without fragile X, with the former being more distractible, more active, and more intense in emotional responsivity than autistic boys who do not have fragile X; whereas the autistic boys with fragile X show greater socially-acceptable adaptiveness to their environment as well as a higher threshold for triggering a response to environmental cues. Boys with fragile X and autism show less variability in overall severity of ratings across different autistic behaviors, relative to boys with autism and no fragile X, on the profiles of autistic behavior [Bailey et al., 1999].

Autistic features in fragile X appear to occur on a continuum, thus representing another area in which phenotypic profiles are variable both between and within groups of males and females. Autistic features have been described in many reports of individuals with fragile X who do not meet full criteria for autism, among both boys [Reiss and Freund, 1992; Bailey et al., 1998b; Mazzocco et al., 1998c] and girls [Mazzocco et al., 1997a]. It is not clear whether this continuum of autistic features is associated with intellectual ability. Among young boys with both diagnoses, the severity of autistic features has been positively correlated with the severity of overall developmental delay [Bailey et al., 1998b]. This is in contrast to reports that, among girls with fragile X, severity of autistic features is not related to IQ score [Mazzocco et al., 1997a]. The range of cognitive ability varies between the groups of males and females in these studies, as do the instruments used to assess severity of autistic features.

Social anxiety

One of the most prominent of the "autistic features" observed in persons with fragile X is social avoidance. The degree of social avoidance ranges from mild to extreme, such as is seen in reported cases of selective mutism in a 12-year-old child with a long history of social anxiety [Hagerman et al., 1999]. Abnormal social and communication behaviors appear positively correlated with anxiety measures in girls with fragile X [Mazzocco et al., 1997a]. In a behavioral analysis of videotaped role-plays with adult strangers, girls with fragile X took more time to initiate conversation than girls in two comparison groups. But there were no group differences in the total duration of silence during role play nor in the frequency of behaviors examined as potential indicators of social anxiety [Lesniak-Karpiak et al., 1999].

Cohen proposed that this social avoidance is linked to hyperarousal, and this hypothesis has received empirical support from psychophysiological studies. In a preliminary study, Miller et al [1999] demonstrated differences in electrodermal response (EDR) ratings between individuals with, or without, fragile X. Individuals with fragile X had heightened EDR ratings for all five sensory modalities examined. Moreover, these ratings were negatively correlated with levels of FMR protein and activation ratio, and positively correlated with

degree of methylation. The authors interpret these findings as potential evidence for either hyperarousal or hyper-reactivity; each hypothesis may lead to important understanding of the basis of anxiety, including social anxiety, in fragile X syndrome in addition to other frequent features of the syndrome (e.g., tactile defensiveness). It is interesting to note evidence that social anxiety—whether linked to hyperarousal or hyper-reactivity—may be either denied or unrecognized by girls with fragile X [Kovar, 1993; Lanham et al., 1999]. Parents of girls with fragile X rate their daughters as more anxious than do parents of girls without fragile X, although the self report ratings among the girls do not differ across groups [Lanham et al., 1999]

Longitudinal studies

The majority of published longitudinal studies of fragile X to date include data from males and females across a wide age span, often including preschoolers through adults. Evidence from these studies indicate that persons with fragile X show a decline in scores on cognitive, language, and adaptive skills measures during the school years. This decline reflects the common widening of the gap between performance of children with developmental delay and that of these children's age-matched peers. In males, cognitive [Dykens et al., 1989] and adaptive behavior [Dykens et al., 1993] scores plateau at approximately age 10 years. Females also show a decline based on group means, although the degree of decline is more variable.

To date, the only published longitudinal studies of preschool age boys with fragile X have been reported by Bailey and colleagues [Bailey et al., 1998a]. The 46 boys included in their 1998 report ranged from 24 to 66 months when initially enrolled, and between 24 to 72 months of age when evaluated. Each child received multiple evaluations (2 to 8) at 6-month intervals. The evaluations were based on the Batelle used to assess cognition, communication, adaptive behaviors, motor function, and personal-social function. Of interest was the variability in development among these 46 boys, the degree of delay evident, the rate of development in the five areas examined, and the degree to which developmental trajectory varied between the five areas examined. The results indicate significant variability in the developmental trajectory between young boys with fragile X. Some boys showed relatively stable courses of development, whereas others demonstrated high or low rates of

growth. The rate (slope) of development ranged from 0.14 to 0.75, representing a wide range and a clear difference from the projected rate of 1.0 expected for the average child. The number of children with deficient scores increased with age, with all children scoring in the deficient range by 66 months of age. The developmental trajectories were similar in slope across the five domains examined; this reflects stable development over time within a domain. What differed was the intercept for each slope, with less delay in motor and adaptive behavior at all ages examined, and poorest performance in communication and cognitive performance at each age as well. An essential finding of Bailey and colleagues' research is the emphasis on developmental age scores (rather than IQ scores), which re-

