

FXTAS: A Progressive Neurologic Syndrome Associated with Fragile X Premutation

Rob Willemsen, PhD*, Edwin Mientjes, PhD, and Ben A. Oostra, PhD

Address

*Department of Clinical Genetics, Erasmus MC, PO Box 1738, 3000 DR, Rotterdam, The Netherlands.
E-mail: r.willemsen@erasmusmc.nl

Current Neurology and Neuroscience Reports 2005, 5:405–410
Current Science Inc. ISSN 1528-4042
Copyright © 2005 by Current Science Inc.

The *FMR1* gene is involved in two different syndromes: Fragile X syndrome and Fragile X-associated tremor/ataxia syndrome (FXTAS). Fragile X syndrome is a childhood disease and is associated with mental retardation as the main clinical characteristic, whereas FXTAS develops in men and women over 50 years of age. FXTAS represents a new form of inclusion disorder with a high prevalence in the general population. The neurologic phenotype of FXTAS includes intention tremor and ataxia. Associated features are dementia, parkinsonism, neuropathy, and autonomic dysfunction. Elevated *FMR1* transcripts have been proposed as the molecular basis of the pathogenic mechanism leading to FXTAS. This review discusses recent developments in the clinical phenotype, prevalence and screening, animal models, and molecular mechanisms of RNA-based pathogenesis in FXTAS.

Introduction

Fragile X syndrome represents the most common inherited form of mental retardation and is almost exclusively caused by an expansion of a CGG repeat in the 5' untranslated region of the *FMR1* gene. In the normal population, the CGG repeat is polymorphic and ranges from 5 to 55 CGGs, with an average length of 30 CGG units [1]. Fragile X patients have more than 200 CGG units that are usually hypermethylated, and the methylation extends to the adjacent promoter region of the *FMR1* gene [2–4]. As a consequence, the gene is transcriptionally silenced and the gene product, the Fragile X mental retardation protein (FMRP), is absent. The lack of FMRP in neurons is the cause of the mental retardation in Fragile X patients [5–7]. Unmethylated expansions of 55 to 200 CGG units, called premutations (PM), are found in both male and female individuals and may expand to a full mutation (FM) only upon maternal

transmission to the next generation. The risk of transition is dependent on the size of the PM; the smallest CGG repeat number known to expand to a FM is 59 repeats [8]. Next to PM alleles, CGG repeats within the “gray zone” of 40 to 54 CGG repeats have been identified and shown to be slightly unstable upon transmission. However, they have never been observed to expand to a FM in a single step.

Individuals with a PM do not exhibit the classical phenotype of Fragile X syndrome and were initially thought to be asymptomatic, although a number of studies have reported mild learning disabilities and social phobias or anxiety disorders in a small subgroup of PM carriers [9,10•]. In addition, approximately 20% of female PM carriers manifest premature ovarian failure [11]. Recent studies have reported male individuals with alleles in the PM range with increased *FMR1* mRNA levels that are up to eightfold higher than normal and with (mildly) reduced FMRP levels [12•,13,14]. The elevated *FMR1* transcript levels were positively correlated with the number of CGG repeats [15]. The question of whether these elevated *FMR1* mRNA levels and slightly reduced FMRP levels result in a mild Fragile X phenotype was challenged by the recent description of older males carrying a PM who exhibit a unique neurodegenerative syndrome characterized by progressive intention tremor and ataxia, named Fragile X-associated tremor/ataxia syndrome (FXTAS) [16,17,18••,19].

Clinical Phenotype

Clinical phenotype in symptomatic male patients

In the initial description of the neurologic phenotype of PM carriers, the action tremor was the first sign related to FXTAS, followed by ataxia; however, later studies point to ataxia as the major symptom [20••]. More advanced cases may be accompanied by progressive memory and executive function deficits, anxiety, parkinsonism, peripheral neuropathy, essential tremor, and autonomic dysfunction (impotence, bowel incontinence, urinary incontinence, and hypertension) [18••]. Significant dementia has been observed in a limited number of patients [20••,21•]. All symptomatic patients say that the onset of symptoms started in their 50s or later with a further progressive course; however, the cognitive decline shows a high

