
The behavioral neurogenetics of fragile X syndrome: Analyzing gene–brain–behavior relationships in child developmental psychopathologies

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Abstract

Analyzing gene–brain–behavior linkages in childhood neurodevelopmental disorders, a research approach called “behavioral neurogenetics,” has provided new insights into understanding how both genetic and environmental factors contribute to complex variations in typical and atypical human development. Research into etiologically more homogeneous disorders, such as fragile X syndrome, in particular, allows the use of more precise metrics of genetic risk so that we can more fully understand the complex pathophysiology of childhood onset neurodevelopmental disorders. In this paper, we review our laboratory’s behavioral neurogenetics research by examining gene–brain–behavior relationships in fragile X syndrome, a single-gene disorder that has become a well-characterized model for studying neurodevelopmental dysfunction in childhood. Specifically, we examine genetic influences, trajectories of cognition and behavior, variation in brain structure and function, and biological and environmental factors that influence developmental and cognitive outcomes of children with fragile X. The converging approaches across these multilevel scientific domains indicate that fragile X, which arises from disruption of a single gene leading to the loss of a specific protein, is associated with a cascade of aberrations in neurodevelopment, resulting in a central nervous system that is suboptimal with respect to structure and function. In turn, structural and functional brain alterations lead to early disruption in emotion, cognition, and behavior in the child with fragile X. The combination of molecular genetics, neuroimaging, and behavioral research have advanced our understanding of the linkages between genetic variables, neurobiological measures, IQ, and behavior. Our research and that of others demonstrates that neurobehavior and neurocognition, genetics, and neuroanatomy are all different views of the same intriguing biological puzzle, a puzzle that today is rapidly emerging into a more complete picture of the intricate linkages among gene, brain, and behavior in developing children. Understanding the complex multilevel scientific perspective involved in fragile X will also contribute to our understanding of normal development by highlighting developmental events throughout the life span, thereby helping us to delineate the boundaries of pathology.

The “Problem”

What is a disorder? What is a symptom? How should a clinician or researcher define the boundaries of typical and atypical cognition,

behavior, and development? Should these boundaries be discrete or should they be “fuzzy,” with overlap between the constructs of “normal” and “abnormal”? Despite many decades of intensive research on cognitive and

The research presented in this article was funded by the Lynda and Scott Canel Fund for Fragile X Research and NIH Grants MH50047, MH64708, and MH01142.

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behavioral disorders occurring during child development, there are relatively few unambiguous answers to these questions at present.

Why are many fundamental questions underlying developmental psychopathology unanswered? There is a growing impression in the field that traditional research efforts aimed at understanding the pathogenesis of phenomenologically defined childhood-onset disorders such as autism, attention-deficit/hyperactivity disorder (ADHD), or dyslexia may be impeded, in part, by the etiological heterogeneity of individuals meeting the widely accepted diagnostic criteria defining these disorders. Without reliable and valid biological markers for the presence of a pathological condition or state, we are often left to ponder, if not pursue, a circular approach to research methodology. We may initially define a disorder according to a consensus of experts in the field agreeing to a diagnostic algorithm that includes the presence or absence of essential symptoms or signs. This definition may even have reasonable psychometric properties from the standpoints of diagnostic reliability and discriminative validity (from other phenomenologically defined disorders). However, the logic underlying this process is, inherently, at risk for circularity. The fact that we can reliably diagnose a disorder does not confer biological validity. Accordingly, the process of revamping or parsing such behaviorally or phenomenologically defined disorders into more etiologically or pathophysiologically meaningful subgroups is essential before we can begin to understand fundamental neurodevelopmental processes, and how these processes are influenced by genetic and environmental factors. Such progress will be particularly important in childhood cognitive and behavioral disorders that are currently defined by broad and incomplete classifications such as “mental retardation” and “learning disabilities.”

Since 1994, our research strategy has focused on explicating multiple levels of scientific inquiry to study children and adults with known or suspected homogenous genetic risk for neuropsychiatric, cognitive, behavioral and developmental dysfunction, an approach we have coined *behavioral neurogenetics* (Baum-

gardner, Green, & Reiss, 1994; Reiss & Freund, 1998; Reiss, Eliez, Schmitt, Patwardhan, & Haberecht, 2000). This research method has provided a powerful tool for scientific inquiry that encompasses quantitative assessments of genetic risk factors, brain structure and function, neurobehavioral and neurocognitive function, psychoneuroendocrinology, and environmental influences in investigating and understanding the neurodevelopmental pathways underlying learning and developmental disabilities. This approach complements more traditional research methods in cognitive-behavioral neuroscience that attempt to elucidate genetic and environmental risk factors starting at the point of a behaviorally defined disorder, as illustrated in Figure 1.

In our approach of behavioral neurogenetics research, we have made two fundamental underlying assumptions. First, the complex pathways beginning with one or more genetic factors affecting brain development or function will be more accessible when studied with genetically homogeneous groups. Examining developmental patterns within relatively homogeneous subgroups of individuals has been realized as an important analytic strategy in a multilevel approach (Cicchetti & Rogosch, 1996); studying the course of developmental processes within distinct homogenous subpopulations is vital before we more fully understand and identify individual patterns of dysfunction (Sroufe & Rutter, 1984).

A second underlying assumption has been that the information derived from studying these prototypic conditions will be relevant to understanding brain-behavior associations in children with similar patterns of cognitive, behavioral, and developmental dysfunction from the general population. As such, our research efforts have been aimed at examining the behavioral neurogenetics of several well-characterized genetic disorders that give rise to identifiable developmental, cognitive, and neuropsychiatry dysfunction in childhood; these include Williams syndrome, Turner syndrome, Klinefelter syndrome, velocardiofacial syndrome (VCFS), and fragile X syndrome. As “experiments of nature,” these disorders have provided researchers with invaluable insights

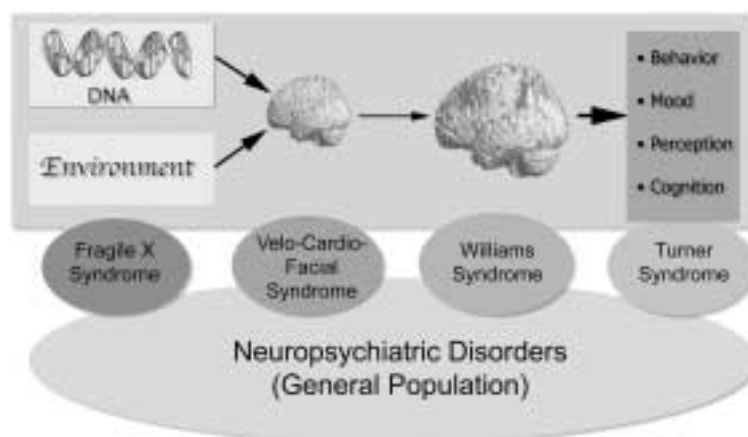


Figure 1. Behavioral neurogenetics research in the genetic conditions as models for understanding neuropsychiatric disorders such as fragile X syndrome.

into the developing human brain and child cognition and behavior that otherwise would not be possible. A major component of this research focuses on defining the cognitive, behavioral, and emotional developmental trajectories in children with these disorders, as well as how functional outcomes are moderated and mediated by risk factors such as age, family, educational environments, and neurobiological functioning.

In this paper, we review our work in fragile X syndrome to demonstrate how a behavioral neurogenetics approach can improve our understanding of the complex linkages between genetic, neurobiological, and behavioral variables contributing to neurodevelopmental and neuropsychiatric dysfunction in children. By mapping fundamental molecular events in fragile X to specific neurobiological correlates and phenotypic features, we open the exciting possibility of establishing direct links between genetic etiology and cognitive and behavioral outcomes.

The opportunity to study a group of children with a homogeneous etiology for cognitive and behavioral disability is rare in developmental psychopathology research. However, in an attempt to map fundamental molecular events to specific changes in brain structure and function, as well as cognitive/behavioral outcome, investigators increasingly are undertaking to study these more homogenous groups,

for example, in autism. This research provides a glimpse of the future of neuropsychiatric investigation in which the complex interplay between genetic risk and environment can be more fully appreciated, described, and elucidated. Thus, while the information gained from behavioral neurogenetics will have specific benefit to children and adolescents with fragile X syndrome, it also will have broader relevance to understanding how genetic–neurobiologic pathways lead to increased risk for distinctive profiles of cognitive and behavioral disability in children.

Fragile X Syndrome: Genetics and Phenotype

The fragile X syndrome, an X-linked semi-dominant disorder, is the most common heritable form of neurodevelopmental disability, second only to Down syndrome among all genetic causes of mental retardation in males and females (Crawford, Acuna, & Sherman, 2001; Freund, Reiss, & Abrams, 1993; Nussbaum et al., 2001). Numerous studies have found fragile X in every ethnic group, with current estimates of prevalence at 1 in 4,000 male births and 1 in 8,000 female births for individuals with the “full mutation” (see below; Crawford, Meadows, Newman, Taft, Pettay, Gold, Hersey, Hinkle, Stanfield, Holmgreen, Yeargin–Allsopp, Boyle, & Sherman,

1999; Turner, Webb, Wake, & Robinson, 1996; Warren & Sherman, 2001).

Genetics

In 1991, Verkerk et al. reported that a single gene on the X chromosome, *FMR1* (fragile X mental retardation 1), was associated with the symptoms of fragile X (Verkerk et al., 1991). Subsequently, it was determined that persons with fragile X showed dramatically increased numbers of triplet CGG repeats in the 5' untranslated region of the first exon of *FMR1* on the long arm of the X chromosome (locus Xq27.3). Unlike many single-gene inheritance patterns, in which the responsible mutation is stable in form from one generation to the next, fragile X syndrome is one of several disorders known to be caused by a dynamic gene mutation, resulting in instability and subsequent expansion of trinucleotide repeats through generations. In normal alleles, the CGG repeats vary from 6 to 50, whereas expansions of ~50–200 repeats are associated with the “premutation” form of the gene seen in carrier females and males. An early analysis of 977 genetically unrelated individuals unselected for mental retardation or fragile X syndrome who were analyzed for *FMR1* mutations revealed an estimated premutation frequency of 1 in 510 X chromosomes (Reiss, Kazazian, Krebs, McAughan, Boehm, Abrams, & Nelson, 1994); several large, population-based studies of the premutation or carrier form of fragile X estimate has since established premutation prevalence at 1 in 246–468 Caucasian females and 1 in 1000 Caucasian males in the general population (Crawford et al., 2001; Warren & Sherman, 2001). Larger expansions of more than 200 (up to 2,000) CGG repeats are considered a “full mutation” and associated with excessive methylation of cytosines in the *FMR1* promoter region. This modification extinguishes transcription of the *FMR1* gene into mRNA, shutting down translation of the fragile X mental retardation protein (FMRP). Excess methylation is believed to be primarily responsible for extinguishing *FMR1* expression (Figure 2). Treatment of cell lines containing the fragile X full mutation with methylation inhibitors reinitiates a

low level of *FMR1* expression (Chiurazzi, Pomponi, Pietrobono, Bakker, Neri, & Oostra, 1999). The massive expansion and methylation characterizing the full mutation also interfere with replication and chromatin condensation, producing the characteristic “fragile” appearance of metaphase X chromosomes under certain culture conditions.

Premutation alleles are unstable and tend to expand when transmitted from parent to child. A premutation can undergo a small expansion or it can develop into a massive expansion to a full mutation. A large expansion only occurs when the premutation is transmitted from a female (Rousseau, Heitz, et al., 1994), a phenomenon known as *genetic imprinting* (Ashley-Koch, Robinson, Glicksman, Nolin, Schwartz, Brown, Turner, & Sherman, 1998; Malter, Iber, Willemsen, de Graaff, Tarleton, Leisti, Warren, & Oostra, 1997). (In this case, *imprinting* refers to the presence of different epigenetic characteristics of male and female germ cell lines.) In addition, the risk of expansion in the child increases as the premutation size in the parent increases. Because the length of an unstable CGG repeat increases during each generation if transmitted by a female, increasing numbers of affected offspring may be seen in later generations of an affected family, a phenomenon known as *genetic anticipation*. However, not all small premutations may be predisposed to expand in subsequent generations. This may be due to AGG triplets embedded within CGG strings, which “anchor” the gene by inhibiting CGG expansion: analysis of premutation alleles in fragile X carriers has shown 70% contain a single AGG interruption and that loss of this triplet is an important mutational event leading to instability and expansion of the *FMR1* gene locus (Dombrowski, Levesque, Morel, Rouillard, Morgan, & Rousseau, 2002; Eichler, Holden, Popovich, Reiss, Snow, Thibodeau, Richards, Ward, & Nelson, 1994).

Phenotype

The fragile X mutation and decreases in FMRP influences developmental pathways that modulate physical appearance, cognitive

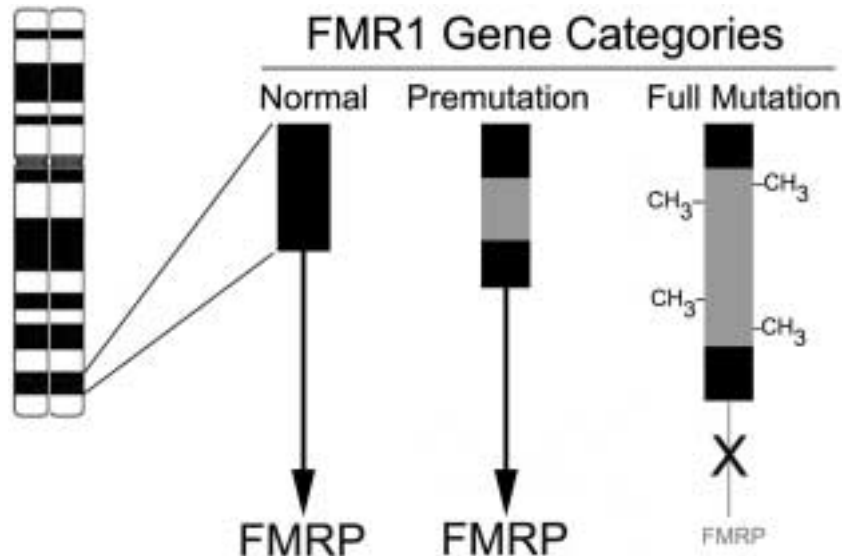


Figure 2. The genetics of fragile X syndrome. The *FMR1* gene in unaffected individuals is characterized by approximately 50 or fewer CGG repeats, whereas the fragile X “premutation” contains ~50–200 repeats and normal methylation patterns. Large expansions of >200 CGG repeats are typically associated with excessive methylation of the gene, thus reducing *FMR1* protein expression. This state is referred to as the “full mutation.”

ability, emotional function, and adaptive behavior. Although quite variable, the physical manifestations of fragile X include can include a long and narrow face, large ears, and mildly prominent jaw (Davids, Hagerman, & Eilert, 1990; Loesch & Hay, 1988; Meryash, Cronk, Sachs, & Gerald, 1984). These features, together with macroorchidism, often are seen in postpubertal males with fragile X (Lachiewicz & Dawson, 1994b). However, before puberty, children may have large heads but few other distinctive features. Children with fragile X show an abnormal trajectory of brain development and are at increased risk for cognitive, developmental, and behavioral problems beginning in infancy. School-age boys with fragile X syndrome show, on average, moderate mental retardation, whereas females with the disorder usually demonstrate a range of cognitive function from normal to mild mental retardation. Approximately 50% of females with the full mutation have IQs ranging from mental retardation to borderline levels (Hagerman, Jackson, Amiri, Silverman, O'Connor, & Sobesky, 1992). Because these clinical findings are not unique to fragile X syndrome and the physical characteristics are

particularly variable in prepubertal children and females, the definitive diagnosis of the disorder is made from both genetic testing (Southern blot) and polymerase chain reaction. These tests determine *FMR1* CGG repeat number and methylation characteristics. The severity of the fragile X phenotype depends mostly on the degree of abnormal methylation of the *FMR1* gene and, in females, the degree of skewing of normal X-chromosome inactivation (Nussbaum et al., 2001).

