

ELECTRONIC LETTER

The G6055A (G2019S) mutation in *LRRK2* is frequent in both early and late onset Parkinson's disease and originates from a common ancestor

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Background: Mutations in the gene *Leucine-Rich Repeat Kinase 2 (LRRK2)* were recently identified as the cause of PARK8 linked autosomal dominant Parkinson's disease.

Objective: To study recurrent *LRRK2* mutations in a large sample of patients from Italy, including early (<50 years) and late onset familial and sporadic Parkinson's disease.

Results: Among 629 probands, 13 (2.1%) were heterozygous carriers of the G2019S mutation. The mutation frequency was higher among familial (5.1%, 9/177) than among sporadic probands (0.9%, 4/452) ($p < 0.002$), and highest among probands with one affected parent (8.7%, 6/69) ($p < 0.001$). There was no difference in the frequency of the G2019S mutation in probands with early v late onset disease. Among 600 probands, one heterozygous R1441C but no R1441G or Y1699C mutations were detected. None of the four mutations was found in Italian controls. Haplotype analysis in families from five countries suggested that the G2019S mutation originated from a single ancient founder. The G2019S mutation was associated with the classical Parkinson's disease phenotype and a broad range of onset age (34 to 73 years).

Conclusions: G2019S is the most common genetic determinant of Parkinson's disease identified so far. It is especially frequent among cases with familial Parkinson's disease of both early and late onset, but less common among sporadic cases. These findings have important implications for diagnosis and genetic counselling in Parkinson's disease.

Parkinson's disease affects more than 1% of people after the age of 65 years, and is the second most common neurodegenerative disorder after Alzheimer's disease.¹ The disease is defined clinically by the association of bradykinesia, resting tremor, muscular rigidity, and postural instability, and pathologically by loss of dopaminergic neurones in the substantia nigra-pars compacta and other brain sites, with formation of ubiquitin containing inclusions (Lewy bodies) in the surviving neurones.¹

The cause of the disease remains unknown in most patients, but a positive family history of Parkinson's disease is found in ~15–25% of cases, and mutations in five genes have been firmly implicated in the aetiology of rare inherited forms of the disease.^{2–3}

An autosomal dominant form of Parkinson's disease (PARK8) was first mapped to chromosome 12 in a Japanese family⁴; this linkage was later confirmed in white families.^{5–6} Recently, mutations in the gene *Leucine-Rich Repeat*

Kinase 2 (LRRK2) (MIM *609007) were identified in PARK8 linked families.^{7–8} The *LRRK2* gene encodes a predicted protein of 2527 amino acids, which has unknown function. This protein, termed dardarin, belongs to the ROCO group within the Ras/GTPase superfamily, and contains several conserved domains: an Roc (Ras in complex proteins) and a COR (C-terminal of Roc) domain, together with a leucine-rich repeat, a WD40 domain, and a tyrosine kinase catalytic domain.⁹

To date, seven *LRRK2* pathogenic mutations have been reported in autosomal dominant Parkinson's disease. Four of these mutations occurred in at least two unrelated families: Y1699C (present in two large kindreds, family "A" of German-Canadian ancestry, and one British kindred)^{7–8}; R1441C (found in family "D" of Western Nebraska origin, and another family)⁸; R1441G (found in several families and a few sporadic cases in the Basque population)⁷; and G2019S, which we and other groups have recently identified.^{10–14}

Mutations in the *LRRK2* gene, particularly G2019S, appear to be relevant for Parkinson's disease, but the frequency of these mutations according to clinical features of the probands—such as onset age and pattern of presentation (familial or sporadic)—has not been assessed in large consecutive series of probands from homogeneous well defined populations. The frequency of known or novel *LRRK2* mutations might be different in different populations; moreover, the previous studies have targeted mainly late onset Parkinson's disease series. Therefore the frequency of mutations remains unknown among early onset patients.

The penetrance of *LRRK2* mutations appears strongly age related, and is probably incomplete^{4–7–8–10–12–14}; these mutations might therefore also be expected in patients with the sporadic presentation (the vast majority of cases of Parkinson's disease). It is therefore urgent to assess the prevalence and associated phenotype of the G2019S and other *LRRK2* mutations in clinically and ethnically well defined series of familial and sporadic Parkinson's disease cases, including early and late onset patients.

