Penetrance of the Fragile X–Associated Tremor/Ataxia Syndrome in a Premutation Carrier Population

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Context Premutation expansions (55–200 CGG repeats) of the fragile X mental retardation 1 (FMR1) gene are frequent in the general population, with estimated prevalences of 1 per 259 females and 1 per 813 males. Several articles have recently described the presence of late-onset neurological symptoms in male carriers of premutation (FMR1) alleles. The main clinical features described in this newly identified syndrome are cerebellar ataxia and intention tremor. Additional documented symptoms include short-term memory loss, executive functional deficits, cognitive decline, parkinsonism, peripheral neuropathy, lower-limb proximal muscle weakness, and autonomic dysfunction.

Objective To study the penetrance of the fragile X–associated tremor/ataxia syndrome (FXTAS) among premutation carriers.

Design, Setting, and Participants Family-based study of 192 individuals (premutation carriers and controls) whose families belong to the Northern or Southern California Fragile X Associations. Data were collected (March 2002–April 2003) through a survey and a standardized neurological examination, which was videotaped and subsequently scored in a blinded fashion.

Main Outcome Measures Penetration of intention tremor and ataxia among adult carriers (aged ≥50 years) of premutation expansions of the FMR1 gene.

Results Data from the survey of 192 individuals demonstrated an age-related penetrance of the combination of reported intention tremor and gait ataxia in male carriers (17%, 38%, 47%, and 75% [lower-bound estimates] for participants aged 50–59, 60–69, 70–79, and ≥80 years, respectively). The male carrier group had an age-adjusted 13-fold increased risk (95% confidence interval, 3.9–25.4; P = .003) of combined intention tremor and gait ataxia when compared with male controls. The clinical examination data from 93 individuals demonstrated that male carriers experienced more difficulties on each of 3 standardized neurological rating scales compared with controls (P < .05). Female carrier scores were also higher than those of female controls (P < .05) on 2 of the 3 neurological rating scales, but no participant was identified with probable or definite FXTAS.

Conclusions The study demonstrates that older male carriers of premutation alleles of the FMR1 gene are at high risk of developing FXTAS. Since male premutation carriers are relatively common in the general population, older men with ataxia and intention tremor should be screened for the FMR1 mutation, especially if these signs are accompanied by parkinsonism, autonomic dysfunction, or cognitive decline, regardless of family history.
Carriers of the premutation typically have a normal IQ, although emotional problems such as anxiety are common.10–11 Approximately 20% of women who carry premutation expansions of the FMR1 gene experience premature ovarian failure, a clinical finding that is unique to premutation alleles.12,13 We recently reported a distinctive form of clinical involvement in some older male carriers of the fragile X premutation.14–17 These carriers, aged in their 50s and older, develop progressive intention tremor and ataxia. These movement disorders are often accompanied by progressive cognitive and behavioral difficulties, including memory loss, anxiety, deficits of executive function, reclusive or irritable behavior, and dementia. More variable features include parkinsonism, peripheral neuropathy, lower-limb proximal muscle weakness, and autonomic dysfunction (urinary/bowel incontinence and impotence). This disorder has been designated fragile X–associated tremor/ataxia syndrome (FXTAS).16

Magnetic resonance imaging in patients with FXTAS reveals approximately symmetric increases in T2-weighted signal intensity in the middle cerebellar peduncles (MCPs) and adjacent cerebellar white matter.15 This unusual radiological finding, visible in the majority of published cases of premutation carriers with the tremor/ataxia disorder, is not observed in controls. The MCP finding thus potentially serves as a major diagnostic feature of FXTAS.16 The most striking neuropathological feature associated with FXTAS, present in all 8 brains examined to date from patients who died with this disorder, is the presence of eosinophilic, intra-nuclear inclusions in both neurons and astrocytes.18,19 These inclusions are found throughout the cortex and brain stem, with the greatest densities of inclusions located in the hippocampus and frontal cortical regions. No inclusions have been detected in the Purkinje cells of the cerebellum, although there is evident Purkinje cell dropout and cerebellar axonal degeneration.10,19 The inclusions are polyglutamine-negative, distinguishing FXTAS from CAG-repeat ataxias. Moreover, no tau or α-synuclein proteins have been found within the inclusions. Intranuclear inclusions that are morphologically similar to the inclusions observed in the FXTAS cases18 have also been generated in a transgenic mouse model with expanded CGG repeats in the Fmr1 gene.20

