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

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Review Article

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

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symptoms of the disease.[8,9] 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.[10] 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 neurocognitive deficits that are not always proportional to the global impairment.[11] In males, these deficits concern visuospatial ability, the processing of sequential information, and attention skills, and a deviant, repetitive, or litany speech pattern.[12-15] In females, specific neurocognitve impairments include attention and concentration skills and visuospatial abilities.[15] Their behavior is characterized by autistic spectrum, attention deficits, hand flapping, hand biting, hyperactivity, and anxiety.[16]

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. FXS also shows the phenomenon known as anticipation, whereby the severity of phenotype appears to worsen through subsequent generations. These features were explained by the discovery of the dynamic nature of the CGG triplet expansions in the transition from premutations to full mutations.[10]

Family physicians are most likely to encounter the undiagnosed child before school age, when formal testing can confirm cognitive deficits. The most suggestive criteria for the diagnosis of FXS are mental retardation, a family history of mental retardation, large or prominent ears, an enlarged face, attention deficit, hyperactivity disorder, and autistic-like behavior.[17] 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.[7]

The molecular biology of FMR1 gene

The FMR1 gene was identified and sequenced in 1991 through an international collaborative effort.[1,18,19] It is located on the X chromosome at cytogenetic band Xq27.3 and collocates to a fragile site termed FRAXA.[18,20,21] In normal individuals, FMR1 spans 38 kb of DNA sequence and encodes a 4.4 kb transcript consisting of 17 exons.[8,22-24] A notable feature of FMR1 is a repeat element of the triplet CGG which is located in the 5′-untranslated region of exon1.

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.[25,26] FMR1 CGG repeat alleles are categorized according to number as normal, intermediate, premutation, and full mutation.[27,28] 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.[29] 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 from parents to offspring.[30] Premutation alleles range from between 60 and 200 repeats and are usually inherited in an unstable 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.[21,31,32] 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.[62,63] Furthermore, in humans, FMRP is highly expressed in neurons, particularly in dendrites, in the adult brain and in fetal testis.[57,61] A higher expression of FMRP has been documented in the human brain compared with other tissues, especially in neuron-rich areas.[57,61] 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.[53,64-66] 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.[53,67,68] 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.[69] 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.[69,70] There is also evidence that FMRP regulates translational neuronal mRNAs pathways.[71,72] 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.[66,68,70,73] 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.[74] In addition, impaired motor learning and abnormal elongated Purkinje cells have been observed in cerebellum and spine cells in Fmr1 knockout mice.[75] 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.[16,76]

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.[16,77,78] In the total population of mentally retarded individuals, the incidence of FXS is 6%.[79,80] 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.[9]

The cost for lifetime care of a moderately affected adult was £20.000 per year in 1995 in the UK.[81] 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.[9] 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.[77]

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.[33,101-103] 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.[16,104,105] 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.[98] 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.[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.[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.[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.

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.[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.[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 acida (GABAa) receptors.[122] It is suggested that the interactions of FMR1 with other genes are underrecognized.[16]

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.[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%.[123,124] Furthermore, intermediate alleles have recently been considered to be associated with specific clinical phenotypes.[125-127]

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

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

The basis for the increased FMR1 mRNA level is not known, but the higher levels may

be due to increased transcription.[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.[136] An RNA toxic gain-of-function model for FXTAS was presented by Hagerman et al.[16] Bodega et al. reported that AGG interruption within the FMR1 CGG tract may influence FMR1 mRNA level and clinical presentation.[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.[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.[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.[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.[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.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.[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.[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.[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.[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.[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.[141,145]

3) The secondary structure of DNA, such as hairpins and tetraplexes, has also been suggested to play a role in expansion.[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.[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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References

