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Genetic comorbidities in Parkinson's disease

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Parkinson's disease (PD) has a number of known genetic risk factors. Clinical and epidemiological studies have suggested the existence of intermediate factors that may be associated with additional risk of PD. We construct genetic risk profiles for additional epidemiological and clinical factors using known genome-wide association studies (GWAS) loci related to these specific phenotypes to estimate genetic comorbidity in a systematic review. We identify genetic risk profiles based on GWAS variants associated with schizophrenia and Crohn's disease as significantly associated with risk of PD. Conditional analyses adjusting for SNPs near loci associated with PD and schizophrenia or PD and Crohn's disease suggest that spatially overlapping loci associated with schizophrenia and PD account for most of the shared comorbidity, while variation outside of known proximal loci shared by PD and Crohn's disease accounts for their shared genetic comorbidity. We examine brain methylation and expression signatures proximal to schizophrenia and Crohn's disease loci to infer functional changes in the brain associated with the variants contributing to genetic comorbidity. We compare our results with a systematic review of epidemiological literature, while the findings are dissimilar to a degree; marginal genetic associations corroborate the directionality of associations across genetic and epidemiological data. We show a strong genetically defined level of comorbidity between PD and Crohn's disease as well as between PD and schizophrenia, with likely functional consequences of associated variants occurring in brain.

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INTRODUCTION

Parkinson's disease (PD) is recognized to be associated with a number of genetic susceptibility factors, including variability at the loci *SNCA*, *LRRK2*, *MAPT*, *BST1*, *GAK*, *HLA-DR*, *ACMSD*, *STK39*, *MCCCI/LAMP3*, *SYT11*, *CCDC62/HIP1R*, *PARK16/1q32*, *STX1B/16p11*, *FGF20/8p22*, *STBD1/4q21*, *GPNMB/7p15*, among others, which have been identified in genome-wide association studies (GWAS). It is likely that other additional genetic risk factors also contribute. PD has been reported to be associated with a number of clinical comorbidities and altered laboratory values, such as affective disorders and serum urate levels. Several of these have themselves been associated with genetic susceptibility factors, for which information is available from the NHGRI GWAS catalog (<http://www.genome.gov/26525384>, (1)).

This offers the opportunity to examine genetic risk profiles associated with these clinical phenotypes as risk factors for PD. Genetic risk profiles may be thought of as the cumulative genetic load of risk alleles related to a particular disease or trait, explaining more of the attributable genetic risk associated with this disorder than a single SNP itself. While many epidemiological studies examine associations between intermediate phenotypes and a specific outcome, in this study we sought to examine how the genetic risk profiles associated with these intermediate phenotypes may be associated with PD risk themselves. We sought to identify genetic comorbidities of PD by testing associations with genetic risk profiles previously associated with intermediate phenotypes of interest. Any disease or trait with a significant risk profile score associated with PD essentially would share some genetic factors in common.

In this study, we identify PD genetic comorbidities such as Crohn's disease and schizophrenia. We also attempt to dissect the individual contributions of these associated loci in their contributions to PD risk. Additionally, we utilize expression and methylation data to infer functional genetic consequences in brain tissues associated with these genetic comorbidities.

RESULTS

We used a large sample series of >5000 PD cases and 9000 controls with genome-wide genotyping data (Table 1). We defined a list of clinical factors that are potentially associated with PD risk or comorbidity. We then examined how SNPs associated with these clinical factors might be associated with PD itself using a measure of the cumulative effect of all SNPs related to that particular factor (Supplementary Material, Tables S1 and S2). We

identified 18 clinical factors suggested by the literature and clinical observation to be possible factors associated with PD for which there were high-quality GWAS results in the NHGRI catalog (<http://www.genome.gov/26525384>, (1)). These include: serum amyloid, bipolar disorder, caffeine intake, Crohn's disease, hypertension, inflammation, serum iron, melanoma, obesity, PD, psoriasis, rheumatoid arthritis, schizophrenia, smoking, type 2 diabetes, ulcerative colitis and serum urate (2–68). As a note, PD was included as a positive control. For these phenotypes of interest, alleles associated with an increase in risk for binary phenotypes and/or alleles associated with increase in the level of continuous phenotypes were summed to create the genetic risk profiles.

Within each of the five IPDGC cohorts participating in this analysis, each risk profile comprising a number of SNPs was tested using logistic regression for an association with PD status, adjusting for sex, age at onset/exam and population substructure. Summary statistics from each cohort were combined through meta-analysis under random effects to account for possible heterogeneity (see Figure 1 and Table 2). This resulted in three genetic risk profiles being significantly associated with PD. These include profiles based on SNPs taken from studies of Crohn's disease, schizophrenia and PD. A single standard deviation increase in the genetic risk profile for PD, equivalent to roughly a 34% increase in the genetic burden associated with known PD risk alleles within a population, was associated with an odds ratio (OR) of 1.29 (95% confidence interval 1.22–1.38, P -value < $2E-16$). Using the same burden scale, a single standard deviation increase from population means in the allelic burdens derived from Crohn's disease and schizophrenia risk profiles was significantly associated with PD status at an OR of 1.06 (95% confidence interval 1.02–1.11, P -value 0.005) and 1.05 (95% confidence interval 1.01–1.10, P -value 0.012), respectively.

