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Original Article

GWAS risk factors in Parkinson's disease: *LRRK2* coding variation and genetic interaction with *PARK16*

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Abstract: Parkinson's disease (PD) is a multifactorial movement disorder characterized by progressive neurodegeneration. Genome-wide association studies (GWAS) have nominated over fifteen distinct loci associated with risk of PD, however the biological mechanisms by which these loci influence disease risk are mostly unknown. GWAS are only the first step in the identification of disease genes: the specific causal variants responsible for the risk within the associated loci and the interactions between them must be identified to fully comprehend their impact on the development of PD. In the present study, we first attempted to replicate the association signals of 17 PD GWAS loci in our series of 1381 patients with PD and 1328 controls. *BST1*, *SNCA, HLA-DRA*, *CCDC62/HIP1R* and *MAPT* all showed a significant association with PD under different models of inheritance and *LRRK2* showed a suggestive association. We then examined the role of coding *LRRK2* variants in the GWAS association signal for that gene. The previously identified LRRK2 risk mutant p.M1646T and protective haplotype p.N551K-R1398H-K1423K did not explain the association signal of *LRRK2* in our series. Finally, we investigated the gene-gene interaction between *PARK16* and *LRRK2* that has previously been proposed. We observed no interaction between *PARK16* and *LRRK2* GWAS variants, but did observe a non-significant trend toward interaction between *PARK16* and *LRRK2* variants within the protective haplotype. Identification of causal variants and the interactions between them is the crucial next step in making biological sense of the massive amount of data generated by GWAS studies. Future studies combining larger sample sizes will undoubtedly shed light on the complex molecular interplay leading to the development of PD.

Keywords: Association studies in genetics, Parkinson's disease/Parkinsonism

Introduction

Genome-wide association studies (GWAS) heralded a new era in the resolution of common genetic risk factors in disease. Parkinson's disease (PD) was long considered the archetypal age-related sporadic disorder with a minimal genetic component. Early GWAS in PD highlighted a known risk locus, *SNCA*, with other candidates failing to replicate [1-3]. However, as the field has progressed with improved analysis platforms and larger patient-control series, a number of novel candidate loci have been proposed including two other familial parkinsonism genes, *MAPT* and *LRRK2* [4-7].

In a recent study by the International Parkinson Disease Genomics Consortium (IPDGC), six pre-

Table 1. Patient characteristics

Variable	Patients with PD	Controls
Irish series	N=362	N=370
Age	58±12 (32 - 87)	66±22 (17 - 97)
Gender		
Male	203 (56%)	134 (36%)
Female	159 (44%)	236 (64%)
Age at PD onset	59±11 (18 - 87)	N/A
US series	N=674	N=724
Age	69±11 (33 - 97)	65±13 (18 - 88)
Gender		
Male	427 (63%)	300 (41%)
Female	247 (37%)	424 (59%)
Age at PD onset	64±12 (28 - 94)	N/A
Polish series	N=345	N=234
Age	65±11 (29 - 88)	56±15 (19 - 96)
Gender		
Male	216 (63%)	126 (54%)
Female	129 (37%)	108 (46%)
Age at PD onset	58±11 (25 - 81)	NA
Combined series	N=1,381	N=1,328
Age	65±12 (29 - 97)	64±17 (17 - 97)
Gender		
Male	846 (61%)	560 (42%)
Female	535 (39%)	768 (58%)
Age at PD onset	61±12 (18 - 94)	NA

The sample mean ± SD (minimum - maximum) is given for age and age at PD onset. Information was unavailable regarding age at PD onset for 94 patients with PD in the Irish series and for 25 patients with PD in the Polish series.

viously reported and five novel loci were nominated for association with PD via a meta-analysis of five datasets from published GWAS in US and European populations [8]. Subsequent meta-analytical studies have nominated a number of additional loci [9-12]. For example Lill and colleagues used data collected via the PDgene.org website, while Do et al. performed a GWAS study using information collected through the 23andMe personal genomics company [10, 11]. Further investigation and replication of the original IPDGC dataset and combined analysis with Do et al. nominated a further five loci [9].

Nomination of genomic regions of association (confirmed by independent replication) is the first step in disease-related gene identification. However, the regions of association are generally large and it is critical that the causal variant(s) responsible for the association signal is identified to provide diagnostic biomarkers, mechanistic insights and rational drug targets. Our recent investigation of the *LRRK2* locus highlighted one coding variant conferring risk to PD (p.M1646T) in Caucasians as well as a protective haplotype p.N551K-R1398H-K1423K [13]. In addition, it is important to not only identify risk loci but also to understand the joint effects and interaction of the individual associations [14]. Therefore herein, we attempt to replicate single variant association from GWAS loci, we investigate whether the previously nominated functional *LRRK2* coding variants account for a proportion of the *LRRK2* GWAS signal, and finally we examine if *LRRK2* variation interacts with the associated *PARK16* SNP as recently reported [15].

Methods

Study subjects

A total of 1,381 patients with PD and 1,328 controls from a US series (674 patients, 724 controls), an Irish series (362 patients, 370 controls), and a Polish series (345 patients, 234 controls) were included in this case-control study. Characteristics of patients with PD and controls are summarized in **Table 1** for each series. Patients were diagnosed with PD using standard criteria. Controls were individuals free of PD or a related movement disorder at the time of examination. All subjects were unrelated within and between diagnosis groups. The Mayo Clinic Institutional Review Board approved the study, each individual site received local IRB approval, and all subjects provided informed consent.

