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Journal Title: Movement Disorders
Volume: Volume 29, Number 9
Publisher: Wiley | 2014-08-01, Pages 1171-1180
Type of Work: Article | Post-print: After Peer Review
Publisher DOI: 10.1002/mds.25895
Permanent URL: <https://pid.emory.edu/ark:/25593/rmfhd>

Final published version: <http://dx.doi.org/10.1002/mds.25895>

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Accessed November 14, 2019 8:24 AM EST



Published in final edited form as:

Mov Disord. 2014 August ; 29(9): 1171–1180. doi:10.1002/mds.25895.

Peptidoglycan Recognition Protein Genes and Risk of Parkinson's Disease

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Abstract

Increased gut permeability, inflammation, and colonic α -synuclein pathology are present in early Parkinson's disease (PD) and have been proposed to contribute to PD pathogenesis. Peptidoglycan is a structural component of the bacterial cell wall. Peptidoglycan recognition proteins (PGRPs)

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Relevant conflicts of interest/financial disclosures: R.A.H. and S.M.G. received fees as expert witnesses from a group of current and former manufacturers of welding consumables.

Full financial disclosures and author roles may be found in the online version of this article.

maintain healthy gut microbial flora by regulating the immune response to both commensal and harmful bacteria. We tested the hypothesis that variants in genes that encode PGRPs are associated with PD risk. Participants in two independent case-control studies were genotyped for 30 single-nucleotide polymorphisms (SNPs) in the four *PGLYRP* genes. Using logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) adjusted for potential confounding variables, we conducted analyses in each study, separately and pooled. One SNP failed the assay, and three had little to no variation. The ORs were similar in both study populations. In pooled analyses, three of seven *PGLYRP2* SNPs (rs3813135, rs733731, rs892145), one of five *PGLYRP3* SNPs (rs2987763), and six of nine *PGLYRP4* SNPs (rs10888557, rs12063091, rs3006440, rs3006448, rs3006458, and rs3014864) were significantly associated with PD risk. Association was strongest for *PGLYRP4* 5' untranslated region (UTR) SNP rs10888557 (GG reference, CG OR 0.6 [95% CI 0.4–0.9], CC OR 0.15 [95% CI 0.04–0.6]; log-additive *P*-trend, 0.0004). Common variants in *PGLYRP* genes are associated with PD risk in two independent studies. These results require replication, but they are consistent with hypotheses of a causative role for the gut microbiota and gastrointestinal immune response in PD.

Keywords

Parkinson's disease; peptidoglycan; PGLYRP; microbiome; gut

Parkinson's disease (PD) is now thought of as a systemic disorder. Non-motor symptoms such as hyposmia and constipation sometimes precede motor symptoms by years to decades,^{1–4} and associated α -synuclein pathology in the autonomic and enteric nervous systems may precede development of pathological conditions in the brain.^{5–8} Some have proposed that PD begins in the gut and moves to the brain by one of several possible mechanisms. These include retrograde axonal transport of a toxic or infectious agent,^{9–11} neuron-to-neuron transmission of α -synuclein protein aggregates,^{6,12,13} or a prion-like seeding process.^{14,15} The gut has the largest mucosal surface of the body and is thus a primary anatomic target for exposure to toxicants and infectious agents. Recent reports suggest that patients with early PD may manifest increased intestinal permeability, bacterial invasion, and high levels of inflammatory cytokines in colonic biopsy specimens.^{16,17}

The gut microbiota, comprising the trillions of organisms that line the length of the gastrointestinal tract, may play a role in PD. Systemic administration of gram-negative bacterial endotoxin (lipopolysaccharide [LPS]) activates microglia in the substantia nigra and induces progressive dopaminergic degeneration in a rodent model of parkinsonism.¹⁸ However, little is known about possible effects of other bacterial components. Peptidoglycan is a major structural component of the bacterial cell wall that serves to protect the plasma membrane. Because it is unique to bacteria, it is recognized as foreign and binds pattern recognition receptors, potentially triggering an innate immune response.¹⁹ Humans have four peptidoglycan recognition proteins (PGRPs), highly conserved innate immunity proteins encoded by *PGLYRPs* 1–4, which are selectively expressed in a range of tissues and are also secreted into the gut.²⁰ The PGRPs modulate the immune response to advantageous and harmful gut bacteria and play a major role in the development and maintenance of a healthy commensal microbiota,²⁰ and variants in *PGLYRP* genes have recently been associated with

risk of inflammatory bowel disease.²¹ We hypothesized that variation in *PGLYRP* genes might affect the risk of PD and tested this hypothesis in two independent study populations.

Patients and Methods

Participants were drawn from two case-control studies of PD: FAME (Farming and Movement Evaluation) and SEARCH (Study of Environmental Association and Risk of Parkinsonism using Case-Control Historical Interviews). Analyses were conducted in each population separately and with pooled data.