All 96 SNPs within these three risk profiles (Crohn's disease, PD and schizophrenia) were tested independently for association with PD using identical statistical models and meta-analytic methods as described for the analyses of risk profiles (Supplementary Material, Table S3). Two SNPs not previously known to be associated with PD passed Bonferroni correction for 99 tests. The first was rs11191580, a SNP in the *NT5C2* gene known to be associated with schizophrenia. This SNP reached genome-wide significance in our testing (risk associated with reference allele T at OR 1.35, 95% confidence interval 1.21–1.50, P -value $3.98E-8$, T frequency 91.2%). The region surrounding rs11191580 was suggested to be associated with PD

Table 1. Descriptive statistics of cohorts contributing to analyses

Cohort	Cases			Controls		
	Age at onset, mean (SD) in years	% Female	<i>n</i>	Age at enrollment, mean (SD) in years	% Female	<i>n</i>
France	57.548 (13.086)	41.523	985	73.736 (5.373)	42.843	1984
Germany	55.715 (11.549)	39.595	740	47.416 (12.376)	47.987	944
Netherlands	55.649 (11.826)	36.446	771	55.721 (5.771)	56.028	2024
NIA—USA	57.812 (13.156)	40.235	937	61.933 (11.599)	24.525	1896
UK	64.167 (12.434)	42.051	1648	53 (0)	48.388	2699

NIA denotes cohorts with data generated at the Laboratory of Neurogenetics at the National Institute on Aging, National Institutes of Health, Bethesda, MD, USA.

