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ARTICLE

Smoking and Parkinson disease

Evidence for gene-by-smoking interactions

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Abstract

Objective

To investigate whether cigarette smoking interacts with genes involved in individual susceptibility to xenobiotics for the risk of Parkinson disease (PD).

Methods

Two French population-based case-control studies (513 patients, 1,147 controls) were included as a discovery sample to examine gene-smoking interactions based on 3,179 single nucleotide polymorphisms (SNPs) in 289 genes involved in individual susceptibility to xenobiotics. SNP–by–cigarette smoking interactions were tested in the discovery sample through an empirical Bayes (EB) approach. Nine SNPs were selected for replication in a population-based case-control study from California (410 patients, 845 controls) with standard logistic regression and the EB approach. For SNPs that replicated, we performed pooled analyses including the discovery and replication datasets and computed pooled odds ratios and confidence intervals (CIs) using random-effects meta-analysis.

Results

Nine SNPs interacted with smoking in the discovery dataset and were selected for replication. Interactions of smoking with rs4240705 in the *RXRA* gene and rs1900586 in the *SLC17A6* gene were replicated. In pooled analyses (logistic regression), the interactions between smoking and rs4240705-G and rs1900586-G were 1.66 (95% CI 1.28–2.14, $p = 1.1 \times 10^{-4}$, p for heterogeneity = 0.366) and 1.61 (95% CI 1.17–2.21, $p = 0.003$, p for heterogeneity = 0.616), respectively. For both SNPs, while smoking was significantly less frequent in patients than controls in AA homozygotes, this inverse association disappeared in G allele carriers.

Conclusions

We identified and replicated suggestive gene-by-smoking interactions in PD. The inverse association of smoking with PD was less pronounced in carriers of minor alleles of both *RXRA*-rs4240705 and *SLC17A6*-rs1900586. These findings may help identify biological pathways involved in the inverse association between smoking and PD.

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Glossary

CAR = constitutive androstane receptor; **CI** = confidence interval; **EB** = empirical Bayes; **FHC** = free health care; **GEI** = gene-environment interaction; **GWAS** = genome-wide association studies; **ICD-9-CM** = *International Classification of Diseases, 9th revision, clinical modification*; **OR** = odds ratio; **PD** = Parkinson disease; **PXR** = pregnane X receptor; **RA** = retinoic acid; **RAR** = retinoic acid receptor; **RXR** = retinoid X receptor; **SNP** = single nucleotide polymorphism; **VGLUT** = vesicular glutamate transporter.

Parkinson disease (PD) is considered a complex disorder, involving both genetic and environmental factors. According to twin studies, PD heritability is modest,[1] with stronger estimates for patients with younger onset.[2] Mendelian genes account for a small proportion of patients and in several instances display reduced penetrance.[3] Genetic susceptibility plays a role in sporadic cases, and genome-wide association studies (GWAS) highlighted several low-penetrance genes generally associated with small effects.[4] Epidemiologic studies show that environmental risk/protective factors play a role in PD[1] and may account for the low penetrance of some genes through gene-environment interactions (GEIs).

Among environmental factors, numerous studies have shown an inverse association of cigarette smoking with PD, with a 40% lower risk in cigarette ever smokers.[5] It is unclear, however, whether this relation is causal and what biological mechanisms may be involved.[6] One possible way to help better understand this puzzling association is to search for biologically plausible genetic modifiers. Evidence of an interaction between cigarette smoking and genetic susceptibility may help identify relevant pathways and improve causal inference. Genes involved in individual susceptibility to xenobiotics represent plausible candidates for this approach.

We conducted a discovery study in a French dataset with replication in an independent US dataset (both relied on population-based studies) and investigated whether cigarette smoking interacted with genes involved in individual susceptibility to xenobiotics.

Methods

Patients with PD and controls

Discovery sample

Data come from 2 French population-based case-control studies performed within a health insurance system (Mutualité Sociale Agricole). In France, PD belongs to a list of 30 chronic diseases for which free health care (FHC) is granted. Patients (age 18–75 years) applying for FHC for PD (February 1998–August 1999) were included in the Terre study.[7,8] Patients (age 18–80 years) from 5 districts who benefited from FHC for PD or bought antiparkinsonian drugs were included in the Partage study (2006–2007).[9,10] Patients were examined by neurologists. Parkinsonism was defined as ≥2 cardinal signs (rest tremor, bradykinesia, rigidity, impaired postural reflexes). Among patients with parkinsonism, 3 criteria were used to define PD: no other cause (e.g., repeated stroke; drug induced), no unresponsiveness to levodopa ≥1 g/d (when applicable), and no prominent/early (within 1 year of onset) signs of more extensive nervous system involvement not explained otherwise (e.g., dysautonomia).[11] Parkinsonism-free population-based controls were individually matched to patients on age, sex, and district of residency. In Terre, controls were recruited among all members who requested reimbursement for health expenses; a maximum of 3 controls were matched to each patient. In Partage, 2 controls per patient were randomly drawn from the electronic list of all members. In both studies, the majority of participants (>90%) were of French ancestry. This population of farmers is not a mobile one, with ~75% of patients and controls born in the same district as both their parents. Because both studies were performed within the same population and participants had similar characteristics, we combined them for the analysis.

