CHAPTER

33

Fragile X Clinical Features and Neurobiology

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OUTLINE

Abbreviations

ADHD Attention deficit hyperactivity disorder
AMPA Alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
ASD Autism spectrum disorders
CGG Cytosine–guanine–guanine
DD Developmental disabilities
DSM-IV-TR Diagnostic and Statistical Manual, fourth edition, text revision
FMR1 Fragile X mental retardation 1
FMRP Fragile X mental retardation protein
FSH Follicle stimulation hormone
FXPOI Fragile X-associated primary ovarian insufficiency
FXS Fragile X syndrome

Neural Circuit Development and Function in the Brain: Comprehensive Developmental Neuroscience, Volume 3 http://dx.doi.org/10.1016/B978-0-12-397267-5.00044-3

FXTAS Fragile X-associated tremor/ataxia syndrome
ID Intellectual disability
KO Knock out
MCP Middle cerebellar peduncle
MDD Major depressive disorder
mGluR Metabotropic glutamate receptor
MMP9 Matrix metalloproteinase 9
MP Metacarpal-phalangeal
MPEP 2-Methyl-6-(phenylethynyl)-pyridine hydrochloride
MS Multiple sclerosis
MVP Mitral valve prolapse
NCS-R National Comorbidity Survey Replication
PCR Polymerase chain reaction
PECS Picture exchange communication system
POF Premature ovarian failure
POI Primary ovarian insufficiency
PWP Prader–Willi phenotype
SCID Structured Clinical Interview for DSM IV
SCL-90 Symptom Checklist-90
SSRIs Selective serotonin reuptake inhibitors

33.1 INTRODUCTION

33.1.1 Fragile X Syndrome and Fragile X-Associated Disorders

Fragile X syndrome (FXS) and the fragile X family of disorders may affect generations of a family in a variety of ways. It is essential for readers to understand the spectrum of involvement from FXS and premutation disorders, as significant advances in the knowledge of these disorders have occurred in recent years. As one enters into a new stage of targeted treatments, identification and understanding of the fragile X-associated disorders are even more important. The study of fragile X-associated disorders serves as a pathway for gaining insight into the molecular pathogenesis of other neurodevelopmental disorders.

FXS is the most common inherited cause of intellectual disability (ID) and the most common single genetic cause of autism. It is also known as Martin–Bell syndrome and, in South America, as Escalante syndrome. The syndrome was first described in 1943 by Martin and Bell, two British physicians who reported a sex-linked ID syndrome in a large family without definitive physical characteristics (Martin, 1943). Sex-linked ID in the sons of otherwise unaffected daughters of a Saskatchewan family was described by Renpenning in 1962 (Renpenning et al., 1962). In 1969, Lubs noted an unusual secondary constriction of the long arm of the X chromosome in a family with four intellectually disabled males and three carrier females (Lubs, 1969). Further investigations were aided by studies showing that observation of these fragile sites on the X chromosome depended on what type of tissue culture medium was used, allowing increased frequency of observation (Sutherland, 1977). The fragile X chromosome and ID were further linked to macroorchidism by Turner (Turner et al., 1978) and Sutherland (Sutherland and Ashforth, 1979). In 1981, Richards et al. reexamined the pedigree described by Martin and Bell in 1943, which did not appear to have physical anomalies. Seven members of the original family reported by Martin and Bell were evaluated, and five were found to carry the fragile X chromosome. Unusual physical features were also noted upon further examination, including large ears, prognathism, and macroorchidism (Richards et al., 1981). Of note, the family described by Renpenning in 1962 was reevaluated by Fox and colleagues, and members of the family were found to have microcephaly and no macroorchidism, both of which are atypical of FXS. It has since been suggested that Renpenning syndrome refers to the condition of mental retardation, microcephaly, and sex-linked inheritance, but without an associated fragile X chromosome (Fox et al., 1980).

The molecular etiology of the FXS, an unstable cytosine–guanine–guanine (CGG) repetitive sequence region of DNA on the X chromosome, was discovered in 1991 (Verkerk et al., 1991; Yu et al., 1991). A mutation in the *fragile X mental retardation-1* (*FMR1*) gene leads to expanded CGG repeats on the 5′ end of the *FMR1* gene in successive generations (Fu et al., 1991). The number of CGG repeats determines whether an individual is categorized as having a full mutation, or a premutation, or is in the gray or intermediate zone (Figure 33.1).

33.1.1.1 Full Mutation

In the general population, the number of CGG repeats ranges from 5 to 44 repeats. Greater than 200 CGG repeats confers the full mutation (Maddalena et al., 2001). In the full mutation, the *FMR1* gene is usually completely methylated and transcription of *FMR1* mRNA is silenced, so that very little fragile X mental

FIGURE 33.1 A family affected by fragile X. *Left*, a young man with fragile X syndrome; *middle*, his mother who is a premutation carrier; *right*: his maternal grandmother who is a premutation carrier with FXTAS. Notice the young man's gaze away from the camera.

retardation protein (FMRP) is produced (Bell et al., 1991; Pieretti et al., 1991). It is a lack of this protein that leads to the syndrome (Loesch et al., 2004). FXS is characterized by features including developmental delay, deficits in short-term memory, and speech delays. Affected individuals may have hyperactivity, attention difficulties, and an autism spectrum disorder (ASD). A sometimes subtle physical phenotype can be associated with the full mutation, including macroorchidism (from puberty onward) and prominent ears. These are discussed in detail later in the chapter.

Studies of the prevalence of FXS have focused on individuals with significant cognitive impairment. These studies resulted in a prevalence of 1 in 3,600 to 1 in 4,000 (Crawford et al., 2002; Turner et al., 1996). The allele frequency in the general population is 1 in 2,500 for both males and females identified in newborn screening (Fernandez-Carvajal et al., 2009b; Hagerman, 2008). Most affected people have the full mutation with >200 CGG repeats; however, in rare cases, the disorder can be caused by a deletion or point mutation in the *FMR1* gene or by an FMRP deficit in the upper range of the premutation.

33.1.1.2 *Premutation Carriers*

Carriers of fragile X, also known as premutation carriers, are defined as having 55–200 CGG repeats (Maddalena et al., 2001). The prevalence of the premutation ranges from 1 in 250 to 810 in males and 1 in 130 to 260 in females (Dombrowski et al., 2002; Fernandez-Carvajal et al., 2009b; Hagerman, 2008). The premutation was previously thought to have no implications for disease. Most individuals with the premutation are cognitively unaffected. However, research has shown effects of the premutation, first in reproduction with the discovery of fragile X-associated primary ovarian insufficiency (FXPOI). Primary ovarian insufficiency (POI) refers to menopause before the age of 40. Neurological symptoms including tremor and ataxia were reported in 2001 in an older male subgroup of fragile X premutation carriers (Hagerman et al., 2001). In children with the premutation, most are unaffected, but a subgroup demonstrates evidence of difficulties including attention deficits and social deficits (Farzin et al., 2006). A pattern of psychiatric problems in a subgroup of premutation carriers is also emerging, which is discussed later in the chapter.

33.1.1.3 *Gray or Intermediate Zone*

Among the general population, noncarriers have 5–54 CGG repeats. However, those with 45–54 repeats have been discovered to have unique features as well. These individuals are known as being in the gray or intermediate zone (Maddalena et al., 2001). They are usually phenotypically unaffected, although there can be elevated *FMR1* mRNA and twice the rate of POI in the general population (Bodega et al., 2006; Bretherick et al., 2005; Loesch et al., 2007). On occasion, alleles in the gray zone can be unstable, and, therefore, this category requires further study for both phenotypic involvement and allelic stability. There have been rare reports of males with gray zone alleles who have had some fragile X features, but whether the features are due to the gray zone allele is not certain (Aziz et al., 2003).

