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***FMR1* Gray Zone Alleles: Association with Parkinson Disease in Women?**

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Abstract

Purpose—Carriers of fragile X mental retardation 1 (*FMRI*) repeat expansions in the premutation range (55–200 CGG repeats), especially males, often develop tremor, ataxia, and parkinsonism.^{1–2} These neurological signs are believed to be due to elevated levels of expanded CGG repeat *FMRI* mRNA. The purpose of this study was to determine the prevalence of *FMRI* repeat expansions in a movement disorder population, comprised of all types of tremor, ataxia or parkinsonism subjects.

Methods—We screened 335 consecutive movement disorders patients with tremor, ataxia, or parkinsonism and 273 controls confirmed to have no movement disorders.

Results—There was no difference in *FMRI* premutation size expansions in the cases compared to controls. Eleven percent of the women with Parkinson disease (PD) had *FMRI* gray zone expansions compared to 4.4% of female controls, odds ratio of 3.2 (95% CI 1.2–8.7). Gray zone expansions in patients with other phenotypes were not overrepresented in comparison with controls.

Conclusions—*FMRI* premutation range expansions are not more common in a mixed movement disorder population compared to controls. Our results, however, suggest that *FMRI* gray zone alleles may be associated with PD in women.

Keywords

Parkinson disease; *FMRI*; FXTAS; genetics; parkinsonism

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Introduction

Expansions of a CGG repeat in the 5' noncoding region of the *fragile X mental retardation 1* (*FMRI*) gene give rise to several distinct clinical phenotypes, depending on the size of the expansion. Large expansions (>200 CGG repeats; full mutation) generally result in fragile X syndrome (FXS); whereas carriers of smaller expansions (55–200 CGG repeats; premutation) may manifest primary ovarian failure (POF)³ or the neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS).¹ Individuals with FXTAS generally have late onset (>55 yr) core features of intention tremor and gait ataxia, with associated parkinsonism, cognitive decline, and peripheral neuropathy.¹ Individuals with FXTAS also have characteristic imaging and neuropathological changes.⁴

An unresolved issue is the clinical significance of CGG repeats that fall below 55 and yet are above the normal upper limit of 40. This range of 41–54 is specifically defined as the “gray zone” in this study. The “gray zone”, also termed “intermediate zone”, was originally defined as the CGG range of carriers who do not have children with fragile X syndrome. Typically, gray zone expansions expand into full expansions over three generations. Gray zone carriers have been believed to have no abnormal neurological features, but more recent reports have suggested an association between gray zone carriers and neurodevelopmental disorders or parkinsonism.^{5–7} Largely because of the lack of a defined pathological phenotype and the low risk of expansion within pedigrees to full fragile X syndrome, gray zone carriers have been studied much less than larger expansion carriers. Whereas the CGG range that constitutes the gray zone is defined here as 41–54, there is variance among its definition. The prevalence of gray zone expansions (41–54 CGG repeats) in the general population is estimated to be between 0.1–2%.^{8–9}

The current study was designed to remove limitations of disease specific diagnostic criteria in previous *FMRI* screening studies. It is possible that low numbers of premutation carriers were identified in screens of existing DNA banks because these patients were diagnosed with Parkinson disease (PD), essential tremor (ET), or other disorders based on criteria that may have excluded the premutation phenotype. For example, many PD DNA repositories use the UK PD Brain Bank Criteria that excludes individuals with parkinsonism who have cerebellar signs.¹⁰ Excluding PD patients with mild cerebellar signs may have resulted in lower ascertainment of *FMRI* premutation carriers than a study design that screens all parkinsonism patients. We hypothesized that *FMRI* premutation range expansions would be elevated in a consecutive population of adult parkinsonism, tremor, and ataxia patients in a movement disorder clinic compared to a control population rate.

