Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene cause some forms of autosomal dominant Parkinson’s disease. We measured the frequency of a novel mutation (Gly2019Ser) in familial Parkinson’s disease by screening genomic DNA of patients and controls. Of 767 affected individuals from 358 multiplex families, 35 (5%) individuals were either heterozygous (34) or homozygous (one) for the mutation, and had typical clinical findings of idiopathic Parkinson’s disease. Thus, our results suggest that a single LRRK2 mutation causes Parkinson’s disease in 5% of individuals with familial disease. Screening for this mutation should be a component of genetic testing for Parkinson’s disease.

Parkinson’s disease is the second most common neurodegenerative disorder, affecting more than 1% of individuals aged 55 years and 3% of those aged over 75 years. It is characterised by resting tremor, bradykinesia, muscular rigidity, postural instability, and a clinically significant response to treatment with levodopa. Mutations in α-synuclein, parkin, PINK1, and DJ1 have already been identified in families segregating Parkinson’s disease. Genetic analyses have detected linkage to several other chromosomal regions, although the genes have not yet been identified. Autosomal dominant families showing linkage to chromosome 12p11.2-q13.1 (PARK8) have been identified with mutations in the leucine-rich repeat kinase 2 (LRRK2) gene that codes for the protein dardarin. To ascertain the frequency of a Gly2019Ser mutation of dardarin that was previously identified in two families, we screened patients with familial Parkinson’s disease.

As part of our ongoing study to identify susceptibility genes of Parkinson’s disease (PROGENI), we recruited 358 multiplex families with at least one pair of living siblings diagnosed with the disease. Only those members of multiplex families reported to be affected with the disease were recruited for the study, with the exception of a few parents, who were recruited when available irrespective of their disease status.

Movement disorder specialists at 59 participating sites for the Parkinson Study Group located throughout North America assessed 767 patients. Mean age at onset of these patients was 60–6 years (SD 13–12; median 63, range 18–87) whereas mean age at time of study participation was 70–5 years (9–85 72, 25–93). The patients were mainly white (720 individuals, 94%), although Hispanic people (35, 5%) also participated. After written informed consent approved by every institution’s independent review board was obtained, a standardised clinical assessment (unified Parkinson’s disease rating scale; UPDRS) and a diagnostic checklist were completed and peripheral blood taken from all study participants. We used responses on the diagnostic checklist to classify individuals as having verified or non-verified Parkinson’s disease.

To verify the role of the Gly2019Ser mutation in the susceptibility of Parkinson’s disease, we screened for this mutation in two different control samples. Individuals included in this study consisted of 262 healthy men from 177 unique, white, twin families (85 dizygotic twins, 84 monozygotic twins, eight singlets), ascertained as part of an ongoing study to identify genes contributing to healthy aging. These men were from the National Academy of Sciences–National Research Council (NAS–NRC) twin panel of World War II veterans born between 1917 and 1927. They completed health screens in 1998 and, at that time, with a median age of 74 years (range 71–82), had not been diagnosed with Parkinson’s disease. Another control sample included 965 North American white individuals. All were examined by a neurologist but did not show any signs of the disease. At assessment, mean age of control individuals was 66·7 years (SD 15·0; median 70·7, range 42–93).

30 ng of genomic DNA from every patient with Parkinson’s disease and twin controls were genotyped for a Gly2019Ser mutation in LRRK2 in a 25 μL reaction by use of a TaqMan single-nucleotide-polymerase assay (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 7000 sequence detection system (Applied Biosystems). The panel of 965 controls was genotyped by either direct sequencing (188) or restriction endonuclease digestion with Sfc I and agarose gel electrophoresis (777). We did a statistical comparison of affected patients with and without the Gly2019Ser mutation using either linear or logistic regression for basic characteristics, Blessed functional activity scale, Schwab and England activities of daily living score, Hoehn and Yahr score, and clinical findings.