Subject Ascertainment

Fame—FAME is a case-control study nested in the Agricultural Health Study (AHS).²² The AHS is a prospective study of private pesticide applicators (mostly farmers) and their spouses recruited between 1993 and 1997 in Iowa and North Carolina (n584,739).²³ Participants were identified from AHS data releases PIREL0506 and AHSREL06 (<http://aghealth.nci.nih.gov/>).

Cases: The AHS cohort members suspected to have PD were identified by self-report. Neurologists assessed suspect case subjects at home. Assessments included a standardized neurological history, examination, and scripted videotaping. Final diagnosis based on National Institute of Neurological Disorders and Stroke/UK Brain Bank criteria^{24,25} was determined by consensus of two movement disorder specialists using all available information, including medical records.

Controls: Potential control subjects were identified by stratified random sampling of nondemented AHS participants and frequency-matched to case subjects by age, sex, and state (Iowa or North Carolina) at a ratio of approximately three per case. Neurologists or technicians trained by neurologists conducted assessments of control subjects. Technician-assessed controls with possible parkinsonism were reassessed by neurologists. Eighty-eight percent (n = 115) of “suspected” cases and 71% (n = 383) of eligible controls agreed to participate.

Search—SEARCH is a case-control study of PD and parkinsonism conducted in eight North American movement disorders centers between July 2004 and May 2007.²⁶

Cases: Nondemented case subjects were consecutively enrolled in six centers and convenience sampled in two. National Institute of Neurological Disorders and Stroke/UK Brain Bank diagnostic criteria for PD were applied by the enrolling movement disorders physician.^{24,25}

Controls: Control subjects without neurodegenerative disorders or dementia were frequency-matched to cases by age, sex, and site. To minimize bias related to demographic or socioeconomic differences, controls were primarily non-blood relatives (68%) or acquaintances (15%) referred by patients in the clinical practice of the enrolling physicians. The remainder had other nonpatient relationships with referring clinics (7%) or were recruited using a commercial list of telephone numbers matching on case subjects' zip codes

(10%). A total of 519 case and 511 control subjects were enrolled. Blood was available for 172 control subjects, because most controls were not evaluated in person. Demographic characteristics were similar in controls with and without blood collection.

Human Subjects—FAME and SEARCH were approved by institutional review boards of all participating institutions. All participants provided written informed consent.

Data Collection

Covariate Assessments—Methods were similar in FAME and SEARCH. Trained interviewers at the Parkinson's Institute collected demographic and risk factor information by telephone. If a participant was deceased or cognitively impaired at interview, a proxy respondent was recruited. Race and ethnicity were self-reported. Use of tobacco was assessed until a reference age, defined, for cases, as age at diagnosis and, for controls, as the median age at diagnosis among cases in the corresponding sex-, state/center-, and age-specific stratum. Tobacco use was defined as smoking at least one cigarette daily for 6 months or longer before reference age.

Genotyping—DNA was extracted from venous blood.²⁷ A custom Illumina GoldenGate array was designed using a candidate gene approach that included 1536 single-nucleotide polymorphisms (SNPs) across 132 genes. We selected tag SNPs enriched with nonsynonymous coding variants, including 8 SNPs in *PGLYRP1*, 8 SNPs in *PGLYRP2*, 5 SNPs in *PGLYRP3*, and 9 SNPs in *PGLYRP4*. Genotyping was conducted by the genomics core at the University of California, San Francisco. Clustering of calls was manually reviewed while blinded to disease status. Subjects with call rates of less than 0.93 were excluded from analyses.

Statistical Analyses

We compared subject characteristics within and between study populations using Fisher's exact test or Pearson's chi-square statistic for categorical data and independent *t* tests or Mann-Whitney-Wilcoxon rank-sum tests for continuous data. We used Pearson's chi-square statistic to test deviation from Hardy-Weinberg equilibrium in controls. Associations between *PGLYRP* SNPs and PD were assessed using unconditional logistic regression. To control for potential confounding, we included reference age (tertile), sex, state (for FAME), race/ethnicity (non-Hispanic white or other), and cigarette smoking in all models. Most FAME participants were non-Hispanic white (97%), so we considered six subjects with missing race/ethnicity to be non-Hispanic white. SEARCH had a higher proportion of non-white subjects (12%), so we excluded three participants with missing race/ethnicity. In constructing the model for the pooled data, we first assessed whether each covariate's effect differed between study populations using a chi-square test.²⁸ Only for smoking was heterogeneity indicated ($P < 0.20$); consequently, we included an interaction term for smoking by study in the pooled model. We also performed analyses in men and women separately, and sensitivity analyses restricted to non-Hispanic whites and excluding case subjects with a history of PD in a first-degree relative.