1. Yu S, Pritchard M, Kremer E, Lynch M, Nancarrow J, Baker E, et al. Fragile X genotype characterized by an unstable region of DNA. Science 1991; 252(5009): 1179-81.
2. Jacquemont S, Hagerman RJ, Hagerman PJ, Leehey MA. Fragile-X syndrome and fragile X-associated tremor/ataxia syndrome: two faces of FMR1. Lancet Neurol 2007; 6(1): 45-55.
3. Loesch DZ, Huggins RM, Hagerman RJ. Phenotypic variation and FMRP levels in fragile X. Ment Retard Dev Disabil Res Rev 2004; 10(1): 31-41.
4. Stevenson RE, Schwartz CE, Arena JF, Lubs HA. X-linked mental retardation: the early era from 1943 to 1969. Am J Med Genet 1994; 51(4): 538-41.
5. Sutherland GR. Heritable fragile sites on human chromosomes. III. Detection of fra(X)(q27) in males with X-linked mental retardation and in their female relatives. Hum Genet 1979; 53(1): 23-7.
6. Tamanini F, Bontekoe C, Bakker CE, van UL, Anar B, Willemsen R, et al. Different targets for the fragile X-related proteins revealed by their distinct nuclear localizations. Hum Mol Genet 1999; 8(5): 863-9.
7. Mostofsky SH, Mazzocco MM, Aakalu G, Warsofsky IS, Denckla MB, Reiss AL. Decreased cerebellar posterior vermis size in fragile X syndrome: correlation with neurocognitive performance. Neurology 1998; 50(1): 121-30.
8. Hessl D, Tassone F, Loesch DZ, Berry-Kravis E, Leehey MA, Gane LW, et al. Abnormal elevation of FMR1 mRNA is associated with psychological symptoms in individuals with the fragile X premutation. Am J Med Genet B Neuropsychiatr Genet 2005; 139B(1): 115-21.
9. Song FJ, Barton P, Sleightholme V, Yao GL, Fry-Smith A. Screening for fragile X syndrome: a literature review and modelling study. Health Technol Assess 2003; 7(16): 1-106.
10. Nolin SL, Brown WT, Glicksman A, Houck GE, Jr., Gargano AD, Sullivan A, et al. Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. Am J Hum Genet 2003; 72(2): 454-64.
11. Bennetto L, Pennington BF, Porter D, Taylor AK, Hagerman RJ. Profile of cognitive functioning in women with the fragile X mutation. Neuropsychology 2001; 15(2): 290-9.
12. Crowe SF, Hay DA. Neuropsychological dimensions of the fragile X syndrome: support for a non-dominant hemisphere dysfunction hypothesis. Neuropsychologia 1990; 28(1): 9-16.
13. Freund LS, Reiss AL. Cognitive profiles associated with the fra(X) syndrome in males and females. Am J Med Genet 1991; 38(4): 542-7.
14. Loesch DZ, Huggins RM, Bui QM, Epstein JL, Taylor AK, Hagerman RJ. Effect of the deficits of fragile X mental retardation protein on cognitive status of fragile x males and females assessed by robust pedigree analysis. J Dev Behav Pediatr 2002; 23(6): 416-23.
15. Sudhalter V, Maranion M, Brooks P. Expressive semantic deficit in the productive language of males with fragile X syndrome. Am J Med Genet 1992; 43(1-2): 65-71.
16. Hagerman RJ. Lessons from fragile X regarding neurobiology, autism, and neurodegeneration. J Dev Behav Pediatr 2006; 27(1): 63-74.
17. Raymond FL. X linked mental retardation: a clinical guide. J Med Genet 2006; 43(3): 193-200.

18. Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 1991; 65(5): 905-14.

19. Bell MV, Hirst MC, Nakahori Y, MacKinnon RN, Roche A, Flint TJ, et al. Physical mapping across the fragile X: hypermethylation and clinical expression of the fragile X syndrome. Cell 1991; 64(4): 861-6.

20. Kremer EJ, Pritchard M, Lynch M, Yu S, Holman K, Baker E, et al. Mapping of DNA instability at the fragile X to a trinucleotide repeat sequence p(CCG)n. Science 1991; 252(5013): 1711-4.

21. Oberle I, Rousseau F, Heitz D, Kretz C, Devys D, Hanauer A, et al. Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. Science 1991; 252(5009): 1097-102.

22. Eichler EE, Richards S, Gibbs RA, Nelson DL. Fine structure of the human FMR1 gene. Hum Mol Genet 1993; 2(8): 1147-53.

23. Cronister AE, Hagerman RJ. Fragile X syndrome. J Pediatr Health Care 1989; 3(1): 9-19.

24. Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. Am J Hum Genet 2000; 66(1): 6-15.

25. Mirkin SM. Expandable DNA repeats and human disease. Nature 2007; 447(7147): 932-40.

26. Mirkin SM. DNA structures, repeat expansions and human hereditary disorders. Curr Opin Struct Biol 2006; 16(3): 351-8.

27. Curlis Y, Zhang C, Holden JJ, Loesch PK, Mitchell RJ. Haplotype study of intermediate-length alleles at the fragile X (FMR1) gene: ATL1, FMRb, and microsatellite haplotypes differ from those found in common-size FMR1 alleles. Hum Biol 2005; 77(1): 137-51.

28. Patsalis PC, Sismani C, Hettinger JA, Holden JJ, Lawson JS, Chalifoux M, et al. Frequencies of "grey-zone" and premutation-size FMR1 CGG-repeat alleles in patients with developmental disability in Cyprus and Canada. Am J Med Genet 1999; 84(3): 195-7.

29. Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 1991; 67(6): 1047-58.

30. Huggins RM, Loesch DZ, Sherman SL. A branching non-linear autoregressive model for the transmission of the fragile X dynamic repeat mutation. Ann Hum Genet 1998; 62(Pt 4): 337-47.