variability between affected individuals. Cognitive functioning may be stable for several decades or patients may suffer from severe dementia within 2 years after onset of the first symptoms [18••]. A logical explanation for this variability is the genetic background of the different individuals. Magnetic resonance imaging (MRI) studies (T2 signal) of the brain of symptomatic adult male PM carriers showed characteristic imaging, including dilated ventricles; hyperintensities of the middle cerebellar peduncle (MCP); cerebellar white matter lateral, superior, and inferior to the dentate nuclei; and volume loss involving the pons, mesencephalon, cerebellar cortex, cerebral cortex, white matter of the cerebral hemispheres, and corpus callosum [18••,22]. On the basis of a detailed clinical and radiological survey, Jacquemont *et al.* [18••] have proposed diagnostic criteria for definite FXTAS that include (next to an *FMR1* PM allele as mandatory criterion) the presence of intention tremor and/or ataxia as major clinical finding, and the MCP features as the major radiologic finding. A recent clinical study reported two cases that fulfilled the diagnostic clinical criteria, but FXTAS was excluded by direct genetic testing [23]. This illustrates that increased T2 signal in the MCP per se is not specific for FXTAS and emphasizes the importance of including the mandatory criterion for the presence of an *FMR1* PM allele to prevent diagnostic errors. Neurohistologic studies on autopsy brains of symptomatic PM carriers demonstrated degeneration in the cerebellum, including Purkinje cell loss, Bergman gliosis, spongiosis of the deep cerebellar white matter, and the presence of eosinophilic intranuclear inclusions in both neurons and astroglia throughout the brain and in ependymal cells [21•,24]. Furthermore, these inclusions showed a positive reaction with antibodies against ubiquitin, components of the proteasome Hsp70, and $\alpha\beta$ -crystallin, which suggests a link with the proteasome degradation pathway [17,21•,24]. Finally, it should be noted that older adult individuals carrying a FM never develop the characteristic clinical symptoms of FXTAS.

Clinical phenotype in symptomatic female patients

Thus far, the description of FXTAS in female PM carriers is limited. It is assumed that female PM carriers are less likely to develop FXTAS because the neurons in which the normal X chromosome is active may have a (partly) protective effect. Indeed, a recent study demonstrated a correlation between the severity of clinical signs and the X-inactivation ratio in two sisters with FXTAS [25•]. Both women, however, had milder symptoms than typical symptomatic male patients and both lacked the cognitive decline. The less severe symptomatology, including lack of dementia, was also observed in two other studies presenting female PM carriers with FXTAS [10•,26]. Interestingly, a neurohistologic study on the autopsy brain from one symptomatic female PM carrier revealed ubiquitin-positive intranuclear neuronal and astrocytic inclusions, as have been described for symptomatic PM male carriers [10•].

Prevalence of FXTAS and Screening

The number of CGG repeats in the general population is polymorphic, with an average of 29 to 30 repeats. PM alleles are frequent in the general population, with previous estimated prevalence of 1:259 females and 1:813 males [27]. In fact, earlier studies actually showed a higher prevalence of PM alleles (1:100 females) [28,29]. The true incidence of this new neurologic syndrome among male Fragile X PM carriers remains to be established; however, initial reports on the penetrance of FXTAS in a family-based study suggest a prevalence of 1:3000 men aged 50 years and older in the general population with a lifetime risk of FXTAS [30•]. This would implicate that FXTAS is one of the most common single-gene forms of gait ataxia and tremor in older males. As a consequence, it can be proposed that older men with ataxia and tremor should be screened for the *FMR1* PM. These studies are in progress and the first results have been reported (Table 1). In summary, successful screening has only been achieved in the group of ataxia patients who initially were referred for testing of the spinocerebellar ataxia (SCA) genes and who were found to be negative. Apparently, because ataxia is one of the main cardinal features of FXTAS, the group of SCA patients most closely resembles the FXTAS phenotype. Notably, despite the typical presence of tremor and associated features like parkinsonism, screening among patients with idiopathic Parkinson's disease (PD), multiple system atrophy (MSA), or essential tremor (ET) did not reveal any individual with a *FMR1* PM allele. Apparently, the contribution of the *FMR1* PM alleles in the pathogenesis of idiopathic PD, MSA, or ET is limited. However, large-scale epidemiologic studies of the prevalence of FXTAS in the general population are required to make conclusive statements about the contribution of the *FMR1* PM alleles to the movement disorders of aging.