Replication sample

Patients with PD (1998–2007) residing in agriculturally highly active Fresno, Tulare, and Kern counties (California) were recruited into the Parkinson Environment and Gene (PEG) study within 3 years of diagnosis; 90% of 31 practicing local neurologists providing PD care assisted in recruiting patients. We identified 563 eligible patients by ICD-9-CM codes from medical providers; 410 were confirmed clinically as having PD by study movement disorder specialists and provided all information needed. Eight hundred forty-five controls ≥65 years of age were identified from Medicare lists or randomly selected from tax assessor residential units in each county. Of 410 patients and 845 controls, 20.7% and 32.1% were not of European ancestry.

Standard protocol approvals, registrations, and patient consents

Ethics committees of Kremlin-Bicêtre/Pitié-Salpêtrière University Hospitals approved the research protocols for French studies. The PEG study was approved by the institutional review board of the University of California at Los Angeles. All participants provided written informed consent.

Cigarette smoking and other covariates

In the French and US studies, participants were interviewed in person about lifetime history of cigarette smoking and categorized as never or ever smokers before PD onset for patients and an index date in controls. In the US dataset, the index date was the interview date, and in the French dataset, it

was the PD onset date in matched patients. The average number of cigarettes smoked per day and number of years of smoking were used to compute pack-years of smoking.

We collected information on family history of PD among first-degree relatives. In the discovery dataset, we obtained information on alcohol drinking (never/occasional, regular, everyday) and passive smoking (at home or workplace) before PD onset (patients) or index date (controls).

Genotyping

DNA was extracted from leukocytes (Terre) and saliva (Partage).[12] A panel of experts designed a microarray (16,500 single nucleotide polymorphisms [SNPs]) including genes involved in individual susceptibility to xenobiotics (metabolism, transport, pharmacologic target, gene regulation, signal transduction) and immunogenetics. SNP selection was performed by including functional and tag SNPs that captured >80% of haplotypic diversity. Genes were defined by their position on human genome build 36. Microarrays were processed by Integragen (Evry, France) using Illumina technology and Infinium iSelect custom genotyping. Analyses are restricted to 4,161 SNPs ≤50 kb from 298 genes involved in individual susceptibility to xenobiotics.

DNA was extracted from blood or saliva (PEG). Genotyping of SNPs selected for replication was performed by Integragen using the BioMark platform (Fluidigm, South San Francisco, CA).

Statistical analysis

From the initial 4,161 SNPs, we excluded 982 with genotyping call rates ≤95% (n = 331), in Hardy-Weinberg disequilibrium among controls ($p < 0.001$, n = 23), and with minor allele frequency <5% (n = 912), leading us to retain 3,179 SNPs (the sum of the 3 groups is >982 because of overlap). Quality control checks were performed with Plink.[13] Because not all participants provided DNA, we broke the matching to retain a larger number of participants for analyses.

Discovery phase

We examined interactions between SNPs and ever smoking (yes/no) rather than with continuous measures (pack-years, duration) because the latter approach may lead to biased estimates if interactions are misspecified.[14] SNP-by-smoking interactions were tested in the discovery dataset through an empirical Bayes (EB) approach.[15] This method has higher statistical power than standard logistic regression; it combines GEI estimates from a standard (unconstrained) logistic regression model including a multiplicative gene-by-environment term and a constrained logistic model that assumes gene-environment independence in controls. The weight given to each estimate depends on the sample size and strength of the gene-environment association among controls. Throughout this article, the interaction parameter is the exponential of the interaction regression coefficient.

We used an additive SNP coding (number of minor alleles). All models were adjusted for sex, age (quartiles), and population stratification (2 first principal components in controls, based on 5,683 independent SNPs among all SNPs on the microarray). We checked that adjusting for study or combining the 2 studies (Terre, Partage) led to similar findings and that there was no heterogeneity. SNPs interacting with smoking at the 0.005 α level were selected for replication. For these SNPs, results from EB analyses were compared to those from standard logistic regression (including a product term between smoking and the SNPs).

Replication phase

For SNPs selected for replication (additive coding), we tested interactions using both the EB approach and standard logistic regression because the latter allows stricter control of type 1 error.[16]

Pooled analyses

We pooled results from the discovery and replication datasets and computed pooled odds ratios (ORs) using random-effects meta-analysis (additive SNPs coding). For SNPs displaying significant interactions with smoking, we estimated individual and joint effects of these SNPs and cigarette smoking, and we performed stratified analyses by both smoking and genotypes. We also examined pooled interactions using quantitative definitions of smoking (cigarettes per day, years, pack-years). Because there were few participants in some of the smoking × SNP categories, we used dominant coding for this analysis.