The penetrance of FXTAS among fragile X premutation carriers is unknown, although the frequency of premutation alleles within the general population suggests that this disorder could represent a significant and currently unidentified contribution to the adult tremor and ataxia patient populations. For example, a recent screen of adult ataxia patients in the United Kingdom found that approximately 5% were previously undiagnosed premutation carriers.21 Therefore, controlled studies are necessary to assess the relationships among the motor, cognitive, and autonomic symptoms and the premutation. To this end, we have conducted a community-based survey and a standardized neurological evaluation on members of families affected by fragile X syndrome in California.

**METHODS**

All aspects of this study were reviewed and approved by the institutional review board of the University of California, Davis.

**Determination of FMR1 Carrier Status**

All parents or grandparents of each fragile X syndrome proband whose genetic status was not previously known through prior genetic testing or pedigree analysis underwent FMR1 genotyping to document their carrier status. Since CGG repeat number was not a covariate in the current investigation, participants underwent genotyping only when their FMR1 status (premutation carrier or normal) was not known. Genotype analysis was used to establish carrier status in 50 of 99 carriers (CGG repeats were available for 42 patients; in 8 cases, individuals were tested in other laboratories and exact repeat numbers were not available). The status of all remaining carriers (49/99) was established by pedigree analysis, which documented obligate carrier status in individual pedigrees through additional genotyped children with fragile X syndrome. Genomic DNA was isolated from peripheral blood leukocytes using standard procedures (Puregene kit, Gentra Inc, Minneapolis, Minn). Southern blot and polymerase chain reaction (PCR) analyses were performed on each sample, per Taylor et al,22 with the exception that for some of the samples the analysis was performed using a digoxigenin-labeled probe (dig-11ddUTP, Roche Diagnostics, Indianapolis, Ind). For Southern blot analysis, the enzymes EcoRI and NruI were used for DNA digestion, and StB12.3 was used for the FMR1-specific probe.23 The number of CGG repeats was determined by PCR analysis using primers 1 and 3.24

**Ascertainment**

This study is based on the ascertainment of all fragile X families in California who are members of either the Northern or the Southern California Fragile X Associations. All member families became affiliated with the associations through a child who was diagnosed as having fragile X syndrome (proband); that is, no family was identified through a premutation carrier with neurological problems. All premutation carriers who were members of families residing in California and who were at least 50 years of age qualified for this study. The control groups for both male and female carriers were composed predominantly of the spouses of the carriers (85% of male controls were spouses; 82% of female controls were spouses). The remaining controls were siblings of the carriers who either tested negative for premutation alleles or who were obligate controls (eg, since men cannot inherit an X chromosome from their father, sons of carrier men are obligate controls). Genetic screening was lim-
Identified Through the Southern and Northern California Fragile X Associations

<table>
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<td>Unreachable carriers and spouses</td>
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<td>Total</td>
<td>64</td>
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</table>

Survey

One hundred twenty-three families belonging to either the Northern or the Southern California Fragile X Association were asked to participate in the study via a letter from their respective association. Upon receiving each family’s permission through the associations, we contacted the families by telephone. A total of 23 families were excluded. Four families refused to participate without providing a reason; in 18 families the child with fragile X syndrome, or a parent, was adopted; and in 1 family the individual had lost contact with all members of the family aged 50 years or older. We were unable to contact an additional 11 families (incorrect telephone information).

In the remaining 89 families, 298 individuals aged 50 years or older were identified on the premutation side (predominantly the mother’s side) of the family. Sixty-four individuals (28 men and 36 women) did not qualify for participation in the study because they were granduncles or grandaunts whose genetic status was unknown. Of the 234 individuals who did qualify for the study, we were unable to contact 21 known carriers (6 men and 15 women) and their 21 spouses (controls). The 6 male carriers were included in a more conservative penetrance calculation. Thus, 192 (82%) participated in the study (40 male carriers, 59 male spouse controls, 59 female carriers, and 34 female spouse controls) (Table 1).