Methods

We examined *FMRI* size among consecutive patients with any form of tremor, cerebellar ataxia, or parkinsonism attending the University of Colorado movement disorders clinic between 08/2006 and 05/2009. Subjects were seen and examined by a movement disorders neurologist (DAH). Diagnostic criteria used for ataxia was impaired tandem gait¹¹ and a score of 4 or higher on the gait portion of the International Cooperative Ataxia Rating Scale.¹² Tremor was defined as at least a 1+ kinetic tremor on three tasks and at least a 2+ tremor on one task on the Clinical Rating Scale for Tremor.¹³ Essential tremor was defined by published criteria.¹⁴ Parkinsonism was defined as two of the following signs: bradykinesia, resting tremor, rigidity, and/or asymmetric onset. Additional diagnostic criteria completed for parkinsonism subjects included criteria for PD¹⁵, multiple system atrophy¹⁶, progressive supranuclear palsy¹⁷, corticobasal degeneration¹⁸, diffuse Lewy body disease¹⁹, and unclassified parkinsonism. Control subjects were recruited from accompanying persons not biologically related to the cases during clinic and were typically

spouses. A portion of the controls were recruited as fragile X research participants and were spouses of known *FMRI* premutation carriers. Controls had no tremor, ataxia, or parkinsonism based on neurological exam and history.

Genomic DNA was isolated from peripheral blood leukocytes using standard procedures (Puregene kit, Gentra, Inc and EZ-1 robot, Qiagen). Repeat sizes were determined by PCR analysis for all samples, including those from subjects with movement disorders and from controls, using standard and similar methods at one of three laboratories in Colorado (ES), UC Davis (FT) or Rush University (EBK).^{20–21} All alleles were sized by polyacrylamide gel electrophoresis or capillary electrophoresis and inter-laboratory reproducibility for allele sizing to within one repeat in the normal and gray zone range had been established among these laboratories during collaborations for past and ongoing projects and was established for a subset of samples from this project. In addition, validation studies in our labs showed good concordance between the automated sequencer based method and the old method of running PCR products on a denaturing sequencing gel and probing with a labeled CGG(10) probe. In the current study, gray zone expansions were defined as 41–54 CGG repeats.

Univariate analysis was used to determine differences in demographics between the groups and to compare prevalence rates of repeat expansions of the *FMRI* gene, with odds ratios (OR) and 95% confidence intervals (CI) reported as appropriate. Multivariate logistic regression was used to determine other variables influenced the prevalence rates within the cases, with the presence of *FMRI* repeat expansion as the dependent variable; and age, gender, race, ethnicity, type of clinic the patient was diagnosed in, or phenotype (parkinsonism, tremor, and/or ataxia) as the independent variables. Sample sizes were estimated by gender individually as the frequency of the premutation in the population differs based on gender. The pre-specified sample size for women with tremor, ataxia, or parkinsonism was 142 female cases and controls, for power of 80% to detect a difference of 6% between the cases and controls. The rate of premutation carriers in the female controls was estimated to be 1/259, which is the established population rate in women.⁹ The sample size for men with tremor, ataxia, or parkinsonism was 131 male cases and controls, for power of 80% to detect a difference of 6% between the cases and controls. The rate of premutation carriers in the male controls was estimated to be 1/813, which is the established population rate in men.⁸ A difference of 6% in the combined phenotype group was predicted by anticipated recruitment in each of these phenotypes and prior data suggesting a rate of 7% *FMRI* expansions in ataxia patients, 8% in parkinsonism patients, and 2% in tremor patients. Significance was set at 0.05. The study was approved by the Colorado Multiple Institutional Review board, Rush University Institutional Review Board, and the University of California Davis Institutional Review Board.

Results

There were no significant age or gender differences between the cases and controls. There were more Hispanic cases than controls (6.6% vs. 1.8%, $p=0.005$). Clinical characteristics of the study population are located in Table 1. The largest phenotypic category was parkinsonism ($n=273$) with 218 subjects diagnosed as having PD. There were 78 subjects who had neurological signs in more than one movement disorder category, for example tremor and ataxia: 30 with parkinsonism and ataxia, 39 with parkinsonism and (kinetic) tremor, 8 with ataxia and (kinetic) tremor, and one who fit the clinical diagnosis of FXTAS.