Of patients with Parkinson’s disease who inherited at least one copy of the Gly2019Ser mutant allele, 34 were heterozygous and one homozygous for this mutation (table). Thus, in our large sample of 767 affected patients, 5% carried the same LRRK2 mutation (95% CI 3·1%–6·1%). Of 358 families, we identified a LRRK2 mutation in at least one member of 20 families (6%, 3·2%–8·0%). No other single mutation identified so far in this or any other gene associated with Parkinson’s disease.

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There are proportions for equivalent numbers might not be the same. 0 = asymptomatic, 5 = wheelchair dependent. The denominator is not the same for all phenotypes because of missing data, *Score range 0–29; 0 = independent, 29 = dependent. † Scored from 0–100; 0 = vegetative, 100 = independent. ‡ Score range 0–5.

<table>
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<tbody>
<tr>
<td>Number of men (%)</td>
<td>20 (57%)</td>
<td>460 (60%)</td>
<td>0.727</td>
</tr>
<tr>
<td>Number with an affected parent (%)</td>
<td>13 (37%)</td>
<td>197 (37%)</td>
<td>0.188</td>
</tr>
<tr>
<td>Age of onset (years, SD)</td>
<td>61.1 (13.9)</td>
<td>66.5 (13.1)</td>
<td>0.824</td>
</tr>
<tr>
<td>Age at exam (years, SD)</td>
<td>75.1 (8.3)</td>
<td>70.3 (9.9)</td>
<td>0.005</td>
</tr>
<tr>
<td>Disease duration (years, SD)</td>
<td>14.0 (12.2)</td>
<td>9.8 (8.4)</td>
<td>0.005</td>
</tr>
<tr>
<td>Blessed functional activity scale†</td>
<td>3.7 (4.0)</td>
<td>4.4 (4.9)</td>
<td>0.300</td>
</tr>
<tr>
<td>Schwab and England activities of daily living scale†</td>
<td>74 (12.7)</td>
<td>75 (22.8)</td>
<td>0.351</td>
</tr>
<tr>
<td>Hoehn and Yahr score‡</td>
<td>2.6 (1.2)</td>
<td>2.6 (1.0)</td>
<td>0.979</td>
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</tbody>
</table>

*Score range 0–29; 0 = independent, 29 = dependent. † Scored from 0–100; 0 = vegetative, 100 = independent. ‡ Score range 0–5.

Table: Comparison of Parkinson’s disease patients with or without the G2019S mutation

14 were heterozygous for the mutation. For five, only one member of the affected sibling pair was identified as a mutation carrier whereas the other was homozygous for the normal allele. No obvious clinical differences distinguished the siblings for the mutation, which is consistent with the complex genetic cause of Parkinson’s disease whereby siblings might inherit different combinations of susceptibility alleles. Despite the apparent autosomal dominant effect of the Gly2019Ser mutation, only 13 (37%) of the families with a mutation reported an affected parent. We postulate that this proportion could be due to the effects of censoring, no diagnosis, or reduced penetrance. Because our sample consisted almost exclusively of individuals affected with Parkinson’s disease, we could not directly estimate the penetrance of the Gly2019Ser mutation using data from unaffected siblings who might have inherited the mutation. However, we speculate that this mutation is probably not fully penetrant since only a third of families reported an affected parent.

In the 20th family, one member of the sibling pair was a heterozygous mutation carrier whereas the other sibling was homozygous for the mutant allele. This individual, the only one to carry two copies of the mutant allele, did not differ in clinical presentation from the other mutation carriers, and did not have early disease onset or more rapid disease progression.

Screening for the Gly2019Ser LRRK2 mutation will probably become a key component of genetic testing for Parkinson’s disease in the near future. Importantly, although we have only screened for one mutation, additional LRRK2 mutations have already been identified in this very large, 51-exon gene. Therefore, the actual rate of LRRK2 mutations in our sample, and familial disease in general, is probably substantially higher than 5%. Moreover, in view of the fact that the late onset of this disease can sometimes mask its familial nature, mutations in LRRK2 will probably be identified in apparently sporadic patients. Mutations in this gene could become the most important cause of susceptibility for Parkinson’s disease identified so far.

