LRRK2 Exon 41 Mutations in Sporadic Parkinson Disease in Europeans

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Background: Mutations in leucine-rich repeat kinase 2 gene (LRRK2), particularly the G2019S mutation in exon 41, have been detected in familial and sporadic Parkinson disease (PD) cases.

Objectives: To assess the frequency of LRRK2 exon 41 mutations in a series of sporadic PD cases from Europe and to determine the clinical features of LRRK2 mutation carriers.

Design: We analyzed European cases of sporadic PD for the presence of LRRK2 exon 41 mutations. These mutations were screened by denaturing high-performance liquid chromatography, and abnormal chromatograph traces were investigated by direct sequencing to determine the exact nature of the variants. Early-onset sporadic PD cases were also screened for parkin mutations. The haplotypes associated with the G2019S mutation were determined. The clinical characteristics of patients carrying LRRK2 mutations were detailed.

Setting: French Network for the Study of Parkinson Disease Genetics.

Patients: Three hundred twenty patients with apparently sporadic PD from Europe.

Main Outcome Measures: Results of genetic analyses.

Results: We found the G2019S mutation in 6 patients and identified 2 new variants (Y2006H and T2031S) in 1 patient each. Their clinical features were similar to those of typical PD. All G2019S mutation carriers shared a common haplotype.

Conclusions: The G2019S mutation is almost as frequent in sporadic cases (1.9%) as in previously reported familial cases (2.9%) in Europe and occurs in the same common founder. We identified 2 novel variants. Although the phenotype of LRRK2 mutation carriers closely resembles that of typical PD, the age at onset was younger (29 years in 1 patient) than previously described, and 3 patients were improved by deep brain stimulation.

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Parkinson disease (PD) is a common progressive neurodegenerative disorder. Ninety percent of patients referred to as having sporadic PD have no affected relatives. Eleven loci and 6 genes are responsible for familial forms of parkinsonism. Mutations in the leucine-rich repeat kinase 2 gene (LRRK2) have been implicated in an autosomal dominant form of PD. LRRK2 encodes a large protein of 2527 amino acids with multiple domains, including a nonreceptor tyrosine kinase–like domain, a Ras-like small guanidine triphosphatase family domain (Roc), and several ankyrin, leucine-rich, and WD40 repeats. Among white populations, a single pathogenic G2019S in exon 41 is responsible for 3% to 6% of familial PD and for 1% to 2% of sporadic PD, which is more frequent in North Africa but is rare in Asia. Because of the high prevalence of this mutation, LRRK2 is of major importance for the diagnosis and genetic counseling of patients with familial PD. Because the penetrance of the mutation is age related, or even reduced, in G2019S mutation carriers, affected subjects who are in fact familial may lack a family history. Therefore, we analyzed 320 apparently sporadic PD cases of European descent for LRRK2 exon 41 mutations.

Method: The PD cohort, consisting of 320 patients with no first-degree relatives with PD, was recruited through the French Network for the Study of Parkinson Disease Genetics. Patients...
included in the study fulfilled at least 3 mandatory diagnostic criteria for clinically defined PD (akinesia, rigidity, resting tremor, asymmetrical onset, or >30% improvement with levodopa therapy), with a good (>30%) response to levodopa treatment and without exclusion criteria (cerebellar signs, ophthalmoplegia, bilateral Babinski signs, dementia in the first 2 years of the disease, use of neuroleptic drugs before onset of PD, and severe and early postural instability or urinary incontinence). Informed written consent was obtained from all individuals included in the study.

Most of the 320 white patients with sporadic PD were of French descent (n=300) but some were also of Italian (n=10), Spanish (n=4), Belgian (n=2), Polish (n=3), and Portuguese (n=1) descent. The mean±SD age of the patients at examination was 56.4±13.4 years (age range, 17-85 years), the mean age at onset was 45.6±12.4 years (age range, 12-72 years), and the mean±SD disease duration was 10.8±7.1 years (range, 1-37 years). The frequency of the LRRK2 G2019S mutation was 0.3% in 174 previously described European control subjects (mean±SD age at examination, 62.7±9.5 years [age range, 27-84 years]).