Finally, we stratified analyses on PD status to investigate the association between smoking and SNPs separately in patients and controls. Under the assumption of gene-environment independence among controls, a significant SNP-smoking association in patients indicates an interaction. This approach is more powerful than a traditional case-control analysis; however, if the gene-environment independence hypothesis among controls does not hold, interaction ORs are biased and type 1 error is inflated.[17]

To assess the overall statistical significance of the SNPs selected for replication in pooled analyses, we compared p values to a significance level that accounts for multiple comparisons. The median number of SNPs in 289 xenobiotic genes is 7 (bottom quartile 4, upper quartile 12). Many were in strong linkage disequilibrium. We estimated the number of independent tests as 2,301, leading to a corrected significance level of 2.2×10^{-5}.[18]

Sensitivity analyses

We performed matched analyses using conditional logistic regression (discovery dataset). We also adjusted the analyses for family history and alcohol drinking/passive smoking (discovery dataset).

Statistical analyses were performed with R version 3.01 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The discovery dataset included 513 patients and 1,147 controls (figure e-1, http://links.lww.com/WNL/A132), and the replication dataset included 410 patients and 845 controls (table 1). In both datasets, family history of PD was more frequent in patients than controls and patients smoked less often than controls (discovery: OR 0.62, 95% confidence interval [CI] 0.47–0.80; replication: OR 0.69, 95% CI 0.53–0.88; pooled: OR 0.66, 95% CI 0.55–0.79, I^2 = 0.0%, p for heterogeneity = 0.568).

Nine of 3,179 SNPs mapping 5 different loci interacted significantly with smoking (EB analysis, α level ≤0.005) and were selected for replication (table 2); table e-1 (http://links.lww.com/WNL/A133) shows the joint distribution of cigarette smoking and these SNPs, and table e-2 shows marginal associations. These SNPs were in Hardy-Weinberg equilibrium in controls (discovery, replication). Table e-3 shows interaction tests for 109 additional SNPs significant at 0.005 < α level ≤ 0.05 in the discovery dataset (not selected for replication).

Table 2 shows the results of interaction tests for 9 SNPs selected for replication and the pooled analyses. Only rs4240705 in the *RXRA* gene was significant in the replication dataset with standard logistic regression (interaction 1.46, 95% CI 1.01–2.13, p = 0.046, table 2). There was no marginal association between rs4240705 and PD in the discovery or replication dataset (table e-2, http://links.lww.com/WNL/A133). In pooled analysis (table 3 and table e-4), the rs4240705 × smoking interaction (1.66, 95% CI 1.28–2.14, $p = 1.1 \times 10^{-4}$) was statistically significant without evidence of heterogeneity (p = 0.366). Smoking was less frequent in patients than controls among rs4240705-AA carriers (OR 0.48, 95% CI 0.36–0.64, $p = 5.1 \times 10^{-7}$), and this association disappeared in rs4240705-G carriers (AG: OR 0.67, 95% CI 0.38–1.16, p = 0.153; GG: OR 1.42, 95% CI 0.48–4.19, p = 0.529). Conversely, while rs4240705-G tended to be less frequent in patients than controls among never smokers (OR additive 0.86, 95% CI 0.74–1.00, p = 0.052), it was significantly more frequent in patients than in controls among ever smokers (OR additive 1.42, 95% CI 1.17–1.73, $p = 4.8 \times 10^{-4}$). Pooled analyses based on smoking duration, pack-years, and number of cigarettes per day are presented as graphs (figure 1A). The inverse association of smoking characteristics with PD was less pronounced in rs4240705-G carriers than rs4240705-AA homozygotes.

The smoking × rs1900586 (*SLC17A6*) interaction was stronger in the discovery dataset with the EB approach (interaction_{EB} 1.85, 95% CI 1.30–2.63, $p = 5.7 \times 10^{-4}$) than

Table 1 Characteristics of patients with PD and controls in the discovery and replication datasets

	Discovery sample (France)		Replication sample (US)	
Characteristics	**Patients (n = 513)**	**Controls (n = 1,147)**	**Patients (n = 410)**	**Controls (n = 845)**
Male sex, % (n)	58 (296)	59 (672)	59 (241)	49 (413)
Median age at study (range), y	72 (37–81)	71 (36–82)	73 (37–92)	67 (35–99)
Median age at onset (range), y	66 (35–80)	—	71 (34–88)	—
Family history of PD, % (n)[a]	11 (55)	4 (50)	17 (67)	8 (64)
Ever cigarette smoking, % (n)	25 (129)	33 (382)	47 (194)	53 (448)
Years of smoking, % (n)				
Nonsmokers	75 (384)	67 (765)	53 (216)	51 (397)
≤29	15 (75)	17 (191)	31 (128)	29 (226)
>29	10 (53)	17 (190)	16 (65)	20 (154)
Average No. of cigarettes per day, % (n)				
Nonsmokers	75 (384)	67 (765)	53 (216)	51 (397)
≤12	13 (69)	17 (199)	20 (81)	19 (149)
>12	12 (59)	16 (182)	27 (112)	30 (231)
Average pack-years of smoking, % (n)				
Nonsmokers	75 (384)	67 (765)	53 (216)	47 (397)
≤15	14 (70)	17 (197)	24 (98)	24 (199)
>15	11 (58)	16 (184)	23 (96)	29 (248)