31. Heitz D, Devys D, Imbert G, Kretz C, Mandel JL. Inheritance of the fragile X syndrome: size of the fragile X premutation is a major determinant of the transition to full mutation. J Med Genet 1992; 29(11): 794-801.

32. Nolin SL, Lewis FA, III, Ye LL, Houck GE, Jr., Glicksman AE, Limprasert P, et al. Familial transmission of the FMR1 CGG repeat. Am J Hum Genet 1996; 59(6): 1252-61.

33. Loesch DZ, Churchyard A, Brotchie P, Marot M, Tassone F. Evidence for, and a spectrum of, neurological involvement in carriers of the fragile X pre-mutation: FXTAS and beyond. Clin Genet 2005; 67(5): 412-7.

34. McConkie-Rosell A, Lachiewicz AM, Spiridigliozzi GA, Tarleton J, Schoenwald S, Phelan MC, et al. Evidence that methylation of the FMR-I locus is responsible for variable phenotypic expression of the fragile X syndrome. Am J Hum Genet 1993; 53(4): 800-9.

35. Hagerman RJ, Hull CE, Safanda JF, Carpenter I, Staley LW, O'Connor RA, et al. High functioning fragile X males: demonstration of an unmethylated fully expanded FMR-1 mutation associated with protein expression. Am J Med Genet 1994; 51(4): 298-308.

36. Rousseau F, Robb LJ, Rouillard P, Der Kaloustian VM. No mental retardation in a man with 40% abnormal methylation at the FMR-1 locus and transmission of sperm cell mutations as premutations. Hum Mol Genet 1994; 3(6): 927-30.

37. Taylor AK, Tassone F, Dyer PN, Hersch SM, Harris JB, Greenough WT, et al. Tissue heterogeneity of the FMR1 mutation in a high-functioning male with fragile X syndrome. Am J Med Genet 1999; 84(3): 233-9.

38. Tassone F, Hagerman RJ, Ikle DN, Dyer PN, Lampe M, Willemsen R, et al. FMRP expression as a potential prognostic indicator in fragile X syndrome. Am J Med Genet 1999; 84(3): 250-61.

39. Abrams MT, Reiss AL, Freund LS, Baumgardner TL, Chase GA, Denckla MB. Molecular-neurobehavioral associations in females with the fragile X full mutation. Am J Med Genet 1994; 51(4): 317-27.

40. Sobesky WE, Taylor AK, Pennington BF, Bennetto L, Porter D, Riddle J, et al. Molecular/clinical correlations in females with fragile X. Am J Med Genet 1996; 64(2): 340-5.

41. Wiegers AM, DeVries LB, Curfs LM, Fryns JP. Identical psychological profile and behaviour pattern in different types of mutation in the FMR-1 region. Clin Genet 1993; 43(6): 326-7.

42. Sherman S, Pletcher BA, Driscoll DA. Fragile X syndrome: diagnostic and carrier testing. Genet Med 2005; 7(8): 584-7.

43. Mannermaa A, Pulkkinen L, Kajanoja E, Ryynanen M, Saarikoski S. Deletion in the FMR1 gene in a fragile-X male. Am J Med Genet 1996; 64(2): 293-5.

