Screen for Excess \textit{FMR1} Premutation Alleles Among Males With Parkinsonism

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\textbf{Background}: Individuals with fragile X–associated tremor/ataxia syndrome frequently have associated features of parkinsonism, often leading to an initial diagnosis of Parkinson disease or other parkinsonism spectrum disorders. Parkinson disease populations may thus include individuals who harbor premutation expansions (55-200 CGG repeats) of the fragile X mental retardation 1 (\textit{FMR1}) gene.

\textbf{Objective}: To screen DNA samples (male) from an Italian Parkinson disease clinic for an excess of premutation expansions of the \textit{FMR1} gene.

\textbf{Design}: DNA samples obtained from 903 unrelated males through consecutive clinic visits were analyzed by an enhanced polymerase chain reaction method for detecting expanded CGG repeats.

\textbf{Setting}: Diagnostic assessments were performed at the Parkinson Institute, Istituti Clinici di Perfezionamento, Milan, Italy. Genotyping was conducted at the University of California Davis School of Medicine.

\textbf{Participants}: A cohort of unrelated males with clinical features of parkinsonism. All but 12 males were of Italian origin, and all reported Caucasian ethnicity.

\textbf{Main Outcome Measure}: CGG repeat number.

\textbf{Results}: Three premutation carriers (61, 69, and 80 CGG repeats) were identified (0.33%), which is not significantly higher than the frequency of premutation alleles in the general population. The outcome of the current study, the largest screen of individuals with parkinsonism to date, supports previous screens of smaller parkinsonism cohorts.

\textbf{Conclusion}: Broad screening for premutation alleles in Parkinson disease populations is unlikely to be productive in the absence of additional clinical or family history data that suggest involvement of the \textit{FMR1} gene.
ics. Initial surveys of several groups of patients with PD or parkinsonism failed to detect an excess of premutation allele. However, the screened groups were relatively small, and some would not have detected even relatively high frequencies of premutation alleles within the samples. To further address this issue, we have screened DNA samples from a cohort of 903 males with clinical features of parkinsonism. This cohort is more than twice as large as any previously screened group.

### METHODS

#### SUBJECTS

A polymerase chain reaction–based screen for premutation alleles of the FMR1 gene was performed on a cohort of 903 unrelated male patients who had received a diagnosis of either primary degenerative parkinsonism or essential tremor and who had contributed samples to the Human Genetic Bank of Patients Affected by Parkinson Disease and Parkinonsisms, Istituti Clinici di Perfezionamento, Milan, Italy (http://www.parkinson.it/dnabank.html). The cohort was selected on the basis of consecutive clinic visits, regardless of family history of parkinsonism or age at onset of the disease. Written informed consent was obtained from all subjects.

Patients fulfilled criteria for the following: 776 for idiopathic PD, 14 for dementia with Lewy bodies, 3 for frontotemporal dementia, 21 for multiple system atrophy, 21 for progressive supranuclear palsy, 9 for corticobasal degeneration, and 26 for essential tremor. For the remaining 33 cases, the clinical diagnosis was still uncertain; those patients are reported as having undefined primary parkinsonism.

The clinical diagnosis of PD was established according to the UK Parkinson’s Disease Society Brain Bank criteria, which require the presence of bradykinesia, the absence of atypical features or other causes of parkinsonism, and at least 1 of the following: resting tremor, rigidity and postural instability, and a positive response to dopaminergic therapy. In the non-PD cases, diagnoses were assigned according to published diagnostic criteria. Among the 776 patients with idiopathic PD, the mean age at onset was 54.01 years (range, 14-83 years; SD, 10.81 years), and the mean disease duration was 11.75 years (range, 1-56 years; SD, 6.3 years).