Abbreviation: PD = Parkinson disease.
[a] Among first-degree relatives.
Cutoffs for smoking-related variables are based on the medians of the variables in exposed controls.

Table 2 SNPs (n = 9) interacting with cigarette smoking for PD risk in the discovery dataset and selected for replication

Chromosome	SNP	Position	Gene	Minor/major allele	Unconstrained logistic regression		EB	
					Interaction parameter (95% CI)	*p* Value	Interaction parameter (95% CI)	*p* Value
Discovery dataset[a]								
2	rs699664	85634047	GGCX	A/G	1.58 (1.11–2.23)	0.010	1.59 (1.19–2.14)	0.002
2	rs7568458	85641686	GGCX	A/T	1.65 (1.17–2.32)	0.004	1.55 (1.15–2.09)	0.004
6	rs3734254	35502988	PPARD	C/T	2.04 (1.36–3.05)	5.1×10^{-4}	1.84 (1.22–2.77)	0.003
9	rs1536474	136437917	RXRA	T/G	1.93 (1.33–2.79)	5.0×10^{-4}	1.76 (1.20–2.58)	0.004
9	rs4240705	136451221	RXRA	G/A	1.85 (1.30–2.63)	5.9×10^{-4}	1.70 (1.18–2.43)	0.004
11	rs1900586	22344016	SLC17A6	G/A	1.73 (1.13–2.64)	0.012	1.85 (1.30–2.63)	5.7×10^{-4}
16	rs212090	16143505	ABCC1; ABCC6	A/T	1.48 (1.06–2.09)	0.024	1.53 (1.15–2.04)	0.003
16	rs12448760	16147040	ABCC1; ABCC6	A/G	1.63 (1.12–2.36)	0.010	1.56 (1.15–2.11)	0.004
16	rs169844	16162267	ABCC1; ABCC6	C/T	1.51 (1.06–2.14)	0.021	1.53 (1.15–2.04)	0.004
Replication dataset[b]								
2	rs699664	85634047	GGCX	A/G	0.89 (0.61–1.28)	0.518	0.97 (0.68–1.40)	0.882
2	rs7568458	85641686	GGCX	A/T	0.86 (0.61–1.22)	0.405	0.94 (0.66–1.34)	0.737
6	rs3734254	35502988	PPARD	C/T	1.12 (0.73–1.74)	0.594	1.02 (0.68–1.51)	0.937
9	rs1536474	136437917	RXRA	T/G	1.14 (0.77–1.67)	0.519	1.04 (0.75–1.46)	0.803
9	rs4240705	136451221	RXRA	G/A	1.46 (1.01–2.13)	0.046	1.33 (0.92–1.93)	0.134
11	rs1900586	22344016	SLC17A6	G/A	1.47 (0.92–2.38)	0.108	1.52 (1.03–2.23)	0.034
16	rs212090	16143505	ABCC1; ABCC6	A/T	1.00 (0.71–1.43)	0.979	1.07 (0.80–1.42)	0.666
16	rs12448760	16147040	ABCC1; ABCC6	A/G	1.06 (0.71–1.56)	0.785	1.12 (0.82–1.54)	0.478
16	rs169844	16162267	ABCC1; ABCC6	C/T	1.14 (0.80–1.64)	0.471	1.11 (0.84–1.48)	0.469
Pooled analysis[c]								
2	rs699664	85634047	GGCX	A/G	1.19 (0.67–2.09)	0.555	1.26 (0.78–2.04)	0.348
2	rs7568458	85641686	GGCX	A/T	1.19 (0.63–2.24)	0.583	1.22 (0.75–1.99)	0.426
6	rs3734254	35502988	PPARD	C/T	1.52 (0.85–2.73)	0.157	1.37 (0.76–2.44)	0.295
9	rs1536474	136437917	RXRA	T/G	1.48 (0.88–2.49)	0.136	1.35 (0.81–2.25)	0.257
9	rs4240705	136451221	RXRA	G/A	1.66 (1.28–2.14)	1.1×10^{-4}	1.51 (1.17–1.95)	0.002
11	rs1900586	22344016	SLC17A6	G/A	1.61 (1.17–2.21)	0.003	1.69 (1.31–2.19)	6.9×10^{-5}
16	rs212090	16143505	ABCC1; ABCC6	A/T	1.22 (0.83–1.79)	0.301	1.28 (0.90–1.83)	0.176
16	rs12448760	16147040	ABCC1; ABCC6	A/G	1.32 (0.86–2.01)	0.204	1.33 (0.96–1.83)	0.086
16	rs169844	16162267	ABCC1; ABCC6	C/T	1.32 (1.01–1.73)	0.046	1.30 (0.95–1.79)	0.099