Genotype was modeled for each SNP by including indicator variables for the number of minor alleles, with major allele homozygotes (no minor alleles) as the reference category. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for each genotype. *P*-values for trend (0, 1, or 2 minor alleles) were calculated assuming a log-additive relationship. Linkage disequilibrium (LD) between SNPs was calculated using Haploview v4.2²⁹ and is expressed as r^2 . We used multiple Web-based bioinformatics tools to predict SNP functional effects, including PROVEAN³⁰ (<http://provean.jcvi.org/index.php>), PolyPhen (<http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm>) (<http://genetics.bwh.harvard.edu/pph2/dokuwiki/start>),^{31,32} TANGO, and FOLDX (<http://snpeffect.switchlab.org>).^{33,34} All other analyses were conducted with SPSS v21.0 (IBM, Armonk, NY, USA).

Results

DNA was available for 100 case and 371 control subjects in FAME, and 418 case and 172 control subjects in SEARCH. Ten subjects with genotype call rates less than 0.93 were excluded from analyses (1 case and 7 controls in FAME, 2 controls in SEARCH). Complete genotyping and covariate data were available for 95 cases and 353 controls in FAME, and 385 cases and 157 controls in SEARCH. Demographic characteristics were similar in subjects with and without complete data (data not shown). Compared with FAME, SEARCH participants were more likely to be female and non-white (Table 1). At enrollment, FAME cases had a longer PD duration than SEARCH cases (7.3 vs. 2.8 years) and were approximately 4 years older, but reference age was similar. Smoking was less common among cases than controls in both studies, although differences were greater in FAME than in SEARCH.

The *PGLYRP* allele frequencies were comparable in both studies, and all SNPs satisfied Hardy-Weinberg equilibrium (Table 2). The rs2304200 in *PGLYRP2* failed the assay; call rates for all other SNPs exceeded 99%. Three SNPs in *PGLYRP1* had allele frequencies less than 1% (rs13343537, rs28722714, rs7245473) and were excluded from analyses.

None of five *PGLYRP1* SNPs was associated with PD risk in FAME, SEARCH, or in pooled analyses. In pooled analyses, three of seven *PGLYRP2* SNPs (rs3813135, rs733731, rs892145), one of five *PGLYRP3* SNPs (rs2987763), and six of nine *PGLYRP4* SNPs (rs10888557, rs12063091, rs3006440, rs3006448, rs3006458, rs3014864) were significantly associated with PD risk (Table 3). Odds ratios were of similar magnitude in FAME and SEARCH for all associated SNPs; and, for most SNPs, minor alleles were associated with reduced risk of PD. Evidence of association was strongest for *PGLYRP4* SNPs rs10888557 (pooled *P*-trend, 0.0004; significant in both FAME and SEARCH), rs12063091 (pooled *P*-trend, 0.009; significant in SEARCH), and rs3014864 (pooled *P*-trend, 0.008; significant in SEARCH). Analyses of two- and three-marker haplotypes did not identify any stronger associations than did analyses of single SNPs. Results were similar in sex-specific strata and in analyses limited to non-Hispanic whites, and in analyses that excluded subjects with a family history of PD. Age at PD diagnosis did not differ by genotype for any associated SNP in either study-specific or pooled analyses (data not shown).

Significant SNPs in *PGLYRP2* were highly correlated with one another (Fig. 1). In contrast, LD varied among significant SNPs in *PGLYRP4*, which spanned approximately 35,000 base pairs and included the 5' untranslated region (UTR), 3' UTR, and nonsynonymous coding SNPs. Among significantly associated nonsynonymous coding SNPs, the PGLYRP2 M270K amino acid substitution conferred by rs892145 was predicted to have a probable functional effect by Poly-Phen and TANGO (decreased aggregation tendency), whereas FOLDX predicted PGLYRP4 V213I (rs12063091) to have slightly enhanced stability. Effects of the synonymous coding and noncoding SNPs on protein expression or function are unknown, but the significantly associated SNPs rs2987763 (*PGLYRP3*), and rs10888557 and rs3014864 (*PGLYRP4*) are located near transcription factor binding sites.

Discussion

Parkinson's disease risk was associated with common variants in *PGLYRP2*, *PGLYRP3*, and *PGLYRP4*, which encode three of the four PGRPs. Although most associations were not statistically significant in the smaller FAME study, ORs were very similar in FAME and SEARCH, and several were significant in pooled analyses. For most SNPs, minor allelic variants were inversely associated with PD risk, with log additive effects.

