

Brief Research Communication

CGG Repeat Length Correlates With Age of Onset of Motor Signs of the Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS)

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Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset neurological disorder among carriers of premutation CGG-repeat expansions within the *FMR1* gene. Principal features of FXTAS include progressive action tremor and gait ataxia, with associated features of parkinsonism, peripheral neuropathy, dysautonomia, and cognitive decline. Although both clinical and neuropathologic features of FXTAS are known to be highly associated with CGG repeat length, the relationship between repeat length and age-of-onset is not known. To address this issue, the ages of onset of action tremor and gait ataxia were documented by history for 93 male carriers. For this cohort, the mean ages of onset were 62.6 ± 8.1 years (range, 39–78 years) for tremor, and 63.6 ± 7.3 years (range, 47–78 years) for ataxia; the mean CGG repeat number was 88.5 ± 14 (range, 60–133). Analysis of the relationship between clinical onset and molecular measures revealed significant correlations between CGG repeat number and onset of both tremor ($P = 0.001$) and ataxia ($P = 0.002$), as well as overall onset ($P < 0.0001$). Our findings indicate that the CGG repeat number is a potential predictor of the age of onset of core motor features of FXTAS. © 2007 Wiley-Liss, Inc.

KEY WORDS: neurodegeneration; dementia; parkinson; ataxia; RNA toxicity

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Fragile X-associated tremor/ataxia syndrome (FXTAS) is a late-onset, progressive, neurological disorder that affects a large subgroup of older male and, less frequently, older female carriers of premutation CGG repeat expansions (55–200 CGG repeats) of the *FMR1* gene [review: Hagerman and Hagerman, 2004]. The major clinical features of FXTAS are progressive action tremor and gait ataxia, with additional forms of clinical involvement including parkinsonism, cognitive decline, autonomic dysfunction, anxiety, and peripheral neuropathy [Hagerman et al., 2001; Jacquemont et al., 2003]. Typical neuroradiological features include characteristic high signal lesions (T2/FLAIR MR imaging) of the middle cerebellar peduncles (“MCP sign”), high signal lesions of cerebral white matter [Brunberg et al., 2002; Jacquemont et al., 2003], and global reductions of cerebral and cerebellar volume [Brunberg et al., 2002; Jacquemont et al., 2003; Cohen et al., 2006]. Post-mortem examination of carriers who died with FXTAS revealed the presence of ubiquitin-positive, intranuclear inclusions in both astrocytes and neurons throughout the brain, including the brainstem and spinal cord [Greco et al., 2002; Tassone et al., 2004; Greco et al., 2006]. Associated neuropathology includes global cerebral and cerebellar atrophy, spongiform white matter disease, and marked Purkinje cell dropout.

Both clinical, radiological and neuropathologic features of FXTAS are significantly correlated with CGG repeat length [Hessl et al., 2005; Loesch et al., 2005; Cohen et al., 2006; Greco et al., 2006; Grigsby et al., 2006]. For example, there is a dramatic increase in the numbers of inclusion-bearing neuronal and astrocytic nuclei with increasing size of the CGG repeat [Greco et al., 2006]. The CGG-repeat dependence of these clinical and neuropathologic features within the premutation range, the absence of symptoms of FXTAS among older adults with full mutation alleles (>200 CGG repeats), elevated *FMR1*

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mRNA in peripheral blood leucocytes of carriers [Tassone et al., 2000a,b], and the presence of *FMR1* mRNA within the inclusions themselves [Tassone et al., 2000b], all support an RNA toxic gain-of-function model for FXTAS pathogenesis [Hagerman et al., 2001; Greco et al., 2002; review: Hagerman and Hagerman, 2004]. Data from both animal and cellular models of the disorder are consistent with this model [Jin et al., 2003; Willemsen et al., 2003; Arocena et al., 2005].