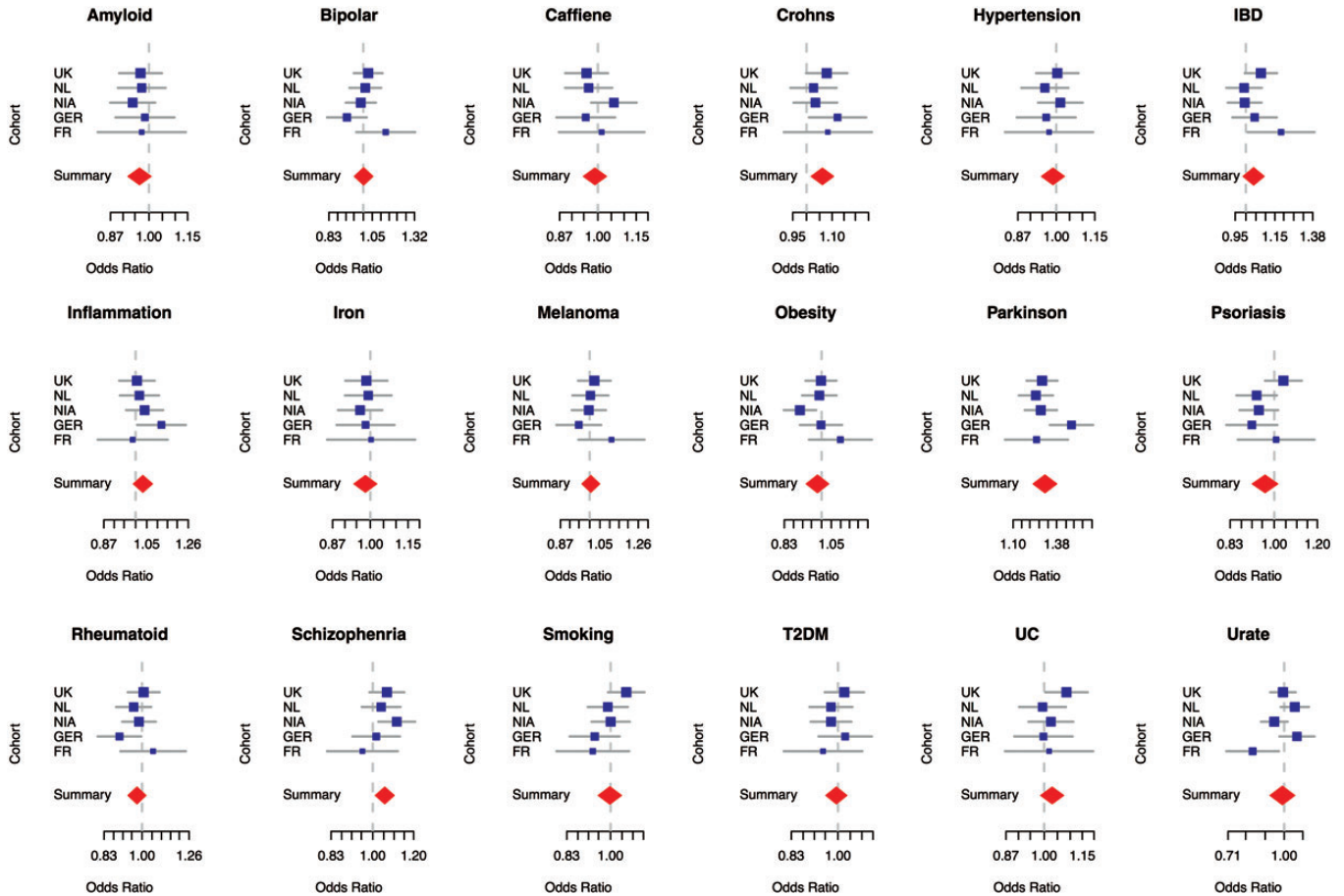


Figure 1. Forest plots from random effects meta-analyses of profiled phenotypes. Cohort-specific OR associated with PD are shown in blue, the size of the square is proportional to the size of the study. Confidence intervals of the summary OR per phenotype are shown as red diamonds, with the centerline of each diamond representing the summary OR for that particular phenotype. The dependent variable in each model is the standardized count of disease risk or quantitative measure increasing alleles per sample associated with either disease status or continuous measures of interest. Abbreviations include Bipolar for bipolar disorder, IBD for inflammatory bowel disease, T2DM for type 2 diabetes, Crohn's for Crohn's disease, UC for ulcerative colitis, Parkinson's for Parkinson's disease, Rheumatoid for Rheumatoid arthritis, Smoking for smoking history/rate, Caffeine for caffeine or coffee consumption levels, amyloid for serum amyloid levels, iron for serum iron levels and urate for serum urate levels.

initially in a previous publication by Simon-Sanchez *et al.* using a portion of the IPDGC data (42). However, the association at this locus was never definitively replicated in a European ancestry population and was therefore not included in the PD genetic risk profiles we have constructed in this report (42). The other SNP passing Bonferroni correction was rs11564258, a SNP near the LRRK2 risk locus from the Crohn's disease risk profile, at an OR of 0.69 associated with the G reference allele (95% confidence interval 0.58–0.83, P -value $5.49E-5$, G frequency 97.5%) showing possible LD with the LRRK2 PD risk locus (49).

To assess the independence of the risk profile associations outside of possible spatial overlaps with PD loci, risk profile score associations were recalculated adjusting for SNPs near PD risk loci as covariates. Keller *et al.*'s summary of PD loci was used, in addition to data mined from the GWAS catalog to further scrutinize putative PD loci (69). To define SNPs used as covariates, they must be within 1 mb of a PD risk locus described in either the downloaded GWAS catalog, Keller *et al.* or Simon-Sanchez *et al.* (1,42,69). When adjusting for SNPs near PD risk

loci as covariates, the schizophrenia risk profile association was reevaluated adjusting for rs1191580 and rs7914558 in NT5C2 and rs3131296 near the HLA-DRA risk locus as additional covariates. The Crohn's disease risk profile association was adjusted for rs11564258 and rs11175593 near LRRK2, rs17309827 near HLA-DRA and rs1736135 near the suspected PD risk locus at USP25. After meta-analyzing the cohort-specific summary statistics adjusted for these SNPs, the association between the Crohn's disease's risk profile remained significantly associated with PD risk (OR 1.05, 95% confidence interval 1.01–1.09, P -value 0.03), while the schizophrenia association was completely attenuated (OR 0.99, 95% confidence interval 0.92–1.06, P -value 0.76). This suggests that there are additional genetic factors outside of known PD loci overlap that contribute to the genetic comorbidity shared by PD and Crohn's disease, while the genetic comorbidity shared by schizophrenia and PD may simply be due to possible overlapping loci, although the association at NT5C2 in PD remains to be definitively replicated.

Systematic review data were extracted for overlapping phenotypes from Noyce *et al.* (1) to evaluate how genetic risk may be

Table 2. Summary statistics from random effects meta-analysis of profile phenotypes

Profiled phenotype	OR	Lower limit of the OR 95% confidence interval	Upper limit of the OR 95% confidence interval	Beta	SE	P-value	I ²	Heterogeneity P-value
Amyloid	0.966	0.927	1.007	-0.034	0.021	0.106	0	0.970
Bipolar	1.002	0.953	1.053	0.002	0.025	0.949	0.270	0.241
Caffeine	0.989	0.949	1.031	-0.011	0.021	0.613	0	0.406
Crohns	1.061	1.018	1.106	0.059	0.021	0.005	0	0.688
Hypertension	0.988	0.948	1.030	-0.012	0.021	0.569	0	0.867
IBD	1.038	0.987	1.092	0.038	0.026	0.143	0.284	0.232
Inflammation	1.032	0.991	1.076	0.032	0.021	0.131	0	0.535
Iron	0.982	0.942	1.024	-0.018	0.021	0.388	0	0.986
Melanoma	1.005	0.965	1.048	0.005	0.021	0.796	0	0.583
Obesity	0.980	0.927	1.036	-0.020	0.028	0.471	0.416	0.144
Parkinson	1.298	1.222	1.380	0.261	0.031	<2E-16	0.466	0.112
Psoriasis	0.962	0.912	1.015	-0.039	0.027	0.155	0.359	0.182
Rheumatoid	0.975	0.934	1.019	-0.025	0.022	0.259	0.065	0.370
Schizophrenia	1.055	1.012	1.100	0.053	0.021	0.012	0	0.424
Smoking	0.997	0.949	1.047	-0.003	0.025	0.906	0.259	0.249
T2DM	0.993	0.953	1.036	-0.007	0.021	0.756	0	0.734
UC	1.030	0.988	1.073	0.029	0.021	0.168	0	0.595
Urate	0.989	0.918	1.066	-0.011	0.038	0.772	0.665	0.018