Variant and genotype information

Seventeen variants that have been previously nominated for association with PD in GWAS were genotyped in this study [6, 8-12]; a summary of these variants is provided in **Table 2**. Genomic DNA extracted from peripheral blood lymphocytes using the Autogen FlexStar (Holliston, MA.) was used for genotyping. GWAS loci genotyping was performed using the Sequenom iPlex platform and data acquisition was obtained using Typer 4.0 software (Sequenom, San Diego, CA). *LRRK2* coding variants were genotyped using a combination of the Sequenom iPlex platform and bidirectional

Table 2. Variants included due to previous associations with PD in GWAS

Variant	Chromosome	Position (bp)[a]	Candidate Gene	MAF in current study
rs2230288	1q22	155206167	*GBA*	1.7%
rs34372695[b]	1q22	156030037	*SYT11*	1.8%
rs708723	1q32	205739266	*PARK16*	32.3%
rs10928513[b]	2q21	135456759	*ACMSD*	44.8%
rs2102808[b]	2q24	169117025	*STK39*	13.7%
rs11711441[b]	3q27	182821275	*MCCC1/LAMP3*	12.2%
rs6599388[b]	4p16	939087	*GAK*	30.7%
rs11724635[b]	4p15	15737101	*BST1*	44.4%
rs6812193	4q21	77198986	*STBD1/SCARB2*	36.5%
rs356219[b]	4q22	90637601	*SNCA*	40.2%
rs3129882[b,c]	6p21	32409530	*HLA*	42.0%
rs156429	7p15	23306020	*GPNMB*	39.0%
rs7077361	10p13	15561543	*ITGA8*	12.5%
rs1491942[b]	12q12	40620808	*LRRK2*	21.3%
rs10847864[b]	12q24	123326598	*CCDC62/HIP1R*	35.1%
rs2942168[b]	17q21	43714850	*MAPT*	19.4%
rs12456492	18q12	40673380	*RIT2*	33.0%

[a]Chromosomal positions are based on the February 2009 (GRCH37/hg19) genome assembly. MAF=minor allele frequency. [b]Indicates a variant examined in a previous larger study by Sharma et al.[16] that included many of the same subjects utilized in the current study. [c]The association of *HLA* rs3129882 with PD has been previously reported in essentially the same patient-control group (~97% overlap) utilized in the current study[17]; these results are reported again in the current study in order to display a more complete replication of GWAS risk factors for PD.

Sanger sequencing approaches; primer sequences are available upon request.

Genotype data for 11 of these variants was included in a previous consortium replication effort of 8,750 patients and 8,955 controls[16] that involved many of the same subjects included in the current study; the analysis of these 11 variants in our study differs from this previous study in that dominant and recessive statistical models were utilized in addition to the additive models utilized in the previous study (see Statistical analysis section). One of these 11 variants, *HLA* rs3129882, has previously been genotyped and assessed for association with PD in essentially the same patient-control group (~97% overlap) utilized in the current study[17]; these results are reported again in the current study in order to display a more complete replication of GWAS risk factors for PD. The remaining 6 GWAS PD risk factors have not been previously reported in the patients or controls utilized in this study.

LRRK2 variants in the p.N551K-R1398H-K1423K protective haplotype were also genotyped, as was the LRRK2 risk substitution p.M1646T; the majority of the patients and controls utilized in the current study were also included in the aforementioned larger original study nominating these *LRRK2* variants for association with PD[13]. In each of the three individual series, all genotype call rates were >95% and there was no evidence of any departures from Hardy Weinberg Equilibrium in study controls (all P≥0.05 after Bonferroni correction).

Statistical analysis

For each individual series and the combined series, associations of GWAS-nominated variants with PD were evaluated using logistic regression models adjusted for age, gender, and series (combined series only). Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated. We considered each GWAS-nominated variant under an additive model (effect of each additional minor allele), a dominant model (presence vs. absence of the minor allele), and a recessive model (presence vs. absence of two copies of the minor allele). Associations of LRRK2 p.N551K, p.R1398H, p.K1423K, and p.M1646T with PD in the combined series were evaluated using logistic

Table 3A. Single variant associations with PD in the combined series (1,381 patients with PD, 1,328 controls)

			Additive model		Dominant model		Recessive model	
Variant	Minor allele	MAF	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
rs2230288	T	1.7%	1.75 (1.12 - 2.74)	0.014	1.75 (1.12 - 2.74)	0.014	N/A	N/A
rs34372695	T	1.8%	1.25 (0.82 - 1.90)	0.30	1.25 (0.82 - 1.90)	0.30	N/A	N/A
rs708723	C	32.3%	0.88 (0.79 - 0.98)	0.023	0.83 (0.70 - 0.98)	0.028	0.86 (0.71 - 1.05)	0.15
rs10928513	T	44.8%	1.12 (1.00 - 1.25)	0.046	1.16 (0.98 - 1.37)	0.089	1.17 (0.96 - 1.41)	0.12
rs2102808	T	13.7%	1.24 (1.06 - 1.46)	0.0079	1.24 (1.03 - 1.47)	0.020	1.90 (1.01 - 3.54)	0.045
rs11711441	A	12.2%	0.84 (0.71 - 0.99)	0.036	0.84 (0.70 - 1.01)	0.072	0.56 (0.29 - 1.08)	0.083
rs6599388	T	30.7%	1.06 (0.94 - 1.19)	0.35	0.98 (0.84 - 1.15)	0.83	1.38 (1.06 - 1.80)	0.015
rs11724635	C	44.4%	0.83 (0.74 - 0.92)	0.0006	0.86 (0.73 - 1.02)	0.082	0.67 (0.55 - 0.82)	5.9×10^{-5}
rs6812193	T	36.5%	0.85 (0.76 - 0.96)	0.0065	0.84 (0.72 - 0.99)	0.034	0.76 (0.60 - 0.95)	0.017
rs356219	G	40.2%	1.44 (1.29 - 1.61)	1.8×10^{-10}	1.67 (1.42 - 1.96)	6.3×10^{-10}	1.54 (1.25 - 1.90)	4.9×10^{-5}
rs3129882[a]	G	42.0%	0.93 (0.83 - 1.04)	0.21	1.07 (0.91 - 1.27)	0.40	0.70 (0.57 - 0.86)	0.0008
rs156429	G	39.0%	1.00 (0.90 - 1.12)	0.93	1.00 (0.85 - 1.17)	0.99	1.02 (0.82 - 1.26)	0.85
rs7077361	C	12.5%	0.87 (0.74 - 1.03)	0.10	0.87 (0.72 - 1.04)	0.14	0.71 (0.38 - 1.33)	0.29
rs1491942	C	21.3%	1.17 (1.02 - 1.34)	0.023	1.12 (0.96 - 1.31)	0.16	1.82 (1.23 - 2.68)	0.0022
rs10847864	T	35.1%	1.20 (1.07 - 1.35)	0.0023	1.34 (1.14 - 1.56)	0.0004	1.12 (0.88 - 1.42)	0.36
rs2942168	A	19.4%	0.72 (0.63 - 0.83)	2.9×10^{-6}	0.68 (0.58 - 0.80)	4.4×10^{-6}	0.62 (0.43 - 0.91)	0.014
rs12456492	G	33.0%	1.03 (0.92 - 1.16)	0.58	1.07 (0.91 - 1.25)	0.40	0.98 (0.76 - 1.26)	0.85