Abbreviations: CI = confidence interval; EB = empirical Bayes; PD = Parkinson disease; SNP = single nucleotide polymorphism.
[a] Interaction parameters, 95% CIs, and *p* values were calculated under an additive coding of the SNPs (number of minor alleles) and adjusted for sex, age in quartiles, and population stratification (2 first principal components).
[b] Interaction parameters, 95% CIs, and *p* values were calculated under an additive coding of the SNPs (number of minor alleles) and adjusted for sex, age in quartiles, and minority status.
[c] Pooled interaction parameters and 95% CIs were computed with random-effects meta-analysis.

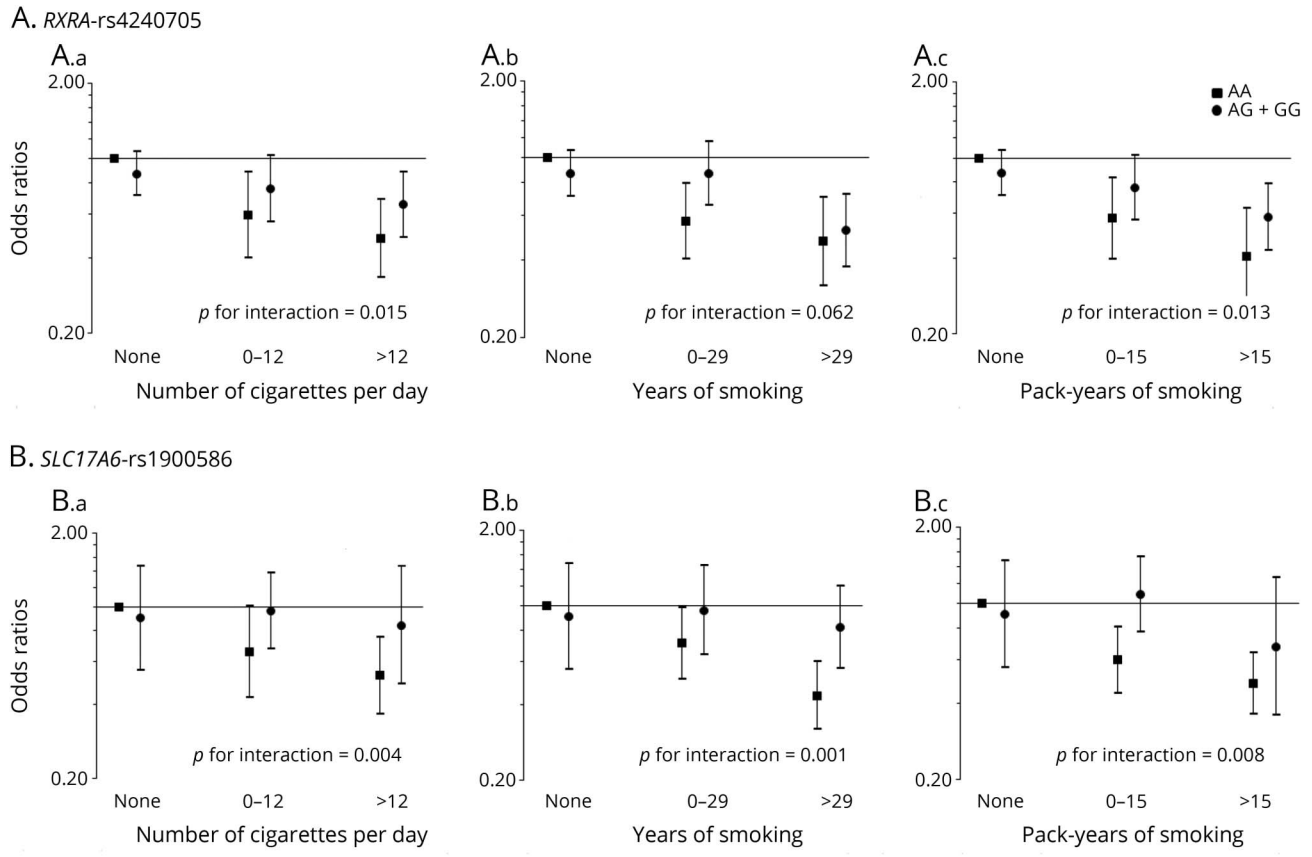
Table 3 Joint effects of cigarette smoking and *RXRA*-rs4240705 in PD