44. Zhong N, Kajanoja E, Smits B, Pietrofesa J, Curley D, Wang D, et al. Fragile X founder effects and new mutations in Finland. Am J Med Genet 1996; 64(1): 226-33.
45. Eichler EE, Macpherson JN, Murray A, Jacobs PA, Chakravarti A, Nelson DL. Haplotype and interspersion analysis of the FMR1 CGG repeat identifies two different mutational pathways for the origin of the fragile X syndrome. Hum Mol Genet 1996; 5(3): 319-30.
46. Kunst CB, Warren ST. Cryptic and polar variation of the fragile X repeat could result in predisposing normal alleles. Cell 1994; 77(6): 853-61.
47. Dombrowski C, Levesque S, Morel ML, Rouillard P, Morgan K, Rousseau F. Premutation and intermediate-size FMR1 alleles in 10572 males from the general population: loss of an AGG interruption is a late event in the generation of fragile X syndrome alleles. Hum Mol Genet 2002; 11(4): 371-8.
48. Gunter C, Paradee W, Crawford DC, Meadows KA, Newman J, Kunst CB, et al. Re-examination of factors associated with expansion of CGG repeats using a single nucleotide polymorphism in FMR1. Hum Mol Genet 1998; 7(12): 1935-46.
49. Lim JH, Luo T, Sargent TD, Fallon JR. Developmental expression of Xenopus fragile X mental retardation-1 gene. Int J Dev Biol 2005; 49(8): 981-4.
50. Kenneson A, Zhang F, Hagedorn CH, Warren ST. Reduced FMRP and increased FMR1 transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. Hum Mol Genet 2001; 10(14): 1449-54.
51. Darnell JC, Fraser CE, Mostovetsky O, Stefani G, Jones TA, Eddy SR, et al. Kissing complex RNAs mediate interaction between the Fragile-X mental retardation protein KH2 domain and brain polyribosomes. Genes Dev 2005; 19(8): 903-18.
52. Darnell JC, Mostovetsky O, Darnell RB. FMRP RNA targets: identification and validation. Genes Brain Behav 2005; 4(6): 341-9.
53. Irwin SA, Idupulapati M, Gilbert ME, Harris JB, Chakravarti AB, Rogers EJ, et al. Dendritic spine and dendritic field characteristics of layer V pyramidal neurons in the visual cortex of fragile-X knockout mice. Am J Med Genet 2002; 111(2): 140-6.
54. Feng Y, Absher D, Eberhart DE, Brown V, Malter HE, Warren ST. FMRP associates with polyribosomes as an mRNP, and the I304N mutation of severe fragile X syndrome abolishes this association. Mol Cell 1997; 1(1): 109-18.
55. Eberhart DE, Malter HE, Feng Y, Warren ST. The fragile X mental retardation protein is a ribonucleoprotein containing both nuclear localization and nuclear export signals. Hum Mol Genet 1996; 5(8): 1083-91.
56. Ashley CT, Sutcliffe JS, Kunst CB, Leiner HA, Eichler EE, Nelson DL, et al. Human and murine FMR-1: alternative splicing and translational initiation downstream of the CGG-repeat. Nat Genet 1993; 4(3): 244-51.
57. Devys D, Lutz Y, Rouyer N, Bellocq JP, Mandel JL. The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. Nat Genet 1993; 4(4): 335-40.
58. Verheij C, Bakker CE, de GE, Keulemans J, Willemsen R, Verkerk AJ, et al. Characterization and localization of the FMR-1 gene product associated with fragile X syndrome. Nature 1993; 363(6431): 722-4.
59. Verkerk AJ, de GE, De BK, Eichler EE, Konecki DS, Reyniers E, et al. Alternative splicing in the fragile X gene FMR1. Hum Mol Genet 1993; 2(8): 1348.
60. Hagerman RJ, Hagerman PJ. Fragile X Syndrome: Diagnosis, Treatment, and Research. Baltimore, MD: Johns Hopkins University Press; 2002.
61. Abitbol M, Menini C, Delezoide AL, Rhyner T, Vekemans M, Mallet J. Nucleus basalis magnocellularis and hippocampus are the major sites of FMR-1 expression in the human fetal brain. Nat Genet 1993; 4(2): 147-53.
62. Hinds HL, Ashley CT, Sutcliffe JS, Nelson DL, Warren ST, Housman DE, et al. Tissue specific expression of FMR-1 provides evidence for a functional role in fragile X syndrome. Nat Genet 1993; 3(1): 36-43.
63. Bakker CE, de Diego OY, Bontekoe C, Raghoe P, Luteijn T, Hoogeveen AT, et al. Immunocytochemical and biochemical characterization of FMRP, FXR1P, and FXR2P in the mouse. Exp Cell Res 2000; 258(1): 162-70.
64. Willemsen R, Mohkamsing S, de VB, Devys D, van den Ouweland A, Mandel JL, et al. Rapid antibody test for fragile X syndrome. Lancet 1995; 345(8958): 1147-8.
65. Mineur YS, Sluyter F, de WS, Oostra BA, Crusio WE. Behavioral and neuroanatomical characterization of the Fmr1 knockout mouse. Hippocampus 2002; 12(1): 39-46.
66. Weiler IJ, Greenough WT. Synaptic synthesis of the Fragile X protein: possible involvement in synapse maturation and elimination. Am J Med Genet 1999; 83(4): 248-52.
67. Beckel-Mitchener A, Greenough WT. Correlates across the structural, functional, and molecular phenotypes of fragile X syndrome. Ment Retard Dev Disabil Res Rev 2004; 10(1): 53-9.
68. Willemsen R, Oostra BA, Bassell GJ, Dictenberg J. The fragile X syndrome: from molecular genetics to neurobiology. Ment Retard Dev Disabil Res Rev 2004; 10(1): 60-7.

69. Laggerbauer B, Ostareck D, Keidel EM, Ostareck-Lederer A, Fischer U. Evidence that fragile X mental retardation protein is a negative regulator of translation. Hum Mol Genet 2001; 10(4): 329-38.

70. Zalfa F, Giorgi M, Primerano B, Moro A, Di PA, Reis S, et al. The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. Cell 2003; 112(3): 317-27.

71. Siomi H, Ishizuka A, Siomi MC. RNA interference: a new mechanism by which FMRP acts in the normal brain? What can Drosophila teach us? Ment Retard Dev Disabil Res Rev 2004; 10(1): 68-74.

