REVIEW ARTICLE

The Spectrum of Fragile X Disorders

Randi Jenssen Hagerman, M.D.,[1,2] and Paul J. Hagerman, M.D., Ph.D.[1,3]

Author affiliations are listed at the end of the article. Dr. R. Hagerman can be contacted at rjhagerman@ucdavis.edu or at University of California, Davis, MIND Institute, 2825 50th St., Sacramento, CA 95817.

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CME

Mutations in *FMR1*, the gene encoding fragile X messenger ribonucleoprotein 1 (FMRP), located at Xq27.3 and discovered in 1991, are the leading single-gene causes of autism and intellectual disability. The name of the disorder is derived from a fragile site that appears as a break on the bottom end of the X chromosome. Subsequent research has shown that variants in this gene encompass two separate groups of conditions.

The vast majority of cases of fragile X syndrome, which leads to intellectual disability and autism, are caused by a CGG-repeat expansion to greater than 200 repeats (termed the full mutation) in the 5′ noncoding region of *FMR1*. The promoter region of full-mutation alleles is usually fully methylated, which silences the gene from transcription and results in the production of little or no messenger RNA (mRNA), and subsequently little or no FMRP, the protein encoded by *FMR1*, from that allele; thus, fragile X syndrome is a loss-of-function condition.

For smaller repeat expansions in the premutation range of 55 to 200 repeats (termed the carrier state), there is no methylation, but instead there is increased transcription of the premutation CGG-repeat–containing mRNA (gain of function) that gives rise to a separate group of conditions called fragile X premutation–associated conditions. There are several of these disorders; among them are an adult-onset neurodegenerative condition, fragile X–associated tremor–ataxia syndrome (FXTAS); fragile X–associated primary ovarian insufficiency (FXPOI); and other disorders caused by gain-of-function toxic effects associated with the expanded repeats in the mRNA, a pathogenic mechanism completely separate from fragile X syndrome (Fig. 1 and Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).

The term "premutation" was first used by Pembrey et al.[1] to describe mothers with genetic profiles who were considered to be clinically unaffected but who had a definitive mutation that resulted in fragile X syndrome in their offspring. Pembrey surmised that these women must have carried a "premutation," a term that predates the discovery of *FMR1* by Verkerk and colleagues.[2] "Premutation," although considered to be an outdated term, is still in use in the context of clinical involvement.

Phenotype of Fragile X Syndrome (Full Mutation)

Fragile X syndrome is the most common inherited cause of intellectual disability and the most common single-gene cause of autism, with approximately 1 in 4000 persons in the general population having clinical features of fragile X syndrome. Most male patients with fragile X syndrome have substantial intellectual disabilities, and approximately 60% have autism. Therefore, persons with intellectual disabilities or autism are appropriate candidates for DNA testing for fragile X syndrome. Female patients are affected to a lesser degree because the normal X chromosomal allele produces FMRP, with the degree of clinical involvement dependent on the fraction of normal alleles that are transcriptionally active and escape X inactivation. Approximately one third of female patients who have a full mutation have intellectual disability (IQ of <70), one third have a borderline IQ (70 to 85), and

KEY POINTS

THE SPECTRUM OF FRAGILE X DISORDERS

- Persons with autism or intellectual disability should undergo DNA testing for fragile X to rule out the diagnosis of fragile X syndrome or premutation involvement.
- The premutation conditions (characterized by 55 to 200 CGG repeats) are caused by RNA toxicity from elevated mRNA levels and not by a lack of FMRP (the protein encoded by *FMR1*), which is the cause of fragile X syndrome.
- When fragile X syndrome is diagnosed, it is important to obtain a medical history of the family tree and to investigate whether other family members are affected by conditions associated with fragile X mutations: tremor–ataxia syndrome, primary ovarian insufficiency, neuropsychiatric disorders, or other conditions of premutation or full mutation.
- Children with fragile X syndrome benefit from a multimodality treatment approach that includes counseling, special education support (including speech and language therapy and occupational therapy), and medication to help with the behavioral features associated with fragile X syndrome.
- In women with early menopause or infertility, DNA testing for fragile X should be included in the medical workup.
- Persons 60 years of age or older who have tremor or ataxia should be considered for DNA testing for fragile X.

one third have a normal or near-normal IQ (>85); however, learning and emotional problems are common even with a normal IQ, as described below.