Summary and synthesis

Fragile X syndrome, an X-linked dominant neurodevelopmental disorder, is the most common heritable form of neurodevelopmental disability, with a prevalence at 1 in 4,000 male births and 1 in 8,000 female births. The disorder is caused by an abnormal expansion of CGG trinucleotide repeats within the *FMR1* gene located on the long arm of the X chromosome. Repeat lengths up to approximately 40–50 triplets are normal. However, expansions of up to approximately 200 triplets are associated with the “premutation” form of the gene and over 200 (up to 2,000)

contain hypermethylated CGG repeats, which results in reduced production of the *FMR1* protein (FMRP). Reductions or loss of FMRP causes a trajectory of abnormal brain development and function, in turn leading to a cascade of cognitive, behavioral, and emotional problems in children with fragile X. Because random X inactivation occurs in females, 40–50% of females (and nearly all males) who inherit the full mutation will exhibit identifiable cognitive symptoms. Some males and females with fragile X have a mixture of cells with ranges of repeats (mosaicism) and therefore, a large range of phenotypic features is observed in affected individuals. Before puberty, boys with fragile X have somewhat large heads but few other features; after puberty, the features may be more distinctive, including a long face with prominent jaw and forehead, large ears, and macroorchidism.

The Development of Behavior and Cognition

From infancy, both female and male children and adolescents with the fragile X full mutation are predisposed to manifesting a characteristic set of cognitive, behavioral, and emotional problems. Taken overall, these cognitive, behavioral, and emotional deficits include cognitive delay with age-related declines in IQ, disturbance in language and communication, reduced trajectory and abnormalities in the development of adaptive behaviors, particular cognitive abnormalities within the domains of executive function and visual–spatial cognition, hyperactivity, and significant problems with hyperarousal and anxiety. Females with the premutation do not usually manifest symptoms; however, recent evidence suggests that those with large repeat sizes (>100) may manifest a milder and more variable phenotype. The trajectories of these impairments from infancy through adolescence and adulthood are complex and variable due to variability in the interplay between complex genetic, environmental, and biological risk factors. As we will discuss, such risk factors play important roles in determining outcomes of cog-

nition, behavior, and emotion in children with fragile X.

Considering the behavioral and intellectual development of individuals with fragile X is a complex endeavor. First, the behavioral and cognitive manifestations of fragile X are variable in males and females, and therefore, developmental trajectories must be presented by gender. Furthermore, because trajectories of cognitive and behavioral development change throughout life in subjects with fragile X, as well as those with typical development, it is important to present each developmental stage as a unique, albeit interrelated, phenomenon.

Infants and toddlers

Although efforts are beginning to identify the developmental trajectory of infants and toddlers with fragile X, there is currently a relative void of information specific to this developmental period compared to school-age children or adults (Keysor & Mazzocco, 2002). Studies that have included infants and toddlers under 2 years of age have not differentiated findings specific to this age group (Bailey, Roberts, et al., 2001). However, recent longitudinal investigations of early development in infants, toddlers, and young children with fragile X aged 24–60 months have described early development and behavior over time (Bailey, Hatton, & Skinner, 1998; Bailey, Hatton, Tassone, Skinner, & Taylor, 2001; Bailey, Skinner, Hatton, & Roberts, 2000; Hatton, Bailey, Hargett–Beck, Skinner, & Clark, 1999).

Early in infancy, fragile X is typically identified through the infant's delayed or abnormal development, but the large variability and subtlety in its expression make identification difficult (Bailey, Roberts, et al., 2001). Parents may first notice concerns about their infant's lack of gross motor coordination (Simko, Hornstein, Soukup, & Bagamery, 1989) and hypotonia (Friefeld & MacGregor, 1993). In a recent study of 41 mothers of infants with fragile X, at least 10% of the infants with fragile X displayed low muscle tone and unusual motor movements, hyperactivity, or irritability (Bailey, Skinner, 2000). In this study, these symptoms of develop-

mental delay were identified by the parents at an average age of 24 months, but it was a year later at a mean age of 35 months before fragile X syndrome was typically diagnosed by genetic testing in the toddler. Mothers first expressed concerns about their infant's development as early as 9 months of age and most often noticed delays in their infant's developmental milestones—first speech, crawling, walking; other reported concerns about infants with fragile X were problems in breathing, perceived pain, a lack of eye contact or focus, a glazed look, or lack of attentiveness. Young boys with fragile X often come to the attention of their pediatrician specifically because of speech delays or abnormal language, frequent ear infections, irritability, sensory regulation problems, and frequent tantrums and hyperactivity (Hagerman, Staley, O'Conner, Lugenbeel, Nelson, McLean, & Taylor, 1996).

An analysis of 26 male infants and toddlers with fragile X (aged 12–36 months; average = 24 months) found that Battelle Developmental Inventory (BDI) scores increased moderately from 12 to 36 months, but over time, the developmental quotient from the BDI continued to lag behind normal age-matched infants and toddlers so that by age 36 months, toddlers with fragile X tested equivalent to a developmental age of 20 months (Roberts, Boccia, et al., 2001). In this study, developmental delays were seen in some infants as early as 12 months (equivalent to developmental age of 9 months). As the male toddler approached 36 months of age, motor skills appeared least delayed, whereas communication skills were most delayed: mean age of crawling, walking, sitting was delayed 2–3 months, whereas mean age of first spoken word was delayed by an average of 17 months. While these signs and symptoms of fragile X in male infants and toddlers are variable, detection of fragile X in female infants and toddlers is particularly difficult due in part to their relatively mild phenotype (Hatton et al., 1997). Based on findings from longitudinal studies of infants and toddlers with fragile X describing behavioral development, Bailey created a screening checklist of behavioral features that may help clinicians identify critical behaviors

important in diagnosis of fragile X in this age group (Bailey, Roberts, et al., 2001).

Preschool children

Few studies have been carried out on the cognitive and behavioral profiles of preschool children with fragile X. Compared with boys, preschool girls with fragile X are less likely to come to professional attention due to lesser severity of symptoms, although some young girls present with developmental delay, which signals attention. In a screening study of 534 preschool children with developmental delay, 3 girls were diagnosed with fragile X; the girls displayed language delays, cognitive delays (15–20 months below their age level of 3.75 years), as well as attentional problems, hyperactivity, tantrums, aggression, self-injurious behavior, and mood swings (Mazzocco, Myers, Hamner, Panoscha, Shapiro, & Reiss, 1998). Preschool boys with fragile X also display similar behavioral and cognitive features to girls with fragile X, but the symptoms are generally more pronounced (Bailey, Hatton, Mesibov, Ament, & Skinner, 2000; Hatton et al., 1997; Hatton et al., 1999; Hatton, Hooper, Bailey, Skinner, Sullivan, & Wheeler, 2002).

Motor. Preschool-age children with fragile X exhibit significant motor delays, with development approximately half the rate expected for typically developing children (Kau, Reider, Payne, Meyer, & Freund, 2000). In one study, motor as well as speech delays were found to be related to levels of FMRP expression (Bailey, Hatton, Tassone, 2001). That is, the higher the FMRP (i.e., more like unaffected individuals), the better the developmental course. When compared to age-, IQ-, and language-level matched controls, preschool children with fragile X showed greater delays and greater variability in motor skills (Kau et al., 2000). When the fragile X group was further divided in terms of full mutation and mosaicism, the latter condition presumably being associated with some preservation of FMRP production, the full mutation group showed a lower, but not significant, mean age equivalent on the motor skills domain of the Vine-

land Adaptive Behavior Scales (VABS) than did the mosaic group.

Cognition. The majority of boys with fragile X present with significant cognitive delay (Rousseau, Heitz, et al., 1994), usually by age 4 years (Bailey, Hatton, & Skinner, 1998). However, an early study of 221 preschool- and school-age boys with fragile X showed that 13% were classified as "high functioning" (IQ \geq 70; Hagerman et al., 1994). In a study of preschool age (16–64 months) boys with fragile X compared to age-matched boys with developmental delay but without fragile X, 44% of the boys with fragile X had overall IQs in borderline-average range, although boys with fragile X scored lower on cognitive assessments with the Stanford–Binet (4th edition) than boys with developmental delay. There was no evidence in this cross-sectional study that overall IQ declined among boys with fragile X across this developmental period, although group size was limited (Freund et al., 1995). In a multicenter cross-sectional analysis, boys with fragile X (aged 1–10), particularly preschool boys, showed age-related increases in adaptive skills (Vineland composite scores), reaching levels that often exceeded IQ expectations. IQ and adaptive behavior were highly correlated in this study, suggesting that, like typically developing children, their courses of development may be in synchrony (Dykens, Ort, Cohen, Finucane, Spiridigliozzi, Lachiewicz, Reiss, Freund, Hagerman, & O'Connor, 1996). The trajectory of cognitive development in preschool-age children with fragile X shows relatively mild to moderate reduction in IQ compared with typically developing children; this is in contrast to that observed in school-age children and early adolescents with this disorder, for which several investigators have observed a decline in IQ scores (see below).

In a longitudinal prospective study of 46 boys with fragile X aged 24–66 months (average, 44 months), Bailey, Hatton, and Skinner (1998) evaluated five domains, cognition, communication, adaptive, motor, and personal–social, using the Battelle Development Inventory. The boys with fragile X varied widely in rate and level of performance, but

overall, their development was significantly delayed, with a slope of 0.48, approximately half the rate expected for typically developing age-matched boys. As boys with fragile X grew older, more of them scored in the deficient range of the cognitive domain of the Battelle inventory, with all boys in the study scoring in the deficient range by 66 months of age. The developmental trajectories were similar in slope across all five domains that were examined, indicating a stable development over time within a domain. As with male toddlers, preschool boys with fragile X tested higher in motor and adaptive skills and lower in communication and cognitive skills at every age. The level of FMRP was correlated with the level of cognitive impairment—the less FMRP, the lower the cognitive domain scores. Wright–Talamante et al. found that there was no significant IQ decline in young males with less than 50% methylation of the full mutation, suggesting that a small to moderate amount of FMRP production partially protects against significant IQ decline (Wright–Talamante, Cheema, Riddle, Luckey, Taylor, & Hagerman, 1996).

In contrast to boys, the developmental trajectory of cognition in girls with fragile X is more variable, with about one-half of the preschool- and school-age girls with the full mutation presenting with mental retardation (full scale IQ $<$ 70; Rousseau, Heitz, et al., 1994). Although remarkably little is known about longitudinal changes in IQ scores among preschool girls with fragile X (Fisch, Simensen, Arinami, Borghgraef, & Fryns, 1994), as we discuss for school-age girls, several specific weaknesses characterize the cognitive profiles of young females with this condition, including deficits in mathematical reasoning, reduced attention, and decreases in short-term memory while verbal skills remain relatively preserved (Dykens et al., 1994).

Behavior and emotion. Although behavioral styles are variable, boys with fragile X between the ages of 2 and 5 years of age are at risk for manifesting problems with motoric hyperactivity, hyperarousal, inattention, gaze avoidance, unusual speech (echolalia, perseveration), stereotypies (hand flapping), exces-

sive mouthing behavior, self-injury (e.g., hand biting), and tactile defensiveness (Bailey, Hatton, et al., 1998; Borghgraef, Fryns, Dielkens, Pyck, & Van den Berghe, 1987; Cohen, Vietze, Sudhalter, Jenkins, & Brown, 1989; Freund et al., 1995; Fryns, 1984; Hagerman, Amiri, & Cronister, 1991; Kau et al., 2000; Lachiewicz, Dawson, & Spiridigliozzi, 2000; Lachiewicz, Spiridigliozzi, Gullion, Ransford, & Rao, 1994; Maes, Fryns, Ghesquiere, & Borghgraef, 2000; Simko et al., 1989). The behavioral style or temperament of 45 preschool boys with fragile X (ages 47–88 months, average = 64 months) evaluated on the Behavioral Style Questionnaire (McDevitt & Carey, 1978) showed that, compared to typically developing boys, those with fragile X were more active yet less intense, approachable, adaptable, and persistent, although there was considerable variability in temperament profiles at this stage of development (Hatton et al., 1999). Over the 6- to 18-month period of this study, boys' scores on temperament dimensions were stable and there was no link between the severity of overall developmental disability and behavior or temperament, suggesting that IQ and temperament are separate constructs.

In the social domain, young boys with fragile X often appear excessively shy and anxious (Baumgardner, Reiss, Freund, & Abrams, 1995); they typically avoid unfamiliar people (Cohen, Sudhalter, Pfadt, Jenkins, Brown, & Vietze, 1991), develop poor eye contact (Cohen, Fisch, Sudhalter, Wolf-Schein, Hanson, Hagerman, Jenkins, & Brown, 1988; Hagerman et al., 1992; Payton, Steele, Wenger, & Minshew, 1989; Teisl, Reiss, & Mazzocco, 1999; Wolff, Gardner, Paccla, & Lappen, 1989), and begin to demonstrate stereotypic movements and qualitative abnormalities of speech, such as cluttering and echolalia (Baumgardner et al., 1995; Lachiewicz et al., 1994; Sudhalter, Cohen, Silverman, & Wolf-Schein, 1990; Teisl et al., 1999). In preschool boys with fragile X, this behavioral profile is seen as early as 3–5 years of age, commensurate with the slowing of adaptive behavior development during this age period. Among maladaptive behaviors, social reticence or withdrawal is often the most problematic for

preschool boys (Freund et al., 1995). In a study of preschool boys with fragile X and age- and IQ-matched control boys (Kau et al., 2000), children were rated by their mothers on the Dimensions of Temperament Scale—Revised, the Child Behavior Checklist, and the Aberrant Behavior Checklist—Community. Compared to age-, IQ-, and language-level matched boys with idiopathic developmental delay, those with fragile X showed increased initial avoidance and decreased social withdrawal.