72. Jin P, Zarnescu DC, Ceman S, Nakamoto M, Mowrey J, Jongens TA, et al. Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. Nat Neurosci 2004; 7(2): 113-7.

73. Lu R, Wang H, Liang Z, Ku L, O'donnell WT, Li W, et al. The fragile X protein controls microtubule-associated protein 1B translation and microtubule stability in brain neuron development. Proc Natl Acad Sci U S A 2004; 101(42): 15201-6.

74. Huber KM, Gallagher SM, Warren ST, Bear MF. Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc Natl Acad Sci U S A 2002; 99(11): 7746-50.

75. Koekkoek SK, Yamaguchi K, Milojkovic BA, Dortland BR, Ruigrok TJ, Maex R, et al. Deletion of FMR1 in Purkinje cells enhances parallel fiber LTD, enlarges spines, and attenuates cerebellar eyelid conditioning in Fragile X syndrome. Neuron 2005; 47(3): 339-52.

76. Berry-Kravis E, Potanos K. Psychopharmacology in fragile X syndrome--present and future. Ment Retard Dev Disabil Res Rev 2004; 10(1): 42-8.

77. McConkie-Rosell A, Abrams L, Finucane B, Cronister A, Gane LW, Coffey SM, et al. Recommendations from multi-disciplinary focus groups on cascade testing and genetic counseling for fragile X-associated disorders. J Genet Couns 2007; 16(5): 593-606.

78. Rousseau F, Rouillard P, Morel ML, Khandjian EW, Morgan K. Prevalence of carriers of premutation-size alleles of the FMRI gene--and implications for the population genetics of the fragile X syndrome. Am J Hum Genet 1995; 57(5): 1006-18.

79. Murray A, Macpherson JN, Pound MC, Sharrock A, Youings SA, Dennis NR, et al. The role of size, sequence and haplotype in the stability of FRAXA and FRAXE alleles during transmission. Hum Mol Genet 1997; 6(2): 173-84.

80. Sherman SL, Meadows KL, Ashley AE. Examination of factors that influence the expansion of the fragile X mutation in a sample of conceptuses from known carrier females. Am J Med Genet 1996; 64(2): 256-60.

81. Pembrey ME, Barnicoat AJ, Carmichael B, Bobrow M, Turner G. An assessment of screening strategies for fragile X syndrome in the UK. Health Technol Assess 2001; 5(7): 1-95.

82. Feng Y, Lakkis L, Devys D, Warren ST. Quantitative comparison of FMR1 gene expression in normal and premutation alleles. Am J Hum Genet 1995; 56(1): 106-13.

83. Tassone F, Hagerman RJ, Gane LW, Taylor AK. Strong similarities of the FMR1 mutation in multiple tissues: postmortem studies of a male with a full mutation and a male carrier of a premutation. Am J Med Genet 1999; 84(3): 240-4.

84. Tassone F, Hagerman RJ, Chamberlain WD, Hagerman PJ. Transcription of the FMR1 gene in individuals with fragile X syndrome. Am J Med Genet 2000; 97(3): 195-203.

85. Hessl D, Rivera S, Koldewyn K, Cordeiro L, Adams J, Tassone F, et al. Amygdala dysfunction in men with the fragile X premutation. Brain 2007; 130(Pt 2): 404-16.

86. Ranum LP, Day JW. Myotonic dystrophy: clinical and molecular parallels between myotonic dystrophy type 1 and type 2. Curr Neurol Neurosci Rep 2002; 2(5): 465-70.

87. Tzeng CC, Tsai LP, Hwu WL, Lin SJ, Chao MC, Jong YJ, et al. Prevalence of the FMR1 mutation in Taiwan assessed by large-scale screening of newborn boys and analysis of DXS548-FRAXAC1 haplotype. Am J Med Genet A 2005; 133A(1): 37-43.

88. Toledano-Alhadef H, Basel-Vanagaite L, Magal N, Davidov B, Ehrlich S, Drasinover V, et al. Fragile-X carrier screening and the prevalence of premutation and full-mutation carriers in Israel. Am J Hum Genet 2001; 69(2): 351-60.

89. Loesch DZ, Bui QM, Huggins RM, Mitchell RJ, Hagerman RJ, Tassone F. Transcript levels of the intermediate size or grey zone fragile X mental retardation 1 alleles are raised, and correlate with the number of CGG repeats. J Med Genet 2007; 44(3): 200-4.

90. Mahadevan MS, Yadava RS, Yu Q, Balijepalli S, Frenzel-McCardell CD, Bourne TD, et al. Reversible model of RNA toxicity and cardiac conduction defects in myotonic dystrophy. Nat Genet 2006; 38(9): 1066-70.

