Published in final edited form as:
Adv Exp Med Biol. 2012 ; 769: 78–114.

UNSTABLE MUTATIONS IN THE *FMR1* GENE AND THE PHENOTYPES

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Abstract

Fragile X syndrome (FXS), a severe neurodevelopmental anomaly, and one of the earliest disorders linked to an unstable ('dynamic') mutation, is caused by the large (>200) CGG repeat expansions in the noncoding portion of the *FMR1* (Fragile X Mental Retardation-1) gene. These expansions, termed full mutations, normally silence this gene's promoter through methylation, leading to a gross deficit of the Fragile X Mental Retardation Protein (FMRP) that is essential for normal brain development. Rare individuals with the expansion but with an unmethylated promoter (and thus, FMRP production), present a much less severe form of FXS.

However, a unique feature of the relationship between the different sizes of CGG expanded tract and phenotypic changes is that smaller expansions (<200) generate a series of different clinical manifestations and/or neuropsychological changes. The major part of this chapter is devoted to those *FMR1* alleles with small (55-200) CGG expansions, termed 'premutations', which have the potential for generating the full mutation alleles on mother-offspring transmission, on the one hand, and are associated with some phenotypic changes, on the other. Thus, the role of several factors known to determine the rate of CGG expansion in the premutation alleles is discussed first. Then, an account of various neurodevelopmental, congnitive, behavioural and physical changes reported in carriers of these small expansions is given, and possible association of these conditions with a toxicity of the elevated *FMR1* gene's transcript (mRNA) is discussed.

The next two sections are devoted to major and well defined clinical conditions associated with the premutation alleles. The first one is the late onset neurodegenerative disorder termed fragile X-associated tremor ataxia syndrome (FXTAS). The wide range of clinical and neuropsychological manifestations of this syndrome, and their relevance to elevated levels of the *FMR1* mRNA, are described. Another distinct disorder linked to the CGG repeat expansions within the premutation range is fragile X-associated primary ovarian insufficiency (FXPOI) in females, and an account of the spectrum of manifestations of this disorder, together with the latest findings suggesting an early onset of the ovarian changes, is given.

In the following section, the most recent findings concerning the possible contribution of *FMR1* 'grey zone' alleles (those with the smallest repeat expansions overlapping with the normal range

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i.e., 41-54 CGGs), to the psychological and clinical manifestations, already associated with premutation alleles, are discussed. Special emphasis has been placed on the possibility that the modest elevation of 'toxic' *FMR1* mRNA in the carriers of grey zone alleles may present an additional risk for some neurodegenerative diseases, such as those associated with parkinsonism, by synergizing with either other susceptibility genes or environmental poisons.

The present status of the treatment of fragile X-related disorders, especially FXS, is presented in the last section of this chapter. Pharmacological interventions in this syndrome have recently extended beyond stimulants and antipsychotic medications, and the latest trials involving a group of GluR5 antagonists aim to ascertain if these substances have the potential to reverse some of the neurobiological abnormalities of FXS.

INTRODUCTION

The trinucleotide expansion of CGG repeats in the 5′ untranslated region (5′-UTR) of the fragile X mental retardation 1 gene (*FMR1*) located at Xq27.3 was sequenced in 1991.[1] Although the phenotype of fragile X syndrome (FXS) was first described by Lubs,[2] it was not until 1991 that those with FXS were found to have >200 CGG repeats described as a full mutation in *FMR1*. Individuals who were carriers of smaller expansions between 55 to 200 CGG repeats (premutation) were originally thought to be unaffected clinically. However, in 1991 an elevated rate of premature ovarian failure (POF) was documented in carriers compared to controls[3] and later confirmed by many other groups (reviewed in refs. 4,5).POF has been renamed the fragile X-associated primary ovarian insufficiency (FXPOI) to emphasize the association with the premutation and also the occasional ability of women to reproduce such that the ovary has not completely failed.[6] Subsequently in 2001, the fragile X-associated tremor ataxia syndrome (FXTAS) was discovered in aging carriers[7,8] and it includes not only tremor and ataxia but also neuropathy, autonomic dysfunction, neuropsychiatric problems and cognitive decline sometimes leading to dementia.[9,10] This chapter delineates the history and development of the spectrum of involvement in these fragile X-associated disorders, with special emphasis on premutation (PM) carriers.

As the role of elevated *FMR1* mRNA in carriers of the PM alleles[11] has been researched and the concept of RNA toxicity leading to FXPOI or FXTAS has been developed[12,13] a variety of additional phenotypes has been described in those carriers. They include developmental problems in a subgroup of young male carriers including autism, autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), shyness, anxiety and seizures.[10,14-18] In many adults with the premutation including both males and females, psychopathology is common including anxiety and depression compared to controls.[19-24] Cognitive changes, particularly executive function deficits, cna begin before the onset of FXTAS in carriers[25] and there is evidence of early white matter disease reflected in diffusion tensor imaging changes before the onset of FXTAS.[26] Most recently autoimmune dysfunction including fibromyalgia and hypothyroidism has been found to be more common in carriers compared to controls.[22,27] Therefore a large spectrum of involvement associated with the premutation constitutes a newly emerging group of disorders leading to new avenues in research and clinical management.

LARGE EXPANSIONS OF CGG REPEAT—THE CAUSE OF FRAGILE X SYNDROME

Fragile X syndrome (FXS) was one of the earliest conditions found to be linked to the unstable mutation. It is the most common inherited form of intellectual disability caused by the large expansion in the *FMR1* gene, where cognitive and behavioural impairments are associated with minor physical features.[28] These expansions defined as 'full mutation' are usually associated with the methylation-coupled inactivation of *FMR1* gene leading to gross deficit of the protein gene product, FMRP. [29,30] Abnormalities of FXS are primarily caused by the depletion of FMRP,[31] and experimental evidence indicates that FMRP is essential for normal brain development.[32-35] More specifically, studies based on rat brains have shown that FMRP is involved in synaptogenesis, especially in the cerebral cortex, cerebellum and hippocampus,[36] and in modifying synaptic structure in response to environmental stimulation.[37-40] FMRP is an RNA binding protein and it carries mRNAs to the synapse where it typically inhibits translation until stimulation occurs and then it allows translation.[41] In the absence of FMRP there is dramatic up-regulation of protein production in the hippocampus.[42] FMRP regulates the translation of hundreds of proteins and they include many of the proteins also associated with autism when they are mutated including Neuroligins 3 and 4, Neurorexins, SHANK3, amyloid precursor protein (APP), Arc, MAPKinase, CYFIP 1 and 2 and many more.[43-48] There is also upregulation of mTOR in animals and humans with FXS[49,50] similar to other forms of autism such as Tuberous Sclerosis.[51] The molecular overlap between FXS and autism is likely the cause of the high rate of autism (30%) and also autism spectrum disorder (30%; ASD) in FXS.[16,52-54] The behavioral phenotype also includes a high rate of anxiety disorders[55] and ADHD.[56-58] Significant mood instability leading to aggression or severe tantrums occurs in about 30 to 40% of adolescents or adults.[28] This problem frequently leads to out of home placement and the use of psychotropic medication.

The physical phenotype of FXS includes a long face and prominent ears but approximately 30% of children will not have these features, although hyperextensible finger joints are seen in the majority along with double jointed thumbs. [28] Large testicles (macroorchidism) begin to occur at about 8 years of age and they reach their maximal size, typically at 2 to 3 times normal (60 ml) at about 15 to 16 years.[59]

Approximately 20% to 40% of individuals with FXS have mosaicism, meaning some cells with the premutation and some cells with the full mutation (size mosaicism) or a mixture of some cells with a lack of methylation and some cells with the full methylation (methylation mosaicism). Occasional individuals have a complete lack of methylation but a CGG repeat number >200. Those with a lack of methylation or those with a majority of their cells with the premutation typically have the highest levels of FMRP and they are often high functioning with an IQ > 70.[31,60] Also, individuals with FXS and FMRP levels >30% to 50% of normal are likely to maintain an IQ above 70 as adults and demonstrate fewer physical features of FXS.[31,61] Although these individuals seem to be better off in childhood and early adulthood, they may also have an elevated level of *FMR1* mRNA because of an increased rate of translation compared to those with a full mutation that is fully

methylated.[62] The higher level of *FMR1* mRNA may put them at risk for neurological disease with aging similar to those with the premutation.[63] Hall et al[63] reported a case of a male with a high level of mosaicism who experience signifiacnt neurodegeration in his 60s, although his diagnosis was consistent with Parkinson's disease (PD). Although there is concern for aging problems in those with mosaicism, a recent report of more than 60 individuals with FXS who were aging demonstrated no difference in the molecular studies in those who developed PD and those who did not.[64] So far there has never been an individual with FXS who developed FXTAS, although PD is more common in aging in FXS than what is seen in the general population.[64]

Females with FXS are typically less affected than males because they have a second X chromosome. Their level of FMRP correlates with their activation ratio (the proportion of cells that have the normal X as the active X).[30,31] Approximately 25 to 30 percent of females have an IQ below 70 but the majority of females have an IQ in the borderline or low normal range.[65] The majority of girls or women with the full mutation experience some anxiety and common diagnoses are selective mutism, social phobia and specific phobia.[55] ADHD is seen in at least 30% of women and executive function deficits are common even when ADHD is not apparent.[66] Even women with a normal IQ and the full mutation can experience attentional problems and anxiety difficulties.[67] Usually girls respond well to stimulant medication to treat ADHD and selective serotonin reuptake inhibitors (SSRIs) to treat anxiety.[68] Counseling with a psychologist is also helpful for these problems especially if the women are under additional stress from having children with FXS and they need support and behavioral management guidance.[69]

SMALL CGG EXPANSION ALLELES AS THE SOURCE OF THE FULL MUTATION ALLELES

Apart from the flurry of studies on psychological and clinical manifestations of FXS, a major interest was in the origin of the large expansions leading to this severe developmental abnormality. According to the earliest reports, the mothers of the affected offspring did not manifest any obvious developmental anomalies reminiscent of those seen in FXS, but had a potential to generate offspring with this syndrome. Hence the term premutation (PM) was coined to reflect this potential, as well as the apparently unaffected status of the carriers of these PM alleles.[70]

The reason for, and the pattern of, these intergenerational differences were explained by the molecular data from large pedigrees which showed that these high risk parents carried small-size expansions of CGG repeat in the *FMR1* gene, which did not cause gene silencing, but further expanded if transmitted from mothers to offspring into the full mutation range, with the rates of expansion increasing proportionally to the size of repeat.[71-77] The detailed risk, as up-dated by Nolin and colleagues[78] in a large multicentre study of female carriers (with correction for ascertainment) were as follows. For PM carriers: 3.7% for the repeat range of 55-59, 5.3% for the range of 60-69, 31.1% for the range of 70-79, 57.8% for the range of 80-89, 80.1% for the range of 90-99, between 94.4 and 100% for the range of 100-139 repeats, and 100% for repeat sizes >139 repeats. It was established that the PM alleles

carried by the fathers were usually stable, and on occasions they might even be reduced in size on transmission to their daughters.

