LRRK2 Mutations in Spanish Patients With Parkinson Disease

Frequency, Clinical Features, and Incomplete Penetrance

Carles Gaig, MD; Mario Ezquerra, PhD; Maria Jose Marti, MD, PhD; Esteban Muñoz, MD, PhD; Francesc Valdeoriola, MD, PhD; Eduardo Tolosa, MD, FRCP

Background: Several pathogenic mutations in the LRRK2 gene have been implicated in familial and sporadic cases of Parkinson disease (PD). The R1441G mutation is frequent in Spanish patients of Basque ethnicity with PD, and the G2019S mutation is a common mutation found in several populations worldwide.

Objectives: To determine the frequency of the LRRK2 G2019S and R1441G mutations in PD patients from the non-Basque northeast region of Spain (Catalonia), and to characterize their family history and clinical features.

Design: We screened patients for the presence of the LRRK2 R1441G and G2019S mutations. These LRRK2 mutations were detected by restriction endonuclease digestion, and samples with an abnormal electrophoresis pattern were sequenced to identify the exact nucleotide change. The clinical features and family history of patients with LRRK2 mutations were studied in detail.

Setting: The northeast region of Spain.

Patients: Three hundred two patients with PD.

Main Outcome Measures: Onset age, clinical features, and family history of PD and LRRK2 mutations.

Results: The R1441G mutation was present in 0.7% of total PD cases. The G2019S mutation was found in 6.4% of familial and 3.4% of sporadic cases. Additionally, we found 1 patient with the R1441C mutation. Age at onset ranged from 33 to 78 years. Clinical features were not different from classic PD, except for 1 patient who presented with monosymptomatic leg rest tremor of 8 years' duration. In addition, a 91-year-old unaffected relative of a patient with the G2019S mutation was found to be a mutation carrier.

Conclusions: The G2019S mutation frequency in PD patients from northeast Spain is similar to that reported in other European regions. The R1441G mutation is very uncommon in Catalonia. The presence of an aged unaffected G2019S mutation carrier supports the previously described occurrence of incomplete penetrance in PD patients with LRRK2 mutations.

Arch Neurol. 2006;63:377-382

RECENTLY, PATHOGENIC MUTATIONS IN THE leucine-rich repeat kinase 2 (LRRK2) gene have been identified in patients with autosomal dominant forms of Parkinson disease (PD). The LRRK2 gene has 51 exons, encodes a large protein of 2527 amino acids (dardarin), and has an unknown function, although there are 5 predicted functional domains: a leucine-rich repeat domain, a Roc (Ras guanosine triphosphatase) domain, a C-terminal of Roc domain, a WD40 domain (β-transducin repeats), and a tyrosine kinase catalytic domain. The clinical features of parkinsonism and the response to levodopa treatment in patients with LRRK2 mutations seem to be indistinguishable from those of classic PD. Features like dementia or atypical signs such as amyotrophy or supranuclear gaze palsy are rarely observed. Until now, several different missense mutations have been reported in the LRRK2 gene. One of them, the G2019S mutation, has been found in a significant proportion of patients with PD; it accounts for about 5% to 6% of familial and 1% to 2% of apparently sporadic cases. The R1441G mutation has been described only in populations located in the Basque country and its neighboring region of northern Spain, Asturias. This mutation has been found in 8% of Basque PD patients and in 2.7% of patients with late-onset PD in Asturias. Two more mutations, located in position 1441 within the Roc domain, have been described in other populations: the R1441C and R1441H mutations, emphasizing the importance of this residue for the LRRK2 pathogenic mechanism. The aims of this study were to determine the frequency of the LRRK2 G2019S
and R1441G mutations in PD patients included in our PD DNA bank, composed of patients from the non-Basque northeast region of Spain (Catalonia), and to characterize in detail their family history and clinical features.

### METHODS

#### PATIENTS

The LRRK2 mutations were screened in 302 nonconsecutive and unrelated PD patients (mean ± SD age at onset, 53.8 ± 13.3 years; range, 8-85 years; 170 [56.3%] males; 132 [43.7%] females). All subjects in this study resided in Catalonia and were recruited in the Neurology Service of the Hospital Clinic of Barcelona from January 1, 1997, to June 30, 2005. They all fulfilled commonly accepted clinical diagnostic criteria for PD. 