Among the three significantly associated *PGLYRP2* coding SNPs that were in strong LD, only rs892145 was predicted to have a high probability of conferring a functional change. However, data on predicted functional effects are limited, and functional changes that might underlie the observed risk associations could result from variability elsewhere in the gene. Associated SNPs in *PGLYRP3* and *PGLYRP4* are located in coding, 3'UTR and 5'UTR regions, and LD was more variable, suggesting that functional effects also may result from altered transcription. However, limited sample size and low SNP density preclude drawing conclusions regarding a direct functional role for any of the associated SNPs. We are not aware of any prior reports of *PGLYRP* genes and PD. Among genome-wide association studies (GWAS) in the publicly accessible GWAS Central database (<http://www.gwascentral.org/index>), Maraganore et al. assessed eight markers in or near *PGLYRP3* and *PGLYRP4*, and a single marker in *PGLYRP2*, none of which were associated with PD,³⁵ but the SNPs we studied were not included in their assay.

The PGRPs bind both gram-negative and gram-positive bacterial peptidoglycan¹⁹ and are also able to recognize and bind LPS to a lesser extent.³⁶ PGLYRP1, 3, and 4 are directly bactericidal,³⁷ whereas PGLYRP2 is a peptidoglycan-cleaving amidase.³⁸ The PGRPs function to maintain beneficial gut flora, and the high expression of PGLYRP3 and 4 in the upper gastrointestinal tract make them particularly important in this regard. Stool from *Pglyrp* knockout mice has a markedly altered bacterial composition, with reduced numbers of *Lactobacillus*/*Lactococcus* species, and increased ability to induce inflammatory cytokine and chemokine production in cultured colonic fibroblasts.³⁹ In a dextran sulfate sodium mouse model of colitis, knockout of any of the four PGRPs, and especially *Pglyrp3*, increased mucosal permeability and tissue damage and markedly increased colonic expression of γ -interferon. This increased sensitivity was transferable between animals by stool gavage, strongly implicating PGLYRP-dependent regulation of gut flora.³⁹

The gut microbiota is a complex, highly evolved system comprising 100 trillion organisms.⁴⁰ As the direct interface with the external environment, it is both a product and determinant of the gut immune response, and it influences interactions with nutrients, xenobiotics, and pathogenic organisms.⁴¹ In addition to its local immune role, the gut microbiota regulates the development and function of the immune system more broadly⁴²; alterations are associated with systemic diseases such as asthma and arthritis.^{43,44} The microbiota has also been shown to modulate brain development and striatal dopaminergic turnover, and even to affect higher cognitive function and behavior via gut–brain bidirectional communication.^{45,46}

Although no evidence has been found to suggest that an altered microbiome is associated with increased risk of PD, convincing data argue that the gut is affected early in the disease process and could potentially play an important causative role.^{6,9,11} Constipation is a near universal symptom in PD⁴⁷; and, in prospective epidemiological studies, having less frequent bowel movements is associated with future risk of PD.^{2–4} In addition, α -synuclein pathological conditions are found throughout the myenteric nervous system in individuals with PD or incidental Lewy bodies.^{6,8,48–50} Importantly, it has been observed in colon biopsy specimens obtained from patients with early PD,^{8,51} and even before disease onset.⁷ Paralleling observations in *Pglyrp*-knockout animal models, relative to controls, PD patients manifest small intestinal bacterial overgrowth,⁵² increased intestinal permeability, mucosal bacterial invasion and oxidative damage, and greater colonic expression of γ -interferon messenger RNA as well as TNF- α , IL-6, and IL-1b.^{16,17} Taken together, these data support a central role for the gut in PD pathogenesis, and findings are consistent with a disrupted gut flora and immune response, as seen in *Pglyrp* knockout animal models. Thus, altered PGRP expression or function could be a causative factor in PD.

Further supporting the plausibility of a causative role for peptidoglycan, LPS, another bacterial product that is released from gram-negative bacterial cell membranes during lysis, produces an animal model of parkinsonism. Intraperitoneal injection of LPS activates microglia and causes specific progressive loss of nigral dopaminergic neurons as well as increased sensitivity to subsequent toxic insults.^{53,54} Although a peptidoglycan model of parkinsonism has not been reported, like LPS, peptidoglycan up-regulates inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and inflammatory cytokines, and it activates microglia in culture.⁵⁵

Another possible relationship between gut immune function and PD is suggested by observations that variants in or near the *LRRK2* gene are associated with increased risk of the inflammatory bowel disorder Crohn's disease, which is thought to result from a dysregulated immune response to the intestinal flora⁵⁶ and has also been associated with variants in *PGLYRP* genes.²¹ Autosomal dominantly inherited variants in the *LRRK2* gene are the most common genetic cause of PD, accounting for 1% to 2% of cases, and several polymorphic variants are also associated with modestly increased risk.⁵⁷ The mechanisms underlying *LRRK2* PD are not known, but *LRRK2* is highly expressed in a range of circulating and tissue-based immune cells. It is up-regulated in intestinal biopsy specimens from patients with Crohn's disease, and its expression in intestinal mucosa is markedly increased in response to bacterial pathogens and γ -interferon.^{58,59}