Fragile X syndrome is usually diagnosed in patients at approximately 3 years of age, when delayed speech milestones become apparent and the child is typically hypotonic, anxious, and hyperactive with tantrums. Autism is often diagnosed before fragile X syndrome owing to the emergence of social deficits and anxiety. Therefore, DNA testing for fragile X is considered to be appropriate for children with autism or intellectual disability. Additional connective-tissue changes may be present, including soft skin owing to the lack of elastin infrastructure in the dermis, prominent and elongated ear pinnae, elongated face, high palate, flat feet, hyperextensible finger joints, and pubescent macroorchidism. Most of the connective-tissue problems are present in childhood, but an elongated face is more commonly noted in adults. Medical problems associated with fragile X syndrome include frequent otitis media in the first 3 to 4 years of life, gastroesophageal reflux, hernias, and occasional joint dislocations; seizures are reported in approximately 16% of male and less than 5% of female patients.[3,4] There is a spectrum of behavioral, cognitive, and physical changes associated with fragile X syndrome, owing in part to FMRP levels in blood, which reflect levels in all tissues, including the brain, and can vary according to the sex of the child or the degrees of methylation and mosaicism.[5] Women and girls with a full-mutation allele produce more FMRP than their male counterparts because of the second X chromosome, which does not have the mutation. Both the FMRP levels in peripheral blood mononuclear cells and the IQ are correlated with the activation ratio, the proportion of cells in which the normal X chromosome is the active X chromosome.[6,7] Persons with both methylated and unmethylated *FMR1* alleles, termed methylation mosaicism, have higher IQs than persons with fully methylated *FMR1* alleles.[5]

Deletions or point mutations in *FMR1* are rare causes of fragile X syndrome that result from changes in the structure and function of FMRP, leading to a phenotype similar to that of fragile X syndrome caused by a full mutation.[8] However, this phenotype is variable and often more severe than that associated with the full mutation. Although persons with this fragile X syndrome phenotype represent approximately 1% of all persons with fragile X syndrome, as whole-genome and whole-exome sequencing become more common, they may be identified more frequently.

PREMUTATION CONDITIONS

FRAGILE X–ASSOCIATED TREMOR–ATAXIA SYNDROME

Premutation is far more common than full mutation, occurring in 1 in 159 to 200 female and about 1 in 400 male persons in the general population.[9] FXTAS, one of the most common single-gene