In the women, one premutation carrier was identified in the controls (1/135) and none in the cases (0/147)($p=0.94$). In the men, two premutation carriers were identified in the cases (2/188) and none in the controls (0/138)($p=0.95$). One of the male premutation carriers had both ataxia and tremor on examination (Table 2) and the male control premutation carrier

developed dementia and ataxia after the study was completed. Twelve female gray zone carriers were identified in the cases (12/147, 8.2%) and 6 in the controls (6/135, 4.4%) ($p=0.19$). Three male gray zone carriers were identified (3/188, 1.6%) in the cases and six in the controls (6/138, 4.3%) ($p=0.4$). Gray zone expansions were particularly elevated in women with a diagnosis of PD (11/98, 11%) compared to the female controls (6/135, 4.4%) ($p=0.019$) (Table 3). Expansions were not significantly elevated in men with a diagnosis of PD (1/120, 0.8%) compared to a rate in the controls of 6/138 (4.3%) ($p=0.24$).

Results from the multivariate logistic regression model in the cases showed that women were more likely than men to have an expansion of any kind (OR=0.21, CI:0.07–0.67; $p=0.008$). In addition, gender was associated with the presence of a gray zone expansion (OR=0.17, CI 0.05–0.6; $p=0.005$) and gray zone expansion carriers were more likely to have been diagnosed by a neurologist or a movement disorder specialist as opposed to a primary care physician (OR=2.3, CI:1.03–5.2; $p=0.04$). Of the gray zone carriers with movement disorders, five women had hysterectomies (at ages 32, 33, 37, 42, and 60) and one started menopause in her early 40s; but none of the women met criteria for premature ovarian failure. Nine of the gray zone carriers with movement disorders had been imaged for their clinical care and none had the middle cerebellar peduncle sign. Clinical features of the *FMRI* repeat expansion carriers are noted in Table 2. Most of the gray zone carriers had features typical for PD, with some of the carriers having motor fluctuations, psychosis, or cognitive impairment. One patient met criteria for kinetic tremor and PD, also having orthostatic tremor on history and examination. None of the carriers had a family history of fragile X syndrome, although eight had a history of movement disorders in the family (Table 2).

Per inclusion criteria, the recruited control subjects did not have movement disorders, but we did not do a detailed neuropathy exam or systematically gather historical information. The female control premutation carrier had a grandchild with dyslexia. One of the female control gray zone carriers had infertility (CGG=45). None of the gray zone carriers on which history was collected had a family history of movement disorders or developmental disorders.

Discussion

This study reports an unexpectedly high prevalence of *FMRI* gray zone alleles in females with PD compared to female controls. We anticipated finding a higher prevalence rate of *FMRI* premutation expansions in our population, given the description of FXTAS and frequent misdiagnosis in these patients.²² However, this was not the case and the original hypothesis of the study was not proven. This finding suggests that despite the estimated prevalence of the disorder in men over age 55 of 1/3000, these individuals are not being evaluated in movement disorder clinics. At present, most of the individuals with FXTAS have been identified through a proband with fragile X syndrome, usually a grandson with intellectual disability and/or autism. It may be possible that families with fragile X children have additional genetic abnormalities that predispose premutation carriers in the family to manifest FXTAS symptoms, whereas premutation carriers in the general population without a family history of fragile X syndrome are less likely to manifest neurological symptoms. Possible explanations may include epigenetic modifications, such as abnormal histone or chromatin configurations, mutations in the *FMRI* antisense transcript, or other secondary mechanisms.