Here, we report the first study of all four so far known recurrent *LRRK2* mutations in a large sample of 629 probands with Parkinson's disease ascertained at a single centre in Italy. We also analyse the haplotypes and the clinical phenotypes associated with the G2019S mutation.

Abbreviations: LD, linkage disequilibrium; SNP, single nucleotide polymorphism

Table 1 Distribution of study sample according to 10 year onset age classes

	Early onset (years)			Late onset (years)			Total
	<30	30–39	40–49	50–59	60–69	≥70	
All cases	7	83	140	224	142	33	629
G2019S heterozygous	–	3	4	4	–	2	13
Familial	1	21	46	65	33	11	177
G2019S heterozygous	–	2	3	3	–	1	9
Sporadic	6	62	94	159	109	22	452
G2019S heterozygous	–	1	1	1	–	1	4

METHODS

Subjects and clinical analyses

We studied 629 probands, representing two consecutive cohorts of Parkinson's disease cases with early onset disease (<50 years old at symptoms onset, n = 230) or late onset disease (≥50 years old at onset, n = 399). The age at which the patient noticed the first symptom was considered to be the age of disease onset. Thirty three relatives affected by Parkinson's disease were also included, giving a total of 662 cases with the disease. All cases were examined and collected at the Parkinson Institute, Istituti Clinici di Perfezionamento, Milan, one of the largest referral centres for diagnosis and treatment of Parkinson's disease in Italy. Most cases were of Italian origin, but one case originated from each of the following countries: Argentina, Colombia, Ethiopia, France, Greece, Iceland, Ireland, Israel, and the United Kingdom.

The mean (SD) age at disease onset was 52.7 (10.9) years in the whole series of 629 probands, and 40.8 (5.6) years and 59.5 (6.6) years in the early onset and late onset groups, respectively. The clinical diagnosis of definite Parkinson's disease was established according to widely accepted criteria,¹⁵ and required the presence of bradykinesia and at least one of the following: resting tremor, rigidity, and postural instability; a positive response to dopaminergic therapy; and the absence of atypical features or other causes of parkinsonism.

Patients were classified as "familial" if at least one relative was reported with a formal diagnosis of Parkinson's disease among the first, second, or third degree relatives. The other probands were classified as "sporadic".

The four mutations were tested—using the same method as for the Parkinson's disease cases—in 440 Italian controls, including 304 elderly individuals free from Parkinson's disease or dementia (spouses of Parkinson's disease cases, outpatients of general practices, and blood donors, average age 66.4 (9.3) years), and 136 inpatients with cerebrovascular disease (average age 64.9 (8.4) years). The whole sample of controls (880 chromosomes) was tested for the G2019S mutation. The remaining mutations were tested in a total of 530 chromosomes. The project was approved from the local ethics authorities, and written informed consent was obtained from all subjects.

Mutation analysis

Genomic DNA was isolated from peripheral blood using standard protocols.¹⁶ The primers and polymerase chain reaction (PCR) protocol used to amplify the *LRKK2* exons (Nos 31, 35, and 41) containing the C4321T (R1441C), C4321G (R1441G), the A5096G (Y1699C), and the G6055A (G2019S) mutation have been reported previously.¹⁰ The consequences of mutations at the protein level were predicted according to the *LRKK2* cDNA sequence (Genbank accession number AY792511).

About 20 ng of pooled PCR product (exons 31, 35, and 41) were purified using ExoSAP-IT (USB) and used in a primer extension reaction (SNaPshot) including the following

primers: for the R1441C and the R1441G mutations in exon 31 (sense strand), 5'-agaatcacagggaagaagaagcgc-3', product size 26 base pairs (bp) (primer length plus one base); for the Y1699C mutation in exon 35 (antisense strand), 5'-taatc-gattgattaatcttgacaaaatcccattggaaa-3', product size 41 bp; for the G2019S mutation in exon 41 (antisense strand), 5'-aatgctgccatcattgcaaagattgctgactac-3', product size 34 bp.