33.2 CLINICAL DESCRIPTION

33.2.1 Clinical Description of FXS

The phenotype of FXS can be quite broad, with a mix of physical, cognitive, and behavioral features. Typically, children with FXS are not diagnosed until 3 years of age, when their behaviors bring them to the attention of their physician (Bailey et al., 2003). ID is a key feature in FXS, as it is the most common inherited cause of ID. Eighty-five percent of males and 25–30% of females are found to have an IQ <70 (de Vries et al., 1996; Hagerman et al., 1992, 2008b; Loesch et al., 2004). Lower FMRP levels are associated with more severe cognitive deficits. Females with FXS usually present with learning disorders and a normal or borderline IQ (Chonchaiya et al., 2009a).

33.2.1.1 *Clinical Phenotype*

The classical physical phenotype associated with males with FXS is of a person with macroorchidism, large and prominent ears, and a long, narrow face. This physical picture is typical for adult males with FXS. The physical features are often not present in the prepubertal child with FXS (Chudley and Hagerman, 1987).

Macroorchidism was the first physical feature identified in FXS. It is present in more than 90% of adult males (Merenstein et al., 1996). The macroorchidism does not appear to affect fertility as males with FXS are fertile (Willems et al., 1992). The sperm of full-mutation males alone contains the premutation (Reyniers et al., 1993). Due to this, fathers with FXS pass on the premutation to all of their daughters, and as they pass on their Y chromosome to their sons, the latter are not affected. Using an orchidometer, Butler et al. compiled growth charts for 185 males with FXS including height, weight, and testicular volume measurements. In the study, the average testicular volume in an adult male with FXS was 45 ml with a 95% confidence interval of 25–70 ml (Butler et al., 1992). The normal testicular volume is around 25–30 ml (Prader, 1966), and a volume greater than 30 ml is considered to be macroorchidism.

Prominent ears were described in 78% of 97 prepubertal boys with the full mutation (Merenstein et al., 1996). The ears can be long and wide and may demonstrate loss

FIGURE 33.2 Image of a young boy with fragile X syndrome. Note the broad forehead, epicanthal folds, and mildly prominent ears with cupping of the pinnae bilaterally.

of the antihelical fold, leading to 'cupping' of the upper pinna (Hagerman, 2002b). If the prominent ears are undesired, a surgical pinning procedure can be done (Figure 33.2).

Facial features were characterized in a retrospective study of children with FXS by Hockey and Crowhurst. Common characteristics in prepubertal children included puffiness around the eyes, strabismus, and hypotonia (Hockey and Crowhurst, 1988). Epicanthal folds were described by Simko et al. (1989). A high palate is a common finding, and often the palate is narrow as well (Simko et al., 1989). Jaw length increases disproportionately to body height in patients with FXS (Loesch and Sampson, 1993), and jaw prominence can be seen in adults, in addition to a long face after puberty. Dental maturity may be advanced in FXS, more so in younger children. Dental maturity was advanced in girls with the premutation as well (Kotilainen and Pirinen, 1999).

Connective tissue abnormalities are seen in FXS, including pes plenus, joint laxity, scoliosis, and hyperextensibility of the finger joints (Hagerman et al., 1984). Davids et al. examined 150 males with FXS and found pes planus in 50% of them. Hyperextensibility of the metacarpal–phalangeal (MP) joints was observed in 73% of patients younger than 11 years of age, in 56% of those 11–19 years old, and in 30% of those older than 20 years of age. Hyperextensibility is defined as an MP joint angle greater than or equal to 90° (Davids et al., 1990). Double-jointed thumbs may also be a physical finding. The skin is soft and 'velvety' in texture in FXS. A single or bridged palmar crease and calluses from hand-biting behaviors may be present. Hypotonia is described in young patients (Hagerman et al., 1983), and it

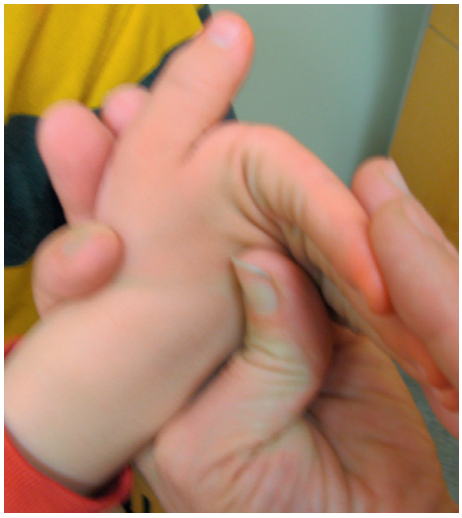

FIGURE 33.3 Hyperextensibility of MP joints with extension to 90°.

FIGURE 33.4 Single palmar crease in a boy with FXS.

FIGURE 33.5 Double-jointed thumb in a boy with FXS.

appears to be due to a general effect of CNS dysfunction. Clonus may be present in adult FXS individuals more frequently than in children with FXS. It should be noted that approximately 30% of individuals with FXS do not have the typical physical features (Figures 33.3, 33.4, and 33.5).

In female individuals with FXS, the spectrum of physical involvement is tempered by the unaffected X chromosome. Phenotypic involvement is closely associated

with the activation ratio (Abrams et al., 1994; Sobesky et al., 1996). The activation ratio refers to the fraction of normal *FMR1* alleles on the active X chromosome. Females with FXS were described in 1971 by Escalante, who noted flat feet and a high palate (Escalante, 1971). Cronister et al. looked at 105 full-mutation females and found an increased incidence of voluntary thumb dislocation and hyperextensible MP joints compared to 90 unaffected controls (Cronister et al., 1991). Mildly prominent ears are a common finding in prepubertal girls. Female individuals with FXS may have the full spectrum of involvement including ID and physical features or, on the other side of the spectrum, have no ID. Angkustsiri et al. reported a girl with the full mutation who had not only gifted intellectual abilities but also mild auditory processing problems and significant anxiety (Angkustsiri et al., 2008).

33.2.1.2 Behavioral Phenotype

Young children with FXS may present with language delays and are diagnosed with developmental delays at an average age of 21 months (Bailey et al., 2003). Hyperactivity is commonly reported, as are irritability, tantrums, perseveration, self-injurious behavior, mood instability, hand flapping, and hand biting (Hagerman, 2002b).

Patients with FXS often seem to have hyperarousability to auditory, visual, or tactile stimuli. This may be due to autonomic dysregulation with both sympathetic hyperarousal to sensory stimuli (Miller et al., 1999) and decreased parasympathetic activity compared to controls (Boccia and Roberts, 2000). Hyperactivity was noted in 47% of boys with FXS by Finelli et al. (1985). Bregman et al. found attention problems in all of 14 boys studied, but only 71% met the criteria for attention deficit hyperactivity disorder (ADHD; Bregman et al., 1988). ADHD may be the initially noted complaint in higher-functioning boys with FXS (Hagerman et al., 1985). Impulsivity is evident in boys with FXS when compared to boys with Down syndrome and unaffected control boys (Munir et al., 2000). Sullivan and colleagues have documented attention deficits in the majority of boys with FXS studied (Sullivan et al., 2006).

Shyness and social anxiety are commonly seen in both males and females with FXS and may improve with age. It may be more of a problem in females with FXS because ADHD symptoms are often more severe in boys, and ADHD may serve to temper the shyness (Merenstein et al., 1996). Psychiatric problems may also occur in individuals with FXS. Franke et al. evaluated psychiatric problems in mothers with the full mutation and in siblings with both the premutation and without the premutation. Fifty-four percent of full-mutation mothers had a psychiatric problem. Anxiety disorders were seen in 46% of those mothers with the full mutation, and social phobia was seen in 31%. Bipolar disorder was present in 15% and major depressive disorder (MDD) in 15%. Schizotypal and schizoid personality disorder was seen in 23% and avoidant personality disorder in 23% (Franke et al., 1998).

