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LRRK2 exonic variants and susceptibility to Parkinson's disease

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Author Contributions

OAR, MJF were the principal investigators and responsible for the concept and design of the study. AIO, JB, OAR, CVG were responsible for technical aspects of study. MGH, ND were responsible for all analysis. OAR, MJF were responsible for drafting of manuscript. All authors participated in study design and approach, sample collection, data acquisition, critical revision and final approval of manuscript.

Conflict of interest

The authors report no conflict of interest

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Abstract

Background—Leucine-rich repeat kinase 2 (*LRRK2*) is known to harbor highly penetrant mutations linked to familial parkinsonism. However, its full polymorphic variability in relationship to Parkinson's disease (PD) risk has not been systematically assessed.

Methods—We examined the frequency pathogenicity of 121 exonic *LRRK2* variants in three ethnic series (Caucasian [N=12,590], Asian [N=2,338] and Arab-Berber [N=612]) consisting of

8,611 patients and 6,929 control subjects from 23 separate sites of the Genetic Epidemiology of Parkinson's Disease Consortium.

Findings—Excluding carriers of previously known pathogenic mutations, new independent risk associations were found for polymorphic variants in Caucasian (p.M1646T, OR: 1.43, 95% CI: 1.15 – 1.78, P=0.0012) and Asian (p.A419V, OR: 2.27, 95% CI: 1.35 – 3.83, P=0.0011) populations. In addition, a protective haplotype was observed at >5% frequency (p.N551K-p.R1398H-p.K1423K) in the Caucasian and Asian series', with a similar finding in the small Arab-Berber series that requires further study (combined 3-series OR: 0.82, 95% CI: 0.72 – 0.94, P=0.0043). Of the two previously reported Asian risk variants p.G2385R was found to be associated with disease (OR: 1.73, 95% CI: 1.20 – 2.49, P=0.0026) but no association was observed for p.R1628P (OR: 0.62, 95% CI: 0.36 – 1.07, P=0.087). Also in the Arab-Berber series, p.Y2189C showed potential evidence of risk association with PD (OR: 4.48, 95% CI: 1.33 – 15.09, P=0.012). Of note, two variants (p.I1371V and p.T2356I) which have been previously proposed as pathogenic were observed in patient and control subjects at the same frequency.

Interpretation—*LRRK2* offers an example where multiple rare and common genetic variants in the same gene have independent effects on disease risk. Lrrk2, and the pathway in which it functions, is important in the etiology and pathogenesis of a greater proportion of patients with PD than previously believed.

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Keywords

Parkinson disease; LRRK2; genetics

INTRODUCTION

Parkinson's disease (PD) is generally considered a late-onset sporadic disorder. Nevertheless, genetic insights have helped to define the molecular etiology and have provided new models to develop neuroprotective interventions. Mutations of the leucine-rich repeat kinase 2 gene (*LRRK2*; Lrrk2) are now recognized as the most frequent genetic determinant of familial and sporadic PD[1]. The *LRRK2* gene (51 exons) encodes a protein (2527 amino acid; Lrrk2) which has five conserved domains: including a Roc (Ras in complex proteins; Rab GTPase) and a catalytic core common to both tyrosine and serine/threonine kinases.

Pathogenic *LRRK2* variability has been identified by sequencing of probands with familial parkinsonism, with results confirmed and occasionally extended within community and/or clinically-based patient-control series[2–6]. Seven definite pathogenic mutations (Lrrk2 p.N1437H, p.R1441C/G/H, p.Y1699C, p.G2019S, and p.I2020T) have been described[7, 8]. These mutations may be relatively frequent in patients from specific ethnicities, although still rare in ethnically-matched control subjects. Lrrk2 p.R1441G is found in more than 8% of patients originating in the Basque region of Northern Spain [9], whereas Lrrk2 p.G2019S is found in 30% of Arab-Berber patients with PD[10, 11]. *LRRK2* polymorphisms (>1% minor allele frequency) have also been associated with PD in Asia, for which the estimated attributable risk is often dependent on the specific ethnicity. Lrrk2 p.R1628P and p.G2385R are each found in 3–4% of individuals of Chinese descent and increase the risk of PD by approximately two-fold[12–15].

However, the large majority of *LRRK2* variants have not been systematically studied. It is possible that *LRRK2* may harbor many more variants that are important for determining PD pathogenicity and clinical risk. To address this possibility, with the Genetic Epidemiology of Parkinson's Disease (GEO-PD) consortium we have examined frequency of 121 *LRRK2* exonic variants in 15,540 subjects including 8,611 patients with PD, and assessed their role in disease susceptibility.

METHODS

Participants

The GEO-PD consortium includes investigators from 35 sites representing 22 countries, and six continents. All GEO-PD sites were invited to participate in this study. A total of 23 sites representing 15 countries and 5 continents agreed to participate in the current study and contributed clinical data for a total of 15,540 individuals (8,611 patients with PD and 6,929 controls). The Caucasian series consisted of 6,995 PD cases and 5,595 controls, the Asian series consisted of 1,376 PD cases and 962 controls, and the Arab-Berber series consisted of 240 PD cases and 372 controls. Patients were diagnosed using either the Gelb or the United Kingdom Parkinson's Disease Brain Bank (the exclusion criterion ">1 affected relative" was not included). Controls were collected at each site as unrelated healthy individuals (not all controls would have been given a detailed neurological examination but would have been asked about prior diagnosis of a neurological disorder or family history). Demographics for each series are shown in Table 1 and the sample size breakdown from each site is provided in Supplemental Table 1. All human biological samples were collected, fulfilling requested ethical approvals, and used in accord with the terms of subjects' informed consent.

