Molecular characterisation and assessment of clinical significance of small fragile X alleles

Mahmoud Shekari Khaniani¹, Sima Mansoori Derakhshan¹

Abstract

BACKGROUND: Fragile X syndrome is a genetic mental retardation syndrome caused by an unstable mutation in the fragile X mental retardation 1 gene (FMR1) on the X chromosome. FMR1 CGG repeat alleles are categorized according to number as normal, intermediate, premutation, and full mutation alleles. Considerable information is available, from reported studies, on the structure of the full mutation alleles.

METHODS: This review focused on the characterization of FMR1 CGG repeat size alleles in the premutation and intermediate ranges.

RESULTS: The premutation and intermediate carriers, previously thought to be clinically unaffected, are recently known to be at increased risk of premature ovarian failure (POF), fragile X-associated tremor/ataxia syndrome (FXTAS), autism, emotional problems, late-onset neurodegenerative deficits, and neurocognitive deficits. A number of studies have suggested that the underlying cause might be RNA toxicity resulting from abnormally high levels of FMR1 mRNA in these alleles.

CONCLUSIONS: It can be concluded that abnormality of FMR1 gene has different clinical presentations, especially in small alleles, and should be considered more by physicians in clinics.

KEYWORDS: FMR1 Gene, FXS, Permutation alleles, POF, FXTAS


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Introduction

Fragile X syndrome (FXS) (OMIM #309550) is a genetic mental retardation syndrome caused by an unstable mutation in the fragile X mental retardation 1 gene (FMR1) on the X chromosome.¹-³ FXS, also known as Martin-Bell syndrome, is an X-linked semi-dominant disorder affecting a high proportion of carrier females with full penetrance in males.⁴,⁵

The molecular advances of FMR1 gene, during recent years, and subsequent molecular-clinical correlations have shown that variation in the clinical phenotype is related to changes in the FMR1 gene; changes such as lack of methylation, the presence of mosaicism, or variation in the activation ratio (the percentage of cells with the normal X as the active X).⁶,⁷ Almost all mutations are trinucleotide CGG repeat expansions in the 5’-untranslated region of FMR1. The most-affected individuals have a full mutation (more than 200 CGG repeats) that usually causes inactivation of FMR1 leading to a deficit in production of FMR1 protein (FMRP).

The clinical presentation of FXS is broad, including mental retardation, learning disabilities, autism, and psychiatric problems. Females generally present with milder

¹- Assistant Professor, Department of Medical Genetics, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
Corresponding Author: Mahmoud Shekari Khaniani, MD, PhD, Email: Mahmoud.khaniani@gmail.com
symptoms of the disease. The first clinical clue in children is usually delayed attainment of one or more developmental milestones. The main clinical manifestation in preadolescent males is mental retardation; in post pubescent males, additional features include increased head size, large ears, macroorchidism, elongated and narrow faces, and mild skeletal defects. The symptoms and signs of mental retardation are variable from mild to severe, and depend on the age group of the selected cases. The phenotype of individuals with the full mutation is characterized by the presence of distinctive neuropsychological deficits that are not always proportional to the global impairment. In males, these deficits concern visuospatial ability, the processing of sequential information, and attention skills, and a deviant, repetitive, or litany speech pattern. In females, specific neuropsychological impairments include attention and concentration skills and visuospatial abilities. Their behavior is characterized by autistic spectrum, attention deficits, hand flapping, hand biting, hyperactivity, and anxiety.

The genetics of FXS show some unusual features. Pedigrees are notable for the presence of phenotypically normal transmitting males who transmit premutations to all daughters who in turn transmit larger mutations to their offspring. Often, half of all male children of these mothers show clinical FXS. Magnetic resonance imaging (MRI) in males has shown the size of the cerebral posterior vermis to be decreased, the hippocampus enlarged, and the fourth ventricle increased.

**The molecular biology of FMR1 gene**

The FMR1 gene was identified and sequenced in 1991 through an international collaborative effort. It is located on the X chromosome at cytogenetic band Xq27.3 and collocates to a fragile site termed FRAXA. In normal individuals, FMR1 spans 38 kb of DNA sequence and encodes a 4.4 kb transcript consisting of 17 exons. A notable feature of FMR1 is a repeat element of the triplet CGG which is located in the 5' untranslated region of exon 1.