ORs, 95% CIs, and *p*-values result from logistic regression models adjusted for age, gender, and series. ORs correspond to an additional minor allele (additive models), presence of the minor allele (dominant models), and presence of two copies of the minor allele (recessive models). N/A is given for variants with no homozygotes of the minor allele in either patients with PD or controls. [a]The association of *HLA* rs3129882 with PD has been previously reported in essentially the same patient-control group (~97% overlap) utilized in the current study[17]; these results are reported again in the current study in order to display a more complete replication of GWAS risk factors for PD. MAF=minor allele frequency; OR=odds ratio; CI=confidence interval.

Table 3B. Single variant associations with PD in the Irish series (362 patients with PD, 370 controls)

			Additive model		Dominant model		Recessive model	
Variant	Minor allele	MAF	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
rs2230288	T	1.8%	4.29 (1.58 - 11.67)	0.0043	4.29 (1.58 - 11.67)	0.0043	N/A	N/A
rs34372695	T	1.5%	2.42 (0.90 - 6.51)	0.079	2.42 (0.90 - 6.51)	0.079	N/A	N/A
rs708723	C	44.7%	0.82 (0.66 - 1.01)	0.066	0.68 (0.49 - 0.95)	0.024	0.88 (0.60 - 1.29)	0.52
rs10928513	T	42.5%	0.84 (0.68 - 1.04)	0.10	0.86 (0.63 - 1.19)	0.37	0.69 (0.47 - 1.02)	0.060
rs2102808	T	14.0%	1.18 (0.87 - 1.59)	0.30	1.17 (0.83 - 1.66)	0.37	1.53 (0.56 - 4.17)	0.40
rs11711441	A	10.8%	0.63 (0.45 - 0.90)	0.011	0.65 (0.44 - 0.95)	0.028	0.20 (0.04 - 0.96)	0.045
rs6599388	T	30.7%	1.02 (0.81 - 1.28)	0.85	0.89 (0.66 - 1.21)	0.46	1.53 (0.92 - 2.54)	0.098
rs11724635	C	44.4%	0.78 (0.63 - 0.96)	0.019	0.68 (0.49 - 0.94)	0.021	0.75 (0.51 - 1.09)	0.13
rs6812193	T	38.2%	0.88 (0.71 - 1.09)	0.25	0.91 (0.67 - 1.25)	0.57	0.73 (0.48 - 1.12)	0.15
rs356219	G	42.1%	1.79 (1.44 - 2.24)	2.7×10^{-7}	2.38 (1.71 - 3.31)	2.7×10^{-7}	1.90 (1.27 - 2.83)	0.0016
rs3129882	G	38.2%	0.87 (0.69 - 1.09)	0.23	1.02 (0.75 - 1.41)	0.88	0.55 (0.35 - 0.87)	0.011
rs156429	G	37.3%	0.97 (0.77 - 1.21)	0.78	1.06 (0.77 - 1.44)	0.73	0.79 (0.50 - 1.23)	0.30
rs7077361	C	12.1%	1.15 (0.82 - 1.61)	0.43	1.17 (0.82 - 1.67)	0.40	0.93 (0.17 – 5.00)	0.93
rs1491942	C	20.8%	1.21 (0.93 - 1.58)	0.15	1.18 (0.86 - 1.61)	0.30	1.87 (0.87 - 4.04)	0.11
rs10847864	T	36.9%	1.08 (0.86 - 1.35)	0.51	1.11 (0.81 - 1.52)	0.51	1.09 (0.70 - 1.71)	0.70
rs2942168	A	19.1%	0.63 (0.48 - 0.83)	0.0009	0.59 (0.43 - 0.82)	0.0016	0.46 (0.22 – 1.00)	0.050
rs12456492	G	32.3%	0.93 (0.74 - 1.18)	0.54	1.03 (0.75 - 1.40)	0.86	0.66 (0.39 - 1.10)	0.11

ORs, 95% CIs, and *p*-values result from logistic regression models adjusted for age and gender. ORs correspond to an additional minor allele (additive models), presence of the minor allele (dominant models), and presence of two copies of the minor allele (recessive models). N/A is given for variants with no homozygotes of the minor allele in either patients with PD or controls. MAF=minor allele frequency; OR=odds ratio; CI=confidence interval.

regression models adjusted for age, gender, and series, where each variant was considered under a dominant model owing to the small number of homozygotes of the minor allele. Haplotype analysis was performed using a score test for association [16].