Executive function defects and difficulties with math are commonly reported in females with FXS. Attention problems are less frequent in females in comparison to males, with around 33% having ADHD (Hagerman et al., 1992). Females tend to have less hyperactivity but impulsivity is usually evident. Selective mutism may be seen in females as well (Hagerman et al., 1999).

33.2.1.3 Autism

Autism is a disorder characterized by deficits in social communication, language, and repetitive movements. It is closely related to FXS, as approximately 2–7% of individuals with autism will be positive for the fragile X mutation (Brown et al., 1986; Reddy, 2005; Wassink et al., 2001). Once a child receives the diagnosis of autism or ASD, fragile X DNA testing is recommended. Around 25% of males with FXS were diagnosed with autism in the 1990s using the Childhood Autism Rating Scale (Bailey et al., 1998). When evaluated with current gold standard diagnostic tools such as the Autism Diagnostic Observation Schedule and the Autism Diagnostic Interview, 30–35% of males meet the diagnostic criteria for autism (Harris et al., 2008; Rogers et al., 2001). Autistic-like features such as hand biting, hand flapping, perseveration, shyness, and poor eye contact have been noted in FXS, but it is the core social and communication deficits that lead to a diagnosis of autism (Baumgardner et al., 1995; Hagerman et al., 1986; Hatton et al., 2006; Kaufmann et al., 2004; Kerby and Dawson, 1994; Lewis et al., 2006). Social communication is not typically a weak point in the majority of patients, except in those with autism. Individuals with FXS are typically sensitive to social cues (Simon and Finucane, 1996).

In females with FXS as well, autism has been diagnosed, but on a less frequent basis than in males. Several studies have been conducted that evaluated the number of females with FXS and autism, and the rate is approximately 4–10% (Dissanayake et al., 2009; Hagerman, 2002a; Leigh et al., 2010).

33.2.1.4 Associated Medical Conditions

Seizures are the most important medical problem associated with FXS. They occur in approximately 20% of individuals and are more common in early childhood (Berry-Kravis, 2002; Hagerman and Stafstrom, 2009; Musumeci et al., 1999). The seizures are usually well controlled by anticonvulsants if treated early, but, in some cases, multiple anticonvulsants are necessary (Berry-Kravis, 2002). All types of seizures can occur, but partial complex seizures are perhaps the most common.

Strabismus has been noted in 8–40% of patients with FXS (Hatton et al., 1998; King et al., 1995; Maino et al., 1991; Storm et al., 1987). The type of strabismus reported has included exotropia, esotropia, and hyperdeviations. Surgery or patching may be necessary for correction, and, if found on physical examination, a referral to an ophthalmologist is recommended.

Otitis media has been reported by Hagerman and colleagues in 63% of 30 boys with FXS in comparison to 15% of their typical siblings and 38% of developmentally disabled children without FXS. Forty-three percent were treated with polyethylene (PE) tubes or prophylactic antibiotics for persistent middle ear effusions (Hagerman et al., 1987). Monitoring for otitis media should be done, as the resulting effects such as hearing loss may worsen language and cognitive defects. The facial structure in FXS is thought to contribute to the propensity for otitis media. Sinusitis was noted in 23% of 43 fragile X full-mutation males (Hagerman, 2002b).

Mitral valve prolapse (MVP) has been observed in FXS and is likely due to connective tissue abnormalities. Loehr et al. found MVP by echocardiogram in 55% of 40 male and female full-mutation individuals, and the prolapse was associated with a click or murmur on physical examination (Loehr et al., 1986). A study of 13 males and females using standard ECG, Holter ECG, and echocardiography found MVP in 77%, tricuspid prolapse in 15%, and mild pulmonary artery dilation in 23%. Other defects were noted including posterior aortic leaflet prolapse, mild aortic regurgitation, and mild pulmonary artery dilation (Puzzo et al., 1990). Any signs or physical examination symptoms suggestive of a cardiac problem warrant a referral to a cardiologist for further evaluation.

In FXS, abnormal growth patterns have been noted, including macrocephaly (Meryash et al., 1984). Short stature is common in both males and females (Loesch et al., 1987). There is evidence of hypothalamic–pituitary dysfunction with enhanced cortisol levels after stress and a blunted thyroid stimulation hormone response to thyrotropin-releasing hormone (Hessl et al., 2004; Wilson et al., 1988; Wisbeck et al., 2000).

The Prader–Willi phenotype (PWP) of FXS is characterized by extreme obesity with a full, round face; small, broad hands and feet; a small penis; and hyperphagia (de Vries et al., 1993; Nowicki et al., 2007). This PWP is phenotypically similar to Prader–Willi syndrome, but occurs without cytogenetic or methylation abnormalities at 15q11–13. It is estimated to occur in <10% of males with FXS, and occasionally occurs in females. CYFIP1 mRNA levels are generally reduced by two- to fourfold in the patients with the PWP compared to individuals with FXS alone and controls (Nowicki et al., 2007).

Children with FXS typically have disordered sleep early in childhood, and this usually improves with time (Kronk et al., 2009). Treatment with melatonin can improve this wakefulness in the middle of the night (Wirojanan et al., 2009).

33.2.2 Premutation Involvement

The authors' understanding regarding involvement in the premutation has evolved over the years: from considering those with the premutation as completely unaffected or 'nonpenetrant' in the 1980s to identification of premature ovarian failure (POF) in approximately 20% of women with the premutation in 1991 (Cronister et al., 1991) and now to the presence of a large spectrum of psychiatric, medical, and neurological problems in a limited number of carriers (Chonchaiya et al., 2009a). Over the last decade, the understanding of the molecular mechanism leading to premutation involvement through RNA toxicity related to elevated levels of *FMR1* mRNA first identified by Tassone et al. (2000a) has grown. Below each type of premutation, the involvement is described in detail.

33.2.2.1 Developmental Involvement in Children with the Premutation

Involvement in children with the premutation was first identified in 1995 with a report of ADHD and social difficulties in high-end premutation carriers (Hagerman et al., 1996). Further reports of developmental problems followed (Aziz et al., 2003; Goodlin-Jones et al., 2004; Tassone et al., 2000b), and it was the problems seen in some young carriers that led to the discovery of elevated mRNA in carriers (Tassone et al., 2000a). Although the original cases were biased by clinic referral, Farzin et al. carried out a controlled study comparing the developmental problems of boys with the premutation who presented clinically as the proband to those who were identified through cascade testing once the proband had been diagnosed and to brothers who did not have the premutation. High rates of ASD (73%) and ADHD (90%) were found in those that were the probands compared to a rate of 8% of ASD and 38% of ADHD in the non-probands, and no ASD and 13% of ADHD in the typical brothers without the premutation (Farzin et al., 2006). Although the rate of ASD was not significantly different in the nonproband boys with the premutation compared to the non-carrier brothers, social deficits as measured by the Social Communication Questionnaire were significantly higher than in the unaffected brothers. Therefore, a subgroup of boys with the premutation is at a higher risk for ASD, social anxiety, and social interactional difficulties, in addition to ADHD.

Similar findings of premutation involvement were seen in the family comorbidity study of Bailey et al. where 1276 families completed an online survey about the children affected by fragile X in the family. Although

most of the patients evaluated by this survey had the full mutation, the survey also included 57 boys and 199 girls with the premutation. Developmental delay was found in 32% of males and 6% of females with the premutation, attention problems in 45% of males and 14% of females, hyperactivity in 30% of males and 3% of females, autism in 19% of males and 1% of females, and anxiety in 36% of males and 31% of females (Bailey et al., 2008). However, this survey may also be biased toward clinical involvement in those with the premutation. Determining the predictors of those at risk with the premutation and the percentage who will be affected by developmental problems will require larger studies of unselected populations, such as those who are identified by newborn screening. Such studies are currently underway with longitudinal follow-up of those in whom the premutation was identified at birth.