All qualifying participants self-reported symptoms on a survey completed by telephone or in person. For the purpose of the survey, symptoms were scored as present if noticed by the respondent or spouse, with clarification of the questions or characterization of the symptoms provided by the interviewing physician as necessary. The participant provided the final answers. Patients signed informed consent approved by the institutional review board before participating. Those contacted by telephone were read the consent form and gave oral consent.

The survey comprised 54 questions exploring 6 domains: (1) tremor: questions were asked regarding the presence, characteristics, and time of onset of tremors; (2) gait and lower extremities: questions were asked related to the onset of balance problems, recent falls, walking distance, and use of a cane, walker, or wheelchair. Sensory issues were also explored with questions about lower extremity numbness, loss of sensation, and pain; (3) cognitive: questions were asked of the participant or his/her spouse regarding short-term memory loss and episodes of confusion and disorientation; (4) emotional: questions explored anxiety, depression, mood instability, and medications used for those symptoms; (5) other medical issues: questions explored any other medical problem and treatment (in particular, heart problems, stroke, autonomic symptoms, hearing loss, diabetes, and all medications taken by the participant); and (6) family history: questions addressed the presence of a family history positive for neurological disorders, including Parkinson disease, Alzheimer disease, other dementias, and multiple sclerosis.

Responses for cognitive or emotional impairment were not used further for this study.

Finally, participants completed the Tremor Disability Rating Scale (TDRS), designed and validated by Louis et al. This rating scale has 31 questions exploring fine motor tasks. The final (summed) score, converted to a percentage, is the extent of impairment related to the loss of fine motor skills. Zero percent represents absence of impairment and 100% reflects inability to perform any fine motor tasks associated with daily living.

Clinical Assessment

To document the findings of the survey, all participants who were geographically available for clinical assessment (n=93; 24 male carriers, 25 male controls, 26 female carriers, and 18 female controls) underwent a detailed neurological examination that included pertinent items of 3 standardized scales: the Clinical Rating Scale for...
Tremors (CRST), the motor section of the Unified Parkinson’s Disease Rating Scale (UPDRS), and the International Cooperative Ataxia Rating Scale (ICARS). Whereas the CRST and ICARS are unvalidated, the UPDRS has undergone extensive clinometric analysis. The neurological examination was videotaped and subsequently rated by a neurologist blinded to the patient’s diagnosis and DNA study results. The clinician administering the video protocol was not blinded in all cases. However, this individual did not appear on the videotape. The duration of each task was standardized, and the administering clinician did not insist on a longer task interval than what is specified by the protocol. The neurologist rating the videotapes did not use the soundtrack except for the 3 tasks requiring vocalizations.

All patients available for clinical testing underwent the video protocol, which included only half (93/192) of the survey participants. Among the 93 individuals participating in the videotape protocol, 36% had reported 1 or more neurological symptoms on the survey, similar to the 30% figure for all 192 survey participants. Moreover, 58% of the male carriers available for the videotaping had self-reported 1 or more neurological symptoms on the survey, identical to the 58% figure for all 40 male carriers participating in the study. Therefore, there does not appear to be a substantial bias in the population participating in the video protocol.

Throughout this article, we use FXTAS to denote probable or definite FXTAS, as defined by Jacquemont et al. Probable FXTAS requires the presence of both tremor and gait ataxia, whereas definite FXTAS requires a positive MCP finding on magnetic resonance imaging as well as at least 1 major clinical finding (tremor or gait ataxia).

Statistical Methods

Only 16 items on the 54-question survey were subjected to further analysis. This reduction was obtained by (1) removing all questions related to cognition and emotional difficulties, as the evaluation of these symptoms requires the use of appropriate psychometric instruments, which were not a part of the current study; and (2) removing all questions that required more than a closed yes/no answer. Logistic regression analyses were performed on the results of the remaining 16 questions, comparing male carriers with male controls and female carriers with female controls. Results are presented as odds ratios (ORs) and 95% confidence intervals (CIs), with P values gauging the significance of the OR for each question. For each item, the analyses are adjusted for age, ie, age is included as a covariate in the regression model. All analyses were 2-tailed.