The introduction of PCR techniques[79] allowed for the recognition of the potential for expansions of the alleles below the PM range (~35 to 54), and the risks for expansion of these small size (intermediate size, or GZ) alleles of 49-54 range were established.[78,80-87] Instability in maternal transmission of GZ alleles over one generation has been reported as 2%-4% on average, with the possibility of expanding to the full mutation within two generations. The lowest small expansion allele reported to expand to the full mutation had 56 CGG repeats.[88]

Unlike for the premutation (PM) alleles, grey zone (GZ) alleles have a much higher instability rate (~16%) if transmitted through the male than the female carriers.[89] It is the potential for expansion that determined the latest guidelines for an overall classification of *FMR1* alleles.[90] The upper bound of normal alleles (that is, showing no meiotic or mitotic instability) was set at ~44 CGG repeats; the range of intermediate size or GZ alleles (showing only minor increase or decrease in repeat number, but never expanding to full mutation) was set between 45 and 54 CGG repeats; and the alleles in the PM range (set between 55 and 200 repeats), if carried by females, showed a gradually increasing risk of expansion to the full mutation. The relevance of this classification in the light of the most recent molecular and clinical findings in carriers of small expansions will be discussed in the next sections.

The above figures represent an average risk for different categories of *FMR1* alleles, but the individual risks may vary widely between different carriers, and between different families. In some families small expansions may be transmitted unchanged throughout several generations, and in others the risk of expansion from PM in the mother to full mutation in the offspring may be high or very high. This variation prompted a search for those molecular and other factors which might predispose to, or protect from, further expansions of the small CGG repeat size alleles. The major modifying factor is the sex of a parent, where, as described above, the risk of expansion of PM alleles is limited to the mothers but for the GZ alleles, it is relatively higher for the fathers than from the mothers.[89] In addition, the data from large pedigrees suggested that the sex of offspring might be another modifying factor, with male offspring manifesting higher rate of large expansions from the parent than female offspring;[91,92] however this concept still remains controversial.[78,93]

Some other molecular factors associated with *FMR1* gene have been found to be influential in expansion of small size CGG repeats. Earlier hypothesis implicated a role for the number and position of AGG triplets. It has been argued that the AGG triplets interspersed within the CGG repeat tract stabilize this repeat, and in their absence increased length of ‘pure’ CGG are generated, which are more prone to replication slippage.[80,94-98] However, more recent data suggested that the role of AGG is a late event in progressive CGG expansion[83] although definitely related to the risk for expansion in the next generation.[99]

The role of molecular markers flanking the CGG repeat in the expansion process has been well established. In earlier studies, these markers comprised dinucleotide (CA)

microsatellites which, in combination, generated polymorphic haplotypes.[94,100] The frequencies of some of these haplotypes differed significantly between normal as opposed to fragile X both full mutation and PM alleles in all populations tested.[97,101,102] The fact that the haplotype associations in the smallest expansion (GZ) alleles showed a pattern similar to this seen in fragile X alleles indicated that these alleles evolved from among GZ alleles.[94,103,104]

Currently, single nucleotide polymorphisms, SNPs, are more widely used and are now recognized as important markers in studies of fragile X, because of greater discriminatory power of large number of SNPs that can be tested to identify haplotypes that segregate with different CGG repeat size allele categories.[104,105] The finding that the haplotypes combining the two SNPs, ATL1-alleles A and G[104,106] and FMRb-alleles A and G[103,106] exhibit strong associations with PM/full mutation, as well as with GZ alleles provided further evidence for the role of GZ alleles along the pathway leading from the normal to the fragile X full mutation alleles.

PHENOTYPIC FEATURES IN PM CARRIERS

The effect of small expansions on the phenotypes is an important problem considering their high population prevalence. The PM (using 55 repeats as the lower bound) is relatively common in the general population affecting 1 in 113 to 259 females and 1 in 260 to 813 males.[83,107-111] Population prevalence of intermediate (GZ) size alleles is spectacularly higher reaching 1 in ~ 30 males;[81,83,84] and may be at least twice as common in females.[112,113] Therefore, any evidence for clinical and neuropsychological involvement in carriers of these alleles would have a significant bearing on the causes of health problems in a previously unrecognized but sizeable group of individuals.

Phenotypic effects of small expansions has taken a long time to be appreciated since the term ‘PM’ was first coined by Pembrey and colleagues in 1985[70] who stated that it ‘causes no harm other then predisposing to the final event’, with ‘final event’ being the full mutation associated with the FXS. It took more than a decade to change the belief in a clear-cut division of carriers of fragile X into two distinct categories: ‘affected’ with FXS, as opposed to unaffected ‘carrier females’ and ‘transmitting males’. Other than the finding of FXPOI in 1991,[3] the earliest studies of small samples of male PM carriers,[114,115] and both male and female carriers[116] demonstrated minor yet significant physical and intellectual impairments. Physical problems found in approximately 25% of PM carriers included prominent ears and hyperextensible finger joints. [117,118] More stringent analysis of cognitive status used genetic statistical models based on pedigree data, which allowed for adjusting the measures obtained from the carriers for these measures in other relatives. This analysis provided evidence for a significance effect of PM on standard cognitive (Wechsler) measures, including performance IQ, Block Design, Perceptual Organization, and Object Assembly, in the adult male carriers and Symbol Search, in the adult female carriers,[119] as illustrated in Figure 1.

A significant effect of PM irrespective of age was alos established, using pedigree models, on some FSIQ-adjusted executive function test scores.[120] These included impairment of the motor planning, inhibition and working memory measured by the Behavioural Dyscontrol

Scale (BDS),[121] in the male carriers, and of visuospatial memory and visuospatial constructional ability, assessed by Rey Complex Figure Design and Recognition Test (RCFT),[122] in the female carriers. Similar findings of significant impairments on tests of executive function (Verbal Fluency, Trail Making Test and Tower of London) and memory were reported in a sample of 20 unrelated male PM carriers.[123] This data may indicate that the CGG expansion alleles within the PM range may affect specific neuronal circuits manifesting as relevant neuropsychological deficits. These deficits may also include, specifically for the female sex, impairment in arithmetics as reported in 39 PM women based on performance on the Wide Range Achievement Test-3,[124] visual pathway deficits involving low social/high temporal frequency contrast sensitivity and frequency-doubling veneer,[125] and motion perception deficits.[126] That the PM alleles affect brain functioning has been also shown in CGG knock-in (KI) female mice where, apart from deficits in processing special information, poor performance on tests of temporal order of presenting two objects, specifically related to the upper-end of PM range (150-200 CGGs) was encountered.[127] This latter finding is reminiscent of the results of a recent study based on 60 female PM carriers, where a (significant) decrease in IQ (Wechsler Scales) verbal and performance scores was more evident for the females in the upper PM range (CGG>100), and with a 10% decrement of FMRP expression.[128]

Although these findings have been largely based on adult males and females, the overlap of the observed cognitive deficits with some of those seen in FXS and their relevance to minor FMRP deficits and the upper end of PM range, indicated a significant neurodevelopmental component in the origin of these changes. The presence of the associated behavioural deficits, including autism spectrum, and attention deficit hyperactivity disorder (ADHD), in boys carrying the PM allele, support this notion.[14,16,129] Another supportive piece of evidence has been provided by demonstration of several minor physical anomalies in unrelated PM male and female carriers, which are typical of the FXS phenotype,[117,118] and in families,[130] as well as of the distinct abnormalities in brain structure on MR images.[131-134] Notably, reduced hippocampal volume was found to be associated with memory deficits in both male and female carriers,[131] or with anxiety-related psychological symptoms in female carriers.[134] In another study, the wide range of structural brain anomalies, including reduced volume of the whole brain, caudate and thalamic nuclei bilaterally were found to be associated with changes in metabolic rates in several brain areas in female carriers.[132] The results of the study, which used fMRI to measure brain responses to fearful faces and fearful social images, demonstrated diminished activation of amygdala and several other brain areas that mediate social cognition in the group of PM adult male carriers compared with age and IQ-matched controls.[135] This reduction was also significantly associated with self-report of psychological symptoms on the Symptom Checklist-90-revised (SCL-90-R). A significant association between reduced activity and volume of the hippocampus during a memory recall task, and psychological symptom severity, was also reported in two other studies based on male[136] and female[134] PM carriers.

It is clear from these and other reports (to be discussed below) that it is difficult to draw the clear demarcation line between the phenotypic abnormalities which can be related to neurodevelopmental changes, and those determined by the late onset progressive brain pathology. While the first category of changes appears to represent a mild version of

physical and neuropsychological anomalies linked to the full mutation that may be related mainly to subtle deficits of FMRP at the upper end of the PM range, [31,137] evidence for the late onset age-dependent changes suggest alternative or additional major pathogenic effects, related to the elevated levels of expanded *FMR1* transcript, which may account for the late neurodegenerative changes, or for both neurodegenerative and neurodevelopmental abnormalities (as detailed in the later sections of this chapter).