Patients included were observed longitudinally in our Movement Disorders Unit. Their family history of PD was periodically evaluated, and when positive, the conservative criteria of Marder et al. were used to assess its degree of certainty. Among the 302 PD patients, 94 had a family history (31.1%; mean ± SD age at onset, 53.1 ± 14.0; range, 15-84 years), whereas 208 cases were sporadic (68.9%; mean ± SD age at onset, 53.8 ± 13.3 years; range, 8-85 years; 170 [56.3%] males; 132 [43.7%] females). Written informed consent was obtained from all participants, and the local ethics authorities approved the project. The clinical data of patients with LRRK2 mutations were reviewed. In some cases, it was possible to obtain blood samples from affected and unaffected relatives.

#### GENETIC ANALYSIS

The DNA from peripheral blood was isolated by using standard methods in all subjects. The LRRK2 G2019S mutation was detected by restriction endonuclease digestion with the SfiI enzyme as previously described, running in an acrylamide gel electrophoresis and subsequently stained with ethidium bromide. The R1441G, R1441C, and R1441H mutations were screened by restriction endonuclease digestion with the BstUI enzyme, and samples with an abnormal electrophoresis pattern were sequenced to identify the exact nucleotide change. Genomic DNA was amplified using polymerase chain reaction and done in 25 µL containing 2.5 µL of polymerase chain reaction buffer, 1.5mM magnesium chloride, 1.25mM concentration of each deoxynucleotide triphosphate (dNTP), 0.4µM forward primer, 0.4µM reverse primer, 2.5 U of Taq DNA polymerase, and 50 ng of genomic DNA. Cycle conditions were as follows: 5 minutes at 94°C, 30 cycles of 30 seconds' denaturation at 94°C, 30 seconds' annealing at 58°C, and 90 seconds' extension at 72°C, with a final extension of 5 minutes at 72°C. The samples were sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Foster City, Calif) and run on an ABI-prism automatic DNA sequencer (Perkin Elmer).

### RESULTS

The LRRK2 mutations were found in 16 patients, which represent 5.3% of all PD subjects studied in this cohort. Thirteen patients carrying the G2019S mutation, 2 patients with the R1441G mutation, and 1 patient with the LRRK2 R1441C mutation were identified. The R1441H mutation was not present in any of our patients. An R1441G mutation was found in a familial PD patient originating from the northwest region of Spain, near Asturias, but the other R1441G mutation carrier had not known ancestors from this geographical area.

Nine patients (56.3%) had a family history of PD. Two other patients (patients 1 and 8) reported tremor in 1 parent, but these families did not meet the conservative criteria of Marder and colleagues for familial PD, and the cases were classified as sporadic. The pedigrees of patients with LRRK2 mutations are shown in the Figure. The family of proband 4 showed incomplete penetrance. The proband’s mother, who was a confirmed mutation carrier, did not have illness symptoms or signs of parkinsonism at age 91 years. Furthermore, a proband’s half-sister, from the same mother but a different father, was affected by PD but was not a mutation carrier.

Two additional patients carrying the G2019S mutation (patients 17 and 18), relatives of probands 4 and 11, respectively, were identified, and their clinical features are also reported herein (Table 2 and Figure). The mean age at illness onset for the 18 patients with LRRK2 mutations (mean ± SD, 57.1 ± 13.2 years; range, 33-78 years) did not differ significantly from PD patients without these mutations (mean ± SD, 53.7 ± 13.3 years; range, 8-85 years). Early onset (<40 years) was present in 2 patients, aged 33 and 35 years, respectively. One relative of patient 7 developed symptoms of PD at age 26 years, but extensive clinical information and DNA from this individual were not available.

### Table 1. Patients With PD With or Without LRRK2 Mutations

<table>
<thead>
<tr>
<th>LRRK2 Mutation</th>
<th>G2019S</th>
<th>R1441G</th>
<th>R1441C</th>
<th>Total†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with PD (n = 302)</td>
<td>268</td>
<td>13 (4.3)</td>
<td>2 (0.7)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Familial PD (n = 94)</td>
<td>85</td>
<td>6 (6.4)</td>
<td>2 (2.1)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Onset ≥40 y (n = 77)</td>
<td>69</td>
<td>5 (6.5)</td>
<td>2 (2.6)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Onset &lt;40 y (n = 17)</td>
<td>16</td>
<td>1 (6.9)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sporadic PD (n = 208)</td>
<td>201</td>
<td>7 (3.4)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Onset ≥40 y (n = 177)</td>
<td>171</td>
<td>6 (3.4)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Onset &lt;40 y (n = 31)</td>
<td>30</td>
<td>1 (3.2)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviation: PD, Parkinson disease.