We tested for interaction, collinearity, and overfitting in the logistic regression and found no violations of regression model assumptions due to collinear variables. The Holm method and the Benjamini false discovery rate procedure were applied to control for familywise (multiple outcomes) error rates for the 16 analyses. Since there were only 2 pairs of siblings in the group of 40 male carriers, any correlation of outcomes between these 2 pairs of family members would not bias the conclusions of our regression analyses. The summed TDRS score was analyzed separately. A nonparametric Mann-Whitney test, stratified by age group, was performed to compare the scores obtained by the carriers and controls for each sex.

The clinical rating scale scores were analyzed in 2 ways. First, the results were analyzed according to the total scores of each of the 3 neurological scales. Regression analyses were performed on the summary scores, comparing the square root–transformed mean score of male carriers with male controls and female carriers with female controls. The square root transformation was used to ensure that the assumptions of homoscedastic and Gaussian errors in the regression model are not violated. For these 3 analyses, adjustment for multiple outcomes was unnecessary. Second, the results were analyzed by 12 different subscores for the 3 neurological scales. Three CRST subscores (postural, action, and rest tremor) were computed for the 3 tremor types identified within this scale. Four ICARS subscores were computed for the 4 domains described within this scale. Six UPDRS subscores were also computed for the 6 clinically distinct disability domains previously identified by factor analysis within the UPDRS motor examination. However, 1 of the subscores (rigidity) was removed because data on this subgroup could not be obtained in a blinded fashion. Comparisons between the carrier and control groups for each sex were made by the nonparametric Wilcoxon rank sum test. The Holm method and the Benjamini false discovery rate procedure were applied to control for familywise error rates on the basis of the 12 analyses and the tandem walk task, as described by Singer et al.

Statistical analyses were performed using S-Plus software, version 6.1 (Insightful Corp, Seattle, Wash). P <.05 was considered statistically significant throughout all analyses.

RESULTS

Questionnaire

Among men, in the premutation carrier group (n = 40), the prevalence of reported gait instability, tremor, and both symptoms combined increased with age from 30 years to 80 years or older. The penetrance of these symptoms in carriers and controls is displayed in Figure 1. To produce more conservative estimates for these penetrance data, we also included the additional 6 male carriers (Table 1) who were unable to participate, stipulating that these cases were all asymptomatic for the lower-bound estimates. The resulting estimates are 17%, 38%, 47%, and 75% of male carriers with self-reported frequencies of combined tremor and gait ataxia within the 50 to 59, 60 to 69, 70 to 79, and 80 years or older age ranges, respectively. The frequency of these reported symptoms was significantly increased in the premutation group rela-
The severity of gait difficulty was also greater in the male carrier group. Thirty-five percent of male carriers reported episodes of unexplained falling as opposed to 3% in the male control group (OR, 12.9; 95% CI, 2.6-62.4; \( P = .001 \) ) (Table 2). Twenty percent of the male carriers reported using a cane, with a mean onset at 71 years of age, as opposed to 5% in the male control group (at 53, 54, and 80 years). Five male carriers (13%) required a wheelchair at a mean age of 77 years as opposed to 1 male control at 53 years. After multiplicity adjustment using 2 different methods at the .05 level,29,31 4 items remained significant: intention tremor, gait instability, falling more than once in the past month without an obvious reason, and the combination of tremor and gait instability.

Among women, the survey did not reveal any significant difference between carriers and controls, either before or after multiplicity adjustment. All frequencies, ORs, and 95% CIs for the survey are reported in Table 2.