GENETIC ANALYSIS

Genomic DNA was extracted from blood samples. A standardized phenol-chloroform method was used.

Denaturing High-Performance Liquid Chromatography Analysis

Exon 41 of LRRK2 was amplified by polymerase chain reaction (PCR) from genomic DNA using the forward 5'-GACAGAATTTTGTAGCCTG-3' and reverse 5'-GAGGTCAGTGGTGATACATC-3' primers and was screened by denaturing high-performance liquid chromatography (dHPLC) (model 3500 HT Transgenicome Wave Nucleic Acid Fragment Analysis System controlled by Navigator software version 1.6.2; Transgenicome Inc, Elancourt, France). Briefly, immediately before dHPLC analysis, heteroduplex formation was induced by denaturing the PCR products at 96°C for 5 minutes and then gradually cooling to 25°C during 30 minutes. The PCR products (5 µL) were automatically loaded onto the column (DNAsep Cartridge; Transgenicome Inc) and were eluted using a linear acetonitrile gradient in a 0.1M triethylammonium acetate buffer at a constant flow rate of 0.9 mL/min. Potential variants in exon 41 of LRRK2 were screened by using 2 predicted optimal melting temperatures of 52.6°C and 59.7°C. A positive control for the G2019S mutation previously identified by sequencing and a confirmed wild-type DNA control were run at the same time as control samples.

Direct Sequence Analysis

The PCR products yielding abnormal chromatograms were purified by digestion (Exo/SAP; 10 U of exonuclease I [New England Biolabs, Saint Quentin en Yvelines, France] and 2 U of shrimp alkaline phosphatase [Roche Diagnostics, Meylan, France]) for 15 minutes at 37°C, followed by enzyme inactivation for 15 minutes at 80°C. They were then sequenced on the forward and reverse strands with the same primers used for the dHPLC analysis (Big Dye Terminator version 3.1 kit), purified by ethanol precipitation and electrophoresed on an automated sequencer (ABI 3730), and analyzed using commercially available software (DNA Sequencing Analysis version 5.1 and Seqscape version 2.1.1; all from Applied Biosystems, Courtaboeuf, France). The consequences of mutations at the protein level were predicted according to the LRRK2 complementary DNA sequence (GenBank accession AF792511).

Haplotype Analysis

Haplotypes associated with the G2019S mutation were analyzed by typing 21 markers (17 chromosome 12q microsatellites and 4 single nucleotide polymorphisms) flanking the G2019S mutation. This method has been previously reported.

Parkin Mutation Analysis

The parkin gene was analyzed in 152 cases of early-onset sporadic PD, as previously reported. Briefly, point mutations and small rearrangements in the promoter region and all 12 coding exons and their flanking regions were detected by dHPLC, and abnormal chromatographic profiles were checked by direct sequencing. Exonic rearrangements were screened by semi-quantitative multiplex PCR technique.

RESULTS

Eight of 320 patients with sporadic PD had abnormal dHPLC elution patterns for LRRK2 exon 41 (Figure). Six patients (1.9%), 5 French and 1 Portuguese, were shown by direct sequence analysis to have the G2019S (c.6095 G>A) mutation. The other 2 mutations were novel substitutions, a heterozygous c.6016 T>C transversion predicted to cause a tyrosine to histidine substitution at amino acid 2006 (Y2006H) in a French patient and a heterozygous c.6091 A>T transversion predicted to cause a T2031S substitution in a Spanish patient. Neither was found on 348 control chromosomes, and both were located in a region that is highly conserved among species ranging down to amphibians.

No LRRK2 mutations were found in 152 patients screened for parkin mutations, although 7 single heterozygous and 3 compound heterozygous parkin mutations were identified. A minimal common haplotype as previously described was shared by all 6 apparently sporadic PD cases carrying the G2019S mutation (Table 1).