The dependent variable in each model is the standardized count of disease risk or quantitative measure increasing alleles per sample associated with either disease status or continuous measures of interest. OR and beta coefficients correspond to a single standard deviation of change in the genetic profile for each phenotype based on allele counts associated with an increase in disease risk or the level of quantitative phenotype. Abbreviations include IBD for inflammatory bowel disease, T2DM for type 2 diabetes, UC for ulcerative colitis, SE denotes standard error of the beta coefficient from logistic regression and I² is the index of heterogeneity.

reflected in epidemiological data (Table 3). Neither Crohn's disease or schizophrenia associations were significantly replicated in the epidemiological data, although the directionality of effect for psychosis aspects of schizophrenia and Crohn's disease mirror genetic risk estimates associated with PD. The directionality of effect in our study of genetic factors relating to PD comorbidity leads support to the effects described in the statistically significant epidemiologically evaluated comorbidities of smoking status, coffee drinking and hypertension as described in the systematic review.

Mining of brain tissue to infer possible biological functionality at loci associated with PD, Crohn's disease and schizophrenia, we examined regional methylation and expression data within ± 1 mb of SNPs of interest in a large series of neurologically normal frontal cortex and cerebellar tissues. While we identified a number of loci significantly associated with methylation and expression changes in the brain tissue samples, our primary interest was to focus on SNPs from overlapping regions identified as risk loci for PD and Crohn's or PD and schizophrenia (Table 4). SNPs in the HLA region used to construct the Parkinson's and schizophrenia profiles were significantly associated with changes in regional methylation status in both the frontal and cerebellar tissue samples, allowing us to infer functional consequence in brain tissues associated with these proximal risk SNPs. Alleles at this locus associated with risk of PD and schizophrenia were concurrently associated with decreased methylation in the frontal cortex (49). We also show that a Crohn's disease-associated SNP near the LRRK2 PD risk locus is also significantly associated with methylation changes in the frontal cortex. This suggests that genetic variants associated with Crohn's disease and schizophrenia (in addition to PD) may alter brain function to some degree.

DISCUSSION

In this study, we identified Crohn's disease and schizophrenia as genetic comorbidities of PD. The genetic risk profiles based on GWAS identified loci for these two diseases showed significant risk associated with PD. The genetic comorbidity associated with schizophrenia was almost entirely accounted for by SNPs in the PD associated loci near NT5C2 and HLA-DRA. On the other hand, Crohn's disease's risk profile and PD remained significantly associated even when adjusting for overlapping SNPs at loci near known PD risk loci USP25, HLA-DRA and LRRK2. This suggests that additional risk loci associated with Crohn's disease are also connected to PD risk, outside of those already near known PD loci. In the near future, deep sequencing of large population based studies should help clarify further genetic comorbidities and provide greater insight into the mechanisms of multiple related diseases.

There seems to be functional connectivity between these diseases based on our analyses of expression and methylation data. SNPs associated with both schizophrenia and Crohn's disease cause significant changes in proximal methylation and expression levels in the brain, allowing for the inference of functional changes occurring in the brain related to these genetic comorbidities. The associated changes in methylation and expression status surrounding these SNPs mirrors their genomic context in previous analyses related to PD (49,70). Future studies with single cell capture designs, deep sequencing and additional brain tissue regions sampled may better quantify possible functional genetic consequences in PD etiology.

We recognize that a lack of clinical data on many of the participants in the study pose problems. In particular, we are concerned about the issue of drug-induced parkinsonism in this

Table 3. Systematic review of epidemiological literature for select traits of interest

Factor	Study/studies (n)	Cases (n)	Controls (n)	OR	Lower limit of the OR 95% confidence interval	Upper limit of the OR 95% confidence interval	SE	P-value	I ²	Heterogeneity P-value
Ever versus never smoking ^a	67	19 518	1 053 664	0.64	0.6	0.69	0.0347	<0.001	49.60%	<0.001
Drinking versus non-drinking coffee ^a	19	5801	723 072	0.67	0.58	0.76	0.0684	<0.001	42.90%	0.025
Hypertension preceding PD ^a	12	5993	187 226	0.74	0.61	0.9	0.0989	0.003	76.50%	<0.001
Diabetes preceding PD ^a	13	20 025	303 543	0.91	0.72	1.15	0.1189	0.423	70.90%	<0.001
Melanoma preceding PD ^b	6	–	–	1.07	0.62	1.84	–	–	49.30%	0.079
Serum iron ^c	10	520	711	NS	NS	NS	–	–	93.40%	<0.0000
Bipolar disorder preceding PD	Shiba <i>et al.</i> (71, 83)	196	196	1	0.1	16	–	–	NA	NA
Psoriasis preceding PD	Rugbjerg <i>et al.</i> (72, 84)	13 695	68 445	1.25	0.51	3.06	–	–	NA	NA
Ulcerative colitis preceding PD	Rugbjerg <i>et al.</i> (72, 84)	13 695	68 445	1.3	0.9	1.8	–	–	NA	NA
Crohn's disease preceding PD	Rugbjerg <i>et al.</i> (72, 84)	13 695	68 445	1.06	0.54	2.1	–	–	NA	NA
Rheumatoid arthritis preceding PD	Rugbjerg <i>et al.</i> (72, 84)	13 695	68 445	0.7	0.5	0.9	–	–	NA	NA
Blood inflammatory markers preceding PD ^d	Chen <i>et al.</i> (73, 85)	84	165	3.4	1.1	10.5	–	–	NA	NA
Obesity preceding PD ^a	3/7 studies described significant associations with obesity preceding PD through a variety of different measures (RR/OR 2.8 ^f , 2.03 ^f , 0.43 ^f , 0.9, 0.8, 0.86, 0.99)									
Urate level preceding PD ^a	3/5 studies described significant associations with elevated urate preceding PD using various cut-offs (ORs 0.4 ^f , 0.6 ^f , 0.71 ^f , 0.43, 1.33) and 1 with preceding gout (OR 0.69 ^f)									
Schizophrenia preceding PD ^{se}	Two studies (Shiba <i>et al.</i> (71, 83): schizophrenia OR 1.0 (CI 0.1–7.0); Rajput <i>et al.</i> (74, 86): psychosis RR 1.5 (CI 0.3–6.7))									
Serum amyloid	No known epidemiological studies comparing serum amyloid levels between PD and controls									

