Association of *LRRK2* exonic variants with susceptibility to Parkinson's disease: a case–control study

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Summary

Background The leucine-rich repeat kinase 2 gene (*LRRK2*) harbours highly penetrant mutations that are linked to familial parkinsonism. However, the extent of its polymorphic variability in relation to risk of Parkinson's disease (PD) has not been assessed systematically. We therefore assessed the frequency of *LRRK2* exonic variants in individuals with and without PD, to investigate the role of the variants in PD susceptibility.

Methods *LRRK2* was genotyped in patients with PD and controls from three series (white, Asian, and Arab–Berber) from sites participating in the Genetic Epidemiology of Parkinson's Disease Consortium. Genotyping was done for exonic variants of *LRRK2* that were identified through searches of literature and the personal communications of consortium members. Associations with PD were assessed by use of logistic regression models. For variants that had a minor allele frequency of 0·5% or greater, single variant associations were assessed, whereas for rarer variants information was collapsed across variants.

Findings 121 exonic *LRRK2* variants were assessed in 15 540 individuals: 6995 white patients with PD and 5595 controls, 1376 Asian patients and 962 controls, and 240 Arab–Berber patients and 372 controls. After exclusion of carriers of known pathogenic mutations, new independent risk associations were identified for polymorphic variants in white individuals (M1646T, odds ratio 1·43, 95% CI 1·15–1·78; p=0·0012) and Asian individuals (A419V, 2·27, 1·35–3·83; p=0·0011). A protective haplotype (N551K-R1398H-K1423K) was noted at a frequency greater than 5% in the white and Asian series, with a similar finding in the Arab–Berber series (combined odds ratio 0·82, 0·72–0·94; p=0·0043). Of the two previously reported Asian risk variants, G2385R was associated with disease (1·73, 1·20–2·49; p=0·0026), but no association was noted for R1628P (0·62, 0·36–1·07; p=0·087). In the Arab–Berber series, Y2189C showed potential evidence of risk association with PD (4·48, 1·33–15·09; p=0·012).

Interpretation The results for *LRRK2* show that several rare and common genetic variants in the same gene can have independent effects on disease risk. LRRK2, and the pathway in which it functions, is important in the cause and pathogenesis of PD in a greater proportion of patients with this disease than previously believed. These results will help discriminate those patients who will benefit most from therapies targeted at LRRK2 pathogenic activity.

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Introduction

Parkinson's disease (PD) is generally thought of as a late-onset sporadic disorder. Nevertheless, genetic insights are helping to define the molecular causes of PD and have provided new models for the development of neuroprotective interventions. Mutations in the leucine-rich repeat kinase 2 gene (*LRRK2*) are now recognised as the most common genetic determinant of familial and sporadic PD.[1] *LRRK2* has 51 exons and encodes the 2527 aminoacid protein LRRK2, which has five conserved domains, including a Roc (Ras in complex proteins, Rab GTPase) domain and a catalytic core common to both tyrosine and serine-threonine kinases.

Pathogenic *LRRK2* variability has been identified by sequencing probands with familial parkinsonism, with results confirmed and occasionally extended within community or clinically-based patient–control series.[2–6] Seven definite pathogenic *LRRK2* mutations (encoding LRRK2 N1437H, R1441C, R1441G, R1441H, Y1699C, G2019S, and I2020T) have been described.[7,8] These mutations can be relatively common in patients from some ethnic origins, but are rare in ethnically matched controls. LRRK2 R1441G has been identified in more than 8% of patients with PD originating from the Basque region of northern Spain,[9] and LRRK2 G2019S has been reported in 30% of Arab–Berber patients

with PD.[10,11] *LRRK2* polymorphisms with more than 1% minor allele frequency have also been associated with PD in Asia, with the estimated attributable risk often dependent on ethnic origin. LRRK2 R1628P and G2385R have each been recorded in 3–4% of individuals who are of Chinese descent and roughly double the risk of PD.[12–15]

However, most *LRRK2* variants have not been systematically studied. *LRRK2* might harbour more variants that are important determinants of PD pathogenicity and clinical risk. To address this possibility, with the Genetic Epidemiology of Parkinson's Disease (GEO-PD) Consortium, we assessed the frequency of *LRRK2* exonic variants in people with and without PD, and assessed the role of the variants in disease susceptibility.

Methods

Participants and procedures

All 35 GEO-PD sites (hospitals and centres), representing 22 countries and six continents, were invited to participate in this study. Patients were diagnosed by use of either the Gelb or the UK Parkinson's Disease Society Brain Bank criteria (the exclusion criterion of more than one affected relative was not included).[16,17] Controls at each site were healthy individuals who were not related to the patients; not all controls were given a detailed neurological examination but all were asked about any previous diagnosis or family history of a neurological disorder. All biological samples were gathered after ethics approval had been obtained from the Mayo Clinic Institutional Review Board Committee, and were used in accordance with the terms of the written informed consent provided by the participants.