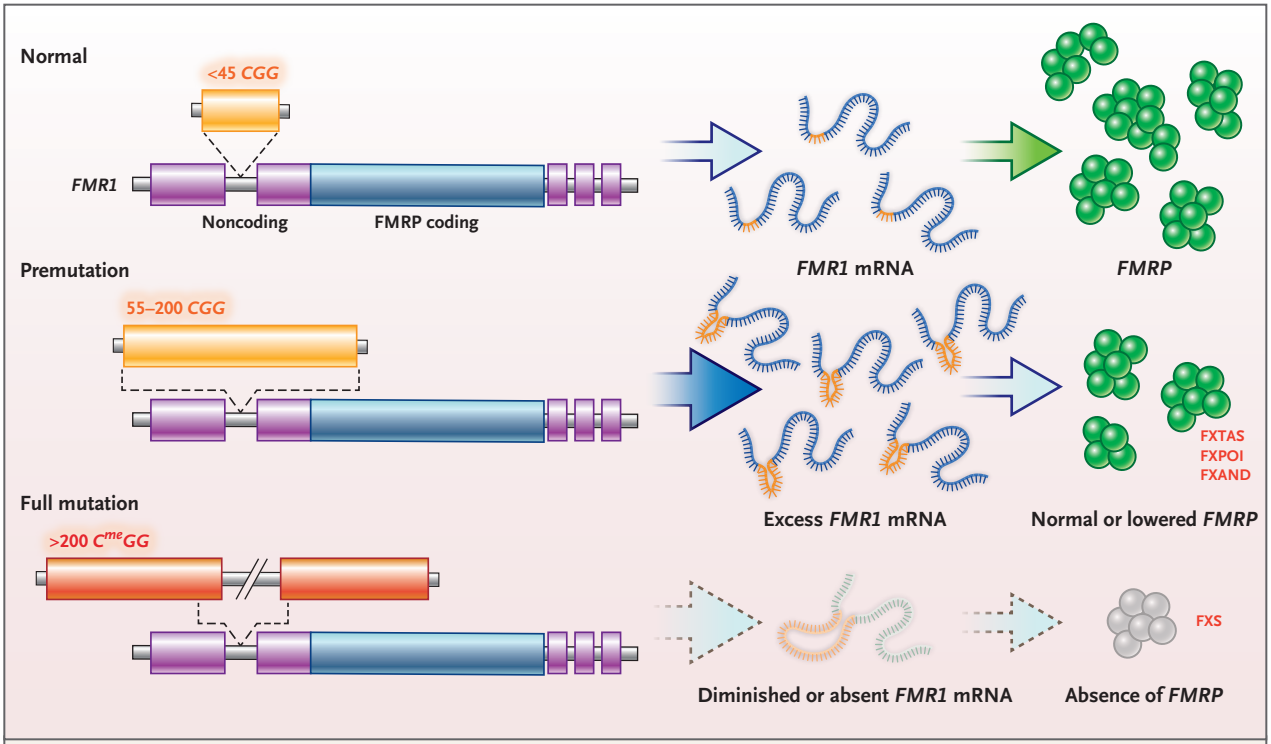

Figure 1. CGG Repeats in *FMR1* Expression.

Premutation alleles comprise between 55 and 200 repeats. The promoter region is generally unmethylated, so protein levels are generally normal or are moderately diminished in the upper premutation range owing to translational inhibition by the repeat itself. However, transcriptional activity is substantially increased, leading to toxicity of the messenger RNA (mRNA) containing the CGG repeats. For alleles with more than 200 CGG repeats, the promoter region is generally hypermethylated ($C^{me}GG$) and transcriptionally silenced, thus preventing the production of *FMR1*, the gene encoding fragile X messenger ribonucleoprotein 1 (FMRP). Key phenotypic features of premutation and full mutation conditions are shown. FXAND denotes fragile X–associated neuropsychiatric disorders, FXPOI fragile X–associated polycystic ovarian insufficiency, FXS fragile X syndrome, and FXTAS fragile X–associated tremor–ataxia syndrome.

forms of adult-onset neurodegeneration, generally manifests with gait ataxia or intention tremor (or both) along with features of progressive cognitive decline, neuropathy, and dysautonomia.[9] Although premutation conditions appear to arise through CGG-repeat RNA toxicity, the mechanisms by which the expanded CGG-repeat RNA triggers the disease process probably involve multiple processes (see Section S1 in the Supplementary Appendix).

The onset of FXTAS generally occurs after 50 years of age, and the mean age at onset is 62 years (overall penetrance of 40%, increasing with age to penetrance of 75% among men 80 years of age or older[10]) and among 8 to 16% of female carriers.[9,11] In addition to the core features of gait ataxia and tremor, dementia develops in 50% of men with FXTAS but is seen in far fewer women with the syndrome.[9] Brain atrophy and white-matter changes on magnetic resonance imaging typically are found in the middle cerebellar peduncles (in 60% of men with FXTAS), in the splenium of the corpus callosum (in 64% of women with the syndrome), and in the periventricular areas.[12] As the disease progresses, atrophy and white-matter disease can extend into frontal, parietal, and occipital areas, and ultimately the ventricles become enlarged (Fig. 2). Peripheral neuropathy that manifests in the legs and feet occurs in most persons with FXTAS, and parkinsonian features such as a resting tremor and shuffling gait occur in approximately 30% of those affected.[13] Neuropathologic features of FXTAS include spongiform changes in the white matter, eosinophilic-positive and tau-negative intranuclear inclusions in neurons, and astrocytes that contain proteins that have undergone ubiquitination and sumoylation (in which small ubiq-