Young girls with fragile X also exhibit higher rates of emotional disturbance and maladaptive behaviors, including problems with depression, social anxiety and withdrawal, and attention deficit (Freund et al., 1993; Hagerman et al., 1992; Lachiewicz, 1992; Lachiewicz & Dawson, 1994a; Mazzocco, Baumgardner, Freund, & Reiss, 1998; Mazzocco, Kates, Baumgardner, Freund, & Reiss, 1997). By using a behavioral screening questionnaire for parents of preschool boys and girls with fragile X, it was found that considering the behavioral features of the child are useful diagnostic indicators of fragile X syndrome, particularly in the absence of a recognizable physical phenotype (Reiss et al., 1992; Teisl et al., 1999).

Autistic behaviors. The topography of behavioral, social, and developmental abnormalities that emerges in the preschool years has led some investigators to suggest that fragile X is a genetic risk factor for autism or autistic behavior. Compared with non-fragile X males who have comparable cognitive disability, boys with fragile X are at increased risk, starting at a young age, for manifesting a profile of maladaptive behaviors that overlaps with the phenomenologically defined *DSM-IV* category of Pervasive Developmental Disorder (Bailey, Hatton, et al., 2000; Bailey, Hatton, Skinner, & Mesibov, 2001; Bailey, Mesibov, Hatton, Clark, Roberts, & Mayhew, 1998; Baumgardner, Reiss, Freund, & Abrams, 1995; Cohen et al., 1988; Cohen, Sudhalter, et al., 1991a; Feinstein & Reiss, 1998; Harris, 1999; Lachiewicz et al., 1994; Rogers, Wehner, & Hagerman, 2001). Approximately 15 to 30% of males with fragile X fulfill DSM criteria for autism, although a much higher

percentage show one or more components of behavior from the autistic domain (Baumgardner et al., 1995; Reiss & Freund, 1992).

The findings suggest that young children with fragile X demonstrate a pattern of development that includes more autistic behaviors than children with developmental delay but has a unique trajectory compared to that for children with idiopathic autism (Rogers et al., 2001). Specifically, in a study of autistic behaviors using *DSM-III-R* criteria for autism, compared with IQ- and age-matched typical boys, young boys with fragile X (mean age = 8.7 years) showed increased dysfunction in peer social play, nonverbal communication (gaze aversion, gesturing), verbal communication (cluttered speech, echolalia, word/phrase perseveration), and repetitive motor behaviors (handflapping, rocking; Reiss & Freund, 1992). By using the Childhood Autism Rating Scale (CARS), Bailey, Mesibov, et al. (1998) showed that 25% of 57 young boys with fragile X (mean age = 5.5 years) scored above the cut-off for autism, suggesting a relatively high incidence of autistic behaviors. In this study, the severity of behavioral delay in boys with fragile X was related to scores on the CARS: the more severely delayed children scored higher (more autistic) on the CARS. Compared with a control group of typically developing boys, those boys with fragile X (aged 3–8 years) and age-matched boys with autism were slower to adapt, less persistent, and more withdrawing than controls; boys with fragile X had a relatively flat profile of behavioral development, whereas autistic boys had significantly greater delays in social and communication skills (Bailey, Hatton, et al., 2000).

In terms of genetic risk factors, recent research suggests that autistic behaviors in children with fragile X may be influenced by additional background genes whose own protein production is influenced by FMRP (see below; Feinstein & Reiss, 1998; Rogers et al., 2001).

School-age children

During the school-age period of development, increased demands on cognition and speech, as well as new and variable social experiences

with teachers and schoolmates, present significant challenges to the child with fragile X. Although development progresses at half or less the normal rate in preschool-age boys with fragile X (up to age 8), the trajectory of development does not appear to plateau or level off until later, usually during the school years and into preadolescence (9–11 years).

Cognition. Although typically developing preschool- and school-age children and most groups of children with mental retardation of mixed etiologies demonstrate a stable IQ, several cross-sectional and longitudinal investigations in school-age children with fragile X indicate that development of cognitive abilities may follow an abnormal trajectory. Slowing or early plateauing of development (as opposed to a loss of skills) leads to declining standardized scores in school-age children with fragile X, perhaps beginning as young as 5 years of age (Bailey, Hatton, et al., 1998; Dykens et al., 1996; Dykens, Hodapp, Ort, Finucane, Shapiro, & Leckman, 1989; Dykens, Hodapp, Ort, & Leckman, 1993; Fisch, Carpenter, Holden, Simensen, Howard–Peebles, Maddalena, Pandya, & Nance, 1999; Fisch, Carpenter, Simensen, Smits, van Roosmalen, & Hamel, 1999; Fisch, Simensen, Tarleton, Chalifoux, Holden, Carpenter, Howard–Peebles, & Maddalena, 1996; Freund & Reiss, 1991; Hagerman, Schreiner, Kemper, Wittenberger, Zahn, & Habicht, 1989; Hodapp, Dykens, Hagerman, Schreiner, Lachiewicz, & Leckman, 1990; Lachiewicz, Gullion, Spiridigliozzi, & Aylsworth, 1987). As more demands are placed on reasoning and cognition in school, the IQ of the child with fragile X begins to decline (Hagerman et al., 1989; Hodapp et al., 1990; Lachiewicz et al., 1987). Compared with school-aged boys with a full mutation, age-matched girls with the full mutation show a wider range in IQ scores, but test–retest IQ scores also may decrease (Fisch, Carpenter, Holden, Howard–Peebles, Maddalena, Borghgraef, Steyaert, & Fryns, 1999).

In both school-age girls and boys with fragile X, the speech and language domain are particularly affected. Compared with girls, boys with fragile X have significantly lower age-equivalent language skills (Fisch, Holden,

Carpenter, Howard-Peebles, Maddalena, Pandya, & Nance, 1999). In a longitudinal analysis of 28 male children with fragile X (aged 4–14 years) examining three domains of adaptive behavior (Vineland Scales), Fisch, Carpenter, Holden, Howard-Peebles, et al. (1999) found that, compared with socialization or daily living skills, communication was the most severely affected skill, which appeared to plateau early. Furthermore, there is some evidence that delays in communication skills are age related. Communication skills age equivalents are about half the chronological age of preteen children with fragile X, a delay that increases for children aged 11 years or older (Dykens et al., 1996). This finding was confirmed in longitudinal studies showing a decline in speech and language skills in children over 10 years of age, with speech and language development at approximately 50% of typically developing children (Bailey, Hatton, et al., 1998; Dykens et al., 1993).

The profile of school-age girls (mean = 11.3 years) and boys (mean = 10 years) with fragile X on the Stanford-Binet Intelligence Scale shows distinct patterns of weaknesses in visual-motor coordination, spatial memory, and arithmetic, but strengths in verbal labeling and comprehension (Freund & Reiss, 1991). Girls with fragile X (7–14 years) show significant relative weaknesses on visuo-construction tasks such as block assembly and drawing (Cornish, Munir, & Cross, 1998). This profile of weaknesses in visual memory and perception, mental manipulation of visual-spatial relationships among objects, visual-motor coordination, processing of sequential information and arithmetical stimuli, and attentional/executive function has been well documented in both male and female children with fragile X (Freund & Rice, 1996; Freund & Reiss, 1991; Hagerman et al., 1992; Hinton, Halperin, Dobkin, Ding, Brown, & Mizejeski, 1995; Mazzocco, Hagerman, Cronister-Silverman, & Pennington, 1992; Mazzocco, Pennington, & Hagerman, 1993; Mizejeski, Jenkins, Hill, Wisniewski, French, & Brown, 1986; Munir, Cornish, & Wilding, 2000a, 2000b; Riddle, Cheema, Sobesky, Gardner, Taylor, Pennington, & Hagerman, 1998; Schapiro et al., 1995; Sobesky, Pennington,

Porter, Hull, & Hagerman, 1994; Theobald, Hay, & Judge, 1987). Relative to intellectual potential, math achievement is particularly deficient in girls with fragile X beginning in the early school years. In a comparison of 5- and 6-year-old school girls with and without fragile X matched for age, full-scale IQ score, and grade in school, verbal scores were comparable but math ability scores for girls with fragile X were significantly lower than the average scores for typically developing girls (Mazzocco, 2001). As we examine later, the neural underpinnings of this deficit in girls with fragile X are beginning to emerge.

Behavior and emotion. In the behavioral domain, prospective studies of small groups of school-age boys with fragile X show that whereas cognitive abilities decline in a non-linear manner, adaptive behaviors (scored by the Vineland Adaptive Behavior Scales) decline steeply and linearly (Fisch et al., 1996). Whereas Vineland domain scores did not reveal a specific profile of adaptive skills development, Aberrant Behavior Checklist scores in school-age boys with fragile X (average = 8.7 years) revealed high levels of hyperactivity, stereotypic movements, and unusual speech (Baumgardner et al., 1995) compared to boys with a comparable level of cognitive disability not due to fragile X. Compared with typically developing boys (mean = 10.3 years), age-matched boys with fragile X were less emotionally stable and less open to new experiences; particularly boys with parents who were less angry and more consistent in planning with the child were more agreeable, open, and less irritable than boys with parents who openly displayed anger and were inconsistent in their planning (van Lieshout, De Meyer, Curfs, & Fryns, 1998). As we discuss in a later section, parental psychological status and quality of home environments are important correlates of behavior in both boys and girls with fragile X.

Social behaviors begin to become more problematic as boys enter the school-age period (Freund, 1995), and school-age boys with fragile X syndrome demonstrate deficits with peers, social avoidance, avoidance of eye contact, and gaze aversion, as well as inattention,

impulsivity, and hyperactivity in school and social situations (Bailey et al., 1998a, 2001a; Baumgardner et al., 1995; Bregman, Leckman, & Ort, 1988; Einfeld, Tonge, & Turner, 1999; Lachiewicz et al., 1994; Reiss & Freund, 1990, 1992). As in preschool boys, school-age boys with fragile X also exhibit autistic behaviors and boys with fragile X and autistic behaviors demonstrate slower growth in developmental age than boys without autistic behaviors (Bailey, Hatton, et al., 2001).

Behavioral symptoms in school-age girls with fragile X particularly include shyness and social avoidance, mild to moderate symptoms of ADHD, and anxiety and depression (Freund et al., 1993; Hagerman et al., 1992; Lachiewicz, 1992; Mazzocco, Kates, et al., 1997). In school-age girls with fragile X, prevalence of significant anxiety symptoms varied from 23 (Lachiewicz & Dawson, 1994a) to 50% (Freund et al., 1993; Mazzocco, Baumgardner, et al., 1998). Although girls and boys with fragile X show deficits in social interactions with their peers, this typically does not extend to relationships with their parents or caregivers, with whom they are generally able to establish strong and developmentally appropriate attachments (Reiss & Freund, 1992). Relative to their own sisters without fragile X, girls with fragile X aged 6–14 years had higher ratings of withdrawn behaviors (Mazzocco, Baumgardner, et al., 1998). Young girls with fragile X were often rated by their parents and teachers as significantly more withdrawn and depressed when compared with control girls, and 38% of the girls with fragile X were diagnosed with mood disorders in a structured interview (Freund et al., 1993). In one study, 47% of girls with fragile X aged 4–11 years demonstrated high Child Behavior Checklist *T* scores (>70) for social withdrawal, and 26% also had high *T* scores on the depression scale (Lachiewicz, 1992).

The social avoidance and anxiety seen in girls with fragile X qualitatively overlaps with that associated with pervasive developmental disorders. Although autistic behaviors were reported more frequently for 6- to 16-year-old girls with fragile X compared to girls without fragile X (Mazzocco, Kates, et al., 1997), the

symptoms were more variable and less severe than in boys with fragile X. Cohen et al. found only 1.7% of 33 school girls with fragile X were autistic (Cohen, Brown, Jenkins, Krawczun, French, Raguthu, Wolf-Schein, Sudhalter, Fisch, & Wisniewski, 1989). This low rate is consistent with the 4:1 predominance of males to females with autism and the limited number of clinical case descriptions of autistic, fragile X females (Bolton, Rutter, Butler, & Summers, 1989; Gillberg, Ohlson, Wahlstrom, Steffenburg, & Blix, 1988; Hagerman, Chudley, Knoll, Jackson, Kemper, & Ahmad, 1986).

Hyperarousal. A particularly notable behavioral characteristic in children with fragile X is a strong propensity for hyperarousal. In particular, boys with fragile X are predisposed to hyperarousal, which is signaled by behaviors such as poor eye contact, tactile defensiveness, hyperactivity, hand flapping, nail biting, and tantrums (Bailey, Hatton, et al., 1998; Borghgraef et al., 1987; Cohen, Vietze, et al., 1989; Freund et al., 1995; Fryns, 1984; Hagerman et al., 1991; Kau et al., 2000; Lachiewicz et al., 1994, 2000; Maes et al., 2000; Simko et al., 1989). Most boys with fragile X are highly sensitive to auditory, tactile, visual, and olfactory stimuli (Cronister & Hagerman, 1989) and may overreact in highly stimulating environments such as a supermarket or mall (Besler & Sudhalter, 1995; Cohen, 1995). Recent studies of heartbeat irregularities showed that, compared with typically developing boys, those with fragile X had higher heart rates during passive phases, as reflected in shorter heart periods, which was a result of increased sympathetic and reduced parasympathetic nervous system activity (Boccia & Roberts, 2000; Roberts, Boccia, Bailey, Hatton, & Skinner, 2001). In addition, children with fragile X also showed greater magnitude, more responses, and lower rates of habituation to skin stimulation, suggesting an abnormal overreaction in the sympathetic nervous system (Miller, McIntosh, McGrath, Shyu, Lampe, Taylor, Tassone, Neitzel, Stackhouse, & Hagerman, 1999). Along with the findings of increased cortisol levels in boys with fragile X discussed later in this review

(Hessl, Glaser, Dyer–Friedman, Blasey, Hastie, Gunnar, & Reiss, 2002; Wisbeck, Huffman, Freund, Gunnar, Davis, & Reiss, 2000), these findings support the neural and physiological basis of hyperarousal in boys with fragile X.