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FMR1 was one of the first genes in which dynamic mutation or trinucleotide repeat expansion was discovered; whereby CGG triplet number increases from a few to thousands in mutant alleles. FMR1 CGG repeat alleles are categorized according to number as normal, intermediate, premutation, and full mutation. The normal or common size allele consists of between 5 and 40 CGG repeats and these appear, as far as can be determined in studies of up to three or four generations, to be transmitted from parent to offspring in a stable manner. There is also a larger intermediate or grey zone class in the overlap between normal and small premutation alleles. These alleles have approximately 40-60 CGG repeats (definitions vary at the lower end from 35 repeats) and may or may not be transmitted in a stable manner during transmission. In rare instances, decrease in size of a premutation when transmission occurs through either a male or a female, has also been observed. Full mutation alleles
range from > 200 to several thousand CGG repeats. These alleles are unstable during transmission and are usually abnormally methylated.28,29,33 There is no linear correlation between the number of CGG repeats greater than 200, and clinical severity. Most males with full mutation are mentally retarded. Although rare, incomplete methylated full mutations have been observed in males with normal IQs.34-36 The level of FMRP is most likely related to the variability of cognitive involvement in males with full mutation.37,38

In males with full mutation, transcription is blocked due to gene silencing resulting from methylation. Approximately 53-71% of females with full mutation have IQs in the borderline or mentally retarded range.37 Those with a normal IQ may have learning disabilities or emotional problems.3 The variable expression in females with a full mutation is not fully understood; however, some studies have found a correlation between IQ and X activation ratios.39,40

An estimated 12% of females and 6% of males with full mutation are mosaics; meaning that some of their cells contain a methylated full mutation, whereas other cells contain an unmethylated premutation.36 Mental retardation is not uncommon among mosaic males.36,41 However, mosaic males with IQs in the normal range have also been reported. The mean IQ score for these males has been shown to be higher than those males who carry only the full mutation.

In addition to the CGG expansion mutations, very rare deletions and point mutations have been reported.18,29,42 Individuals with these appear to be phenotypically indistinguishable from those with CGG expansion.43

The FMR1 CGG tracts are usually interrupted by one or two AGG triplets every 9, 10, 11, or 12 CGG repeats in the normal range, whereas fragile X alleles show the loss of one or both of these interruptions in the CGG expansion.44 Investigation of the distribution of AGG triplets within the normal FMR1 CGG tract showed 4.5% with none, 29.5% with one, 64.5% with two, and 1.5% with more than two AGG interruptions.45 In several studies, the majority of normal FMR1 alleles reported had one or two AGG interruptions, whereas in premutation and full mutation usually no interruptions were detected.46-48 This suggests that the purity of CGG tract may influence the stability of FMR1.

### The molecular biology of FMR1 protein (FMRP)

FMRP is a ribosome-associated RNA-binding protein, which is necessary for normal brain development.49-53 FMRP is predominantly located in the cytoplasm where it is bound to mRNAs and associates with translating polyribosomes.54 It appears to shuttle between the cytoplasm and the nucleus.55

FMRP was characterized as using different monoclonal and polyclonal antibodies. Multiple FMRP bands were detected with molecular weights ranging from 67 to 80 kDa; consistent with different isoforms generated by alternative splicing.56-58 Because of alternative splicing of the precursor mRNA at three different locations in the FMR1 gene, the gene can give rise to as many as 12 different molecules and thus 12 possible proteins, which differ in various internal segments yet are the same at the N- and C-terminus.56,58,59

The FMR1 gene is highly conserved among different species.18 Moreover, the murine homolog Fmr1 shows about 95% nucleic acid sequence identity and 97% identity in amino acid sequence with the human FMRP.56 The murine Fmr1 gene also contains a CGG repeat that is polymorphic between different mouse strains, with an average repeat length of 10 CGG repeats.60 The expression pattern of FMR1 at the mRNA and protein level is almost identical in various tissues of humans and mice.57,61,62 This makes the mouse a relevant animal
model in which to study the FMR1. Consequently, knockout and transgenic mice have been developed for molecular and phenotypic study of FMR1 gene.