Molecular Mechanisms Underlying FXTAS

The molecular basis of FXTAS has been linked to the elevated *FMR1* mRNA levels in cells from carriers of the PM [16,21•,31]. Such an RNA gain-of-function model has also been proposed for several triplet repeat-related ataxias (SCA 8, 10, and 12) and myotonic dystrophy (DM1 and DM2) [32]. The pathogenic untranslated repeat mutations in DMs have helped us attain major insights into the underlying molecular mechanisms. The CUG and CCUG repeat expansions within the untranslated part of the DM transcripts sequester CUG-binding proteins and three different forms of muscleblind protein, and the depletion of free muscleblind protein results in altered splicing and abnormal function of several genes, including the insulin receptor, cardiac troponin, chloride channel, Tau protein, and myotubularin [32].

A similar mechanism of a dominant RNA gain-of-function has been proposed for FXTAS (Fig. 1), in which the elevated *FMR1* transcripts, containing an expanded CGG

Table 1. Screening for *FMR1* premutation alleles in male patient cohorts

Study / year	Cohort	Frequency of premutation (> 55 CGG)	Repeat size	Subjects with PM <i>FMR1</i> alleles, %
Macpherson et al. [33] / 2003	SCAs*	2/59	66, 87	3.4
Di Maria et al. [34] / 2003	SCAs*	2/28	84, 86	7.1
Milunsky and Maher [35] / 2004	SCAs*	1/167	80	0.6
Van Esch et al. [36] / 2005	SCAs*	5/122	80–111	4.1
Brussino et al. [37] / 2005	SCAs†	6/143	83–109	4.2
Tan et al. [38] / 2004	SCAs‡	0/30	-	NA
Garcia Arocena et al. [39] / 2004	ET	0/40	-	NA
Tan et al. [38] / 2004	ET, APD	0/49	-	NA
Deng et al. [40] / 2004	ET, PD	0/412	-	NA
Tan et al. [41] / 2005	PD	0/121	-	NA
Toft et al. [42] / 2004	Parkinsonism	0/414	-	NA
Tan et al. [38] / 2004	MSA	0/12	-	NA
Garland et al. [43] / 2000	MSA	0/40	-	NA

*SCA 1, 2, 3, 6, and 7 were excluded, and age at onset was older than 50 years.

†SCA 3, 6, and 7 were excluded, and age at onset was older than 50 years.

‡SCA 1, 2, 3, 6, 7, 8, 10, and 12 were excluded, and age at onset was older than 50 years.

APD—atypical Parkinson's disease; ET—essential tremor; MSA—multiple system atrophy; NA—not available; PD—Parkinson's disease; PM—premutation; SCA—spinocerebellar ataxia.

repeat, result in the sequestration or misfolding of important cellular proteins, including CGG-binding proteins, with a cumulative cytotoxic effect that may lead to the formation of intranuclear inclusions [16,31]. Whether the inclusion formation precedes neurodegeneration or has a protective effect, as described for the intranuclear inclusions in Huntington's disease, remains unclear [44]. Interestingly, a recent study by Tassone et al. [45] has demonstrated the presence of *FMR1* transcripts within the inclusions isolated from autopsy brain tissue. The origin and constitution of the inclusions in FXTAS is poorly understood, but a possible explanation for the elevated *FMR1* mRNA levels is the increased transcriptional activity as compensatory mechanism for the diminished translational efficiency of the *FMR1* message, because no significant increase in *FMR1* mRNA stability was observed [14]. The presence of ubiquitin, molecular chaperones, and components of the proteasome suggests a link with the proteasome degradation pathway and shares common features also described for the polyglutamine disorders (eg, Huntington's disease) [46,47]. The molecular mechanism in polyglutamine disorders is based on a gain-of-function of the mutant proteins, because the repeat is present in the coding region of the gene, whereas the pathogenic mechanism in FXTAS is explained by an RNA gain-of-function mediated by an expanded CGG repeat in the noncoding region of the *FMR1* gene. The cellular consequences of perturbation of the ubiquitin-proteasome degradation pathway in the polyglutamine disorders may include transcriptional dysregulation of important genes, potentially leading to neuronal cell death. The model that aggregation-prone proteins can impede proteasome activity mediating neuronal dysfunction and death has also been proposed as a general model for a variety of the neurodegenerative diseases [48].