Reactions were carried out in 10 µl containing 1 µl SNaPshot multiplex ready reaction mix (Applied Biosystems, Foster City, California, USA); 2.5 µM R1441C/R1441G, 7.5 µM Y1699C, 2.5 µM G2019S extension primer, and 1 µl ½ term buffer (200 mM TrisHCl; 5 mM MgCl₂, pH 9). Additional thermal cycling was undertaken for 40 cycles of 10 seconds at 95°C, five seconds at 50°C, and 30 seconds at 60°C. Removal of the 5'-phosphoryl groups was done using 1 unit of shrimp alkaline phosphatase (SAP) (Roche Diagnostics, Monza, Italy) for 30 minutes at 37°C.

One microlitre of SNaPshot product was diluted in 10 µl Hi-Di formamide (Applied Biosystems) containing GeneScan-120 LIZ size standard (Applied Biosystems), denatured for five minutes at 95°C, cooled on ice, and loaded on an ABI3100 Genetic Analyzer (Applied Biosystems). Fragments were analysed using GeneMapper V3.0 software (Applied Biosystems).

Negative and positive controls for the G2019S and R1441C mutations were included in all experiments. Positive controls were not available for the R1441G and the Y1699C mutation. All the mutations identified in the SNaPshot screening were confirmed by direct sequencing using a second DNA aliquot.

In one case carrying the G2019S mutation and one control, total RNA was isolated from blood cells and cDNA was prepared using standard protocols. A 251 bp fragment of the *LRKK2* cDNA spanning exons 41–42 was amplified using the following primers: forward 5'-cacgtagctgatggtttgagatacc-3'; reverse 5'-ccaatgaataaacatcagcctgt-3'.

Haplotype analysis

Nineteen intragenic and flanking markers (13 microsatellites and six single nucleotide polymorphisms (SNP)) were typed, including both known exonic and a newly discovered *LRKK2* intronic SNP (IVS13+104G/A) in linkage disequilibrium (LD) with the G2019S mutation. Microsatellites were selected from the Marshfield integrated map and from Kachergus *et al*¹⁴; they were amplified by PCR using fluorescently labelled F-primers according to standard methods; fragments were loaded on an ABI3100 and analysed using the GeneMapper version 3.0 software (Applied Biosystems). Exonic and intronic *LRKK2* SNPs were typed by direct sequencing using the primers and PCR conditions reported previously.¹⁰

The frequency of the IVS13+104G/A SNP was assessed in 100 chromosomes from Italian Parkinson's disease cases and 200 chromosomes of Italian controls.

We included in the haplotype analysis 12 families with the G2019S mutation detected in this series, the four families reported by us previously,¹⁰ and another two unpublished families (IT-023 and TH-08, from Italy and Morocco,

Table 2 Frequency of the G2019S mutation according to familial aggregation

Proband category	n (%)	Male/female	Onset (years) (mean (SD))	Range	G2019S	%
All probands (early and late onset)	629 (100%)	369/260	52.7 (10.9)	23 to 82	13	2.1%
All familial probands (1st, 2nd, 3rd degree affected relatives)	177 (28.1%)	103/74	52.6 (10.9)	23 to 82	9	5.1%*
All sporadic probands	452 (71.9%)	266/186	52.7 (11.0)	23 to 80	4	0.9%
All early onset probands	230 (100%)	149/81	40.8 (5.6)	23 to 49	7	3.0%
Familial early onset	68 (29.6%)	43/25	41.5 (5.5)	23 to 49	5	7.4%†
Sporadic early onset	162 (70.4%)	106/56	40.6 (5.7)	23 to 49	2	1.2%
All late onset probands	399 (100%)	220/179	59.5 (6.6)	50 to 82	6	1.5%
Familial late onset	109 (27.3%)	60/49	59.6 (6.9)	50 to 82	4	3.7%‡
Sporadic late onset	290 (72.7%)	160/130	59.4 (6.5)	50 to 80	2	0.7%
Proband with "dominant" PD (1st or 2nd degree affected relatives)	114	68/46	50.1 (10.7)	23 to 74	8	7.0%§
Proband with one affected parent	69	42/27	51.0 (9.7)	23 to 71	6	8.7%§
Proband with affected 2nd degree relative only	42	24/18	49.2 (11.7)	32 to 74	2	4.8%NS
Proband with affected siblings and 2nd degree relative	3	2/1	41.0 (15.6)	32 to 59	0	—
Proband with affected siblings only	49	27/22	56.9 (10.5)	36 to 82	1	2.0%NS
Proband with affected 3rd degree relative only	14	7/7	58.1 (6.9)	57 to 65	0	—

* $p < 0.002$ v the frequency among the 452 sporadic probands (Fisher exact test).