91. Osborne RJ, Thornton CA. RNA-dominant diseases. Hum Mol Genet 2006; 15 Spec No 2: R162-R169.

92. Galvao R, Mendes-Soares L, Camara J, Jaco I, Carmo-Fonseca M. Triplet repeats, RNA secondary structure and toxic gain-of-function models for pathogenesis. Brain Res Bull 2001; 56(3-4): 191-201.

93. Jin P, Zarnescu DC, Zhang F, Pearson CE, Lucchesi JC, Moses K, et al. RNA-mediated neurodegeneration caused by the fragile X premutation rCGG repeats in Drosophila. Neuron 2003; 39(5): 739-47.

94. Willemsen R, Hoogeveen-Westerveld M, Reis S, Holstege J, Severijnen LA, Nieuwenhuizen IM, et al. The FMR1 CGG repeat mouse displays ubiquitin-positive intranuclear neuronal inclusions; implications for the cerebellar tremor/ataxia syndrome. Hum Mol Genet 2003; 12(9): 949-59.
95. Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, et al. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. Neurology 2001; 57(1): 127-30.
96. Hagerman RJ, Leavitt BR, Farzin F, Jacquemont S, Greco CM, Brunberg JA, et al. Fragile-X-associated tremor/ataxia syndrome (FXTAS) in females with the FMR1 premutation. Am J Hum Genet 2004; 74(5): 1051-6.
97. Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, et al. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. Am J Hum Genet 2003; 72(4): 869-78.
98. Jacquemont S, Farzin F, Hall D, Leehey M, Tassone F, Gane L, et al. Aging in individuals with the FMR1 mutation. Am J Ment Retard 2004; 109(2): 154-64.
99. Jacquemont S, Hagerman RJ, Leehey MA, Hall DA, Levine RA, Brunberg JA, et al. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. JAMA 2004; 291(4): 460-9.
100. Grigsby J, Brega AG, Jacquemont S, Loesch DZ, Leehey MA, Goodrich GK, et al. Impairment in the cognitive functioning of men with fragile X-associated tremor/ataxia syndrome (FXTAS). J Neurol Sci 2006; 248(1-2): 227-33.
101. Bacalman S, Farzin F, Bourgeois JA, Cogswell J, Goodlin-Jones BL, Gane LW, et al. Psychiatric phenotype of the fragile X-associated tremor/ataxia syndrome (FXTAS) in males: newly described fronto-subcortical dementia. J Clin Psychiatry 2006; 67(1): 87-94.
102. Cohen S, Masyn K, Adams J, Hessl D, Rivera S, Tassone F, et al. Molecular and imaging correlates of the fragile X-associated tremor/ataxia syndrome. Neurology 2006; 67(8): 1426-31.
103. Adams JS, Adams PE, Nguyen D, Brunberg JA, Tassone F, Zhang W, et al. Volumetric brain changes in females with fragile X-associated tremor/ataxia syndrome (FXTAS). Neurology 2007; 69(9): 851-9.
104. Berry-Kravis E, Potanos K, Weinberg D, Zhou L, Goetz CG. Fragile X-associated tremor/ataxia syndrome in sisters related to X-inactivation. Ann Neurol 2005; 57(1): 144-7.
105. Zuhlke C, Budnik A, Gehlken U, Dalski A, Purmann S, Naumann M, et al. FMR1 premutation as a rare cause of late onset ataxia--evidence for FXTAS in female carriers. J Neurol 2004; 251(11): 1418-9.
106. Hagerman PJ, Greco CM, Hagerman RJ. A cerebellar tremor/ataxia syndrome among fragile X premutation carriers. Cytogenet Genome Res 2003; 100(1-4): 206-12.
107. Sherman SL. Premature ovarian failure in the fragile X syndrome. Am J Med Genet 2000; 97(3): 189-94.
108. Coulam CB. Premature gonadal failure. Fertil Steril 1982; 38(6): 645-55.
109. Allingham-Hawkins DJ, Babul-Hirji R, Chitayat D, Holden JJ, Yang KT, Lee C, et al. Fragile X premutation is a significant risk factor for premature ovarian failure: the International Collaborative POF in Fragile X study-preliminary data. Am J Med Genet 1999; 83(4): 322-5.
110. Sullivan AK, Marcus M, Epstein MP, Allen EG, Anido AE, Paquin JJ, et al. Association of FMR1 repeat size with ovarian dysfunction. Hum Reprod 2005; 20(2): 402-12.
111. Cook EH, Jr., Courchesne RY, Cox NJ, Lord C, Gonen D, Guter SJ, et al. Linkage-disequilibrium mapping of autistic disorder, with 15q11-13 markers. Am J Hum Genet 1998; 62(5): 1077-83.
112. Rogers SJ, Wehner DE, Hagerman R. The behavioral phenotype in fragile X: symptoms of autism in very young children with fragile X syndrome, idiopathic autism, and other developmental disorders. J Dev Behav Pediatr 2001; 22(6): 409-17.
113. Szatmari P, Paterson AD, Zwaigenbaum L, Roberts W, Brian J, Liu XQ, et al. Mapping autism risk loci using genetic linkage and chromosomal rearrangements. Nat Genet 2007; 39(3): 319-28.
114. Gillberg C. Chromosomal disorders and autism. J Autism Dev Disord 1998; 28(5): 415-25.
115. Clifford S, Dissanayake C, Bui QM, Huggins R, Taylor AK, Loesch DZ. Autism spectrum phenotype in males and females with fragile X full mutation and premutation. J Autism Dev Disord 2007; 37(4): 738-47.
116. Kerby DS, Dawson BL. Autistic features, personality, and adaptive behavior in males with the fragile X syndrome and no autism. Am J Ment Retard 1994; 98(4): 455-62.
117. Bailey DB, Jr., Hatton DD, Skinner M, Mesibov G. Autistic behavior, FMR1 protein, and developmental trajectories in young males with fragile X syndrome. J Autism Dev Disord 2001; 31(2): 165-74.
118. Bailey DB, Jr., Hatton DD, Mesibov G, Ament N, Skinner M. Early development, temperament, and functional impairment in autism and fragile X syndrome. J Autism Dev Disord 2000; 30(1): 49-59.
119. Bailey DB, Jr., Mesibov GB, Hatton DD, Clark RD, Roberts JE, Mayhew L. Autistic behavior in young boys with fragile X syndrome. J Autism Dev Disord 1998; 28(6): 499-508.
120. Brown V, Jin P, Ceman S, Darnell JC, O'donnell WT, Tenenbaum SA, et al. Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. Cell 2001; 107(4): 477-87.