Animal Models of FXTAS

A murine and a fly model have been generated to study the pathogenesis of FXTAS. The knock-in mouse model, in which the endogenous mouse CGG repeat (8 CGGs) was replaced by a human CGG repeat carrying 98 CGG units, shows moderate CGG repeat instability upon both maternal and paternal transmission [49,50••]. To date, the repeat size has expanded close to the critical 200 CGG repeats (Unpublished data). The brains of expanded repeat mice were analyzed neurohistologically and biochemically at different ages from neonatal until final stage of life (1 to 72 weeks) [50••]. Biochemically, elevated *Fmr1* mRNA levels (2 to 4 times normal) were already detectable in the first week of life. Neuropathologic analysis of the brains showed the presence of neuronal ubiquitin-positive intranuclear inclusions throughout the brain (Fig. 2). The inclusions became visible at 30 weeks of age and an increase was observed in both the number and the size of the inclusions during the course of life, which correlates with the progressive character of FXTAS. Next to ubiquitin, Hsp40 and the 20S catalytic core complex of the proteasome could be demonstrated as constituents of the inclusions. Strikingly, in contrast to brains from symptomatic FXTAS patients, inclusions were totally absent in astrocytes. Furthermore, a correlation was found between the occurrence of inclusions within specific brain regions and the clinical features in symptomatic PM carriers. Very recently, the expanded CGG repeat mouse was assessed for cognitive, behavioral, and neuromotor performance at different ages (20, 52, and 72 weeks). The results clearly indicate an age-dependent decline of visual-spatial learning capacities, a potential increase of anxiety levels, and mild neuromotor disturbances in the expanded CGG repeat mouse model [51]. Importantly, this mouse model will facilitate molecular studies to further analyze the pathogenesis of FXTAS from onset of symptoms until the final stage of the disease.