Genotyping

LRRK2 exonic variants were identified through searches of available literature up to April 1st 2010, personal communications of Consortium members and from unpublished data (Table 2). Genotyping was performed on a Sequenom MassArray iPLEX platform (San Diego, CA) at the Mayo Clinic Florida laboratory of Neurogenetics (except for the groups from Paris, France, and Belgium who supplied genotype data and positive control genomic DNA[2, 3]); all primer sequences are provided in Supplemental Table 2. In total 8 iPLEX variant combinations were used to incorporate 123 *LRRK2* coding variants (Table 2). Positive control DNA was included for each variant; where positive genomic control DNA was unavailable a synthetic positive control DNA sequence was generated by a mismatch primer PCR method. A chi-square test followed by Bonferroni correction was used to test for deviation from Hardy Weinberg equilibrium (HWE) in controls for each site. Direct DNA sequencing was employed to confirm genotyping for all variants with a frequency below 0.3% ($n<50$).

Statistical Analysis

All analyses were performed separately for the Caucasian, Asian and Arab-Berber series'. For common variants with a minor allele frequency (MAF) of 0.5% or greater, single variant associations with PD were evaluated utilizing fixed effects logistic regression models, where genotypes were dichotomized as presence versus absence of the minor allele (dominant model) due to the fact that *LRRK2* mutations cause an autosomal dominantly inherited form of PD and also given the lack of rare homozygotes for many of the variants; additive models were also examined. Models were adjusted for site in the Asian and Caucasian series'. Sensitivity of results to the use of random effects models was also examined[16]. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated. Between-site heterogeneity was assessed using likelihood ratio tests for variant by site interaction in logistic regression

analysis, and also by estimating the I^2 statistic, which is a measure of the proportion of total variation of ORs across sites due to heterogeneity beyond chance[17].

For variants with a MAF below 0.5% (rare variants), though we estimated the proportion of carriers separately in patients and controls, no statistical tests were used to evaluate associations with PD due to insufficient power. Instead, we collapsed information across rare variants, acknowledging that this has the potential limitation of mixing groups of variants with protective and risk effects, and evaluated the association between presence of any rare variant and PD in logistic regression analysis adjusted by site[18]. In exploratory analysis when collapsing across variants, we also employed SIFT prediction freeware to examine only those substitutions predicted as not tolerated.

Haplotype analysis was performed using score tests for association with adjustment made for site[19]; haplotypes of frequency <0.5% were not considered. Any patient with a copy of the minor allele for any of the pathogenic variants that were observed in the study population (p.R1441C, p.R1441H, or p.G2019S) was excluded from all disease-association analysis in order to prevent confounding by these pathogenic variants; these patients were not excluded for any other portion of the analysis. Linkage disequilibrium (LD) between variants was assessed using r^2 values in study controls, separately for each series. Single variant associations with age at onset were examined using linear regression models, adjusting for site in the Caucasian and Asian series'; regression coefficients and 95% CIs were estimated.

We adjusted for multiple testing using the single-step minP method[20], with 10,000 within-site permutations of outcome labels in order to determine the level of significance that controls the family-wise error rate at 5%. After this adjustment, $P \leq 0.0033$ was considered significant in the Caucasian and $P \leq 0.0038$ in the Asian logistic regression disease-association analysis, while $P \leq 0.0035$ was considered significant in the Caucasian and $P \leq 0.0037$ was considered significant in the Asian linear regression age at onset association analysis. Note that adjusted significance cutoff levels differ between the Caucasian and Asian series due to the different number of tests performed in each, and the different correlation structures between variants within them. For the relatively small Arab-Berber series no adjustment for multiple testing was made, and as such these results are considered of a more exploratory nature. All statistical analyses were performed using the SAS software package (version 9.2; SAS Institute; Cary, North Carolina) or S-Plus (version 8.0.1; Insightful Corporation, Seattle, Washington).

RESULTS

A total of 123 *LRRK2* variants were selected for genotype analysis; however two variants (p.R793M and p.L2466H) failed to assay by iPLEX and were subsequently dropped from the study. All other variants (n=121) were genotyped through the entire patient-control series (n=15,540). All genotype call rates for the series' were >95%. Deviation from HWE among controls for each site (all $P>0.05$), was observed for p.N2081D in the Norwegian series, which was caused by two patients with a rare homozygote genotype and thus were retained in the analysis. However, Lrrk2 p.N289N and p.P1262A were dropped from the Arab-Berber analysis due to significant variation from HWE due to an increased number of rare minor allele homozygotes which may be due to the consanguineous nature of the population.

Of the 121 *LRRK2* exonic variants assessed, 4 were nonsense, 89 missense and 28 silent. In total, 48 variants (including 4 of the 7 known pathogenic mutations) were not observed in the sample of 15,540 patients and controls, suggesting these are rare mutations in the

populations originally examined. For the majority of variants the pair-wise LD was weak (r^2<0.3), with higher values observed with D′ given the relatively low minor allele frequency for many of these variants (Supplemental Tables 3a–f).

PD susceptibility for common variants

The results of single variant disease-association analysis are displayed in Table 3, separately for the Caucasian, Asian, and Arab-Berber series. In the Caucasian series, significant associations with PD were identified for Lrrk2 p.K1423K (OR: 0.83, 95% CI: 0.74 – 0.92, *P*=0.0006) and p.M1646T (OR: 1.43, 95% CI: 1.15 – 1.78, *P*=0.0012). Country-specific ORs and 95% CIs for the risk factor p.M1646T are displayed in Figure 1a. As shown in Supplemental Table 4, between-site heterogeneity in effects was low for p.M1646T (I^2=0%, *P*=0.44) and moderate for p.K1423K (I^2=34%, *P*=0.069).