The finding of age-dependent deficit in the ability to inhibit prepotent responses,[138] and in working memory tasks, particularly executive control of memory,[139,140] in a sample of 40 adult PM male carriers, may additionally illustrate an overlap between these 2 dimensions. The progress in severity of cognitive decline was shown to be more striking in the older age group of carrier males aged >50 years, with 33.3% penetrance of dementia for the average age of 63.4 years, which represented sixfold increase compared with noncarriers, and further increasing with age and allele size.[141] However, a critical review of neuropsychological status of male and female PM carriers[142] recommends caution in the interpretation of these data and the use of larger samples obtained from several sources.

Although no psychiatric pathology has been found in the earliest study of female carriers,[143] it has been widely reported among numerous subsequent studies of adult carrier males and females. In a large comprehensive study by Franke et al in 1998[144] an overall rate of a lifetime diagnosis of affective disorders was found as high as 55.7%; of anxiety disorders, 41%; of panic disorder, 11.5%; and 18% was a lifetime risk of social phobia, which was independent on the effect of having children with FXS. Although distinct schizoaffective disorders have only been reported in rare individuals,[145] schizotypal features and avoidant personality disorder occurred in respectively 8.2% and 4.9% of carriers.[144] Later studies provided confirmatory evidence for the high prevalence of mood, especially depressive disorders, in female PM carriers. The Spanish study based on a small sample of PM carriers and mothers of FXS children showed higher scores for depression (based on SCL-90-R and Beck Depression Inventory) compared with noncarrier mothers of mentally retarded children.[146] In the much larger study by Roberts et al in 2009,[147] 93 PM females were compared to 2,159 controls from the National Comorbidity Survey Replication (NCS-R) US dataset. The results based on Structured Clinical Interview for the DSM-IV (SCID-1) showed a significant increase in the risk of lifetime major depressive disorder (43% in carriers compared with 31.9% controls), which is close to the earlier estimate of nearly 60% risk of all affective disorders by Franke et al.[144] The risk of lifetime panic disorder and current agoraphobia were not high (8.6% and 3.2%, respectively), but they were significantly elevated compared with control results. Interestingly, the risks were higher in the lower end of PM range, which suggested that the link of the affective disorders as seen in the carriers with minor FMRP depletion is unlikely.

Significantly higher levels of obsessive-compulsive disorder were reported in the later study of 122 female and 26 male carriers of PM alleles.[20] Increase in depression was reported in 34 PM carrier mothers of FXS children who were compared, using SCL-90 and Beck Depression Inventory, with 39 noncarrier mothers of mentally retarded child and 39 mothers from the general (Spanish) population.[146] Notably, the results of a survey of 199 female and 57 male PM carriers from North Carolina enrolling in a national survey of families of

children with FXS showed that the range of problems such as anxiety, depression or learning difficulties in females, and attention problems, aggression and anxiety in males, tend to cluster in the same individuals.[148] These confounding effects may be additional reasons for difficulties in the phenotypic risk estimates for the carriers, and thus for conflicting results from different studies, which may have already been affected by small sample sizes, inadequate psychometric measures, and biases related to preselection of the tested or control samples. In the large study by Hunter and colleagues in 2008,[149] the effect of small CGG expansions on mood and anxiety was investigated using regression models (adjusting for potential confounders) in a sample of 119 male and 446 female PM carriers aged 18-50 ascertained from either fragile X families or the general population. The results have shown that repeat length was (marginally) associated with depression and negative affect in males, and with negative affect in females, which suggested that, although PM carriers may be at risk of emotional morbidity, the effect of repeat size appears subtle. However, considering the later finding by Roberts et al[19] that the lower end of the range of PM alleles may have the largest effect on the risk of mood disorder, the linear model[149] analysis may not have been the most appropriate one for probing this kind of a relationship.

TOXICITY OF THE ELEVATED *FMR1* TRANSCRIPT CONTRIBUTES TO ABNORMAL PHENOTYPES

Search for possible pathophysiological mechanisms involved in abnormal phenotypes or specific disorders related to PM alleles resulted in a discovery of an elevated level of *FMR1* transcript (mRNA) in peripheral blood lymphocytes of PM carriers,[11] which was up to 10-fold higher than normal in carriers of the higher end (>100) alleles, and 2-4 times higher than normal in carriers of the lower end (55-100) alleles. The original finding was confirmed and expanded in several later studies.[150-155] This elevation results from increased transcriptional activity rather than an increased mRNA stability,[11,151,156] and direct evidence for an increased transcription rate has later been obtained using a nuclear run-on experiment.[157] The elevated *FMR1* transcript levels were found to be associated with the size of CGG repeat expansion within the PM-size range.[11,150,152,153,155] The data obtained from 63 male and 61 female carriers (with the effect of inactivation accounted for in the females) showed that this relationship was linear and consistent in both sexes.[150] However, in the more recent study of 90 PM females using piecewise regression model, the relationship between mRNA levels and CGG repeat was shown to be much stronger above the threshold of 90-100 repeats after correction for the activation ratio (AR); whereas without the correction, a significant regression was only limited to the lower portion (<100) of PM range.[158] These findings have also provided evidence for skewed inactivation in females carrying PM alleles with >100 repeats, and these data emphasize the need for applying the relevant correction in correlation analyses involving molecular and phenotypic changes. A recently proposed statistical method of covariate adjusted correlation analysis between CGG repeat number and *FMR1* mRNA, accommodating both additive and multiplicative effects of activation ratio nonparametrically,[159] will allow the more accurate evaluation of the effect of this ratio on molecular measures in females.

FRAGILE X-ASSOCIATED TREMOR/ATAXIA SYNDROME (FXTAS)

Clinical Manifestations and Prevalence in Males

The fragile X-associated tremor/ataxia syndrome (FXTAS), a recently identified form of late-onset (>50 years) neurodegenerative disorder, was first described in a sample of five older PM carrier males who were the grandfathers of children with FXS.[7] The major clinical features of this progressive disorder comprise intention or postural/ action tremor, cerebellar gait and limb ataxia, and parkinsonism.[7-9,160-164] Although severe rigidity typical of Parkinson's disease is uncommon, mild rigidity in the upper limbs was reported in 71% of FXTAS patients, 40% out of this group manifested mild resting tremor, and 57% showed bradykinesia.[165] The three scales recognized as the measures of tremor (Clinical Rating Scale for Tremor, CRST), ataxia (International Cerebellar Ataxia Scale, ICARS), and parkinsonism (Unified Parkinson's Disease Rating Scale, UPDRS) were used in several different studies of male PM carriers, and these scores were found significantly higher compared with age and sex-matched controls.[162,163,165,166] Gait ataxia usually starts from balance problems manifesting as difficulty with tandem walking and progresses till the person is unable to walk without support or is wheel-chair bound. The tremor most often involves upper extremities, with postural/action tremor initially, and resting tremor at the later stages. Autonomic dysfunction and neuropathy have also been reported,[7,160,165,167] and the latter has been confirmed by the results of a tibial nerve conduction study showing significantly slower velocity and prolonged F-wave latency in typical FXTAS patients compared with controls and unaffected PM carriers.[168] The analysis of the progression of the major motor signs conducted in 55 patients showed that tremor was usually the first sign of the disorder occurring at the median age of 60 years; median delay of onset of ataxia was 2 years; onset of falls, 6 years; dependence on walking aid, 15 years; and death, 21 years.[163] Approximately 60% of male PM carriers manifesting tremor/ataxia also show increased T2 signal intensity in white matter of the MCP on MR images.[169] Other MR imaging findings include widespread cerebral, cerebellar and brainstem atrophy, as well as patchy or confluent areas of hyperintensity on T2-weighted images in periventricular and deep white matter of the cerebral hemispheres and corpus callosum.[8,165,166,170] Patchy or confluent T2 hyperintensities were recently reported in the basis pontis of a female affected with FXTAS,[171] and our own observations in the affected male carriers suggest that these changes may have been underreported.[172] The pattern of white matter pathology as seen on MR images and histological preparations in FXTAS is distinct from that associated with hypertensive vascular disease,[173] with the changes being in the form of various degrees of spongiosis and loss of axons and myelin in the areas shown as having T2 hyperintensities. An example of the typical white matter and other changes as recorded on MR images of a PM carrier affected with FXTAS is given in Figure 2.

The frequencies of these characteristic abnormalities are significantly higher in the affected carriers than in age and sex matched controls.[169,170,174] Confirmatory evidence for the relevance of these changes to the effect of PM alleles have been provided by the MRI volumetric studies, showing significant volume loss ofthe cerebellum, brainstem, thalamus, hippocampus and cerebral cortex in the affected carriers compared with age-matched normal controls.[133,169,173,174]

The characteristic neurohistological changes of FXTAS are ubiquitin-positive intranuclear inclusions abundant in both neuronal and astrocytic nuclei throughout the cerebrum, cerebellum (apart from Purkinje cells) and brainstem, being most numerous in the hippocampal formation (~50%, contrasting with only 5%-10% in cerebral cortex); the inclusions were also found in autonomic neurons and astrocytes of the spinal cord.[176] Although they contain about 20 different proteins, they are negative for PAS, tau, silver, polyglutamine and alpha synuclein,[173,176] which distinguishes them from the intranuclear inclusions of the CAG repeat (polyglutamine) disorders, and obviously from cytoplasmic inclusions associated with multiple system atrophy or Lewy body dementia (Fig. 3). The same inclusions were also found in neuronal nuclei of a KI mice carrying expanded (~100 CGG) repeat in the mouse gene.[177] Although the inclusions were not originally seen in the astrocytes in the KI mouse they have subsequently been documented in astrocytes in addition to neurons as in PM carriers.[178]