Executive function. One of the most consistent neuropsychological findings in school-age children with fragile X is a deficit in executive functioning, involving goal-directed, future-oriented behaviors involving working memory, planning, and inhibition (Hagerman et al., 1992; Mazzocco, Hagerman, Cronister–Silverman, & Pennington, 1992; Mazzocco, Hagerman, & Pennington, 1992; Sobesky et al., 1994; Thompson, Gulley, Rogeness, Clayton, Johnson, Hazelton, Cho, & Zellmer, 1994). In studies of high-functioning girls with fragile X (IQ > 70), the largest group differences from control girls involved tasks of executive function, which are conceptual problem solving, flexibility in thinking, inhibition, and concept formation (Mazzocco et al., 1993). These group differences remained significant even when statistically accounting for the effects of IQ. In a study of molecular and phenotypic correlations in females with fragile X, it was found that the X inactivation ratio (a metric that parallels FMRP levels) was strongly and positively correlated with executive function (Sobesky, Taylor, Pennington, Bennetto, Porter, Riddle, & Hagerman, 1996), suggesting that executive functioning is sensitive to levels of FMRP. The defects in executive functioning may help to explain some of the other weaknesses in females with fragile X—problems in attentional function, organization, and memory and behavioral problems such as hyperactivity or impulsivity.

Boys with fragile X also show a deficit in executive functioning, which may help explain some cognitive and behavioral problems. As in girls, deficits in executive function in boys with fragile X are consistent with problems in attentional control and impulsivity, difficulty with maintaining one topic and a tangential conversational style, and deficits in memory. In a study of working-memory tasks, Kaufmann et al. found that boys with

fragile X were able to learn simple tasks but showed impairment on more complex tasks requiring memorization (Kaufmann et al., 1990), suggesting that more difficult tasks requiring the use of working memory to guide behavior may be difficult for males with fragile X.

Adolescents

An early study of adolescent and adult women with the fragile X premutation showed no differences from control subjects with respect to cognitive abilities or profile, neuropsychological function, psychiatric diagnoses or symptoms, and self-rated personality profiles (Reiss, Freund, Abrams, Boehm, & Kazazian, 1993). However, this issue has recently regained attention, as evidence indicating that individuals with large premutations may manifest some of the signs and symptoms of fragile X (Johnston, Eliez, Dyer–Friedman, Hessl, Glaser, Blasey, Taylor, & Reiss, 2001). In males and females with the fragile X full mutation, however, there appears to be steady cognitive growth until late childhood and early adolescence (10–15 years of age), at which point mental age plateaus and IQ declines (Dykens, Hodapp, Ort, et al., 1989).

Cognition. IQs of children with fragile X that initially begin to decline in middle childhood continue to decline through adolescence (Hagerman et al., 1989; Lachiewicz et al., 1987), and adolescent males with higher initial IQ scores are more likely to manifest IQ decline than those with initial lower levels of intelligence (Dykens, Hodapp, Ort, et al., 1989). It was suggested that the decline in IQ in fragile X might be explained by inherent properties of cognitive tests, which for older children may place greater emphasis on skills that are known to be specific weaknesses in this disorder (Hagerman et al., 1989). However, findings from studies of adaptive behavior development in fragile X, which rely on informant (usually parent) report, also indicate atypical developmental trajectory. Particular weaknesses are seen in communication skills in adolescents with fragile X. From the preschool to adolescent years and into adulthood, individuals with fragile X consistently show de-

creased trajectories in speech and language skills, approximately half those of normal adolescents.

Behavior and emotion. As noted above, adaptive behavior skills also decline as the child enters the adolescent years. In a multicenter study of adaptive behavior profiles in males with fragile X, Dykens et al. (1993, 1996) found that boys between 1 and 10 years old showed significant age-related gains in adaptive skills, but older boys between 11 and 20 years old showed more variability in adaptive skills and there was no relationship between age and the changes in adaptive skills. As these males with fragile X reached early adulthood (21–40 years), age-equivalent adaptive scores stabilized. Relative strengths were seen in daily living skills and weaknesses in communication were evident only among older males with fragile X (Dykens et al., 1993, 1996).

In the social domain, adolescent and young adult women with the fragile X full mutation manifest anxiety in social interaction and are at high risk for developing major depression (Freund, Reiss, Hagerman, & Vinogradov, 1992; Hagerman & Sobesky, 1989; Reiss, Hagerman, Vinogradov, Abrams, & King, 1988; Sobesky et al., 1994, 1996; Thompson et al., 1994; Thompson, Rogeness, McClure, Clayton, & Johnson, 1996). The relationship between neurobehavioral functioning and CGG repeat length was studied in female carriers of the fragile X premutation (56–166 repeats; Johnston et al., 2001). Compared with those individuals with smaller (<100 repeats) alleles, those females with larger (>100 repeats) alleles scored significantly higher on the Interpersonal Sensitivity and Depression subscales of the Symptom Checklist-90-R (Derogatis, 1994). The behaviors encompassing these dimensions include withdrawn behavior and depressed mood. Females with a premutation have been reported to show schizotypal traits, emotional difficulties, social anxiety and increased prevalence with mood disorders (Franke, Leboyer, Gansicke, Weiffenbach, Bincalana, Cornillet-Lefebvre, Croquette, Frosster, Schwab, Poustka, Hautzinger, & Maier, 1998; Sobesky et al., 1996; Thompson et al.,

1996). Although social anxiety and attentional dysfunction are likely to be “core” deficits directly correlated with reduced FMRP in females, the development of depression may be a secondary complication associated with social neglect or rejection by peers and increasing self-awareness of behavioral, emotional, and cognitive differences from others (Fopma-Loy, 2000).

As in females with fragile X, males with fragile X are usually socially interested but avoidant and many have problems relating to their peers. For example, adolescent boys with fragile X commonly avert their gaze when meeting new people. Wolff et al. (1989) illustrated the unique and commonly observed pattern of gaze aversion in adolescent and adult males with fragile X. Of 18 adolescents and adults in the study, 14 demonstrated gaze aversion with avoidant behavior; in this longitudinal study, 6 boys with fragile X who were under 8 years of age did not demonstrate gaze aversion, but nearly all the adolescent males over 12 showed this unique greeting behavior. This greeting behavior has been linked to the excessive anxiety seen in boys with fragile X in social situations (Kerby & Dawson, 1994). As is discussed in a later section, recent neuroimaging research has examined the neural basis of gaze aversion.

Executive function. Adolescent females with the fragile X full mutation continue to show significant and consistent deficits on tasks of executive functioning, which are not totally accounted for by their lower IQs. In particular, adolescent girls and young adult women with fragile X are at risk for demonstrating impulsivity and attentional inefficiency and have difficulty with the organizational aspects of their memory. In a neurocognitive study, women with the fragile X full mutation performed worse than those with the premutation or those without fragile X on tests of executive function, spatial ability, and visual memory, but the defects seen in executive functioning were more obvious than visuospatial deficits (Bennetto, Pennington, Porter, Taylor, & Hagerman, 2001).

Males in this age group also show problems in executive functioning, which may be

associated with continued problems with working memory and planning activities (Hagerman et al., 1992). Preliminary evidence from our laboratory suggests that individuals with fragile X use a cognitive strategy that relies on verbal working-memory processing in performing a behavioral inhibition task, providing support to a hypothesis that subjects with fragile X may have the adaptive ability to overcome some executive functioning weaknesses through verbal mediation strategies (Freund & Reiss, 1991).

Summary and synthesis

In summary, both female and male children and adolescents with the fragile X full mutation are at significant risk for developing a characteristic profile of behavioral, cognitive, and emotional problems beginning in infancy that is qualitatively similar, but quantitatively different, in females and males with the full mutation. In contrast, individuals with the premutation usually show normal functioning unless repeat length is greater than 100, in which case manifestations may be highly variable. As early as 9 months, mothers of children with fragile X, usually boys, may notice that their infants show a delay in developmental milestones and abnormal tone and motor coordination. On average, delays are noticed in the infant at 24 months, but on average, children with fragile X are not diagnosed until a year later, usually because as a result of persistent speech delays or behavioral abnormalities. As boys with fragile X reach preschool age, there is significant variability in development, but overall, their rate of development ranges from one third to one half that expected for typically developing boys. Expressive language is more adversely affected than receptive language, whereas scores for motor and adaptive function are relatively higher compared with communication and cognitive functioning. The pattern of adaptive development in boys with fragile X shows significant growth from 1 to 10 years of age, gains that may be most robust in the toddler and preschool years and less marked in the school-age years. Cognitive and adaptive behavioral development slows in children with fragile X beginning as early as

5 years of age. It reaches a plateau in middle to late childhood or early adolescence, generally by age 10, as evidenced by declining IQ scores and a lack of consistent gains during these years. Beginning in the preschool years and extending into the school and adolescent years, boys with fragile X show pervasive deficits in conversational language skills with increasing discrepancy between language level and chronological age. Patterns of behavioral, social, and developmental abnormalities that emerge in preschool boys suggest that fragile X is a risk factor for autistic behavior. In particular, the presence of a nervous system that is poorly modulated (e.g., hyperarousal, problems with inhibition and habituation) may contribute to the development of the autistic "features" observed in children with fragile X.

Girls with fragile X are more variable in their development; whereas those with the full mutation may show mildly to moderately severe quantitative and qualitative abnormalities, those with the premutation are much more likely to show trajectories similar to typically developing girls. In preschool and school-age girls, the presence of social anxiety, shyness, and avoidant behavior appears to be a risk factor for the emergence of depression in late childhood and adolescence and beyond. In the school environment especially, peer neglect or rejection as well as increasing self-awareness of differences from normal children predispose girls with fragile X to poor self-esteem and various emotional problems. Our work and that of others leads us to believe that shyness and anxiety are linked and are central manifestations of the genetic "risk" from the *FMR1* mutation in females. As children with fragile X reach adolescence, cognition and adaptive behavior skills continue to decline. Executive functioning, particularly involving working memory, inhibition, and planning, also fail to develop at expected rates during the adolescent years.

Prospective longitudinal studies of large groups of children with fragile X are needed to better understand the topography of typical and atypical development in fragile X syndrome. This information is vital because (a) elucidating the biological and environmental factors that influence cognitive and behavioral

outcomes will identify areas of function sensitive to intervention; (b) obtaining precise information about development will help determine whether specific interventions lead to meaningful changes in functioning; and (c) understanding specific domains of suboptimal development may provide clues for the development of new, early interventions.

Neurological Basis of Phenotype, Behavior, and Cognition

Neuron and synapse level

In the 10 years following the discovery of the *FMR1* gene (Verkerk et al., 1991), advances in genetics and cell research have provided scientists with a wealth of information about the mechanisms of *FMR1* mutation and the functions of FMRP. Several research groups have begun to decipher the molecular signals important for *FMR1* gene expression at the neuronal level that begin to explain the range of cognitive and behavioral features of fragile X (Brown, Jin, Ceman, Darnell, O'Donnell, Tenenbaum, Jin, Feng, Wilkinson, Keene, Darnell, & Warren, 2001; Darnell, Jensen, Jin, Brown, Warren, & Darnell, 2001; Schaeffer, Bardoni, Mandel, Ehresmann, Ehresmann, & Moine, 2001; Zhang, Bailey, Matthies, Renden, Smith, Speese, Rubin, & Broadie, 2001).

FMRP function in neurons. In this section, we review what is currently known about FMRP's function at the neuronal level. How FMRP regulates normal neuronal development and its absence leads to the observed array of neurological dysfunctions continues to be an ongoing research effort (Feng, 2002).

At the neuronal level, FMRP is found primarily in the cell body, in dendrites, and in synapses (Devys, Lutz, Rouyer, Bellocq, & Mandel, 1993; Feng, Gutekunst, Eberhart, Yi, Warren, & Hersch, 1997; Weiler, Irwin, Klintsova, Spencer, Brazelton, Miyashiro, Comery, Patel, Eberwine, & Greenough, 1997). In neurons, FMRP is predominately associated with actively translating ribosomes during protein synthesis (Khandjian, Corbin, Woerly, & Rouseau, 1996). Analysis of the amino acid sequence of FMRP has revealed molecular do-

main characteristic of RNA binding proteins, suggesting that FMRP's function is involved in binding mRNAs during protein synthesis (Feng, 2002); in vitro, FMRP binds mRNA selectively and is associated with polyribosomes and the rough endoplasmic reticulum (Jin & Warren, 2000). Further, analysis of FMRP binding sites indicates that it contains both a nuclear localization signal and an export signal (Eberhart, Malter, Feng, & Warren, 1996), suggesting FMRP shuttles between cytoplasm and nucleus, possibly to transport specific mRNAs to translating ribosomes (Feng et al., 1997; Tamanini, Willemsen, van Unen, Bontekoe, Galjaard, Oostra, & Hoogeveen, 1997). FMRP's functional role as an mRNA binding protein is so critical to normal development that a de novo point mutation in one of its RNA binding sites leads to a severe form of fragile X syndrome (De Boulle, Verkerk, Reyniers, Vits, Hendrickx, Van Roy, Van den Bos, de Graaff, Oostra, & Willems, 1993).

FMRP is believed to regulate synaptic activity by transporting mRNAs transcribed from a number of other genes to neuronal dendrites in response to neural stimulation. In animals, behavioral and environmental stimulation produces elevated FMRP expression in brain neurons (Irwin, Galvez, & Greenough, 2000; Irwin, Swain, Christmon, Chakravarti, Weiler, & Greenough, 2000). Further, the localization of FMRP to dendrites and dendritic spines suggests that FMRP is involved in regulation of proteins involved in dendritic structure or function (Feng et al., 1997; Weiler et al., 1997); therefore, it is believed that FMRP plays an important role in regulating protein translation at postsynaptic sites that are critical for synaptic development and function during memory and learning (Worley, 1998).

Evidence from animal models of fragile X and in humans with fragile X indicates that during development, absent or reduced FMRP disrupts the normal adaptive formation and elimination (pruning) of dendritic spines, a process known as neuronal plasticity. In normal animals during early development or in those reared in a sensory-deprived environment, neurons exhibit abnormally long dendritic spines with immature morphologies and elevated spine numbers. Post mortem studies

in subjects with fragile X show cortical neurons with similar morphological abnormalities of dendritic processes (Hinton, Brown, Wisniewski, & Rudelli, 1991; Rudelli, Brown, Wisniewski, Jenkins, Laure-Kamionowska, Connell, & Wisniewski, 1985). A recent qualitative examination of brain autopsy tissues from both temporal and visual cortical areas of individuals with fragile X revealed significantly more immature dendritic spines that are longer and morphologically abnormal compared with brain tissues from control subjects (Irwin, Patel, Idupulapati, Harris, Crisostomo, Larsen, Kooy, Willems, Cras, Kozłowski, Swain, Weiler, & Greenough, 2001).