LRRK2 exonic variants were identified through searches of available literature up to April 1, 2010, from personal communications with consortium members, and from in-house sequencing studies that had identified novel variants (unpublished data; table 1). DNA was sourced from blood and was stored in a –20°C freezer. All samples were de-identified with an anonymous code from each site and only a minimal clinical dataset. Data were collected in batches but analysed as a single dataset. Genotyping was done on a MassArray iPLEX platform (Sequenom, San Diego, CA, USA) at the Mayo Clinic neurogenetics laboratory, FL, USA (except for the groups from Paris, France, and Antwerp, Belgium, who supplied genotype data and positive control genomic DNA[2,3]); all primer sequences are provided in the webappendix pp 1–4). Eight iPLEX variant combinations were used to incorporate 123 *LRRK2* coding variants (table 1). Positive control DNA was run for each variant; in the absence of a positive genomic control DNA, a synthetic positive control DNA sequence was generated by use of mismatch-primer PCR. A χ^2 test followed by Bonferroni correction was used to test for deviation from the Hardy–Weinberg equilibrium (HWE) in controls for

	Exon	Accession number	cDNA	Aminoacid	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	..
chr12:38958213	17	rs35611877	2198insA	L708fsX718	Ankyrin
chr12:38958223	18	..	2134A>G	M712V	Ankyrin
chr12:38958236	18	..	2147C>T	A716V	Ankyrin
chr12:38958256	18	rs10878307	2167A>G	I723V	Ankyrin
chr12:38963966	19	rs34410987	2264C>T	P755L	Ankyrin
chr12:38964080	19	rs35173587	2378G>T	R793M	Ankyrin
chr12:38964130	19	rs72546337	2428A>G	I810V	Ankyrin
chr12:38964183	19	rs76890302	2481T>C	S827S	Ankyrin
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

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	Exon	Accession number	cDNA	Aminoacid	Domain
(Continued from previous page)					
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
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
chr12:39000188	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	R1725X	COR
chr12:39002116	36	..	5183G>T	R1728L	COR
chr12:39002116	36	rs145364431	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	E1874X	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

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each site. Direct DNA sequencing was used to confirm genotyping for all variants with a frequency of less than 0·3% (n<50 carriers).

Statistical analysis

All analyses were undertaken separately for the patients in the white, Asian, and Arab–Berber series. For common variants with a minor allele frequency of 0·5% or greater, single variant associations with PD were assessed by use of fixed-effects logistic regression models, in which genotypes were dichotomised as presence versus absence of the minor allele (dominant model), because *LRRK2* mutations cause an autosomal dominantly inherited form of PD and homozygotes for many of the variants are rare; additive models were also assessed. Models were adjusted for site in the white and Asian series. Sensitivity of results to the use of random-effects models was also assessed.[18] Odds ratios (ORs) and 95% CIs were estimated. Between-site heterogeneity was assessed with likelihood ratio tests for variant by site interaction in a logistic regression analysis, and also by estimation of the I^2 statistic (a measure of the proportion of total variation in ORs between sites due to heterogeneity beyond chance).[19]

For variants with a minor allele frequency of less than 0·5% (rare variants), although we estimated the proportion of carriers separately in patients and controls, no statistical tests were used to evaluate associations with PD because of insufficient power. Instead, we collapsed information for rare variants, acknowledging that this has the potential limitation of mixing groups of variants with protective and risk effects, and evaluated the association between the presence of any rare variant and PD in a logistic regression analysis adjusted by site.[20] In an exploratory analysis, when collapsing data across variants, we also used the Sorts Intolerant From Tolerant (SIFT) prediction program[21] to assess only those substitutions predicted to be not tolerated.

Haplotype analysis was done by use of score tests for association with adjustment for site;[22] haplotypes of less than 0·5% frequency were not assessed. Any patient with a copy of the minor allele for any of the pathogenic variants that were noted in the study population (R1441C, R1441H, or G2019S) was excluded from all disease-association analyses to prevent confounding by the pathogenic variants; these patients were not excluded for any other portion of the analysis. Linkage disequilibrium between variants was assessed by use of r^2 values in study controls, separately for each series. Single variant associations with age at onset were assessed with linear regression models, adjusting for site in the white and Asian series; regression coefficients and 95% CIs were estimated.

We adjusted for multiple testing by use of the single-step minP method,[23] with 10 000 within-site permutations of outcome labels to assess the level of significance that controls the family-wise error rate at

5%. After this adjustment, in the logistic regression disease-association analysis p≤0·0033 was judged to be significant in the white series and p≤0·0038 in the Asian series, whereas in the linear regression age at onset association analysis p≤0·0035 was judged to be significant in the white series and p≤0·0037 in the Asian series. The adjusted significance cutoff levels differed between the white and Asian series because of the different number of tests undertaken in each series, and the different correlation structures between variants within them. For the fairly small Arab–Berber series, no adjustment for multiple testing was made, and as such the results were judged to be exploratory. All statistical analyses were done by use of SAS software (version 9.2) or S-Plus (8.0.1).

Role of the funding source

The funding agencies did not play any part in the design of the study, collection, analysis, or interpretation of data, writing of the report, or the decision to submit the report for publication. The principal investigators (OAR and MJF) had access to all the data in this study. The corresponding author had final responsibility for the decision to submit.