There was an ethnicity difference between our cases and controls, with more Hispanics represented in our cases. This is likely to lead to an underestimation of prevalence rates of *FMRI* repeat expansions because populations rates of expansions in Hispanic populations

are lower than in Caucasian populations.^{23–24} However, many of these population prevalence studies have small sample sizes making the implications of our results unclear.

The overrepresentation of female PD gray zone carriers is an unexpected and distinctive finding and may represent an important genetic link to PD in women. The 11% prevalence of gray zone expansion in PD women exceeds that found in our control sample as well as the published rates for the general population of 0.5–2.3%.^{8–9} Although each subgroup analysis was planned prior to the start of the study in each of the subgroups, we did not pre-specify an adjustment of the significance level for multiple testing. We reduced the number of planned analyses overall as we did not reach sufficient sample size for analysis in the ataxia and tremor subgroups, but an inflated probability of a false-positive result remains. If the significance levels were adjusted for the six subgroup analyses we performed using the Bonferroni method ($\alpha=0.008$), none of our subgroup analyses would have reached significance. Thus, our results should be considered with caution.

Parkinsonism is part of the FXTAS phenotype, with male *FMRI* premutation carriers having a parkinsonism subscore on the FXTAS rating scale twice as high as controls ($p=0.0026$).² Further, some premutation carriers identified in fragile X families have a PD phenotype rather than a FXTAS phenotype, meeting criteria for PD and responding to dopaminergic medication.²⁵ However, screening studies for the *FMRI* premutation in PD cohorts have not yielded many cases, suggesting that the premutation genotype accounts for only a negligible proportion of idiopathic PD.^{26–29} Data collected of gray zone rates in parkinsonism patients, however, is limited. Hedrich et al evaluated 208 women with parkinsonism and found a rate of 7.2% of females with gray zone alleles, compared to a control rate of 4.6% in females.²⁷ A screen of women with parkinsonian disorders in Italy showed a rate of 2.3% carriers of 45–54 CGG repeats among 595 women.³⁰ The full gray zone range of 41–54, however, was not examined in this sample. Further, there were no controls in this study, making it difficult to directly compare this report with our results. Most recently, a screen was conducted in Australia and Tasmania that showed men with parkinsonism ($n=228$) had a rate of 7.5% *FMRI* gray zone alleles compared to a control rate ($n=578$) of 3.3%.⁷ Differences in geographic ethnic or racial distributions may account for why the Australian sample had higher rates compared to our male PD population. For example, rates of *FMRI* carriers are higher in Israeli populations, with 1:113 females having a premutation³¹ and lower in Asian populations, with no carriers found in 946 individuals screened.³²

Interestingly, a study looking at *FMRI* repeats in hallucinating PD patients revealed two *FMRI* gray zone carriers ($n=56$) with marked cognitive decline suggesting a possible association.³³ Intermediate repeats have been defined in other trinucleotide repeat disorders. In Huntington disease (HD), the intermediate allele range (27–35 CAG repeats) is defined as the range of CAG repeat sizes that will expand into a HD phenotype in one transmission.³⁴ By definition, HD intermediate alleles are not causative of the HD phenotype. Between 36–39 CGG repeats, there is incomplete penetrance and over 40 CGG repeats, individuals are affected. However, there have been several case reports of individuals with intermediate alleles having neurological signs consistent with HD.³⁵ These findings in intermediate HD allele carriers are controversial, with proposed explanations of the presence of HD-like phenocopies, mutations in as-yet unidentified genes, misdiagnoses, or mistakes in sample processing. The issue of whether there are neurological signs in HD intermediate carriers may be clarified by ongoing studies in this area. Although intermediate alleles have also been reported in spinocerebellar ataxia type 10, it appears that the intermediate allele range is really a range of reduced penetrance.³⁶