**Tremor Disability Rating Scale**

In men, the mean score obtained on the TDRS (reflecting the mean degree of impairment in fine motor tasks associated with daily living) by the carrier group was 7% among men aged 50 to 59 years and 41% among those aged 80 years or older. In the male control group, the degree of impairment was steady at between 1% and 2% throughout all age ranges. We performed a non-parametric Mann-Whitney test to compare carriers and controls. The age groups of 50 to 59 years and 80 years or older showed significantly higher TDRS scores among the male carriers than among the controls (\( P = .01 \) and \( P < .01 \), respectively). The severity of impairment was greater in the male carrier group across all age ranges. A logistic regression analysis was performed comparing impaired premutation carriers with impaired controls after adjusting for age. Impairment was considered as a 2-level factor (normal or mild, score \( \leq 10 \); severe, score > 10). Carriers and controls differed significantly after adjusting for age (OR, 6.45; 95% CI, 1.26-33; \( P = .02 \)).

The scores on the TDRS for women did not differ significantly between the premutation and control groups.

The TDRS has been validated with a control population (aged \( \geq 65 \) years) and the mean score was 1.6%,32 which reflects a quasi absence of impairment. This score is not significantly different from our control population (mean scores, 2% for the male control group and 0.3% for the female control group).

**Standardized and Videotaped Clinical Protocol**

The distribution of the scores for all 3 neurological scales is represented in Figure 2. Male premutation carriers showed significant impairment on the 3 scales that measured tremor (CRST), cerebellar dysfunction (ICARS), and parkinsonism (UPDRS). Regression analysis indicated that the average score on a CRST item (ranging from 0 to 3) was significantly higher in the male carrier group relative to the control group (the relationship was defined through an estimated regression coefficient of 0.18; 95% CI, 0.02-0.35; \( P = .04 \) ). Significant differences were also observed on the 1CARS (coefficient=0.2; 95% CI, 0.05-0.37; \( P = .01 \) ) and UPDRS scales (coefficient=0.2; 95% CI, 0.06-0.37; \( P = .01 \) ). In all 3 scales, age was a significant covariate (\( P < .01 \) for the CRST, \( P = .01 \) for the ICARS, and \( P < .01 \) for the UPDRS).

We investigated a total of 12 subscores within the 3 neurological scales, as well as an additional tandem walking task described by Singer et al,34 to identify tasks that were the most sensitive to the neurological phenotype of the male carriers (Table 3). The subscores were compared between the carrier and control groups using the Wilcoxon rank sum test. We examined the 3 components of tremor on the CRST3b.
rest, postural, and kinetic tremors (Table 3). In contrast with the total CRST score, male carriers did not show significant impairment in any of the 3 subscores.

Similarly, we analyzed the 4 categories of the original ICARS rating tool:28 postural/gait/stance, limb movement, speech, and oculomotor movements (Table 3). Male carriers were significantly more impaired relative to male controls in all subgroups except oculomotor movements.

Based on previously published factor analysis data on the UPDRS,32,33 we examined 5 stable factors: right bradykinesia, left bradykinesia, midline bradykinesia/balance, rest tremor, and postural tremor. Only 1 subscore, midline/gait/balance, was significantly different between male carriers and controls. Right bradykinesia was nearly significant (P = .06). The tandem walking task described by Singer et al.34 not part of any of the 3 scales, was analysed as a 13th subscore. It showed significant impairment in the male carrier group compared with controls (Table 3). However, after multiple-outcomes correction using the methods of both Holm35 and Benjamini et al31, only 1 item (tandem walking) remained significant at the .05 level.

The distribution of the scores among women for all 3 neurological scales is represented in Figure 2. Only 3 women, aged 69, 81, and 84 years, showed very mild intention tremor and/or gait ataxia. However, the regression analysis indi-
Table 3. Summary of CRST, ICARS, and UPDRS Components by Carrier Status Among Men*