Recent studies by Chen and colleagues of premutation neuronal cell cultures from the *FMR1* knock-in (KI) mouse have demonstrated a deficit of neuronal cell branching and larger synaptic connections compared to neurons without the premutation. In addition, enhanced neuronal cell death in culture was seen by 21 days of division compared to neurons without the premutation (Chen et al., 2010). These findings suggest that premutation neurons may be more vulnerable to early cell death, which could lead to developmental problems of connectivity, such as ASD in early development, or perhaps neurodegeneration in later life. Additionally, neurons with the premutation may be a population genetically vulnerable to environmental toxins that could increase neuronal cell death. The authors have recently published four cases of carriers who were exposed to environmental neurotoxins from chemical plants in close proximity to their home and whose neurological symptoms began early in life (Paul et al., 2010). These neurological problems included multiple sclerosis (MS) symptoms in early adulthood in two women and fragile X-associated tremor/ataxia syndrome (FXTAS) symptoms beginning in their 40s to early 50s. This is earlier than the mean age of onset of FXTAS symptoms at age 60 as reported by Leehey et al. (2007) and described below.

33.2.2.2 *Fragile X-Associated Tremor/Ataxia Syndrome*

The FXTAS was first reported in five cases of older males with the premutation described in 2001 (Hagerman et al., 2001). All these patients had an intention or action tremor combined with ataxia leading to frequent falls. They progressed to needing a cane and eventually a wheelchair as weakness, autonomic dysfunction, and fatigue developed. The brain imaging demonstrated brain atrophy and white matter disease, usually in the periventricular region and in the middle cerebellar peduncles (MCP) sign of the cerebellum (Brunberg et al., 2002). Subsequently, criteria for the diagnosis of FXTAS were outlined in 2003 (Jacquemont et al., 2003) and then modified in 2004 (Hagerman and Hagerman, 2004). The modifications included the presence of eosinophilic intranuclear inclusions in neurons and astrocytes reported by Greco et al. (2002, 2006) that are unique to FXTAS. These inclusions occur throughout the central and the peripheral nervous system with the highest rate in the hippocampus and limbic system (Greco et al., 2006; Hunsaker et al., 2011). Molecular studies of the inclusions have demonstrated the presence of elevated *FMR1* mRNA in addition to other proteins (Iwahashi et al., 2006). The inclusions may help to arrest the cellular dysregulation that occurs in the presence of elevated mRNA, and they are also seen in the KI premutation mouse (Brouwer et al., 2008; Wenzel et al., 2010).

Recently, inclusions have been seen in peripheral tissue including the myenteric plexi of the gastrointestinal system, in the thyroid gland, in the adrenal gland, and in the testicles, particularly in the Leydig cells that make testosterone (Gokden et al., 2009; Greco et al., 2007; Louis et al., 2006). Impotence and testosterone deficiency are common and often occur even before the onset of FXTAS (Hagerman et al., 2008a). A report by Jacquemont et al. (2004) surveyed all the positive families in California, and a study was carried out on all the premutation carriers. It was found that in male carriers, 17% in their 50s were affected by tremor and ataxia, 38% in their 60s, 42% in their 70s, and 75% in their 80s. These rates were significantly different compared to age-matched male relatives who did not have the premutation. The female carriers were not significantly different from controls in this study.

Subsequently, the authors and others have reported on older female carriers who have developed FXTAS (Berry-Kravis et al., 2007; Coffey et al., 2008; Hagerman and Hagerman, 2004; O'dwyer et al., 2005; Rodriguez-Revenga et al., 2009). Typically, fewer females with the premutation develop FXTAS (8–16%) compared to males (Coffey et al., 2008; Rodriguez-Revenga et al., 2009), and when they do develop FXTAS, they have less brain atrophy, less white matter disease, and only 13% have the MCP sign (Adams et al., 2007). Seritan and colleagues reported the presence of dementia in approximately 50% of male carriers with FXTAS, but it is rare in female carriers with FXTAS (Seritan et al., 2008).

Cognitive problems usually begin with memory deficits and then progress to executive function deficits by the time the patients present with tremor and ataxia problems (Brega et al., 2008; Grigsby et al., 2006, 2007, 2008; Koldewyn et al., 2008; Loesch et al., 2003). Many patients may have a disinhibited sense of humor, leading to problems in public, which can be a burden to families. The dementia that develops is a frontal subcortical

dementia initially, and, on occasion, the initial symptom of FXTAS can be cognitive disorientation (Bourgeois et al., 2009). Treatment of the cognitive loss includes the use of medications important for Alzheimer disease such as memantine, since glutamate toxicity may be a factor in the pathophysiology of this disease. Treatment of early depression or anxiety as described below is also important.

33.2.2.3 *Primary Ovarian Insufficiency*

Approximately 16–20% of carriers can experience POI, and in carriers this is called FXPOI. As the CGG repeat number increases, the prevalence of POI is increased until about 120 repeats, and then the prevalence will drop to less than 16% (Sullivan et al., 2005). There is evidence that RNA toxicity affects the viability of the granulosa cells that support the ovum, although RNA toxicity may also decrease the viability of the ovum directly. Women with the premutation may also demonstrate mild elevations of follicle stimulation hormone (FSH) compared to controls even before they go into FXPOI (Welt et al., 2004). Of note, POI was previously referred to as premature ovarian failure (POF), but was renamed POI because a limited number of patients may become pregnant after experiencing the lack of menses (Welt, 2008). The American College of Obstetrics and Gynecology has recommended that all women who present with POI should be screened by fragile X DNA testing to see if they have the premutation (ACOG Committee Opinion, 2006). Approximately 1–7.5% of those with spontaneous POI have the premutation, and 13% of those who have a familial history of POI turn out to have the premutation (Welt, 2008). Once FXPOI is diagnosed, there are treatments including hormonal support and treatment of the emotional problems that often accompany this diagnosis (Nelson, 2009; Wittenberger et al., 2007).

33.2.2.4 *Psychiatric Manifestations of Premutation Carriers*

Emotional problems are common in carriers of the premutation both with and without FXTAS. A study carried out by Rodriquez-Ravenga et al. evaluated 34 women with the premutation and a child with FXS compared to 39 women without the premutation but with a child with developmental disabilities (DD) compared to age-matched control women without the premutation and without a child with DD. They used psychiatric questionnaires including the Symptom Checklist-90 (SCL-90) and the Beck Inventory to assess psychiatric problems. They found higher rates of psychiatric problems in women with the premutation and in women with a child with DD compared to controls, and this likely reflects the increased emotional stress that occurs in raising a child with FXS or DD. However, the women with the premutation had higher scores on the depression subtest than the other two groups of women (Rodriguez-Revenga et al., 2008). Similar results were seen utilizing the SCL-90 in 144 women and 68 men with the premutation (Hessl et al., 2005). Compared to published normative data, there was a higher rate of obsessive–compulsive symptoms in carriers of both sexes compared to controls. In addition, elevations in the *FMR1* mRNA levels correlated with increased symptoms on the obsessive–compulsive scale and on the psychoticism scale in males both with and without FXTAS (Hessl et al., 2005).

Roberts et al. utilized the Structured Clinical Interview for DSM IV (SCID) for mood and anxiety disorders in 93 women with the premutation. They found a significant increase in the rate of lifetime major depressive disorder (MDD) in premutation women (43%) that was significantly different from the rate of MDD in the National Comorbidity Survey Replication (NCS-R) data set. Forty-eight percent of these women had their first episode before the birth of their child with FXS, and the mean age of the first episode was 27 years. In the overall group, 31% had sought help from a professional, and 35% were on psychiatric medication. The premutation group also had four times the rate of lifetime panic disorder without agoraphobia and current agoraphobia without depressive disorder compared to the NCS-R. There was an inverse correlation between the CGG repeat number and the prevalence of depression, suggesting that the lowered FMRP levels at the upper end of the premutation range was protective for MDD (Roberts et al., 2009). Sobesky and colleagues in their studies of women with the full mutation found a protective benefit of lowered FMRP in that executive function deficits in the full mutation interfered with their understanding of psychiatric problems (Sobesky et al., 1996). The same issue may occur at the upper end of the premutation range.