NS, non-significant but figures not provided; NA, not available; RR, relative risk.

^aSummary data from meta-analysis undertaken by Noyce *et al.* (1).

^bSummary data from meta-analysis undertaken by Liu *et al.* (86). NB: After exclusion of the only negative study OR 1.44 (CI 1.06–1.96); association of existing PD with melanoma OR 3.61 (CI 1.49–8.77).

^cSummary data from meta-analysis undertaken by Mariani *et al.* (87); data are for patients with established PD; Mariani *et al.* also report reduced transferrin and transferrin saturation.

^dOR refers to highest quintile of IL-6 compared with the lowest (*P* for trend 0.03). Associations with high-sensitivity c-reactive protein (CRP), fibrinogen and tumor necrosis factor were non-significant. NB: In established PD, Song *et al.* (75, 87) reported increased high-sensitivity CRP compared with controls OR 2.04 (CI 1.18–3.52).

^eRisk of bias through drug-induced parkinsonism due to neuroleptic treatment.

^fStatistically significant result (where confidence interval not given).

study and in other GWAS of both schizophrenia and PD. There is a long history of drug-induced parkinsonism that is often acute in onset (76), and misdiagnosis could affect the power and validity of our current study. However, as part of the Queen Square Brain Bank diagnostic criteria utilized to diagnose a majority of samples contributed to the IPDGC consortium effort, we believe that the possible effect of drug-induced PD is minimal, as a concurrent diagnosis of schizophrenia was an exclusionary criteria at study inception.

We recognize that there are some shortcomings in this analysis, first and foremost is the lack of available data relating to intermediate phenotypes in our PD consortium. The IPDGC datasets contain little to no information regarding the 18 intermediate exposures that would be necessary to conduct a true Mendelian randomization (7, 77–80). A previous study of PD risk focused

on serum iron levels as the intermediate exposure has attempted to extrapolate risk estimates from non-overlapping populations and conduct an indirect Mendelian randomization based on summary statistics from disparate cohorts in a complex and well-conducted study, although this was logistically prohibitive in this survey of multiple outcomes due to data availability and standardization issues across intermediate exposures (81). Due to logistic constraints, we opted to use this risk profiling method instead, which could be applied within our consortium. While this approach does not infer causality, it does elucidate comorbid factors, and the associations between intermediate factors are directly comparable using this method. In addition, this approach can successfully identify comorbid intermediate factors associated with PD, as this study has >95% power to detect an effect at the magnitude of the schizophrenia and Crohn's disease

Table 4. Local changes in mRNA expression and CpG methylation are associated with SNPs of interest for Crohn's disease, PD and schizophrenia