*Data are presented as number (percentage) unless otherwise indicated.

†Combination of the 3 mutations.
The clinical features of patients with LRRK2 mutations after a mean disease duration of 13 years are shown in Table 2 and Table 3 (mean follow-up at our hospital, 6.3 years). The parkinsonism in patients with LRRK2 mutations usually presented with unilateral rest tremor or motor slowness. During the course of the illness, the 3 cardinal parkinsonian signs of rest tremor, bradykinesia, and rigidity were present in most patients, with frequent persistence of the asymmetry of parkinsonian motor signs (Table 3). Only 1 case (patient 18; Table 2) differed from this clinical picture. At age 57 years this patient developed rest tremor of the left leg, which has remained mild and intermittent and been restricted to the left leg for 8 years, without the presence of other parkinsonian signs. Dementia was present in only 1 patient (patient

Figure. Pedigrees of familial patients with LRRK2 mutations. Each proband is indicated by an arrow. Black symbols indicate affected family members; gray symbols, relatives who have only tremor; slash, deceased; circles, females; squares, males; asterisk, genotyped individual, with m for mutation carriers and wt for wild-type LRRK2.
16; Table 2) with the R1441G mutation, who developed visual hallucinations and delusions with cogni-
tive decline (Mini-Mental State Examination score, 12/30) and fluctuating attention after 15 years of ill-
ness. Atypical features of PD such as supranuclear gaze palsy or early dysautonomia were not observed in any of our patients.

Response to levodopa was positive in all cases. Treat-
ment-induced motor complications appeared fre-
cently after a mean illness duration of 7 years but in some cases appeared after 10 or as early as 2 years. All patients with treatment-induced motor complications had motor fluctuations, mainly wearing-off but also on-off phenomena (Table 3). Three patients (patients 2, 3, and 11; Table 2) were treated with bilateral subthalamic nu-
clei deep brain stimulation after 7 to 11 years of illness, with sustained control of motor PD symptoms and func-
tional improvement in all cases.

COMMENT

We have found the LRRK2 G2019S mutation in 4.3% of our PD patients, accounting for 6.4% of familial and 3.4% of apparently sporadic cases. This is the first study that reports the frequency of the LRRK2 G2019S mutation in Spanish PD patients. The proportion of LRRK2 G2019S mutation carriers in both familial and isolated cases of PD is similar to or slightly higher than that reported previously in other studies performed in different populations, where this mutation accounts for about 2% to 6%
of familial and 0.6% to 2.0% of apparently sporadic cases.7-10,13,16

The R1441G mutation was present in 0.7% of our PD patients. This mutation was originally identified in 8% of Spanish families originating from the Basque region1 and later in patients from a neighboring region, Asturias, with a lower frequency (2.7%).11 This mutation has not been found in other world populations, including Portuguese PD patients in the Iberian peninsula,16 suggesting that it is geographically restricted to northern Spain by a founder effect in the Basque people, a relatively homogeneous and historically isolated ethnic group. Haplo-type analysis of this chromosomal region in PD patients supports this hypothesis.1,11 We have found a lower R1441G mutation frequency in Catalonia when compared with Basque or Asturian regions, supporting the existence of a geographical gradient for this mutation as previously suggested.11 In our population, 1 patient with an R1441G mutation originated from a region near Asturias, whereas the other patient carrier had not known Basque or Asturian ancestors. Therefore, the presence of this mutation in Catalonia could be explained by the existence of recent or ancient migration from the Basque population. Supporting the hypothesis of an ancient migration is the description of an ancestral gene flow between the Basque people and other populations of Europe, especially with the Catalan people, during the past few thousand years.17