Most FXTAS cases identified to date have been ascertained through families with a known fragile X syndrome (full mutation) proband. The majority of these male carriers with FXTAS harbor an *FMR1* allele in the middle premutation range (80–100 CGG repeats), much larger than the preponderance of premutation alleles in the general population where approximately 80% of alleles have fewer than 70 repeats [Jacquemont et al., 2006]. Although this finding could have been due to ascertainment bias within the fragile X families, the same size bias was observed in screening studies of movement disorders populations [Di Maria et al., 2003; Van Esch et al., 2005; Jacquemont et al., 2006] where a family-based ascertainment bias is not expected. The comparable allele-size bias for those affected by FXTAS, for both family-based and non-family-based studies, is also consistent with a CGG-repeat size effect on the penetrance and/or severity of FXTAS [Jacquemont et al., 2006].

An RNA gain-of-function mechanism, in which the degree of clinical involvement increases with increasing CGG repeat length, also predicts an earlier onset of clinical involvement for larger repeats, although this effect has not been documented thus far. To investigate this possibility, the ages of onset of action tremor and gait ataxia were documented by history for 93 male carriers. In accordance with the model, we observe highly significant correlations between the ages of onset of both tremor and ataxia and the size of the CGG repeat.

Participants in the U.S. were recruited from known fragile X families, either through the Fragile X Research and Treatment Center of the University of California, Davis, M.I.N.D. Institute (Sacramento, CA), through the Departments of Pediatrics and Neurology, Rush University Medical Center (Chicago, IL), or through the Department of Neurology, UCHSC. European participants were recruited through the Azienda Ospedaliera San Giovanni Battista of Turin (Italy). All subjects were recruited in accordance with approved IRB protocols.

A total of 93 male carriers of the *FMR1* premutation (55–200 CGG repeats) were included in this study. Carrier status was confirmed by PCR and Southern blot DNA analysis for all subjects, who all had documented presence of either gait ataxia only (13/93 cases), action tremor only (17/93 cases), or both tremor and ataxia (63/93 cases) by exam at the time the medical history was taken.

For the purpose of this study, age-of-onset was defined as the age when the subject, his spouse, or other relative(s) first noticed the symptoms of tremor or gait problems (falls, balance, or walking difficulty), and is based on a medical history taken from the subject and/or spouse at the time of the neurological examination. When the subject had cognitive or memory difficulties, the history was taken from the spouse. In cases where both the subject and the spouse were present at the evaluation, the history of onset obtained from the subject was compared to that from the spouse to ensure that the most reproducible history was obtained.

Genomic DNA was isolated from peripheral blood leucocytes (5 ml of whole blood using standard methods; Puregene Kit, Gentra, Inc., Minneapolis, MN). For Southern blot analysis, 5–10 μ g of isolated DNA was digested with EcoRI and NruI. The probe used for Southern blot hybridization was the *FMR1* specific dig-labeled StB12.3 [Tassone et al., 2004]. Genomic DNA was also amplified using a betaine-PCR method [Saluto

et al., 2005], and primers c and f [Fu et al., 1991]. Analysis and calculation of the repeat size for both Southern blot and PCR analysis were carried out using an Alpha Innotech FluorChem 8800 Image Detection System.

All *FMR1* mRNA levels were quantified using TaqMan-based RT-PCR, as described in Tassone et al. [2000b], using the 7900 Sequence detectors (Applied Biosystems, Foster City, CA).

The percent FMRP-positive lymphocytes was determined on blood smears by immunocytochemistry as previously described [Willemsen et al., 1995; Tassone et al., 1999b].

Spearman's correlation test was employed to evaluate the association between the individual molecular parameters (CGG repeat number, *FMR1* mRNA level, FMRP level) and the clinical features (onsets of action tremor and gait ataxia). Spearman's test is a non-parametric counterpart of the commonly used Pearson's test, which measures bivariate correlations. It operates on the ranks of the data rather than on the actual data values, thus reducing the distortions inherent in the Pearson's correlation (e.g., outliers, unequal variance, non-normality, and non-linearity).