<table>
<thead>
<tr>
<th></th>
<th>Carriers (Median)</th>
<th>Controls (Median)</th>
<th>P Value†</th>
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</thead>
<tbody>
<tr>
<td>CRST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Action/intention</td>
<td>11 (0–49)</td>
<td>8 (0–16)</td>
<td>.06</td>
</tr>
<tr>
<td>Postural tremor</td>
<td>2 (0–14)</td>
<td>1 (0–5)</td>
<td>.24</td>
</tr>
<tr>
<td>Rest tremor</td>
<td>0 (0–15)</td>
<td>0 (0–1)</td>
<td>.09</td>
</tr>
<tr>
<td>ICARS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limb ataxia</td>
<td>8 (0–25)</td>
<td>4 (0–12)</td>
<td>.02</td>
</tr>
<tr>
<td>Postural/gait/stance</td>
<td>5 (1–32)</td>
<td>3 (0–8)</td>
<td>.02</td>
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<tr>
<td>Oculomotor</td>
<td>1 (0–3)</td>
<td>2 (0–3)</td>
<td>.30</td>
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<tr>
<td>Speech</td>
<td>2 (0–6)</td>
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<tr>
<td>UPDRS</td>
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<td></td>
</tr>
<tr>
<td>Midline/gait/balance</td>
<td>4 (0–32)</td>
<td>2 (0–8)</td>
<td>.01</td>
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<tr>
<td>Bradykinesia</td>
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<tr>
<td>Right</td>
<td>4 (0–10)</td>
<td>2 (0–7)</td>
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<tr>
<td>Left</td>
<td>4 (0–12)</td>
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<tr>
<td>Tandem walking</td>
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<td>.003‡</td>
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</table>

Abbreviations: CRST, Clinical Rating Scale for Tremor; ICARS, International Cooperative Ataxia Rating Scale; UPDRS, Unified Parkinson’s Disease Rating Scale.
*Summary data and analyses for women are not shown because of absence of significant differences between carriers and controls on all subscores.
†Analyzed by Wilcoxon rank sum test. This nonparametric analysis did not allow for age correction. The comparison of the age distribution using the χ² (parametric) and Kolmogorov-Smirnov (nonparametric) tests indicated no significant difference between the 2 groups (P values of .55 and .99, respectively).
‡This item remained statistically significant at the .05 level after multiplicity correction on the basis of 13 different analyses, using both the Holm method29 and the Benjamini false discovery rate.31

The data from the survey reveal that gait instability was the most common symptom reported by male carriers, involving 17% (aged 50-59 years) to 100% (aged ≥80 years) of that group, in agreement with an earlier report.15 The penetrance and severity of intention tremor is higher in the male carrier group relative to controls across all age ranges (OR, 6.8; 95% CI, 2.3-20; P = .01). Again, the CIs are wide, and larger groups will be needed to produce more accurate ORs. In the male control group, the mean self-reported penetrance of intention tremor was 10%, which is similar to the results of epidemiological studies of essential tremor for the same age range.32 In contrast, 48% of the male premutation group self-reported intention tremors (Table 2). Intention tremor is also a major finding in the study by Berry-Kravis et al.38

COMMENT

The current investigation provides initial estimates for the penetrance of self-reported gait difficulties and intention tremor among carriers of the fragile X premutation. The penetrance of this tremor/ataxia disorder (corresponding to probable FXTAS as diagnosed clinically)16 is defined as the fraction of carriers who self-reported intention tremor and gait instability combined. The data obtained from the survey, representing a total ascertainment of families enrolled in the Northern and Southern California Fragile X Associations, yielded a lower-bound estimate of the age-related penetrance of probable FXTAS in male carriers that ranged from 17% (aged 50-59 years) to 75% (aged ≥80 years) and averaged 39% for the male carrier group (aged ≥50 years) as a whole. However, the elevated penetrance of the group aged 80 years or older is based on a small sample size (n=4) (Table 1). Applying the correction of Zhang and Yu36 to the age-adjusted OR (2.1; 95% CI, 4.3-103.3) for combined tremor and gait ataxia (Table 2), the relative risk of combined major clinical symptoms defining the diagnosis of probable FXTAS is 13.2 times higher (95% CI, 3.9-25.4; P = .003) in the male carrier group than in the male control group. However, the CIs are large because of small sample size, especially in participants aged 80 years or older. Larger samples are required to generate more accurate penetrance data and ORs. The severity of symptoms was variable within the carrier group, and 25% of the participating male carriers experienced significant disability related to ambulation (requiring a wheelchair) and/or fine motor skills (significant writing impairment on the TDRS), as opposed to 3% in the male control group.