Cognitive Deficits and Psychiatric Symptoms

The earlier studies of cognitive functioning of men with FXTAS showed marked impairment of executive cognitive abilities, with only slightly lowered performance on standard cognitive IQ scores.[179,180] Moreover, significantly higher scores on the Neuropsychiatric Inventory (NPI) scales (total, aggression, depression, apathy and irritability) in the smaller sample of FXTAS males compared with normal controls were reported and interpreted as indicative of fronto-subcortical dementia.[181] In another study of 15 patients with FXTAS, twelve were diagnosed with dementia, and seven others with mood and anxiety disorders.[182] A larger study of 68 adults with FXTAS, including 50 males and 18 females, found dementia in approximately 50% of men that was similar in severity to Alzheimer's dementia.[183] However, none of the 18 females withFXTAS demonstrated dementia or significant cognitive impairment.[183] These data, considered in context with disease duration, indicated that deficits in executive cognitive functioning (ECF), working memory and speed, as well as capacity of information processing, combined with obvious personality changes, typically represent the early stages of FXTAS. Amongst the most affected aspects of executive functioning is the initiation of purposeful goal directed activity, and the inhibition of inappropriate or irrelevant behaviour. Standard cognitive measures using Wechsler scale usually reveals deficits of working memory represented by Arithmetic and Digit Span scores, whereas other verbal IQ subtests, especially language and verbal comprehension, are relatively unaffected. The Folstein Mini Mental State Examination (MMSE) usually remains within normal limits until the late stages of a disorder because it does not include the assessment of executive functioning. In the latest stages of the disorder, however, severity of dementia is fast progressing as the result of diffuse deficits in other cognitive functions. That these deficits are related to FXTAS has been shown by the results of a study based on 109 men divided into FXTAS+ carriers, FXTAS- carriers, and noncarriers. This data showed that, while FXTAS- carriers performed worse than controls on some aspects of ECF, FXTAS+ carriers performed worse than controls on working memory, recall of information, declarative learning and memory, information processing speed and temporal sequencing, as well as on ECF tests.[25] On the other hand, there is obvious overlap, especially in ECF impairments, between FXTAS and asymptomatic carriers, with the former showing more severe and widespread deficits. This overlap may be

further illustrated by the latest study based on analysis of functional magnetic resonance imaging (fMRI) BOLD signals obtained during the performance of verbal working memory from 15 FXTAS+, 15 FXTAS− and age matched normal control individuals.[26] The results have shown that activation of prefrontal cortex, which may underlie executive and memory deficits, was reduced in both carrier samples compared to normal controls; in addition, FXTAS+ group showed reduced activation of the right ventral inferior frontal cortex.

A large scale comprehensive longitudinal study is still required to establish the sequence of different aspects of cognitive and neuropsychiatric changes in carriers.[9] This is because their relevance to FXTAS has not yet been clearly established, considering that, although neuropsychiatric manifestations often follow the establishment of motor disability, some, such as anxiety or depression, also occur in carriers not affected with FXTAS, or prior to the onset of neurological changes.

Prevalence and Atypical Manifestations

The initial clinical and genetic descriptions of male PM carriers preselected for tremor and ataxia was shortly followed by estimates of prevalence of this syndrome by screening of unselected population of PM carriers over 50 years of age ascertained through fragile X families with at least one child diagnosed with the FXS. The results of two parallel studies conducted in the US[165] and Australia[166] provided compelling evidence for the association between the PM carrier status and neurological involvement. The US study showed age-related increase in prevalence of tremor/ataxia, ranging from 17% to 75% between the age of 51 and 85, with the overall prevalence of 37% for this age range. A similar study based on Australian families generated an overall prevalence of 41.7% for the comparable age range. In both studies, an excess of manifestations of tremor/ataxia in the sample of PM carriers was significant compared to the sample of noncarriers matched for age. In a more recent study of 44 male PM carriers aged >50 years and ascertained through 151 fragile X Spanish families from Barcelona 45.5% of respondents surveyed over the phone reported tremor, balance problems, falls and/or gait ataxia.[22] However, the extensive variability of neurological manifestations needs to be considered while interpreting the prevalence results, since these estimates were biased towards considering syndromic manifestations rather than any less specific clinical changes indicating a significant neurological involvement. Shortly after the first description of FXTAS stringent diagnostic criteria were recommended, which included intention tremor or gait ataxia, and MCP sign (‘definite’ FXTAS), with less stringent criteria including problems of executive function deficits and brain atrophy incorporated into possible or probable FXTAS.[8] Thus, the combination of intention tremor and gait ataxia, and parkinsonism, was classified as ‘probable’ FXTAS and the combination of intention tremor or gait ataxia and cerebral white matter lesion and atrophy, as ‘possible’ FXTAS. Modifications of the FXTAS diagnostic criteria was made with addition of the FXTAS inclusions inthe definite criteria.[12]

However, it appears that neurological involvement in PM carriers does not always meet those criteria, as illustrated by a number of published cases,[166,184-186] and those known through personal communications or our unpublished observations. Some carriers manifested typical MCP change with minimal or no clinical signs of tremor/ataxia, and

others showed cerebral white matter lesions without the MCP sign, combined with some atypical neurological manifestations, psychiatric manifestations or autoimmune disease such as fibromyalgia and/or hypothyroidism[22,27] or multiple sclerosis.[187,188] Severe and fast progressing dementia was a major feature in a 62-year-old PM carrier, who at the later stages of his condition manifested typical FXTAS signs including gait ataxia and white matter degeneration including MCP sign.[189] A more recent study in France demonstrated a high rate of cognitive changes even before the onset of tremor or ataxia in carriers compared to controls.[141]

Another atypical form reported in a 58-year-old female PM carrier presented with dementia and parkinsonism, and typical ubiquitin positive intranuclear inclusions in neurons and astrocytes, but no MCP sign or ataxia.[190] The occurrence of low symptomatic or atypical manifestations determined by the same major etiological factor as a typically diagnosed case of FXTAS warrants the term ‘FXTAS spectrum’, which better reflects the fact that neurological, cognitive and psychiatric involvements associated with neurodegenerative changes in PM carriers extend beyond the classical definition of FXTAS. This new definitionalso covers the cases of co-occurrence ofsome of the clinical features ofFXTAS with other neurodegenerative disorders, such as Parkinson's disease with Lewy bodies and Alzheimers disease, which will cause a more rapid decline in function than is seen in FXTAS without other neurodegenerative disorders. However, there are still clinical problems associated with the PM that may not be on the FXTAS spectrum, such as the autoimmune disease, but do seem to be related to the RNA toxicity and as the molecular mechanisms are clarified, a deeper understanding of these effects will be known.

The observed variability of manifestations is predictable considering complex mechanisms which might be involved in the origin of late onset neurological involvement, where the toxicity of the expanded *FMR1* transcript may interact with the number of other genetic or environmental effects throughout the lifespan (as detailed in Pathology section below), all contributing to the late-onset neurodegenerative changes, with FXTAS representing the most severe form of involvement. The occurrence of features of both FXTAS and Alzheimer's disease in a carrier of PM[189] is a good illustration of such combined effects towards more severe and complex manifestations; on the other hand, some other factors may be protective in nature thus determining less severe or vestigial manifestations, as well as the fact that at least 30% of individuals carrying PM alleles do not show any neurological involvement, even at advanced age. FXTAS appears to be clustered in families and a family without any neurological manifestations appears to have a lower incidence of involvement in subsequent members. Although the rates of FXTAS are low in daughters of men who have had FXTAS, other symptoms such as sleep disturbances, psychopathology and intermittent tremor are increased in these women as they age compared to women who do not have a father with FXTAS.[191]

Genotype-Phenotype Relationships

Confirmatory evidence for the link between the effect of *FMR1* small expansion alleles and specific neurodegerative processes has been provided by the results of genotype-phenotype correlations. The earliest data showed that *FMR1* CGG repeat length was significantly

associated with the total brain volume loss in a sample of male PM carriers aged >50 years.[175] This association was also reported in a sample of male carriers affected with FXTAS.[169] A strong significant association was foundbetween CGG repeat number and the percentage of neurons and astrocytes with intranuclear inclusions in the cortical grey matter and hippocampus;[173] a similar association was found between the number of repeats and the proportion of inclusion-bearing neurones in dentate gyrus and inferior colliculus in the expanded (CGG)*n* mice.[192] Some important relationships have also been reported between the number of CGG repeats and clinical outcomes. A negative correlation was found between CGG repeat number and the age of death[173] and age of onset of motor signs in FXTAS.[193] In a sample of male PM carriers who were not preselected for FXTAS diagnosis, CGG repeat size was found associated with the degree of motor impairment represented by all three motor rating scales,[194] and both neuropathic features[195] and abnormal nerve conduction.[168] Only a few of those relationships were also significant in female carriers.

Relationships between the phenotypic changes and elevated *FMR1* mRNA levels in PM carriers are less obvious than those with the size of CGG repeat expansion. This is not unpredictable since, while there is close correspondence between the size of CGG expansion betweenblood and brain cells,[196] there are blood-brain discrepancies between the levels of mRNA transcript.[154] Nevertheless, significant relationships between the elevated *FMR1* mRNA and psychological symptoms measured by SCL-90-R Global Severity Index was reported in 68 male and 144 female PM carriers both with and without a diagnosis of FXTAS,[20] with the relationships predominantly concerning obsessive-compulsive disorder and psychoticism. Moreover, in another study, a (negative) correlation was found between the levels of *FMR1* mRNA and the right ventral inferior frontal activity on fMRI during performance on working verbal memory test in 30 female PM carriers (15 with and 15 without FXTAS) compared with 12 matched healthy controls.[26] However, since the studies concerned with such relationships have been scarce, global interpretation of the findings so far is limited.

Since the discovery of FXTAS, there have been several studies from different locations assessing the prevalence of this syndrome in neurological disorders including essential tremor, cerebellar ataxia, Parkinson's disease, and multiple system atrophy (MSA).[197-210] Overall, the prevalence of PM alleles amongst groups of patients with late onset movement disorders has been lower than expected.[211] The diagnostic groups with the highest prevalence of PM carriers were males over 50 with cerebellar ataxia, with the overall prevalence of 17/872, 2%, ranging from 0%-7%[197-199,201,208] and individuals with multiple system atrophy-cerebellar type (MSA-C), with an overall prevalence of 2% with a range of 0% to 3.95%.[204,208] Surveys of male patients originally diagnosed with atypical,[200] or typical[203,205,212] Parkinson's disease (PD) have failed to show a statistically significant excess of PM carriers. Three carriers were found among 776 patients with idiopathic PD[213] and, although this was at least a three-fold greater rate than the normal population prevalence, it was not statistically significant. However, a significant excess of PM male carriers in the sample comprising typical and atypical idiopathic PD was reported in the latest screening study.[214]

Clinical Manifestations and Prevalence of FXTAS in Females

FXTAS has also been reported in female PM carriers.[22,206,215-220] However, it occurs at a much lower rate (~8% to 16.5%) than in male carriers, and clinical manifestations and cognitive decline tend to be milder and occur at a later age than in men; this is largely because ofthe protective role ofthe second X chromosome.[211,216-218] In another study[22] based on a sample of 85 Spanish females aged >50 years, FXTAS symptoms were encountered in 14 (16.5%) individuals using less stringent diagnostic criteria.