In the Asian series, significant associations with PD were observed for Lrrk2 p.A419V (OR: 2.27, 95% CI: 1.35 – 3.83, *P*=0.0011), p.N551K (OR: 0.73, 95% CI: 0.60 – 0.89, *P*=0.0017), p.R1398H (OR: 0.73, 95% CI: 0.59 – 0.89, *P*=0.0020), and p.G2385R (OR: 1.73, 95% CI: 1.20 – 2.49, *P*=0.0026). Country-specific ORs and 95% CIs for these variants are displayed in Figure 1b and 1c; between-site heterogeneity was very low for each aforementioned association in the Asian series (all I^2=0%, all *P*≥0.42, Supplementary Table 4). Of note, Lrrk2 p.R1628P was not associated with PD in the Asian series, with a non-significant protective effect observed (OR: 0.62, 95% CI: 0.36 – 1.07, P=0.087). Upon further examination of this unexpected finding, the protective effect was driven by the Taiwanese series where it was most common (MAF: 3.8%, OR: 0.56, 95% CI: 0.32 – 1.01, P=0.054). Albeit not approaching significance, the risk effect for p.R1628P was observed in the South Korean series (MAF: 0.2%, OR: 1.33, 95% CI: 0.24 – 7.32, P=0.74), at the Seoul site in particular (MAF: 0.2%, OR: 2.47, 95% CI: 0.28 – 22.15, P=0.42). Lrrk2 p.R1628P was not observed in the Japanese series. Also of note, the previously suggested association of p.S1647T with PD in Asian populations[14] was not supported in our study (OR:0.97, 95% CI: 0.82 – 1.15, P=0.73).

In a more exploratory analysis for the smaller Arab-Berber series, significant associations (*P*≤0.05, without correction for multiple testing) with PD were observed for p.K1423K (OR: 0.42, 95% CI: 0.21 – 0.86, *P*=0.011) and p.Y2189C (OR: 4.48, 95% CI: 1.33 – 15.09, *P*=0.012). Larger Arab-Berber series' are needed to confirm these associations.

Results seen in single variant disease-association analysis in each series remained similar when adjusting for age and gender for the subjects (94.9%) for whom this information was available (Supplemental Table 5) and under an additive model (Supplemental Table 6). Effect sizes were also similar when adjusting simultaneously for other variants significantly associated with PD in a given series, and also when adjusting for p.R1628P in the Asian series where previous association has been demonstrated (Supplemental Table 7), providing evidence that these associations are independent of one another. When utilizing a random effects model for the Caucasian and Asian series', results were generally similar though slightly weaker (Supplementary Table 4) to those of a fixed effects model. Haplotype analysis across the series showed a significant overall association with disease in the Caucasian (*P*=0.0016) and Asian ($P=2 \times 10^{-24}$) series', with a trend in the Arab-Berber series (*P*=0.056). Haplotype associations appear to be driven by variants independently implicated in disease (Supplemental Tables 8a, b and c).

It is worth highlighting associations of Lrrk2 p.N551K, p. R1398H and p.K1423K noted across series (Figure 1c). Lrrk2 p.N551K, p. R1398H and p.K1423K are in strong LD and constitute a common (>5% frequency) protective haplotype that is inversely associated with risk.

Age of onset and common variants

Results of all common single variant associations with age at onset are shown in Supplemental Table 9. We did not identify any associations that withstood a multiple testing correction in the Caucasian and Asian series'. In the small Arab-Berber series, p.L153L was associated with an approximately 4-year earlier age at onset (*P*=0.038), which requires confirmation in larger samples.

Rare variants

A descriptive summary of rare variants (MAF <0.5%) is provided in Table 4 where the proportion of carriers is presented separately for cases and controls in each series. The pathogenic variants p.R1441H was observed in one Asian patient, p.R1441C was observed only in the Caucasian series (10 patients) and p.G2019S was observed in all three series. The ages of the eight p.G2019S control carriers of these pathogenic variants ranged from 48 to 76 years (Median: 64 years). As previously stated, due to the strong confounding potential of these three variants on disease-association analyses, any patient with a copy of these risk alleles was excluded in such analysis, including the summaries presented in Table 4. A number of other possible rare risk variants (p.E334K, p.R1325Q and p.T1410M) and protective variants (p.A221V, p.A1151T and p.D1375E) with notable differences in frequency between patients with PD and controls were observed. Of note, when collapsing across rare variants, the presence of any rare variant was not associated with PD in the Caucasian series (OR: 1.01, 95% CI: 0.81 – 1.25, *P*=0.95), Asian series (OR: 1.03, 95% CI: 0.57 – 1.85, *P*=0.92), or Arab-Berber series (OR: 0.78, 95% CI: 0.28 – 2.20, *P*=0.64). Additionally, no association was observed when collapsing across only those variants predicted as not tolerated using the SIFT prediction program[21] (Caucasian series [OR: 0.89, 95% CI: 0.55 – 1.43, P=0.62], Asian series [OR: 1.05, 95% CI: 0.37 – 2.99, P=0.93], or Arab-Berber series [PD cases: 0.0%, Controls: 0.6%, Fisher's exact P=1.00]). A summary of variants where no carriers were observed in any of the three series' is provided in Supplemental Table 10 and a complete list of genotype and allele frequencies per site are provided in Supplementary Table 11.

DISCUSSION

Our study, one of the largest to date in the study of the genetics of PD, shows that a single gene, *LRRK2*, harbors a large number of both rare and common variants that confer susceptibility to PD in diverse populations. Although population stratification is an inherent caveat of these types of large-scale collaborative efforts (and a potential limitation of the present study in the absence of genome-wide population control markers), these findings exemplify the confluence and independent effects of rare and common variation on gene loci that have a major influence in shaping both familial and sporadic disease.

Of the 121 variants that we assessed approximately one third (n=48) were not observed in any study participant. This includes 4 previously documented pathogenic mutations (Lrrk2 p.N1437H, p.R1441G, p.Y1699C and p.I2020T) illustrating their rarity in the population samples assessed. Twenty-six variants were at a greater than 0.5% frequency in any one of the three different series', and only thirteen were observed at >0.5% frequency in all three. This highlights the importance of studying genetic variability in large samples separately in different ethnic groups, since both frequencies and genetic effects may vary substantially[22].