SNP name	Chromosome	SNP position (bp, build 37)	Major allele, minor allele	MAF	Imputation quality (RSQ from MiniMach)	Effect, based on minor allele dosage	Standard error	P-value	CpG or mRNA probe	Assay	Tissue sampled	Distance from SNP to probe (bp)	Probe-associated gene	Phenotype
rs2274910	1	160852046	C,T	0.2776	1	-0.744	0.09	1.05E-16	cg10106388	CpG methylation	CRBLM	19384	CD244	Crohns
rs10181042	2	61224259	C,T	0.4224	0.9946	0.414	0.074	1.82E-08	ILMN_1754501	mRNA expression	FCTX	166006	NA	Crohns
rs9858542	3	49701983	G,A	0.3273	0.8245	-0.485	0.095	3.05E-07	cg12788313	CpG methylation	FCTX	24144	MST1	Crohns
rs9858542	3	49701983	G,A	0.322	0.6	-0.553	0.099	2.06E-08	ILMN_1690454	mRNA expression	CRBLM	140324	FAM212A	Crohns
rs2188962	5	131770805	C,T	0.4244	1	0.388	0.087	8.62E-06	cg20512303	CpG methylation	CRBLM	177896	PDLIM4	Crohns
rs2188962	5	131770805	C,T	0.4244	1	-0.388	0.087	8.67E-06	cg22598563	CpG methylation	CRBLM	206884	P4HA2	Crohns
rs359457	5	173279842	T,C	0.4171	0.999	0.463	0.074	4.26E-10	ILMN_1722025	mRNA expression	CRBLM	105114	CPEB4	Crohns
rs359457	5	173279842	T,C	0.4171	0.999	0.672	0.075	2.31E-19	ILMN_1722025	mRNA expression	FCTX	105114	CPEB4	Crohns
rs13194053	6	27143883	T,C	0.1683	0.9872	0.516	0.109	2.26E-06	cg14345882	CpG methylation	FCTX	779090	BTN3A2	Schizophrenia
rs2021722	6	30174131	C,T	0.2031	0.9775	0.429	0.107	5.96E-05	cg17475918	CpG methylation	CRBLM	283056	HLA-E	Schizophrenia
rs3131296	6	32172993	C,T	0.1409	0.9875	-0.597	0.126	2.13E-06	cg25764570	CpG methylation	FCTX	234246	HLA-DRA	Schizophrenia
rs2395163	6	32387809	T,C	0.2251	1	0.42	0.102	3.46E-05	cg07363637	CpG methylation	FCTX	540863	SLC44A4	Parkinson
rs2395163	6	32387809	T,C	0.2251	1	0.428	0.102	2.46E-05	cg21415604	CpG methylation	FCTX	439376	C4B	Parkinson
rs3129882	6	32409530	A,G	0.4151	1	0.473	0.089	1.00E-07	cg25764570	CpG methylation	FCTX	2291	HLA-DRA	Parkinson
rs2301436	6	167437988	C,T	0.4308	0.8426	-0.35	0.081	1.50E-05	ILMN_1671565	mRNA expression	CRBLM	94917	RNASET2	Crohns
rs4077515	9	139266496	C,T	0.4247	0.9333	-0.901	0.076	1.67E-32	ILMN_1811301	mRNA expression	CRBLM	56817	INPP5E	Crohns
rs4077515	9	139266496	C,T	0.4247	0.9333	-0.904	0.076	6.37E-33	ILMN_1811301	mRNA expression	FCTX	56817	INPP5E	Crohns
rs7914558	10	104775908	G,A	0.3991	0.9932	-0.696	0.086	5.20E-16	cg00035347	CpG methylation	FCTX	177700	NT5C2	Schizophrenia
rs7914558	10	104775908	G,A	0.3993	0.8939	-0.439	0.08	3.36E-08	ILMN_1682165	mRNA expression	CRBLM	72030	NT5C2	Schizophrenia
rs102275	11	61557803	T,C	0.3279	0.9763	-0.423	0.075	1.70E-08	ILMN_1670134	mRNA expression	CRBLM	9609	FADS1	Crohns
rs102275	11	61557803	T,C	0.3279	0.9763	-0.315	0.075	2.57E-05	ILMN_1670134	mRNA expression	FCTX	9609	FADS1	Crohns
rs694739	11	64097233	A,G	0.4073	0.8502	-0.911	0.081	2.75E-29	ILMN_1772208	mRNA expression	CRBLM	27286	CCDC88B	Crohns
rs11175593	12	40601940	C,T	0.0189	0.9679	1.324	0.299	9.86E-06	cg04575343	CpG methylation	FCTX	102678	SLC2A13	Crohns
rs12817488	12	123296294	G,A	0.4249	0.8039	-0.368	0.093	7.46E-05	cg05384917	CpG methylation	CRBLM	95341	GPR109B	Parkinson
rs2942168	17	43714850	G,A	0.2538	0.9091	0.522	0.104	5.73E-07	cg19832721	CpG methylation	CRBLM	534966	KIAA1267	Parkinson
rs2942168	17	43714850	G,A	0.247	0.3054	-0.87	0.149	5.30E-09	ILMN_1709549	mRNA expression	CRBLM	201405	PLEKHM1	Parkinson
rs2942168	17	43714850	G,A	0.247	0.3054	-2.287	0.164	3.66E-44	ILMN_2393693	mRNA expression	CRBLM	130367	PLEKHM1	Parkinson
rs393152	17	43719143	A,G	0.2551	0.997	0.497	0.099	5.76E-07	cg19832721	CpG methylation	CRBLM	530673	KIAA1267	Parkinson
rs393152	17	43719143	A,G	0.2483	0.335	-0.829	0.142	5.25E-09	ILMN_1709549	mRNA expression	CRBLM	205698	PLEKHM1	Parkinson
rs393152	17	43719143	A,G	0.2483	0.335	-2.18	0.156	3.41E-44	ILMN_2393693	mRNA expression	CRBLM	134660	PLEKHM1	Parkinson
rs8070723	17	44081064	A,G	0.2547	1	0.496	0.099	5.77E-07	cg19832721	CpG methylation	CRBLM	168752	KIAA1267	Parkinson
rs8070723	17	44081064	A,G	0.2592	0.946	-0.509	0.083	8.29E-10	ILMN_1709549	mRNA expression	CRBLM	567619	PLEKHM1	Parkinson
rs8070723	17	44081064	A,G	0.2592	0.946	-1.265	0.091	1.07E-43	ILMN_2393693	mRNA expression	CRBLM	496581	PLEKHM1	Parkinson
rs199533	17	44828931	G,A	0.2386	0.8935	0.487	0.108	5.91E-06	cg19832721	CpG methylation	CRBLM	579115	KIAA1267	Parkinson
rs199533	17	44828931	G,A	0.2449	0.7188	-0.4	0.099	5.37E-05	ILMN_1698680	mRNA expression	FCTX	234735	ARL17A	Parkinson
rs281379	19	49214274	A,G	0.4943	0.9789	0.484	0.086	1.71E-08	cg13881341	CpG methylation	CRBLM	44941	FUT1	Crohns
rs281379	19	49214274	A,G	0.4943	0.9789	0.389	0.086	5.73E-06	cg116155702	CpG methylation	CRBLM	45936	FGF21	Crohns
rs4809330	20	62349586	G,A	0.3435	1	-0.388	0.077	4.23E-07	ILMN_1794643	mRNA expression	CRBLM	17792	ZGPAT	Crohns
rs181359	22	21928641	G,A	0.2118	0.8549	-0.698	0.111	3.49E-10	cg22262140	CpG methylation	CRBLM	58335	FLJ36046	Crohns
rs181359	22	21928641	G,A	0.2118	0.8549	-0.481	0.111	1.51E-05	cg22262140	CpG methylation	FCTX	58335	FLJ36046	Crohns