Although its results are biologically plausible, our study had some limitations. Its relatively small size could have resulted in chance associations with *PGLYRP* SNPs, and we did not adjust results for multiple comparisons. Thus, *P*-values should be interpreted cautiously. However, we tested an a priori hypothesis, and although most associations were not significant in FAME, the magnitude and direction of associations were very similar in FAME and the much larger SEARCH population. Similar findings in two distinct populations argues against a chance association. In addition, although we adjusted for known potential confounding variables and conducted sensitivity analyses, we cannot rule out possible confounding by unrecognized factors that might be related to both *PGLYRP* genotype and PD risk. Finally, we were unable to adjust for population substructure because of the limited number of markers in our array; however, the study populations were relatively homogeneous, and results of analyses restricted to non-Hispanic whites were very similar.

In summary, we found that multiple common SNPs in three of the four genes encoding peptidoglycan recognition proteins, *PGLYRP2*, *3*, and *4*, were significantly associated with the risk of PD. Results were similar in two independent study populations and were significant in pooled samples. The gut is a site of early involvement in PD. Because PGRPs influence the host immune response to gut bacteria and the makeup of the gut microbiota, they could play a role in PD cause and pathogenesis. Further characterization of these mechanisms may lead to novel early approaches to delay or prevent onset of PD.

Acknowledgments

Funding agencies: This study was supported in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences, NIEHS (grants Z01-ES044007 and Z01-ES049030), NCI (grant Z01-CP010119), NIEHS grants R01-ES10803 and U54 ES012077, the Michael J. Fox Foundation, Parkinson's Unity Walk, and James and Sharron Clark. SEARCH was supported by an unrestricted grant from a group of current and former manufacturers of welding consumables awarded to The Parkinson's Institute.

We thank Drs. Meike Kasten, Anabel Chade, Hubert Fernandez, Franca Cambi, Diana Tracy, and Amanda Deligtisch for examination fieldwork, Dr. Marie Richards for statistical support, and the participants of the FAME and SEARCH studies.

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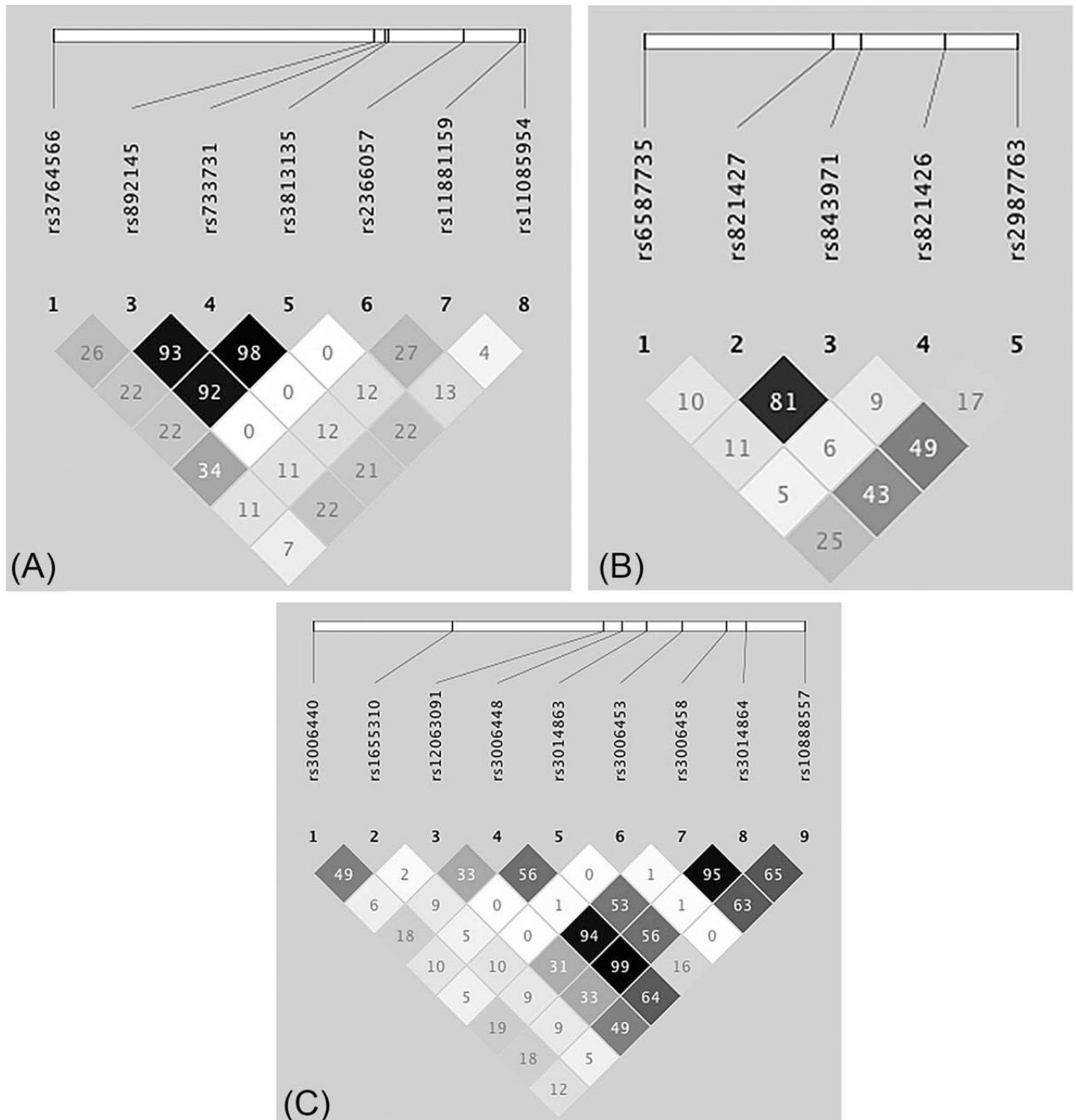