In the combined series, we examined the degree of linkage disequilibrium of *LRRK2* rs1491942 with the three variants in the protective LRRK2 p.N551K-R1398H-K1423K haplotype and also with LRRK2 p.M1646T by estimating r^2 values in study controls; the asso-

Table 3C. Single variant associations with PD in the US series (674 patients with PD, 724 controls)

			Additive model		Dominant model		Recessive model	
Variant	Minor allele	MAF	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
rs2230288	T	1.9%	1.16 (0.65 - 2.06)	0.62	1.16 (0.65 - 2.06)	0.62	N/A	N/A
rs34372695	T	2.2%	0.94 (0.55 - 1.61)	0.83	0.94 (0.55 - 1.61)	0.83	N/A	N/A
rs708723	C	44.2%	0.99 (0.85 - 1.16)	0.92	1.00 (0.79 - 1.26)	0.97	0.98 (0.75 - 1.29)	0.90
rs10928513	T	43.7%	1.12 (0.96 - 1.30)	0.15	1.13 (0.90 - 1.43)	0.29	1.20 (0.92 - 1.57)	0.19
rs2102808	T	13.3%	1.28 (1.02 - 1.60)	0.036	1.28 (0.99 - 1.64)	0.056	1.82 (0.77 - 4.31)	0.18
rs11711441	A	13.2%	0.93 (0.74 - 1.17)	0.53	0.92 (0.71 - 1.18)	0.51	0.96 (0.41 - 2.25)	0.92
rs6599388	T	29.3%	1.08 (0.91 - 1.27)	0.39	1.04 (0.83 - 1.29)	0.74	1.32 (0.90 - 1.93)	0.16
rs11724635	C	44.8%	0.85 (0.72 - 0.99)	0.036	0.86 (0.68 - 1.09)	0.22	0.73 (0.55 - 0.96)	0.024
rs6812193	T	36.1%	0.85 (0.73 – 1.00)	0.057	0.85 (0.68 - 1.06)	0.15	0.75 (0.54 - 1.04)	0.088
rs356219	G	39.6%	1.29 (1.10 - 1.51)	0.0014	1.50 (1.19 - 1.88)	0.0005	1.25 (0.93 - 1.68)	0.13
rs3129882	G	41.2%	1.01 (0.87 - 1.19)	0.86	1.07 (0.85 - 1.34)	0.57	0.95 (0.71 - 1.26)	0.71
rs156429	G	40.2%	1.00 (0.85 - 1.17)	0.96	0.92 (0.74 - 1.16)	0.49	1.13 (0.84 - 1.51)	0.42
rs7077361	C	13.3%	0.76 (0.60 - 0.96)	0.019	0.76 (0.59 - 0.98)	0.033	0.51 (0.22 - 1.21)	0.13
rs1491942	C	21.6%	1.16 (0.96 - 1.40)	0.11	1.08 (0.86 - 1.35)	0.51	2.19 (1.27 - 3.78)	0.0047
rs10847864	T	34.5%	1.37 (1.16 - 1.62)	0.0002	1.65 (1.31 - 2.07)	1.5×10^{-5}	1.19 (0.84 - 1.68)	0.34
rs2942168	A	21.6%	0.66 (0.55 - 0.80)	1.6×10^{-5}	0.58 (0.46 - 0.73)	2.9×10^{-6}	0.72 (0.44 - 1.17)	0.19
rs12456492	G	31.8%	1.07 (0.90 - 1.26)	0.46	1.02 (0.82 - 1.27)	0.84	1.28 (0.88 - 1.85)	0.19

ORs, 95% CIs, and *p*-values result from logistic regression models adjusted for age and gender. ORs correspond to an additional minor allele (additive models), presence of the minor allele (dominant models), and presence of two copies of the minor allele (recessive models). N/A is given for variants with no homozygotes of the minor allele in either patients with PD or controls. MAF=minor allele frequency; OR=odds ratio; CI=confidence interval.

Table 3D. Single variant associations with PD in the Polish series (345 patients with PD, 234 controls)

			Additive model		Dominant model		Recessive model	
Variant	Minor allele	MAF	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
rs2230288	T	0.9%	1.61 (0.39 - 6.68)	0.51	1.61 (0.39 - 6.68)	0.51	N/A	N/A
rs34372695	T	1.0%	1.28 (0.36 - 4.61)	0.71	1.28 (0.36 - 4.61)	0.71	N/A	N/A
rs708723	C	39.2%	0.68 (0.52 - 0.89)	0.0055	0.66 (0.45 - 0.96)	0.029	0.55 (0.33 - 0.91)	0.019
rs10928513	T	31.4%	0.94 (0.71 - 1.25)	0.69	0.96 (0.68 - 1.38)	0.84	0.83 (0.43 - 1.60)	0.58
rs2102808	T	14.4%	1.31 (0.89 - 1.92)	0.17	1.24 (0.83 - 1.86)	0.29	N/A	N/A
rs11711441	A	11.4%	0.76 (0.51 - 1.14)	0.18	0.77 (0.50 - 1.17)	0.22	0.43 (0.06 - 2.90)	0.39
rs6599388	T	33.9%	1.07 (0.82 - 1.39)	0.61	0.94 (0.66 - 1.34)	0.72	1.57 (0.90 - 2.76)	0.11
rs11724635	C	43.8%	0.85 (0.66 - 1.09)	0.20	1.06 (0.72 - 1.54)	0.77	0.55 (0.35 - 0.86)	0.0084
rs6812193	T	35.4%	0.81 (0.63 - 1.05)	0.11	0.73 (0.51 - 1.05)	0.093	0.81 (0.48 - 1.36)	0.42
rs356219	G	39.2%	1.47 (1.14 - 1.91)	0.0034	1.48 (1.03 - 2.14)	0.034	2.08 (1.24 - 3.49)	0.0055
rs3129882	G	48.5%	0.85 (0.66 - 1.10)	0.23	1.25 (0.83 - 1.88)	0.28	0.51 (0.34 - 0.78)	0.0019
rs156429	G	38.1%	1.01 (0.79 - 1.31)	0.91	1.01 (0.71 - 1.46)	0.94	1.03 (0.63 - 1.67)	0.91
rs7077361	C	11.2%	0.73 (0.50 - 1.07)	0.11	0.69 (0.45 - 1.07)	0.10	0.69 (0.21 - 2.33)	0.55
rs1491942	C	21.2%	1.12 (0.82 - 1.52)	0.48	1.18 (0.82 - 1.70)	0.37	0.94 (0.40 - 2.24)	0.89
rs10847864	T	34.2%	1.10 (0.85 - 1.43)	0.47	1.21 (0.84 - 1.73)	0.31	1.00 (0.59 - 1.69)	0.99
rs2942168	A	14.3%	1.03 (0.73 - 1.46)	0.85	1.24 (0.82 - 1.88)	0.30	0.35 (0.12 - 0.98)	0.045
rs12456492	G	36.9%	1.12 (0.86 - 1.46)	0.40	1.31 (0.90 - 1.90)	0.16	0.92 (0.55 - 1.53)	0.74