Brain changes as seen on MR images are also less severe in FXTAS females than in their male counterparts. Although a significant reduction in brain volumes and increased severity of white matter disease were found in a FXTAS group of 15 females compared with 20 age-matched controls,[169] these changes were less pronounced than in a FXTAS group of 36 males; the MCP sign occurred in only 13% of affected females compared with 58%, in males, and there was no significant association between the volumetric measures and either the severity of FXTAS manifestations, or CGG repeat expansion. However, females presenting with typical symptoms of FXTAS had greater medical comorbidity than males.[27] In this comprehensive study of 146 PM female carriers aged 20-75 years and 69 age-matched controls, 18 carriers (12.3%), who met the criteria of FXTAS diagnosis, had significantly higher prevalence by history of thyroid disorders (50%), hypertension (61.1%), fibromyalgia (43.8%), diagnosed peripheral neuropathy (52.9%), and persistent muscular pain (76.5%) compared to controls. In non-FXTAS group of carriers, the frequencies of chronic muscle pain, paraesthesias in extremities and history of tremor, were significantly elevated compared with normal controls. The size of CGG repeat and the levels of mRNA were both significantly higher in the FXTAS than in non-FXTAS groups, but the ARs were surprisingly not different. The more recent similar study based on a larger sample of 280 female PM carriers from Spain (with 195 between 4-50 years of age and 85 older than 50 years) found lower cormorbidity than reported in the American sample with thyroid disease (15.9%), and muscle pain (24.4%).[22] However females, just as their male counterparts, may present with atypical clinical manifestations of FXTAS, such as in the case of a 58-year-old PM female recently described,[190] who developed progressive dementia and parkinsonian signs, but neither action-intention tremor nor ataxia or MCP sign. Magnetic resonance imaging, however, showed extensive subcortical *wmd*, consistent with the distribution of *wmd* on post-morten examination, which showed white matter spongiosis in this region, as well as widespread ubiquitin-positive intranuclear inclusions in both neurons and astrocytes.

To date there has been no systematic study of cognitive status of females affected with FXTAS. But the analysis ofpsychological symptoms obtained from the SCL-90-R showed a significantly elevated somatization, obssessive-compulsive symptoms, depression, and overall symptom severity in a sample of 22 women affected with FXTAS.[20]

Pathogenesis of FXTAS

The associations of FXTAS and of the other related neurological manifestations with CGG repeat expansion within the PM range, the elevation of the expanded *FMR1* mRNA, as well as the presence of this mRNA in the intranuclear inclusions, gave rise to the hypothesis of a toxic ‘gain-of-function’ effect of excessive *FMR1* mRNA on brain tissue.[7,151,173,176] This

concept originated from pathogenesis of myotonic dystrophy,[221] where another (CUG) repeat expansion in the gene's promoter leads to sequestration of Muscleblind-like 1 (MBNL1) protein found in the intranuclear inclusions, leading to dysregulation and splicing of several other mRNAs and thus clinical involvement through deficit of the relevant proteins.[222,223] According to this model hypothesised for small CGG expansions in *FMR1* mRNA, the 'toxic' effect of elevated transcript leads to dysregulation of specific candidate proteins, such as purine-rich element binding protein (Pur α), or nuclear ribonucleoprotein A2/B1 (hnRNP A2/B1), which are sequestrated from their normal function and deposited in cells' nuclei causing their premature death. In support of this claim, the lack of Pur α protein in knock-out (KO) mice resulted in severe loss of neurones in the hippocampus and cerebellum, and development of tremor and seizures at two weeks of age,[224] whereas over-expression of this protein alleviated manifestations of neurodegeneration in a fly FXTAS model.[225] However, it has also been speculated that the elevated toxic RNA may not be directly involved in the sequestration of these proteins, but may act as a trigger of stress responses leading to over-expression of candidate proteins involved in neuroprotection.[13,178,225] Increased stress responses to toxic gain of function of *FMR1* mRNA with expanded CGG repeat have most recently been shown in cultured human fibroblasts and CNS tissue obtained from PM carriers.[226] These data provide evidence for a somewhat modified model of a toxicity of the expanded mRNA, in which increased expression of this transcript results in altered expression and disruption of the nuclear Lamin A/C architecture and induction of the expression of stress response genes. Since this type of cellular pathology may be induced within a very short time frame, the authors suggest that *FMR1* mRNA-induced abnormal skeletal organization in neural cells may take place in early development, thus underlying both neurodevelopmental and late-onset clinical conditions relevant to the PM carrier status. The risk and the type of manifestations of the latter may be determined by the presence and the nature of additional genetic effects varying between individuals, as well as some environmental factors such as pollutants.[227]

Identification of the contents of intranuclear inclusions has been an important step towards better understanding of the mechanisms leading to FXTAS.[151,228,229] Unlike in many other disorders associated with unstable repeat expansions, there was lack of a principal protein, and ubiquinated proteins represented only a minor component in the inclusions' deposits, which indicated that their formation is unlikely to result from accretion of specific abnormal proteins. The minor proteins deposited included lamin A/C, two RNA directly binding proteins: Pur α, hnRNP A2/B1 and MBL1, along with the *FMR1* mRNA. Recent evidence of the sequestration of Sam 68, an important RNA splicing protein,[230] and the recent evidence of DROSHA sequestration in the inclusions suggests that dysregulation of miRNAs is involved in the toxicity of the premutation.[231]

Although precise mechanisms leading from the elevated RNA to neurodegeneration still remain speculative, sufficient evidence fordirect RNA toxicity hasbeenprovidedby the results ofanimal and cell based studies (reviewed in: Garcia-Arocena and Hagerman 2010[13]). The earliest findings in *Drosophila melanogaster* showed that human PM-size CGG repeat transcripts ectopically expressed in neural eye tissue caused neurodegeneration in a dosage- and repeat length-dependent manner.[232] Furthermore, the ubiquitin-positive

intranuclear neuronal inclusions and neurodegeneration similar to that in human PM carriers affected with FXTAS were observed in the *FMR1* KI mice;[177] these mice also manifested cognitive decline, neuromotor, and behavioural disturbances associated with the elevated mRNA.[233] Further evidence for the RNA with the expanded CGG repeats to cause neurodegeneration in FXTAS was provided by the findings showing that this RNA expressed in Purkinje neurons of experimental mice outside the context of *FMR1* mRNA resulted in neuronal pathology presenting as intranuclear inclusions, neuronal death and behavioural changes.[234] In the most recent study of PM KI mice the presence of ubiquitin-positive intranuclear inclusions were found in neurons throughout the brain in cortical and subcortical regions increasing in number and size with advanced age;[127] importantly, these inclusions were also present in protoplasmic astrocytes including Bergman glia in the cerebellum, which might have some relevance to a particularly severe white matter neurodegeneration in cerebellar peduncles.

The typical inclusions have also been found in the anterior and posterior pituitary gland in humans as well as in KI PM mice and throughout the limbic system including the amygdala[235-237] which imply the possibility of a dysfunction of hypothalamus-pituitary-adrenal gland axis, and may explain some of neuroendocrine and psychiatric problems seen in carriers with and without obvious FXTAS. The inclusions have also been located in the Leydig and mucoid cells in the testicles obtained from human PM carriers which may explain the low testosterone levels and frequent onset of impotence before the tremor and ataxia of FXTAS.[235]

Another important step towards understanding of pathophysiological mechanisms of broader neuropathology linked to the RNA with expanded CGG repeats has been made in the latest study based on female KI mice heterozygous for the PM allele, where cultured neurones manifested dendritic changes and lower cell viability.[238] This finding, combined with the recent report of abnormal cellular distribution of lamin A/C isoforms in embryonic fibroblasts of KI mice with the expanded CGG repeat in the murine *FMR1* gene[226] point to both neurodegenerative and neurodevelopmental effects of the expanded-repeat mRNA; notably, in the same study, altered lamin A/C expression/organization and increased stress response were also found in cultured skin fibroblasts and CNS tissue from male PM carriers either with or without FXTAS. These data provide strong evidence for biological processes underlying the postulated link between early onset or mid-life pathologies associated with elevated levels of *FMR1* transcript, such as behavioural and cognitive deficits, psychiatric symptoms or FXPOI (described later in this chapter), on the one hand, and late-onset neurodegenerative disorders, on the other.

Additional difficulty in understanding the individual steps leading from the toxic RNA to changes at the cellular and clinical level may be related to the likely involvement of other factors and processes interacting with, and modifying the effects of the expanded toxic RNA. After all, only certain proportions of PM carriers are affected with either neurodevelopmental or neurodegenerative conditions, or present the symptoms ofboth of them. Clearly, individual genetic make-up, including background genes of minor effects, contributes to the variability in penetrance at the individual as well as the family level. The role of environmental exposure, especially to neurotoxic agents, postulated in context with

the severity of expression ofFXTAS,[166] and recently demonstrated by several clinical examples[227] should not be underestimated. This aspect is especially relevant considering the latest evidence suggesting the involvement of mitochondrial dysfunction in pathogenesis of neurological involvement in PM carriers based on fibroblast and brain samples,[239] and on blood leucocytes from PM and GZ allele carriers.[240] Finally, the contribution of the recently identified antisense *FMR1* mRNA, which is also elevated in PM carriers[241,242] to neurotoxicity, needs to be further investigated. Our preliminary data[240] have shown that the elevated levels of this transcript may play a pivotal role in the severity of neurological involvement in the carriers of small expansion *FMR1* alleles.