FIG. 1. PGLYRP SNP linkage disequilibrium as R^2 in pooled sample. **A:** PGLYRP2; **B:** PGLYRP3; **C:** PGLYRP4. SNP, single-nucleotide polymorphism.

TABLE 1

Subject characteristics

	FAME		SEARCH	
	Cases	Controls	Cases	Controls
Number	95	353	385	157
Reference age ^a Mean (SD), range	61.9 (9.5), 45–87	61.6 (7.5), 45–80	61.6 (9.8), 30–86	61.6 (9.9), 32–91
Enrollment age Mean (SD), range	69.1 (8.7), 48–89	69.1 (8.2), 42–88	64.5 (9.7), 30–87	65.1 (9.5), 37–92
PD duration at enrollment, years Mean (SD), range	7.3 (5.1), 0–21	na	2.8 (2.0), 0–8	na
Male (%)	70 (74%)	268 (76%)	227 (59%)	95 (61%)
Non-Hispanic white	92 (97%)	345 (98%)	340 (88%)	138 (88%)
Cigarette smoker (%)	22 (23%)	124 (35%)	153 (40%)	69 (44%)

^aReference age is defined as PD diagnosis age for cases, and median case diagnosis age in corresponding sex-, state/center-, and age-specific strata for controls.

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TABLE 2

PGLYRP SNP coordinates and minor allele frequencies

Gene	SNP	Coordinate (36.3)	Gene Location	_MAF in controls		
				FAME	SEARCH	Minor allele
<i>PGLYRP1</i> Chromosome 19	rs12982353	51221296	Flanking_5UTR	0.32	0.36	A
	rs13343537 ^c	51214673	Coding ^a	0	0	na
	rs2041991	51216136	Intron	0.38	0.40	T
	rs2041993	51216807	Intron	0.35	0.37	A
	rs2072561	51217707	Intron	0.06	0.05	G
	rs2072563	51218488	Flanking_5UTR	0.32	0.36	A
	rs28722714 ^c	51217844	Coding ^b	0.004	0.006	A
	rs7245473 ^c	51218293	Flanking_5UTR	0.003	0.003	T
<i>PGLYRP2</i> Chromosome 19	rs11085954	15455019	Flanking_5UTR	0.14	0.15	T
	rs11881159	15454813	Flanking_5UTR	0.18	0.20	C
	rs2304200 ^c	15441658	Coding ^d	No call	No call	No call
	rs2366057	15452052	Flanking_5UTR	0.48	0.46	T
	rs3764566	15431931	Flanking_3UTR	0.32	0.30	T
	rs3813135	15448345	Coding ^d	0.38	0.40	C
	rs733731	15448185	Coding ^d	0.38	0.40	A
	rs892145	15447672	Coding ^d	0.37	0.40	A
<i>PGLYRP3</i> Chromosome 1	rs2987763	151552658	Flanking_5UTR	0.47	0.41	A
	rs6587735	151532151	Flanking_3UTR	0.44	0.37	A
	rs821426	151548696	Intron	0.16	0.20	G
	rs821427	151542601	Intron	0.35	0.38	A
	rs843971	151544047	Coding ^d	0.37	0.39	A
	rs10888557	151591674	Flanking_5UTR	0.11	0.17	C
	rs12063091	151579668	Coding ^d	0.06	0.10	T
	rs1655310	151570554	Intron	0.35	0.34	T
<i>PGLYRP4</i> Chromosome 1	rs3006440	151562231	Flanking_3UTR	0.49	0.45	A
	rs3006448	151580777	Coding ^d	0.16	0.21	C

Gene	SNP	Coordinate (36.3)	Gene Location	MAF in controls		Minor allele
				FAME	SEARCH	
	rs3006453	151584347	Coding ^a	0.06	0.07	C
	rs3006458	151586996	Coding ^a	0.16	0.22	T
	rs3014863	151582203	Coding ^b	0.10	0.11	A
	rs3014864	151588208	Flanking_5UTR	0.16	0.21	G

^a Nonsynonymous.