ORs, 95% CIs, and *p*-values result from logistic regression models adjusted for age and gender. ORs correspond to an additional minor allele (additive models), presence of the minor allele (dominant models), and presence of two copies of the minor allele (recessive models). N/A is given for variants with no homozygotes of the minor allele in either patients with PD or controls. MAF=minor allele frequency; OR=odds ratio; CI=confidence interval.

ciation of *LRRK2* rs1491942 with PD while adjusting for p.N551K, p.R1398H, p.K1423K, and p.M1646T in logistic regression analysis was also investigated in order to evaluate the independence of associations with PD.

Interactions of *PARK16* rs708723 with *LRRK2* rs1491942 and the three aforementioned *LRRK2* variants in the protective haplotype in the combined series were evaluated using logistic regression models adjusted for age,

gender, and series. LRRK2 p.M1646T was not evaluated in any interaction analysis owing to its lower frequency.

A relatively large number of statistical tests were performed in evaluation of associations with PD for variants previously nominated via GWAS (48 - 49 tests per series with 17 variants and 3 potential statistical models). In order to adjust for multiple testing and control the family-wise error rate at 5% in this primary analysis, we employed the single-step minP method [17] separately for each series with 10,000 permutations of patient and control labels, after which *p*-values ≤0.0018 (Irish series), ≤0.0015 (US series), ≤0.0019 (Polish series), ≤0.0016 (combined series) were considered as statistically significant. *P*-values ≤0.05 were considered as statistically significant in all remaining analysis. All statistical analyses were performed using R Statistical Software (version 2.14.0; R Foundation for Statistical Computing, Vienna, Austria).

Results

Replication of GWAS loci

To confirm the effect of the PD GWAS loci in disease risk, we attempted replication in our PD series. Single variant associations with PD according to each model of inheritance are displayed in **Table 3A** for the combined series, while association results for individual Irish, US, and Polish series are presented in **Table 3B-D**. In the combined group of 1,381 patients with PD and 1,328 controls, variants that were significantly associated with PD after correction for multiple testing included *BST1* rs11724635 under an additive model (OR: 0.83, P=0.0006) and a recessive model (OR: 0.67, P=5.9 x 10^{-5}), *SNCA* rs356219 under an additive model (OR: 1.44, P=1.8 x 10^{-10}), a dominant model (OR: 1.67, P=6.3 x 10^{-10}), and a recessive model (OR: 1.54, P=4.9 x 10^{-5}), *HLA-DRA* rs3129882 (as previously reported[17]) under a recessive model (OR: 0.70, P=0.0008), *CCDC62/HIP1R* rs10847864 under a dominant model (OR: 1.34, P=0.0004), and *MAPT* rs2942168 under an additive model (OR: 0.72, P=2.9 x 10^{-6}) and a dominant model (OR: 1.44, P=4.4 x 10^{-6}). Additionally, though not quite statistically significant after multiple testing correction, *LRRK2* rs1491942 was associated with PD under a recessive model (OR: 1.82, P=0.0022). All of these associations were relatively consistent in magnitude across the three individual series' except those involving *LRRK2* rs1491942 and *MAPT* rs2942168. For *LRRK2* rs1491942, the association with PD was observed in the Irish series (OR: 1.87, P=0.11) and the US series (OR: 2.19, P=0.005) but not in the Polish series (OR: 0.94, P=0.89). For *MAPT* rs2942168, associations under an additive model were also observed in the Irish series (OR: 0.63, P=0.0009) and the US series (OR: 0.66, P=1.6 x 10^{-5}) but not the Polish series (OR: 1.03, P=0.85); results regarding *MAPT* rs2942168 were similar under a dominant model.

Other variants showing significant evidence of an association with PD in the combined series prior to correction for multiple testing were *GBA* rs2230288, *PARK16* rs708723, *ACMSD* rs10928513, *STK39* rs2102808, *MCCC1/LAMP3* rs11711441, *GAK* rs6599388, and *STBD1/SCARB2* rs6812193. Of these associations, the strongest were observed for *STK39* rs2102808 under an additive model (OR: 1.24, P=0.008) and *STBD1/SCARB2* rs6812193 under an additive model (OR: 0.85, P=0.007), both of which were consistent across the three individual series. There was no evidence of an association with PD in the combined series for *SYT11* rs34372695, *GPNMB* rs156429, *ITGA8* rs7077361, or *RIT2* rs12456492 (all P≥0.10).

No role for LRRK2 coding variants in the GWAS association signal

Associations of LRRK2 p.N551K, p.R1398H, p.K1423K, and p.M1646T with PD are displayed in **Table 4**. There was no statistically significant evidence of an association between any of these *LRRK2* variants and PD in the combined series (all P≥0.38), though the direction of effects (protective for p.N551K, p.R1398H, and p.K1423K; risk for p.M1646T) is similar to what has been previously observed in our larger series and by others [13, 18]. The 3-variant p.N551K-R1398H-K1423K haplotype was also not significantly associated with PD (OR: 0.97, 95% CI: 0.71 - 1.34, P=0.71). There was very low correlation of *LRRK2* rs1491942 with LRRK2 p.N551K, p.R1398H, p.K1423K, and p.M1646T (all r^2<0.01), and additionally the association of *LRRK2* rs1491942 with PD was consistent with adjusting for each of the four aforementioned *LRRK2* variants (**Table 5**), both of which indicate that the