To evaluate the reliability of the self-reported symptoms on the survey, we performed a Wilcoxon rank sum test comparing the scores obtained on the survey with scores of the neurological evaluation. Participants who reported tremor scored higher (median, 14; interquartile range [IQR], 11-24) on the CRST than those who reported no tremor (median, 7; IQR, 3-12; P < .01). The participants who reported gait instability scored higher (median, 20; IQR, 11-35) on the ICARS than those who reported no gait instability (median, 11; IQR, 6-15; P < .01). Participants who reported combined tremor and gait instability scored higher (median, 37; IQR, 24-66) on the combined CRST and ICARS than those who reported no tremor and no gait instability (median, 18; IQR, 9-27; P < .01).
The penetrance data in this study are all derived from the survey. To correlate the data obtained by the self-report method with the degree of clinical involvement, we analyzed the scores obtained on both the survey and the neurological evaluation using nonparametric regression. Significant correlations were demonstrated between self-reported tremor and the CRST score (100% increase in the summary scores of the CRST; \( P < .01 \)) and between self-reported gait instability and the ICARS score (75% increase in the summary scores of the ICARS; \( P < .01 \)). The high level of concordance between the survey and the clinical evaluation established that the self-report method in this study was clinically meaningful. Clinical follow-up of the 24 male carriers who had participated in the video protocol revealed that 10 of the 11 carriers who had self-reported the combination of tremor and gait instability demonstrated these symptoms on the clinical examination. One participant was asymptomatic on the clinical examination but claimed to have intermittent symptoms. This suggests that a small fraction of participants overreported symptoms or that an intermittent and mild presentation predates the appearance of clinically recognizable symptoms.

The results of the clinical assessment reported here confirm the preliminary findings of a study by Berry-Kravis et al\(^{38} \) for a smaller group of individuals (n = 37). Those investigators reported significantly higher scores in male carriers vs male controls on the ICARS and CRST. For their study, significance was reached on the UPDRS only when comparing the male carrier group with the 3 other groups combined (male controls, female carriers, and female controls), most likely because of the small sample size. The current study, with larger sample sizes, demonstrates a significant difference between male premutation carriers and controls for the UPDRS.

The clinical data also reveal that a limited number of subscores are sensitive enough to distinguish carriers from controls (Table 3). The subscores with significant \( P \) values reflect either cerebellar symptoms (ICARS) or gait difficulties (midline/gait/balance in the UPDRS and tandem walking\(^{31} \)). None of the CRST subscores (postural, intention, or action tremor) was significantly different between groups. In addition, after correction for multiple outcomes, none of the individual subtests, other than tandem walking, retained significance at the \( .05 \) level. Larger populations will be necessary to assess significance for each of the subtests.

The statistical analysis demonstrates that the overall scores of the UPDRS and ICARS are significantly higher in female carriers than controls (\( P = .02 \) for UPDRS and ICARS); however, the mean scores of the female carrier group did not differ from those of the male control group. The subclinical neurological involvement of female carriers of the premutation thus needs to be evaluated further with larger clinical studies. We have documented the rare occurrence of FXATS symptoms in women outside of California.\(^{39-41} \) However, the occurrence of FXATS in women appears to be far less common than in men.

The skewed sex ratio observed in this study supports the concept of an X-linked disorder,\(^{14,16,38} \) although earlier reports\(^{42-49} \) failed to reach a consensus regarding the sex ratios of intention tremor, gait ataxia, and parkinsonism in the general population. For essential tremor, several studies have reported contradictory results regarding sex ratio.\(^{37,42-48} \) Most surveys with a personal (as opposed to medical record-based) screening for parkinsonism found no significant sex bias.\(^{43-49} \)