Contributors
W C Nichols designed and undertook assays for mutation screening, and contributed to the hypothesis and preparation of the manuscript. N Parkrutz undertook statistical analyses comparing the clinical phenotype of those with and without the PARK8 mutation and contributed to the manuscript. D Hernandez undertook genotyping of the 965 controls. C Paisán-Ruiz and S Jain independently identified the Gly2019Ser mutation. C Halter maintained the family history database, screened and scheduled patients, and processed blood samples. V Michaels helped in the study design and undertook assays for the mutation. T Reed maintained the database including demographic and clinical information for the twin control samples. A Rudolph maintained clinical and treatment data from the study visits of patients with Parkinsonian disease. C W Shults assisted in developing the assessments of patients and controls. A Singleton provided mutation information and developed the hypothesis. T Foroud participated in the design and coordination of the study, the processing of data, the hypothesis, and preparation of the manuscript. The Parkinson Study Group-PROGENI investigators are a group of movement disorder specialists and their
A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson’s disease


Mutations in the LRRK2 gene have been identified in families with autosomal dominant parkinsonism. We amplified and sequenced the coding region of LRRK2 from genomic DNA by PCR, and identified a heterozygous mutation (Gly2019Ser) present in four of 61 (6·6%) unrelated families with Parkinson’s disease and autosomal dominant inheritance. The families originated from Italy, Portugal, and Brazil, indicating the presence of the mutation in different populations. The associated phenotype was broad, including early and late disease onset. These findings confirm the association of LRRK2 with neurodegeneration, and identify a common mutation associated with dominantly inherited Parkinson’s disease.

Parkinson’s disease is the second most common neurodegenerative disease after Alzheimer’s disease, with a prevalence of more than 1% after the age of 65 years. The condition is defined clinically by resting tremor, bradykinesia, and muscular rigidity, and pathologically by brain dopaminergic neuronal loss, with inclusion formation (Lewy bodies) in surviving neurons. The cause of the disease remains unknown in most cases. About 15–20% of patients have a positive family history of Parkinson’s disease in first-degree relatives, suggesting that genes have a role. However, until recently, causative mutations had been identified only in rare cases of Parkinson’s disease, usually of early-onset, and sometimes with atypical clinical or pathological features.1

Linkage of an autosomal dominant form of parkinsonism (PARK8) to chromosome 12 was shown in a Japanese family,1 and later confirmed in two white families. Recently, mutations in a gene termed LRRK2 (leucine-rich repeat kinase 2) were identified in families with PARK8.2,3 The ranges of clinical and pathological characteristics associated with LRRK2 mutations are broad, and include typical late-onset Parkinson’s disease with Lewy-body pathology, showing that mendelian mutations are associated with the classic form of Parkinson’s disease. In other cases, Lewy bodies are absent, and unusual inclusions or pathological findings usually associated with different neurodegenerative diseases are present.2

The LRRK2 gene encodes a large protein of 2527 amino acids and unknown function. The protein, dardarin,1 belongs to a group within the Ras/GTPase superfamily, termed ROCO, characterised by the presence of two conserved domains named Roc (Ras in complex proteins) and COR (C-terminal of Roc), together with other domains including a leucine-rich repeat region, a WD40 domain, and a tyrosine kinase catalytic domain.3

We recruited a consecutive series of 61 families with Parkinson’s disease and a family history compatible with autosomal dominant inheritance. 51 families were from Italy, nine from Brazil, and one from Portugal. The clinical diagnosis of definite Parkinson’s disease was established according to widely accepted criteria.5

We isolated genomic DNA from peripheral blood from 61 patients with Parkinson’s disease. We directly sequenced in both strands. PCR reactions were performed with forward and reverse primers flanking the coding region of LRRK2. PCR products were directly sequenced in both strands.

