Clinical Heterogeneity of the LRRK2 G2019S Mutation

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Background: Several pathogenic mutations have been reported in the leucine-rich repeat kinase 2 gene (LRRK2) that cause parkinsonism. The “common” LRRK2 G2019S kinase domain substitution has been reported to account for approximately 5% of familial and 1% of sporadic Parkinson disease.

Objective: To observe the clinical heterogeneity presented by LRRK2 kinase mutation carriers.

Design, Setting, and Participants: We screened 130 patients with pathologically confirmed Parkinson disease and 85 controls for 3 LRRK2 kinase domain pathogenic substitutions: I2012T, G2019S, and I2020T.

Main Outcome Measures: Detailed clinical phenotypes for individuals who screened positive for LRRK2 mutations.

Results: Five LRRK2 G2019S carriers were identified, of whom 4 had Parkinson disease (clinically and pathologically confirmed), and the fifth was a control subject who died at age 68 years after an acute myocardial infarction with no evidence of neurodegenerative abnormalities. There was no evidence of the I2012T or I2020T mutation in these participants.

Conclusions: The underlying disease mechanisms of LRRK2 G2019S–associated parkinsonism are similar to those of typical Parkinson disease. The identification of a control subject raises important questions concerning genetic diagnosis and counseling.

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ATHOGENIC MUTATIONS IN the leucine-rich repeat kinase 2 gene (LRRK2) have been identified in PARK8-linked autosomal-dominant parkinsonism (PARK8, Online Mendelian Inheritance in Man [OMIM] No. 609007).1-3 Parkinsonism (OMIM No. 168600) is a clinical syndrome characterized by bradykinesia, resting tremor, muscle rigidity, and postural instability.4 Parkinson disease (PD) is the most common cause of parkinsonism and is the second most prevalent neurodegenerative disorder after Alzheimer disease. The pathognomonic features of PD are a profound deficiency of dopamine neurons in the basal ganglia, degeneration of the substantia nigra pars compacta, and the presence of Lewy bodies and Lewy neurites.5 Parkinson disease was considered an environmental disorder; however, the subsequent identification of rare mutations in 7 genes that cause familial forms of parkinsonism has revealed a clinical, pathologic, and genetically heterogeneous syndrome.6 The most frequent mutation observed to date is an LRRK2 6055G→A transition, resulting in a glycine-to-serine substitution at amino acid position 2019 (G2019S).3 This common variation has been observed in patients with familial (approximately 5%) and apparent sporadic (approximately 1%) PD, whereas it has been generally absent in the control populations screened to date.3,6-8 The G2019S mutation lies in the highly conserved activation segment of the LRRK2 mitogen-activated protein kinase kinase kinase (MAPKKK) domain and may therefore affect LRRK2 kinase activity. Interestingly, G2019S is located adjacent to I2020T, a substitution identified in a German family with typical late-onset PD and in the original PARK8-linked Sagamihara kindred.9 A further pathogenic substitution at position I2012T highlights the functional importance of this region in parkinsonism.10

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We screened the University of Miami/National Parkinson Foundation Brain Endowment Bank for patients with a clinical and pathologic diagnosis of PD for the kinase domain LRRK2 substitutions I2012T, G2019S, and I2020T. A detailed description of the recruitment procedures, clinical data collection, diagnostic criteria for PD and other neuropsychiatric complications, tissue handling, and neuropathologic evaluations of the Brain Endowment Bank cases (n=130) is provided elsewhere. Information regarding a history of parkinsonism or another neurologic disorder in a first-degree relative was obtained from the Brain Endowment Bank’s database. We also screened 85 individuals with no clinical or pathologic evidence of neurologic disease. Samples of DNA were obtained from frozen brain tissue for all the participants using standard protocols and were genotyped for the exon 41 LRRK2 6053T→C (I2012T), 6059G→A (G2019S), and 6097T→C (I2020T) mutations using a “by-design” probe on a sequence detection system (ABI PRISM 7900HT; Applied Biosystems Inc, Foster City, Calif), the analysis performed using SDS 2.2.2 sequence detection software (Applied Biosystems Inc). Polymerase chain reaction amplification and genotyping were performed following the manufacturer’s protocol (Applied Biosystems Inc). Positive and negative controls were included on all assay plates. Positive or ambiguous results were confirmed or resolved with direct sequencing using sense and antisense primers on a genetic analyzer (ABI PRISM 3100; Applied Biosystems Inc). No individuals harboring the I2012T or I2020T substitutions were identified. This study was approved by the institutional review boards at Mayo Clinic and the University of Miami.