Expression of Fmr1 transcripts was found in early mouse embryos with enrichment in the brain and gonads. Furthermore, in humans, FMRP is highly expressed in neurons, particularly in dendrites, in the adult brain and in fetal testis. A higher expression of FMRP has been documented in the human brain compared with other tissues, especially in neuron-rich areas. Moreover, the more recent studies using mouse brains have shown that FMRP is involved in synaptogenesis, especially in the cerebral cortex, cerebellum, and hippocampus, and in modifying synaptic structure in response to environmental stimulation.

It therefore appears that cognitive impairment, which is a core deficit in the FXS, is primarily caused by the deficit of FMRP. The precise physiological function of FMRP is unknown. The important and key findings in the brain of Fmr1 knockout mice and FXS patients are elongated, weak, and immature synaptic connections. These findings have led to the hypothesis that FMRP is involved in synaptogenesis and spine maturation through its role in transport and/or translational efficiency of neuronal mRNAs, including its own mRNA. It has been observed that FMRP acts as a translational repressor that is involved in synaptic plasticity through regulation of local protein synthesis of specific mRNAs in response to synaptic stimulation. There is also evidence that FMRP regulates translational neuronal mRNAs pathways.

In addition, it organizes the translation of inhibitory messages that are important for synaptic functional changes or synaptic plasticity through stimulation of the metabotropic glutamate system. The variation in CGG repeat number observed in normal individuals does not appear to interfere with the biology of FMRP.

The metabotropic glutamate receptor 5 (mGluR5) pathway is an important pathway for cognitive development. This pathway, in the absence of FMRP, causes weakened synaptic connections and eventually synaptic elimination. In addition, impaired motor learning and abnormal elongated Purkinje cells have been observed in cerebellum and spine cells in Fmr1 knockout mice. These findings have led to recent studies that have focused on novel pharmacological therapies for FXS and fragile X-associated tremor/ataxia syndrome (FXTAS) through manipulation of mGluR5.

Full mutation alleles (200 repeats) are associated with gene methylation and transcriptional silencing, which are the fundamental causes of FXS. This is a cognitive and behavioral problem with some clinical manifestation. FXTAS is a progressive neurodegenerative disorder occurring in some older permutation (55-200 CGG repeat) male carriers.

### Epidemiology of fragile X syndrome

The prevalence of FXS in the general population is about 1 in 4000 in males and 1 in 6000 in females. In the total population of mentally retarded individuals, the incidence of FXS is 6%. FXS is commonly detected among individuals with learning difficulties and the prevalence of FXS in these individuals ranges widely due to different selection criteria. The prevalence of FXS among individuals with learning difficulties is on average 2.3% (ranging from 0.3% to 16%) in males, and 0.7% (ranging from 0 to 8%) in females.

The cost for lifetime care of a moderately affected adult was £20,000 per year in 1995 in the UK. FXS does not reduce life expectancy and the costs of managing an affected individual over a lifespan have been estimated to be about £380,000 in the UK. At the present, there is no cure for FXS and treatment is supportive, requiring a multidisciplinary team. Their treatment...
includes anxiety-reducing measures, behavior modification, and medications to manage associated psychiatric disorders. Individual education plans are necessary for school-age children. It is important to diagnose affected patients as early as possible to provide early intervention and supportive care (i.e., specific developmental therapy and an individualized education plan), and to inform parents for further family planning.