FRAGILE X-ASSOCIATED PRIMARY OVARIAN INSUFFICIENCY

Another distinct disorder linked to the *FMR1* PM alleles is Primary Ovarian Insufficiency (POI). This term was proposed[243] to reflect, apart from the cessation of menstrual periods before age 40, a spectrum of diminished ovarian functions occurring in female carriers of the *FMR1* PM alleles. The more specific and currently used term of 'Fragile X-associated Primary Ovarian Insufficiency' (FXPOI) was introduced to describe any degree of POI attributable to the *FMR1* aetiology.[244] FXPOI affects approximately 20% of PM carriers, consistently in many population/ethnic groups,[245-249] representing 20-fold increase compared with the risk of 1% seen in the general population. Estimated frequencies of PM carriers among females reporting to infertility clinics with familial or sporadic FXPOI were: 11.5% with 95% CI: 5.4%-20.8%, for familiar sample; and 3.2% with 95% CI: 1.4%-6.2%, in sporadic sample, compared with the population frequency of these carriers of ~0.4%, as reviewed by De Caro et al.[244]

Notably, data obtained[4] from over 500 women with a wide range of CGG repeats from the normal range into the high end of the PM range demonstrated a significant association between CGG repeat number and prevalence of FXPOI that was nonlinear. For those with a repeat <40 the prevalence of POI was 0.9%, for those with 41-58 repeats it increased to 2.2%, for those with 59-79 repeats it was 5.9%, for those with 80-99 repeats it reached 18.6%, but for those with ≥100 repeats the prevalence decreased to 12.5%. These authors suggested that perhaps those with a high CGG repeat number may have some ovarian target cells with a full mutation, or some cells have early methylation at a lower CGG repeat number that protects them from the toxicity of the PM. An overall effect of the CGG repeat size on age of menopause has been reported,[4] with low end CGG repeats demonstrating menopause 2.5 years earlier than controls, and medium to high end PM carriers demonstrating menopause 4 years earlier than low- end carriers. Nonlinear association between the CGG repeat number and the risk of FXPOI and age of menopause was later confirmed in different samples.[250-252] In the 2007 study based on data from 948 women with the wide range of repeats sizes, the mean age of menopause for carriers of 59-79 repeats and >100 repeats was similar (48.5 and 47.5, respectively), but was ~3 years lower (44.9) in carriers with 80-100 repeats. Although the highest decrease in menopausal age was in carriers of CGG expansions with the 80-100 range, it was reduced across the total PM range compared with 52.3 + 0.5 years found in the sample of noncarriers.

Although the *FMR1* PM represents a common major gene affecting the age of menopause in particular, and the female reproductive system in general, other factors modifying this effect should be considered. Smoking has been shown to aggravate this major effect in carriers of PM by 1 year. The identity of other major genes possibly interacting with the effect of *FMR1* has not yet been established. However, the effect of the background genes (residual genetic variance) in the presence of the major effect of the *FMR1* PM, was recently estimated in a study based on 230 fragile X families and 219 families from the general population.[253] The analysis used a random effect version of the Cox proportional hazards model allowing for shared polygenic (additive) effects and the effects of confounders.[254] The results confirmed significant major effect of *FMR1* PM, with the greatest effect of mid-size group, and provided evidence of a substantial additive (background) genetic component even after adjusting for the major effect; estimated additive genetic variance ranged from 0.55 to 0.96 (depending on different model assumptions), with P-values ranging from 0.0002 to 0.0027. Examples from single families further illustrate a significance of shared genetic effect on the risk of menopausal anomalies. In one family[255] premature menopause occurred in all six sisters carrying PM; in another family[256] two sisters, one PM carrier and one noncarrier, presented with POI. The reported examples of menopausal problems co-existing with the other *FMR1* PM- associated conditions such as FXTAS or psychiatric involvement in one and the same carrier,[220] or between two generations of carriers191 are also relevant to a significance of shared family effect, which should be considered in individual risk estimates and counselling.

The features reflecting pathology of the reproductive system in PM females extend beyond, though are not irrelevant to, the earlier menopausal age. The earliest and most comprehensive endocrine study of the hormonal profile of female PM carriers demonstrated that those carriers that were cycling normally had endocrine dysfunction.[243] These authors studied 11 normally ovulating PM carriers (ages 23 to 41 years) and demonstrated a significantly shortened cycle, elevated follicle stimulating hormone (FSH) throughout the cycle (91% with elevations >2 SD above the mean), elevated Inhibin -B in the follicular phase, and elevated Inhibin- A and progesterone in the luteal phase compared to controls. These findings suggested a decreased number of follicles and granulosa cell dysfunction or decreased cell number in the corpus luteum compared to controls. In addition, 45% (5 of 11) of these carriers had a history of infertility as defined by 1 year of unprotected intercourse without a pregnancy. This study demonstrated sub-clinical ovarian dysfunction in PM females who do not have POI. For women at the premenopausal stage there was a CGG repeat, and AR effect on the FSH level, but only when controls and carriers were included together. Consistent finding of an increase in FSH serum levels in PM carrier who were still cycling was reported in the other studies.[257,258] This effect remained after adjusting for smoking and use of contraceptives[257] or age and shared family effects.[258] However, significantly increased FSH levels were limited only to women aged 30-39, which suggested late onset of ovarian dysfunction.[4] But the results of the later study showed that this dysfunction may occur at an earlier age. Using another biomarker of ovarian reserve, mullerian-inhibiting substance (AMH), which, unlike FSH, is only expressed in growing follicles, a significant reduction of the AMH levels was shown for all age groups within the 18-50 years range in the higher (>70 repeats) versus lower (<70 repeats) risk PM carriers.[244]

Some other abnormalities within the FXPOI spectrum include irregular menses,[245,252] shorter cycles,[243,252] and irregular length and skipped cycles in the mid-size repeat PM carriers;[252] in the same group of carriers these authors also found increased twinning. Moreover, the findings of significant decrease of mineral bone density[259] inPM carriers compared with normal controls, and a significant increase in osteoporosis, with the highest increase of 11.9% in the mid-range PM carriers based on large samples,[260] are of particular concern.

The mechanisms involved in ovarian insufficiency in small expansion carriers are still unknown. The fundamental question to be answered is what stage of individual growth/development is most relevant to the damaging effect of the processes related to these expansions. Do these events lead to a smaller ovarian endowment already present at birth? Do they lead to alteration of recruitment of follicles later in life? Or do they lead to follicular atresia and thus premature degeneration? It is also unknown if these processes directly influence the ovarian tissue, or act via an altered hypothalamo-pituitary-gonadal axis, especially considering that the typical intranuclear inclusion formation has been found in neurons of the anterior and posterior pituitary gland in a male PM carrier.[235] Although these changes have not yet been investigated in female carriers, it has been postulated that these degenerative changes in gonadotropic cells lead to dysfunction of hypothalamic-pituitary-gonadal axis and thus to abnormal follicular recruitment.[244] Alternatively, FXPOI may result from direct damage to the ovaries either by increased FMRP levels during specific stages of development, or by the ‘gain of function’ toxic effect of elevated *FMR1* mRNA leading to premature ovarian degeneration later in life.[244] The recent important finding of an overproduction of FMRP among carriers of 80-89 repeat alleles[261] may give some support to the hypothesis of this protein's related damage to ovaries, since most clinical manifestations of FXPOI have been most prominent in carriers of medium size repeat PM alleles.

The lack of convincing evidence for any anomalies in the age of menarche or around menarcheal age in PM carriers[259,262] suggest that the changes related to the effect of this PM occur at later stages of reproductive life. However, one study[252] found a small but significant difference in the age of menarche only in a subgroup of low repeat PM carriers (12.18 ± 1.38 in the carriers compared with 12.44 ± 1.51 in noncarriers), although overall the age of menarche was not different from controls. Considering scarcity and inconsistency of the data concerning adolescent period in female carriers, more studies are required to identify the onset and the progress of FXPOI, which might be related to the specific effect of *FMR1* PM. Evidence that it may extend over to the adolescence period has been provided by data showing that the onset of menopause was below the age 30 in 30% of PM carriers,[247,263] and in rare cases, it was at the age of [18,256,264] and the lowest reported ages were between 13 and 17 years in three carriers.[263]

DO THE INTERMEDIATE SIZE (GZ) ALLELES CAUSE ABNORMAL PHENOTYPES?

Understanding of phenotypic effects of GZ alleles is important considering their bridging position between the common and fragile X (PM and full mutation) allele categories, as

indicated by haplotype associations (discussed above). Most importantly, given their high population frequency of~3-4 per 100 males,[83,84,97,112,265] even a small effect contributing to the developmental or late-onset conditions, if substantiated, is likely to be borne by a considerable number of individuals.

The GZ alleles, with the originally recommended range of 45-54 CGGs,[90] but varying between different studies (reviewed by Mitchell et al[84]) have, until recently, been included in the normal category with regard to phenotypic expression. However, the breakthrough has come with a demonstration of the elevated *FMR1* transcript in a sample of 32 Australian GZ male carriers identified in the SEN (special educational needs) population.[266]

This study also showed that, if the regression is applied across a wider range of *FMR1* alleles, including both GZ and PM allele categories, the sharp increase in mRNA levels that is proportional to the number of CGG repeats seen in the GZ range, slows down in the PM range (Fig. 4). The onset of this disturbance indicated by this study's data is at approximately 39 repeats; however more observations, especially at the upper end of the 'normal' alleles, are required to estimate this threshold more precisely. In the earliest study of small CGG expansions that included only two GZ carriers, increased transcription and a slight decrease in translation of the gene was also reported.[155]

Findings from another study, based on human cell lines transfected with the *FMR1* 5′-UTR containing CGG repeat lengths ranging in size from 0 to 99 repeats and a downstream reporter gene, confirmed a transcription defect in GZ class of alleles.[156] Although a different reporter gene (luciferase and not *FMR1*) gene was used, the results demonstrated an increase in transcription levels for constructs possessing either PM or GZ alleles, further supporting the later findings based on human subjects.[266] If, indeed, the elevated levels of *FMR1* mRNA cause neurodevelopmental and neurodegenerative changes in PM carriers, one can postulate that the elevated levels observed in carriers of GZ alleles, even if more moderate, could cause conditions reminiscent of those seen in a significant proportion of PM carriers.