The gender effect seen in this study is unexpected as FXS and FXTAS typically manifest in males due to the X linked inheritance. This female gender effect may be related to other

disorders associated with expansion in the *FMR1* gene. There is a robust literature regarding the link of estrogen to differential prevalence rates of PD between men and women. Women who have a shorter fertile life from menopause, hysterectomy, or oophorectomy; have a higher risk of parkinsonism.^{37–39} This is thought to be due to the neuroprotective effects of estrogen within the dopaminergic pathways.⁴⁰ *FMR1* is the most significant gene associated with premature ovarian insufficiency or POF. Both premutation and gray zone expansions in the gene are associated with POF, which is defined as menopause prior to the age of 40.^{41–42} A shortened fertile life in these women related to the *FMR1* expansion may have caused a loss of endogenous estrogen and increased the risk of parkinsonism. However, many of our gray zone carriers in this study had hysterectomies and the stated age of menopause could have been confounded by recall bias, making it unclear if our carriers had shortened fertility.

The rate of gray zone expansions was higher in our control population than rates reported in large population studies. It is unclear whether this difference is due solely to study design or to some other biological reason. In our study, the controls were in the age range of the study population, whereas prior population gray zone screens were typically performed in newborns. An improved study design would also include longitudinal follow up of the control population to ensure a continued normal neurological examination. This lack of follow up is a flaw of most cross-sectional genetic screening studies.

In individuals in the *FMR1* premutation range, there is elevation of *FMR1* mRNA levels and a slight depletion in fragile X mental retardation protein levels.⁴³ There is also an increase in mRNA levels starting at 39 CGG repeats.⁴⁴ However, an association between mRNA levels and clinical signs has not been shown in the premutation carriers despite a strong correlation between the length of the CGG repeat and severity of clinical involvement.^{2,45} Further study, screening consecutive parkinsonism patients, is required to confirm the findings of this study. As our carriers lack distinctive clinical characteristics, targeted screening is not recommended. We also do not recommend testing parkinsonism patients for *FMR1* repeat expansions until follow up studies confirm our findings and the phenotype is better defined.

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References

1. 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. [PubMed: 14747503]
2. Leehey MA, Berry-Kravis E, Goetz CG, et al. *FMR1* CGG repeat length predicts motor dysfunction in FXTAS. *Neurology*. 2008; 70:1397–1402. [PubMed: 18057320]