Results

Data were gathered from June, 2008, to October, 2010. 23 sites from the GEO-PD Consortium, representing 15 countries and five continents, agreed to participate in this study and contributed clinical data from 8611 patients with PD and 6929 controls. We studied individuals in three series: white (6995 patients and 5595 controls), Asian (1376 patients and 962 controls), and Arab–Berber (240 patients and 372 controls). Table 2 shows the demographics for each series, and webappendix p 5 shows the sample size breakdown for each site. 123 *LRRK2* variants were selected for genotype analysis, but two (R793M and L2466H) did not assay by use of iPLEX and were dropped from the study. The other 121 variants were genotyped in the entire patient–control series (n=15 540); genotyping was successful in all individuals. Call rates for all genotypes in the series were greater than 95%. Deviation from HWE in the controls for each site (all p>0·05) was noted for LRRK2 N2081D in the Norwegian series and was attributable to two patients with a rare homozygous genotype; all patients were retained in the analysis. However, N289N and P1262A were excluded from the analysis of the Arab–Berber series because of significant variation from HWE due to an increased number of rare minor allele homozygotes, which might have been attributable to the consanguineous nature of the population.

Four of 121 *LRRK2* exonic variants were nonsense, 89 missense, and 28 silent. 48 variants, including four of the seven known pathogenic mutations, were not identified in the 15 540 patients and controls. For most of the variants, the pair-wise linkage disequilibrium was weak (r^2<0·3), with higher values noted with D' because of the low minor allele frequency for many of these variants (webappendix pp 6–17).

	Exon	Accession number	cDNA	Aminoacid	Domain
(Continued from previous page)					
chr12:39020473	41	rs35870237	6059T>C	I2020T	MAPKKK
chr12:39020505	41	rs78029637	6091A>T	T2031S	MAPKKK
chr12:39026899	42	rs111739194	6187delCTCTA	L2063X	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
chr12:39047119	50	rs55633591	7435A>G	N2479D	WD40

Chr12=chromosome 12. Roc=Ras in complex. COR=C-terminal of Ras. MAPKKK=mitogen-activated protein kinase kinase kinase. LRR=leucine-rich repeat.

***Table 1*: *LRRK2* exonic variants investigated in the study**

	Patients	Controls
White series	n=6995	n=5595
Age (years)	69 (12; 18–107)	65 (15; 19–107)
Men	4036 (58%)	2669 (48%)
Age at onset (years)	58 (12; 18–96)	NA
Asian series	n=1376	n=962
Age (years)	63 (13; 20–91)	59 (11; 23–98)
Men	681 (49%)	319 (33%)
Age at onset (years)	54 (12; 20–89)	NA
Arab–Berber series	n=240	n=372
Age (years)	66 (12; 27–87)	58 (11; 31–92)
Men	116 (48%)	190 (51%)
Age at onset (years)	57 (13; 20–82)	NA

Data are mean (SD; range) or number (%), unless otherwise indicated. Information about sex was not available for six patients and eight controls in the Asian series, and 16 patients and 249 controls in the white series. Information about age was not available for eight patients and eight controls in the Asian series, 482 patients and 289 controls in the white series, and six patients and four controls in the Arab–Berber series. Information about the age at onset was not available for 14 patients in the Asian series and 801 patients in the white series. 71 controls in the Taiwan case–control series overlapped with a previous study of R1628P.[15] NA=not applicable.

***Table 2*: Characteristics of participants**

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E Mutez MD); **INSERM, U837, Lille, France** (Prof M-C Chartier-Harlin, E Mutez); **Department of Neurology, Laboratory of Neurogenetics, Faculty of Medicine, University of Thessaly, Larissa, Greece** (E Dardiotis MD, G M Hadjigeorgiou MD); **Institute of Biomedical Research and Technology, Centre for Research and Technology Thessaly (CERETETH), Larissa, Greece** (E Dardiotis, G M Hadjigeorgiou); **INSERM, U708, Neuroepidemiology, Paris, France** (Prof A Elbaz MD); **Université Pierre et Marie Curie-Paris 6, UMR S708, Neuroepidemiology, Paris, France** (Prof A Elbaz); **IRCCS Casa Sollievo della Sofferenza Hospital, Mendel Laboratory, San Giovanni Rotondo, Italy** (A Ferraris MD, Prof E Maria Valente MD); **Michael J Fox Foundation for Parkinson's Research, New York, NY, USA** (B Fiske PhD); **Department of Neurology, Royal Victoria Hospital, Belfast, UK** (Prof J M Gibson MD); **Research and Development, GlaxoSmithKline Pharmaceuticals, Harlow, UK** (R Gibson PhD); **Department of Neurology, Juntendo University School of Medicine, Tokyo, Japan** (Prof N Hattori MD, H Tomiyama MD); **Clinical and Molecular Epidemiology Unit, Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, Greece** (Prof J P A Ioannidis MD); **Stanford Prevention Research Center, Stanford University School of Medicine, Stanford, CA, USA** (Prof J P A Ioannidis); **Department of Neurology, Medical University of Silesia, Katowice, Poland** (B Jasinska-Myga MD, Prof G Opala MD); **Department of Neurology** (Prof B S Jeon MD) **and Department of Laboratory Medicine** (Prof S S Park MD), **Seoul National University Hospital, Seoul, South Korea; Ilsong Institute of Life Science and Department of Neurology, Hallym University, Anyang, South Korea** (Prof Y J Kim MD); **Section of Clinical and Molecular Neurogenetics at the Department of Neurology, University of Lübeck, Lübeck, Germany** (Prof C Klein MD, V Tadic MD); **Department for**