Additional clues for the role of FMRP in neuronal development come from animal models of fragile X. In the fruit fly *Drosophila melanogaster* knockout model of the *FMR1* homolog (*dfxr*), neurons exhibit enlarged synaptic terminals with structural defects and altered neurotransmission (Zhang et al., 2001). Also, neuronal tissue from the fragile X mouse model (*Fmr-1* knockout mice) shows more immature dendritic spines with greater spine densities and impairments in spine maturation and pruning compared with neuronal tissue from the normal mouse (Comery, Harris, Willems, Oostra, Irwin, Weiler, & Greenough, 1997).

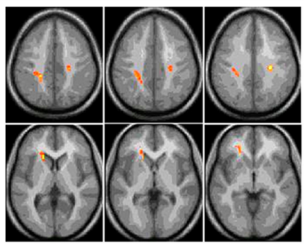
From the evidence in both animals and human, Feng recently suggested two roles for FMRP in neurons: FMRP is required for synapse formation and maturation, which is under the control of signals during development; and/or FMRP, in response to neurotransmitter release, controls the localization and/or translation of its bound mRNA(s) at postsynaptic sites (Feng, 2002). Thus, structural and functional abnormalities in the fragile X brain may result from translational misregulation of FMRP-bound mRNAs within the synapse as well as from structural synaptic defects acquired during development. How FMRP regulates those neuronal functions critical for normal development has, until recently, remained largely unknown.

FMRP targets. Discovering the specific FMRP mRNA targets important to neuronal development has been at the forefront of recent research. By identifying unique and characteris-

tic binding structures in mRNA (G-quartets), Darnell et al. found several mRNAs to which FMRP binds that are misregulated in neurons of subjects with fragile X (Darnell et al., 2001). Independently, using microarray or "DNA chip" analysis, Brown et al. identified 432 mRNAs in the mouse brain that were normally associated with FMRP; of these, 251 mRNAs also appeared in lymphoblast cells from fragile X patients (Brown et al., 2001). Of the 12 overlapping FMRP-associated mRNAs found in both experiments, 8 contained the characteristic G-quartet binding structure, and it was shown that these mRNAs were abnormally expressed and regulated in neurons of patients with fragile X. Three mRNAs were found particularly critical for normal neuronal development. One mRNA codes for microtubule-associated protein (MAP1B), which is involved in synaptic maturation through the extension of axons and dendrites; another mRNA codes for somaphorin 3F, which is critical for axon path finding; and a third mRNA codes for the protein ID3, involved in normal neuronal plasticity (Brown et al., 2001). It is interesting that one mRNA codes for the protein MINT, which affects craniofacial development, the absence of which may account for the long face and prominent forehead/jaw in patients with fragile X.

Thus, during normal development, FMRP is produced at synapses in response to synaptic activation and FMRP is increased in the brain undergoing active synaptogenesis in response to motor learning or enriched environments. In the individual with fragile X, reductions or absence of FMRP cause developmental changes at the neuronal level, chiefly abnormalities of dendritic spines, which are related by impairments in spine maturation and a failure of normal synaptic pruning. Recent evidence has implicated several important mRNA targets of FMRP in this process, including those involved in neuronal plasticity and development, synaptic maturation and axon path finding. These neurodevelopmental processes lead to both structural and functional abnormalities that can be visualized with brain imaging methodologies as discussed in the next section.

The work on *FMR1* function, FMRP, and neuronal development was the subject of re-



DTI: Prefrontal-striatal white matter tracts disrupted



Lateral Ventricle
fraX > TYP = DD
Motor/sensory, reasoning, memory, intelligence

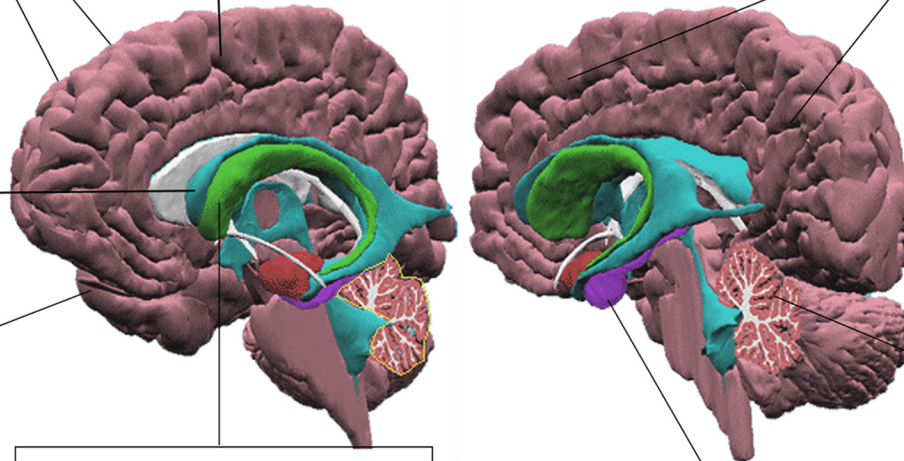


Cerebrum
fraX ≥ TYP > DD
Higher brain functions—thought and action.

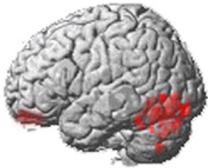
Total and prefrontal gray volume positively correlated with IQ in TYP not in fraX



Math, Visuo-Spatial WM, Exec. Funct.: Prefrontal, Parietal activation differences



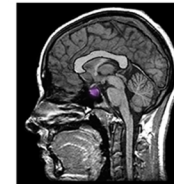
Face, Gaze Processing: Superior temporal gyrus Activation



Sup. temporal gyrus structure: fraX < TYP
Important for language function face and gaze processing



Caudate Structure: fraX >> TYP > DD
Attention and working memory
Motor
Mood
Impulse control
Response to environmental cues
Somatosensory
Eye movement



Hippocampus Structure: fraX > TYP; fraX > DD
Learning and memory
Episodic memory
New associations
Visual and language-related
Spatial problem solving
Detection of novel stimuli



Cerebellar Vermis Structure: FraX < TYP = DD
Visual-spatial processing
Implicit/procedural learning
Perceptual timing
Attention/executive function
Higher-order language

cent reviews (Churchill, Grossman, Irwin, Galvez, Klintsova, Weiler, & Greenough, 2002; Irwin et al., 2001; Kaytor & Orr, 2001; O'Donnell & Warren, 2002; Oostra, 2002; Oostra & Chiurazzi, 2001).

Neural systems level

Over the past 15 years, a series of brain imaging studies in our laboratory have consistently established links between reduced or absent FMRP and abnormalities of specific neuroanatomical regions in subjects with fragile X (primarily school-age children or older) as compared to IQ matched (non-fragile X) and typically developing subjects (Eliez, Blasey, Freund, Hastie, & Reiss, 2001; Kates, Abrams, Kaufmann, Breiter, & Reiss, 1997; Mostofsky, Mazzocco, Aakalu, Warsofsky, Denckla, & Reiss, 1998; Reiss, Abrams, Greenlaw, Freund, & Denckla, 1995; Reiss, Aylward, Freund, Joshi, & Bryan, 1991; Reiss, Freund, Tseng, & Joshi, 1991; Reiss, Lee, & Freund, 1994; Reiss, Patel, Kumar, & Freund, 1988). Figure 3 shows a compilation of our findings from this neuroimaging research. Our studies and others have described abnormalities such as increased cerebral and ventricular volumes (Eliez et al., 2001; Reiss, Abrams, et al., 1995; Reiss, Abrams, Singer, Ross, & Denckla, 1996; Reiss, Freund, et al., 1991; Schapiro et al., 1995; Wisniewski, Segan, Mizejeski, Sersen, & Rudelli, 1991), enlarged caudate nucleus (Reiss, Abrams, et al., 1995; Reiss, Freund, Baumgardner, Abrams, & Denckla, 1995) and hippocampal volumes (Kates et al., 1997; Reiss, Aylward, et al., 1991), and decreased cerebellar vermis area (Mazzocco, Kates, et al., 1997; Mostofsky et al., 1998; Reiss, Aylward, et al., 1991; Reiss, Freund, et al., 1991) and superior temporal gyrus volume (Reiss, Lee, & Freund, 1994). Further, diffusion tensor imaging (DTI)

neuroimaging evidence shows that prefrontal–striatal white matter connectivity is disrupted in the brain of individuals with fragile X (Barnea-Goraly et al., 2002), and that prefrontal–parietal areas show activation differences during math computation (Menon, Rivera, White, Glover, & Reiss, 2000; Rivera, Menon, White, Glaser, & Reiss, 2002), and working memory tasks (Kwon, Menon, Eliez, Warsofsky, White, Dyer–Friedman, Taylor, Glover, & Reiss, 2001; Menon, Kwon, Eliez, Taylor, & Reiss, 2000). Taken together, these neuroimaging findings implicate disruptions in specific neural systems in individuals with fragile X that are associated with the neurobehavioral and neurocognitive phenotype.

Structural neuroimaging: Magnetic resonance imaging (MRI)

Caudate. Both females and males with fragile X show highly significant increases in caudate nucleus volume compared to IQ and healthy controls (Reiss, Abrams, Greenlaw, Freund, & Denckla, 1995; Reiss, Freund, et al., 1995). In a more recent MRI study in both children and adolescents with fragile X, Eliez also found that both males and females had significantly larger caudate volumes than controls, although males with fragile X had significantly larger caudate volumes than females with fragile X (Eliez et al., 2001). This finding supports the hypothesis that females represent an intermediate status of the *FMR1* gene mutation on brain development.

The caudate is a subcortical nucleus that functions as a component of neural systems through which the cerebral cortex affects behavior. Although well known for involvement in movement, the caudate nucleus also is believed to play an important role in cortical–subcortical loops related to emotion and cognition via connections with nonmotor areas of the frontal cortex, including the dorsolateral

Figure 3. The compilation of structural and functional MRI findings from our neuroimaging laboratory (prior to 2000) showing brain regions altered in subjects with fragile X compared with controls. DD, individuals with developmental disability comparable to fragile X (fraX); TYP, typically developing individuals. Brain image copyright the Digital Anatomist, University of Washington. The three-dimensional reconstructions of the right hemisphere and deep structures are used with permission of the Digital Anatomist Project at the University of Washington.

prefrontal, medial and lateral orbitofrontal, and anterior cingulate regions (Cummings, 1993; DeLong, 2000; Masterman & Cummings, 1997). Disturbances of these frontal-subcortical circuits are known to produce disturbance in attention and spatial working memory, motor programming/movement selection, regulation of mood, social behavior, impulse control, and flexibility in behavioral response to environmental cues (Cummings, 1993; Masterman & Cummings, 1997). Thus, disruption of circuits involving prefrontal-striatal connections would be consistent with some of the cognitive and behavioral abnormalities observed in fragile X: attention deficit, hyperactivity, stereotypic and perseverative language and motor behavior, and problems with impulse control (Abrams, Reiss, Freund, Baumgardner, Chase, & Denckla, 1994; Reiss, Abrams, et al., 1995). An important finding is that in control subjects, larger caudate is associated with higher IQ, whereas in the subjects with fragile X, larger caudate is correlated with lower IQ (Reiss, Abrams, et al., 1995). This suggests that the developmental process leading to increased caudate volume in subjects with fragile X reflects aberrant neural organization.

Hippocampus. We previously reported increased hippocampal volume from two independent investigations in our laboratory (Kates et al., 1997; Reiss et al., 1994). The volume changes were greater in subjects with fragile X than normally developing controls or control subjects with developmental delay but without fragile X. The hippocampus is involved in encoding and retrieving episodic memories and encodes and consolidates visual and language-related associations. The hippocampus also is used in spatial problem solving and movement-related cues to guide spatial behavior. Further, this structure is involved in the detection of novel stimuli in a developing child.

Superior temporal gyrus. Age-related decreases in superior temporal gyrus volume have been reported in individuals with fragile X (Reiss, Aylward, et al., 1991), an area important in processing complex auditory and language stimuli. The superior temporal re-

gion also is implicated in the interpretation of faces and gaze (Campbell, Heywood, Cowey, Regard, & Landis, 1990; Hoffman & Haxby, 2000; Wicker, Michel, Henaff, & Decety, 1998), and alterations in this region and structures associated with the face/gaze neural circuit may be associated with the observed behavior of gaze aversion in subjects with fragile X as described below.

Cerebellar vermis. An early neuroimaging study from our laboratory (Reiss, Patel, et al., 1988) showed a significantly decreased posterior cerebellar vermis and pons and increased fourth ventricular area in subjects with fragile X, compared with control subjects. We theorized that vermis alterations could account for some of the behavioral and cognitive abnormalities observed in males with fragile X, particularly those overlapping with autism. In a further investigation of subjects with fragile X compared with males with other causes of developmental disability and normally developing males, the finding of a significantly decreased posterior cerebellar vermis volume and increased fourth ventricle volume was replicated in subjects with fragile X compared with males in the comparison groups (Reiss, Aylward, et al., 1991). This result was further confirmed in a study of young females with fragile X who were compared with age- and IQ-matched, typically developing females (Reiss, Freund, et al., 1991). Results from other laboratories using smaller numbers of subjects have replicated our findings of increased brain volumes (Schapiro et al., 1995) and decreased cerebellar vermis size (Guerreiro, Camargo, Kato, Marques-de-Faria, Ciasca, Guerreiro, Netto, & Moura-Ribeiro, 1998).

The cerebellar vermis is normally involved in processing sensory information (Rao, Mayer, & Harrington, 2001) and modulating attention, emotion, and coordinating movement; it also may play a role in language (Bobee, Mariette, Tremblay-Leveau, & Caston, 2000; Critchley, Corfield, Chandler, Mathias, & Dolan, 2000; Levisohn, Cronin-Golomb, & Schmahmann, 2000; Mostofsky et al., 1998; Parsons, Denton, Egan, McKinley, Shade, Lancaster, & Fox, 2000; Richter, Lee, & Pardo, 2000; Riva & Giorgi, 2000).

White matter connectivity in fragile X—DTI. Given its role in the regulation of multiple brain proteins, reduction in FMRP is likely to influence brain structure and function via different mechanisms. For example, recent evidence has shown that one of the proteins that is regulated by FMRP is involved in axon path finding (Brown et al., 2001; Darnell et al., 2001), a process that has direct influences on axonal directionality, cohesiveness, and connectivity. To study the integrity of white matter tracts in subjects with fragile X, we used DTI, a recently developed MRI technique enabling us to visualize and measure the orientation of white matter tracts in vivo (Basser & Pierpaoli, 1996; Pierpaoli, Jezzard, Basser, Barnett, & Di Chiro, 1996). Using this technique, we studied 10 females with fragile X and 10 age-matched healthy females (Barnea-Goraly et al., 2002).