3. Sullivan AK, Marcus M, Epstein M, et al. Association of FMR1 repeat size with ovarian dysfunction. *Human Reproduction*. 2005; 20(2):402–412. [PubMed: 15608041]
4. 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. [PubMed: 12638084]
5. Ennis S, Murray A, Youings S, et al. An investigation of FRAXA intermediate allele phenotype in a longitudinal sample. *Ann Hum Genet*. 2006; 70:170–180. [PubMed: 16626328]
6. Sherman S, Marsteller F, Abramoqitz A, Scott E, Leslie M, Bergman J. Cognitive and behavioral performance among FMR1 high-repeat allele carriers surveyed from special education classes. *Am J Med Genet*. 2002; 114:458–465. [PubMed: 11992571]
7. Loesch DZ, Khaniani MS, Slater HR, et al. Small CGG repeat expansion alleles of FMR1 gene are associated with parkinsonism. *Clin Genet*. 2009; 76:471–476. [PubMed: 19796183]
8. Dombrowski C, Levesque S, Morel ML, Rouillard P, Morgan K, Rousseau F. 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 Med Genet*. 2002; 11(4):371–378.
9. Rousseau F, Rouillard P, Morel M, Khandjian EW, Morgan K. Prevalence of carriers of premutation-size alleles of the FMR1 gene - and implications for the population genetics of the fragile X syndrome. *Am J Hum Genet*. 1995; 57:1006–1018. [PubMed: 7485149]
10. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry*. 1992; 55(3):181–184. [PubMed: 1564476]
11. Siesling S, Zwiderman AH, van Vugt JP, Kieburz K, Roos RA. A shortened version of the motor section of the Unified Huntington's Disease Rating Scale. *Mov Disord*. 1997; 12(2):229–34. [PubMed: 9087982]
12. Trouillas P, Takayanagi T, Hallett M, et al. International Cooperative Ataxia Rating Scale for pharmacological assessment of the cerebellar syndrome. *Journal of Neurological Sciences*. 1997; 145:205–211.
13. Fahn, S.; Tolosa, E.; Marin, C. Clinical Rating Scale for Tremor. In: Jankovic, J.; Tolosa, E., editors. *Parkinson's disease and movement disorders*. Baltimore: Urban & Schwarzenberg; 1987. p. 225-234.
14. Louis ED, Ford B, Lee H, Andrews H. Does a screening questionnaire for essential tremor agree with the physician's examination? *Neurology*. 1998; 50:1351–1357. [PubMed: 9595986]
15. Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson's disease. *Arch Neurol*. 1999; 56:33–39. [PubMed: 9923759]
16. Gilman S, Low PA, Quinn N, et al. Consensus statement on the diagnosis of multiple systems atrophy. *J Neurol Sci*. 1999; 163:94–98. [PubMed: 10223419]
17. Tolosa E, Valldeoriola F, Cruz-Sanchez F. Progressive supranuclear palsy: clinical and pathological diagnosis. *Eur J Neurol*. 1995; 2:258–273.
18. Lang, AE.; Riley, DE.; Bergeron, C. Cortical-basal ganglionic degeneration. In: Calne, DB., editor. *Neurodegeneration diseases*. Philadelphia: WB Saunders; 1994. p. 877-894.
19. McKeith IG, Galasko D, Kosaka K, et al. Consensus guidelines for the clinical and pathological diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. *Neurology*. 1996; 47:113–124.
20. Tassone F, Pan R, Amiri K, Taylor AK, Hagerman PJ. A rapid polymerase chain reaction-based screening method for identification of all expanded alleles of the fragile X (FMR1) gene in newborn and high-risk populations. *J Molec Diagnostics*. 2008; 10:43–9.
21. Levinson G, Maddalena A, Palmer FT, et al. Improved sizing of fragile X CCG repeats by nested polymerase chain reaction. *Am J Med Genet*. 1994; 51:527–534. [PubMed: 7943035]
22. Hall DA, Berry-Kravis E, Jacquemont S, et al. Initial Diagnoses Given to Persons with the Fragile X-associated Tremor/Ataxia Syndrome (FXTAS). *Neurology*. 2005; 65:299–301. [PubMed: 16043804]
23. Kunst CB, Zerylnick C, Karickhoff L, et al. *FMR1* in global populations. *Am J Hum Genet*. 1996; 58:513–22. [PubMed: 8644711]