All patients with PD were previously tested for the G2019S mutation and 10 were found to be carriers. The parkin gene was tested in all PD patients with an age at onset of 40 years or younger and 8 were homozygous for the mutated gene. Such patients were not excluded from this analysis. All but 12 patients were of Italian origin. The other patients were from Turkey, South Africa, Sri Lanka, the United States, Yugoslavia, Romania, Ireland, Iran, France, Albania, and Argentina. All patients reported Caucasian ethnicity.

### CASES IDENTIFIED AS PREMUTATION CARRIERS

**Case 1 (C-0230)** is a 65-year-old man with an 18-year history of PD. The onset of symptoms occurred when he was aged 48 years, with bradykinesia and rigidity on the right side. He had a good response to treatment with dopaminergic agents. He first experienced wearing-off motor fluctuations 9 years after his initial presentation and peak-dose dyskinesias after 14 years.

He progressively developed severe motor fluctuations and dyskinesia and was considered for subthalamic nucleus deep brain stimulation 14 years after initial symptoms. Neurological examination was assessed both 12 hours after last medication intake (off) and immediately after medication intake (on), with an improvement in Unified Parkinson Disease Rating Scale motor score of 29 to 15 (−49%). Brain MRI showed mild corticosubcortical atrophy but no white matter signal abnormalities. Brain perfusion single-photon emission computed tomography showed bilateral striatal perfusion reduction. Neuropsychological evaluation revealed no specific cognitive deficits but disclosed psychotic symptoms, such as auditory hallucinations, jealousy, and persecutory delusions.

The neuropsychological follow-up assessment (1 year later) revealed mild difficulties with memory and frontal lobe–related abilities but also showed improvement of psychiatric symptoms. Therefore, the patient underwent deep brain stimulation surgery and showed a significant clinical improvement, with reduction of daily time spent in off condition and dyskinesia. Hallucinations were not reported after surgery, but delusions occasionally occurred.

**Case 2 (B-0556)** is a 50-year-old man with a 16-year history of PD. The onset of symptoms occurred at 34 years of age with resting tremor on the right hand and slowly progressive bradykinesia and rigidity. Dopaminergic treatment was instituted 6 years after the first symptoms appeared. He always showed a good clinical response to medication, experiencing wearing-off fluctuations 11 years from the onset, but no other motor complications.

At 48 years of age, neuropsychological assessment revealed mild deficits of frontal lobe functions in the patient. Psychotic features, such as jealousy and persecution delusions, were reported and partially improved with quetiapine treatment. Brain imaging investigation results, such as MRI and perfusion single-photon emission computed tomography results, are not available.

**Case 3 (G-0444)** is a 63-year-old man who first complained of micrography, bradykinesia, and rigidity on the right side at 43 years of age. The diagnosis of PD was established and dopaminergic treatment was started with good response. He developed motor fluctuations and dyskinesias 7 years following symptom onset. Clinical, radiological, and molecular findings are presented in Table 1.

Neurological examination at 60 years was assessed both in off and on states, with an improvement in Unified Parkinson Disease Rating Scale motor score from 31 to 25 (−20%). The neuropsychological tests detected mild frontal deficits characterized by reduction in attention and presence of perseverative behavior. Impulsiveness, slight apathy, and depressed mood were also observed.

Brain MRI showed moderate subcortical and cerebellar atrophy. Brain perfusion single-photon emission computed tomography revealed bilateral cortical hypoperfusion in the left posterior parietal and occipital areas and asymmetric blood flow reduction in the striatum bilaterally, mainly on the left side.

### MRI ASSESSMENT

Following the identification of FMR1 premutation alleles, a neuroradiologist reexamined MRIs of cases 1 and 3 (patients C-0230 and G-0444). Cerebellar atrophy was noted in case 3. However, neither case demonstrated the high-signal lesions (T2-weighted) of the middle cerebellar peduncles (MCP sign) that are characteristic of FXTAS.