Documentation of clinical history was obtained for a total of 93 male carriers with confirmed premutation (CGG) repeat expansions and with clear evidence on exam of one or both of the core motor features of FXTAS (action tremor and/or gait ataxia). For each subject, the age of symptom onset was determined separately for action tremor and for gait ataxia. The mean age of tremor onset was 62.6 ± 8.1 years ($n=80$; range, 39–78 years), the mean age of ataxia onset was 63.6 ± 7.2 years ($n=76$; range, 47–78 years), and the mean age of onset of either symptom (initial symptom when both are present) was 61.6 ± 7.9 years ($n=93$). Within this subject group, the mean CGG repeat number was 88.5 ± 14 (range, 60–133), the mean *FMR1* mRNA expression level in peripheral blood leucocytes was 3.4 ± 1.08 (range, 1.98–5.79), and the mean FMRP expression was 81.6 ± 10 (% FMRP-positive lymphocytes; range, 60–98). The FMRP expression levels were not significantly lower than levels found in controls [Tassone et al., 2000b].

Analysis of the relationship between clinical onset and molecular measures (Spearman's rho) revealed significant correlations between CGG repeat number and onset of tremor ($P=0.001$), ataxia ($P=0.002$), and the initial onset of either tremor or ataxia ($P<0.0001$), which encompasses the earlier of the two individual symptom onsets for those individuals who experience both tremor and ataxia (Table I; Fig. 1). By contrast, there was neither apparent correlation between age of onset and mRNA levels, nor was there any significant association between FMRP expression and any of the clinical measures. This last (negative) observation is not surprising in view of the fact that FMRP expression is comparable to control levels in most FXTAS patients; however, it may also reflect the non-quantitative nature of the protein test itself.

Based on an estimated lifetime risk of one in 3,000 males [Jacquemont et al., 2004], FXTAS is a common single-gene cause of tremor, ataxia, and cognitive decline among older

TABLE I. Correlations Between Molecular Measures and the Ages of Onset of Action Tremor and/or Gait Ataxia (Pearson's Correlation Coefficients)

Measure	Symptom onset		
	Ataxia	Tremor	Either ^a
CGG	-0.3441 (0.0025) ^c	-0.3607 (0.0011)	-0.4420 (<0.0001)
mRNA ^b	-0.2017 (0.14)	-0.2138 (0.11)	-0.2649 (0.0347)

^aEarlier of ataxia or tremor onset when both are present.

^b*FMR1* mRNA levels relative to normal controls.

^c*P* values are indicated in parenthesis.

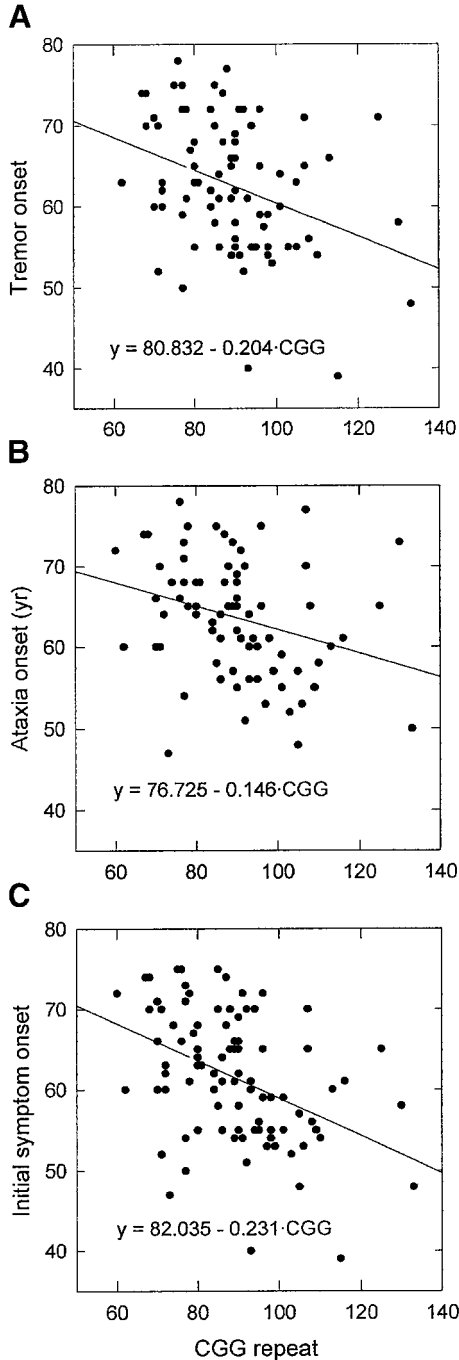


Fig. 1. Plots of the ages of onset of tremor (A), ataxia (B), and initial symptom (C); earlier of tremor or ataxia, when both are present as a function of the length of the CGG repeat. Regression functions and lines are displayed for each dataset.