References
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done in 25 μL containing 1× Invitrogen PCR buffer, 1-5 mmol/L MgCl2, 0.01% W1 detergent, 25 μmol/L of each dNTP, 0-4 μmol/L forward primer, 0.4 μmol/L reverse primer, 2-5 units of Taq DNA polymerase (Invitrogen Corporation, Carlsbad, CA, USA), and 50 ng genomic DNA. Cycle conditions were: 5 min at 94°C; 30 cycles of 30 s denaturation at 94°C; 30 s annealing; and 90 s extension at 72°C; final extension 5 min at 72°C (primers and annealing temperatures reported in the weblink, http://image.thelancet.com/extras/04let12084webtable.pdf). Direct sequencing of both strands was done with Big Dye Terminator chemistry version 3.1 (Applied Biosystems, Foster City, CA, USA). Fragments were loaded on an ABI3100 automated sequencer and analysed with DNA Sequencing Analysis (version 3.7) and SeqScape (version 2.1) software (Applied Biosystems).

We predicted the consequences of mutations at the protein level according to the LRRK2 cDNA sequence deposited in Genbank (accession number AY792511).
Novel variants that co-segregated with disease were tested in a panel of 250 chromosomes from healthy Italian people aged older than 60 years, by use of allelic specific oligonucleotideisation. For the Gly2019Ser mutation, PCR products containing LRRK2 exon 41 were blotted into Hybond-N+ membranes (Amersham Biosciences, Amersham Biosciences, Buckinghamshire, UK). The blots were hybridised for 1 h at 37°C in 5 × sodium chloride/sodium phosphate/EDTA (SSPE), 1% sodium dodecyl sulphate, and 0.05 g/L single-strand salmon sperm DNA with either the normal or mutated sequence oligonucleotides (wild-type allele: tgactacggcattg; mutant allele: gactacagcattgc). Filters were washed in buffer containing 0.045 mol/L sodium chloride, 0.0045 mol/L sodium phosphate/EDTA (SSPE), 1% sodium dodecyl sulphate, and 0.1% sodium dodecyl sulphate, at 37°C.

By sequencing the whole LRRK2 coding region in the probands from 15 families, we identified two heterozygous carriers of an exon 41 mutation, 6055G→A (numbered from the A of the ATG-translation initiation codon), predicted to replace the glycine at position 2019 of the dardarin protein with serine (Gly2019Ser; electro- codon), predicted to replace the glycine at position 2019 (numbered from the A of the ATG-translation initiation codon), predicted to replace the glycine at position 2019.

The mutation co-segregated with Parkinson’s disease in the families (figure 1A), and was absent in the 250 control chromosomes. In these two probands, we detected several polymorphisms but no further variants that co-segregated with Parkinson’s disease and were absent in control chromosomes.

Direct sequencing of exon 41 in the remaining 46 probands identified another two heterozygous carriers, bringing the prevalence of the Gly2019Ser mutation to four of 61 autosomal dominant families (6-6%, 95% CI 0.4-12.8).

16 individuals in these four families had Parkinson’s disease, but accurate clinical information was available for only ten of them (table). These individuals had a broad range of age of disease onset (table; average 50-5 years, range 38-68, n=10), including two patients with onset before age 40 years. All patients responded well to levodopa. Dementia and additional neurological signs were not present. Asymmetric onset and complications typically associated with long-term treatment with levodopa (motor fluctuations and choreic dyskinesias) were noted in some patients, lending support to the accuracy of the clinical diagnosis of typical Parkinson’s disease. The broad range of ages of onset suggests that factors other than the mutation identified have a role in modifying the disease. Clinical features in patients who carried the Gly2019Ser mutation were similar to those of patients who did not (data not shown).

Several unaffected family members carried the mutation, but were younger than the latest age of onset observed in these families (figure 1A). These individuals are still at risk of developing Parkinson’s disease. This finding indicates an age-dependent (perhaps incomplete) penetrance for this mutation, as reported for other LRRK2 mutations.5 The families carrying the Gly2019Ser allele lived in Italy (two families), Portugal, and Brazil, suggesting that this mutation is present in different populations.

Further evidence for the pathogenic role of the mutation is provided by the observation that the Gly2019 residue is not only conserved among the dardarin protein homologues, but is also part of a motif of three amino acids (AspTyrGly or AspPheGly) that is required by all human kinase proteins (figure 1B and C).