24. Barros-Nunez P, Rosales-Reynoso MA, Sandoval L, et al. Genetic variation of the *FMR1* gene among four Mexican populations: Mestizo, Huichol, Purepecha, and Tarahumara. *Am J Hum Biol*. 2008; 20:259–63. [PubMed: 18172867]
25. Hall DA, Howard K, Hagerman RJ, Leehey MA. Parkinsonism in *FMR1* premutation carriers may be indistinguishable from Parkinson Disease. *Park Rel Dis Parkinsonism Relat Disord*. 2009; 15:156–159.
26. Hall DA, Hagerman RJ, Hagerman PJ, Jacquemont S, Leehey MA. Prevalence of *FMR1* repeat expansions in movement disorders: a systematic review. *Neuroepidemiology*. 2006; 26:151–155. [PubMed: 16493202]
27. Hedrich K, Pramstaller P, Stubke K, et al. Premutations in the *FMR1* gene as a modifying factor in *Parkin*-associated Parkinson's Disease? *Mov Disorders*. 2005; 20:1060–1062.
28. Deng H, Jankovic J. Premutation alleles associated with Parkinson disease and essential tremor. *JAMA*. 2004; 292:1685–1868. [PubMed: 15479932]
29. Kraff J, Tang H, Cilia R, et al. Screen for excess *FMR1* premutation alleles among males with parkinsonism. *Arch Neurol*. 2007; 64:1002–1006. [PubMed: 17620491]
30. Cilia R, Kraff J, Canesi M, Pezzoli G, Goldwurm S, et al. Screening for the presence of *FMR1* premutation alleles in women with parkinsonism. *Arch Neurol*. 2009; 66:244–249. [PubMed: 19204162]
31. Toledano-Alhadeff 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(2):351–360. [PubMed: 11443541]
32. Otsuka S, Sakamoto Y, Siomi H, et al. Fragile X carrier screening and *FMR1* allele distribution in the Japanese population. *Brain Dev*. 2010; 32(2):110–114. [PubMed: 19211207]
33. Kurz MW, Schlitter AM, Klenk Y, et al. *FMR1* alleles in Parkinson's disease: relation to cognitive decline and hallucinations, a longitudinal study. *J Geriatr Psychiatry Neurol*. 2007; 20(2):89–92. [PubMed: 17548778]
34. Semaka A, Creighton S, Warby S, Hayden MR. Predictive testing for Huntington disease: interpretation and significance of intermediate alleles. *Clin Genet*. 2006; 70(4):283–294. [PubMed: 16965319]
35. Groen JL, de Bie RM, Foncke EM, Roos RA, Leenders KL, Tijssen MA. Late-onset Huntington disease with intermediate CAG repeats: true or false? *J Neurol Neurosurg Psychiatry*. 81(2):228–230. [PubMed: 20145031]
36. Alonso I, Jardim LB, Artigas O, et al. Reduced penetrance of intermediate size alleles in spinocerebellar ataxia type 10. *Neurology*. 2006; 66(10):1602–1604. [PubMed: 16717236]
37. Ragonese P, D'Amelio M, Salemi G, et al. Risk of Parkinson disease in women effect of reproductive characteristics. *Neurology*. 2004; 62:2010–2014. [PubMed: 15184606]
38. Benedetti MD, Maraganore DM, Bower JH, et al. Hysterectomy, menopause, and estrogen use preceding Parkinson's disease: an exploratory case-control study. *Mov Disorders*. 2001; 16:830–837.
39. Rocca WA, Bower JH, Maraganore DM, et al. Increased risk of parkinsonism in women who underwent oophorectomy before menopause. *Neurology*. 2008; 70(3):200–209. [PubMed: 17761549]
40. Ragonese P, D'Amelio MD, Savettieri G. Implications for estrogens in Parkinson's disease. *Ann N Y Acad Sci*. 2006; 1089:373–382. [PubMed: 17261781]
41. Bretherick KL, Fluker MR, Robinson WP. *FMR1* repeat sizes in the gray zone and high end of the normal range are associated with premature ovarian failure. *Hum Genet*. 2005; 117:376–382. [PubMed: 16078053]
42. Bodega B, Bione S, Dalpra L, Toniolo D, Ornaghi F, Begetti W, et al. Influence of intermediate and uninterrupted *FMR1* CGG expansions in premature ovarian failure manifestation.
43. Tassone F, Hagerman RJ, Taylor AK, Mills JB, Wood S, Gane L, et al. Clinical involvement and protein expression in individuals with the *FMR1* premutation. *Am J Med Genet*. 2000; 91:144–152. [PubMed: 10748416]

44. Loesch DZ, Bui QM, Huggins RM, Mitchell RJ, Hagerman RJ. Transcript levels of the intermediate size or grey zone fragile X mental retardation 1 alleles are raised, and correlate with the number of CGG repeats. *J Med Genet.* 2007; 44:200–204. [PubMed: 16905681]
45. Garcia-Arocena D, Hagerman PJ. Advances in understanding the molecular basis of FXTAS. *Hum Molec Genet.* 2010; 19:R83–89. [PubMed: 20430935]