The newly identified associations warrant further discussion. Lrrk2 p.M1646T in the COR domain was identified in the Caucasian series (OR 1.43) and the effect was consistent across many diverse countries (Figure 1a). This variant was not observed in Asian descent participants and was rare in the Arab-Berber series. Conversely, Lrrk2 p.A419V (OR 2.27)

was consistently more common in patients than controls in Asian sites (Figure 1b). Although we cannot exclude the possibility of a non-coding element in LD, the N-terminal region of the protein appears functionally relevant to disease development. Lrrk2 p.M1646T is the first disease-associated common variant to have been identified in Caucasian populations, whereas the p.A419V is now the third risk-factor that appears specific to individuals of Asian ancestry along with p.R1628P and p.G2385R[12, 14, 15]. Interestingly, in the present study Lrrk2 p.R1628P was not significantly associated with risk in our Asian series. This variant was only common within the Taiwanese series, where a non-significant protective effect was observed. Our lack of replication of the previously reported risk effect for R1628P is likely due to a combination of the low frequency of this variant, the small sample size of the Taiwanese series, natural sampling variation and population heterogeneity, given the results of previous larger studies of ethnic Han Chinese populations (of note Lrrk2 p.G2385R did display association)[14, 15],

The identification of a common three variant haplotype (p.N551K-p.R1398H-p.K1423K) across series that appears to act in a protective manner is also important. It suggests the reduced penetrance associated with *LRRK2*-parkinsonism may be due to variants acting in *cis*- or *trans*- with the pathogenic variant and that activity can be exploited to modify symptomatic onset in patients (Figure 1c), and that therapeutic strategies that lower risk in Lrrk2 parkinsonism may protect against symptomatic onset in idiopathic PD[14, 23]. The previous report of a protective effect for p.N551K and p.R1398H demonstrated a reduced kinase activity for the p.R1398H variant suggesting this ROC domain substitution may be the most likely functional allele on the haplotype[14].

Although our study identifies association with common variation only, it also highlights the wealth of rare variants in the *LRRK2* gene which may contribute to disease risk. It is increasingly appreciated that genetic loci that contribute to disease risk may do this through variants that span the whole range of MAF, from rare mutations to very frequent SNP alleles[24]. Despite the very large sample size, we documented only 3 of the 7 previously described pathogenic *LRRK2* mutations. Hence, the search for mutations underlying familial PD should include an analysis of single pedigrees, with evaluation in very large population studies. Single pedigrees may yield some false-positives and these can be filtered out with large population samples. For example, two variants (p.I1371V and p.T2356I) have been previously proposed as pathogenic and used to attribute clinical and functional features to *LRRK2*-parkinsonism[25, 26]. However, in the present study both variants were observed in patient and control subjects at the same frequency (Table 4). Conversely, we observed a number of other possible rare risk (p.E334K, p.R1325Q and p.T1410M) and protective (p.A211V, p.A1151T and p.D1375E) variants, however given their low frequency even larger meta-analytical approaches are necessary to fully define their role.

This study focused on exonic variants as to date all pathogenic variants identified in *LRRK2* have been single nucleotide missense changes. However, silent, synonymous variants were also included as they can result in alternative splicing, and may influence the rate of protein domain folding and secondary modifications (protein translation is a function of codon usage and t-RNA abundance)[27]. Neither copy number variants nor other risk factors in non-coding regions that regulate *LRRK2* expression or alter splicing were examined in the present work.

As genome-wide association and whole genome sequencing studies continue to yield new loci for susceptibility to diverse diseases, our study suggests that it is important to revisit loci where rare or common variants have been identified, since they may harbor a trove of many more independent signals of genetic risk in different populations[28–30]. Furthermore, *LRRK2* sequencing studies in under-represented populations (e.g. South American continent,

sub-Saharan Africa, Middle East and Western Asia) will undoubtedly reveal novel ethnic-specific risk variants and may clarify the role of the rare/absent variants in the present study. *LRRK2* variants were recently reported as part of the 1000 genome project including novel exonic variants supporting this hypothesis[31].

Massively-parallel resequencing (targeted genomic capture of the specific regions e.g. *LRRK2*, exome, transcriptome and whole-genome sequencing) will identify many more variants in candidate genes that may predispose to disease. Characterization of each will require this type of collaborative international effort to define pathogenicity, the frequency of variants in different populations and their contribution to disease pathogenesis through genotype-phenotype assessment.

Panel: Research in context

Systematic review—We searched PubMed for the terms “LRRK2” and “Genetics Parkinson’s disease” and identified all LRRK2 coding variations published up until April 2010. In addition we also contacted our global network of collaborators and the members of the Genetic epidemiology of Parkinson’s disease consortium (GE-OPD) for unpublished variants.

Interpretation—The study focuses on the role of *LRRK2* variation in Parkinson’s disease and has identified a common risk-factor in Caucasian population (p.M1646T), the third common risk factor in Asian populations (p.A419V) and a common global protective haplotype (p.N551K-p.R1398H-p.K1423K). This work complements the recent meta-analysis of PD GWAS, which suggests a possible association at the *LRRK2* locus. We define some of the actual genetic variation likely to be driving association observed in recent GWAS efforts and nominate potential functionally- and clinically-relevant variants. We show modulation of the underlying toxic effect is possible given the protective nature of the p.N551K-p.R1398H-p.K1423K haplotype. Perhaps most importantly, the study demonstrates a greater role for *LRRK2* in typical, idiopathic PD than previously believed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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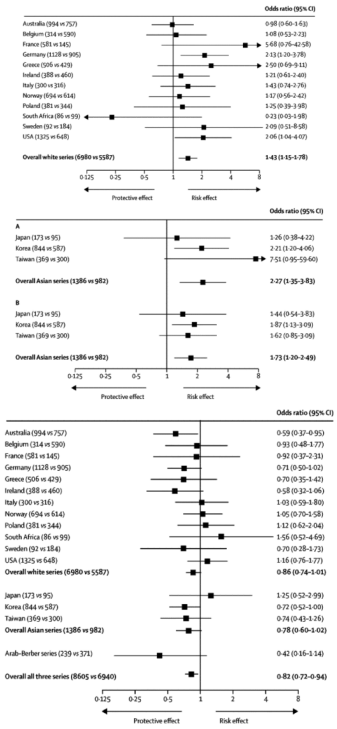