		White series				Asian series				Arab–Berber series			
	Aminoacid	MA	MAF	OR (95% CI)	p value	MA	MAF	OR (95% CI)	p value	MA	MAF	OR (95% CI)	p value
rs2256408	R50H	G	+	+	+	..	..	..	..	G	1·7%	2·05 (0·82–5·14)	0·13
rs10878245	L153L	T	39·6%	0·98 (0·91–1·06)	0·57	C	31·2%	1·04 (0·88–1·23)	0·65	C	47·1%	0·81 (0·55–1·19)	0·28
rs34594498	A419V	T	+	+	+	T	1·9%	2·27 (1·35–3·83)	0·0011	..	..	..	..
rs7308720	N551K	G	6·7%	0·88 (0·79–0·98)	0·025	G	11·9%	0·73 (0·60–0·89)	0·0017	G	8·0%	0·83 (0·49–1·39)	0·47
rs10878307	I723V	G	7·4%	0·94 (0·84–1·04)	0·23	G	1·1%	1·36 (0·74–2·49)	0·32	G	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	+	+	+	..	..	..	..	C	0·9%	0·62 (0·13–2·99)	0·55
rs7966550	L953L	C	12·8%	0·98 (0·90–1·07)	0·66	C	17·6%	0·80 (0·66–0·95)	0·012	C	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	+	+	+	G	+	+	+	G	0·5%	4·45 (0·81–24·56)	0·086
rs7133914	R1398H	A	6·6%	0·89 (0·80–0·99)	0·034	A	11·5%	0·73 (0·59–0·89)	0·0020	A	8·7%	1·00 (0·61–1·64)	1·00
rs11175964	K1423K	A	6·6%	0·83 (0·74–0·92)	0·0006	A	11·5%	0·75 (0·62–0·92)	0·0064	A	5·4%	0·42 (0·21–0·86)	0·011
rs35507033	R1514Q	A	0·9%	1·13 (0·85–1·49)	0·41	..	..	..	..	A	+	+	+
rs33958906	P1542S	T	2·8%	0·90 (0·77–1·06)	0·21	..	..	..	..	T	1·0%	2·27 (0·72–7·13)	0·16
rs1427263	G1624G	C	34·7%	1·06 (0·98–1·14)	0·15	A	46·7%	0·92 (0·77–1·11)	0·40	C	31·7%	0·96 (0·67–1·39)	0·84
rs33949390	R1628P	C	+	+	+	C	1·2%	0·62 (0·36–1·07)	0·087	..	..	..	..
rs11176013	K1637K	A	45·0%	1·02 (0·94–1·11)	0·60	G	44·6%	0·96 (0·80–1·16)	0·68	A	46·0%	1·07 (0·70–1·63)	0·76
rs35303786	M1646T	C	1·6%	1·43 (1·15–1·78)	0·0012	..	..	..	..	C	+	+	+
rs11564148	S1647T	A	29·9%	0·93 (0·86–1·00)	0·048	A	28·3%	0·97 (0·82–1·15)	0·73	A	27·6%	0·81 (0·55–1·19)	0·29
rs10878731	G1819G	T	45·2%	1·06 (0·98–1·15)	0·16	C	43·3%	0·99 (0·83–1·19)	0·95	T	46·2%	1·07 (0·70–1·64)	0·75
rs33995883	N2081D	G	2·6%	1·24 (1·05–1·47)	0·013	G	+	+	+	G	4·7%	0·92 (0·49–1·73)	0·79
rs10878405	E2108E	A	31·4%	0·96 (0·89–1·03)	0·27	A	29·6%	1·01 (0·85–1·20)	0·92	A	28·1%	0·75 (0·51–1·10)	0·14
rs35658131	Y2189C	G	+	+	+	..	..	..	..	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	G	1·8%	0·96 (0·62–1·49)	0·85	G	8·4%	1·14 (0·7–0 1·83)	0·60
rs3761863	M2397T	C	34·4%	1·06 (0·98–1·14)	0·17	C	43·9%	0·88 (0·73–1·05)	0·16	C	39·8%	1·33 (0·85–2·07)	0·21

ORs and p values result from logistic regression models, where adjustment was made for the site in the Asian and white series. ORs correspond to the presence of the MA. After adjustment for multiple testing, p≤0·0038 was judged to be significant in the Asian series, and p≤0·0033 was judged to be significant in the white series. No adjustment for multiple testing was made in the Arab–Berber series, for which p≤0·05 was judged to be significant. MA=minor allele. MAF=MA frequency. OR=odds ratio. +=a variant with a MAF of less than 0·5% and therefore not included in the logistic regression analysis. ··=a variant not noted in the series.

***Table 3*: Common single *LRRK2* variant associations with Parkinson's disease**

Table 3 shows the results of the disease-association analysis of single *LRRK2* variants. In the white series, significant associations with PD were noted for K1423K and M1646T. Figure 1 shows the country-specific ORs and 95% CIs for the risk factor M1646T. The between-site heterogeneity was low for M1646T (I^2=0%, p=0·44) and moderate for K1423K (I^2=34%, p=0·069) in the white series.