121. Miyashiro KY, Beckel-Mitchener A, Purk TP, Becker KG, Barret T, Liu L, et al. RNA cargoes associating with FMRP reveal deficits in cellular functioning in Fmr1 null mice. Neuron 2003; 37(3): 417-31.
122. Musumeci SA, Hagerman RJ, Ferri R, Bosco P, Dalla BB, Tassinari CA, et al. Epilepsy and EEG findings in males with fragile X syndrome. Epilepsia 1999; 40(8): 1092-9.
123. Mitchell RJ, Holden JJ, Zhang C, Curlis Y, Slater HR, Burgess T, et al. FMR1 alleles in Tasmania: a screening study of the special educational needs population. Clin Genet 2005; 67(1): 38-46.
124. Youings SA, Murray A, Dennis N, Ennis S, Lewis C, McKechnie N, et al. FRAXA and FRAXE: the results of a five year survey. J Med Genet 2000; 37(6): 415-21.
125. Loesch DZ, Godler DE, Khaniani M, Gould E, Gehling F, Dissanayake C, et al. Linking the FMR1 alleles with small CGG expansions with neurodevelopmental disorders: preliminary data suggest an involvement of epigenetic mechanisms. Am J Med Genet A 2009; 149A(10): 2306-10.
126. Khaniani MS, Kalitsis P, Burgess T, Slater HR. An improved Diagnostic PCR Assay for identification of Cryptic Heterozygosity for CGG Triplet Repeat Alleles in the Fragile X Gene (FMR1). Mol Cytogenet 2008; 1: 5.
127. Loesch DZ, Khaniani MS, Slater HR, Rubio JP, Bui QM, Kotschet K, et al. Small CGG repeat expansion alleles of FMR1 gene are associated with parkinsonism. Clin Genet 2009; 76(5): 471-6.
128. Castellvi-Bel S, Fernandez-Burriel M, Rife M, Jimenez D, Mallolas J, Sanchez A, et al. Detection of the fragile X syndrome protein for the evaluation of FMR1 intermediate alleles. Hum Genet 2000; 107(2): 195-6.
129. Mazzocco MM, Sonna NL, Teisl JT, Pinit A, Shapiro BK, Shah N, et al. The FMR1 and FMR2 mutations are not common etiologies of academic difficulty among school-age children. J Dev Behav Pediatr 1997; 18(6): 392-8.
130. Mornet E, Chateau C, Simon-Bouy B, Serre JL. The intermediate alleles of the fragile X CGG repeat in patients with mental retardation. Clin Genet 1998; 53(3): 200-1.
131. Sherman SL, Marsteller F, Abramowitz AJ, Scott E, Leslie M, Bregman J. Cognitive and behavioral performance among FMR1 high-repeat allele carriers surveyed from special education classes. Am J Med Genet 2002; 114(4): 458-65.
132. Crawford DC, Meadows KL, Newman JL, Taft LF, Pettay DL, Gold LB, et al. Prevalence and phenotype consequence of FRAXA and FRAXE alleles in a large, ethnically diverse, special education-needs population. Am J Hum Genet 1999; 64(2): 495-507.
133. Bretherick KL, Fluker MR, Robinson WP. FMR1 repeat sizes in the gray zone and high end of the normal range are associated with premature ovarian failure. Hum Genet 2005; 117(4): 376-82.
134. Bodega B, Bione S, Dalpra L, Toniolo D, Ornaghi F, Vegetti W, et al. Influence of intermediate and uninterrupted FMR1 CGG expansions in premature ovarian failure manifestation. Hum Reprod 2006; 21(4): 952-7.
135. Tassone F, Beilina A, Carosi C, Albertosi S, Bagni C, Li L, et al. Elevated FMR1 mRNA in premutation carriers is due to increased transcription. RNA 2007; 13(4): 555-62.
136. Beilina A, Tassone F, Schwartz PH, Sahota P, Hagerman PJ. Redistribution of transcription start sites within the FMR1 promoter region with expansion of the downstream CGG-repeat element. Hum Mol Genet 2004; 13(5): 543-9.
137. Morris A, Morton NE, Collins A, Lawrence S, Macpherson JN. Evolutionary dynamics of the FMR1 locus. Ann Hum Genet 1995; 59(Pt 3): 283-9.
138. Moutou C, Vincent MC, Biancalana V, Mandel JL. Transition from premutation to full mutation in fragile X syndrome is likely to be prezygotic. Hum Mol Genet 1997; 6(7): 971-9.
139. Malter HE, Iber JC, Willemsen R, de GE, Tarleton JC, Leisti J, et al. Characterization of the full fragile X syndrome mutation in fetal gametes. Nat Genet 1997; 15(2): 165-9.
140. Bontekoe CJ, Bakker CE, Nieuwenhuizen IM, van der Linde H, Lans H, de LD, et al. Instability of a (CGG)98 repeat in the Fmr1 promoter. Hum Mol Genet 2001; 10(16): 1693-9.
141. Eichler EE, Holden JJ, Popovich BW, Reiss AL, Snow K, Thibodeau SN, et al. Length of uninterrupted CGG repeats determines instability in the FMR1 gene. Nat Genet 1994; 8(1): 88-94.
142. Chong SS, Eichler EE, Nelson DL, Hughes MR. Robust amplification and ethidium-visible detection of the fragile X syndrome CGG repeat using Pfu polymerase. Am J Med Genet 1994; 51(4): 522-6.
143. Nancarrow JK, Holman K, Mangelsdorf M, Hori T, Denton M, Sutherland GR, et al. Molecular basis of p(CCG)n repeat instability at the FRA16A fragile site locus. Hum Mol Genet 1995; 4(3): 367-72.
144. Zhong N, Ju W, Pietrofesa J, Wang D, Dobkin C, Brown WT. Fragile X "gray zone" alleles: AGG patterns, expansion risks, and associated haplotypes. Am J Med Genet 1996; 64(2): 261-5.
145. Eichler EE, Hammond HA, Macpherson JN, Ward PA, Nelson DL. Population survey of the human FMR1 CGG repeat substructure suggests biased polarity for the loss of AGG interruptions. Hum Mol Genet 1995; 4(12): 2199-208.
146. Usdin K. NGG-triplet repeats form similar intrastrand structures: implications for the triplet expansion diseases. Nucleic Acids Res 1998; 26(17): 4078-85.