As reported in previous studies,2-6 the clinical features of parkinsonism in our patients with LRRK2 mutations were similar to classic PD. Age at illness onset was variable, ranging from 33 to 78 years, although mean age at onset was similar to that of idiopathic PD. Onset was frequently asymmetrical, with rest tremor or motor slowness. A significant proportion of our patients had minimal or no rest tremor despite long disease duration. As noted in other studies, dementia was rare in patients with LRRK2 mutations.24 A clinical presentation different from classical PD occurred in 1 G2019S mutation carrier, who had monosymptomatic, mild, intermittent rest tremor in 1 leg for several years without developing additional parkinsonian symptoms or signs. This suggests that in some cases, the LRRK2 mutation phenotype may have a benign course. All patients had a good response to levodopa but frequently developed treatment-induced motor complications, especially fluctuations and freezing of gait, that were disabling for many of them. Three of our patients were treated with chronic bilateral electrical stimulation of the subthalamic nuclei, with an excellent outcome.

Family history of PD was present only in 56.3% of our LRRK2 mutation carriers. Negative family history for this subset of PD patients (43.7%) may be related to several factors, including incomplete penetrance,10 undiagnosed PD in a relative, appearance of a mutation de novo, false paternity, and early death of family members before illness development. We found an unaffected 91-year-old relative who carries the G2019S mutation, supporting the existence of incomplete penetrance in this family. Previous studies have reported a penetrance between 70% and 100% in different families with LRRK2 mutations.3,10 In 1 study, the penetrance of the mutation was found to be age dependent, increasing from 17% at age 50 years to 85% at age 70 years.3 However, the 91-year-old unaffected carrier reported herein, together with the previously reported 89-year-old unaffected subject carrying a similar mutation,10 suggests that other factors besides age are important for LRRK2 incomplete penetrance.

Identification of affected relatives of G2019S PD patients but who do not carry this mutation, as in 1 of our patients, has also been observed in other studies.6,8 Nichols et al9 reported the presence of such phenocopies in 5 of 19 affected sibships with the G2019S mutation. As suggested by Singleton,20 these phenocopies may indicate that some risk factors might be present in other family members, independently of their G2019S status, and consequently increase the lifetime risk of PD.

In light of the high frequency of LRRK2 mutations in PD, it seems likely that the identification of many patients and unaffected relatives with LRRK2 mutations will be possible in the near future. This will facilitate the search of genetic and environmental factors that could condition disease susceptibility and age at disease onset. Furthermore, the detection of presymptomatic mutation carriers will allow researchers to perform studies of putative neuroprotective treatments.

Accepted for Publication: October 5, 2005.

Correspondence: Eduardo Tolosa, MD, FRCP, Hospital Clinic, Service of Neurology, Villarroel 170, Barcelona 08036, Spain (etolosa@clinic.ub.es).

Author Contributions: Study concept and design: Gaig and Ezquerra. Acquisition of data: Gaig, Ezquerra, Marti, Muñoz, Valdeorriola, and Tolosa. Analysis and interpretation of data: Gaig, Ezquerra, and Tolosa. Drafting of the manuscript: Gaig, Ezquerra, and Tolosa. Critical revision of the manuscript for important intellectual content: Marti, Muñoz, Valdeorriola, and Tolosa. Obtained funding: Tolosa. Administrative, technical, and material support: Gaig and Ezquerra. Study supervision: Tolosa. Drs Gaig and Ezquerra contributed equally to this work.

Funding/Support: This project was supported by grants from the Distinció per la Recerca de la Generalitat de Catalunya (Barcelona, Spain) and grant C03/06 from Centro en Red de Investigaciones en Enfermedades Neurologicas, Institut d’Investigacions Biomèdiques August Pi i Sunyer–Instituto de Salud Carlos III, Redes Tematicas de Investigación Cooperativa (Barcelona) to Dr Tolosa.

Acknowledgment: We thank the patients and their families for their participation in this study. We acknowledge technical support provided by Manel Fernandez.

REFERENCES


**Call for Papers**

The ARCHIVES launched a new ARCHIVES Express section in the September 2000 issue. This section will enable the editors to publish highly selected papers within approximately 2 months of acceptance. We will consider only the most significant research, the top 1% of accepted papers, on new important insights into the pathogenesis of disease, brain function, and therapy. We encourage authors to send their most exceptional clinical or basic research, designating in the cover letter a request for expedited ARCHIVES Express review. We look forward to publishing your important new research in this accelerated manner.

Roger N. Rosenberg, MD