adults [Hagerman and Hagerman, 2004; Jacquemont et al., 2004]. However, this estimate is based on the population frequency ($\sim 1/800$) of premutation alleles among males within the general population [Dombrowski et al., 2002] and the estimated penetrance of $\sim 30\%$ for male carriers over 50 years of age in known fragile X families. A recent meta-analysis of the allele distribution among males with FXTAS suggests that alleles with more than ~ 70 CGG repeats are more likely to lead to neurological symptoms [Jacquemont et al., 2006]. This

observation, coupled with the clinical and neuropathologic data indicating a positive association between repeat size and age of death and inclusion density [Greco et al., 2006], suggests that the overall penetrance of FXTAS in the general population is lower than the above estimate, perhaps by two- to threefold. However, CGG-repeat-dependence of eventual penetrance does not speak directly to the issue of the age of onset, since one could imagine either earlier onset with increasing CGG repeat length, reflective of greater eventual penetrance (negative association), or a threshold onset (no association).

The current results provide evidence for a significant negative (inverse) correlation between CGG repeat number and the onset of two principal neurological features of FXTAS, providing further evidence that CGG repeat length drives the severity of the disorder. The absence of any clear threshold age is consistent with reports of individuals with onset of neurological symptoms as early as the late 30s [Hagerman et al., 2004], although such cases are uncommon. Our results are also in accordance with a recent post-mortem study of 11 males who died with FXTAS [Greco et al., 2006], which demonstrated a strong positive correlation between the number of CGG repeats and the fraction of neuronal and astrocytic nuclei harboring inclusions. Two previous reports did not find any association between CGG repeat length and age of onset [Jacquemont et al., 2003; Grigsby et al., 2006]. However, those studies had smaller cohorts of affected individuals (25 and 20, respectively), with a smaller range of CGG repeat sizes than in the current study.

Recently, Grigsby et al. [2006] reported a correlation between CGG repeat length and increased cognitive and functional impairment in premutation carriers. Reductions in total brain volume and increases in the volume of white matter disease (area of increased signal on T2/FLAIR MRI) are both associated with FXTAS [Brunberg et al., 2002], and both are correlated with the size of the CGG repeat [Loesch et al., 2005; Cohen et al., 2006]. Because corresponding, albeit smaller, volumetric changes were found among unaffected premutation carriers, it is likely that these changes begin before the disease process becomes clinically apparent [Cohen et al., 2006]. The dependence of the magnitude of the volumetric changes on CGG repeat size suggests that the disease may become clinically apparent at younger ages for those with larger repeats, consistent with the current results.

Although age-of-onset of clinical involvement is clearly correlated with the CGG repeat length, there was no evident correlation with *FMR1* mRNA levels. This finding is not surprising, as the transcript levels were measured in peripheral blood leukocytes. Although elevated levels of *FMR1* message were reported in different brain regions from a premutation male affected by FXTAS [Tassone et al., 2004], the increase in the *FMR1* transcript levels, in the premutation carrier relative to the control subject, was much less pronounced in brain tissue compared to peripheral blood leukocytes. Further, while no differences were observed in cerebellar cortex, a wide range of expression levels were observed in other brain regions, suggesting a broad *FMR1* expression heterogeneity across the different brain regions. Thus, it is conceivable that lack of association between message levels and clinical outcome in FXTAS is due to such variability. However, all such levels will be driven by the genotype (CGG repeat length), which is generally consistent between blood and brain [Tassone et al., 1999a, 2004].

In conclusion, we have demonstrated a significant correlation between the age of onset of clinical symptoms of FXTAS and the number of CGG repeats. Such knowledge both reinforces our understanding of the pathogenesis of FXTAS, and helps us to refine our estimate of prevalence and severity of this disorder within the general population.

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