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flect a "steady and consistent rate of progress over time" among preschoolers, despite the reported declines in IQ scores. No clear temperament profile was observed among the boys who participated in this longitudinal study [Bailey et al., 1999], although boys with fragile X were described as being more active, less adaptable, less persistent, and less approachable relative to a comparison group of boys; these differences were not associated with severity of overall developmental delay [Hatton et al., 1999]. (It is important to consider this lack of differentiating temperamental profiles occurred between boys with fragile X and published normative data for boys in a similar age group, in contrast to the aforementioned temperamental differences that emerged between boys with

autism and boys with fragile X.) Bailey and colleagues are examining potential factors that may affect the developmental trajectories and temperament qualities described; these factors include opportunity for early intervention and characteristics of the home environment.

van Lieshout and colleagues [1998] also have addressed the role of home characteristics, specifically family stress and parental behavior, in a cross-sectional study of personality characteristics among boys with fragile X. One group in their study was comprised of 32 boys with fragile X (no girls with fragile X were included), aged 3 to 18 years. Relative to children with Williams or Prader Willi syndrome and children with no known disorder, the boys with fragile X did not differ on measures of extroverted behavior. The boys with fragile X were less emotionally stable and less open to new experiences than the group of children with no known disorder, but not to a greater extent than children in the other two syndrome groups. Levels of motor activity decreased with age among the boys with fragile X; no other age-related findings emerged for this group. Parental effects were noted, with mothers demonstrating more control than fathers for all three syndrome groups, although parent control was not associated with the personality dimensions examined. Nor were family stress or marital conflict associated with personality dimensions. For children with fragile X, lower parental anger and higher parental consistency were associated with higher degrees of "conscientiousness," defined as "planful . . . orientation . . .with high levels of involvement and persistence." A relation was seen between greater levels of parental anger and lower agreeableness, lower openness, and higher irritability.

IS THERE A PREMUTATION PHENOTYPE?

A hypothesis frequently addressed in research on the *premutation* is that a phenotype similar to, but milder than, that described above for the full mutation will be evident in persons with the premutation. Many studies have been carried out to address this hypothesis, as it pertains to physical, cognitive, or psychosocial features associated with the premutation. These studies include screening studies, clinical case reports, and experimentally controlled studies. Additionally, prior to identification of the FMR1 mutation, studies of "obligate carriers" or "transmitting males" were carried out; the findings from these studies have since been interpreted as relevant

to this research question. The findings from each of these methods of inquiry must be interpreted within the context from which they were drawn. Moreover, it is important to consider the challenges in defining a premutation, as described above, when interpreting the finding from these studies.

Screening studies

The screening studies have involved examining whether the rate of premutation genes in special needs populations differs from rates reported in the general population. Findings from these studies provide evidence for a lack of difference in these rates. For instance, a single premutation among 1,017 school age children without mental retardation who were clinically referred for academic difficulties [Mazzocco et al., 1997b]; and 3 children with a premutation among 534 preschoolers clinically referred for developmental delay and/or language delay [Mazzocco et al., 1998b]. Crawford et al. [1999a] found no males with the premutation and two females with the premutation alleles in a group of more than 2,000 children with special education school placements, a rate that did not differ from that obtained in their control sample. Patsalis et al. [1999a] reported 2/866 premutation carriers among mentally retarded individuals in Greece and Cyprus. The frequency rates reported in these studies are comparable to those reported for the general population, as described earlier; and suggest that the premutation is not an etiology of the delays represented in these select populations.

The frequency of intermediate (gray zone) alleles in special needs populations has also been examined. The hypothesis tested with this methodology is that, if intermediate alleles affect development, their prevalence should be greater among special needs populations relative to the prevalence observed in the general population. Only one study published to date is in support of this hypothesis [Murray et al., 1996], whereas several other studies support the alternative hypothesis of no difference in prevalence rates. Mornet et al.[1998] found comparable frequencies (2% to 3%) of intermediate alleles among controls and patients with mental retardation. Similarly, Crawford et al. [1999] found comparable levels across children in special education school placements and controls in the United States. In other studies of special populations, rates are comparable to those observed in population screening studies. The frequency of intermediate

alleles was comparable across studies of school age children with academic difficulty [Mazzocco et al., 1997b], preschoolers with language delay [Mazzocco et al., 1998b], consecutive live male births [Holden et al., 1995], and a randomly selected sample of over 10,000 women [Rousseau et al., 1994]. Related to these findings are reports that normal variation in size of the fragile X gene is not associated with variation in intelligence [Daniels et al., 1994; Mazzocco and Reiss, 1997].