Herein we present the detailed clinical phenotypes of 4 patients with PD and 1 control individual who had positive test results for the G2019S mutation. These individuals were unrelated, originated from the northeastern United States, and carried the mutation on the same haplotype as previously reported for G2019S; the 4 patients with PD had a pathologic diagnosis of Lewy body disease.

REPORT OF CASES

CASE 1

This right-handed white woman was diagnosed as having PD at age 50 years after developing typical left-hand pill-rolling rest tremor and stiffness. She reported an extensive family history of movement disorders: her sister was diagnosed as having early-onset PD (exact age unknown), and her maternal grandmother and uncle were diagnosed as having familial benign “tremors” reminiscent of essential tremor. The patient experienced significant benefit from treatment with levodopa and a variety of dopamine agonists (bromocriptine mesylate, pergolide mesylate, and pramipexole dihydrochloride) and progressed slowly during the first 10 years of disease. During the next year (approximately 11 years after disease diagnosis), she experienced significant deterioration of motor functions, mainly limb tremors and rigidity. At the same time, severe levodopa-induced motor adverse effects were observed, initially with wearing-off and later with disabling on-off variations and peak-dose dyskinesias. The patient was never diagnosed as having dementia or major depression. She remained wheelchair bound for the last few years of her life and died at age 65 years of Hoehn and Yahr stage V PD. The pathologic diagnosis was brainstem Lewy body disease.

CASE 2

This right-handed white man with no family history of neurodegenerative disease was diagnosed as having PD at age 79 years. He had a 6-year history of progressive bilateral gait disorder due to lower limb stiffness. He also developed shuffling gait and imbalance with 2 severe falls and complained of an occasional mild bilateral, mainly kinetic hand tremor, and handwriting difficulties in the form of micrographia. There was also loss of facial expression, hypophonia, and moderate bradykinesia. Initial treatment with low doses of levodopa (300 mg/d) was unsuccessful, however, the patient responded to higher doses of levodopa (up to 800 mg/d) and selegiline hydrochloride (10 mg/d). He started experiencing symptoms of depression, which were successfully controlled with venlafaxine hydrochloride. Although the caregiver reported some mild memory loss, this was not sufficient for a formal diagnosis of dementia. The patient died, 3 years after the diagnosis of PD, of an acute myocardial infarction at age 82 years (Hoehn and Yahr stage III). The pathologic diagnosis was brainstem Lewy body disease.

CASE 3

This right-handed white woman with no documented family history of neurodegenerative disease was diagnosed as having PD at age 77 years after gradually developing typical right-hand pill-rolling rest tremor of moderate severity in 1 year. The tremor remained unilateral, involving the right limbs (upper and, 6 months later, lower), for 2 years and was well controlled using a regimen of levodopa (up to 600 mg/d), a catechol-O-methyltransferase inhibitor (entacapone), and a dopamine agonist (pramipexole dihydrochloride, 3 mg/d). Two years after disease diagnosis, she was diagnosed as having depression, which was adequately controlled with amitriptyline hydrochloride treatment. Although the tremor improved, the patient started noticing ambulation difficulties, with shuffling gait (approximately 4 years after disease diagnosis). After falling and fracturing her right wrist, the patient started complaining of short-term memory deficits significantly interfering with her activities of daily living; however, no formal diagnosis of dementia was documented in her medical records. The patient’s ambulatory difficulties increased, further limiting her activities of daily living. She died of end-stage chronic obstructive pulmonary disease at age 86 years (Hoehn and Yahr stage III-IV) without developing any severe cognitive deficit or levodopa-induced motor adverse effects. The pathologic diagnosis was transitional Lewy body disease.