Bourgeois et al. carried out an SCID on 85 individuals with the fragile X premutation, 47 with the FXTAS (33 males, 14 females, mean age 66) and 38 without FXTAS (16 males, 22 females, mean age 52). They found that in FXTAS, 30 cases (65%) met the lifetime Diagnostic and Statistical Manual, fourth edition, text revision (DSM-IV-TR) criteria for mood disorder, and 24 cases (52%) met the lifetime DSM-IV-TR criteria for anxiety disorder. Among the non-FXTAS subjects, there were 15 cases (42%) of lifetime mood disorder and 18 cases (47%) of lifetime anxiety disorder. When compared to age-specific NCS-R data, the lifetime prevalence of any mood disorder, MDD, any anxiety disorder, panic disorder, specific phobia, and post-traumatic stress disorder (PTSD) was significantly higher in subjects with FXTAS. The lifetime rates of social phobia in individuals with the premutation without FXTAS were significantly higher than NCS-R data (Bourgeois et al., 2011). Therefore, individuals with the premutation may manifest psychiatric

problems well before the onset of neurological problems. The psychiatric problems are likely related to the RNA toxicity that is apparent in the limbic system before the onset of FXTAS. Hormonal difficulties may also exacerbate the psychiatric problems that are common in carriers, as noted earlier.

33.2.2.5 *Associated Medical Conditions in Carriers*

A recent study of 146 women with the premutation demonstrated that approximately 8% who were not initially referred for this problem suffered from FXTAS (Coffey et al., 2008). In addition, in those with FXTAS, about 61% had hypertension, 50% had thyroid problems, usually hypothyroidism, 22% had seizures, and 43% had fibromyalgia; all these problems were higher than that found in age-matched controls without the premutation. Neuropathy problems were diagnosed in 53% in those with FXTAS, which is expected, but many women without FXTAS but with the premutation had neurological symptoms. These problems include symptoms of numbness and tingling in 34%, muscle pain in 20%, and intermittent tremor in 9% of women without FXTAS. These problems were more frequent than in age-matched controls without the premutation. MS occurs in 2–3% of carriers, which is a higher prevalence than what is seen in the general population of women (Zhang et al., 2009). One carrier who experienced a rapid decline over 15 years of MS demonstrated both active MS lesions in the CNS with inflammation and inclusions of FXTAS (Greco et al., 2008). There appears to be more autoimmune disease in carriers, and this is currently being investigated.

33.3 GENETICS AND NEUROBIOLOGY

33.3.1 Genetics – *FMR1* Gene

The genetic cause of FXS, the *FMR1* gene mutation, was elucidated in 1991 (Verkerk et al., 1991). The gene is 38 kB in length (Penagarikano et al., 2007). It has an unstable CGG region at the 5′ promoter region, and the fragile site is located at band Xq27.3 (Harrison et al., 1983). The fragile site was named FRAXA and was the first fragile site described on the X chromosome.

33.3.1.1 *Trinucleotide Repeat Disorder*

FXS was one of the first disorders associated with a trinucleotide repeat expansion. These trinucleotide repeat expansions may occur in coding regions in syndromes such as in Kennedy disease (spinal–bulbar muscular atrophy). The expansion can also occur in noncoding regions, as is the case in FXS. The reason for the trinucleotide repeat expansion is unclear. One theory is that it occurs during an unequal exchange of genetic material during meiosis or mitosis (Penagarikano et al., 2007).

Premutation alleles are unmethylated. The premutation alleles are unstable and may undergo expansions during oogenesis (Fu et al., 1991; Oberle et al., 1991). This leads to the phenomenon of premutation carrier progeny having either the premutation or the full mutation. It is known that all mothers of children with the full mutation either are premutation carriers or have the full mutation themselves. In general, premutation sizes of 90–100 CGG repeats usually expand to the full mutation (Nolin et al., 2003). There has been no documentation of a normal-sized allele expanding to a full mutation in one generation. However, there has been one instance reported of a gray zone allele expanding to a full mutation in two generations. The maternal grandfather had 52 CGG repeats (gray zone), his daughter had 56 CGG repeats (premutation), and his grandson had the full mutation with 538 CGG repeats (Fernandez-Carvajal et al., 2009a).

As stated previously, having greater than 200 CGG repeats is considered a full mutation. It is sometimes reported as a single number or may be reported as a range of repeat sizes. The *FMR1* gene, its upstream CpG island, and surrounding sequence are hypermethylated, which inactivates the gene (Sutcliffe et al., 1992). With complete methylation, there is little or no *FMR1* mRNA produced. Methylation of the gene works to inactivate transcription directly by inhibiting the binding of transcription factors and also works indirectly by inducing condensation of the chromosome, which then prevents transcription factors from binding (Penagarikano et al., 2007). This leads to the halting of production of *FMR1* mRNA and the gene's product, FMRP. It is the loss of this protein that leads to the phenotype in FXS (Pieretti et al., 1991).

Mosaicism may refer to the size of the allele or the pattern of methylation. Approximately 12% of individuals with the full mutation are considered to be size mosaics, with a combination of premutation or full-size alleles. Six percent of full-mutation individuals are methylation mosaics, with both methylated and unmethylated alleles present on Southern blot (Rousseau et al., 1994). The percentage of methylation can have significant effects on the phenotype. Merenstein and colleagues examined methylation in 218 full-mutation male patients, and those with complete methylation had the lowest IQ scores and greatest physical involvement (Merenstein et al., 1996).

There are additional ways to cause the fragile X phenotype besides the trinucleotide expansion. More than 15 different deletions affecting the *FMR1* gene and point mutations that can lead to the phenotype have been reported (De Boulle et al., 1993; Gedeon et al., 1992; Hammond et al., 1997). The phenotype of FXS can also occur in an individual with the premutation who has significantly low levels of FMRP.

Contraction of the allele occurs when an individual transmits a smaller-sized allele to offspring. This has been documented in mother-to-daughter transmission of the *FMR1* gene (Brown et al., 1996) and also in approximately one-third of father-to-daughter transmissions (Fisch et al., 1995; Nolin et al., 1996).

33.3.1.2 *FMRP and Upregulation of Other Proteins*

The FMRP has a maximum length of 632 amino acids and a molecular mass of 80 kDa (Ashley et al., 1993). It is involved in a variety of cellular processes and is expressed in different types of tissues but primarily in neurons and in the testes (Devys et al., 1993; Khandjian et al., 1995). FMRP has been shown to interact as a selective RNA-binding protein (Bassell and Warren, 2008). FMRP shuttles between the nucleus and cytoplasm and is localized to dendrites (Feng et al., 1997). It is involved in regulating the translation of a subset of mRNAs at synapses. It has been hypothesized that FMRP is involved in chromatin remodeling in the nucleus as well (Krueger and Bear, 2011). Usually, FMRP inhibits translation, but this inhibition is released with activation of metabotropic glutamate receptors (mGluRs). Activation of the mGluR5 pathway leads to long-term depression and weak synaptic connections (Bear et al., 2004). In the absence of FMRP, there is upregulation of the mGluR5 pathway leading to a reduction of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors in the dendrite. Without FMRP, there is dysregulated translation of other mRNAs, which leads to altered synaptic plasticity (Brown et al., 2001).