In the Asian series, significant associations with PD were noted for LRRK2 A419V, N551K, R1398H, and G2385R (table 3). Figure 2 and figure 3 show the country-specific ORs and 95% CIs for A419V and G2385R, and for the N551K-R1398H-K1423K haplotype; between-site heterogeneity was very low for each of these associations in the Asian series (all I^2=0%, all p≥0·42, webappendix p 18). Notably, LRRK2 R1628P was not associated with PD in the Asian series (table 3), with a non-significant protective effect noted for this variant in the Taiwanese series (minor allele frequency 3·8%, OR 0·56, 95% CI 0·32–1·01; p=0·054). Although not significant, the predicted risk effect for R1628P was noted in the South Korean series, particularly at the Seoul site (0·2%, 2·47, 0·28–22·15; p=0·42). R1628P was not noted in the Japanese series. The previously suggested association of S1647T with PD in Asian populations[14] was not supported by the results of our study (0·97, 0·82–1·15; p=0·73).

In an exploratory analysis of the small Arab–Berber series, significant associations (p≤0·05, without correction for multiple testing) with PD were noted for K1423K and Y2189C (table 3). Larger Arab–Berber series are needed to confirm these associations.

For patients with available information (95%), results for the analysis of the association of single variants with disease in each series remained similar after adjustment for age and sex (webappendix p 19) and by use of an additive model (webappendix p 20). Effect sizes were also similar after simultaneous adjustment for other variants that were significantly associated with PD in a particular series, and after adjustment for R1628P in the Asian series in which a previous association had been shown (webappendix p 21), providing evidence that these associations are independent of one another. With a random-effects model for the white and Asian series, results were generally similar though slightly weaker (webappendix p 18) than those obtained with a fixed-effects model.

Haplotype analysis showed a significant overall association with disease in the series of white (p=0·0016) and Asian (p=2×1^{-24}) individuals, but was non-significant in the Arab–Berber series (p=0·056). Haplotype associations seemed to be attributable to the variants independently implicated in disease (webappendix pp 22–24). When the three series were assessed together, LRRK2 N551K, R1398H, and K1423K, which are in strong linkage disequilibrium and constitute a common (>5% frequency) haplotype, were associated with a protective effect (combined OR 0·82, 95% CI 0·72–0·94; p=0·0043; figure 3).

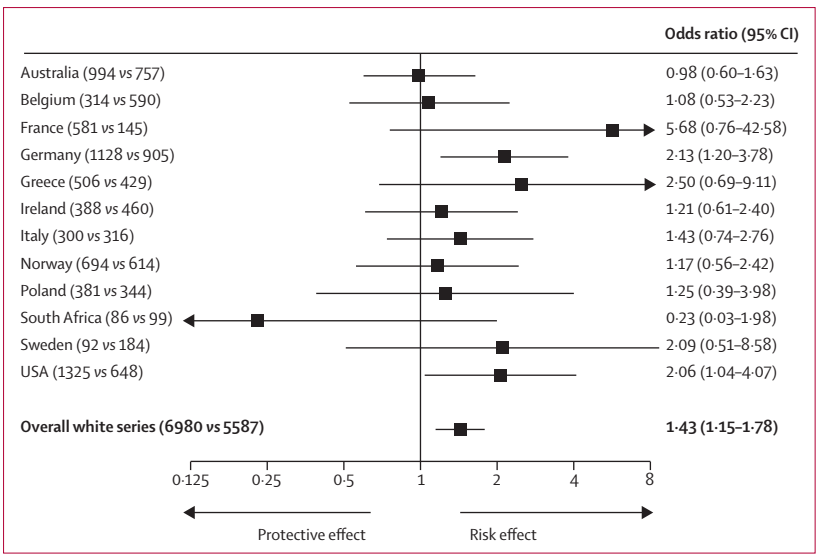

Figure 1: **Forest plot of LRRK2 variant M1646T in individuals with versus without Parkinson's disease in the white series**

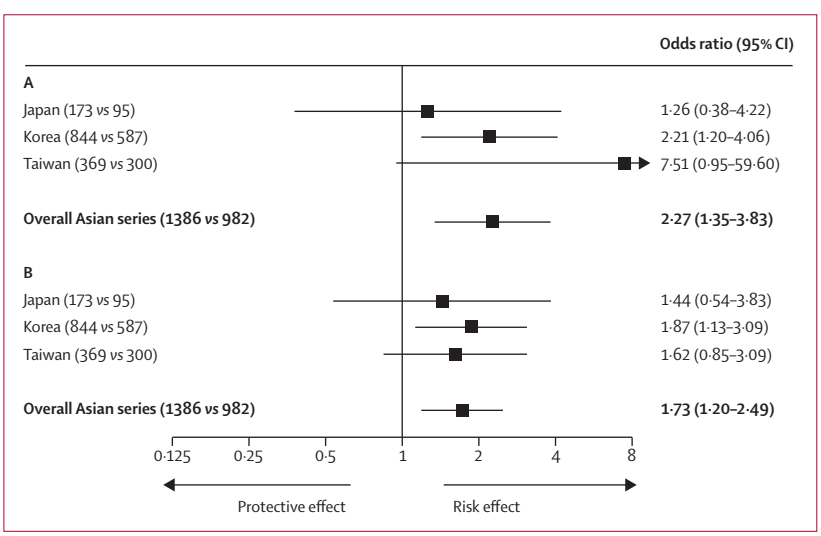

Figure 2: **Forest plots of LRRK2 variants A419V (A) and G2385R (B) in individuals with versus without Parkinson's disease in the Asian series**

Results of all common single variant associations with age at onset are shown on webappendix p 25. We did not identify any associations that withstood multiple testing correction in the white and Asian series. In the Arab–Berber series, L153L was associated with age at onset roughly 4 years earlier (p=0·038), which needs confirmation in larger samples.