### MOLECULAR MEASURES

Genomic DNA was isolated from peripheral blood lymphocytes using standard phenol-chloroform extraction methods. DNA from each patient was amplified using an enhanced poly-
merase chain reaction technique containing the osmolyte, betaine, and primers c and f, as described by Saluto et al.\textsuperscript{18} with only minor modifications to optimize betaine concentration (~2.2 M). Amplified DNA was then visualized on 2% agarose gels and ethidium bromide stained. Gray zone and premutation alleles were accurately sized on acrylamide gels. Hybridization was performed with a digoxigenin–end-labeled oligonucleotide probe, (CGG)\textsubscript{10}. Repeat sizes were determined using an Alpha Innotech FluorChem 8800 Image Detection System (Alpha Innotech Corp, San Leandro, California). Details of the method are presented in an article by Tassone et al.\textsuperscript{19}

### MAIN OUTCOME MEASURE

The sole outcome measure of this study is the number of CGG repeats, determined from the size of the polymerase chain reaction product. CGG repeats are considered to be premutation expansions if the size of the expansion exceeds 54 CGG repeats but is less than or equal to 200 CGG repeats.

### RESULTS

We screened 903 male patients with a diagnosis of primary parkinsonism or essential tremor for the presence of premutation expansions (55-200 CGG repeats) of the FMR1 gene. Three individuals, all with the diagnosis of PD, were identified as carriers of premutation alleles of 61 (case 2), 69 (case 1), and 80 CGG repeats (case 3). The frequency of premutation alleles in this cohort was therefore 0.33% (3 of 903 patients), a value that is not significantly higher than values reported for the general population\textsuperscript{12,20,21} and is consistent with previous, smaller screens of parkinsonism cohorts (Table 2). In addition, 18 gray-zone alleles (45-54 CGG repeats) were identified. Again, they were not significantly higher than values for the general population. The remaining 882 males harbored a normal allele (<45 CGG repeats).

The 3 male carriers of the FMR1 premutation did not show any unusual clinical features that would distinguish them from (noncarrier) patients with idiopathic PD. None of the 3 cases revealed features resembling FXTAS. In particular, they did not show cerebellar findings such as action tremor or gait ataxia, dysautonomia, or peripheral neuropathy. Relevant cognitive deficits were not present, though mild frontal lobe–related deficits were reported, which is typical in advanced PD. Magnetic resonance images, available for 2 of 3 premutation carriers, did not demonstrate the FXTAS MCP finding\textsuperscript{6,27} though 1 individual did display mild cerebellar cortical atrophy. In addition, the premutation carriers did not have a family history positive for neurological or psychiatric disease, cognitive deficits, or development delays.

Thus, it is likely that these cases represent coincidental findings. This suggestion is reinforced both by the relatively early ages at onset (34, 43, and 48 years; mean, 41.7 years) for the 3 FMR1 premutation carriers, which are much earlier than the mean age at onset for symptoms of FXTAS (62.6 years [SD, 8.1 years] for tremor; 63.6 years [SD, 7.3 years] for ataxia).\textsuperscript{28} On the other hand, the early age at onset, which is generally associated with a stronger genetic predisposition, is an interesting finding and suggests that the FMR1 premutation may still have a role in the genetic susceptibility to common idiopathic PD.

### Table 1. Clinical, Radiological, and Molecular Findings in PD Patient Carriers of the Fragile X Premutation

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at Onset, y</th>
<th>Family History of Movement Disorders or Neurodegenerative Diseases</th>
<th>Disease Duration, y</th>
<th>Clinical Presentation</th>
<th>Levodopa Response</th>
<th>Cerebellar Disorder</th>
<th>Other Features</th>
<th>Magnetic Resonance Imaging</th>
<th>No. of CGG Repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>None</td>
<td>18</td>
<td>Bradykinesia, rigidity</td>
<td>Good, &gt; 45%</td>
<td>None</td>
<td>Psychosis (hallucination, delusion)</td>
<td>Mild corticosubcortical atrophy</td>
<td>69</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>None</td>
<td>16</td>
<td>Resting tremor</td>
<td>Good, &gt; 45%</td>
<td>None</td>
<td>Psychosis (delusions)</td>
<td>NA</td>
<td>61</td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>None</td>
<td>19</td>
<td>Bradykinesia, rigidity</td>
<td>Moderate, 20%</td>
<td>None</td>
<td>None</td>
<td>Moderate subcortical and cerebellar atrophy</td>
<td>80</td>
</tr>
</tbody>
</table>