Compared with controls, subjects with fragile X showed lower fractional anisotropy (FA) values, mostly in frontal–striatal and parietal sensorimotor tracts. FA is a commonly employed metric in DTI studies as it is correlated with the directional coherence of white matter fiber tracts. In conjunction with our MRI findings of abnormal caudate volumes in fragile X and functional MRI (fMRI) studies showing abnormalities in prefrontal areas (discussed below), these DTI findings support a hypothesis that dysfunctional prefrontal–striatal (caudate) networks underlie some of the neurocognitive and neurobehavioral deficits in fragile X syndrome. The finding of abnormal white matter tracts leading to the parietal lobes is relevant to the observed cognitive weaknesses in arithmetic reasoning and visuospatial processing in subjects with fragile X. Thus, aberrations in white matter in subjects with fragile X as detected with DTI suggests that reduced or absent FMRP disrupts axon directionality and coherence, possibly due to misregulation of protein(s) by FMRP in neurons (e.g., MAP 1B, related to axon extension and semaphorin 3F, related to axon path finding) as well as by disruption of dendrite maturation. Because synapse formation is concurrent with dendrite and axon growth beginning in early infancy (Huttenlocher & Dabholkar,

1997), longitudinal DTI and MRI studies in infants and young children with fragile X will be critical in helping us elucidate the plasticity and development of frontal–striatal and parietal networks in fragile X syndrome.

Functional neuroimaging with fMRI. Although we can offer hypotheses implicating dysfunction of brain regions seen from these structural studies to account for the pathogenesis of neurobehavioral abnormalities in individuals with fragile X, we must confirm the link between neuroanatomy and neurobehavior with studies of brain function. Because fragile X is a disorder whose initial manifestations are observed in infancy, knowledge of early variation in functional neuroanatomy is critical to improve our knowledge of specific gene–brain–behavior linkages in this condition. Yet, as of 1995, only one functional (positron emission tomography) brain study had been carried out in fragile X using a limited sample (Schapiro et al., 1995). In the intervening years, we have published results from several studies in fragile X using fMRI (Kwon et al., 2001; Menon, Kwon, et al., 2000; Rivera et al., 2002; Tamm, Menon, Johnston, Hessel, & Reiss, 2002) and several others are currently in process. These studies are described below and summarized in Table 1. The data from these fMRI studies and preliminary results from our laboratory have been invaluable in helping us elucidate the neurodevelopmental pathways that underlie disruption of brain function in fragile X.

Arithmetic reasoning/computation. Among the fundamental cognitive deficits seen in children with fragile X, problems in arithmetic reasoning and computation are well documented (Bennetto et al., 2001; Curfs, Borghgraef, Wiegers, Schreppers–Tijdink, & Fryns, 1989; Dykens, Hodapp, & Leckman, 1989; Kemper, Hagerman, Ahmad, & Mariner, 1986; Kemper, Hagerman, & Altshul–Stark, 1988). Accordingly, brain activation during arithmetic processing in subjects with fragile X was studied using fMRI (Rivera et al., 2002). Females with fragile X were compared with normally developing control females who attempted to solve arithmetic equations with

Table 1. Summary of functional MRI studies in fragile X (FX)

Brain Function	Principal Findings/Conclusions	References
Arithmetic processing	<p>Unlike controls, subjects with FX could not modulate brain activation in response to increasing difficulty of the math task.</p> <p>In the control group, brain activation increased in parietal, prefrontal, and cerebellar regions as a function of task difficulty. (FX increased only in prefrontal regions.)</p> <p>In the FX group, <i>FMRI</i> protein (FMRP) level was positively correlated with brain activation for the three-operand task in (math-related) prefrontal and parietal regions.</p>	Menon, Rivera, et al. (2000); Rivera et al. (2002)
Working memory, visuospatial	<p>Typical controls subjects show activation of the prefrontal cortex with additional regions recruited for the two-back condition. FX subjects show scattered prefrontal and parietal cortex activation in the one-back task; activation is not increased during the two-back condition.</p> <p>Correlation between FMRP levels and brain activation provides direct evidence between gene expression and cognition.</p>	Kwon et al. (2001); Menon, Kwon, et al. (2000)
Executive function	<p>In performing cognitive interference tasks, females with FX demonstrate a markedly different pattern of activation than controls.</p> <p>Compared with controls, FX subjects have longer reaction times and adopt a strategy trading speed for accuracy. Females with FX had reduced activation in brain regions important for modulating goal-directed behavior.</p> <p>Deficits in cognitive interference processing during cognitive interference may arise from inability to recruit and modulate lateral prefrontal and parietal resources.</p>	Tamm et al. (2002)

two ($1 + 3 = 4$) or three ($2 + 3 - 1 = 4$) operands. Subjects with fragile X showed significant impairment in behavioral performance on the three-operand but not the two-operand arithmetic equations. Significant brain activation was observed bilaterally in the prefrontal and parietal cortices for unaffected subjects and in the bilateral prefrontal and left angular gyrus for subjects with fragile X, for both trial types. Subjects with fragile X exhibited less overall activation than did unaffected subjects in both types of trials; and, unlike the unaffected group, they did not show increased extent of activation in association with greater task difficulty. During the three-operand trials, activation in bilateral prefrontal and motor/premotor, and left supramarginal and angular gyri were positively correlated with FMRP, suggesting that decreased *FMRI* protein expres-

sion underlies deficits in math performance in persons with fragile X and that reduction in this critical neuronal protein impedes the normal process of neural recruitment associated with tasks of increasingly difficulty.

Working memory. As we have seen, problems with working memory have been reported to be an important component of cognitive dysfunction in fragile X syndrome (Cornish, Munir, & Cross, 2001; Munir et al., 2000a). To understand the neurological foundations of working memory in fragile X, we used fMRI to study females with fragile X and typically developing females who performed standard one-back and two-back working memory tasks (Kwon et al., 2001; Menon, Kwon, et al., 2000). Compared with controls, subjects with fragile X showed significantly reduced performance on the two-back test but

not the one-back test. Whereas control subjects showed increased brain activation between the two working memory tasks, subjects with fragile X showed no change in activation between the two tasks. Significant positive correlations were found in control subjects between frontal and parietal activation and performance (as percent correct) on the two-back task but not on the one-back task. However, in subjects with fragile X, significant positive correlations were found during the two-back task between FMRP expression and activation in prefrontal and supramarginal gyri (Table 2). Thus, subjects with fragile X syndrome are unable to modulate activation in prefrontal and parietal cortex in response to an increasing working memory load, and these deficits are related to a lower level of FMRP expression.

Executive function: Cognitive interference.

To study functional neuroanatomical changes during cognitive interference, we used a variant of the Stroop interference task, in which processing of one stimulus interferes with the simultaneous processing of another (e.g., the word BLUE printed in red ink; Tamm et al., 2002). Compared with controls, females with fragile X had longer reaction times during the interference condition of this task, and adopted a strategy trading speed for accuracy. Compared to females with fragile X, controls showed more activation in the anterior cingulate gyrus, frontal–striatal circuits, left and right supramarginal gyri, left and right posterior hippocampus, and cerebellar vermis. However, subjects with fragile X showed more activation in the left middle and inferior frontal gyri as well as the right angular gyrus. Further, between-group analyses revealed that females with fragile X had reduced activation in the left orbitofrontal gyrus, thought to be involved in modulating goal-directed behavior. Overall, these findings suggest that deficits in cognitive interference processing during a Stroop-like task in females with fragile X may arise from their inability to appropriately recruit and modulate prefrontal and parietal resources.

Gaze aversion. As we have seen, socially mediated gaze aversion is one of the more common behavioral features in children and

adults with fragile X syndrome (Bregman, Leckman, & Ort, 1988; Cohen, Vietze, et al., 1989; Cohen, Vietze, Sudhalter, Jenkins, & Brown, 1991; de Vries, van den Ouweland, Mohkamsing, Duivenvoorden, Mol, Gelsema, van Rijn, Halley, Sandkuijl, Oostra, Tibben, & Niermeijer, 1997; Einfeld, Molony, & Hall, 1989; Wolff et al., 1989). Although many hypotheses have been suggested to explain why subjects with fragile X avoid eye contact (e.g., hyperarousal, anxiety, shyness), the neural basis of this behavior has not been studied. Preliminary evidence from our laboratory results suggest that gaze aversion may be partly related to reduced brain activation in subjects with fragile X. In response to face and gaze information, individuals with fragile X have normal or slightly increased levels of activation in brain regions involved in interpreting gaze direction in a social context but deficient activation in brain areas associated with gaze processing, chiefly the superior temporal sulcus, an area involved in interpreting gaze direction in a social context.

Summary and synthesis

Converging evidence from molecular studies with subjects with fragile X and from fragile X animal models has shown how reductions in FMRP lead to the complex sequences of molecular events resulting in suboptimal cognitive performance. FMRP functions an mRNA binding protein, transporting messenger ribonucleoprotein complexes between nucleus and cytoplasm of the neuron. These FMRP-associated mRNAs, which have been identified as important to neuronal plasticity and development, synaptic maturation, and axon pathfinding, translate ribosomes in dendrites during critical developmental periods of activity-dependent synaptic function, maturation, and plasticity. When FMRP levels are reduced or absent, as occurs in fragile X or the mouse and fruit fly knockout models of this condition, abnormal morphologies of cortical dendritic processes are observed. The resultant disorganization in neuronal circuitry of subjects with fragile X produces the observable profile of cognitive, emotional, and behavioral abnormalities in this disorder. Determin-

Table 2. Correlations between genetic, environmental, and neurobiological functions in fragile X (FX) syndrome

Variable	IQ Measures	Brain Volume	Brain Function	Behavior
FMRP levels	↓ FMRP, ↓ IQ in M and F (fM) with FX ^{a-d}	↓ FMRP, ↓ cerebellar vermis in M and F (fM) with fM ^e ↓ FMRP, ↑ caudate nucleus in M and F with fM and ↑ lateral ventricle in M with fM; caudate and lateral ventricle volumes correlate negatively with IQ ^f	Math processing: ↓ FMRP, ↓ activation of bilateral prefrontal cortex and L supramarginal + angular gyri in F with fM ^g Working memory tests: ↓ FMRP, ↓ activation R inferior and bilateral middle frontal gyri + bilateral supramarginal gyri in F with fM ^{h,i}	↓ FMRP, ↑ social withdrawal, anxious/depressed behavior in F with fM ^j ↓ FMRP, ↑ distractibility in F with fM ^b No correlations with adaptive behavior in M, F with fM ^k
CGG repeat length (RL)	No correlations in M or F with fM ^{d,l,m}			↑ >100 RL, ↑ scores on Interpersonal Sensitivity and Depression Subscales (SCL-90-R) in F with pM; no correlation for ≤100 RL in F with pM ^l ↑ RL, ↑ attention problems, anxiety/withdrawal in F with fM ⁿ
Mean parent IQ (MPIQ)	↑ MPIQ, ↑ IQ in F with fM ^d ↑ MPIQ, ↑ performance IQ in M with fM, and IQ index scores in F with fM ^b			
Quality of home environment (QH)	↑ QH, ↑ in F with fM and typically developing F ^{b,o}			↑ QH, ↓ autistic behaviors in M with fM ^j ↑ QH, ↑ adaptive behavior in M with fM ^k

Note: The FMRP level is a direct measure of the peripheral *FMR1* protein levels. Activation R, the proportion of active (unmethylated, FMRP producing) *FMR1* genes to total *FMR1* genes, is highly correlated with FMRP and is combined under FMRP levels; fM, FX full mutation; pM, FX premutation; F, females; M, males; L, left; R, right; ↑ increased; ↓ decreased.

^aKaufmann et al. (1999). ^bDyer–Friedman et al. (2002). ^cAbrams et al. (1994). ^dReiss, Freund, et al. (1995). ^eMostofsky et al. (1998). ^fReiss, Abrams, et al. (1995). ^gRivera et al. (2002). ^hMenon, Kwon, et al. (2002). ⁱKwon et al. (2001). ^jHessl et al. (2002). ^kGlaser et al. (2002). ^lJohnston et al. (2001). ^mMazzocco et al. (1997). ⁿFreund et al. (1993). ^oKuo et al. (2002).

ing how these changes at the neuronal and tissue level are manifested in structure and function of the brains from subjects with fragile X is a major objective of our neuroimaging research.

Structural MRI studies have begun to localize neuroanatomical differences seen in individuals with fragile X. Although no differences have been observed in brain symmetry or in neocortical lobe volumes in subjects with fragile X, both males and females with the disorder show anatomical abnormalities of several brain regions. Notably, significant volume increases are seen in the caudate nucleus and hippocampus, and decreases are seen in superior temporal gyrus and cerebellar vermis. These structural findings, particularly those seen in the caudate, are robust, and suggest correlations with neurobehavior and neurocognition in fragile X. Abnormalities on DTI, particularly in prefrontal–caudate pathways, suggest developmental abnormality leading to aberrant neural connectivity during development that may need to be overcome to establish “normal” function in fragile X. The abnormalities in white matter tracts may be related to FMRP’s function in regulating axonal path finding.

Functional imaging results suggest that, although individuals with fragile X are generally activating appropriate brain regions during cognitive processing, unlike controls, they cannot recruit the additional resources “on demand” in response to increasing task difficulty. The functional deficits are found to correlate with the level of FMRP expression (higher FMRP levels were associated with more normal brain activation). These studies may provide a metric for measuring responses to new treatments for fragile X.

Further functional and structural neuroimaging studies from our neuroimaging laboratory in subjects with fragile X are underway; our goal is to demonstrate a statistically significant association between the specific genetic marker of fragile X and brain activation and to understand more precisely the timing and nature of these neurobiological disruptions during early development in children with fragile X.

Environmental and Biological Interactions Influencing Outcomes

As we have seen, studies over the past two decades have established that children with fragile X are at increased risk for the development of a relatively specific profile of cognitive, emotional, and behavioral abnormalities. It is also recognized that considerable individual variability exists in the severity of these abnormalities, and therefore, it is important to elucidate the full range of this variability and to identify factors, other than reduced FMRP, that contribute to phenotypic variation in children with fragile X (Finegan, 1998). *FMRI* is only one gene and, accordingly, the general genetic background of the individual plays a significant role in influencing outcomes (Reiss, Freund, et al., 1995). Certainly, environmental risk factors such as home and school environment also influence development and outcomes of children with fragile X, and studies designed to elucidate how functional outcomes are moderated and mediated by risk factors such as family and educational environments as well as neural function are vital to understanding and optimizing development in children with fragile X.