The neurological disorder with the clinical presentation most closely resembling FXATS is multiple system atrophy (MSA). Both MSA and FXATS present with variable features of parkinsonism, gait ataxia, and autonomic dysfunction. Both also share the very distinctive neuroradiological sign of an increased T2 signal of the MCPs and adjacent cerebellar white matter. Multiple system atrophy is a relatively rare disorder. Prevalence in individuals aged 65 years or older is estimated to be approximately 4.4 per 100000.\(^{30} \) Therefore, a separate etiology leading to MSA in more than 1 of these patients would be extremely unlikely. However, the molecular underpinnings of FXATS, related to the \( FMR1 \) gene, may be contributing to the population of patients currently diagnosed as having MSA.\(^{31} \)

The relatively small number of individuals (n = 234) initially qualifying for this study reflects the distribution of the \( FMR1 \) premutation alleles in a family: 1 of 4 grandparents is a carrier, and only 1 of 2 siblings of this grandparent is a carrier. No genetic screening was conducted with siblings of the probands’ parents/grandparents; only granduncles/grandaunts who were identified as obligate carriers or who had been previously tested qualified for this study (this latter situation arises when granduncles/grandaunts also had grandchildren with fragile X syndrome).

There are at least 2 sources of known or potential ascertainment bias in the current family-based study. First, all premutation carriers were identified through families with at least 1 child with fragile X syndrome. This mode of ascertainment would bias the allele distribution toward larger premutation alleles, since there is a steeply increasing likelihood for allelic expansion into the full mutation range as the allele size increases from 60 CGG repeats to approximately 90 repeats; 59 repeats is the smallest allele known to expand to the full mutation range within 1 generation.\(^{52} \) Alleles in the gray zone (41-54 repeats) are highly unlikely to result in full mutation alleles in 2 generations (grandchildren of the affected carriers).\(^{32,51} \) The smallest allele we have observed thus far for a neurologically affected adult carrier within a known fragile X family is 69 CGG repeats,\(^{16} \) and the smallest allele observed with the neuropathological changes associated with FXATS is 70 repeats.\(^{18,19} \) Both of these observations are close to the expected lower limit of approximately 59 CGG repeats for ascertainment within a fragile X syndrome–affected family.\(^{52} \) Second, for premutation alleles much greater than 100 CGG repeats, there is a greater chance...
of emotional, behavioral, and cognitive involvement in carriers, which may reduce their chances of having offspring or their willingness/availability for participation in screening studies. We have not yet investigated the penetrance of FXTAS as a function of CGG repeat length. However, this last issue is of great mechanistic importance and is currently under study.

Based on the expectation that a measurable fraction of adults seen in adult neurology clinics with intention tremor and/or ataxia will be undiagnosed carriers, several groups have initiated screens of selected patients in movement disorders clinics. Macpherson et al. recently reported that 3 individuals (5%) within a group of 59 ataxia patients with no family history of fragile X syndrome possessed gray zone or premutation alleles (51, 66, and 81 CGG repeats). Those results provide significant evidence of association between the ataxia phenotype and expanded FMR1 alleles. Further studies of tremor and/or ataxia populations are thus warranted for the purpose of better defining the neurological consequences of expanded FMR1 alleles in the absence of biasing influences of ascertainment through known fragile X families.

CONCLUSION

The current study demonstrates that male carriers of the fragile X premutation are at high risk of developing FXTAS, a neurological disorder characterized by gait ataxia, intention tremor, parkinsonism, and cognitive decline. The penetrance of this disorder increases with age, suggesting that the preponderance of male carriers may eventually develop neurological dysfunction.

With a published prevalence of 1 in 813 (95% CI, 1/527 to 1/1781) for male carriers of premutation (FMR1) alleles in the general population, the prevalence of FXTAS in men aged 50 years or older may approach approximately 1 in 3000, which would make this recently identified disorder one of the more common single-gene forms of tremor and ataxia in the aging population. Patients with late-onset ataxia and intention tremor (eg, MSA, prevalence of approximately 1/200000[59]; or atypical Parkinson disease, approximately 1/3000[60]) should therefore be screened for premutation expansions of the FMR1 gene.

Author Contributions: Dr P. Hagerman had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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A belief is not true because it is useful.
—Henri Frederic Amiel (1821-1881)