Table 4. Associations of LRRK2 p.M1646T, p.N551K, p.R1398H, and p.K1423K with PD

	US series (674 patients, 724 controls)			Irish series (362 patients, 370 controls)			Polish series (345 patients, 234 controls)			Combined series (1,381 patients, 1,329 controls)		
Variant	MAF	OR (95 CI)	*P*-value	MAF	OR (95 CI)	*P*-value	MAF	OR (95 CI)	*P*-value	MAF	OR (95 CI)	*P*-value
p.M1646T (rs35303786)	1.4%	1.54 (0.78, 3.02)	0.21	2.3%	0.82 (0.39, 1.71)	0.60	1.0%	0.75 (0.22, 2.58)	0.64	1.5%	1.16 (0.74, 1.81)	0.53
p.N551K (rs7308720)	7.0%	1.03 (0.75, 1.41)	0.87	5.6%	0.55 (0.33, 0.92)	0.022	6.0%	1.10 (0.63, 1.94)	0.73	6.4%	0.90 (0.71, 1.14)	0.38
p.R1398H (rs7133914)	6.8%	1.07 (0.77, 1.47)	0.69	6.5%	0.61 (0.38, 0.99)	0.044	6.0%	1.10 (0.63, 1.94)	0.73	6.5%	0.92 (0.73, 1.17)	0.50
p.K1423K (rs11175964)	6.6%	1.07 (0.77, 1.49)	0.68	6.3%	0.57 (0.35, 0.92)	0.022	6.0%	1.10 (0.63, 1.94)	0.73	6.4%	0.92 (0.72, 1.16)	0.47

ORs, 95% CIs, and *p*-values result from logistic regression models adjusted for age, gender, and series (combined series only). LRRK2 p.M1646T, p.N551K, p.R1398H, and p.K1423K were considered under a dominant model in all analysis.

Table 5. Associations of individual *LRRK2* variants with PD when adjusting for other *LRRK2* variants

Association/Model adjustment	OR (95% CI)	*P*-value
Association of *LRRK2* rs1491942 with PD under an additive model adjusting for:		
LRRK2 p.N551K	1.15 (1.00, 1.32)	0.042
LRRK2 p.R1398H	1.17 (1.02, 1.33)	0.026
LRRK2 p.K1423K	1.17 (1.02, 1.34)	0.024
LRRK2 p.M1646T	1.16 (1.01, 1.33)	0.032
LRRK2 p.N551K, p.R1398H, p.K1423K, and p.M1646T	1.17 (1.02, 1.34)	0.025
Association of *LRRK2* rs1491942 with PD under a recessive model adjusting for:		
LRRK2 p.N551K	1.80 (1.22, 2.66)	0.0026
LRRK2 p.R1398H	1.81 (1.23, 2.68)	0.0023
LRRK2 p.K1423K	1.82 (1.23, 2.70)	0.0024
LRRK2 p.M1646T	1.80 (1.22, 2.66)	0.0027
LRRK2 p.N551K, p.R1398H, p.K1423K, and p.M1646T	1.80 (1.21, 2.68)	0.0029

ORs, 95% CIs, and *p*-values result from logistic regression models adjusted for age, gender, series, and the given *LRRK2* variants. LRRK2 p.N551K, p.R1398H, p.K1423K, and p.M1646T were considered under a dominant model in all analysis.

Table 6. Interaction between *PARK16* rs708723 and *LRRK2* rs1491942

LRRK2 rs1491942 model/genotype	*PARK16* rs708723 model/genotype	Sample genotype count and frequency	Test of association		Test of interaction
			OR (95% CI)	*P*-value	
Additive model	Additive model				
GG	TT	520 (19.4%)	1.00 (reference)	N/A	
GG	CT	815 (30.4%)	0.85 (0.68, 1.06)	0.15	
GG	CC	322 (12.0%)	0.73 (0.55, 0.97)	0.031	
CG	TT	309 (11.5%)	1.02 (0.76, 1.36)	0.90	
CG	CT	430 (16.1%)	0.85 (0.66, 1.11)	0.24	OR: 1.09 95% CI: 0.90 - 1.31 P=0.36
CG	CC	162 (6.1%)	0.85 (0.60, 1.23)	0.39	
CC	TT	42 (1.6%)	1.52 (0.78, 2.96)	0.22	
CC	CT	49 (1.8%)	1.73 (0.92, 3.23)	0.088	
CC	CC	28 (1.0%)	1.69 (0.76, 3.79)	0.20	
Recessive model	Additive model				
GG or CG	TT	829 (30.9%)	1.00 (reference)	N/A	
GG or CG	CT	1245 (46.5%)	0.84 (0.70, 1.01)	0.064	
GG or CG	CC	484 (18.1%)	0.76 (0.61, 0.96)	0.022	OR: 1.22 95% CI: 0.73 - 2.05 P=0.44
CC	TT	42 (1.6%)	1.51 (0.78, 2.91)	0.22	
CC	CT	49 (1.8%)	1.71 (0.92, 3.18)	0.087	
CC	CC	28 (1.0%)	1.68 (0.76, 3.74)	0.20	
Additive model	Dominant model				
GG	TT	520 (19.4%)	1.00 (reference)	N/A	
GG	CT or CC	1137 (42.5%)	0.81 (0.66, 1.00)	0.055	
CG	TT	309 (11.5%)	1.02 (0.76, 1.36)	0.90	OR: 1.09 95% CI: 0.82 - 1.45 P=0.57
CG	CT or CC	592 (22.1%)	0.85 (0.67, 1.09)	0.20	
CC	TT	42 (1.6%)	1.52 (0.78, 2.96)	0.22	
CC	CT or CC	77 (2.9%)	1.71 (1.03, 2.85)	0.038	
Recessive model	Dominant model				
GG or CG	TT	829 (31.0%)	1.00 (reference)	N/A	
GG or CG	CT or CC	1729 (64.6%)	0.82 (0.69, 0.97)	0.023	OR: 1.38 95% CI: 0.61 - 3.13 P=0.44
CC	TT	42 (1.6%)	1.51 (0.78, 2.91)	0.23	
CC	CT or CC	77 (2.9%)	1.70 (1.03, 2.80)	0.036	

ORs and *p*-values result from logistic regression models. For tests of association, *LRRK2* rs1491942 and *PARK16* rs708723 were combined into one variable, and the model was adjusted for age, gender, and series. For tests of interaction, models included *LRRK2* rs1491942, *PARK16* rs708723, the interaction between these two variants, age, gender, and series. OR=odds ratio. CI=confidence interval.

association signal for the GWAS-nominated rs1491942 is independent of the *LRRK2* coding variants.