rs4240705	Ever smoking	OR (95% CI)[a]	*p* Value	I^2, %	*p* Value for heterogeneity
Discovery dataset					
AA	No	1.00 (Referent)	—	—	—
AA	Yes	0.49 (0.32–0.73)	4.9×10^{-4}	—	—
AG	No	1.05 (0.80–1.36)	0.742	—	—
AG	Yes	0.53 (0.36–0.77)	7.9×10^{-4}	—	—
GG	No	0.58 (0.38–0.88)	0.011	—	—
GG	Yes	1.41 (0.81–2.44)	0.220	—	—
Interaction parameter[b]	Logistic regression	1.85 (1.30–2.63)[b]	5.9×10^{-4}	—	—
	EB[c]	1.70 (1.18–2.43)[b]	0.004	—	—
Replication dataset					
AA	No	1.00 (Referent)	—	—	—
AA	Yes	0.48 (0.32–0.71)	3.0×10^{-4}	—	—
AG	No	0.72 (0.50–1.04)	0.081	—	—
AG	Yes	0.64 (0.44–0.93)	0.019	—	—
GG	No	1.04 (0.58–1.87)	0.904	—	—
GG	Yes	0.83 (0.48–1.45)	0.521	—	—
Interaction parameter[b]	Logistic regression	1.46 (1.01–2.13)[b]	0.046	—	—
	EB[c]	1.33 (0.92–1.93)[b]	0.134	—	—
Pooled analysis[d]					
AA	No	1.00 (Referent)	—		
AA	Yes	0.49 (0.36–0.65)	5.1×10^{-7}	0.0	0.944
AG	No	0.89 (0.62–1.28)	0.529	62.6	0.102
AG	Yes	0.58 (0.45–0.76)	5.6×10^{-5}	0.0	0.488
GG	No	0.75 (0.42–1.32)	0.317	60.4	0.112
GG	Yes	1.08 (0.64–1.82)	0.766	43.5	0.183
Interaction parameter[b]	Logistic regression	1.66 (1.28–2.14)[b]	1.1×10^{-4}	0.0	0.366
	EB[c]	1.51 (1.17–1.95)[b]	0.002	0.0	0.352

Abbreviation: CI = confidence interval; EB = empirical Bayes; OR = odds ratio; PD = Parkinson disease.
[a] ORs, 95% CIs, and *p* values were computed with standard logistic regression and adjusted for sex, age in quartiles, and population stratification (2 first principal components) for the discovery sample or minority status for the replication sample.
[b] Interaction parameters were computed under an additive coding of the single nucleotide polymorphism by including a multiplicative term between ever smoking and the number of minor alleles.
[c] Interaction parameters computed with the EB approach.
[d] ORs, interaction parameters, and their 95% CI from the 2 datasets were pooled with the use of random-effects meta-analysis to compute pooled ORs and interaction parameters, 95% CIs, I^2, and *p* for heterogeneity.

with standard logistic regression (interaction 1.73, 95% CI 1.13–2.64, $p = 0.0116$, table 2); it was the strongest EB hit in this dataset. The smoking × rs1900586 interaction was replicated in the US dataset with the EB approach (interaction$_{EB}$ 1.52, 95% CI 1.03–2.23, $p = 0.034$); it was weaker and not significant with standard logistic regression (interaction 1.47, 95% CI 0.92–2.38, $p = 0.108$). rs1900586-G was more frequent in patients than controls in the discovery dataset but not in the replication dataset (table e-2, http://links.lww.com/WNL/A133). In pooled analyses (table 4 and table e-5), the EB interaction was the strongest signal (interaction$_{EB}$ 1.69, 95% CI 1.31–2.19, $p = 6.9 \times 10^{-5}$) without heterogeneity ($p = 0.461$) and the closest to the corrected significance level of 2.2×10^{-5}. Smoking was less frequent in patients than controls among rs1900586-AA carriers (OR 0.54, 95% CI 0.43–0.67, $p = 2.5 \times 10^{-8}$), and this

Figure 1 Interaction between smoking characteristics and *RXRA*-rs4240705 and *SLC17A6*-rs1900586 for the risk of PD



Pooled analysis of the interaction between smoking characteristics (years of smoking, pack-years of smoking, number of cigarettes per day) and RXRA-rs4240705 (A.a–A.c) and SLC17A6-rs1900586 (B.a–B.c) for the risk of PD. Interaction tests were computed with an ordinal coding of the smoking variables and a dominant model for the SNPs. PD = Parkinson disease; SNP = single nucleotide polymorphism.

association disappeared in rs1900586-G carriers (AG: OR 0.97, 95% CI 0.70–1.34, p = 0.842; GG: OR 0.95, 95% CI 0.35–2.59, p = 0.916). Conversely, the frequency of rs1900586-G was not significantly different between patients and controls in both never smokers (OR additive 0.94, 95% CI 0.67–1.33, p = 0.742) and ever smokers (OR additive 1.47, 95% CI 0.89–2.41, p = 0.132). In pooled analyses, the inverse association of smoking characteristics with PD was restricted to rs1900586-AA homozygotes (figure 1B).