**Fragile X premutation carriers**

Offspring of premutation carrier females can show small increases or decreases of the CGG repeat, or can show large expansions into the full mutation range. The daughters of transmitting males are obligatory carriers of mutations. Premutations have recently been associated with clinical abnormality. Premutation carriers, previously thought to be clinically unaffected, are now known to be at increased risk of premature ovarian failure (POF) (OMIM #311360), fragile X-associated tremor/ataxia syndrome (FXTAS) (OMIM #300623), autism (OMIM #209850), emotional problems, late-onset neurodegenerative deficits, and neurocognitive deficits. The basis for the variable clinical presentation among individuals with premutations is not known. FMRP levels have generally been thought to be normal for smaller alleles (< 100 repeats) in the premutation range, and moderately decreased for larger permutations.\(^2,8,57,82,83\) Therefore, at the level of FMRP production, the model and clinical presentation are qualitatively consistent. While FMRP levels appear to be low in the upper premutation range, due to a defect in the translation efficiency of the FMR1 gene, the mRNA levels are actually high.\(^84,85\)

It is hypothesized that the presence of elevated levels of expanded-repeat FMR1 mRNA has a toxic “gain-of-function” effect as has been proposed for the etiology of myotonic dystrophy.\(^86\) The presence of a pathology involving the premutation allele would be of great importance given the high prevalence of these alleles in the general population. The lower limit of the premutation range remains imprecise. The American College of Medical Genetics recommends that the premutation range be defined as 55-200 CGG repeats. On the basis of this definition the prevalence of the premutation is 1 in 813 males and 1 in 259 females.\(^47,78\) However, this estimate may reflect geographical differences. For example it is lower in the Asian population and higher in populations of Mediterranean origin (1/157).\(^87,88\)

The clinical manifestations of premutations, POF, and FXTAS may result from this “FMR1 mRNA poisoning” effect.\(^89-91\) It has been proposed that expansion in CGG repeat number results in elevated levels of FMR1 transcripts, which interfere with the binding of several RNA processing factors, generating novel forms of mRNA, and thus leading to functional changes in the corresponding proteins and progressive cell death.\(^92\) This mechanism has been supported by both Drosophila and premutation mouse models.\(^93,94\)

**Fragile X-associated tremor/ataxia syndrome (FXTAS)**

Fragile X-associated tremor/ataxia syndrome (FXTAS) is a progressive neurological condition occurring in some older premutation range carriers, especially males.\(^76,95-98\) This neurodegenerative disorder is completely distinct clinically and molecularly from the neurodevelopmental disorder, FXS. The core clinical features of FXTAS are progressive cerebellar gait ataxia and intention tremor. Associated manifestations include neuropsychiatric abnormalities, Parkinsonism, autonomic dysfunction, and peripheral neuropathy.\(^97,99\) Cognitive changes range from memory deficit to global dementia. In addition, anxiety and depression were reported with this disorder.\(^100,101\) Global brain atrophy associated with the presence and severity of tremor and ataxia, and CGG repeat size has been reported.\(^31,101-103\) FXTAS affects
predominantly male premutation carriers, although female carriers do occasionally have the clinical and neuropathologic features of FXTAS. The neurological symptoms are generally milder and less progressive in females than in males, presumably due to a variable degree of protection provided by the expression of FMR1 from the normal X chromosome in a percentage of cells. It is estimated that at least one-third of males (and a smaller number of females) who are carriers for premutation alleles will develop FXTAS, suggesting that as many as 1 in 3000-5000 males in the general population may have a lifetime risk of developing FXTAS.16,104,105 Thus, the disorder is likely to be the most common single gene form of tremor and ataxia in aging populations.2 More recent studies suggest that the penetrance and severity of the neurological disorder is related to the number of CGG repeats. The penetrance and severity of the neurological presentations among carriers of large premutation alleles is greater than among carriers of smaller premutation alleles.2 At present, most FXTAS is determined from study of family history of FXS or populations with movement disorders. FXTAS is thought to result from cellular toxicity FMR1 mRNA, a mechanism that is entirely distinct from that operating in FXS. Consistent with the RNA toxicity model, FXTAS is not seen in individuals with FXS full mutation, as the FMR1 mRNA is almost always reduced or absent in these individuals due to transcriptional silencing. The neuropathological hallmark of FXTAS is an intranuclear inclusion, present in both neurons and astrocytes throughout the CNS.106 FXTAS is also not associated with hypermethylation of the FMR1 promoter, transcriptional silencing, or absence of FMRP, which are typical of FXS.