† $p = 0.025$; frequency in familial v sporadic early onset probands.

‡ $p < 0.001$ v the frequency among the 452 sporadic probands.

§No significant difference compared with the frequency among the 452 sporadic probands.

NS None of the differences between early onset and late onset groups (familial, sporadic, or all) was statistically significant.

PD, Parkinson's disease.

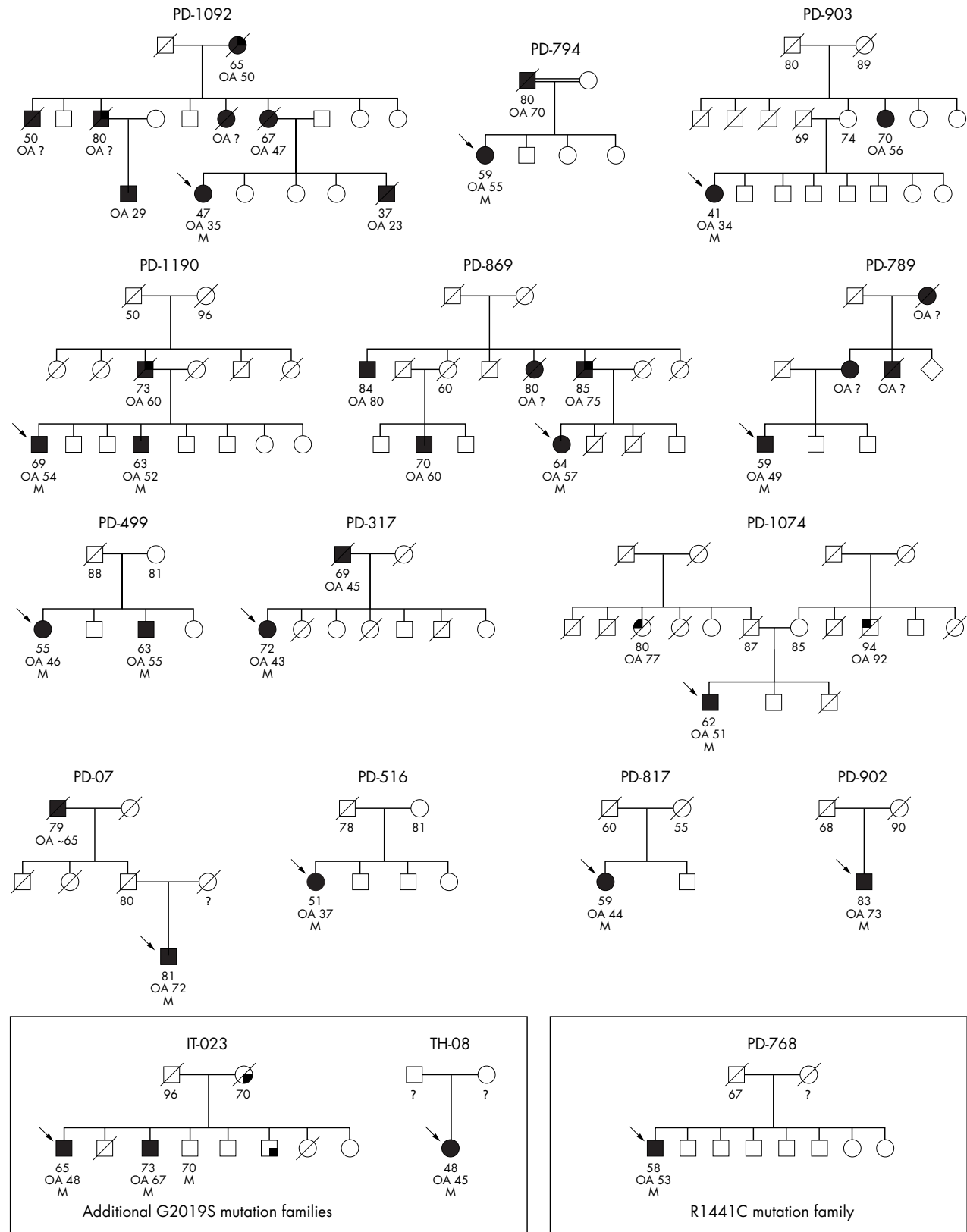


Figure 1 Simplified pedigrees of families with *LRRK2* mutations. Full black symbols: individuals affected by Parkinson's disease; symbols with black upper corner: individuals affected by senile dementia; symbols with black lower corner: individuals with tremor only. To protect confidentiality the order of individuals in sibships was altered. The first number below symbols indicates age at examination or age at death (years). OA, age at disease onset (years). Question mark indicates that information is not available (individuals who lost contacts with their family). M, carrier of heterozygous G2019S mutation. In family PD-768, M indicates the carrier of the R1441C mutation. No further individuals were known to be affected by Parkinson's disease among the more distant relatives, including the families of the sporadic Parkinson probands. Extended versions of these pedigrees are available on request.

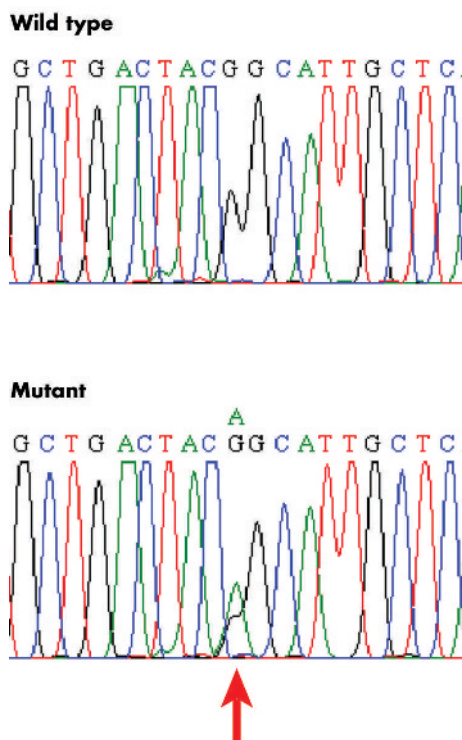


Figure 2 Electropherogram of part of *LRRK2* cDNA sequence from one Parkinson's disease patient and one control. The position of the heterozygous G6055A mutation (G2019S) is indicated (arrow).

respectively) identified by us from unrelated series of patients. Haplotypes were constructed manually. In four families phase could be assigned unambiguously for most markers by genotyping of trios of parents and child. In the remaining families, the phase was estimated using PHASE version 2.1.¹⁷ Haplotypes with known phase were included to improve the performance of the program. Statistical analysis was undertaken using contingency tables and the Student's *t* test, as appropriate.

RESULTS

Frequency of mutations