Table e-6 (http://links.lww.com/WNL/A133) shows pooled analyses of the association of both SNPs with smoking stratified by case-control status. There was an association in patients, with larger estimates for rs1900586 than for rs4240705. There was a borderline inverse association for rs4240705 (additive coding) among controls that was mostly explained by the French study (OR 0.80, 95% CI 0.64–0.99), while no association was seen in the US study (OR 0.92, 95% CI 0.74–1.14).

Sensitivity analyses

We obtained similar results in pooled analyses adjusted for family history (not shown). In matched analyses (discovery dataset), interactions were even stronger than in unmatched analyses (rs4240705: 1.96, 95% CI 1.36–2.82, p = 0.0003; rs1900586: 1.83, 95% CI 1.16–2.89, p = 0.0096). In the discovery dataset, there was no significant interaction between the SNPs and alcohol drinking (rs4240705, p = 0.730; rs1900586, p = 0.541) and passive smoking (rs4240705, p = 0.792; rs1900586, p = 0.160); adjustment for these variables did not change our conclusions (not shown).

Discussion

To examine gene-by-smoking interactions in PD, we focused on genes involved in individual susceptibility to xenobiotics and replicated interactions of smoking with SNPs in the *RXRA* and *SLC17A6* genes in an independent population-based study. For both SNPs, the inverse association between smoking and PD was lost among carriers of the minor allele. These findings may provide clues about biological mechanisms underlying the smoking-PD association, more precisely those relating to vitamin A metabolism and glutamatergic transmission.

We investigated GEIs in population-based case-control studies with detailed environmental data by using an approach more targeted than genome-wide and less restrictive than candidate

Table 4 Joint effects of cigarette smoking and *SLC17A6*-rs1900586 in PD

rs1900586	Ever smoking	OR (95% CI)[a]	*p* Value	I^2, %	*p* Value for heterogeneity
Discovery dataset					
AA	No	1.00 (Referent)	—	—	—
AA	Yes	0.49 (0.36–0.67)	1.2×10^{-5}	—	—
AG	No	1.15 (0.87–1.52)	0.321	—	—
AG	Yes	1.02 (0.69–1.52)	0.916	—	—
GG	No	0.91 (0.35–2.38)	0.841	—	—
GG	Yes	1.40 (0.55–3.57)	0.478	—	—
Interaction parameter[b]	Logistic regression	1.73 (1.13–2.64)	0.012	—	—
	EB[c]	1.85 (1.30–2.63)	5.7×10^{-4}	—	—
Replication dataset					
AA	No	1.00 (Referent)	—	—	—
AA	Yes	0.58 (0.43–0.79)	4.2×10^{-4}	—	—
AG	No	0.65 (0.43–0.98)	0.042	—	—
AG	Yes	0.70 (0.47–1.03)	0.074	—	—
GG	No	1.20 (0.41–3.50)	0.737	—	—
GG	Yes	0.67 (0.26–1.71)	0.399	—	—
Interaction parameter[b]	Logistic regression	1.47 (0.92–2.38)	0.108	—	—
	EB[c]	1.52 (1.03–2.23)	0.034	—	—
Pooled analysis[d]					
AA	No	1.00 (Referent)	—		
AA	Yes	0.53 (0.43–0.66)	2.5×10^{-8}	0.0	0.447
AG	No	0.88 (0.51–1.54)	0.662	80.2	0.025
AG	Yes	0.84 (0.58–1.22)	0.369	43.1	0.185
GG	No	1.03 (0.50–2.10)	0.938	0.0	0.706
GG	Yes	0.97 (0.47–2.00)	0.935	15.6	0.276
Interaction parameter[b]	Logistic regression	1.61 (1.17–2.21)	0.003	0.0	0.616
	EB[c]	1.69 (1.31–2.19)	6.9×10^{-5}	0.0	0.461

Abbreviation: CI = confidence interval; EB = empirical Bayes; OR = odds ratio; PD = Parkinson disease.
[a] ORs, 95% CIs, and *p* values were computed with standard logistic regression and adjusted for sex, age in quartiles, and population stratification (2 first principal components) for the discovery sample or minority status for the replication sample.
[b] Interaction parameters were computed under an additive coding of the single nucleotide polymorphism by including a multiplicative term between ever smoking and the number of minor alleles.
[c] Interaction parameters were computed with the EB approach.
[d] ORs, interaction parameters, and their 95% CIs from the 2 datasets were pooled with the use of random-effects meta-analysis to compute pooled ORs and interaction parameters, 95% CIs, I^2, and *p* for heterogeneity.

gene approaches. We hypothesized that genes involved in individual susceptibility to xenobiotics may be involved in PD etiology and focused on these genes. This approach allows reducing the multiple testing burden and may help identify small effect sizes that may be missed in GWAS.