The table shows only significantly associated SNP-probe pairs after Bonferroni correction for multiple testing. Abbreviations include CRBLM for cerebellum, FCTX for frontal cortex and bp for base pair.

associations (OR ~ 1.05 , $\alpha < 0.05$). On the other hand, this method cannot definitively show a true negative effect for the other 16 intermediate risk factors investigated in this manuscript (particularly those with robust epidemiological associations such as smoking and caffeine intake) as the genetic contributions of known associated SNPs may be so small, and then further diluted in their biological relationship to PD risk, that these effects may be of virtually undetectable magnitude at this current period in genetic analyses.

MATERIALS AND METHODS

Data mining of PD-related traits from GWAS

The entire NHGRI GWAS catalog was downloaded on August 15, 2012. Phenotypes for each GWAS were indexed describing the diseases and quantitative traits associated with these studies. Based on a recently published systematic review of PD-related factors and consultation, we assembled a list of possible comorbidities of interest from the GWAS catalog using the disease/trait index (Supplementary Material, Table S1). From these indexed disease/trait descriptions, we extracted all keywords described in Supplementary Material, Table S1 by text mining to identify studies of relevant phenotypes for this analysis.

To extract relevant SNPs from only high-quality studies, a series of filters were used for SNP inclusion in the profiles based on data from the GWAS catalog. Minimum sample sizes for included GWAS SNPs had to be at least 1000 cases and 1000 controls for binary phenotypes in both discovery and replication analyses, and at least 1000 samples for continuous phenotypes. In addition, since all PD GWAS samples were of European ancestry, these sample size filters were based on European ancestry participants within the reported GWAS only. These SNPs must also have achieved a discovery P -value of $\leq 5E-8$ as reported in the GWAS catalog. The contributing GWAS study must also have made data available regarding allelic direction of effect for the most significant SNP per locus.

Once all high-quality studies and their reported SNPs were extracted, additional quality control was necessary prior to constructing phenotype-specific risk scores. For all GWAS SNPs associated with a specific phenotype of interest, duplicate SNPs were excluded, and SNPs within 250 kb of another SNP associated with the same phenotype were excluded if the r^2 between the two SNPs was > 0.5 . For SNPs in linkage disequilibrium related to the same phenotype, the more significant SNP based on discovery phase P -value was kept for construction of the risk profile scores. r^2 estimates of linkage disequilibrium were based on European ancestry samples from the most recent 1000 Genomes Project data freeze (Phase 1 alpha, version 3 available from (1)). In addition, all SNPs had to be successfully imputed in the IPDGC cohorts participating in this analysis, see below for details.

IPDGC datasets

Genotyping data from five IPDGC cohorts with genome-wide genotyping were extracted for use in this study. These cohorts have been described in Table 1, and in greater detail in previous publications (49,70). In brief, standard genome-wide association quality control was used including inclusion criteria such as:

minimum 95% call rate per sample, Hardy–Weinberg equilibrium P -values $> 1E-6$, minor allele frequency $> 1\%$, missingness per SNP $< 5\%$. Additional quality control parameters for GWAS samples included European ancestry consistent with HapMap3 samples based on multidimensional scaling, X chromosome heterogeneity reflecting self-reported gender per sample, and the exclusion of cryptically related samples at the first cousin level or closer relation. In addition, SNPs were removed for palindromic alleles (A/T or G/C combinations), differential missingness between cases and controls at P -value of $< 1E-4$ and differential missingness by haplotype P -value of $< 1E-4$. All quality control of raw genotype data was conducted using PLINK and R (6, R Development Core Team, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, URL <http://www.R-project.org>, 2006).

After quality control, all IPDGC datasets were imputed using the minimac implementation of the Markov Chain based haplotyper with reference haplotypes from the European ancestry samples available in the August 2010 release from the 1000 Genomes project (82). After imputation, imputed dosages were filtered based on minimum imputation quality of 0.30 (RSQR metric from MACH) and a minor allele frequency of 1% for each cohort.