**Premature ovarian dysfunction and FMR1 gene**

Premature ovarian failure (POF) is defined as cessation of menstrual periods at age 40 or younger.107,108 It is estimated that around 1% of premenopausal women in the general population will experience POF. Environmental, hormonal, metabolic, and genetic factors are involved. It has been reported that FMR1 premutation carriers exhibit a significant association with POF.109 Allingham-Hawkins et al. showed that 63/395 (16%) of premutation carriers had experienced menopause before the age of 40, and interestingly there was no POF among 128 full mutation carriers.109 In general, women who carry a premutation allele have a 20% risk of developing POF.107 Premutation carriers have an increased level of follicle-stimulating hormone (FSH), an indicator of ovarian reserve. Sullivan et al. reported a linear association between CGG repeat number and the age at menopause among carriers of low and medium repeat alleles (59-99 repeats) that appears to plateau or decrease around 100 CGG repeats.110 An association between POF and intermediate alleles is under debate. It is important that women carrying FMR1 premutations receive genetic and fertility counseling for family planning.

**Autism and the FMR1 gene**

Autism is a developmental disorder comprising a ‘triad’ of deficits; impaired social interaction, impaired communication, and restricted interests and repetitive behaviors. Autism is currently considered to be a multifactorial disorder that involves a strong genetic influence.111,112 Identification of molecular factors that contribute to the development of autism is currently an area of intense research. It is shown as part of the behavioral phenotype in several genetic disorders, including FXS, phenylketonuria (PKU), tuberous sclerosis, Rett syndrome, and duplications in chromosome 15 in the q arm.113,114 Mutations in FMR1 are the most common single genetic cause of autism-spectrum disorders with prevalence.
estimates in FMR1 full mutation and premutation populations reported as ~40% and ~18%, respectively.\textsuperscript{115} 50% to 90% of individuals with FXS have been reported to have some symptom of autism, such as hand flapping, hand biting, poor eye contact, perseveration in speech, and tactile defensiveness.\textsuperscript{112,116} There is a clear correlation between FMRP concentrations and mean scores on the child autism rating scale, with more severe autism as FMRP concentrations diminish. It has been recently reported that individuals with FXS and autism had a lower IQ than non-autistic individuals with FXS alone.\textsuperscript{117-119} Therefore individuals with autism should be routinely studied with FMR1 DNA testing to rule out a CGG expansion. In conclusion, current literature suggests some overlap between autism and FMR1 gene mechanisms. Understanding the alteration of FXS and autism may present strategies for relating autism to more single genetic syndromes. Finally, production of FMRP, which couples activation of group-1 metabotropic glutamate receptors to modifications of mRNA translation in dendritic spines, is a case in point.

\textbf{Fragile X mutations and other diseases}

Because of FMRP’s role as a translational repressor and its role in binding a pool of synaptic mRNAs, abnormalities of FMR1 transcription or translation have the potential to elicit other clinical abnormalities. A number of studies have reported the effects of FMR1 mutations on the regulation of other genes. The absence of FMRP or elevated FMR1 mRNA may affect the function of other genes.\textsuperscript{120,121} Anxiety is a common phenotypic feature of FMR1 mutations which may be related to the dysregulation of the glucocorticoid receptor whose message binds to FMRP.\textsuperscript{121} Epilepsy occurs in 13-18% of boys and 4% of girls with FXS perhaps through contribution of FMRP in the regulation process of the Gamma-aminobutyric acid (GABAa) receptors.\textsuperscript{122} It is suggested that the interactions of FMR1 with other genes are underrecognized.\textsuperscript{16}

\textbf{Intermediate FMR1 CGG alleles}

The significance of intermediate alleles (also known as ‘grey zone’ alleles) of CGG number is much less clinically understood. There are various definitions of intermediate alleles. Based on the standardization of the American College of Medical Genetics (ACMG) the range from ~45 to ~54 CGG repeats is intermediate, and based on the European Molecular of Genetics Quality Network (EMQN) the CGG range from ~50 to ~59 repeats describes intermediate alleles. Alleles in this range can be considered normal in the sense that such alleles have not been observed to expand to a full mutation in one generation, although initially minor increases in repeat number can be observed in these alleles. A number of recent studies have demonstrated the elevation of FMR1 mRNA levels in intermediate and premutation CGG allele carriers, with a lower threshold at 40 repeats.\textsuperscript{24,50,89} On this basis, intermediate alleles have been classified as 40-60 repeats throughout this study.