Table 4 provides a descriptive summary of rare variants (minor allele frequency <0·5%) in patients and controls in each series. The pathogenic variant R1441H was noted in an Asian patient, R1441C in only ten patients from the white series, and G2019S in all three series (table 4). The

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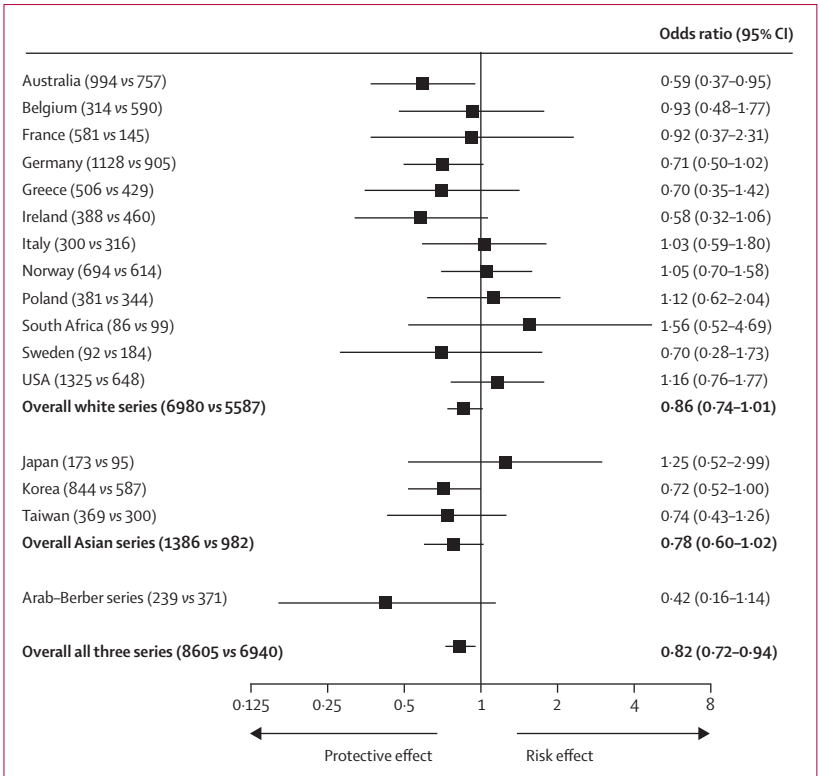

Figure 3: **Forest plot of protective LRRK2 haplotype N551K-R1398H-K1423K in individuals with versus without Parkinson's disease in the white, Asian, and Arab–Berber series**

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median age of the eight control carriers of G2019S was 64 years (range 48–76 years). 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 from the analysis. Other possible rare risk variants (E334K, R1325Q, and T1410M) and protective variants (A221V and A1151T) with differences in frequency between patients with PD and controls were noted. When data for all rare variants were combined, the presence of any rare variant was not associated with PD in the white series (OR 1·01, 95% CI 0·81–1·25; p=0·95), Asian series (1·03, 0·57–1·85; p=0·92), or Arab–Berber series (0·78, 0·28–2·20; p=0·64). Additionally, no association was noted in the white series (0·89, 0·55–1·43; p=0·62), Asian series (1·05, 0·37–2·99; p=0·93), or Arab–Berber series (no PD cases, two [<1%] controls, Fisher's exact p=1·00) when the data were combined only for those variants predicted by use of the SIFT program to be not tolerated.[24] Webappendix p 26 provides a summary of variants for which there were no carriers in any of the three series.

Discussion

The results of our study, one of the largest so far of the genetics of PD, show that a single gene, *LRRK2*, harbours many rare and common variants that confer susceptibility to PD in diverse populations (panel). Although population stratification is an inherent caveat of this type of large-scale collaborative effort (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 variations on gene loci that have a major effect in shaping both familial and sporadic disease.

About a third of variants we assessed were not identified in any study participant. These included four previously documented pathogenic mutations (LRRK2 N1437H, R1441G, Y1699C, and I2020T), showing that they are rare mutations in the population samples we assessed. 26 variants were recorded at a frequency greater than 0·5% in any of the three series, and only 13 were noted at a frequency greater than 0·5% in all three series. This finding draws attention to the importance of studying genetic variability in large samples and in different ethnic groups, because frequencies and genetic effects might vary substantially.[26]

The newly identified associations warrant further discussion. M1646T in the COR (C-terminal of Ras) domain of LRRK2 was identified in the white series, and the effect was consistent in many countries (figure 1). This variant was not identified in participants of Asian descent and was rare in the series of Arab–Berber participants. LRRK2 A419V was consistently more common in patients than in controls in Asian sites (figure 2). Although we cannot exclude the possibility of a non-coding element in linkage disequilibrium, the N-terminal region of the protein seems functionally relevant to disease development. LRRK2 M1646T is the first common-risk factor to have been identified in white populations, whereas A419V is now the third risk factor reported to be specific to individuals of Asian ancestry, along with R1628P and G2385R.[12,14,15] LRRK2 R1628P was not significantly associated with risk in our Asian series. This variant was common only in the Taiwanese series, in which a non-significant protective effect was noted. Our inability to replicate the previously reported risk effect of R1628P is likely to be due to a combination of the low frequency of this variant, natural sampling variation, and population heterogeneity, in view of the results of previous studies of ethnic Han Chinese populations (of note, G2385R did show association).[14,15]