The clinical characteristics of the 8 LRRK2 mutation carriers were similar to those of typical PD (Table 2). The age at onset ranged from 29 to 56 years, and the disease duration at examination ranged from 4 to 27 years. The mean±SD age at onset (41.9±8.3 years [age range, 29-56 years]) tended to be earlier than in the other patients (45.6±12.5 years [age range, 12-72 years]). The disease severity, evaluated by the Unified Parkinson Disease Rating Scale while receiving treatment, tended to increase with the disease duration but remained moderate except for the patient with the longest disease duration (27 years), who had a score of 60 while receiving treatment. Depression was noted in 2 patients and psychosis in 1 patient. Camptocormia was observed in 1 patient. None of the patients were demented. The response to levodopa therapy was good and sustained even after 23 years of treatment. Three patients were treated successfully by deep brain stimulation (DBS) in the subthalamic nucleus. The parkinsonian symptoms of one of these patients still responded well after 7 years of DBS. However, he had severe axial symptoms, such as swallowing difficulties, postural instability, and gait disturbances, as well as a behavioral disorder associated...
with visual hallucinations. All unaffected parents were much older (mean±SD age, 75.5±10.3 years [age range, 60-94 years]) than their affected child at the time they were examined or died.

**COMMENT**

Eight patients (2.5%) with mutations in exon 41 of LRRK2 were identified by dHPLC and sequencing in a series of 320 apparently sporadic PD cases. The G2019S mutation, which was most commonly reported, was detected in 6 patients (1.9%). The LRRK2 G2019S mutation was initially observed in 1% of the cases of sporadic PD from England. Subsequent studies have shown that the frequency of this mutation in patients with sporadic PD varies greatly according to the population studied, ranging from less than 0.1% in Asians to 41% in North Africans. It is also high in Ashkenazi Jews, among whom this mutation was reported to be 13.3% in patients with sporadic PD. In the European population, its frequency is not significantly greater in familial cases (5/174 [2.9%]) than in sporadic cases (6/320 [1.9%]). The genotypes of markers flanking the G2019S mutation were compatible with the common haplotype identified in European and North African carriers, which results from a founder effect. Therefore, de novo G2019S mutations that would account for the absence of affected parents are unlikely.

The 2 novel mutations, Y2006H and T2031S, may also be pathogenic because they were not found on 348 control chromosomes and affected residues in the kinase domain of the Lrrk2 protein that are conserved among species. However, we were not able to analyze the parents. Therefore, it is possible that these 2 novel LRRK2 variants are de novo mutations.

Together with the published I2020T mutation shared by 3 kindreds, including the Sagamihara (Japan) family that has allowed mapping of this locus to chromosome 13q21-q22, these 2 novel mutations extend the phenotypic spectrum of LRRK2 mutations to include additional phenotypic features like dementia, multiple cranial nerve palsies, and prominent autonomic symptoms in some cases.
12, 13 four missense mutations have been found in exon 41 of LRRK2. Recent in vitro overexpression studies16,17 have shown that the Lrrk2 protein was capable of autophosphorylation and that mutants, including the G2019S mutation, increased Lrrk2 autophosphorylation, hinting at a dominant gain of function mechanism. Therefore, it will be interesting to determine the effect of the 2 newly identified mutations on the protein.

The phenotype of LRRK2 mutation carriers closely resembles that of typical PD except for the age at onset, which is earlier than previously reported.3 The G2019S carrier identified to date, to our knowledge. The Mini-Mental State Examination scores were normal in all patients, indicating the absence of dementia even after a disease duration of more than 20 years in 3 patients. Improvement with levodopa therapy and DBS was demonstrated in all patients, indicating the absence of dementia even after a disease duration of more than 20 years in 3 patients. Improvement with levodopa therapy and DBS was shown that the Lrrk2 protein was capable of autophosphorylation, hinting at a dominant gain of function mechanism. Therefore, it will be interesting to determine the effect of the 2 newly identified mutations on the protein. The absence of family histories for these patients cannot be explained by a censor effect because the unaffected parents were more than 28 years older than the age at onset of their affected offspring when the parents were either interviewed or died. Larger series of patients must be studied to differentiate between age-dependent penetrance and true reduced penetrance of the G2019S mutation.

This study confirms that patients with apparently sporadic PD have LRRK2 G2019S mutations with the same frequency as patients with familial PD. We report 2 novel mutations in exon 41 affecting the nonreceptor tyrosine kinase–like domain of the Lrrk2 protein and possibly its kinase activity. Patients who have typical PD should benefit from DBS.
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References