Research attempting to identify factors that influence outcome in fragile X has been limited to examining associations between cognitive or behavioral function and genetic variables such as mutation category or direct and indirect measures of *FMR1* expression in blood (Abrams et al., 1994; Bailey, Hatton, Tassone, et al., 2001; Kaufmann, Abrams, Chen, & Reiss, 1999; Mazzocco, Sonna, Teisl, Pinit, Shapiro, Shah, & Reiss, 1997; Reiss, Freund, et al., 1995; Rousseau, Heitz, et al., 1994; Tassone, Hagerman, Ikle, Dyer, Lampe, Willemssen, Oostra, & Taylor, 1999; Tassone, Hagerman, Taylor, Mills, Harris, Gane, & Hagerman, 2000; Taylor, Safanda, Fall, Quince, Lang, Hull, Carpenter, Staley, & Hagerman, 1994). Studies focused on cognitive function have generally shown that a small to moderate proportion of intellectual ability in children with fragile X can be predicted by these genetic variables. A study of the association between FMRP and behavior in fragile X (Tas-

sone, Hagerman, Ikle, et al., 1999) showed a negative correlation between FMRP and 10 behaviors associated with fragile X, but only in males with mosaicism.

However, at present, it is apparent that genetic measures are necessary, but not sufficient, to explain variation in cognitive, emotional, and behavioral outcome in children with fragile X. There are several reasons why genetic measures may be limited in predicting outcomes in children with fragile X syndrome. First, the most commonly used genetic measures are based on tissues of mesodermal origin (usually blood leukocytes), while relatively inaccessible brain tissue is of ectodermal origin (Abrams, Kaufmann, Rousseau, Oostra, Wolozin, Taylor, Lishaa, Morel, Hoogeven, & Reiss, 1999; Tassone, Hagerman, Gane, & Taylor, 1999; Willemsen, Anar, De Diego Otero, de Vries, Hilhorst-Hofstee, Smits, van Looveren, Willems, Galjaard, & Oostra, 1999). Also, leukocytes rapidly and continually turn over throughout an individual's life span, whereas neurons do not generally turn over after birth. If having increasing FMRP production confers functional advantage to leukocyte progenitors, then individuals who are mosaic for different *FMR1* mutation types (e.g., premutation and full mutation lines, varying allele sizes, and/or methylation) may manifest increasing "selection" of more "fit" cell lines over time (Mornet, Jokic, Bogyo, Tejada, Deluchat, Boue, & Boue, 1993; Rousseau, Heitz, Oberle, & Mandel, 1991; Rousseau, Robb, Rouillard, & Der Kaloustian, 1994). In our ongoing work, we are attempting to characterize this process and understand how it relates to age and mutation type.

It also is highly likely that a significant proportion of the variance in cognitive, emotional, and behavioral function in children with fragile X is attributable to other biological influences (genes whose expression are regulated by FMRP or the hundreds or thousands of other genes that regulate brain maturation and function) or environmental influences such as the prenatal and postnatal environment. For example, as with most neuropsychiatric or developmental disorders, psychological characteristics of the parents, in-

cluding a background genetic predisposition to learning problems or psychopathology (independent of the *FMR1* mutation), may contribute to cognitive and behavioral variation in children with fragile X (Jeffries, Reiss, Brown, Meyers, Glicksman, & Bandyopadhyay, 1993; Reiss, Freund, et al., 1995). Examination of the home and school environment also may yield important information about nongenetic influences on child cognitive and behavioral function and outcome. Although developing specific, biological treatments to prevent or reverse the deleterious effects of fragile X is a common goal in the field, identifying and measuring environmental influences (e.g., effectiveness of educational or therapeutic services, characteristics of parents) will help us develop more effective temporally proximate interventions. Even when specific biological treatments become available, reduced FMRP in the brain affects early development when the nervous system undergoes its most rapid growth and maturation and therefore reduction of cognitive/behavioral symptoms may be less successful in older individuals. Thus, knowledge of early environmental influences on outcome is likely to have long-term benefit to individuals and families affected by fragile X.

Influences on cognitive, behavioral, and emotional outcomes

Cohort and methods. To study cognitive, behavior, and emotional outcomes, we conducted in-home evaluations of 120 children (aged 6–17 years) with fragile X (40 girls, 80 boys; mean age = 10.7 years), their unaffected siblings (62 girls, 58 boys; mean age = 11.2 years), and their parents. Independent predictor values were (a) *biological/demographic* variables, including age, gender, FMRP levels, and mean parental IQ; and (b) *environmental* variables, including family income, quality of the home environment (Wechsler, 1991), parental psychopathology (Derogatis, 1994), and effectiveness of educational and therapeutic services (Dyer-Friedman, Glaser, Hessel, Johnston, Huffman, Taylor, Wisbeck, & Reiss, 2002). The principal dependent variables included WISC-III IQ and index scores (Wech-

sler, 1991), Vineland Adaptive Behavior Scale (Sparrow, 1984), domain scores and scores from the CBCL checklist (Achenbach, 1991), and the Autism Behavior Checklist (Krug et al., 1993). The results from the studies using this cohort are described below (Dyer-Friedman et al., 2002; Glaser et al., 2002; Hessler, Dyer-Friedman, Glaser, Wisbeck, Barajas, Taylor, & Reiss, 2001; Kuo, Reiss, Freund, & Huffman, 2002).

Cognitive outcome. Using this cohort of 120 families, Dyer-Friedman et al. (2002) measured the genetic and environmental factors influencing the cognitive outcomes in children with fragile X. Girls with fragile X, on average, performed in the borderline intellectual functioning range for full scale IQ ($M = 75.48$), whereas boys with fragile X performed in the moderate mental retardation range ($M = 46.35$, floor effect in 43%). Girls with fragile X in this study demonstrated relative strengths in verbal domains. However, there was evidence of an age-related decline in FSIQ and verbal skills for boys with fragile X in this cross-sectional sample. Multiple regression analyses showed that the cognitive outcomes for girls with fragile X were most strongly predicted by the mean IQ of their parents with a small proportion of the variance accounted for by the quality of their home environment. FMRP was associated with girls' levels of distractibility. Mean parental IQ was associated with boys' performance IQs, whereas FMRP was associated with boys' full scale IQs. The quality of boys' home environments accounted for more of the variance in their cognitive outcomes than it did for girls. Thus, both biological/genetic factors and environmental factors were significant predictors of IQ in children with fragile X syndrome; however, the influence of specific factors differed between girls and boys.

Adaptive behavior outcome. Using the cohort described above, Glaser et al. (2002) studied how biological and environmental factors influenced the development of adaptive behavior in children with fragile X. Boys with fragile X had Vineland domain scores in the low range whereas girls with fragile X scored in

the moderately low to low range. For boys with fragile X, older age and lower IQ predicted decreased Composite, Communication, and Socialization (standardized) Vineland domain scores, supporting the hypothesis that the rate of growth of adaptive behavior skills declines in school-age boys with fragile X. As was found in cognitive outcome factors, the quality of the home environment also was related to increased domain scores in boys with fragile X: a better quality of home environment translated to higher scores on adaptive behavior testing. For girls with fragile X, adaptive behavior was most strongly associated with IQ. Adaptive behavior was not significantly associated with FMRP in either boys or girls with fragile X (see Table 2). These results provide the first evidence that both biological and environmental factors contribute significantly to adaptive behaviors development in typically developing sibling controls and boys with fragile X.

Emotional and behavioral outcome. Hessler et al. measured the influence of environmental and genetic factors on behavior problems and autistic symptoms in boys and girls with fragile X syndrome with this cohort (Hessler et al., 2001). Generally, both boys and girls with fragile X exhibited social, attention, and thought problems in the borderline to clinical range on the CBCL. Boys with fragile X had moderate levels of autistic behavior similar to those of a sample of children with severe mental retardation but well below that of children diagnosed with autism. Mild levels of autistic behavior were seen in girls with fragile X with as much variability as boys.

In this study, behavior problems in boys with fragile X were consistently associated with environmental factors, but not with FMRP or IQ. Specifically, maternal reports of more effective educational and therapeutic services were associated with fewer behavioral problems and autistic symptoms, whereas parental psychopathology was significantly associated only with internalizing problems. Autistic behaviors increased linearly in boys with fragile X as the quality of their home environment decreased.

In contrast to boys with fragile X, genetic,

rather than environmental, factors were associated with behavior problems in girls with fragile X. Although FMRP was more strongly associated with internalizing types of problems, IQ was more strongly associated with externalizing behavioral problems, which decreased linearly as levels of FMRP decreased. Overall, IQ and FMRP accounted for 34% of the variance in total behavior problems among girls with fragile X. In contrast to boys with fragile X, for the most part, genetic rather than environmental factors were associated with behavior problems in girls. Although FMRP was more strongly associated with internalizing types of problems, IQ was more strongly associated with externalizing problems. Finally, IQ was the only significant predictor of autistic behavior in girls with fragile X, accounting for approximately 33% of the variance.

In girls with fragile X, FMRP was significantly associated with withdrawn and anxious/depressed behavior, but not with social, attention, or thought problems on the CBCL. Also, increased effectiveness of therapeutic services was associated with girls' decreased attention and thought problems. Boys with fragile X did not change results pertaining to FMRP such that FMRP was not associated with any behavioral scores. In boys, the more effective educational and therapeutic services were significantly correlated with less withdrawn behavior, less anxious/depressed behavior, and fewer attention and thought problems, a result similar to that seen in girls with fragile X.

These findings are among the first to link FMRP expression to behavior and further take into consideration emphasize the importance of home and school environments in influencing behavior in children with fragile X. The results highlight several points at which intervention might be effective. First, the association between the effectiveness of educational and therapeutic services and behavioral outcome indicates that from the mother's perspective, the fit between the child's developmental needs and the services he or she receives is important. Second, parental psychopathology was associated with behavior problems in children with fragile X and their

siblings, so counseling may be useful. Third, home and educational environment strongly influence autistic behaviors in boys with fragile X such that a more structured, enriched home environment and targeted behavioral intervention may reduce these behaviors.

Cortisol and behavior. Despite the relatively consistent links between *FMR1* gene function and outcomes in children with fragile X, there is still considerable variability in stress-related behavioral problems, ranging from high levels of distress, often in novel social situations, to normal functioning. As we have seen, this variability can be partly explained by nongenetic factors, such as characteristics of the home environment and the effectiveness of educational and therapeutic services (Hessl et al., 2001). However, other individual characteristics of children or the environments in which they live may help to better account for these individual differences, leading to more effective methods of assessment and treatment of stress-related symptoms.

One such characteristic, the function of the hypothalamic–pituitary–adrenal (HPA) axis, may help to explain some of the variability in stress-related symptoms among children with fragile X. Reports of endocrine abnormalities in children with fragile X (Bregman, Leckman, & Ort, 1990; Butler & Najjar, 1988; Loesch, Huggins, & Hoang, 1995), observations of neurobehavioral features such as hyperarousal and social anxiety, and evidence of neuroanatomical abnormalities such as hippocampus enlargement (Kates et al., 1997; Reiss, Freund, et al., 1995), suggests that an abnormal HPA axis function may be a component of the fragile X syndrome. Specifically, children with fragile X have a higher incidence of precocious puberty and elevated gonadotrophin levels (Butler & Najjar, 1988; Moore, Chudley, & Winter, 1990), and experience less pubertal growth than normal children, despite normal prepubertal growth (Loesch et al., 1995). This abnormal growth pattern may be due to a premature activation of the HPA in children with fragile X.

Based on the well-characterized profile of autonomic and behavioral overreactivity and hyperarousal observed in our studies and those

of others, we hypothesized that children with fragile X would have higher levels of the adrenal hormone, cortisol, in comparison to their unaffected siblings, and accordingly, we investigated the extent of abnormal activation of the HPA axis in children with fragile X and its relevance to neurobehavioral and neuroanatomical abnormalities (Hessl et al., 2002). In this study, 109 children (70 males and 39 females) and their unaffected siblings (51 males and 58 females) completed an in-home evaluation including a cognitive assessment and structured social challenge task. Multiple samples of salivary cortisol were collected throughout the *evaluation day* (including pre- and postsocial challenge) and on two *typical*, nonschool days. Measures of *FMRI* gene, child intelligence, the quality of the home environment, parental psychopathology, and the effectiveness of educational and therapeutic services also were measured. Regression analyses were conducted to determine whether adrenocortical activity was associated with behavioral problems after controlling for significant genetic and environmental factors.

Both the fragile X and sibling groups exhibited the expected diurnal decline in cortisol. On *typical days*, a significant main effect of diagnosis and a diagnosis by gender interaction showed that, in comparison with their siblings, children with fragile X, especially males, have higher levels of salivary cortisol. On *evaluation days*, children with fragile X showed increased cortisol reactivity during cognitive evaluation and when meeting research staff. We found no correlation between cortisol level and IQ within the fragile X group. Increased cortisol was significantly associated with behavioral problems in boys and girls with fragile X but not in their unaffected siblings, suggesting that the HPA axis may have an independent association with behavioral problems in children with this disorder. These findings replicate and extend previous results from our lab obtained from a different, smaller sample of children (Wisbeck et al., 2000) in which highly significant family effects on salivary cortisol were detected.

Predictors of behavior problems included child intelligence, *FMRI* gene function, and adrenocortical activity, as well as parental

psychopathology, the home environment, and the effectiveness of educational and therapeutic services. It is interesting that the level of salivary cortisol predicted as much, or more of the variance in behavior problems as the level of protein expressed by the *FMRI* gene. Thus, the results highlight many sources of individual differences in behavior problems among children with fragile X, suggesting that multidimensional assessment may be necessary to best predict the outcomes of individual affected children. However, a large proportion of the variance in behavior problems of children with fragile X, especially boys, remains unexplained. Unknown characteristics of children and their families may be influential. The use and effectiveness of medication, parenting practices, the presence or absence of other siblings affected by fragile X, and other biological or genetic factors also may be associated with the frequency and severity of behavioral and psychiatric problems in these children.

In summary, the scope and quality of information collected in these studies provides a unique opportunity to understand the developmental trajectory of cognitive, adaptive behavior, and emotional/behavioral domains as well as more precisely elucidate those environmental and biological factors that most influence outcomes in children with fragile X.

How the Study of Fragile X Can Inform Developmental Theory

During the past 25 years, researchers and clinicians have made dramatic gains in the fields of neuroscience, human development, and developmental psychopathology. Despite these advances, less progress has been made in understanding the relationship between neurobiological, behavioral, and cognitive development of atypical and typical populations. To what extent can neurodevelopmental disorders in childhood be interpreted within models of normal development? How can the study of developmental disorders inform us about typical development of the brain, cognition, and behavior? Through our behavioral neurogenetics approach to studying homogeneous popu-

lations such as fragile X syndrome, we are closer to answering these questions today.