Risk profile construction

For each of the 18 phenotypes of interest with high-quality SNPs available, risk profiles were constructed. This was accomplished by summing all imputed allele dosages from extracted SNPs for each phenotype per cohort. The allelic dosages were quantified as 0–2 dosages for each SNP relating to the specific allele that was associated in previous reports with either a risk increase for a disease phenotype or an increase in the level of a continuous phenotype. All risk profiles were relatively normally distributed and were then Z transformed so that effect estimates would be easily comparable. Z transformation was undertaken using the standard formula where individual profile scores were subtracted by the mean of the population for that score and then divided by their standard deviation.

Analyses of risk profiles

For each of the 18 profiles calculated within each of the contributing cohorts, logistic regression was used to generate summary statistics, using the profile scores as the independent variable and adjusting for age (at onset in cases and exam in controls), sex and principal components 1 and 2 derived from genotyped SNPs. The use of principal components based on an LD pruned SNP set in each cohort as a covariate allows for population substructure to be accounted for in the regression models. Summary statistics for each cohort were meta-analyzed to generate aggregate effect estimates for all profiles using random effects meta-analyses. For PD, schizophrenia and Crohn's disease related SNPs, single SNP analyses were also performed using identical statistical methods as for the risk profile-based analyses to facilitate an in-depth examination of the individual SNPs comprising these three scores.

Expression and methylation quantitative trait analyses

Data for this aspect of analyses were made possible through collaborative efforts of the North American and United Kingdom Brain Expression Consortia (83–85). Frozen frontal cortex and cerebellar samples were obtained from >399 self-reported European ancestry samples without determinable neuropathological evidence of disease. Genomic DNA was extracted with phenol–chloroform. Bisulfite converted DNA and assayed at >27 000 sites on the Illumina Infinium HumanMethylation27 BeadChips. MRNA expression levels were assayed using Illumina HumanHT-12 v3 Expression Beadchips. In brief, individual probes were excluded from analyses if the *P*-value for detection was >0.01 or there was <95% completeness of data per probe, and samples were excluded if <95% of probes were detected. Probes were also removed if an analyzed SNP was within the probe or the probe mapped ambiguously to multiple locations in the genome. Expression data were cubic spline normalized and log₂-transformed prior to analyses.

Each tissue sample was genotyped using the Illumina Human-Hap550 v3, Human610-Quad v1 or Human660W-Quad v1 Infinium Beadchips, shared SNPs were extracted prior to QC and imputation. Standard GWAS quality control was undertaken with inclusion criteria such as: minimum call rate 95% for both participants and SNPs, minor allele frequency (MAF) > 0.01, HWE > 1E–7, no first-degree relatives in the sample collection (identity by descent score <0.125 in PLINK) and European ancestry confirmed by multidimensional scaling analyses.

Data were imputed using MiniMac (<http://genome.sph.umich.edu/wiki/Minimac>) to the most recent data freeze of 1000 Genomes haplotypes (<http://www.sph.umich.edu/csg/abecasis/MaCH/download/1000G.2012-03-14.html>) using default settings. All imputed SNPs were filtered for a minimum imputation quality of 0.30. After quality control, data were available for >10 million SNPs, with expression data on 399 samples (9814 probes from the frontal cortex and 9587 probes in cerebellum) and methylation data on 292 samples (27465 CpG sites in the frontal cortex tissue samples and 27419 CpG sites in the cerebellum).

Linear regression models were utilized to estimate associations between allele dosages of per SNP and gene expression or methylation levels adjusted for covariates of gender, age at death, the first two component vectors from multidimensional scaling, post-mortem interval, brain bank and batch in which preparation or hybridization were performed, using MACH2QTLv1.11 (<http://www.sph.umich.edu/csg/abecasis/MaCH/download/>). Analyses were carried out separately for each brain region and each array type. Only probes within ±1 mb of SNPs of interest in this study were analyzed to test only cis associations. From these analysis results, data were mined for SNPs comprising the Crohn's disease, PD and schizophrenia risk profiles. Multiple test correction was based on simple Bonferroni correction stratified by brain region and assay type.

Systematic review of epidemiological data

Validation of the findings of associations of a genetic profile PD with genetic risk profiles for other conditions or behaviors was sought by comparison with published data from epidemiological studies on their clinical associations. A recent systematic review and meta-analysis reported the combined results from case–

control and cohort studies on association of PD with preceding smoking, coffee drinking, hypertension, diabetes, raised serum urate and obesity. For full details of the search strategy, inclusion and exclusion criteria and analysis of data see Noyce *et al.* (1). A separate meta-analysis on association of PD with preceding as well as existing diagnosis of melanoma (86) included publications until June 2010 and no further relevant papers were published from October 2010 to March 2011; and another meta-analysis on association of existing PD with iron levels included publications until 2011 (87). For the other factors that had not fulfilled inclusion criteria in these meta-analyses, individual publications that reported case–control or cohort studies in the general PD population up to March 31, 2011 were identified. From these publications, the number of studies, number of cases and controls, OR with 95% confidence intervals, standard errors, *I*² statistic, and *P*-values for risk and heterogeneity (in the meta-analyses) were extracted or calculated.

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