The identification of a common three-variant haplotype (N551K-R1398H-K1423K) that seems to act in a protective manner (figure 3) is also important. It suggests that the reduced penetrance that is noted in patients with *LRRK2*-associated parkinsonism might be due to variants acting in cis or trans with the pathogenic variant and that LRRK2 activity can be exploited to modify symptom onset in patients. Any future therapeutic strategies that lower risk in *LRRK2*-associated parkinsonism might protect against symptomatic onset in idiopathic PD.[14,27] The previous report[14] of a protective effect with N551K and R1398H showed a reduced kinase

		White series		Asian series		Arab–Berber series	
	Aminoacid	Patients (n=6995)	Controls (n=5595)	Patients (n=1376)	Controls (n=962)	Patients (n=240)	Controls (n=372)
rs2256408	R50H	7 (0·10%)	1 (0·02%)	..	..	+	+
rs75054132	A75A	..	..	..	..	0	1 (0·27%)
rs33995463	L119P	21 (0·31%)	23 (0·44%)	..	..	0	2 (0·55%)
rs41286468	L122L	5 (0·08%)	7 (0·13%)	..	..	..	..
rs112794616	A211V	4 (0·06%)	11 (0·21%)	..	..	0	1 (0·27%)
rs56108242	C228S	2 (0·03%)	2 (0·04%)	..	..	..	..
rs28365216	N238I	..	..	3 (0·22%)	2 (0·22%)	..	..
rs72546315	H275H	3 (0·04%)	2 (0·04%)	..	..	1 (0·43%)	0
rs17490713	N289N	1 (0·01%)	2 (0·04%)	..	..	NA	NA
rs41286466	A312A	26 (0·38%)	15 (0·28%)	1 (0·7%)	0	0	4 (1·10%)
rs78501232	E334K	14 (0·21%)	4 (0·07%)	..	..	..	..
rs113065049	V366M	1 (0·02%)	0	..	..	..	..
rs34594498	A419V	5 (0·07%)	3 (0·06%)	+	+	..	..
rs35847451	S416S	12 (0·18%)	16 (0·29%)	..	..	..	..
rs75711334	L488L	1 (0·01%)	0	..	..	..	..
rs79996249	K544E	2 (0·03%)	2 (0·04%)	..	..	..	..
rs78154388	S663P	2 (0·03%)	2 (0·04%)	..	..	..	..
rs72546319	V674V	0	2 (0·04%)	..	..	0	1 (0·27%)
rs58559150	Q923H	1 (0·01%)	2 (0·04%)	..	..	+	+
rs75148313	S973N	1 (0·01%)	2 (0·04%)	..	..	..	..
rs113217062	I1006M	1 (0·01%)	0	..	..	..	..
rs76535406	S1096C	0	2 (0·04%)	..	..	..	..
rs35808389	L1114L	5 (0·07%)	1 (0·02%)	..	..	..	..
rs74985840	A1151T	1 (0·01%)	5 (0·09%)	..	..	..	..
rs80179604	S1228T	5 (0·07%)	4 (0·07%)	..	..	..	..
rs4640000	P1262A	1 (0·01%)	1 (0·02%)	..	..	NA	NA
rs72546338	R1325Q	10 (0·15%)	3 (0·06%)	4 (0·29%)	1 (0·11%)	..	..
rs17466213	I1371V	7 (0·10%)	4 (0·07%)	1 (0·07%)	0	+	+
rs72546327	T1410M	5 (0·07%)	1 (0·02%)	..	..	..	..
rs113589830	D1420N	1 (0·01%)	0	..	..	..	..
rs111435410	A1430A	2 (0·03%)	1 (0·02%)	..	..	..	..
rs112998035	R1441R	..	..	1 (0·07%)	0	..	..
rs33939927*	R1441C	10 (0·15%)	0	..	..	..	..
rs34995376*	R1441H	..	..	1 (0·07%)	0	..	..
rs74681492	P1446L	..	..	10 (0·74%)	6 (0·62%)	..	..
rs111501952	V1450I	..	..	2 (0·15%)	1 (0·11%)	..	..
rs113431708	R1483Q	1 (0·01%)	0	..	..	..	..
rs35507033	R1514Q	+	+	..	..	0	1 (0·27%)
rs33949390	R1628P	7 (0·10%)	0	+	+	..	..
rs35303786	M1646T	+	+	..	..	3 (1·25%)	2 (0·54%)
rs111503579	L1653L	2 (0·03%)	1 (0·02%)	4 (0·30%)	9 (0·93%)	..	..
rs79909111	S1721S	1 (0·02%)	1 (0·02%)	..	..	..	..
rs263192805	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 (0·71%)	3 (0·06%)	1 (0·07%)	1 (0·11%)	72 (30·25%)	4 (1·10%)
rs111739194	L2063STOP	1 (0·02%)	2 (0·04%)	..	..	..	..
rs33995883	N2081D	+	+	2 (0·15%)	0	+	+
rs34869625	G2170G	20 (0·30%)	21 (0·39%)	..	..	1 (0·60%)	0
rs35658131	Y2189C	1 (0·01%)	2 (0·04%)	..	..	+	+
rs113511708	T2356I	7 (0·1%)	5 (0·09%)	..	..	..	..