^b Synonymous.

^c Excluded from analyses.

SNP, single-nucleotide polymorphism; MAF, minor allele frequency.

TABLE 3

PGLYRP SNP associations with risk of PD

Gene	SNP	Genotype	FAME OR (95% CI) ^a	SEARCH OR (95% CI) ^a	Pooled OR (95% CI) ^{a,b}
PGLYRP1	rs12982353	GG	1.1 (0.6–1.7)	0.9 (0.6–1.4)	1.0 (0.8–1.4)
		AG	0.9 (0.4–2.1)	0.7 (0.4–1.4)	0.8 (0.5–1.3)
		AA	1.0	0.5	0.6
		<i>p</i> -trend			
	rs2041991	AA	1.3 (0.8–2.2)	0.9 (0.6–1.4)	1.0 (0.8–1.4)
		AT	1.0 (0.5–2.2)	0.7 (0.4–1.3)	0.8 (0.5–1.3)
		TT	0.7	0.3	0.6
		<i>p</i> -trend			
	rs2041993	GG	1.3 (0.8–2.1)	0.9 (0.6–1.3)	1.1 (0.8–1.4)
		AA	1.0 (0.5–2.2)	0.8 (0.4–1.5)	0.9 (0.5–1.5)
	AG	0.6	0.5	0.9	
	<i>p</i> -trend				
rs2072561	TT	0.9 (0.4–1.8)	1.5 (0.8–2.7)	1.2 (0.8–1.9)	
	GG	4.4 (0.3–75)	nc	0.9 (0.1–14)	
	AG	0.9	0.4	0.5	
	<i>p</i> -trend				
PGLYRP2	rs2072563	GG	1.0 (0.6–1.7)	1.0 (0.7–1.5)	1.0 (0.8–1.4)
		AA	0.9 (0.4–2.1)	0.7 (0.4–1.4)	0.8 (0.5–1.3)
		AG	0.9	0.5	0.6
		<i>p</i> -trend			
	rs11085954	CC	1.0 (0.6–1.7)	1.1 (0.7–1.8)	1.1 (0.8–1.5)
		CT	1.7 (0.3–10.2)	0.5 (0.2–1.4)	0.7 (0.3–1.7)
		TT	0.7	0.7	0.9
		<i>p</i> -trend			
	rs11881159	TT ref	1.5 (0.9–2.4)	0.9 (0.6–1.4)	1.1 (0.8–1.5)
		CC	1.7 (0.6–5.2)	1.6 (0.5–5.0)	1.6 (0.8–3.5)
	CT	0.1	0.8	0.24	
	<i>p</i> -trend				
rs2366057	CC ref	1.4 (0.8–2.4)	0.7 (0.5–1.1)	0.9 (0.7–1.3)	
	TT	1.5 (0.8–2.8)	1.0 (0.6–1.7)	1.2 (0.8–1.7)	
	CT	0.2	0.8	0.6	
	<i>p</i> -trend				
rs3764566	CC ref	0.8 (0.5–1.3)	1.0 (0.7–1.5)	0.9 (0.7–1.3)	
	TT	0.9 (0.4–1.9)	2.3 (1.1–4.8)	1.5 (0.9–2.4)	
	CT	0.5	0.09	0.34	
	<i>p</i> -trend				
rs3813135	TT ref	0.7 (0.4–1.2)	0.7 (0.5–1.1)	0.7 (0.5–0.99)	
	CT				