CASE 4

This right-handed white woman with no documented family history of neurodegenerative disease was diagnosed as having PD at age 45 years after developing typical intermittent right-hand pill-rolling rest tremor at age 41 years. Shortly thereafter, she started experiencing bilateral slow-
ness and mild gait difficulties, with foot dragging. Her symptoms remained stable, mild, and responsive to medication for several years. The patient received moderate doses of levodopa (700–800 mg/d) together with a dopamine agonist, anticholinergics, and amantadine hydrochloride. Dosage adjustments were frequently required because she started to experience wearing-off phenomena and peak-dose dyskinesias. Her disease remained benign and did not limit her activities of daily living for more than 10 years after diagnosis. Subsequently, she started experiencing more severe dyskinesias, off-off phenomena, and motor (freezing, postural instability, gait limitations, and swallowing difficulties) and nonmotor deterioration. Approximately 25 years after disease diagnosis, the patient had depression treated with a variety of antidepressants, visual and auditory hallucinations, and orthostatic hypotension. For the last few years of her life, the patient remained wheelchair bound and had substantial cognitive impairment, fulfilling DSM-IV criteria for dementia. She died at age 72 years with Hoehn and Yahr stage V PD. The pathologic diagnosis was brainstem Lewy body disease and secondary vascular dementia.

CONTROL CASE

This right-handed white man with no family or personal history of neurologic or neurodegenerative disease died at age 68 years after an acute myocardial infarction. No clinically evident extrapyramidal syndrome was reported in the medical records of the patient. No disease-specific neurodegenerative abnormalities were observed.

The 5 individuals described in this article demonstrate the heterogeneity of disease that exists among LRRK2 G2019S carriers (Table). Several interesting features are noteworthy. All 4 patients with PD responded to levodopa therapies, with subsequent development of levodopa-induced motor complications. Three patients with sporadic disease displayed signs of depression, which is a common symptom in patients with idiopathic PD.13 These findings suggest that LRRK2 G2019S–associated parkinsonism, using the present criteria, may not be distinguishable from sporadic PD.13,14

Age at onset varied from 41 years (early onset, patient 4) to 79 years (late onset, patient 2). The familial case presented at 50 years of age, and although all 5 cases seem to be unrelated, they share the same common haplotype, indicative of an ancient common founder for G2019S carriers.12,15 Patient 3, with late-onset PD, is reported to have developed mild cognitive impairment and had a pathologic diagnosis of transitional Lewy body disease. Although the presence of cortical Lewy bodies may contribute to cognitive decline, cortical lesions have been reported in patients with PD without obvious cognitive impairment.19 In contrast, patient 4 was reported to have developed substantial cognitive impairment, although no cortical Lewy bodies were observed. However, cognitive decline and dementia have not been common features of G2019S parkinsonism, and in this case, the cognitive problems may be related to the presence of concurrent vascular pathologic abnormalities.14,17

Variability in age at onset, the extent of Lewy body pathologic features, and the reduced penetrance strongly suggest that other factors, genetic and environmental, may affect the phenotypic presentation of LRRK2-associated disease. LRRK2 G2019S–associated parkinsonism seems closest to a clinical phenotype of idiopathic PD with associated Lewy body pathologic features. Families A and D were the first kindreds identified with LRRK2 mutations, Y1699C and R1441C, respectively; have been most extensively characterized, clinically and pathologically; and have notable differences.12 Affected individuals in family A present with parkinsonism, but dystonia, dementia, amyotrophy, and postural tremor are often featured, whereas family D presents with clinical PD. Brain autopsy of affected individuals reveals pleomorphic abnormalities in families, including only nigral neuronal loss, brainstem and more widespread cortical Lewy body disease, mild medial temporal neurofibrillary tangle abnormalities, and mild amyloid angiopathy.

It is important to note that the control with an LRRK2 G2019S mutation died at age 68 years with no diagnosis of PD. He did not have any evidence of neurodegenerative abnormalities. The penetrance of disease in LRRK2 G2019S carriers has been previously estimated, but it is evident that some carriers may not display parkinsonism in their lifetime, highlighted by the report of an asymptomatic octogenarian individual.18 Further epidemiologic characteristics of LRRK2-associated disease remain