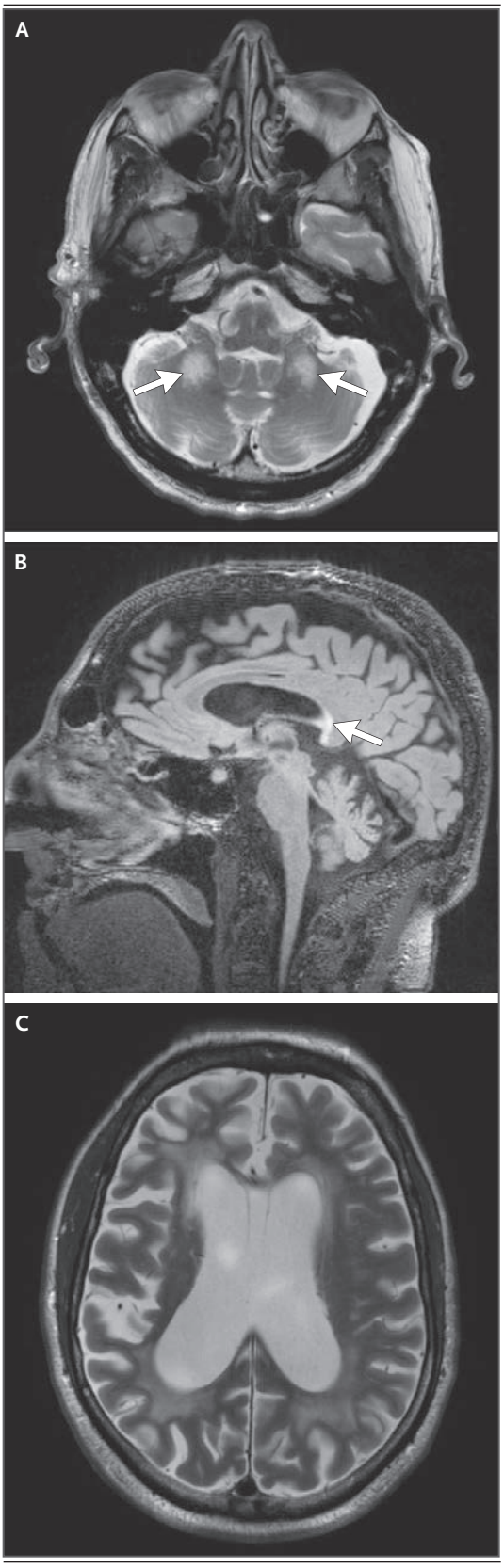

Figure 2. Features of Fragile X–Associated Tremor–Ataxia Syndrome (FXTAS) on MRI.

Shown are a T2-weighted MRI scan of the middle cerebellar peduncle with signal change (Panel A, arrow; axial view), signal change in the splenium of the corpus callosum (Panel B, arrow; sagittal view) on fluid-attenuated inversion recovery (FLAIR) imaging, and enlarged ventricles and white matter changes in a 65-year-old man with the syndrome (Panel C, axial view) on T2-weighted MRI.

uitin-like modifiers attach to proteins), neurofilaments, and other components.[14] Proteomic analyses in persons with FXTAS suggest the presence of proteolytic degradation of nuclear proteins, probably a result of increased oxidative stress.[14] RNA and repeat associated non-AUG (RAN) toxicity in premutation carriers leads to mitochondrial dysfunction, enhanced oxidative stress, and calcium ion dysregulation — effects that eventually lead to neuronal and astrocyte death.[9,15] Neuropathological studies are described in Section S2.

FRAGILE X—ASSOCIATED PRIMARY OVARIAN INSUFFICIENCY

FXPOI manifests in approximately 20% of female premutation carriers before 40 years of age and is the most common hereditary form of ovarian failure or insufficiency (usually menopause before age 40). Although the disorder was formerly termed fragile X–associated primary ovarian failure, there have been several cases in which the carrier was able to conceive after a diagnosis of ovarian failure; therefore, it was renamed fragile X–associated primary ovarian insufficiency. For unknown reasons, the disorder is most likely to occur in a range of CGG repeats between 75 and 100.[16,17] There is no specific treatment, but hormone replacement therapy has been recommended.[18] DNA testing for fragile X is appropriate in women with infertility or ovarian insufficiency if a family history of ovarian insufficiency exists, because premutation occurs in up to 7% of women in this group. Diagnosis of FXPOI in one family member can lead to the identification of other persons in the family with premutation or full-mutation involvement, such as FXTAS in her father or mother or fragile X syndrome in her children, nephews, or nieces. An established diagnosis of FXPOI or fragile X syndrome provides the opportunity for genetic counseling and alternative options for reproduction. Pregnancy termi-

nation has reduced the prevalence of fragile X syndrome in Australia and Israel, where screening for *FMR1* mutations has been common.[19]