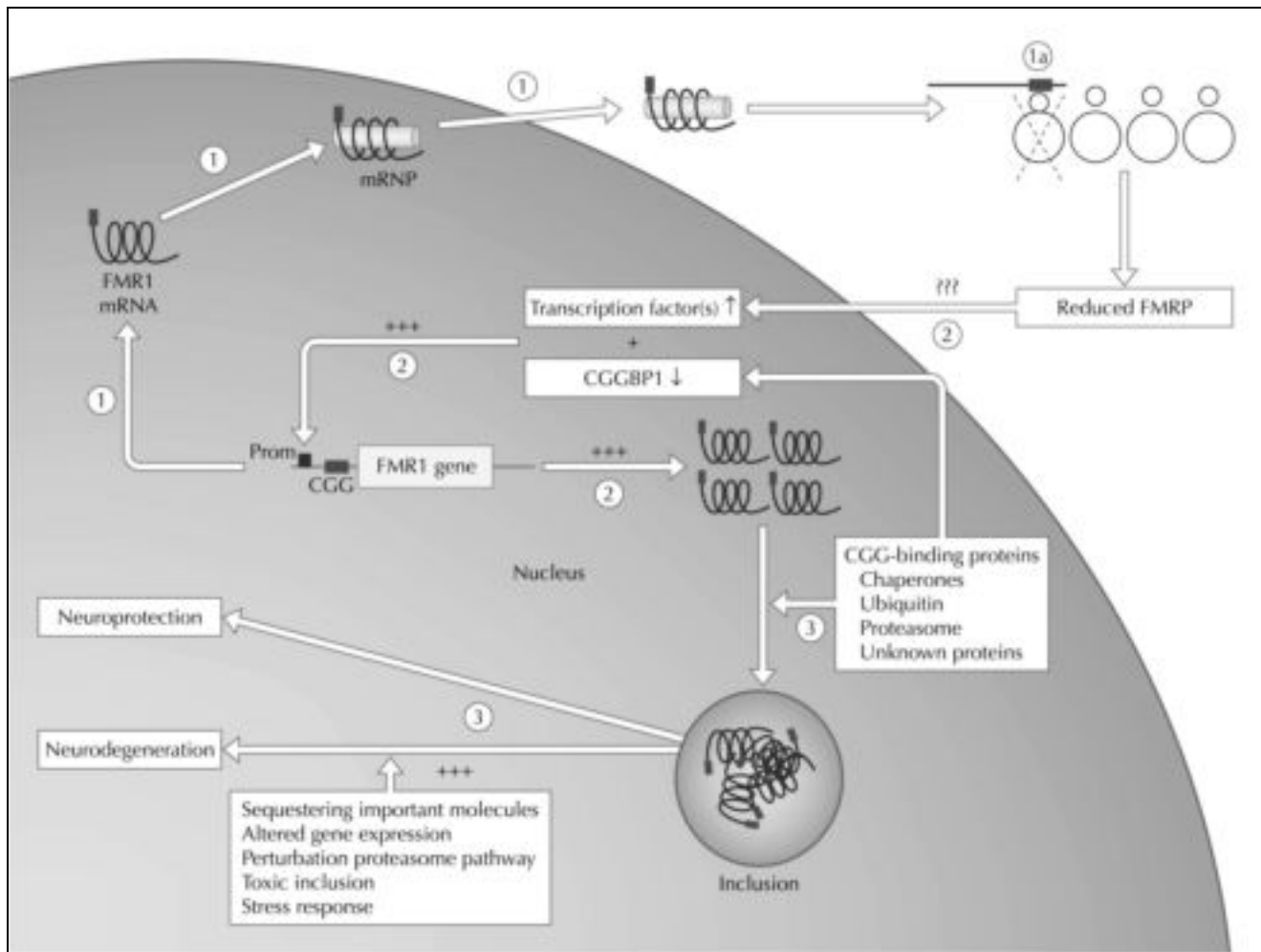


Figure 1. Proposed model for the molecular neuropathology in Fragile X-associated tremor/ataxia syndrome (FXTAS). **1**, *FMR1* transcripts containing an expanded CCG repeat are normally incorporated in a mRNP particle and translocated out of the nucleus. **1a**, The long CCG repeat in the *FMR1* transcript impedes 40S ribosomal subunit migration, resulting in hampered translation. Consequently, the nerve cell produces reduced levels of Fragile X mental retardation protein (FMRP), the gene product of the *FMR1* gene. **2**, In response to lowered FMRP levels, an unidentified feedback mechanism may increase the level of specific transcription factors, which results in increased transcription of the *FMR1* gene. Enhanced transcription leads to elevated *FMR1* mRNA levels. Alternatively, long CCG tracts in the *FMR1* transcript may sequester high quantities of CCG-binding proteins, and lowered CGGBP1 levels result in an increased *FMR1* transcription. **3**, The nerve cell attempts to clear itself from elevated *FMR1* transcript levels by employing molecular chaperones and components of the ubiquitin-proteasome degradation pathway. If elevated *FMR1* transcript levels resist refolding/degradation, then intranuclear inclusions will be formed. Ultimately, the formation of inclusions will trigger neurodegeneration by activation of neurotoxic signaling pathways. Here, several mechanistic pathways can influence this process. Alternatively, the formation of inclusions may protect the nerve cell against the toxic effects of elevated levels of *FMR1* mRNA, preventing cell death. (Adapted from Oostra and Willemsen [31]; with permission.)

The *Drosophila* model, in which PM CCG repeats (90 CCGs) were expressed as part of the 5' UTR of the *FMR1* gene, revealed the presence of ubiquitin-positive inclusions and a progressive degeneration of photoreceptors in the eye [52••]. In the fly model, inclusions were present in both nuclei and the cytoplasm of cells from the retina. Another discrepancy with the human neuropathologic studies is the presence of more than one nuclear aggregate per nucleus and that the composition of the aggregates that appeared to be more electron dense. This RNA-mediated phenotype showed a dosage- and repeat length-dependent progression. Thus, the CCG repeat itself can cause neurodegeneration and is not related with the function of FMRP. Overexpression of the molecular

chaperone Hsp70 could suppress the neurodegeneration illustrating similarities with the features of protein misfolding disorders, including polyglutamine diseases.

These observations in the expanded-repeat mice as well as in *Drosophila* models support a direct role of the elevated levels of expanded CCG-containing transcripts in the formation of the inclusions and neurodegeneration.

Therapy

No effective therapy is available for FXTAS; however, existing treatments to improve tremor and anxiety may be clinically beneficial. Future research will focus on the

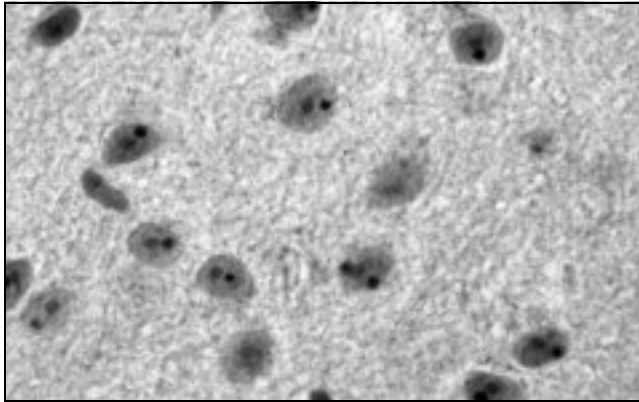


Figure 2. Distribution of neuronal ubiquitin-positive intranuclear inclusions in the colliculus inferior from the expanded-repeat mouse at the age of 72 weeks by an indirect immunoperoxidase staining.

composition of the inclusions to understand the mechanism of RNA-mediated neurodegeneration. Identification of the underlying molecular abnormalities may lead in the future to pharmacologic intervention.