(Continues on next page)

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See **Online** for webappendix

		White series		Asian series		Arab-Berber series	
	Aminoacid	Patients (n=6995)	Controls (n=5595)	Patients (n=1376)	Controls (n=962)	Patients (n=240)	Controls (n=372)
(Continued from previous page)							
rs79546190	V2390M	1 (0·01%)	1 (0·02%)	..	..	..	..
rs78964014	E2395K	1 (0·01%)	0	..	..	..	..
rs60545352	M2408I	1 (0·01%)	0	..	..	0	2 (0·54%)

Data are number (%). PD=Parkinson's disease. +=a variant that was noted with a minor allele frequency of at least 0·5% and as such was analysed as a common variant. ..=a variant that was not noted in the series. NA=a variant that was out of the Hardy–Weinberg equilibrium in the specific series. *Pathogenic variants for which the number (%) of carriers is summarised for the entire sample; any carriers of these pathogenic variants were removed from the summaries provided for each of the remaining non-pathogenic variants.

***Table 4:* *LRRK2* rare variants**

activity for the R1398H variant, suggesting this Roc domain substitution might be the most likely functional allele on the haplotype.

Although the results of our study have identified an association of PD only with common variants, they also draw attention to the many rare variants in *LRRK2* that could contribute to disease risk. Genetic loci that contribute to disease risk might do so through variants that span the whole range of minor allele frequencies, from rare mutations to frequent single nucleotide polymorphisms.[28] Despite the very large sample size, we noted only three of seven previously described pathogenic *LRRK2* mutations. Hence, the search for mutations contributing to familial PD should include an analysis of single pedigrees, with further assessment in very large population studies. Single pedigrees might result in some false-positive results, which can be filtered out with large population samples. For example, two variants (I1371V and T2356I) have been proposed as pathogenic and to account for the clinical and functional features of *LRRK2*-associated parkinsonism.[29,30] However, in our study, both variants were noted in patients and controls at the same frequency (table 4). Conversely, we noted other possible rare risk (E334K, R1325Q, and T1410M) and protective (A211V and A1151T) variants; however, because of their low frequency, large meta-analytical approaches are necessary to define their roles fully.

In this study, we focused on exonic variants because all pathogenic variants identified in *LRRK2* so far have been single nucleotide missense changes. However, silent, synonymous variants were also included because they can result in alternative splicing and, since protein translation is a function of codon use and transfer RNA abundance, could affect the rate of protein domain folding and secondary modifications.[31] Neither copy number variants nor other risk factors in non-coding regions that regulate *LRRK2* expression or alter splicing were assessed in our study.

As new loci for susceptibility to diverse diseases are continuously being discovered in genome-wide association and whole-genome sequencing studies, the results of our study show the importance of revisiting loci at which rare or common variants have been identified, since they could harbour many more independent signals of genetic risk in different populations.[25,32,33] Furthermore, *LRRK2* sequencing studies in under-represented populations (eg, from South America, sub-Saharan Africa, Middle East, and western Asia) will undoubtedly show novel ethnic-group-specific risk variants and could clarify the role of variants that were rare or absent in our study. *LRRK2* variants, including novel exonic variants, were reported as part of the 1000 Genome Project, lending support to this hypothesis.[34]

Large-scale parallel resequencing (targeted genomic capture of the specific regions—eg, gene-specific, exome, transcriptome, and whole-genome sequencing) is likely

***Panel:* Research in context**

Systematic review

We searched PubMed with the terms "*LRRK2*" and "Genetics Parkinson's disease" and identified all *LRRK2* coding variations reported up until April 1, 2010. We also contacted our global network of collaborators and the members of the Genetic Epidemiology Of Parkinson's Disease (PD) Consortium for unreported variants.

Interpretation

By focusing on the role of *LRRK2* variation in PD, we have identified a common risk factor in the white population (M1646T), the third common risk factor in Asian populations (A419V), and a common global protective haplotype (N551K-R1398H-K1423K). This work complements the meta-analysis of PD genome-wide association,[25] which suggests a possible association at the *LRRK2* locus. We define some of the genetic variation that is likely to be contributing to the association noted in recent genome-wide association efforts and nominate potential functionally and clinically relevant variants. We show modulation of the underlying toxic effect is possible because of the protective nature of the N551K-R1398H-K1423K haplotype. The identification of common variants that affect risk clearly shows a greater role for *LRRK2* in idiopathic disease than previously thought.

to identify many more variants in candidate genes that might predispose to PD. Characterisation of each variant will require this type of collaborative international effort to define their pathogenicity, frequency in different populations, and contribution to disease pathogenesis through genotype–phenotype assessment.

Contributors

OAR and MJF were the principal investigators and were responsible for the concept and design of the study. AIS-O, JAB, OAR, and CVG were responsible for the technical aspects of the study. MGH and NND were responsible for all the analyses; OAR and MJF were responsible for drafting the report. All authors participated in study design and approach, sample collection, data acquisition, and critical revision and final approval of the report.

Conflicts of interest

JOA, MJF, and ZKW report holding a patent on *LRRK2* genetic variability and MJF has received royalties for licensing of genetically modified *LRRK2* mouse models. DMM declares a patent pending entitled *Methods to treat PD*. CK and RK declare receiving payment in their role as consultants for Centogene and Takeda Pharmaceutical, respectively. All other authors declare that they have no conflicts of interest.

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A full list of GEO-PD consortia is provided in webappendix pp 27–30.

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