However, in contrast with the PM alleles, establishing the pathogenic role of the GZ allele is not straightforward. *First*, these alleles have been relatively poorly defined with respect to repeat size, and the range of this category has differed between different studies (reviewed in refs. 83,84,97,112,267). *Second*, measurement of CGG repeat number is not consistently performed across all laboratories, and comparisons between studies may not be valid. *Third*, unlike for PM alleles, GZ alleles cannot, except for rare cases, be identified through cascade testing of fragile X families, so that the prevalence and the type of phenotypic involvements cannot be determined in an unbiased manner. On the other hand, the results obtained from screening population groups with specific disorder or developmental delay would be biased and thus inconclusive. Therefore, the most stringent approach would be to test an entirely unbiased population, such as a series of consecutive newborns initially screened for *FMR1* CGG size, and their phenotypes subsequently assessed. Such an undertaking, while ideal, would be logistically daunting. The next best is to study a well defined population of affected individuals, and to compare the CGG_n profile with that of an appropriate control group.

The earliest systematic study based on screening of a well defined population preselected for significant learning problems (SEN) suggested phenotypic (neurodevelopmental) effects of GZ alleles.[267] The results provided evidence for an increased frequency of these alleles in this tested population compared with that in control X chromosomes. However, the studies have notbeen consistent, with some confirming this association,[82,89] and others failing to do so.[96,268270] One study[82] found that 4.4% of 3,732 UK SEN males had GZ alleles, which represented a significant increase compared with non-SEN samples. Our own study conducted in the population of 1253 SEN male students from Tasmanian schools also found increased frequency of GZ alleles (3.45%) compared with 2.43% in 578 consecutive male births in the same state.[84] Although the frequency figures are similar to those obtained in the earlier studies, the SEN-control difference was insignificant. The possibility of an increased risk of learning orbehavioural problems in carriers of GZ alleles has also been suggested by the fact these carriers are sometimes identified among children with learning or behavioural deficits attending genetic or psychiatric clinics. A summary of physical and neuropsychological test results from a sample of such children, combined with the carriers identified through comprehensive testing of fragile X families (the total of 10 boys aged 4-15 years, including 6 PM and 4 GZ carriers), was presented.[15] A majority of participants manifested psychiatric (autism spectrum and/or hyperactivity), psychological (expressive language and/or other minor cognitive impairments), and several physical characteristics (all normally associated with the full mutation). However, no relationship was found between clinical affectedness and either CGG repeat size or FMRP levels. Although descriptions of this kind are informative, such data are not amenable to statistical comparison with normal samples, because of ascertainment bias caused by preselection of participants for the traits examined in the study. Nevertheless, the results of a screening of a small but well defined sample of children diagnosed with autism spectrum disorders (ASDs) suggested a significant role of GZ alleles in the riskforthis condition, and also suggested possible mechanisms involved in their effect.[271] It is clear from these results that the question whether or not GZ alleles are associated with neurodevelopmental problems is still unresolved and requires further population-based study, proper controls and more sensitive and specialized assessment tools.

The stronger evidence for phenotypic effects of GZ alleles has been provided by the data showing a significant association of these alleles with FXPOI spectrum of abnormalities.[272,273] Grouping the alleles into sizes of 35-54 and 55+ repeats, of the women having suffered POI, 14.2% (15 of 106) had an allele in the range of 35-54, whereas in the general population the figure was 6.5% (21 of 322).[272] The increase in the risks is significant, and the data speak for an effect of the CGG repeat length, whether GZ or PM, upon ovarian function.[249] In the later study of small samples (27 POI and 32 control women), six POI (22%) women and one control woman (3%) carrying *FMR1* alleles of >40 repeats, were identified, and this represented a significant increase in the POI sample.[274] An interesting new perspective on this issue has been provided by the latest finding, though based on a small samples of 23 tested and 11 control individuals that the fall in ovarian reserve represented by the FSH and AMH markers is already apparent in carriers of repeats >30.[275] In another study, the rise in serum concentration of FSH was associated with CGG

repeat size within the normal range but exceeding 30 CGGs, both in 80 females with POI and 70 controls.[276]

Since FXTAS has been another late-onset condition found associated with a toxicity of elevated RNA in PM carriers, a similar, though less apparent, association may be expected for GZ carriers with elevated expanded *FMR1* mRNA. In rare studies, the small samples of patients with Parkinson's disease spectrum were screened for the GZ alleles and, although there was a trend towards the increased prevalence (reaching 7%), no significant excess of these alleles relative to their population prevalence has been reported.[205,212,213] However, a significant relationship between the size of CGG repeat within the GZ range and cognitive decline and hallucinations was reported in one of those studies.[213] Among 507 patients (253 male, 254 female) with MSA, 3.6% of males and 3.9% of females had a GZ of size 40-54, comparing with 3.2% and 4.2%, respectively, in controls;[204] in a smaller French series of 77 patients, five had a GZ of size 41-54, for a fraction of 6.5%, this being somewhat higher, although not significantly so, than the control figure of 3.4% (4/117).[208]

In the latest study,[214] 228 Australian males affected with parkinsonism were screened for GZ, as well as PM alleles. Importantly, this sample included broader diagnostic group of the primary parkinsonian syndromes according to the classification in Schapira et al.[277] The frequencies of either of these alleles were compared with the frequencies in a population-based sample of 578 Guthrie spots from consecutive Tasmanian male newborns (controls). Apart from a significant excess of PM, there was also a more than 2-fold increase in GZ carriers, with odds ratio (OR) = 2.36, and 95%confidence intervals (CI): 1.20-4.63, compared with controls.

These results have suggested that both PM and GZ alleles may contribute to the risk of parkinsonism, and that the ‘toxic’ effect of expanded mRNA linked to the PM alleles may also occur when the expansions are smaller. It is possible that the modest elevation of ‘toxic’ RNA, such as is the case in the GZ and lower-end PM carriers, may present an additional risk for neurodegeneration by synergizing with other susceptibility genes for parkinsonism. This notion is supported by common occurrence of parkinsonian manifestations amongst patients primarily diagnosed as FXTAS (as detailed in previous section).

Detailed clinical and genetic testing was performed in 14 carriers of the small expansion *FMR1* alleles encompassing the GZ and the lower-end PM range.[240] This was in order to determine whether these alleles present a significant modifying factor for the manifestations of parkinsonian disorders. The results were compared with the same data obtained from 24 noncarriers. Both GZ and PM carriers presented with more severe symptoms than a sample of noncarriers matched for age, diagnosis, and treatment. The Parkinson's (UPDRS) motor score and the measures of cognitive decline (MMSE and/or ACE-R scores) were significantly correlated with the size of the CGG repeat, and the (elevated) levels of antisense *FMR1* and Cytochrome C1 mRNAs in blood leucocytes. In addition, the carriers showed a significant depletion of NADH dehydrogenase (ND) subunit 1 (ND1) gene mitochondrial DNA in whole blood. These data have shown that both small CGG expansion *FMR1* alleles may play a significant role in the acquisition of the parkinsonian phenotype, possibly through the cytotoxic effect of elevated sense and/or antisense *FMR1* transcripts

involving mitochondrial dysfunction and leading to progressive neurodegeneration. Notably, evidence for mitochondrial involvement was also presented in another study based on fibroblasts and brain samples from PM carriers affected with FXTAS.[239]

In conclusion, although the results on phenotypic involvements of GZ alleles are still sketchy, there is sufficient evidence to postulate their role in the aetiology of certain human conditions affecting the brain and reproductive system. Since the expansion size and thus the elevation of the *FMR1* transcripts is less than in the PM carriers, the effect may be more subtle and more difficult to detect, especially as the carriers cannot be, as is the case with PM carriers, ascertained through fragile X families. Moreover, if the effect of *FMR1* mRNA toxicity in GZ carriers is not sufficient to be the sole determinant of a disease such as FXTAS, it may increase their risk of other disorders that are likely to involve mitochondrial dysfunction, such as Parkinson's disease or multiple sclerosis. This hypothesis requires testing via further comprehensive studies based on large samples.

Apart from the possible effect of small CGG expansions/elevation of *FMR1* mRNA on the phenotype, the results of the latest studies[239,240,278] have thrown new light on the ambiguity currently associated with the definition of GZ alleles. We determined a threshold of ~39 CGGs for the onset of a progressive increase in *FMR1* transcription.[266] Although more data are required to narrow the error margins, it appears that the lower bound for the GZ currently defined as 45CGGs[90] should be revisited in the light of these findings. Moreover, these data also suggested a continuous scale of involvement, especially at the lower end of PM range, rather than the clear-cut separation between GZ and PM categories recommended[90] solely on the basis of the potential for CGG expansion. It appears both from the elevation of the *FMR1* transcript in GZ alleles, and the observed effects of these alleles on the phenotype that the difference between these two categories is quantitative in nature rather than qualitative. The phenotypic effects reported so far in GZ carriers, including POI, late onset neurological involvement, autism, and possibly other behavioural problems and learning problems are similar to these seen in PM carriers. The elevation of the *FMR1* transcript, as well as the linear relationship between the levels of mRNA and the number of CGGs, are other common features of both the GZ and PM *FMR1* alleles and thus the mechanism of this elevation must also be similar.

PRESENT STATUS OF TREATMENT OF FRAGILE X-RELATED DISORDERS

Over the years a variety of pharmacological interventions has been developed for treatment of FXS related to the full mutation68 and also for PM associated disorders including FXTAS.[10,279] Typically treatment ofboth FXS and FXTAS involves multiple professionals and multimodality intervention that have been described at length elsewhere.[10,280]

For the psychopharmacology interventions in those with FXS both stimulants and alpha agonists, such as guanfacine and clonidine, can be helpful for treatment of ADHD.[68] However, the stimulants are usually not started until after 5 years of age because of frequent side effects in those under 5 years. Selective serotonin reuptake inhibitors (SSRIs) are commonly used to treat anxiety and hyperarousal and early treatment may help with language and socialization benefits.[68] In addition, the atypical antipsychotic aripiprasole

(Abilify) has been frequently used with generally good success for anxiety and aggression.[281] Aripiprazole will also stabilize mood in addition to treating ADHD symptoms, but often a lower dose is better tolerated without activation.[68]

The most exciting development in the treatment ofFXS is the introduction of targeted treatments that can reverse some of the neurobiological abnormalities that have been identified in the animal models of FXS. The up-regulation of the metabotropic glutamate receptor 5 system (mGluR5) leading to long term depression (LTD) described by Huber et al[282] and Bear et al[283] has lead to the use of mGluR5 antagonists that have been shown to be helpful in the animal models of FXS[40,284,285] and are now demonstrating benefit in individuals with FXS.[286] At least three mGluR5 antagonists have been developed and 2 are undergoing assessments in adults with FXS in phase II trials with plans for broad phase III trials in the near future. Another way of decreasing the level of glutamate at the synapse is with the use of a $GABA_B$ agonist, specifically Arbaclofen, the right isomer of baclofen. Studies in mice and a phase II trial in children and adults aged 6 to 40 years suggest benefit in those with FXS plus autism and FXS with low sociability.[287] These promising results in FXS and preliminary results in those with autism without FXS have lead to phase III trials in both disorders.