**Table 1.** Clinical, Radiological, and Molecular Findings in PD Patient Carriers of the Fragile X Premutation

**Abbreviations:** NA, not available; PD, Parkinson disease.

### Table 2. Screens for Premutation FMR1 Alleles Within Cohorts of Patients With Parkinson Disease or Parkinsonism

<table>
<thead>
<tr>
<th>Source (Center)</th>
<th>Premutation Allele Frequency</th>
<th>Male</th>
<th>Female</th>
<th>No. of CGG Repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annesi\textsuperscript{22} (Istituto Dermopatico dell’Immacolata, Rome, Italy)</td>
<td>0/203</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Toft\textsuperscript{23} (Mayo Clinic, Jacksonville, Florida)</td>
<td>0/414</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Deng\textsuperscript{24} (Baylor College, Houston, Texas)</td>
<td>0/216</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Tan\textsuperscript{25} (Singapore General Hospital, Singapore)</td>
<td>0/79</td>
<td>0/42</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Hedrich\textsuperscript{26} (University of Lubeck, Lubeck, Germany)</td>
<td>1/265</td>
<td>1/108</td>
<td>Males, 55 Females, 63</td>
<td></td>
</tr>
<tr>
<td>Current study (University of California, Davis)</td>
<td>3/902</td>
<td>NA</td>
<td>61, 69, 80</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Screens for Premutation FMR1 Alleles Within Cohorts of Patients With Parkinson Disease or Parkinsonism

**Abbreviation:** NA, not applicable.
In a screen of 903 cases with a prior diagnosis within the category of parkinsonism or essential tremor, there was no increase in the number of premutation FMR1 alleles over that which is expected in the general population, an observation that is not surprising in view of the differences in diagnostic features between patients with idiopathic PD and related disorders and patients with FXTAS. However, our study does underscore that testing for expanded FMR1 alleles within the parkinsonism movement disorder population is not justified in the absence of additional findings, such as cerebellar gait ataxia, MRI findings positive for MCP signs, or a family history of cognitive impairment, developmental delay, autism, or premature ovarian failure,\(^{29,31}\) which are all indicators of greater potential for expanded FMR1 alleles.

There is one important caveat with the current findings, as with nearly all of the high-risk screens performed to date, namely, the selection bias intrinsic to the movement disorders population itself. In a retrospective study of known patients with FXTAS, only approximately 4% of patients were initially seen and had their syndrome diagnosed by a movement disorders specialist,\(^{11}\) and so only a small fraction of the FXTAS cases would be expected to be found within cohorts of the type screened here and those presented in Table 2. Of the remaining 96% of cases identified by Hall et al.,\(^{11}\) approximately 70% were seen by general neurologists, and another 26% were seen by adult primary care physicians. Thus, there is a need for broader screens of older adult populations, both within and beyond the general neurology clinic.

A screen for expanded CGG-repeat alleles of the FMR1 gene within a cohort of 903 males with clinical presentations, including parkinsonism, detected 3 carriers of premutation alleles (61, 69, and 80 CGG repeats), a frequency (3 of 903 [0.33%]) that is not significantly higher than the frequency of premutation alleles in the general population (0.13%-0.39%).\(^{20,21}\) The outcome of our study, the largest screen to date of individuals with parkinsonism, suggests that broad screening for premutation alleles in PD populations is unlikely to be productive in the absence of additional clinical or family history data suggestive of involvement of the FMR1 gene.

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