Gene	SNP	Genotype	FAME OR (95% CI) ^a	SEARCH OR (95% CI) ^a	Pooled OR (95% CI) ^{a,b}
		CC	0.8 (0.4–1.7)	0.7 (0.4–1.2)	0.7 (0.4–1.1)
		<i>p</i> -trend	0.3	0.1	0.05
	rs733731 GG ref	AG	0.7 (0.5–1.2)	0.7 (0.5–1.1)	0.7 (0.5–0.98)
		AA	0.8 (0.4–1.7)	0.7 (0.4–1.2)	0.7 (0.4–1.1)
		<i>p</i> -trend	0.4	0.08	0.05
	rs892145 TT ref	AT	0.7 (0.4–1.2)	0.7 (0.5–1.1)	0.7 (0.5–1.0)
		AA	0.8 (0.4–1.7)	0.6 (0.3–1.0)	0.6 (0.4–1.0)
		<i>p</i> -trend	0.3	0.047	0.023
PGLYRP3	rs2987763 TT ref	AT	1.1 (0.6–1.9)	1.2 (0.8–1.8)	1.2 (0.8–1.6)
		AA	1.5 (0.8–2.9)	1.7 (1.0–3.0)	1.6 (1.1–2.5)
		<i>p</i> -trend	0.2	0.06	0.022
	rs6587735 GG ref	AG	1.0 (0.6–1.8)	1.0 (0.7–1.5)	1.0 (0.7–1.4)
		AA	1.1 (0.5–2.1)	1.7 (0.96–3.2)	1.4 (0.9–2.1)
		<i>p</i> -trend	0.8	0.1	0.18
	rs821426 TT ref	GT	0.8 (0.4–1.3)	0.9 (0.6–1.3)	0.8 (0.6–1.2)
		GG	0.4 (0.1–2.0)	0.5 (0.2–1.2)	0.5 (0.2–1.03)
		<i>p</i> -trend	0.17	0.2	0.059
	rs821427 GG ref	AG	0.9 (0.4–1.8)	0.8 (0.6–1.3)	0.9 (0.6–1.2)
		AA	0.9 (0.6–1.5)	0.6 (0.4–1.2)	0.7 (0.4–1.2)
		<i>p</i> -trend	0.7	0.16	0.17
	rs843971 GG ref	AG	0.9 (0.6–1.5)	0.8 (0.5–1.2)	0.8 (0.6–1.2)
		AA	0.8 (0.4–1.6)	0.7 (0.4–1.3)	0.8 (0.5–1.2)
		<i>p</i> -trend	0.5	0.24	0.18
PGLYRP4	rs10888557 GG ref	CG	0.5 (0.2–0.99)	0.7 (0.4–1.1)	0.6 (0.4–0.9)
		CC	Small	0.2 (0.04–0.7)	0.15 (0.04–0.6)
		<i>p</i> -trend	0.016	0.01	0.0004
	rs12063091 CC ref	CT	0.7 (0.3–1.6)	0.5 (0.3–0.9)	0.6 (0.4–0.9)
		TT	Small	0.4 (0.02–6.0)	0.2 (0.02–2.6)
		<i>p</i> -trend	0.2	0.02	0.009
	rs1655310 CC ref	CT	1.0 (0.6–1.7)	0.8 (0.6–1.2)	0.9 (0.7–1.2)
		TT	0.9 (0.4–1.9)	0.8 (0.4–1.6)	0.8 (0.5–1.4)

Gene	SNP	Genotype	FAME OR (95% CI) ^a	SEARCH OR (95% CI) ^a	Pooled OR (95% CI) ^{a,b}
rs3006440	GG ref	<i>p</i> -trend	0.9	0.4	0.4
		AG	0.9 (0.5–1.7)	1.3 (0.8–2.0)	1.2 (0.8–1.7)
		AA	1.4 (0.7–2.6)	1.8 (1.0–3.1)	1.6 (1.1–2.4)
		<i>p</i> -trend	0.3	0.04	0.026
rs3006448	AA ref	AC	0.8 (0.5–1.5)	0.8 (0.5–1.2)	0.8 (0.6–1.1)
		CC	0.2 (0.03–1.8)	0.4 (0.1–0.98)	0.3 (0.1–0.8)
		<i>p</i> -trend	0.17	0.03	0.012
		CT	0.8 (0.4–1.8)	0.8 (0.5–1.5)	0.8 (0.5–1.3)
rs3006453	TT ref	CC	Small	Small	Small
		<i>p</i> -trend	0.5	0.3	0.2
		GT	0.8 (0.5–1.4)	0.8 (0.5–1.2)	0.8 (0.6–1.1)
		TT	0.2 (0.03–1.7)	0.4 (0.2–1.2)	0.4 (0.2–0.9)
rs3014863	GG ref	<i>p</i> -trend	0.13	0.06	0.014
		AG	0.8(0.4–1.6)	0.9 (0.5–1.4)	0.8 (0.6–1.2)
		AA	0.6 (0.1–4.9)	1.5 (0.2–14.0)	0.89 (0.2–3.5)
		<i>p</i> -trend	0.5	0.7	0.4
rs3014864	TT ref	GT	0.8 (0.5–1.5)	0.7 (0.5–1.1)	0.8 (0.6–1.1)
		GG	0.2 (0.03–1.8)	0.4 (0.1–0.97)	0.3 (0.1–0.8)
		<i>p</i> -trend	0.17	0.02	0.008

^a Logistic regression models adjusted for age, sex, race, smoking.

^b Additionally adjusted for study, and study*smoking.

SNP, single-nucleotide polymorphism; ref, reference; OR, odds ratio; CI, confidence interval.

Bold text: statistically significant result.