Individuals with FXS have more dendritic spines, which are thinner and longer compared to those in unaffected individuals. Bagni and Greenough presented a description of how FMRP may lead to the synaptic alterations, including regulation of translation and protein interactions including Cytoplasmic FMR1-interacting protein 1 (CYFIP1), Cytoplasmic FMR1-interacting protein 2 (CYFIP2), myosin VA, and nucleolin (Bagni and Greenough, 2005). The effect of the FMRP defect on synapses has been demonstrated in a *Drosophila* model of FXS, where the loss-of-function models show defects in synaptic structure and disturbed neurotransmission. The *dfxr* (*Drosophila* fragile X-related) gene was shown to inversely regulate expression of a microtubule-associated protein, Futsch. Therefore, dFXR was proposed to function as a translational repressor of Futsch (Zhang et al., 2001). Subsequent work has led to the understanding of FMRP as a master protein that regulates hundreds of mRNAs usually through inhibition of translation (Darnell et al., 2011). The synaptic structural defect can be seen in the fragile X mouse model as well. Cruz-Martin and colleagues observed a delayed downregulation in dendritic spine turnover from immature filopodia to mature spines during the early postnatal period compared to wild-type mice. This suggests a developmental delay in the maturation of dendritic spines in FXS (Cruz-Martin et al., 2010).

The decrease in FMRP may explain the increased prevalence of developmental delays and autism. FMRP regulates the translation of proteins associated with autism including neuroligins, neurorexins, the SHANK family of proteins, PTEN, and CYFIP (Bassell and Warren, 2008; Darnell et al., 2005, 2011).

The extracellular signal-related kinase (ERK) pathway seems to be affected in the fragile X mouse model, and the ERK1/2 pathway activation is delayed in individuals with FXS (Weng et al., 2008). Osterweil and colleagues found that basal protein synthesis in the hippocampus of the knockout (KO) mouse was reduced to wild-type levels when treated with an mGluR5 inhibitor as well as an ERK 1/2 inhibitor (Osterweil et al., 2010). The ERK pathway may also be a target for therapies in FXS.

Besides upregulation of the mGluR5 pathway, the lack of FMRP is associated with upregulation of the mammalian target of rapamycin (mTOR) pathway and downregulation of the PTEN pathway, the GABA receptors, and the dopamine pathway (Bassell and Warren, 2008; D'hulst and Kooy, 2007; Wang et al., 2008). These pathways may be the targets of treatment for FXS, which is discussed later. The review by Krueger and Bear can be referred to for further details regarding milestones in the pathophysiology of FXS and implications for treatment approaches as well (Krueger and Bear, 2011).

33.3.1.3 *Inheritance*

The inheritance pattern of FXS is X-linked with variable penetrance. Since it is an X chromosome-linked disorder, it affects more males than females.

33.4 TESTING

33.4.1 DNA Testing

The first method for testing for FXS was cytogenetic testing. The newest standard method of screening for FXS is through DNA testing, which includes a polymerase chain reaction (PCR), and Southern blot. The advantage of PCR is that it uses smaller amounts of DNA, is less expensive, and has a faster result time in comparison to the Southern blot. Its drawback is that it cannot detect longer DNA sequences, which is why both PCR and Southern blots are typically used. The number of CGG repeats is typically reported, as is methylation status in the full mutation. Using both methods, testing is 99% sensitive (McConkie-Rosell et al., 2005). Testing methods do not typically look for conventional mutations.

Guidelines for testing have been issued by the American College of Medical Genetics (Sherman et al., 2005; Table 33.1).

TABLE 33.1 Individuals for Whom Fragile X Testing Should be Considered

Individuals with mental retardation, developmental delay, or autism, especially if they have any physical or behavioral characteristics of fragile X syndrome, a family history of fragile X syndrome, or male or female relatives with undiagnosed mental retardation
Individuals seeking reproductive counseling who have a family history of fragile X syndrome or a family history of undiagnosed mental retardation
Fetuses of known carrier mothers
Affected individuals or their relatives in the context of a positive cytogenetic fragile X test result who are seeking further counseling related to the risk of carrier status among themselves or their relatives

Source: Sherman S, Pletcher BA, and Driscoll DA (2005) Fragile X syndrome: Diagnostic and carrier testing. Genetics in Medicine *7: 584–587.*

Other conditions that may be related to the fragile X family of disorders should also be evaluated with fragile X testing. For women experiencing ovarian dysfunction, the American College of Medical Genetics recommends testing with elevated FSH levels, especially if they have a family history of POF, or FXS, or relatives with undiagnosed mental retardation. Testing for the fragile X premutation and FXTAS is recommended for those individuals who are experiencing late-onset intention tremor and cerebellar ataxia of unknown origin, especially if they have a family history of movement disorders or FXS, or relatives with undiagnosed mental retardation (Sherman et al., 2005).

A new method for quantifying FMRP has been discovered, which uses an ELISA assay (Iwahashi et al., 2009). It is now possible to look more quantitatively at exactly how much protein a particular individual with FXS is able to make.

33.4.2 Newborn Screening/Blood Spot

Population-based screening was examined by Palomaki (1994), and subsequent studies showed the efficacy of screening programs in identifying carriers and affected fetuses (Pesso et al., 2000; Toledano-Alhadef et al., 2001). Fetal testing through amniotic cells or chorionic villi is also possible when one of the parents is known to be a carrier.

Different methods for screening large populations for FXS have been developed using newborn blood spots and PCR. Coffee et al. described screening 36124 deidentified newborn males with an assay. The assay had 100% specificity and sensitivity for detecting *FMR1* methylation in males and detected excess *FMR1* methylation in 82% of females with full mutations. They identified 64 males with *FMR1* methylation and found 7 to have the full mutation (Coffee et al., 2009). Another method utilizing nested PCR was developed by Tassone et al., and this can identify both males and females with the full mutation or the premutation (Tassone et al., 2008). This methodology was implemented in Spain where a total of 10000 newborn blood spots were screened. So far, this study has reported on 5267 male blood spots where 199 gray zone alleles, 21 premutation alleles, and two full-mutation alleles (1 in 2633) have been identified (Fernandez-Carvajal et al., 2009b). Modifications to the Tassone technique have been carried out and recently reported (Filipovic-Sadic et al., 2010). Newborn screening is currently occurring in numerous centers around the United States.

33.4.3 Cascade Testing

Identifying affected members of a family is important, as changes in the fragile X gene can affect multiple family members through its unstable trinucleotide expansion. Usually, a proband is identified, which is often an affected child with FXS. Other members of the family should be identified and counseled about being tested.

33.4.4 Genetic Counseling

Genetic counselors are important members of the treatment team, particularly in helping patients and their families understand the diagnosis. Counseling sessions provide key educational opportunities. Important issues to be discussed should include discussion of the clinical presentation and inheritance patterns, treatments, therapies, follow-up recommendations, referrals for medical, educational, and mental health treatments, and contact information for support groups (McConkie-Rosell et al., 2005). Degree of involvement in both the premutation and the full mutation should be reviewed by the genetic counselor for the whole family tree. There are typically multiple family members involved whenever a proband is identified (Chonchaiya et al., 2009b; McConkie-Rosell et al., 2007).

33.5 TARGETED TREATMENTS

With better understanding of the molecular pathways affected by the lack of FMRP, targeted treatments are currently being studied as described below.

33.5.1 mGluR5 Antagonists

FMRP normally modulates dendritic maturation involving inhibition of the mGluR system 1- and 5-mediated translation in neurons (Aschrafi et al., 2005; Weiler et al., 2004). The mGluR5 system has been found to be upregulated in the fragile X KO mouse, with a

resulting enhanced long-term depression in the hippocampus (Huber et al., 2002). Long-term depression is the phenomenon of a decrease in synaptic effectiveness, thus leading to weakened synaptic connections (Bear and Abraham, 1996).