The frequency of intermediate alleles in various population samples is approximately 2-3%.\textsuperscript{123,124} Furthermore, intermediate alleles have recently been considered to be associated with specific clinical phenotypes.\textsuperscript{125-127}

The first report of a clinical phenotype associated with intermediate alleles was learning difficulties in special educational needs (SEN) children.\textsuperscript{123,124,128,129} Although other studies presented negative or non-significant findings, they showed increased prevalence of intermediate alleles in mentally impaired patients.\textsuperscript{128,129-132}

Two studies reported a significant increase of intermediate FMR1 alleles in POF populations, but another demonstrated only a 2% increase.\textsuperscript{110,133,134}

The basis for the increased FMR1 mRNA level is not known, but the higher levels may
be due to increased transcription.\textsuperscript{135} Furthermore, it has been reported that transcription of FMR1 gene can be initiated at multiple sites downstream of the CGG triplet repeat and these sites change with the expansion of CGG repeat number.\textsuperscript{136} An RNA toxic gain-of-function model for FXTAS was presented by Hagerman et al.\textsuperscript{16} Bodega et al. reported that AGG interruption within the FMR1 CGG tract may influence FMR1 mRNA level and clinical presentation.\textsuperscript{134} Interestingly, Bodega et al. reported that all the POF patients studied showed intermediate alleles without AGG interruptions in their CGG repeat tracts.

### Molecular screening of FMR1 mutations and genetic counseling issues

Advances in genetic testing methods and understanding of the molecular basis of FXS during the last decade have produced different strategies for identifying probands with FXS. As a relatively common and morbid condition with no new mutations (i.e. no observed transitions from the normal CGG repeat range to premutations) and the availability of effective prenatal testing, FXS fulfills the criteria for population screening. However, the lack of simple cost-effective testing has precluded this. The purposes of widespread testing of children with mental retardation, autism spectrum, and behavioral or learning disorders are: (1) to diagnose FXS patients at an early stage to provide every possible improvement in clinical management, (2) to identify carrier mothers who can be provided with family planning information and counseling for prenatal diagnosis to avoid recurrence in a subsequent pregnancy, and (3) to identify other carrier relatives through cascade testing among families which is an established approach to test relatives of FXS patients for carrier status.\textsuperscript{77} These interventions aim to achieve maximum benefits for health, education, and improved quality of life for probands, parents, and other family members.

There are several possible strategies for targeted screening for FMR1 mutations. However, it must be kept in mind that observance of the principle of individual privacy may have ethical implications for relatives in terms of disclosure when a carrier is detected, given that there are no new mutations in FXS and the risk for all first degree relatives is 1 in 2. Possible approaches are preconception screening of females of reproductive age, neonatal screening using Guthrie blood spots, and routine testing of children with learning difficulty or development delay. With the exception of newborn testing in Israel, only the latter of these is performed at present in diagnostic laboratories and even this is very restricted. The others have issues of economics, ethical, individual privacy, data confidentiality, and unknown public acceptability which need to be addressed before introduction.

Additionally, the known associations with FMR1 premutations suggest testing individuals with:

- Idiopathic cerebella ataxia, action tremor, and cognitive decline patients with onset over 50 years of ages. Ascertainment of children with FXS should also be possible through grandfathers with FXTAS, but due to the relatively recent discovery of this condition, little experience exists of this approach.
- Infertile women particularly those with increased FSH levels and women with premature ovarian failure.