Certainly, the study of normal development has provided researchers with a foundation that has furthered our understanding of anomalous growth and development, which, for example, has helped us to determine the causes of birth defects and understand the progress of complex human disease. However, the reverse is also true: researchers have gained valuable information about normal development through the study of abnormal development. By studying atypical populations, developmental theories can be affirmed, augmented, and challenged. A comprehensive understanding of abnormal development can elucidate the consequences of alternate developmental pathways, help us define the range and variability of responses to challenges, and specify the limits of behavioral and biological plasticity in affected individuals. In particular, studying childhood neurodevelopmental disorders at multiple levels allows us to better understand typical development by highlighting specific developmental domains or events throughout a child's life span, thereby helping to delineate the boundaries of pathology. In our research approach, we have focused our study of neurodevelopment on disorders with specific genetic etiologies as a means of developing greater insights into neurodevelopmental pathways that might otherwise be obscured or diluted by studying more behaviorally defined disorders. Fragile X has proved researchers with an invaluable "experiment of nature" to examine developmental processes in a more homogenous population with respect to aberrant neurodevelopmental.

As we have seen, in fragile X, a single gene defect on the X chromosome triggers a cascade of highly complex events that leads the neural system down a path to its ultimate manifestations of increased risk for problems in behavior, learning, and development. Since the discovery of the genetic basis of fragile X syndrome in 1991, researchers and clinicians from many disciplines have had a rare opportunity to study the complex interplay between genes, neurodevelopment, cognitive and behavioral development, and environmental effects on developmental outcomes. In addition,

recent technological advances have greatly enhanced and refined this analysis on many levels. Particularly, in the past decade, we have seen the development of more sophisticated imaging equipment and software for MRI and DTI, increasingly sensitive tools for genetic analysis, informative fragile X animal models, as well as more reliable and valid behavioral and cognitive instruments.

What, then, has fragile X taught us about developmental theory? At the level of the gene, studies of individuals with fragile X, as well as the mouse and fruit fly knockout models of this condition, have recently provided us with valuable new insights into the genetic control of neural development. Specifically, we have seen that the fragile X mental retardation protein, FMRP, increases in those brain regions undergoing active synaptogenesis in response to motor learning or being reared in complex and enriched environments. In normal neurodevelopment, FMRP associates with several mRNAs that are integrally involved in critical neurodevelopmental processes such as neuronal plasticity and development, synapse and dendrite formation and maturation, and axon path finding. In the absence of FMRP, such processes unfold in an aberrant manner very early in neurodevelopment, leading to impairments in dendritic spine maturation and a failure of normal synapse pruning and axon formation. At the neural systems level, such neurodevelopmental impairments result in specific alterations in brain structure and function.

Our structural MRI studies of individuals with fragile X have revealed significant volume increases in the caudate nucleus and hippocampus, and decreases in the superior temporal gyrus and cerebellar vermis. These structural findings, particularly those seen in the caudate, are robust, and suggest correlations with neurobehavior and neurocognition in fragile X. For example, the caudate, through its connections with the frontal cortex, coordinates attention and working memory, regulation of mood, impulse control, and flexibility in behavioral responses to environmental cues, functions that show impairment in the child with fragile X. Further, our recent DTI analyses have uncovered abnormalities in

white matter tracts, particularly in prefrontal–caudate pathways, suggesting aberrant neural tracts and connectivity that may need to be eventually overcome to establish “normal” function in individuals with fragile X. These abnormalities in white matter tracts may be related to FMRP’s function in regulating axon extension and path finding during neural development.

In our fMRI studies, we have seen distinct alterations in brain activation patterns from children with fragile X compared with typically developing children in tasks of executive functioning, visuospatial processing, and math ability. While individuals with fragile X are generally activating appropriate brain regions during cognitive processing, unlike typically developing subjects, they cannot recruit the additional resources “on demand” in response to increasing task difficulty. These functional deficits correlate with the level of FMRP expression: higher FMRP levels are associated with more normal brain activation. By identifying specific regions involved in these cognitive tasks, we can more clearly understand how neurodevelopmental changes in fragile X are manifested in cognitive functioning and, most importantly, our results provide an important metric for directing and following the responses to new treatments for fragile X.

In terms of neurocognitive and neurobehavioral development, we observe that, compared with typically developing children, those with fragile X show early delays in functioning. These delays are characterized particularly by age-related declines in IQ, disturbance in language and communication, reduced trajectory in the development of adaptive behaviors, cognitive abnormalities within the domains of executive function and visuospatial cognition, hyperactivity, and significant problems with hyperarousal and anxiety. Boys are less variable than girls in expressing such cognitive and behavioral symptoms. On average, delays are noticed in the infant at 24 months. As boys with fragile X reach preschool age, their rate of development ranges from one-third to one-half that expected for typically developing boys. Cognitive and adaptive behavioral development slows in boys with fragile X beginning as early as 5 years

of age, reaching a plateau in middle to late childhood or early adolescence, generally by age 10, as evidenced by declining IQ scores and a lack of consistent gains during these years. Beginning in the preschool years and extending into the school and adolescent years, boys show pervasive deficits in conversational language skills with increasing discrepancy between language level and chronological age. We have seen that the patterns of behavioral, social, and developmental abnormalities that emerge in preschool boys suggests that fragile X is a risk factor for autistic behavior. In particular, the presence of a nervous system that is poorly modulated (e.g., hyperarousal, problems with inhibition and habituation) may contribute to the development of the autistic “features” observed in children with fragile X.

Girls with fragile X are more variable in their development: whereas those with the full mutation may show mildly to moderately severe quantitative and qualitative abnormalities, those with the premutation are much more likely to show trajectories similar to typically developing girls.

The trajectories of these impairments from infancy through adolescence and adulthood are complex and variable due to variability in the interplay between complex genetic, environmental, and biological risk factors. The resulting heterogeneity in individual patterns of development and symptom manifestations in fragile X underscore that both genetic expression and environmental factors influence expression in this disorder. Differences in FMRP localization among females and FMRP levels in both males and females due to polymorphisms in genes whose mRNAs are bound by FMRP likely contribute to this heterogeneity. As we have seen, the child’s environment strongly influences the expression of problem behaviors (including autistic symptoms) and cognitive ability. Recently, Grossman et al reviewed the contributions that environment and experience has made on psychopathology in fragile X and other disorders, suggesting that there may be multiple genetically or environmentally influenced routes to common developmental outcomes as well as multiple outcomes in a common genetic syndrome

(Grossman, Churchill, McKinney, Kodish, Otte, & Greenough, 2003).

Thus, we can appreciate how behavioral neurogenetics, as a multilevel systems approach, has led us to an improved understanding of the complex linkages among genetic, neurobiological, cognitive, and behavioral variables that contribute to neurodevelopmental dysfunction. According to such an integrated "systems neuroscience" (Cicchetti & Dawson, 2002) approach, the brain can be conceptualized as developing and operating in a highly plastic, self-organizing environment, which is less constrained by predetermined boundaries than previously thought (Posner, Rothbard, Farah, & Bruer, 2001). In this scheme, distributed groups of neurons maintain functional interconnections based on experience in addition to a genetically predetermined scheme (Courchesne, Chisum, & Townsend, 1994; Gottlieb, Wahlsten, & Lickliter, 1998; Johnson, 1998; Thelen & Smith, 1998). Thus, in addition to genotypic variability influencing behavior and cognition, social experiences also significantly affect neural structure and function throughout development (Cicchetti & Tucker, 1994; Dawson, Hessler, & Frey, 1994; Francis, Diorio, Liu, & Meaney, 1999; Gottlieb, 1992; Kandel, 1998; Meaney, Diorio, Francis, Widdowson, LaPlante, Caldji, Sharma, Seckl, & Plotsky, 1996). Our studies of individuals with fragile X have partly affirmed this. We have seen the complex interplay between genetic events, brain activation and structure, and behavior and cognition, as shown in Table 2. For example, decreases in FMRP are associated with decreases in brain activation during math processing, working memory tasks, and cognitive processes as well as changes in specific brain structures, particularly caudate. Other molecular/genetic changes such as increased CGG amplification size and decreased FMRP levels are associated with decreases in a child's IQ; increased *FMRI* methylation is associated with brain structural changes. Further, decreases in FMRP also correlate with an increase in stereotypy, communication, and attention problems and a decrease in peer socialization. Decreases in vermis and increases in caudate are linked with decreases in IQ and increases in stereotypy.

Interrelationships between cognition and behavior are seen in females with fragile X in which an increase in anxiety is associated with lower math ability and decreases in attention are associated with decreased social skills. Finally, outcomes of children with fragile X are strongly influenced by their environment. For example, increased mean parental IQ and socioeconomic status correlate with increased IQ of the child. Particularly in males with fragile X, we see a significant interplay between brain, behavior, and environment through a premature activation of the HPA axis and cortisol production, expressed in boys as hyperarousal.

Ultimately, however, to better understand the topography of typical and atypical development in fragile X syndrome, we need prospective longitudinal studies of large groups of children with fragile X. This research is vital because (a) elucidating the biological and environmental factors that influence cognitive and behavioral outcomes will identify areas of function sensitive to intervention, (b) obtaining precise information about development will help determine whether specific interventions lead to meaningful changes in functioning, and (c) understanding specific domains of suboptimal development may provide clues for us to formulate early interventions in children with fragile X.

In summary, we have today a clearer understanding of atypical development in fragile X and how the perturbations in a specific genetic locus alters brain development and thereby alters the development of psychological functions. This study has begun to provide us with an integrated scientific explanation for the disorder, and achieving such explanations has important consequences for both basic developmental science and clinical practice. Understanding how language and speech develops abnormally in fragile X syndrome (as with other neurogenetic disorders like Turner syndrome, VCFS, and Williams syndrome) will help us understand how it develops normally in typically developing children. The same is true for other domains such as executive function, social cognition, and emotion regulation. In clinical practice, having an integrated scientific explanation of a disorder such as fragile X inevitably leads to improve-

ments in diagnosis and early detection as well as new treatments.

Implications for Future Research

Progress in fragile X research over the last decade—from the detailed characterization of the *FMR1* gene and FMRP function at the neuronal level to the characterization of brain and behavioral abnormalities—truly has been dramatic and exciting for the field of developmental psychopathology. The capability of utilizing behavioral neurogenetics research strategies has required technical advances that have only recently become available. In particular, recent advances in molecular genetics and brain imaging have greatly facilitated our knowledge of abnormal and normal brain–behavior development in genetic disorders such as fragile X, and today a more profound understanding of the developmental psychopathology of fragile X is within our grasp.

Fragile X also serves as an important reminder of the complexities involved in elucidating the pathogenesis of neurogenetic and neurodevelopmental disorders. Despite the clearly identified genetic cause and well-characterized neuropsychological, molecular, cellular, and neuroanatomic features, the disorder is far from being completely understood. Gaps remain in our understanding of the developmental relationships between *FMR1* gene function and the complex patterns of cognitive and behavioral abnormalities and abnormalities in brain structure and function in fragile X. In particular, we still do not understand the precise timing and nature of gene–brain–behavior disruptions in children with fragile X. Likewise, although it is clear that mutations of the *FMR1* gene, in general, increase risk for cognitive, behavioral and emotional dysfunction, knowledge of how functional outcome is moderated and mediated by factors such as age, the family and educational environments, and neural function has only recently begun to emerge. Information of this nature is vital to understanding and optimizing development in children with fragile X. For example, are infants and preschoolers with fragile X born with the “full measure” of the genetic risk, or do problems in behavior

and cognition accumulate through a progression of worsening anatomic and functional connectivity in key pathways in the brain? Do particular genetic and environmental influences accumulate during development that further alter brain function later in life? When are children with fragile X most likely to develop maladaptive behaviors or plateau in their cognitive trajectory? What factors have a positive influence on cognitive and behavioral development? What factors other than reduced FMRP contribute to the severity of maladaptive behaviors and cognitive impairment? When is intervention most needed and most effective? What are the best cognitive, behavioral, and functional metrics for following response to interventions?

Answering these questions will be key in developing a more complete framework from which to design and implement biological and environmental interventions for children with fragile X. In developing such a framework, we must first characterize the complex developmental trajectories in infants and young children with fragile X. Although most investigators agree that IQ and adaptive behavior scores decline in young children with fragile X, the precise timing and neurobiological basis of this important clinical feature are unknown. Particularly, our research must have a focus on infants and young preschool children for whom dynamic changes in brain development and organization are taking place.

Our ongoing studies using longitudinal, prospective designs with multiple time assessments are designed to elucidate the early signs of aberrant brain development in infant and preschoolers with fragile X. Our goals are to understand the *developmental trajectories* of (a) brain structure and function, specifically patterns of brain size and shape and white matter connectivity; (b) cognitive, behavioral, and emotional development as they relate to morphological brain characteristics; (c) HPA function; and (d) *FMR1* gene expression (FMRP) in relation to brain–behavior changes. In assessing such developmental trajectories, we must compare developing children with fragile X with age- and gender-matched controls, children with idiopathic de-

velopmental disability, and those with development disabilities with specific etiologies and with non-fragile X, autistic children.

This research will provide new and much-needed information to help us characterize the pattern and timing of changes in brain size, shape, and connectivity during early brain development in children with fragile X. A more complete picture of these developmental patterns will help us see the cross-sectional and longitudinal relationships between specific brain characteristics and the pattern of selected cognitive characteristics and behavioral abnormalities known to be abnormal in young children with fragile X.

Further study of fragile X as a model system will likely provide us with important new insights into the pathogenesis of related developmental disorders and abnormalities of behavior and cognition in young children. By studying individuals with fragile X, we can trace the pathway that begins with the single genetic mutation out to its ultimate manifestations in behavior, learning, and development.

By measuring and accounting for these genetic influences, we are, therefore, better able to begin to understand the roles of other factors such as the environment, and neuroendocrine function in the outcomes of children with fragile X. The answers to the questions posed here will provide us with the information necessary to construct well-informed clinical trials in the near future, in which the important biological and environmental influences can be assigned etiological significance with confidence. In our ongoing and future research, elucidating how the brain is structurally and functionally different in children with fragile X and how the children with this disorder behave, learn, and experience emotions differently will help us determine specific treatments and predict outcomes in these children. Our ultimate goal is to improve the lives of those affected by fragile X syndrome and gain deeper insights into the causes of other behavioral, developmental, and learning problems that occur in children.

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