The use of minocycline for 1 month after birth in the KO mouse led to benefits in behaviour and cognition in addition to improvements in the immature dendritic structure that is typical for FXS.[288] Minocycline appears to work by lowering matrix metalloproteinase 9 (MMP9) levels in the KO mouse which are elevated compared to wild type mice. The results of the Bilousova trial stimulated use of minocycline by families of children with FXS and a subsequent survey of parents regarding the benefits and side effects of minocycline suggested that about 70% of children improved on minocycline particularly in language and behaviour.[289] These preliminary results are being followed by a controlled trial of minocycline in children with FXS between the ages of 3.5 years and 16 years. Side effects of minocycline include graying of the tooth enamel when used under 8 years old although occasional occurrence of graying of skin and nails has been reported in older individuals. Minocycline can rarely cause pseudotumor cerebri, a lupus-like rash and seropositivity of the antinuclear antibody (ANA), so regular follow-up of these patients is necessary.

Lastly the use of nutritional supplements including antioxidants have demonstrated benefit in the treatment of the KO mouse with improvements in behaviour and testicular size and this is related to the findings of oxidative stress in the KO mouse.[290] Melatonin which can be helpful for the sleep disturbances in FXS[291] also seems to have an antioxidant effect in the mouse.[292]

The targeted treatments will hopefully strengthen synaptic connections in FXS, but these patients will also need to use educational interventions before the intellectual disabilities of FXS can be reversed.[293] However, the future looks bright for the treatment of FXS and further studies have been planned. The treatment of PM disorders is currently symptomatic, including the treatment of FXTAS[10,279] although neuroprotective agents such as memantine are currently being studied. Further research into the molecular mechanisms leading to RNA toxicity will certainly lead to more trials in both animal models and humans.

CONCLUSION

The fragile X field has changed remarkably since the discovery of the *FMR1* gene in 1991. The nonpenetrant status of the premutation carrier has expanded into an array of clinical involvement influencing psychiatric, endocrinologic, neurocognitive and neurologic health. The clinician must be alert to clinical involvement in all generations of a family identified with a proband. Screening studies of high risk populations will identify many more individuals affected by the mutations in *FMR1* and the premutation will be an important additive component to many common problems of aging including motor and cognitive decline. We are living in an age of targeted treatments and the portal disorder of FXS will open the doors to new treatment endeavors in autism and other disorders that have overlapping molecular involvement because of FMRP's ubiquitous presence and influence. This is an exciting time for clinicians focused on targeted treatments and FXS is likely to be the first neurodevelopmental disorder whose phenotype will be reversed.

Acknowledgments

This study was supported by the National Institutes of Child Health and Human Development Grant HD 036071 to Dr RJ Hagerman and Dr DZ Loesch, NINDS RL1AG032115 to Dr Hagerman and NHMRC project grant No 330400, to Dr DZ Loesch. We thank Jacky Au for his excellent work regarding the preparation of this manuscript.

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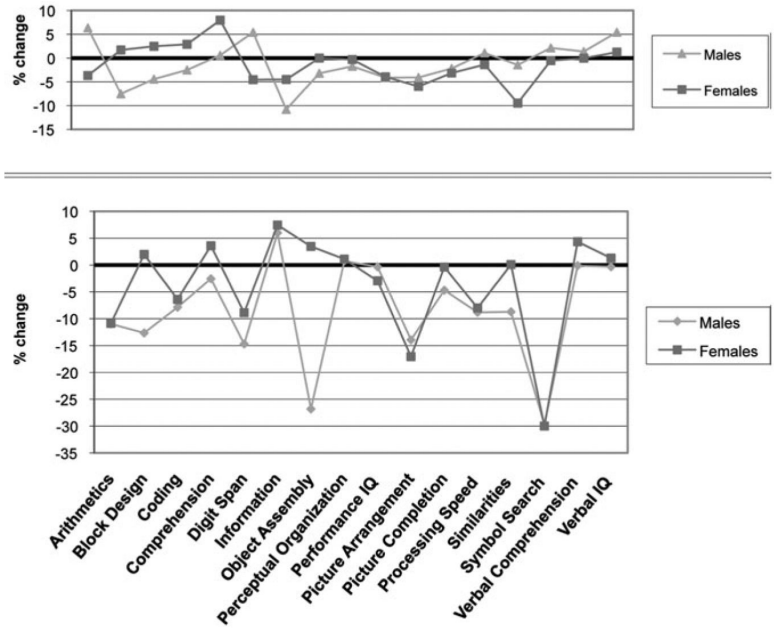

Figure 1.

Standardized percent deviation of FSIQ-adjusted summary and subtest (Wechsler) scores from the normal mean due to the effect of premutation (upper figure), and full mutation (lower figure) separately for males and females, based on pedigree models. The baseline represents the FSIQ of a 100.[119] Reproduced from Loesch DZ et al. Am J Med Genet A 2003; 122A(1):13-23.[119]

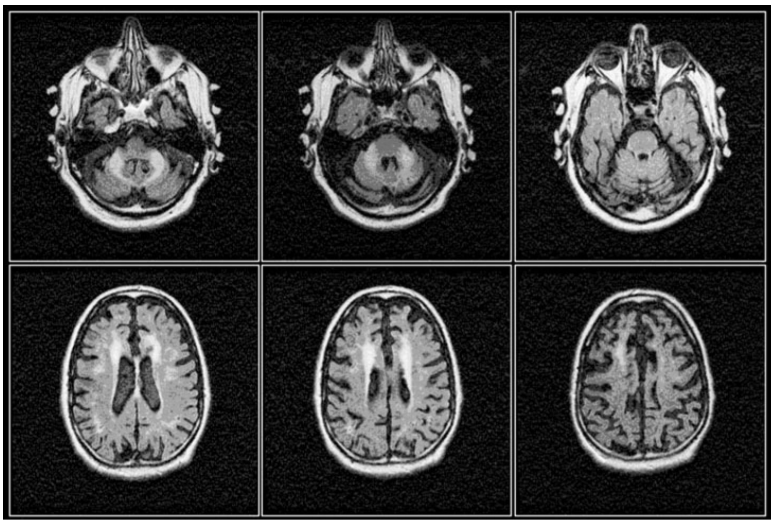

Figure 2.

Axial FLAIR images of the brain of the 64 year old male carrier of 80 CGG repeats with typical manifestations of FXTAS comprising marked cerebellar ataxia, intention tremor, autonomic failure (originally diagnosed with multiple system atrophy, MSA). Marked bilateral T2 hyperintense signals within the middle cerebellar peduncles (MCP sign), combined with symmetrical T2 hyperintense signals within the corona radiate and considerable cerebral and cerebellar atrophy are evident from the images.[166] Reproduced from Loesch DZ et al. Clinical Genetics 2005; 67(5):412-417.

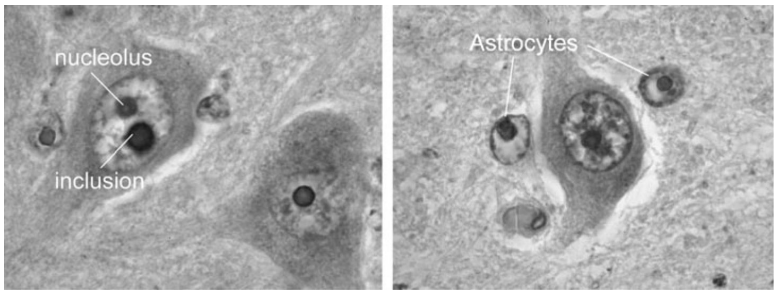

Figure 3.

Eosinophilic inclusions in the nucleus of both neurons (left panel) and astrocytes (right panel) from a patient with FXTAS. Picture courtesy of Claudia Greco MD and Paul Hagerman MD, PhD.

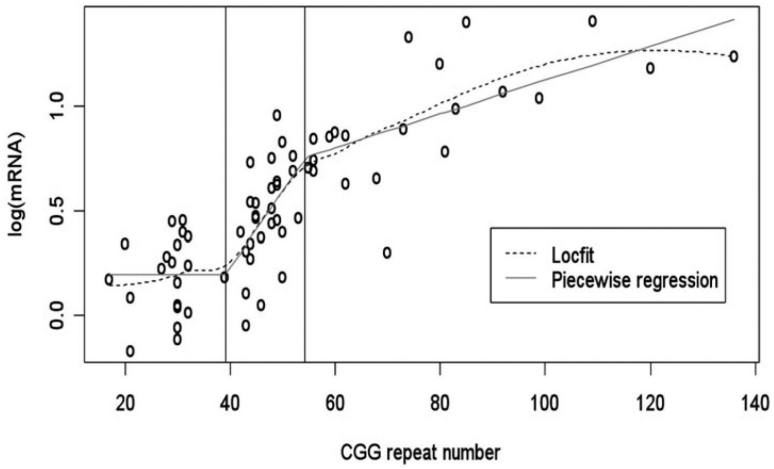

Figure 4.

Nonparametric local fit (Locfit) and piecewise regression fit to the log-transformed mRNA levels versus CGG repeat numbers for data from all three allelic categories: Normal, GZ and PM.[266] Reproduced from Loesch DZ et al. J Med Genet 2007; 44(3):198-204.[266]