<table>
<thead>
<tr>
<th>Patient No./Sex/Type of PD</th>
<th>Age at Onset of PD, y</th>
<th>Age at Death, y</th>
<th>Duration of PD, y</th>
<th>Clinical Diagnosis</th>
<th>Hoehn &amp; Yahr Stage</th>
<th>Pathologic Diagnosis</th>
<th>Secondary Pathologic Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/familial</td>
<td>50</td>
<td>65</td>
<td>15</td>
<td>PD</td>
<td>V</td>
<td>LBD, brainstem</td>
<td>None observed</td>
</tr>
<tr>
<td>2/M/sporadic</td>
<td>79</td>
<td>82</td>
<td>3</td>
<td>PD</td>
<td>III</td>
<td>LBD, brainstem</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>3/F/sporadic</td>
<td>77</td>
<td>86</td>
<td>9</td>
<td>PD with mild cognitive impairment</td>
<td>III-IV</td>
<td>LBD, transitional</td>
<td>Pulmonary disease</td>
</tr>
<tr>
<td>4/F/sporadic</td>
<td>41</td>
<td>72</td>
<td>31</td>
<td>PD with orthostatic hypotension, dementia</td>
<td>V</td>
<td>LBD, brainstem</td>
<td>Vascular dementia</td>
</tr>
<tr>
<td>5/M/control</td>
<td>68</td>
<td>68</td>
<td>NA</td>
<td>Healthy</td>
<td>NA</td>
<td>No pathologic abnormalities</td>
<td>Myocardial infarction</td>
</tr>
</tbody>
</table>

**Table.** Clinical Findings and Pathologic Diagnoses of the 5 Individuals With the LRRK2 G2019S Mutation

Abbreviations: LBD, Lewy body disease; NA, not applicable; PD, Parkinson disease.
important to assess to provide carriers and their families with accurate genetic counseling.

The frequency of LRRK2 6055G→A (G2019S) mutation carriers presents a unique opportunity for detailed studies of PD biomarkers in presymptomatic individuals, including clinical tests of olfaction, depression, and sleep as well as metabolomic and proteomic studies. The common haplotype of carriers also provides a collaborative opportunity to identify disease modifiers through pedigree-based linkage analysis. On the basis of this background, epidemiologic and environmental exposures may be more meaningfully explored, including sex differences, occupation, associations with smoking, coffee and alcohol, and pesticide exposure. Cellular and animal models are being created that will examine these factors and that may recapitulate the clinicopathologic features observed in many patients with PD and LRRK2 MAPKKK mutations.

The LRRK2 I2012T, G2019S, and I2020T substitutions are adjacent to or within a putative kinase activation loop and are in close proximity to divalent cation-binding sites. In addition, all substitutions create potential protein phosphorylation sites. These variants could result in loss or gain of kinase function, may be targeted for LRRK2 autophosphorylation, and may affect LRRK2 folding or substrate specificity. Interestingly, α-synuclein phosphorylation at serine residue 129 has recently been implicated in the protein’s propensity to aggregate or cause dopaminergic neuronal loss (Figure). Progressive degeneration of human mesencephalic neuron-derived cells, triggered by dopamine-dependent oxidative stress, has been shown to depend on the mixed-lineage kinase pathway. If, as recently demonstrated in vitro, LRRK2 G2019S and I2020T substitutions increase kinase activity and downstream secondary messenger signaling, which promotes Lewy body abnormalities in vivo, it may be important to reassess clinical trials of mixed-lineage kinase inhibitors as neuroprotectants in parkinsonism. Such compounds may be of symptomatic benefit and may even be sufficient to prevent disease in asymptomatic carriers.

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Figure. LRRK2 G2019S, α-synuclein, and Lewy body pathologic features. A, The hinge and the activation loop of the LRRK2 mitogen-activated protein kinase kinase kinase (MAPKKK) domain are shown in magenta, and the G2019S and adjacent I2020T substitutions are in yellow. The catalytic site is highlighted in green, and the adenosine triphosphate binding, in blue. The homologic model for the kinase domain (residues 1880-2138) was predicted using the Swiss-Model Repository (http://www.expasy.ch/) with a large family of kinase structures as templates. B, A model for human α-synuclein protein (residues 1-140) highlighting the N-terminal amphipathic helix and the C-terminal acidic tail. Models were generated from the primary sequence in aqueous solution using the program 3D-PSSM. C, Hematoxylin-eosin stain of an intracellular Lewy body in the locus ceruleus.
References