Figure 1.
Forest plots of associated variants

Table 1

Characteristics for Caucasian, Asian and Arab-Berber series

Series	PD patients	Control subjects
Caucasian	n=6,995	n=5,595
Age	69 ± 12 (18 – 107)	65 ± 15 (19 – 107)
Gender		
Male	4036 (58%)	2669 (48%)
Female	2959 (42%)	2926 (52%)
Age at onset	58 ± 12 (18 – 96)	N/A
Asian	n=1,376	n=962
Age	63 ± 13 (20 – 91)	59 ± 11 (23 – 98)
Gender		
Male	681 (49%)	319 (33%)
Female	695 (51%)	643 (67%)
Age at onset	54 ± 12 (20 – 89)	N/A
Arab-Berber	n=240	n=372
Age	66 ± 12 (27 – 87)	58 ± 11 (31 – 92)
Gender		
Male	116 (48%)	190 (51%)
Female	124 (52%)	182 (49%)
Age at onset	57 ± 13 (20 – 82)	N/A

- The sample mean ± SD (minimum – maximum) is given for age and age at onset. Information was unavailable regarding gender in the Asian series (6 Cases, 8 Controls) and Caucasian series (16 Cases, 249 Controls). Information was unavailable regarding age in the Asian series (8 Cases, 8 Controls), Caucasian series (482 Cases, 289 Controls), and Arab-Berber series (6 Cases, 4 Controls). Information was unavailable regarding age at onset in the Asian series (14 Cases) and Caucasian (801 Cases). N/A is not applicable.

Table 2

List of LRRK2 variants examined in the present study.

Position	Exon	rs#	cDNA	Amino Acid	Domain
chr12:38905228	1		28G>A	E10K	
chr12:38905349	1	rs2256408	149G>A	R50H	
chr12:38905627	2	rs72546335	155C>T	S52F	
chr12:38905696	2	rs75054132	224G>A	A75A	
chr12:38915703	4	rs33995463	356T>C	L119P	
chr12:38915711	4	rs41286468	364T>C	L122L	
chr12:38918058	5	rs10878245	457T>C	L153L	
chr12:38918147	5	rs35517158	546A>G	K182K	
chr12:38920612	6	rs112794616	632C>T	A211V	
chr12:38920663	6	rs56108242	683G>C	C228S	
chr12:38923625	7	rs28365216	713A>T	N238I	
chr12:38923737	7	rs72546315	824C>T	H275H	
chr12:38929923	8	rs17490713	867T>C	N289N	
chr12:38929949	8	rs57355477	893T>C	A298A	
chr12:38929992	8	rs41286466	936G>T	A312A	
chr12:38931342	9	rs78501232	1000G>A	E334K	
chr12:38931397	9	rs36016791	1055delC	A352fsX357	
chr12:38931430	9	rs72546336	1088A>G	N363S	
chr12:38931438	9	rs113065049	1096G>A	V366M	
chr12:38933053	11	rs34594498	1256C>T	A419V	
chr12:38937411	12	rs35847451	1383C>T	S461S	
chr12:38939594	13	rs75711334	1464A>T	L488L	
chr12:38939673	13	rs34090008	1543insG	P514fsX529	
chr12:38943875	14	rs35328937	1561A>G	R521G	
chr12:38943944	14	rs79996249	1630 A>G	K544E	
chr12:38943967	14	rs7308720	1653C>G	N551K	
chr12:38954669	15	rs77424631	1647G>A	G558G	
chr12:38958002	17	rs78154388	1987T>C	S663P	
chr12:38958037	17	rs72546319	2022A>C	V674V	

Position	Exon	rs#	cDNA	Amino Acid	Domain
chr12:38958213	17	rs35611877	2198insA	L708fsX718	ANK
chr12:38958223	18		2134A>G	M712V	ANK
chr12:38958236	18		2147C>T	A716V	ANK
chr12:38958256	18	rs10878307	2167A>G	I723V	ANK
chr12:38963966	19	rs34410987	2264C>T	P755L	ANK
chr12:38964080	19	rs35173587	2378G>T	R793M	ANK
chr12:38964130	19	rs72546337	2428A>G	I810V	ANK
chr12:38964183	19	rs76890302	2481T>C	S827S	ANK
chr12:38967530	20		2611A>G	K871E	
chr12:38973693	21	rs58559150	2769G>C	Q923H	
chr12:38973713	21		2789A>G	Q930R	
chr12:38974935	22	rs17519916	2830G>T	D944Y	
chr12:38974962	22	rs7966550	2857T>C	L953L	
chr12:38975535	23	rs75148313	2918G>A	S973N	
chr12:38975635	23	rs113217062	3018A>G	I1006M	LRR
chr12:38975638	23	rs55783828	3021C>T	S1007S	LRR
chr12:38978415	24	rs111341148	3200G>A	R1067Q	LRR
chr12:38978502	24	rs76535406	3287C>G	S1096C	LRR
chr12:38978548	24	rs78365431	3333G>T	Q1111H	LRR
chr12:38978557	24	rs35808389	3342A>G	L1114L	LRR
chr12:38979194	25	rs34805604	3364A>G	I1122V	LRR
chr12:38979281	25	rs74985840	3451G>A	A1151T	LRR
chr12:38979324	25		3494T>C	L1165P	LRR
chr12:38982935	26		3574A>G	I1192V	LRR
chr12:38984073	27	rs72546324	3647A>G	H1216R	LRR
chr12:38984109	27	rs80179604	3683G>C	S1228T	LRR
chr12:38984109	27	rs60185966	3683G>T	S1228I	LRR
chr12:38985860	28	rs4640000	3784C>G	P1262A	LRR
chr12:38988536	29	rs77018758	3960G>C/T	R1320S	
chr12:38988550	29	rs72546338	3974G>A	R1325Q	
chr12:38988687	29	rs17466213	4111A>G	I1371V	Roc