CLINICAL CASE REPORTS

Several cases have been reported that implicate effects of the premutation. Hagerman [1996a] described three boys with a large fragile X premutation (130–210 repeats) who showed a mild version of the fragile X syndrome phenotype. In their report of 15 individuals who had developmental disabilities and a fragile X premutation, Lachiewicz and colleagues [1998] addressed whether their report provided evidence that the premutation increases the risk of developmental disabilities. Aziz and colleagues [1998] described six boys with either a fragile X pM (55–200 repeats) or an "intermediate" sized allele (41–54 repeats), and each demonstrated behavioral and intellectual features consistent with the syndrome phenotype, including autistic-like behaviors and other social difficulties. In contrast, there are also reports of individuals with the premutation who do not manifest a clear fragile X phenotype. Mazzocco and Holden [1996] found that three sisters, each of who inherited two fragile X pM alleles, did not show cognitive disability. Less conclusive evidence is drawn from a report of a compound heterozygote with one fM and one pM whose psychological profile was similar to that of other females with the fM [Linden et al., 1999], including borderline intellectual functioning, rather than to that of males with fragile X syndrome.

It may be that clinical case studies of children "affected" by their premutation may not represent the population of premutation carriers per se. The presence of "effects" in these cases may be "coincidences" [Feng et al., 1995] resulting from inadvertently selected samples that result when samples are clinically ascertained. For instance, Myers [1998] described a case suggestive of premutation effects, in the context of an experimental study described below. That case involved a child with no family history of fragile X, whose premutation was diagnosed because fragile X syndrome was being considered as an explanation for

her mental retardation. This child was clinically ascertained, as were many of the individuals referred to in the aforementioned case reports. Thus case reports need to be interpreted in terms of ascertainment bias—in terms of *who* was screened for the premutation and *why*. The degree to which we can generalize data from case reports is dependent on the relative frequency of the observed features in individuals without the premutation.

An additional concern regarding the interpretation of premutation effects pertains to the accuracy of a premutation diagnosis. Data regarding inter-tissue homogeneity of premutation or full mutation expansion size are quite mixed, with some studies indicating very consistent expansion size and methylation patterns across tissues, including brain tissue [Reyniers et al., 1999; Tassone et al., 1999a], and one study showing marked heterogeneity across different brain regions [Taylor et al., 1999]. Leukoctye-derived DNA has been shown to be a good, but imperfect, measure of expansion size in olfactory neuroblasts [Abrams et al., 1999]. When an individual with a large premutation is found to be "affected," it is important to consider the challenges sometimes faced in differentiating large premutations, small full mutations, methylation mosaicism, and gene instability that may lead to mosaicism within and across tissue.

Group Based Studies

Although more conclusive by nature than case reports, experimental studies of premutation effects are also inconsistent. The most consistent body of findings are those studies pertaining to physical effects of the premutation, including premature menopause; but even these findings are challenged [Kenneson et al., 1997]. The frequency of ovarian failure prior to age 40 is reported to be as high as 28% [Partington et al., 1996], a figure comparable to the 19% rate reported by Schwartz et al. [1994]. In a recent collaborative survey, rates were comparable: 63 of 395 (16%) premutation carriers and 1 of 237 non carriers reported ovarian failure before age 40 years [Allingham-Hawkins et al., 1999]. Despite the counter-arguments, these data provide strong evidence of some effect associated with the premutation. Factors associated with early menopause may also be related to the reports of higher incidence of dizygotic twinning in women with the premutation [Tizzano and Baiget, 1992; Turner et al.,1994; Healey et al., 1997; Martin et al., 1997]. There is also some evidence that females

with a premutation show mild physical features of the fragile X syndrome phenotype [Hull and Hagerman, 1993; Hagerman, 1996a; Riddle et al., 1998]. Riddle and colleagues [1998] examined physical features among over 100 women with the premutation and over 100 controls, and found that the former group had significantly greater jaw and ear prominence, although group means on these variables were not as high as reported for the group of 41 women with the full mutation.

The literature regarding effects of the pM on cognitive and psychosocial development is less consistent. In many of these studies, groups of individuals to whom psychological measures were administered were not ascertained from clinical samples. The hypotheses typically tested are that the psychological profiles among individuals with a premutation will be qualitatively similar to those observed among individuals with the full mutation, although potentially milder. This hypothesis has not been supported in most studies of cognitive function. The initial studies of women with or without the premutation [Mazzocco et al., 1992,1993] and a follow up of that initial work with 41 women in the full mutation group [Riddle et al., 1998], yielded negative findings. Women with the premutation have not shown the deficits in measures of visual-spatial skills, attention, mathematics, or executive function that are apparent in women with the full mutation. Women with the premutation have not demonstrated the linguistic deficits reported among women with the full mutation [Simon and Keenan, 1998]. Myers et al. [1998] studied 14 pairs of children, with or without the premutation, and hypothesized that deficits in performance IQ (PIQ), mathematics subtests, and visual motor integration tasks would emerge, if the pM affects development. Differences on parent ratings would also be expected in the areas of withdrawn, anxious, or depressed behaviors, social and attention problems, stereotypies, and hyperactivity. There was no difference in psychological scores between the two groups of children on cognitive and behavioral measures. Taken together, the findings from these studies are consistent with the hypothesis that the premutation does not affect a child's psychological development.