Conclusions

FMR1 gene expression is involved in two important disorders with distinct entities. Fragile X syndrome, a neurodevelopmental disorder and the most prevalent cause of heritable mental retardation, is caused by the total lack of the *FMR1* gene product FMRP. An expansion of the CGG repeat over 200 units leads to transcriptional silencing of the *FMR1* gene. Thus, in male carriers of the FM, the pathogenic mechanism is caused by a loss-of-function disease mechanism. In contrast, male carriers of the PM show increased transcription of the *FMR1* gene that results in elevated levels of *FMR1* mRNAs and causes a new neurologic syndrome called FXTAS. Although precise estimates for the prevalence of FXTAS in the general population need to be determined, initial family-based studies point to a prevalence of 1:3000 for men aged over 50 years in the general population. The molecular basis of FXTAS is unknown; however, a dominant RNA gain-of-function has been proposed. Compelling evidence suggests that mutant RNA initiates the formation of neuronal intranuclear inclusions in which important cellular proteins are sequestered. Depletion of these proteins may result in disturbed cellular processes leading to cell death.

Acknowledgments

We would like to thank all our colleagues and collaborators for stimulating discussions. Supported by grants from the National Fragile X Foundation, Prinses Beatrix Fonds, and NIH R01 HD38038.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- Of importance
 - Of major importance
1. Fu YH, Kuhl DP, Pizzuti A, et al.: Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell* 1991, 67:1047–1058.
 2. Verkerk AJ, Pieretti M, Sutcliffe JS, et al.: Identification of a gene (*FMR-1*) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 1991, 65:905–914.
 3. Oberlé I, Rousseau F, Heitz D, et al.: Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science* 1991, 252:1097–1102.
 4. Sutcliffe JS, Nelson DL, Zhang F, et al.: DNA methylation represses *FMR-1* transcription in fragile X syndrome. *Hum Mol Genet* 1992, 1:397–400.
 5. Pieretti M, Zhang FP, Fu YH et al.: Absence of expression of the *FMR-1* gene in fragile X syndrome. *Cell* 1991, 66:817–822.
 6. Verheij C, Bakker CE, de Graaff E, et al.: Characterization and localization of the *FMR-1* gene product associated with fragile X syndrome. *Nature* 1993, 363:722–724.
 7. Willemsen R, Oostra BA, Bassell GJ, Dichtenberg J: The fragile X syndrome: from molecular genetics to neurobiology. *Ment Retard Dev Disabil Res Rev* 2004, 10:60–67.
 8. Nolin SL, Brown WT, Glicksman A, et al.: Expansion of the fragile X CGG repeat in females with premutation or intermediate alleles. *Am J Hum Genet* 2003, 72:454–464.
 9. Hagerman RJ: The physical and behavioural phenotype. In *Fragile-X Syndrome: Diagnosis, Treatment and Research*. Edited by Hagerman RJ, Hagerman P. Baltimore: The Johns Hopkins University Press; 2002:3–109.
 10. Hagerman RJ, Leavitt BR, Farzin F, et al.: Fragile-X-associated tremor/ataxia syndrome (FXTAS) in females with the *FMR1* premutation. *Am J Hum Genet* 2004, 74:1051–1056.
- First description of five female carriers of the *FMR1* PM who presented with FXTAS, including neuropathology of one patient showing the presence of intranuclear inclusions.
11. Sherman SL: Premature ovarian failure among fragile x premutation carriers: parent-of-origin effect? *Am J Hum Genet* 2000, 67:11–13.
 12. Tassone F, Hagerman PJ: Expression of the *FMR1* gene. *Cytogenet Genome Res* 2003, 100:124–128.
- Review paper summarizing the results of different studies about elevated *FMR1* mRNA levels in the different groups. Dysregulation of *FMR1* gene expression is discussed and the underlying molecular mechanisms are discussed.
13. Kenneson A, Zhang F, Hagedorn CH, Warren ST: Reduced *FMRP* and increased *FMR1* transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. *Hum Mol Genet* 2001, 10:1449–1454.
 14. Primerano B, Tassone F, Hagerman RJ, et al.: Reduced *FMR1* mRNA translation efficiency in Fragile X patients with premutations. *RNA* 2002, 8:1–7.
 15. Allen EG, He W, Yadav-Shah M, Sherman SL: A study of the distributional characteristics of *FMR1* transcript levels in 238 individuals. *Hum Genet* 2004, 114:439–447.
 16. Hagerman PJ, Hagerman RJ: The fragile-X premutation: a maturing perspective. *Am J Hum Genet* 2004, 74:805–816.
 17. Hagerman PJ, Hagerman RJ: Fragile X-associated tremor/ataxia syndrome (FXTAS). *Ment Retard Dev Disabil Res Rev* 2004, 10:25–30.
 18. Jacquemont S, Hagerman RJ, Leehey M, et al.: Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. *Am J Hum Genet* 2003, 72:869–878.
- This paper describes a large series of FXTAS patients ($n = 26$) who were analyzed in a clinical, radiologic, and molecular manner, and diagnostic criteria were proposed to help clinicians to recognize FXTAS.