Table 1

Characteristics of the Study Population

		Male (n=188 [*])	Female (n=147 [*])	Controls Male (n=138)	Controls Female (n=135)	p-value ^{**}
Percentage		56%	44%	48%	51%	0.08
Mean age		62.1 ± 11	63.5 ± 12	64.7 ± 10	63 ± 9.2	0.29
Ethnicity						
	Hispanic	12	10	1	4	0.005
	Non- Hispanic Nomic	176	137	137	131	
Race						0.06
	White	176	130	134	130	
	Black	2	5	1	1	
	American Indian	8	10	2	3	
	Asian	2	2	1	1	
Parkinsonism	All causes	155	118			
	Parkinson Disease	120	98			
	Progressive Supranuclear Palsy	5	5			
	Corticobasal Degeneration	2	0			
	Multiple System Atrophy Parkinsonism	1	0			
	Unclassified Parkinsonism	29	13			
Ataxia	All causes	37	29			
	Multiple System Atrophy Cerebellar	1	4			
Tremor		32	34			

* A small proportion of cases are classified into more than one phenotypic category

** Comparing cases and controls

Table 2

Clinical Characteristics of the *FMR1* Repeat Expansion Carriers in Cases: 15 gray zone (41–54 CGG repeats) and 2 premutation carriers (55–200 CGG)

Age	Gender	CGG Repeat	Diagnosis	Age of Onset	Age of Diagnosis	UPDRS motor	Dopaminergic response	Family History	Other symptoms
68	F	41, 23	PD, tremor	56	66	11	yes	Tremor: uncle, cousin	Orthostatic tremor
76	F	41, 30	PD	74	76	20	yes	no	
64	F	41, 32	PD	57	59	27	yes	no	Celiac disease
71	F	42, 30	PD	56	56	46	yes	Infertility: sister	Focal hand dystonia
66	M	43	PD	55	55	8	yes	no	REM sleep behavior disorder
36	F	43, 37	ataxia	32	36	18	not tried	no	Toe cramping
78	F	43, 20	PD	66	66	11	yes	PD: father, tremor: brother	Motor fluctuations
57	M	44	parkinsonism	52	56	23	yes	ET: father, tremor: brother	
56	F	44, 29	PD	32	32	25	yes	PD: mother	Psychosis
53	F	45, 28	PD	45	46	9	yes	Unknown	
59	F	46, 20	PD	47	50	24	unknown	no	
59	F	47, 24	PD	44	58	8	yes	no	Prominent anxiety
63	M	48	parkinsonism	55	57	31	yes	no	Kinetic tremor, mild cognitive impairment
79	F	48, 30	PD	69	71	16	yes	PD: father	Motor fluctuations
70	F	51, 21	PD	66	67	14	yes	PD: brother, sister	Mild cognitive impairment
48	M	60	PD	45	46	11	yes	Tremor: uncle	Blepharospasm, head tremor
69	M	109	ataxia, tremor	58	58	35	mild improvement	Tremor: two children	Head tremor

UPDRS, Unified Parkinson's Disease Rating Scale; PD, Parkinson disease

Table 3
Prevalence of *FMR1* Expansions Compared to Controls for Gray Zone Expansions (41–55 CGG repeats)

Cases		Controls		Univariate Logistic Regression			
n	(%)	n	(%)	OR	95% CI	p-value	
Movement Disorders							
Male	3/188	1.6	6/138	4.3	0.52	0.1–2.4	0.4
Female	12/147	8.2	6/135	4.4	1.97	0.7–5.4	0.19
Parkinsonism							
Male	3/155	1.9	6/138	4.3	0.81	0.2–3.7	0.78
Female	11/118	9.3	6/135	4.4	2.34	0.9–6.2	0.09
Parkinson Disease							
Male	1/120	0.8	6/138	4.3	0.27	0–2.3	0.24
Female	11/98	11.2	6/135	4.4	3.23	1.2–8.6	0.019

OR, odds ratio; CI, confidence intervals