Position	Exon	rs#	cDNA	Amino Acid	Domain
chr12:38988701	29	rs28365226	4125C>A	D1375E	Roc
chr12:38989178	30	rs7133914	4193G>A	R1398H	Roc
chr12:38989214	30	rs72546327	4229C>T	T1410M	Roc
chr12:38989243	30	rs113589830	4258G>A	D1420N	Roc
chr12:38989254	30	rs11175964	4269G>A	K1423K	Roc
chr12:38989275	30	rs111435410	4290C>T	A1430A	Roc
chr12:38989294	30	rs74163686	4309A>C	N1437H	Roc
chr12:38990503	31	rs33939927	4321C>T	R1441C	Roc
chr12:38990503	31	rs33939927	4321C>G	R1441G	Roc
chr12:38990504	31	rs34995376	4322G>A	R1441H	Roc
chr12:38990505	31	rs112998035	4323C>T	R1441R	Roc
chr12:38990506	31		4324G>C	A1442P	Roc
chr12:38990519	31	rs74681492	4337C>T	P1446L	Roc
chr12:38990530	31	rs111501952	4348G>A	V1450I	Roc
chr12:38990569	31	rs35363614	4387insA	R1462fsX1468	Roc
chr12:38990584	31		4402A>G	K1468E	Roc
chr12:38990630	31	rs113431708	4448G>A	R1483Q	Roc
chr12:38994045	32	rs35507033	4541G>A	R1514Q	COR
chr12:38994128	32	rs33958906	4624C>T	P1542S	COR
chr12:38994170	32	rs17491187	4666C>A	L1556I	COR
chr12:38995335	33	rs721710	4793T>A	V1598E	COR
chr12:39000067	34		4838T>C	V1613A	COR
chr12:39000101	34	rs1427263	4872C>A	G1624G	COR
chr12:39000112	34	rs33949390	4883G>C	R1628P	COR
chr12:39000140	34	rs11176013	4911A>G	K1637K	COR
chr12:39000166	34	rs35303786	4937T>C	M1646T	COR
chr12:39000168	34	rs11564148	4939T>A	S1647T	COR
chr12:39,000,188	34	rs111503579	4959A>G	L1653L	COR
chr12:39001183	35	rs35801418	5096A>G	Y1699C	COR
chr12:39001350	35	rs79909111	5163A>G	S1721S	COR
chr12:39002106	36	rs11564176	5173C>T	R1725STOP	COR

Position	Exon	rs#	cDNA	Amino Acid	Domain
chr12:39002116	36		5183G>T	R1728L	COR
chr12:39002116	36	ss263192805	5183G>A	R1728H	COR
chr12:39002455	37	rs111910483	5385G>T	L1795F	COR
chr12:39002527	37	rs10878371	5457T>C	G1819G	COR
chr12:39003324	38		5605A>G	M1869V	COR
chr12:39003325	38	rs35602796	5606T>C	M1869T	COR
chr12:39003329	38		5610G>T	L1870F	COR
chr12:39003339	38		5620G>T	E1874STOP	COR
chr12:39015100	39	rs77428810	5822G>A	R1941H	MAPKKK
chr12:39020430	41		6016T>C	Y2006H	MAPKKK
chr12:39020449	41	rs34015634	6035T>C	I2012T	MAPKKK
chr12:39020469	41	rs34637584	6055G>A	G2019S	MAPKKK
chr12:39020473	41	rs35870237	6059T>C	I2020T	MAPKKK
chr12:39020505	41	rs78029637	6091A>T	T2031S	MAPKKK
chr12:39026899	42	rs111739194	6187delCTCTA	L2063STOP	MAPKKK
chr12:39026953	42	rs33995883	6241A>G	N2081D	MAPKKK
chr12:39028521	43	rs10878405	6324G>A	E2108E	MAPKKK
chr12:39028553	43	rs12423862	6356C>T	P2119L	MAPKKK
chr12:39031648	44	rs111691891	6422C>T	T2141M	
chr12:39031736	44	rs34869625	6510C>A	G2170G	WD40
chr12:39031792	44	rs35658131	6566A>G	Y2189C	WD40
chr12:39036195	46	rs12581902	6782A>T	N2261I	WD40
chr12:39043509	48	rs113511708	7067C>T	T2356I	WD40
chr12:39043595	48	rs34778348	7153G>A	G2385R	WD40
chr12:39043597	48	rs33962975	7155A>G	G2385G	WD40
chr12:39043610	48	rs79546190	7168G>A	V2390M	WD40
chr12:39044912	49	rs78964014	7183G>A	E2395K	WD40
chr12:39044916	49	rs111272009	7187insGT	T2356fsX2360	WD40
chr12:39044919	49	rs3761863	7190C>T	M2397T	WD40
chr12:39044953	49	rs60545352	7224G>A	M2408I	WD40
chr12:39047081	50		7397T>A	L2466H	WD40

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Position	Exon	rs#	cDNA	Amino Acid	Domain
chr12:39047119	50	rs55633591	7435A>G	N2479D	WD40