Accurate diagnosis of FXS, FXTAS, and POF requires molecular identification of the associated common mutations in FMR1. Testing is performed by measuring CGG repeat number and promoter methylation status. Two main approaches are used; PCR and Southern blot analysis. PCR analysis utilizes flanking primers to amplify a fragment containing the CGG repeats. Several PCR-based diagnostic and screening methods have been developed with variable performance.\textsuperscript{126} PCR is the most suitable screening tool, but the efficiency of the PCR
reaction deteriorates with high numbers of CGG repeats due to replication slippage and problems associated with high GC-rich sequence denaturation. This, and the fact that no information is afforded about FMR1 methylation, are limitations of the PCR approach. Therefore, PCR analysis is restricted to accurate determination of CGG repeat number in the normal, intermediate, and small premutation FMR1 alleles.

FMR1 analysis by Southern blotting is a definitive diagnostic test that allows both size of the CGG repeat and methylation status to be investigated simultaneously. Southern blot analysis is performed only on samples from males which fail to amplify by PCR and from females that show a single normal allele (apparently homozygous normal females) to exclude the presence of an unamplifiable large allele. It is usually performed using the restriction enzyme EcoRI combined with a methylation-sensitive enzyme, such as EagI or NruI. Larger than normal fragments, which are usually unmethylated in premutation carriers and methylated in full mutation carriers, are indicative of mutations.

The mechanisms of FMR1 CGG repeat expansion

The increase in CGG repeat number occurs during transmission from mother to offspring.\textsuperscript{20,32,137} The mechanism of instability and the exact timing of CGG repeat are still unknown. Recently, the investigation of instability of the FMR1 gene has been a major goal in FXS research.

The CGG expansion appears to occur during meiosis or early embryonic development. Several studies have supported the claim that CGG expansion occurs prezygotically and instability occurs in the oocyte.\textsuperscript{138,139} Furthermore, repeat number is stable during long term in vitro cell culture. The exact timing of the repeat expansion in the oocytes of premutation females is still under debate. Malter et al. have also demonstrated that spermatogenesis is unable to maintain full mutation sized alleles, and especially full mutation males show normal size alleles in their sperm.\textsuperscript{139} It has been suggested that the mouse model for fragile X syndrome may offer a suitable approach to finding both the timing and the mechanism of expansion.\textsuperscript{140}

A number of mutational pathways have been proposed to explain the expansion process from a stable allele over several generations.

1) Eichler et al. reported for the first time that the alleles were shown to be unstable after losing one or two AGG interruptions within the CGG tract.\textsuperscript{141} Further studies presented that most premutation alleles have at most one AGG interruption at the 5’ end of the repeat, or none at all.\textsuperscript{46,142-144} Furthermore, the position of the CGG repeat within its sequence background might influence instability and the purity of the 3’ end of the CGG repeat and flanking markers suggested possible cis-acting factors that influence CGG repeat stability.\textsuperscript{45} Polarized variability within a continuous tract of CGG repeats (3’ end) and the fact that changes involve differences of multiples of three base pairs clearly favor slipped strand mispairing as a likely mechanism for mutation.\textsuperscript{29}

2) The risk of expansion to full mutation increases with the size of the maternal CGG repeat and the large CGG tracts are more likely to expand.\textsuperscript{141,145}

3) The secondary structure of DNA, such as hairpins and tetraplexes, has also been suggested to play a role in expansion.\textsuperscript{146}

4) It has also been hypothesized that the background haplotype of these alleles plays a role in their susceptibility to CGG expansion. Linkage disequilibrium between the CGG repeat and its flanking polymorphic markers has been reported in different geographic and ethnic populations.\textsuperscript{27,147-152}

Conclusion

Dynamic mutations which increase the number of CGG triplet repeats in the 5’-untranslated region of the gene FMR1 are
associated with a group of clinical disorders with quite different phenotypes. This review has provided preliminary evidence for the role of intermediate and permutation FMR1 alleles in the pathology of some clinical manifestations, such as Parkinsonism, neurodegenerative disorder, idiopathic autism, and neurodevelopment disorder. Recent evidence has suggested that the underlying cause might be ‘RNA toxicity’ resulting from abnormally high levels of FMR1 mRNA in these alleles. Finally, it can be concluded that abnormality of FMR1 gene has different clinical presentations and should be considered more by physicians in clinics.

Conflict of Interests
Authors have no conflict of interest.

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