19. Hagerman PJ, Greco CM, Hagerman RJ: A cerebellar tremor/ataxia syndrome among fragile X premutation carriers. *Cytogenet Genome Res* 2003, **100**:206–212.
20. Hagerman RJ, Leehey M, Heinrichs W, et al.: Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. *Neurology* 2001, **57**:127–130.
- This is the first reported evidence of FXTAS in PM males. Patients normally present with either intention tremor or ataxia.
21. Greco CM, Hagerman RJ, Tassone F, et al.: Neuronal intranuclear inclusions in a new cerebellar tremor/ataxia syndrome among fragile X carriers. *Brain* 2002, **125**:1760–1771.
- First neurohistologic report on the brains of four carriers of the PM. All cases showed intranuclear inclusions in both neurons and astroglia throughout the brain.
22. Brunberg JA, Jacquemont S, Hagerman RJ, et al.: Fragile X premutation carriers: characteristic mr imaging findings of adult male patients with progressive cerebellar and cognitive dysfunction. *Am J Neuroradiol* 2002, **23**:1757–1766.
23. Storey R, Billimoria P: Increased T2 signal in the middle cerebellar peduncles on MRI is not specific for fragile X premutation syndrome. *J Clin Neurosci* 2005, **12**:42–43.
24. Tassone F, Hagerman RJ, Garcia-Arocena D, et al.: Intranuclear inclusions in neural cells with premutation alleles in fragile X associated tremor/ataxia syndrome. *J Med Genet* 2004, **41**:E43.
25. Berry-Kravis E, Potanos K, Weinberg D, et al.: Fragile X-associated tremor/ataxia syndrome in sisters related to X-inactivation. *Ann Neurol* 2004, **57**:144–147.
- This report presents data about two PM carrier sisters in whom severity of clinical signs correlated with the X-inactivation ratio. It is hypothesized that female PM carriers who predominantly express the X chromosome with the PM may demonstrate significant signs of FXTAS.
26. Zuhlke C, Budnik A, Gehlken U, et al.: FMR1 premutation as a rare cause of late onset ataxia. Evidence for FXTAS in female carriers. *J Neurol* 2004, **251**:1418–1419.
27. Dombrowski C, Levesque S, Morel ML, et al.: Premutation and intermediate-size FMR1 alleles in 10 572 males from the general population: loss of an AGG interruption is a late event in the generation of fragile X syndrome alleles. *Hum Mol Genet* 2002, **11**:371–378.
28. Pessio R, Berkenstadt M, Cuckle H, et al.: Screening for fragile X syndrome in women of reproductive age. *Prenat Diagnosis* 2000, **20**:611–614.
29. Toledano-Alhadeef H, Basel-Vanagaite L, Magal N, et al.: Fragile-X carrier screening and the prevalence of premutation and full-mutation carriers in Israel. *Am J Hum Genet* 2001, **69**:351–360.
30. Jacquemont S, Hagerman RJ, Leehey MA, et al.: Penetrance of the fragile x-associated tremor/ataxia syndrome in a premutation carrier population. *JAMA* 2004, **291**:460–469.
- This family-based study demonstrates that male carriers of the PM are at high risk of developing FXTAS. The penetrance of FXTAS increases with age, and calculations on the prevalence of FXTAS in the general population point to 1:3000 men aged older than 50 years.
31. Oostra BA, Willemsen R: A fragile balance: FMR1 expression levels. *Hum Mol Genet* 2003, **12**(Suppl 2):R249–R257.
32. Ranum LP, Day JW: Pathogenic RNA repeats: an expanding role in genetic disease. *Trends Genet* 2004, **20**:506–512.
33. Macpherson J, Waghorn A, Hammans S, Jacobs P: Observation of an excess of fragile-X premutations in a population of males referred with spinocerebellar ataxia. *Hum Genet* 2003, **112**:619–620.