The G2019S mutation was not detected in 880 control chromosomes, whereas it was identified in heterozygous state in 13 of the 629 probands (overall frequency 2.1%, $p < 0.01$ *v* controls). The distribution of probands according to onset age classes and pattern of familial aggregation is presented in tables 1 and 2.

The carriers of the G2019S mutation included nine of 177 familial probands (5.1%) and four of 452 sporadic probands (0.9%) ($p < 0.002$ familial *v* sporadic). The frequency of the G2019S mutation among the familial Parkinson's disease probands remained five times higher than among the sporadic probands when early onset or late onset groups were considered separately (table 2).

Considering together the familial and the sporadic sample, seven of 230 early onset probands (3.0%), and six of the 399 late onset probands (1.5%) carried the G2019S mutation (table 2). The frequency of carriers among early onset cases remained about twofold higher than among late onset cases when either the whole sample or only familial or sporadic Parkinson's disease was considered; however, the differences between early and late onset groups did not reach statistical significance.

Among 600 probands tested, there was one heterozygous for the R1441C mutation but none carrying the R1441G or

the Y1699C mutation. These mutations were not observed in controls.

The simplified pedigrees are shown in fig 1. These include the families of the 13 probands with the G2019S mutation and one with R1441C mutation identified in this study, and two unpublished families with the G2019S mutation (IT-023 and TH-08), identified from other Parkinson's disease cohorts, that were included in the haplotype study. Thirteen probands with the G2019S mutation were from Italy, one (PD-1092) was from Greece and another (TH-08) from Morocco.

In three families (PD-499, PD-1190, and IT-023), DNA was available from one affected relative; the G2019S mutation was found in heterozygous state in all these three secondary cases. The lack of DNA samples from other affected or unaffected relatives precludes further detailed analyses of co-segregation and penetrance of the mutation. The cDNA analysis from blood cells documented the expression of the mutant G2019S allele (fig 2).