Interaction between LRRK2 and PARK16

To evaluate the interaction between *PARK16* rs708723 and *LRRK2* rs1491942, we performed logistic regression analyses. The results are displayed in **Table 6**, where we considered *PARK16* rs708723 under additive and dominant models and *LRRK2* rs1491942 under additive and recessive models owing to the significant associations with PD that were observed in these scenarios. There was no statistically significant evidence of an interaction between these two variants (all interaction P≥0.36); the protective effect of *PARK16* rs708723 on risk of PD was observed across *LRRK2* rs1491942 genotypes, and the risk effect of *LRRK2* rs1491942 was seen across genotypes of *PARK16* rs708723. To mirror the presentation of results by MacLeod et al. [15], the association between *LRRK2* rs1491942 under an additive model and PD risk was similar for individuals with (OR: 1.19, P=0.038,

Table 7. Interactions of *PARK16* rs708723 with *LRRK2* p.N551K, p.R1398H, and p.K1423K

LRRK2 variant/ genotype	*PARK16* rs708723 model/genotype	Sample genotype count and frequency	Test of association		Test of interaction
			OR (95% CI)	*P*-value	
LRRK2 p.N551K	Additive model				
CC	TT	757 (28.6%)	1.00 (reference)	N/A	
CC	CT	1116 (42.1%)	0.92 (0.76, 1.11)	0.40	
CC	CC	447 (16.9%)	0.84 (0.66, 1.07)	0.16	OR: 0.77 95% CI: 0.55 - 1.09 P=0.14
CG or GG	TT	107 (4.0%)	1.28 (0.84, 1.96)	0.25	
CG or GG	CT	163 (6.2%)	0.70 (0.49, 0.99)	0.044	
CG or GG	CC	59 (2.2%)	0.71 (0.41, 1.23)	0.22	
	Dominant model				
CC	TT	757 (28.6%)	1.00 (reference)	N/A	
CC	CT or CC	1563 (59.0%)	0.90 (0.75, 1.07)	0.24	OR: 0.61 95% CI: 0.37 - 1.02 P=0.057
CG or GG	TT	107 (4.0%)	1.28 (0.84, 1.96)	0.25	
CG or GG	CT or CC	222 (8.4%)	0.70 (0.52, 0.96)	0.025	
LRRK2 p.R1398H	Additive model				
GG	TT	751 (28.3%)	1.00 (reference)	N/A	
GG	CT	1116 (42.1%)	0.91 (0.75, 1.09)	0.30	
GG	CC	446 (16.8%)	0.81 (0.64, 1.03)	0.083	OR: 0.87 95% CI: 0.61 - 1.22 P=0.41
GA or AA	TT	109 (4.1%)	1.19 (0.78, 1.80)	0.42	
GA or AA	CT	171 (6.5%)	0.72 (0.51, 1.02)	0.062	
GA or AA	CC	57 (2.2%)	0.79 (0.46, 1.38)	0.41	
	Dominant model				
GG	TT	751 (28.3%)	1.00 (reference)	N/A	
GG	CT or CC	1562 (58.9%)	0.88 (0.73, 1.05)	0.15	OR: 0.71 95% CI: 0.43 - 1.18 P=0.19
GA or AA	TT	109 (4.1%)	1.19 (0.78, 1.80)	0.42	
GA or AA	CT or CC	228 (8.6%)	0.74 (0.55, 1.00)	0.052	
LRRK2 p.K1423K	Additive model				
GG	TT	749 (28.5%)	1.00 (reference)	N/A	
GG	CT	1109 (42.2%)	0.92 (0.76, 1.11)	0.39	
GG	CC	448 (17.0%)	0.81 (0.64, 1.03)	0.091	OR: 0.85 95% CI: 0.60 - 1.20 P=0.36
GA or AA	TT	104 (4.0%)	1.21 (0.79, 1.85)	0.39	
GA or AA	CT	166 (6.3%)	0.72 (0.51, 1.02)	0.062	
GA or AA	CC	54 (2.1%)	0.79 (0.45, 1.39)	0.41	
	Dominant model				
GG	TT	749 (28.5%)	1.00 (reference)	N/A	
GG	CT or CC	1557 (59.2%)	0.89 (0.74, 1.06)	0.19	OR: 0.69 95% CI: 0.41 - 1.15 P=0.15
GA or AA	TT	104 (4.0%)	1.21 (0.79, 1.85)	0.39	
GA or AA	CT or CC	220 (8.4%)	0.74 (0.54, 1.00)	0.051	

ORs and *p*-values result from logistic regression models. For tests of association, the two given variants were combined into one variable, and the model was adjusted for age, gender, and series. For tests of interaction, models included each of the two variants, their interaction, age, gender, and series. OR=odds ratio. CI=confidence interval.

N=1,806) and without (OR: 1.11, P=0.40, N=871) the *PARK16* protective allele, and similarly the association between *LRRK2* rs149-1942 under a recessive model and risk of PD was comparable for individuals with (OR: 2.05, P=0.0031) and without (OR: 1.51, P=0.22) the protective *PARK16* allele.

Interactions of *PARK16* rs708723 with LRRK2 p.N551K, p.R1398H, and p.K1423K are exam-

ined in **Table 7**. There were no significant interactions of *PARK16* rs708723 with LRRK2 p.N551K, p.R1398H, or p.K1423K in relation to risk of PD, though non-significant trends toward interaction were observed. This was most evident for LRRK2 p.N551K, where the protective effect of the minor allele for *PARK16* rs708723 was strongest in individuals with a copy of the minor allele for p.N551K, and vice versa (Interaction OR: 0.61, P=0.057). Similar non-significant trends were observed for p.R1398H and p.K1423K (**Table 7**).