Our data provide independent confirmation that LRRK2 mutations cause human neurodegeneration, and identify a single common mutation associated with autosomal dominant Parkinson’s disease. Precise information about the penetrance of this mutation will be important for clinical practice. Since penetrance is age-dependent, this mutation might be found in patients with negative family history. These findings have implications for the diagnosis and counselling of patients with Parkinson’s disease.

### Table: Clinical features of the ten individuals with Parkinson’s disease in families with the mutation

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Onset age (years)</th>
<th>Duration (years)</th>
<th>UPDRS</th>
<th>Rest tremor</th>
<th>Rigidity</th>
<th>Asymmetric onset</th>
<th>Levodopa response</th>
<th>Motor fluctuations</th>
<th>Dyskinesias</th>
<th>ULC</th>
<th>Dementia</th>
<th>Odyautonomia</th>
<th>Others</th>
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<tr>
<td>1</td>
<td>67</td>
<td>6</td>
<td>23</td>
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<td>D</td>
<td>-</td>
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<td>5, D</td>
</tr>
</tbody>
</table>

UPDRS unified Parkinson’s disease rating scale, motor score under the effect of medication (maximum 108). D=early morning dystonia, NA=not available. Patient codes: 1=LISB-01, 2=LISB-02, 3=LISB-03, 4=LISB-04, 5=LISB-05, 6=RM-548, 7=RM-547, 8=NE-101, 9=ROMA-314, 10=SIO-36.
A common LRRK2 mutation in idiopathic Parkinson’s disease


Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene have been shown to cause autosomal dominant Parkinson’s disease. Few mutations in this gene have been identified. We investigated the frequency of a common heterozygous mutation, 2877510G→A, which produces a glycine to serine aminoacid substitution at codon 2019 (Gly2019Ser), in idiopathic Parkinson’s disease. We assessed 482 patients with the disorder, of whom 263 had pathologically confirmed disease, by direct sequencing for mutations in exon 41 of LRRK2. The mutation was present in eight (1.6%) patients. We have shown that a common single Mendelian mutation is implicated in sporadic Parkinson’s disease. We suggest that testing for this mutation will be important in the management and genetic counselling of patients with Parkinson’s disease.

Although Parkinson’s disease is a common neurodegenerative condition, the disease trait is rarely inherited in a simple Mendelian fashion. However, the study of families with inherited Parkinson’s disease has greatly improved our knowledge of the genetic and molecular basis of this incurable disorder.¹ We have shown that a form of autosomal dominant Parkinson’s disease (PARK8) was caused by mutations in the LRRK2 gene (MIM 609007) in a British family and several Basque families.² We have subsequently identified a common missense mutation in four patients with familial Parkinson’s disease (unpublished data). These individuals harbour a heterozygous 2877510G→A change that causes a Gly2019Ser substitution (GenBank AAV63975) adjacent to a previously reported Iso2020Thr mutation in a highly conserved region of the predicted kinase domain.³

This finding prompted us to investigate the frequency of the Gly2019Ser mutation in idiopathic Parkinson’s disease. We screened 482 patients with sporadic Parkinson’s disease (263 had pathologically confirmed disease) by direct sequencing for mutations in exon 41 of LRRK2. We did not screen this series for any other mutations in LRRK2. All patients and controls were of white ancestry, predominantly from the south-east of England, and were recruited through the National Hospital for Neurology and Neurosurgery. Patients with Parkinson’s disease were diagnosed clinically or pathologically from the Queen Square Brain Bank for Neurological Disease, satisfying rigorous accepted diagnostic criteria.⁴ Of the 345 controls, 102 samples were from unaffected and unrelated relatives of patients with Huntington’s disease, and had been obtained for linkage analysis studies. The remaining 243 samples were from unrelated patients who had been genetically diagnosed with non-parkinsonian disorders (eg, mitochondrial myopathies, inherited neuropathies). The study was approved by the Joint Research Ethics Committee of the Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, UK. Informed written consent was obtained from all patients.