Haplotype analysis

The results of the haplotype analysis are reported in fig 3. An extended shared region was present in the patients from all the families with phase assigned. For all patients with uncertain phase, the genotypes were compatible with the presence of the same haplotype (fig 3), as also predicted by the results of the PHASE program. These findings strongly suggest that the mutant G2019S allele was inherited from a common founder. The minimum size of the shared region is ~160 kb, defined by markers D12S2514 and D12S2518, while the maximum size is defined in our dataset by markers D12S2519 (~80kb from D12S2518) and D12S2080 (~570 kb from D12S2514).

Clinical features

Clinical features were similar in patients who carried the G2019S mutation and those who did not (table 3). Among the 15 cases detected from the consecutive cohort in this study (13 probands and two affected relatives) the first symptom at onset was rest tremor in five cases, bradykinesia in nine, and rigidity in one. Body distribution of signs and symptoms at onset was asymmetrical in all but one case. Bradykinesia and rigidity were present in all 15 cases on examination, while in nine cases rest tremor was documented at some time during the disease course. Decreased postural reflexes were documented in 11 cases. Response to levodopa was good in all. Motor fluctuations were observed in 13 cases, and levodopa induced dyskinesias in 12 of these. Two cases showed dystonic features. Freezing of gait was noted in 12. Severe autonomic dysfunction was not observed. Psychiatric disturbances were common: four cases had psychotic phenomena (hallucinations, delusions); two had depression years after the onset of motor symptoms, another three cases had depression at the time of onset, and in one case depression occurred seven years before the onset of motor symptoms.

Dementia was present in only one case. Sleep disturbances were also common, present in nine cases. In one case, amelioration of symptoms after sleep was noted (sleep benefit). Three cases were treated with deep brain stimulation, and one with thalamolysis.

In the patient carrying the R1441C mutation, Parkinson's disease started with asymmetrical rest tremor, later followed by bradykinesia, rigidity, and postural instability. Freezing of gait, levodopa induced motor fluctuations, and dyskinesias also developed. Depression occurred three years before the onset of motor symptoms.

Marker / SNP	Chromosome Position	Italy		Brazil		Portugal		Italy		Italy		Italy		Italy		Morocco		Greece		Italy		Italy	
		Roma 314	Ne 101	PDBR24-4	PDBR24-4	LIBS08	PD449	PD794	PD803	IT23RM535	PD1190	PD817	PD902	PD317	PD869	PD1092	TH8	PD516	PD7	PD1074			
D12S1621	30832 K	190	190	[186]	190	190	190	190	190	190	186	190	192	192	190	?	192	190	?	190	190	190	
D12S2060	33306 K	179	179	[183]	179	179	179	179	179	179	179	191	183	183	179	179	183	183	183	183	183	183	
D12S2514	38874 K	[288]	[288]	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	288	
rs732374	38929 K	[t]	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	
IVS13+104_gB	38939 K	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	
D12S2515	38974 K	222	226	222	222	222	222	222	222	222	222	222	222	222	222	222	222	222	222	222	222	222	
D12S2516	38988 K	251	[251]	251	251	251	251	251	251	251	251	251	251	251	251	251	251	251	251	251	251	251	
D12S2522	39000 K	c	c	[c]	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	
rs1896252	39000 K	a	[a]	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	
rs1427262	39000 K	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	[g]	
rs11176013	39000 K	a	[a]	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	
rs11564148	39000 K	a	[a]	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	
Gly2019Ser	39020 K	a	[a]	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	
D12S2518	39034 K	[152]	[152]	[152]	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	152	
D12S2519	39116 K	131	131	[131]	131	131	131	131	131	131	131	131	131	131	131	131	131	131	131	131	131	131	
D12S2520	39120 K	257	257	[257]	257	257	257	257	257	257	257	257	257	257	257	257	257	257	257	257	257	257	
D12S2521	39128 K	356	356	356	356	356	356	356	356	356	356	356	356	356	356	356	356	356	356	356	356	356	
D12S2522	39132 K	295	295	[295]	295	295	295	295	295	295	295	295	295	295	295	295	295	295	295	295	295	295	
D12S2523	39147 K	317	317	[317]	317	317	317	317	317	317	317	317	317	317	317	317	317	317	317	317	317	317	
D12S1048	39310 K	[210]	210	210	210	210	210	210	210	210	210	210	210	210	210	210	210	210	210	210	210	210	
D12S1668	39490 K	241	241	241	241	241	241	241	241	241	241	241	241	241	241	241	241	241	241	241	241	241	