OTHER FRAGILE X PREMUTATION–ASSOCIATED CONDITIONS

Fragile X–associated neuropsychiatric disorders include depression, anxiety, insomnia, obsessive–compulsive behavior, chronic fatigue, and chronic pain.[20,21] One or more of these disorders occurs in approximately 50% of female carriers who present clinically or have participated in studies; persons should meet *Diagnostic and Statistical Manual of Mental Disorders*, fifth edition (DSM-5), criteria for specific psychiatric disorders and have a premutation for there to be a diagnosis of fragile X–associated neuropsychiatric disorders. The term "fragile X premutation–associated conditions" — a name that includes "condition" instead of "disorder" — is used if the emotional problem is less severe than a disorder defined by the DSM-5, and it is the term preferred by many fragile X carriers.

Neuropsychiatric symptoms often occur in childhood in persons who are carriers,[9] most commonly anxiety in both male and female carriers[22]; attention deficit–hyperactivity disorder (ADHD) is also common in both childhood and adulthood.[23,24] Among patients with the premutation who are probands presenting with ADHD and anxiety or autism, reported rates of fragile X–associated neuropsychiatric disorders are higher than those in the broader group of carriers who do not present clinically.[24] Therefore, the overall prevalence of these problems is not known because unbiased samples are difficult to obtain.

Medical disorders occur at a higher rate among premutation carriers than in the general population. These conditions include hypertension, migraine headaches, and autoimmune disorders (including autoimmune thyroiditis and fibromyalgia; cases of lupus and multiple sclerosis also have been reported).[9,25]

DIAGNOSTIC TESTING AND GENETIC COUNSELING

There are two key reasons to make the diagnosis when signs are suggestive of a fragile X disorder. First, these disorders are inherited in an X-linked pattern, and there have been no spontaneous disease-forming repeat expansions, but the premutation and the full mutation are usually widespread in the family tree. Therefore, testing and genetic counseling provide information regarding the risk of having children affected by fragile X syndrome or premutation involvement. If a man has the premutation, he will pass the premutation, by means of his one X chromosome, to any daughters he may have; the CGG repeat will never expand to the full mutation during paternal transmission. In contrast, a female carrier has an approximately 50% chance of passing the expanded-repeat allele mutation to her offspring; if the transmitted expansion has more than 90 CGG repeats, the premutation will expand to the full mutation in her child. However, in the normal and premutation repeat ranges, the CGG repeats are usually punctuated by 1 to 3 AGG sequences, with a spacing of 9 or 10 CGG repeats, and these interruptions are associated with a lower risk that the mother's mutation will expand to a full mutation.[26] The second reason for diagnostic testing is that treatment — including lifestyle changes, as discussed below — may lower the risk of clinical disorders associated with the premutation, and medications can help alleviate tremor. Medications that are used to treat tremor include primidone, propranolol, gabapentin, and sometimes carbidopa–levodopa (if parkinsonian features are present).[9,27] The use of memantine and intravenous allopregnanolone have been studied in FXTAS, without substantial reversal of symptoms. Although alternative treatments such as deep-brain stimulation can alleviate tremor, some case studies have shown that ataxia has worsened after surgery.[28]

TREATMENT OF FRAGILE X SYNDROME

The Food and Drug Administration has not approved treatments specifically for fragile X syndrome. Stimulant medications may be used to treat patients with ADHD, and a selective serotonin-reuptake inhibitor can be used in the treatment of anxiety, which is present in almost all persons with fragile X syndrome.[29] Anxiety can lead to aggression in 30 to 40% of male and approximately 20% of female persons with fragile X syndrome, but use of an atypical antipsychotic, such as aripiprazole, has been reported to reduce aggression and anxiety.[30]

The absence or deficiency of FMRP in fragile

X syndrome can dysregulate many pathways because the protein regulates the translation of hundreds of mRNAs in the central nervous system. In the absence of FMRP, the mGluR5 pathway is overly active, and multiple ionic channels become dysregulated. Enhanced excitability in the nervous system results in patients having difficulty in habituating to sensory stimuli.[31] Although animal models of fragile X syndrome have shown a response to treatment with an mGluR5 antagonist, studies have failed to show a benefit of these drugs in patients with fragile X syndrome.[32]

Some promise has been shown in studies of cannabidiol (CBD) in a gel form, possibly owing to secondary effects on the GABA system. In a 12-week randomized, controlled trial of topical CBD, the agent was not effective in the overall group of patients with fragile X syndrome.[33] However, a secondary analysis that included patients who had more than 90% methylation efficacy suggested that CBD was associated with greater reductions in social avoidance, anxiety, and disruptive behaviors than placebo. An international multicenter study of CBD in fragile X syndrome has recently completed the active phase (ClinicalTrials.gov number, NCT04977986).