Genomic DNA was extracted from peripheral blood leukocytes or brain cortex tissue by a semi-automated method (Kurabo, Osaka, Japan). PCR products were generated with 50 ng DNA template in 2·5 μL buffer,
2·5 mmol/L each dNTP (Promega, Southampton, UK), 1·5 μL MgCl₂, 50 μmol/L each primer (panel) and 1 unit AmpliTaq Gold (Applied Biosystems, Warrington, UK) in a total volume of 25 μL. PCR conditions were: 94°C for 11 min followed by 30 cycles of 94°C for 30 s, 60°C for 45 s, and 72°C for 1 min, with a final extension step of 72°C for 7 min. 2·5 μL of purified PCR product (Millipore, Watford, UK) was sequenced with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer’s instructions, and resolved on an ABI 3100 automated sequencer (Applied Biosystems). All mutations were confirmed in a second DNA sample. The frequency of mutations in cases versus controls was compared with a 2×2 contingency table; the reported p value was obtained with Fisher’s exact test, since some cell counts were below five.

Of the 482 patients screened we identified eight (1·6%) with heterozygous Gly2019Ser mutations. This mutation was not found in any control samples (two-sided p=0·024). Of the eight mutations described, three were in patients with pathologically confirmed disease. Histopathological examination showed the pathological hallmarks of idiopathic Parkinson’s disease with nigral cell loss, pigment incontinence, and Lewy bodies. Lewy bodies were also present in the limbic cortices in two of these three patients. The third patient showed signs of pathological aging, with neocortical senile plaques of the diffuse type and occasional neurofibrillary tangles.

Two of the patients with mutations had a family history of Parkinson’s disease in one or more first-degree relatives, and one had an affected second-degree relative. The remaining five had no relevant family history. Age of onset was 41–70 years (mean 57·4 years). Symptoms at onset were typical of idiopathic Parkinson’s disease characterised by unilateral bradykinesia and rigidity, with tremor present in some but not all patients (three had tremor, two did not, information not available for three). One individual presented with unilateral foot dystonia. All patients were responsive to levodopa, and had treatment-related dyskinesias.

Despite the progress in understanding the genetic basis of familial Parkinson’s disease—including identification of mutations in α-synuclein, parkin, PINK1, and DJ1—little progress has been made in furthering our knowledge of the genetic factors underlying more common forms of idiopathic sporadic Parkinson’s disease. Until recently, Parkinson’s disease has not been considered as a disease with a pronounced genetic contribution. The commonly held view is that major Mendelian genetic factors are only rarely causal.

Our data lend support to the hypothesis that mutations in LRRK2, and particularly the mutation we describe, should be considered, even in cases of Parkinson’s disease without a strong positive family history. The presence of this mutation in patients without an overt family history raises important issues regarding genetic counselling. The initial reports of mutations in mendelian genes are skewed towards description of mutations with the highest penetrance. However, due to the absence of family history in five patients with the Gly2019Ser mutation, the penetrance of this mutation cannot be accurately estimated, suggesting reduced penetrance or a de-novo mutation. These factors should be discussed with patients and their families before proceeding with testing. However, since this mutation was common in our large case series, we believe that it will quickly become important in clinical service. The overall frequency of LRRK2 mutations remains to be established—our results may underestimate the true prevalence.

Contributors
AJ Lees, KP Bhatia, NP Quinn, DG Healy, and NW Wood clinically assessed patients. K Shaw took blood samples and ensured individual consent. Pathological examination and confirmation of post-mortem cases was by T Revesz and J Holton. WP Gilks, P Abou-Sleiman, and S Jain did the molecular analysis and collection of results. Clinical and pathological review of patients with the mutation was by J Lynch and S Gandhi. P Abou-Sleiman was responsible for statistical analysis of the data. All authors, including A Singleton and V Bonifati, contributed to the study concept and discussion of results, which was under the overall supervision of NW Wood.

Conflict of interest statement
We declare that we have no conflict of interest.

References