Figure 3 Haplotypes of the *LRRK2* locus in 18 cases carrying the G2019S mutation. The minimum ~160 kb of DNA shared by all patients is in blue. The G2019S mutation and the IVS13+104G/A SNP, in linkage disequilibrium (LD) with the mutation among Parkinson's disease cases, are reported in red. Variations in 4 bp observed at tetranucleotide marker D12S2515 are probably the consequence of mutations occurring in this microsatellite, thereby defining subclasses within the ancestral haplotype. Both alleles are shown for this marker, in individuals who are not carrying the consensus allele (222 bp) in LD with G2019S. The marker D12S2518 contributed to the haplotype build but was not polymorphic in our entire dataset. For some other markers, genotypes with phase unknown or not informative are indicated between square brackets.

DISCUSSION
Frequency of *LRRK2* mutations in Parkinson's disease

This is the first comprehensive study of *LRRK2* recurrent mutations targeting large groups of Italian cases with early onset and late onset Parkinson's disease, with familial as well as sporadic presentation. We found a frequent occurrence of the G2019S mutation. On the other hand, the R1441C, R1441G, and Y1699C mutation were rare, suggesting they are not a relevant cause of Parkinson's disease in the Italian population. In addition to Italy, Portugal, and Brazil, and the countries reported by others,¹⁰⁻¹⁴ we expand the presence of the G2019S mutation to Parkinson's disease cases from Greece and Morocco.

In the initial studies, the G2019S mutation was found in ~3-6% of selected samples with familial Parkinson's disease (autosomal dominant families, and sibling pairs) from several European and North American countries, and in ~1% of sporadic Parkinson's disease cases from the United Kingdom, while it was absent in more than 4000 control individuals.¹⁰⁻¹⁴ However, the frequency may vary considerably between populations—recent studies suggest a very high prevalence in North African and a very low prevalence in Asian populations.^{18 19}

The pathogenic role of the G2019S mutation is further supported by the observation that the G2019 residue is extremely conserved in human kinase domains and in all dardarin homologues.²⁰

Here we report the frequency of G2019S in a large sample of clinically and ethnically well defined patients, showing that G2019S is significantly more frequent among the cases with familial Parkinson's disease than among those with sporadic disease, further supporting the pathogenic role of this mutation in Parkinson's disease inheritance. The phenotype associated with the mutation encompasses early and late onset Parkinson's disease, and we show here for the first time that this mutation is also common among cases with onset before the age of 50 years. However, as late onset disease represents the vast majority of cases, it is anticipated that a larger number of patients with this mutation will be identified among the cases with late onset classical Parkinson's disease.

Origin of the G2019S mutation from a common founder

Our haplotype analysis strongly suggests that the G2019S mutation is transmitted from a single ancient founder. This confirms the results of a previous study,¹⁴ and refines the size of the shared region on the 3' end of the *LRRK2* gene, excluding markers D12S2519 and D12S2520. More importantly, in our data the ~160 kb minimum shared region spans the promoter and most of the *LRRK2* gene, suggesting that variation at the promoter or other *cis*-acting regulatory elements are not important determinants of the phenotypic variation observed among G2019S carriers. However, variants at regulatory elements in the other allele might play a modifier role. In the previous study the minimum shared region was reduced to 145 kb from marker D12S2515 to D12S2521, thereby excluding the promoter and the first 21 exons and 20 introns of *LRRK2*.¹⁴ However, our data suggest that D12S2515 is a highly unstable microsatellite, and the observed data in this study and the previous study¹⁴ are also compatible with mutations occurring in this

Table 3 Clinical features in carriers and non-carriers of the G2019S mutation

	Carriers	n	Non-carriers	n
Onset age (years)	50.5 (11.6)	15	52.7 (10.9)	615
Onset age, women (years)	43.9 (8.7)*	8	53.9 (10.7)	254
Onset age, men (years)	58.0 (10.1)	7	51.9 (11.0)	361
Disease duration† (years)	11.4 (5.8)	15	10.4 (6.3)	615
Disease duration, women (years)	12.1 (7.8)	8	10.3 (5.9)	254
Disease duration, men (years)	10.6 (2.2)	7	10.5 (6.6)	361

Values are mean (SD).