34. Di Maria E, Grasso M, Pigullo S, et al.: Further evidence that a tremor/ataxia syndrome may occur in fragile X premutation carriers: findings from two clinical series. *Am J Hum Genet* 2003, **73**:555.
35. Milunsky JM, Maher TA: Fragile X carrier screening and spinocerebellar ataxia in older males. *Am J Med Genet* 2004, **125A**:320.
36. Van Esch H, Dom R, Bex D, et al.: Screening for FMR1 premutations in 122 older Flemish males presenting with ataxia. *Eur J Hum Genet* 2005, **13**:121–123.
37. Brussino A, Gellera C, Saluto A, et al.: FMR1 gene premutation is a frequent genetic cause of late-onset sporadic cerebellar ataxia. *Neurology* 2005, **64**:145–147.
38. Tan EK, Zhao Y, Puong KY, et al.: Fragile X premutation alleles in SCA, ET, and parkinsonism in an Asian cohort. *Neurology* 2004, **63**:362–363.
39. Garcia Arocena D, Louis ED, Tassone F, et al.: Screen for expanded FMR1 alleles in patients with essential tremor. *Mov Disord* 2004, **19**:930–933.
40. Deng H, Le W, Jankovic J: Premutation alleles associated with Parkinson disease and essential tremor. *JAMA* 2004, **292**:1685–1686.
41. Tan EK, Zhao Y, Puong KY, et al.: Expanded FMR1 alleles are rare in idiopathic Parkinson's disease. *Neurogenetics* 2005, **6**:51–52.
42. Toft M, Aasly J, Bisceglia G, et al.: Parkinsonism, FXTAS, and FMR1 premutations. *Mov Disord* 2004, **20**:230–233.
43. Garland EM, Vnencak-Jones CL, Biaggioni I, et al.: Fragile X gene premutation in multiple system atrophy. *J Neurol Sci* 2000, **227**:115–118.
44. Arrasate M, Mitra S, Schweitzer ES, et al.: Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 2004, **431**:805–810.
45. Tassone F, Iwahashi C, Hagerman PJ: FMR1 RNA within the intranuclear inclusions of fragile X-associated Tremor/Ataxia syndrome (FXTAS). *RNA Biology* 2004, **1**:103–105.
46. Davies SW, Turmaine M, Cozens BA, et al.: Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* 1997, **90**:537–548.
47. Petersen A, Larsen KE, Behr GG, et al.: Expanded CAG repeats in exon 1 of the Huntington's disease gene stimulate dopamine-mediated striatal neuron autophagy and degeneration. *Hum Mol Genet* 2001, **10**:1243–1254.
48. Taylor JP, Hardy J, Fischbeck KH: Toxic proteins in neurodegenerative disease. *Science* 2002, **296**:1991–1995.
49. Bontekoe CJ, Bakker CE, Nieuwenhuizen IM, et al.: Instability of a (CGG)(98) repeat in the Fmr1 promoter. *Hum Mol Genet* 2001, **10**:1693–1699.
50. Willemsen R, Hoogveen-Westerveld M, Reis S, et al.: The FMR1 CGG repeat mouse displays ubiquitin-positive intranuclear neuronal inclusions; implications for the cerebellar tremor/ataxia syndrome. *Hum Mol Genet* 2003, **12**:949–959.
- Generation of a knock-in, expanded repeat mouse model to study the pathogenesis of FXTAS. Elevated levels of FMR1 mRNAs and ubiquitin-positive inclusions were reported, which supports a direct role of the Fmr1 gene, by either GCC expansion per se or by elevated transcript levels in the pathogenesis of FXTAS.
51. Van Dam D, Errijgers V, Kooy RF, et al.: Cognitive decline, neuromotor and behavioural disturbances in a mouse model for Fragile-X-associated tremor/ataxia syndrome (FXTAS). *Behav Brain Res* 2005, In press.
52. Jin P, Zarnescu DC, Zhang F, et al.: RNA-mediated neurodegeneration caused by the fragile x premutation rccg repeats in *Drosophila*. *Neuron* 2003, **39**:739–747.
- Drosophila melanogaster* was used as a model to study the pathogenesis of FXTAS using a portion of the human FMR1 5' UTR containing the PM rCGG repeat. Both inclusions and neurodegeneration were observed, supporting evidence of RNA-mediated neurodegeneration.