Discussion

The results of this study provide evidence that confirm associations with PD for a number of variants that have previously been nominated as risk-modifying susceptibility factors for PD in GWAS. The strongest associations with PD were observed for variants in *BST1*, *SNCA*, *HLA*, *CCDC62/HIP1R*, *MAPT*, and *LRRK2* all of which were significant after correction for multiple testing with the exception of the *LRRK2* variant which was almost significant. Additionally, prior to multiple testing correction there was significant (P≤0.05) evidence of associations with PD for variants in the *GBA*, *PARK16*, *ACMSD*, *STK39*, *MCCC1/LAMP3*, *GAK*, and *STBD1/SCARB2* genes. Although there is some degree of overlap between these findings and those reported in the aforementioned larger consortium replication effort that utilized more than 17,000 patients and controls from 19 different countries[16], it is of interest that the many of the previously replicated significant associations (*ACMSD*, *STK39*, *MCCC1/LAMP3*, *BST1*, *SNCA*, *LRRK2*, *CCDC62/HIP1R*, *MAPT*) were still observed when considering only subjects from the US, Ireland, and Poland. Additionally, the significant associations with PD that we identified for variants in *GBA*, *PARK16*, and *STBD1/SCARB2* have been previously unreported in our series. We also observed a very low degree of correlation between *LRRK2* rs1491942 and LRRK2 p.N551K, p.R1398H, p.K1423K, and p.M1646T, indicating that the associations of these variants with PD are independent of one another. Finally, no significant interaction was noted between *PARK16* rs708723 and *LRRK2* rs1491942, though we did observe a non-significant interaction between *PARK16* rs708723 and LRRK2 p.N551K which requires further study.

Of the 13 variants showing significant evidence of an association with PD prior to multiple testing correction, the magnitude of the effect was similar to previous studies for all variants with the exception of *HLA-DRA* rs3129882, where we observed the risk of PD was lower for individuals with two copies of the minor allele as previously reported [19]. This highlights one limitation of GWAS, where due to the extremely large amount of variants that are included, the only statistical model that is usually considered is an additive model, and this may not be the most appropriate model for a given variant. Indeed, the strongest associations that we observed in this study for *GAK* rs6599388, *BST1* rs11724635, *HLA* rs3129882, and *LRRK2* rs1491942 were under a recessive model.

Variants in *SYT11*, *GPNMB*, *ITGA8*, and *RIT2* did not show evidence of an association with PD even before correction for multiple testing. Given the much smaller sample size of this study compared to the aforementioned GWAS, these results should be interpreted carefully, and the possibility of Type II error (i.e. false-negative association) is important to consider. It should be noted that for *ITGA8* rs7077361, the estimated OR of 0.87 that was observed in this study is nearly identical to what has been observed in previous studies, and 95% confidence limits for *GPNMB* rs156429 and *RIT2* rs12456492 are generally consistent with the findings of previous studies. *SYT11* rs34372695 is rare with a MAF of 1.8% in the combined series, and this may in part explain the lack of a significant association between this variant and PD in the current study, where 95% confidence limits are also consistent with previous findings.

Common variation in the *SNCA*, *MAPT* and *LRRK2* genes are well-established to affect susceptibility to disease and were known prior to GWA approaches. Consistent replication of the other loci now nominated from GWAS is required to determine true associations and effect sizes. It is also crucial to discern ethnic-specific effects which have been previously observed for a number of loci including *LRRK2* [13, 20]. Studies have started to publish replication results for some of these loci [21-23]. Sharma and colleagues in the Genetic Epidemiology of Parkinson's disease consor-

tium attempted to replicate 11 variants from the initial IPDGC study in a large patient (n=8750)-control (n=8955) series of Caucasian and Asian descent [23]. This study replicated nine of the loci in either series with no evidence observed for the *HLA* and *ACMSD* association with disease.

Pihlstrom et al. examined 18 loci in a Scandinavian series of 1345 unrelated PD patients and 1225 control subjects collected in Norway and Sweden [22]. Only four loci showed significant association following statistical correction, although *P*-values <0.05 were observed for eleven loci. Lack of association was observed for *BST1*, *PARK16* and *LRRK2* candidates, which may reflect some ethnic specificity given previous associations in Asian series. Interestingly, Liu and colleagues recently examined the association of 17 loci in 1,737 subjects (989 patients and 748 controls) of Chinese descent [21]. It was observed that nine of the selected variants were monomorphic and four other had very low minor allele frequencies. The only variants that showed association were in the *SNCA* and *BST1* loci. This study highlights the ethnic-specific frequencies of nominated variants and the need for further studies in under-represented populations.

Our recent studies of *LRRK2* coding variation in PD susceptibility identified a common protective haplotype (p.N551K-p.R1398H-p.K1423K) and risk factor (p.M1646T) in Caucasian populations [13]. In the present study we assessed whether the coding susceptibility variants accounted for a proportion of the GWAS *LRRK2* signal (rs1491942). Our results suggest that the *LRRK2* signal is independent of the coding variation and is therefore driven by non-coding variation most likely in regulatory regions affecting gene/transcript expression. In addition, the study by MacLeod and colleagues showed the *PARK16* candidate protein RAB7L1 may act in a pathway with LRRK2 [15]. They observed that overexpression of RAB7L1 rescued a neurodegeneration phenotype in LRRK2 mutant neurons. The study also suggested a genetic interaction between the *PARK16* and *LRRK2* loci. Our study examined the potential interaction and although we did not show an interaction with our GWAS SNPs at these loci, we did observe a trend with the *LRRK2* common protective variants and the *PARK16* GWAS SNP. These findings suggest that further gene-gene interaction studies are warranted and it is crucial to determine if the *RAB7L1* gene is accounting for the *PARK16* association signal.

The characterization of population-based genetic susceptibility factors for PD will be an important step forward in our understanding of the disease. Sequencing studies will help pinpoint those functional variants affecting disease risk, which can be used as diagnostic and prognostic markers. In addition, examining how these variants interact will be important to generate accurate predictions of risk and direct therapeutic interventions strategies.

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