†Years from the age at onset of symptoms to the age at last examination.

* $p < 0.02$ v G2019S het. male carriers (Student's *t* test).

polymorphic marker instead of recombination events. We propose that alleles at D12S2515 define a cluster of subhaplotypes in the context of the ancestral G2019S bearing haplotype. The presence of the newly discovered IVS13+104A variant in all carriers of the mutant haplotype supports the contention that the shared region extends beyond the D12S2515 marker. We did not observe the IVS13+104A variant in any Italian Parkinson's disease cases, which do not carry the G2019S mutation (50 cases tested), and we have observed it in only three of 100 Italian controls (allele frequency ~1.5%) (the three controls were also sequenced and confirmed to be non-carriers of G2019S). The low frequency of the haplotype carrying the IVS13+104A variant in the general population also strongly suggests that G2019S originated from a single ancestor. The evidence of a common founder for this mutation in cases from many populations suggests that the mutant allele is very ancient.

The clinical phenotype associated with G2019S

The phenotype associated with the G2019S mutation in this and other studies is broad, encompassing a wide range of onset ages (from 34 to 73 years in this study), and a wide spectrum of penetrance, resulting in pattern ranging from sporadic presentation to autosomal dominant, highly penetrant familial aggregation. Pedigree inspection in our sporadic mutant probands (five carrying G2019S and one carrying R1441C) reveals that four of the 12 parents died before the age of 73 years, the latest onset age known in our patients with these mutations, including both parents of proband PD-817; information was unavailable for three parents, including both parents of proband TH-08. For the remaining five parents (both parents for probands PD-1074 and PD-516) the age at death or at examination was later than 73, and these might represent examples of non-penetrance of the G2019S mutation. For two more probands (PD-07 and PD-903) with unaffected parents but affected second degree relatives, the "transmitting" parent also died or is still alive at an age greater than 73. These observations strongly suggest that the penetrance and phenotype associated with this mutation might be markedly modified by other genetic or non-genetic factors. Future studies must address this issue, which complicates the genetic counselling of Parkinson's disease patients with *LRRK2* mutations.

In this study, the average disease onset and duration showed no differences between the patients who carried the G2019S mutation and those who did not (table 3). However, female patients carrying the mutation ($n = 8$) had an age of onset that was almost 10 years earlier than male patients with the mutation ($n = 7$) ($p < 0.02$, Student's *t* test) (table 3); if the other carriers of the same mutation detected in our previous study,¹⁰ with accurate onset age data available, are considered together, the difference remains significant (women 47.1 (10.3) years, $n = 17$; men 56.5 (10.5) years, $n = 11$; $p < 0.03$). Larger numbers of cases are needed to substantiate this observation; however, it is possible that the penetrance of the G2019S mutation is higher or the onset

earlier in female carriers. Further studies are also needed to assess prospectively the rate of progression of the disease associated with this and other *LRRK2* mutations.

Dementia is within the phenotypical spectrum of *LRRK2* mutations.^{8, 21} The fact that dementia is rare in carriers of the G2019S mutation in this and previous studies suggests that the phenotype associated with this mutation is that of classical Parkinson's disease. However, our study targeted patients with the pure Parkinson's disease phenotype; the presence of the G2019S and other *LRRK2* mutations should be investigated among patients with Parkinson's disease-dementia, or dementia with Lewy bodies.

Conclusions

Our study delineates the G2019S mutation in *LRRK2* as the most important single genetic determinant of Parkinson's disease so far identified and provides sound evidence that this mutation originated from a common founder. G2019S is especially frequent among cases with familial Parkinson's disease of both early and late onset, but it also occurs—albeit more rarely—among patients with sporadic Parkinson's disease. Understanding the mechanisms of the disease caused by G2019S and other *LRRK2* mutations might provide important clues for the dissection of the Parkinson's disease pathogenesis and for designing novel therapeutic strategies. The identification of a first, frequent genetic determinant of Parkinson's disease also has important implications for the diagnosis and genetic counselling of this disease.

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