Lack of inhibition of the mGluR5 system also results in increased internalization of AMPA receptors (Snyder et al., 2001). AMPA receptors in the synapse have been correlated to synaptic protrusion. The mGluR systems are excitatory systems involved in many different areas, and the resulting upregulation may explain some of the physical and neurological aspects of FXS including seizures, electroencephalographic abnormalities including spike wave discharges, anxiety, tactile defensiveness, and difficulty with coordination (Bear et al., 2004).

Features of the fragile X KO mouse have been thought to be due to the increased activity of the mGluR5 system. The effects of decreasing mGluR5 receptors have been evaluated in the KO mouse model of fragile X. In 2007, Dolen et al. created a fragile X KO mouse that also had a 50% decrease in the number of mGluR5 receptors. The mouse demonstrated rescue of phenotypic features such as dendritic spines and a normalization in audiogenic seizures. Macroorchidism was the one feature studied that was not rescued by decreasing mGlur5 receptors (Dolen et al., 2007). mGluR5 antagonists have also been shown to rescue the *Drosophila* model of fragile X (McBride et al., 2005).

mGluR5 antagonists are being studied as a targeted treatment for FXS. In the *FMR1* KO mouse, treatment with MPEP (2-methyl-6-(phenylethynyl)-pyridine hydrochloride), an mGluR5 antagonist, was shown to decrease the startle response and rescue the protrusion phenotype of hippocampal neurons (de Vrij et al., 2008). Trials of mGluR5 antagonists in patients with FXS have also begun. One example is fenobam, a highly potent and selective mGluR5 antagonist. In 2009, Berry-Kravis and colleagues completed a pilot single-dose trial of fenobam and observed calm behavior in 9 out of 12 patients studied with no significant adverse effects. There was an improvement in 6 out of 12 patients in prepulse inhibition testing, which is a measure of impulse control and sensorimotor gating (Berry-Kravis et al., 2009). The use of the mGluR5 antagonists is likely to be beneficial in treating seizures because seizures are ameliorated in the KO animal model with mGluR5 antagonists (Hagerman and Stafstrom, 2009; Yan et al., 2005). AFQ056, an mGluR5 antagonist, was studied in 30 male individuals with FXS aged 18–35 in a double-blind, crossover study. Improvements were seen in seven patients who had fully methylated *FMR1* promoter regions and no response in patients with partial promoter methylation. The most common adverse event was mild to moderately severe fatigue or headache (Jacquemont et al., 2011).

33.5.2 GABA Agonists

GABA dysregulation has been observed in the fragile X KO mouse, and amygdala hyperexcitability has been normalized in the knockout mouse with treatment using the GABA agonist, gaboxadol (Olmos-Serrano et al., 2010). Studies of the GABA B agonist arbaclofen, which is the R isomer of baclofen, are currently being carried out, including a double-blind crossover study in children and adults with FXS from 5 years of age and older. Arbaclofen works at a presynaptic receptor and lowers the level of glutamate at the synapse. It also lowers the level of excess protein produced at the synapse in FXS. A trial of arbaclofen in 63 patients with FXS has shown efficacy in those with autism or significant social deficits (Berry-Kravis et al., 2010).

33.5.3 Minocycline

Minocycline is a second-generation semisynthetic tetracycline derivative (Shetty, 2002). It was first introduced in 1967 and is generally well tolerated (Smith and Leyden, 2005). Minocycline is commonly used as an antibiotic in the treatment of acne vulgaris, and its class of medications, tetracyclines, are the treatment of choice for certain bacterial infections such as Rocky Mountain spotted fever and brucellosis. It has been investigated as a neuroprotective agent in neurological diseases including Huntington's disease, amyotrophic lateral sclerosis, and MS (Kim and Suh, 2009). The mechanism of action by which minocycline exerts its neuroprotective effects is not precisely known, but likely has to do with its anti-inflammatory and anti-apoptotic effects. Minocycline's effects are reported to be through inhibition of cytochrome *C*, caspases 1 and 3, cytokines, and the suppression of metalloproteinase activity (Chen et al., 2000; Stirling et al., 2005). The latter effect is the key to minocycline's efficacy in the treatment of FXS.

As stated before, the lack of FMRP in FXS leads to upregulation of other proteins, one of these being matrix metalloproteinase 9 (MMP9). Matrix metalloproteinases are involved in extracellular degradation of proteins and are important for synaptic structure and plasticity (Sternlicht and Werb, 2001). Elevated MMP9 activity has been proposed as one mechanism for impaired dendritic spine maturation in FXS. Bilousova et al. demonstrated that minocycline treatment lowers MMP9 levels and matures synaptic connections in cultured hippocampal cells and that minocycline treatment for 1 month rescued synaptic abnormalities in the *FMR1* KO mouse. The treated mice showed improvements in anxiety on elevated plus maze and exploratory behavior on Y maze (Bilousova et al., 2009).

Minocycline treatment trials for FXS are currently underway. Utari and colleagues described 50 males and

females with FXS who were treated clinically with minocycline for 2 weeks or more. Using parent impressions, behavioral and cognitive changes were noted including improvements in language (54%), attention (50%), social communication (44%), and anxiety (30%). The side effects described included pigmentation of the nails (in one patient), loss of appetite, gastrointestinal upset, loose stools, and headache (Utari et al., 2010). An open-label study by Paribello and colleagues showed significant improvement in the Aberrant Behavior Checklist irritability subscale scores, Clinical Global Impression Scale – Improvement scores, and visual analog for behavior scores after 8 weeks of treatment with minocycline (Paribello et al., 2010). Currently, trials of minocycline are underway including a double-blind controlled trial in children and adolescents with FXS.

Side effects of minocycline include graying of teeth and gums, particularly in those younger than 8 years of age, gastrointestinal upset, sun sensitivity, and, in rare cases, pseudotumor cerebri and a lupus-like syndrome. These side effects should be monitored in any child or adult undergoing minocycline treatment. For children treated for longer than 3–6 months, an antinuclear antibody blood test should be done and, if positive, consideration should be given as to whether to continue with the medication in the long term.

33.5.4 Riluzole

Riluzole is a medication that is considered to have inhibitory effects on the glutamatergic system and is thought to potentiate the GABA system. It is approved for use in amyotrophic lateral sclerosis in adults. It has been associated with improvements in treatment-resistant depression (Zarate et al., 2004) and, in combination with other medications, was helpful in treatment-resistant obsessive–compulsive disorder (OCD) (Grant et al., 2007). In a 6-week, open-label study in six adults with FXS, riluzole was well tolerated; although it was associated with corrected peripheral ERK activation, riluzole was associated with a clinical response in only one of the six participants (Erickson et al., 2011).

33.5.5 Symptomatic Treatments

A number of currently available medications are useful in the treatment for individuals with FXS and are discussed in detail below.

33.5.5.1 For Mood Stability

Mood instability is seen in the majority of boys with FXS, and perhaps the best medication for this problem is a low dose of aripiprazole (Chonchaiya et al., 2009a; Hagerman et al., 2009). Aripiprazole is an atypical antipsychotic that has fewer problems with weight gain than risperidone, although most children do gain weight on this medication. In the authors' experience, aripiprazole is helpful for anxiety and for ADHD symptoms in addition to tantrums. Usually, just 0.5–2 mg works best when given at bedtime to younger children. Rarely does the dose need to go over 5 mg, and sometimes a higher dose leads to an increase in irritability or activation.

Additional mood stabilizers include anticonvulsants such as valproate or lamotrigine, and these medications can be helpful for aggression. Lithium may also be considered a targeted treatment for FXS because it works by lowering the mGluR5 system. An open trial of lithium was helpful in a small cohort of children and adults with FXS who had a poor response to other medications (Berry-Kravis et al., 2008).