147. Richards RI, Shen Y, Holman K, Kozman H, Hyland VJ, Mulley JC, et al. Fragile X syndrome: diagnosis using highly polymorphic microsatellite markers. Am J Hum Genet 1991; 48(6): 1051-7.
148. Richards RI, Holman K, Kozman H, Kremer E, Lynch M, Pritchard M, et al. Fragile X syndrome: genetic localisation by linkage mapping of two microsatellite repeats FRAXAC1 and FRAXAC2 which immediately flank the fragile site. J Med Genet 1991; 28(12): 818-23.
149. Oudet C, Mornet E, Serre JL, Thomas F, Lentes-Zengerling S, Kretz C, et al. Linkage disequilibrium between the fragile X mutation and two closely linked CA repeats suggests that fragile X chromosomes are derived from a small number of founder chromosomes. Am J Hum Genet 1993; 52(2): 297-304.
150. Macpherson JN, Bullman H, Youings SA, Jacobs PA. Insert size and flanking haplotype in fragile X and normal populations: possible multiple origins for the fragile X mutation. Hum Mol Genet 1994; 3(3): 399-405.
151. Chiurazzi P, Destro-Bisol G, Genuardi M, Oostra BA, Spedini G, Neri G. Extended gene diversity at the FMR1 locus and neighbouring CA repeats in a sub-Saharan population. Am J Med Genet 1996; 64(1): 216-9.
152. Chiurazzi P, Macpherson J, Sherman S, Neri G. Significance of linkage disequilibrium between the fragile X locus and its flanking markers. Am J Med Genet 1996; 64(1): 203-8.