Table 3

Common single variant associations with PD

		Caucasian series			Asian series			Arab-Berber series		
SNP	**MA**	**MAF**	**OR (95% CI)**	**P-value**	**MAF**	**OR (95% CI)**	**P-value**	**MAF**	**OR (95% CI)**	**P-value**
rs2256408 R50H	G	+	+	+	---	---	---	1.7%	2.05 (0.82, 5.14)	0.13
rs10878245 L153L	C*	39.6%	0.98 (0.91, 1.06)	0.57	31.2%	1.04 (0.88, 1.23)	0.65	47.1%	0.81 (0.55, 1.19)	0.28
rs34594498 A419V	T	+	+	+	1.9%	2.27 (1.35, 3.83)	0.0011	---	---	---
rs7308720 N551K	G	6.7%	0.88 (0.79, 0.98)	0.025	11.9%	0.73 (0.60, 0.89)	0.0017	8.0%	0.83 (0.49, 1.39)	0.47
rs10878307 I723V	G	7.4%	0.94 (0.84, 1.04)	0.23	1.1%	1.36 (0.74, 2.49)	0.32	9.0%	1.09 (0.68, 1.75)	0.71
rs34410987 P755L	T	---	---	---	0.6%	0.56 (0.27, 1.18)	0.13	---	---	---
rs58559150 Q923H	C	+	+	+	---	---	---	0.9%	0.62 (0.13, 2.99)	0.55
rs7966550 L953L	C	12.8%	0.98 (0.90, 1.07)	0.66	17.6%	0.80 (0.66, 0.95)	0.012	12.4%	0.92 (0.60, 1.41)	0.70
rs77018758 R1320S	T	---	---	---	1.2%	1.20 (0.69, 2.11)	0.51	---	---	---
rs17466213 I1371V	G	+	+	+	+	+	+	0.5%	4.45 (0.81, 24.56)	0.086
rs7133914 R1398H	A	6.6%	0.89 (0.80, 0.99)	0.034	11.5%	0.73 (0.59, 0.89)	0.0020	8.7%	1.00 (0.61, 1.64)	1.00
rs11175964 K1423K	A	6.6%	0.83 (0.74, 0.92)	0.0006	11.5%	0.75 (0.62, 0.92)	0.0064	5.4%	0.42 (0.21, 0.86)	0.011
rs35507033 R1514Q	A	0.9%	1.13 (0.85, 1.49)	0.41	---	---	---	+	+	+
rs33958906 P1542S	T	2.8%	0.90 (0.77, 1.06)	0.21	---	---	---	1.0%	2.27 (0.72, 7.13)	0.16
rs1427263 G1624G	C*	34.7%	1.06 (0.98, 1.14)	0.15	46.7%	0.92 (0.77, 1.11)	0.40	31.7%	0.96 (0.67, 1.39)	0.84
rs33949390 R1628P	C	+	+	+	1.2%	0.62 (0.36, 1.07)	0.087	---	---	---
rs11176013 K1637K	G*	45.0%	1.02 (0.94, 1.11)	0.60	44.6%	0.96 (0.80, 1.16)	0.68	46.0%	1.07 (0.70, 1.63)	0.76
rs35303786 M1646T	C	1.6%	1.43 (1.15, 1.78)	0.0012	---	---	---	+	+	+
rs11564148 S1647T	A	29.9%	0.93 (0.86, 1.00)	0.048	28.3%	0.97 (0.82, 1.15)	0.73	27.6%	0.81 (0.55, 1.19)	0.29
rs10878731 G1819G	C*	45.2%	1.06 (0.98, 1.15)	0.16	43.3%	0.99 (0.83, 1.19)	0.95	46.2%	1.07 (0.70, 1.64)	0.75
rs33995883 N2081D	G	2.6%	1.24 (1.05, 1.47)	0.013	+	+	+	4.7%	0.92 (0.49, 1.73)	0.79
rs10878405 E2108E	A	31.4%	0.96 (0.89, 1.03)	0.27	29.6%	1.01 (0.85, 1.20)	0.92	28.1%	0.75 (0.51, 1.10)	0.14
rs35658131 Y2189C	G	+	+	+	---	---	---	1.1%	4.48 (1.33, 15.09)	0.012
rs3477838348 G2385R	A	---	---	---	3.3%	1.73 (1.20, 2.49)	0.0026	---	---	---
rs33962975 G2385G	G	15.7%	0.97 (0.89, 1.06)	0.49	1.8%	0.96 (0.62, 1.49)	0.85	8.4%	1.14 (0.70, 1.83)	0.60
rs3761863 M2397T	C	34.4%	1.06 (0.98, 1.14)	0.17	43.9%	0.88 (0.73, 1.05)	0.16	39.8%	1.33 (0.85, 2.07)	0.21

+ Indicates a variant with a MAF <0.5% that was not included in logistic regression analysis but was observed.

--- Indicates a variant that was not observed in the given series.

* Indicates a differing minor allele between the 3 series. For rs10878252 p.L153L, the minor allele was C in the Asian and Arab-Berber series and T in the Caucasian series. For rs1427263 p.G1624G, the minor allele was C in the Caucasian and Arab-Berber series and A in the Asian series. For rs11176013 p.K1637K, the minor allele was A in the Caucasian and Arab-Berber series and G in the Asian series. For rs10878731 p.G1819G, the minor allele was T in the Caucasian and Arab-Berber series and C in the Asian series. Odds ratios and *P*-values result from logistic regression models, where adjustment was made for site in the Asian and Caucasian series'. Odds ratios correspond to presence of the minor allele. After multiple testing adjustment, $P \leq 0.0038$ was considered significant in the Asian series, and $P \leq 0.0033$ was considered significant in the Caucasian series. No adjustment for multiple testing was made in the Arab-Berber series, where $P < 0.05$ was considered significant.