33.5.5.2 For ADHD Symptoms

ADHD symptoms respond well to stimulants with approximately 66% demonstrating a positive response if the medications are given after 5 years of age (Hagerman et al., 2009). The alpha agonists including clonidine and guanfacine can also be helpful for calming the hyperarousal and tantrum behavior (Hagerman et al., 1998, 2009). These medications are often more helpful than stimulants for the child under 5 years of age. Clonidine can also be helpful for sleep disturbance, although melatonin should be tried first for sleep problems (Wirojanan et al., 2009).

33.5.5.3 For Anxiety

Anxiety is almost a universal problem for individuals with FXS and for many carriers as described above. The use of selective serotonin reuptake inhibitors (SSRIs) is very helpful for these problems. Fluoxetine is the most activating of the SSRIs, but that activation may be helpful to boost language in those with selective mutism. Sertraline has had the most common use in young children, and it deserves further study regarding its ability to enhance language in toddlers and to decrease the rate of autism (Hagerman et al., 2009). Many patients may benefit from a combination of a stimulant for ADHD and an SSRI for anxiety.

33.6 BEHAVIORAL INTERVENTIONS

33.6.1 Educational

Effective treatment for FXS consists of a combination of medication therapies as discussed previously and behavioral interventions. With the variable phenotype of FXS and premutation involvement, individualized therapy recommendations are essential. There have been very few studies examining educational and behavioral

therapies in FXS (Hall, 2009). Formal testing for language abilities and cognitive measures may be helpful in formulating an educational and therapy plan. Children with FXS will qualify for specialized educational methods including an individualized education plan.

Work in related disorders such as autism has shown that behavioral interventions can help, especially when started at a young age (Dawson et al., 2010). For those with FXS plus autism, well-established treatment models such as the Denver model (Dawson et al., 2010) and discrete trial training (Applied Behavior Analysis) (Smith et al., 2007) are recommended. Early identification of children with FXS is important, as therapies may be implemented sooner. Additionally, for patients with social deficits such as ASD or social anxiety, social skills groups may be helpful.

Behavior problems have been one of the greatest concerns in parent surveys (Bailey et al., 2000). Different types of therapies have been initiated, including sensory integration therapy and psychological counseling for behavioral interventions. Heightened sensitivity to sensory stimuli is related to hyperarousal and the autonomic dysfunction previously described. Occupational therapists trained in sensory integration therapy as well as fine motor therapy can be a valuable resource for the patients and their families for behavioral problems (Scharfenaker et al., 2002). Eye contact may be improved through behavioral intervention and was shown to be enhanced using a method of percentile scheduling with reinforcement in three out of six fragile X males studied (Hall et al., 2009). However, therapy to improve eye contact is controversial because it may worsen anxiety. A parent-initiated sleep therapy was evaluated in a population of five autistic children and seven children with FXS. The program reduced settling down to sleep and night awakenings (Weiskop et al., 2005).

Speech and language therapy can be essential to the development of a child with FXS as most can have speech delays. An audiology evaluation may be helpful, especially if hearing loss is suspected or recurrent otitis media is a problem. A conductive hearing loss due to fluid accumulating behind the ear is common in FXS. If this occurs as a child during the particularly crucial formative years of speech, it can further delay language development, so aggressive treatment with ear, nose and throat (ENT, also known as otolaryngology) evaluation and PE tubes is recommended (Hagerman, 2002a). Physical therapy can be used to help with the coordination difficulties in FXS and in premutation carriers with FXTAS.

For all of the above therapies, it must be emphasized that while the hours spent working with a therapist are fundamental, a home therapy program that families can continue to implement is needed to achieve the most benefit from the therapies.

33.6.2 Computer Work

Technological advances have been very helpful in providing a way for those with FXS to improve deficiencies. Assistive technology refers to technology that can help enhance functional abilities. For example, computerized learning systems have been created such as Writing with Symbols, which is a word prediction program. Assistive technology may also refer to picture cards, such as those used in the Picture Exchange Communication System. These can enhance communication, particularly for those who have speech delays (Hess et al., 2009). These devices can be obtained through the school or through private purchase. Children with FXS are often adept at computer use, making these interventions enjoyable as well as educational. There are also assistive technology specialists who can provide support with these interventions. There are current trials to assess the utility of assistive technology alone and in combination with medications.

For patients with FXTAS, using computer-assisted devices such as video games or the Nintendo Wii system with a balance board may be helpful to improve balance. Studies examining this are beginning, and the Wii system was found to be useful in improving balance and lower limb muscle strength in a pilot study of unaffected women (Nitz et al., 2010).

33.7 SUMMARY AND FUTURE PERSPECTIVES

33.7.1 Summary

In summary, FXS and its associated disorders have a rich history as a group of disorders caused by a CGG trinucleotide repeat sequence in a single gene, *FMR1*. Although the fragile X family of disorders has an identified single gene mutation cause, the resulting clinical phenotype can be quite diverse. Individuals with FXS may display ID, attention problems, autistic features or autism, as well as physical features including macroorchidism and prominent ears. Females with FXS may have learning disabilities, social anxiety, or impulsivity, in addition to variable physical features. Premutation carriers may develop FXTAS, with cognitive problems, tremor, and difficulties with balance. FXPOI may also occur in female premutation carriers in addition to psychiatric problems, hypertension, autoimmune dysfunction, and mild neurological problems such as neuropathy. Testing is widely available using PCR and Southern blotting in blood and also in amniotic cells or other tissue. Newborn screening is being studied around the world to increase early identification and treatment. Genetic counseling is important, given the possible implications of a diagnosis for both patients and their extended families.

A greater understanding of how the lack or deficiency of FMRP brings about the phenotype of FXS is developing, including the involvement of pathways such as the mGluR5 pathway, GABA pathways, AMPA receptors, PTEN pathway, and mTOR pathway. Treatments have been developed that use the understanding of these pathways to provide reversal of the neurobiological deficits in FXS. mGluR5 antagonists, GABA agonists, and minocycline as well as medications to help anxiety, aggression, and mood stabilization are currently being employed. Educational and behavioral interventions are also important components of a treatment regimen.

33.7.2 Future Perspectives

Treatment for FXS will likely combine both targeted treatments to reverse the neurobiological abnormalities and educational and learning paradigms to strengthen the synaptic connections that are facilitated by the targeted treatments. New treatments are being assessed now in cultured neurons for both premutation and full-mutation disorders. The use of mGluR5 antagonists has been shown to be helpful in a model of autism in the mouse (Silverman et al., 2010), and this suggests that these targeted treatments for FXS will also be helpful for autism. Currently, studies of arbaclofen are being conducted in autism, and trials of mGluR5 antagonists will be initiated in autism in the near future. Fragile X is leading the way for new targeted treatments in autism and perhaps for other neurodevelopmental disorders that have similar molecular dysfunctions. The future is bright for reversing the intellectual disabilities and the autism in FXS.

SEE ALSO

Cognitive Development: Developing Attention and Self Regulation in Infancy and Childhood; Statistical Learning Mechanisms in Infancy; The Development of Visuospatial Processing; The Effects of Stress on Early Brain and Behavioral Development; The Neural Architecture and Developmental Course of Face Processing; The Neural Correlates of Cognitive Control and the Development of Social Behavior. **Diseases**: Autisms; Excitation-Inhibition | Epilepsies; Language Impairment; The Developmental Neurobiology of Repetitive Behavior.

Acknowledgments

This work was supported by the National Fragile X Foundation and the MIND Institute, National Institute of Health grants HD036071 and HD055510; Neurotherapeutic Research Institute (NTRI) grants DE019583, and DA024854; National Institute on Aging grants AG032119 and AG032115; National Center for Research Resources UL1 RR024146; support from the Health and Human Services Administration of Developmental Disabilities grant 90DD05969.

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Relevant Websites

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http://www.fragilex.org/ – National Fragile X Foundation.
http://www.ucdmc.ucdavis.edu/mindinstitute/research/fxrtc.html – UC Davis Medical Center MIND Institute Fragile X Research and Treatment Center.