These results from the initial work with women, and the current study of children, are preliminary in view of the small sample sizes employed. Not all findings support the hypothesis of no premutation effects. In a study employing a larger sample of 29 women with the premutation who had an affected child, and 17 non-fragile X mothers of autistic children, the women with the premutation failed to demonstrate a higher incidence of affective disorders. No such increase in affective disorders was observed between these two participant groups [Franke et al., 1996]. The comparison group of mothers with autistic children was small; and thus these nevertheless important findings should be interpreted as preliminary. There is much work to be completed to more thor-

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The most consistent evidence for premutation effects, to date, is that the premutation is associated with premature menopause (in a significant but minority group of women with the premutation) and mild outward features associated with the syndrome. The evidence for cognitive or psychosocial effects is, to date, far less supportive of a "premutation effects" hypothesis than are these findings.

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oughly identify the effects of the fragile X premutation.

Studies of "Obligate Carriers" or "Transmitting Males"

An additional source of evidence of premutation effects is drawn from the body of literature on "obligate carriers" or "transmitting males." In many cases, these individuals are presumed to be carriers of the premutation. This presumption is not unreasonable, particularly for transmitting males; most males affected by fragile X have mental retardation and are unlikely to marry and reproduce.

However, current knowledge allows us to expand the possibility of mutation categories present in a male with an FMR1 mutation; premutation versus full mutation is not the finite range of possibilities. Case reports have demonstrated instances of males with methylation mosaicism, and allegedly "transmitting males" may have some expression of full mutation genes, as was discussed earlier. These examples illustrate the necessity of DNA or protein studies in drawing inferences about effects of the fragile X premutation.

SUMMARY

Fragile X syndrome, a relatively recently identified neurodevelopmental disorder, results from a single gene mutation on the X chromosome. Cytogenetic testing has been replaced by DNA testing as the method of choice for identification and screening, although studies regarding the efficacy of protein-level screening have been, and are being, explored [de Vries et al., 1998; Tassone et al., 1999b]. Premutation genes are in general associated with no effects, although the controversy regarding possible effects is not yet resolved. The most consistent evidence for premutation effects, to date, is that the premutation is associated with premature menopause (in a significant but minority group of women with the premutation) and mild outward features associated with the syndrome. The evidence for cognitive or psychosocial effects is, to date, far less supportive of a "premutation effects" hypothesis than are these findings. Factors that may interact with the premutation in determining risk for deleterious effects have not been identified; it remains to be seen whether such factors will emerge from future research. One potential confound to this controversy is the fact that the differentiation between the premutation and full mutation genes is not always straightforward.

There is no debate over the notion that the full mutation typically leads to effects, although the specificity of these effects is not completely understood. Research from this decade has helped to address specific aspects of this phenotype, including the early course of development in males with fragile X, the influence of home and family environments, the nature of observed math difficulties, the social and autistic features seen in boys and girls, and the potential role of hyperarousal or hyper-reactivity in the development of the behavioral phenotype. These advances towards understanding the nature of social difficulties

will have an impact on determining the most successful interventions. Studies in these areas, and the ongoing research on the role of FMR protein, will contribute towards ongoing advances in our understanding of fragile X syndrome and its mechanisms.

A critical question that remains concerns who should be screened for fragile X syndrome. The variable phenotype—including variability in physical, social, and cognitive features, as described in this review—is one that prohibits clear-cut screening guidelines designed to avoid high rates of both false positives and false negatives. Clearly, it is important to consider the possibility of fragile X in any case of idiopathic mental retardation. However, testing *only* individuals with a family history of mental retardation leads to missing the first generation in which the full mutation expansion occurs. "Cascade" testing among those who have a relative with fragile X has been described as ineffective at detecting carriers and new cases of fragile X [Wildhagen et al., 1999], but anecdotal reports from clinical practice indicate that this method is quite effective at achieving these goals. Reliance on physical features as indices of risk for fragile X is also inadequate. What is indicated by the studies carried out in this decade is the need to include behavioral features in screening decisions versus the physical features often absent in young children with fragile X [Riddle et al., 1998; Bailey et al., 1999; Teisl et al., 1999]. ■

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