The phosphodiesterase-4D inhibitor zatolmilast (BPN14770) is an investigational treatment for fragile X syndrome that inhibits the enzyme that breaks down cyclic AMP (cAMP).[34] It is hypothesized that as cAMP levels rise, neuronal connectivity is enhanced. A study involving 30 adults with fragile X syndrome showed improvement in language and daily functioning as measured with caregiver ratings and improvements in scores on the National Institutes of Health Toolbox Cognition Battery of Oral Reading Recognition, Picture Vocabulary, and Cognition Crystallized Composite assessments.[34] This agent is currently in phase 3 trials (NCT05358886 [adults with fragile X syndrome] and NCT05163808 [adolescents with the syndrome]).

Metformin, used as a treatment for type 2 diabetes, has been shown to be efficacious in murine and *Drosophila* models of fragile X syndrome[35,36] and could theoretically be of benefit in the treatment for fragile X syndrome, although results of a controlled trial are not yet available (NCT03479476). Metformin lowers the activity of the mTOR (mammalian target of rapamycin) pathway, which is up-regulated in fragile X syndrome.[36] The drug has been studied in limited open-label trials involving patients with fragile X syndrome,[37,38] and a follow-up study involving 26 patients with fragile X syndrome who were treated for 1 to 3 years has shown stabilization of typical declines in IQ and adaptive behavior.[39]

GENOMIC INTERVENTIONS AND FUTURE DIRECTIONS

The development of targeted treatments for fragile X syndrome and premutation conditions awaits potential gene therapy or direct delivery of FMRP. CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats and associated Cas9 endonuclease) technology has been used to eliminate methylation and lower the CGG-repeat number in human induced pluripotent stem cells (iPSCs) to reactivate *FMR1*.[40,41] One group has used nuclease-dead Cas9 combined with Tet 1 as an editing tool to demethylate cytosines, eliminating the methylation and reactivating the gene after insertion in iPSCs with a lentivirus in vitro.[42] This approach rescued the abnormal phenotype of the fragile X neurons, which, when transplanted into the brain of a mouse with the fragile X mutation, resulted in functioning neurons.[42] Other investigators have developed a modified short amino acid sequence FMRP that is tagged with a TAT (transactivator of transcription protein, which acts as a cell-penetrating peptide) peptide tail.[43,44] These investigators showed that the TAT–FMRP protein can enter the central nervous system after a peripheral intravenous infusion to normalize the fragile X syndrome phenotype of the knockout mouse.[43,44] Alternatively, an adeno-associated virus has been shown to carry FMRP into brain cells after it was injected into the cerebrospinal fluid in knockout mice.[45,46] Antisense oligonucleotides have also shown potential in animal models for the targeted treatment of FXTAS, including modulation of FMRpolyG production and activity, reducing the toxicity of the CGG-repeat RNA.[47,48]

SUMMARY

The spectrum of fragile X disorders is complex — both with respect to the varied forms of clinical presentation (typically encompassing cognitive neurodevelopmental and behavioral features of fragile X syndrome, but also ovarian insufficiency in women who carry the premutation allele [FXPOI] and neuropsychiatric [fragile X–associ-

ated neuropsychiatric disorder] and neurodegenerative [FXTAS] conditions in older premutation carriers) and to the entirely distinct molecular mechanisms that underlie the clinical presentations across the fragile X spectrum. Premutation disorders should be distinguished from full-mutation disorders so that the family of a child with fragile X syndrome will not be confused and will not worry that FXTAS will develop in their child. Understanding the mechanisms underlying the disorders of the fragile X spectrum will guide further the development of targeted treatments.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

AUTHOR INFORMATION

[1] MIND Institute, University of California Davis Health, Sacramento; [2] Department of Pediatrics, School of Medicine, University of California, Davis, Sacramento; [3] Department of Biochemistry and Molecular Medicine, School of Medicine, University of California, Davis, Davis.

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