Table 4

Descriptive summary of rare variants

		No. (%) of carriers					
		Caucasian series		Asian series		Arab-Berber series	
SNP	Amino Acid	PD cases	Controls	PD cases	Controls	PD cases	Controls
rs2256408	R50H	7 (0.1%)	1 (0.02%)	---	---	+	+
rs75054132	A75A	---	---	---	---	0 (0.0%)	1 (0.3%)
rs33995463	L119P	21 (0.3%)	23 (0.4%)	---	---	0 (0.0%	2 (0.6%)
rs41286468	L122L	5 (0.1%)	7 (0.1%)	---	---	---	---
rs112794616	A211V	4 (0.1%)	11 (0.2%)	---	---	0 (0.0%)	1 (0.3%)
rs56108242	C228S	2 (0.03%)	2 (0.04%)	---	---	---	---
rs28365216	N238I	---	---	3 (0.2%)	2 (0.2%)	---	---
rs72546315	H275H	3 (0.05%)	2 (0.04%)	---	---	1 (0.6%)	0 (0.0%)
rs17490713	N289N	1 (0.01%)	2 (0.04%)	---	---	NA	NA
rs41286466	A312A	26 (0.4%)	15 (0.3%)	1 (0.1%)	0 (0.0%)	0 (0.0%)	4 (1.0%)
rs78501232	E334K	14 (0.2%)	4 (0.07%)	---	---	---	---
rs113065049	V366M	1 (0.02%)	0 (0.0%)	---	---	---	---
rs34594498	A419V	5 (0.07%)	3 (0.06%)	+	+	---	---
rs35847451	S416S	12 (0.2%)	16 (0.3%)	---	---	---	---
rs75711334	L488L	1 (0.01%)	0 (0.0%)	---	---	---	---
rs79996249	K544E	2 (0.03%)	2 (0.04%)	---	---	---	---
rs78154388	S663P	2 (0.03%)	2 (0.04%)	---	---	---	---
rs72546319	V674V	0 (0.0%)	2 (0.04%)	---	---	0 (0.0%)	1 (0.3%)
rs58559150	Q923H	1 (0.01%)	2 (0.04%)	---	---	+	+
rs75148313	S973N	1 (0.01%)	2 (0.04%)	---	---	---	---
rs113217062	I1006M	1 (0.02%)	0 (0.0%)	---	---	---	---
rs76535406	S1096C	0 (0.0%)	2 (0.04%)	---	---	---	---
rs35808389	L1114L	5 (0.07%)	1 (0.02%)	---	---	---	---
rs74985840	A1151T	1 (0.01%)	5 (0.1%)	---	---	---	---
rs80179604	S1228T	5 (0.07%)	4 (0.07%)	---	---	---	---
rs4640000	P1262A	1 (0.01%)	1 (0.02%)	---	---	NA	NA

		No. (%) of carriers					
		Caucasian series		Asian series		Arab-Berber series	
SNP	Amino Acid	PD cases	Controls	PD cases	Controls	PD cases	Controls
rs72546338	R1325Q	10 (0.15%)	3 (0.06%)	4 (0.3%)	1 (0.1%)	---	---
rs17466213	I1371V	7 (0.1%)	4 (0.07%)	1 (0.1%)	0 (0.0%)	+	+
rs72546327	T1410M	5 (0.07%)	1 (0.02%)	---	---	---	---
rs113589830	D1420N	1 (0.02%)	0 (0.0%)	---	---	---	---
rs111435410	A1430A	2 (0.03%)	1 (0.02%)	---	---	---	---
rs112998035	R1441R	---	---	1 (0.1%)	0 (0.0%)	---	---
rs33939927 *	R1441C	10 (0.2%)	0 (0.0%)	---	---	---	---
rs34995376 *	R1441H	---	---	1 (0.07%)	0 (0.0%)	---	---
rs74681492	P1446L	---	---	10 (0.8%)	6 (0.6%)	---	---
rs111501952	V1450I	---	---	2 (0.1%)	1 (0.1%)	---	---
rs113431708	R1483Q	1 (0.01%)	0 (0.0%)	---	---		---
rs35507033	R1514Q	+	+	---	---	0 (0.0%)	1 (0.3%)
rs33949390	R1628P	7 (0.1%)	0 (0.0%)	+	+	---	---
rs35303786	M1646T	+	+	---	---	3 (1.3%)	2 (0.6%)
rs111503579	L1653L	2 (0.03%)	1 (0.02%)	4 (0.3%)	9 (1%)	---	
rs79909111	S1721S	1 (0.02%)	1 (0.02%)	---	---	---	---
ss263192805	R1728H	1 (0.01%)	3 (0.05%)	---	---	---	---
rs35602796	M1869T	5 (0.07%)	2 (0.04%)	---	---	---	---
rs77428810	R1941H	2 (0.03%)	1 (0.02%)	---	---	---	---
rs34637584 *	G2019S	48 (0.7%)	3 (0.06%)	1 (0.07%)	1 (0.1%)	72 (30.2%)	4 (1.1%)
rs111739194	L2063STOP	1 (0.02%)	2 (0.04%)	---	---	---	---
rs33995883	N2081D	+	+	2 (0.1%)	0 (0.0%)	+	+
rs34869625	G2170G	20 (0.3%)	21 (0.4%)	---	---	1 (0.6%)	0 (0.0%)
rs35658131	Y2189C	1 (0.01%)	2 (0.04%)	---	---	+	+
rs113511708	T2356I	7 (0.1%)	5 (0.1%)	---	---	---	---
rs79546190	V2390M	1 (0.01%)	1 (0.02%)	---	---	---	---
rs78964014	E2395K	1 (0.01%)	0 (0.0%)	---	---	---	---
rs60545352	M2408I	1 (0.01%)	0 (0.0%)	---	---	0 (0.0%)	2 (0.6%)

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*Indicates a pathogenic variant, where the number (%) of carriers is summarized for the entire sample. Any carriers of these pathogenic variants were removed for the summaries provided for each of the remaining non-pathogenic variants.

+ Indicates a variant that was observed with a MAF ≥ 0.5% and as such was analyzed as a common variant.

--- indicates a variant that was not observed in the given series. NA highlights a variant that was out of HWE in the specific series.