We highlighted 2 loci, *RXRA* and *SLC17A6*, that provided suggestive evidence for an interaction with cigarette smoking in PD. The SNPs that interacted with smoking are tag SNPs not expected to affect protein structure and may be in linkage disequilibrium with another unknown variant; alternatively, they may influence gene expression. Retinoic acid (RA) is a vitamin A metabolite involved in complex signaling pathways in the CNS that acts by binding to 2 major groups of nuclear receptors, RA receptor (RAR) and retinoid X receptor (RXR). The dopaminergic system is

a well-established RA target.[19] In an animal PD model, one of the major RA isoforms (9cRA) with high affinity for RXR showed a protective effect against neurodegeneration in DA neurons.[20] RA also carried beneficial effects in a rotenone-induced PD rat model.[21] The promoter of the D2 dopamine receptor contains a functional RAR/RXR binding motif,[22] and RAR/RXR defects reduce dopamine receptors levels in mice.[23] Ligands that activate RXR selectively protect dopaminergic neurons from stress caused by the PD-model toxin 6-hydroxydopamine and hypoxia; these protective effects were seen only in Nurr1-expressing dopaminergic cells.[24,25] IRX4204, a second-generation RXR agonist, promotes the survival of dopaminergic neurons in mesencephalic cultures in a dose-dependent manner and attenuates neurochemical and motor deficits in a rat PD model.[26] These findings led to the development of a phase 1 clinical study of this agonist.[27] The complexity of the retinoid signaling pathways is further demonstrated by the relation of RXR with vitamin D: vitamin D binds to the vitamin D receptor that dimerizes with RXR.[28] This pathway may be relevant because vitamin D deficiency may be associated with increased PD risk.[29] Together, these studies support a role of RXR in PD. Regarding the relation between RA and xenobiotics in general and smoking in particular, *RXRA* forms heterodimers with pregnane X receptor (*PXR*) and constitutive androstane receptor (*CAR*) that bind to promoter sequences of their target genes; PXR and CAR represent the major nuclear receptors involved in gene regulation of phase I/II enzymes and transporters of xenobiotics.[30] Cigarette smoking interferes with RA metabolism and signaling[31] and induces RAR/RXR expression changes in lung cancer.[32] One epigenetic study of non–small cell lung cancer observed different RXR methylation patterns by smoking status, suggesting that smoking may be associated with *RXR* demethylation in tumor cells.[33]

SLC17A6 codes for the vesicular glutamate transporter (VGLUT) 2. Glutamate is the major excitatory neurotransmitter of the brain, and disruption of glutamate neurotransmitters is documented in PD.[34] Four VGLUTs have been identified; their function is to package glutamate into presynaptic vesicles. Glutamate transporter dysfunction increases extracellular glutamate, which has been shown to cause toxicity in the CNS.[35] Glutamatergic transmission is impaired in PD, with lower glutamatergic transmission in the cerebral cortex compared to controls.[36] Normalization of glutamate transmission has been a target for the development of PD therapies.[37] VGLUT2 loss in mesostriatal dopamine neurons leads to perturbations of reward and addictive behaviors in mice.[38] In humans, SLC17A6 expression within the ventral tegmental area is significantly elevated in alcoholics who smoked compared with nonsmoking alcoholics and nonalcoholic controls, thus suggesting that exposure to both alcohol and nicotine increased glutamatergic transmission.[39]

The inverse association of rs4240705 with smoking in controls invalidates the use of the case-only approach for this SNP and underlines the interest of the EB approach. This association was due mainly to the French dataset and may be a chance finding; previous GWAS on smoking behavior did not identify associations with either of the 2 SNPs (https://www.ebi.ac.uk/gwas/search?query=smoking).

For a given association strength, the identification of GEI requires ≈4 times the number of participants compared with marginal genetic associations,[40] with the added difficulty of collecting detailed environmental data. The main limitation of our work lies in the size of the population-based case-control studies on which it relied. This may explain why the statistical significance of interaction estimates failed to reach the corrected significance level of 2.2×10^{-5} and some heterogeneity across the discovery and replication datasets in analyses of joint effects. There are, however, few population-based PD studies with adequate controls that have collected environmental data for GEI analyses. Therefore, the biologically plausible smoking × gene interactions highlighted here require further investigation in additional datasets. If confirmed, the components of cigarette smoke that interact with the 2 genes remain to be identified.

Using a systematic approach, preselection of a large number of SNPs/genes, and replication in an independent study, we identified interactions between smoking and *RXRA*-rs4240705 and *SLC17A6*-rs1900586 in PD. These findings may help identify biological pathways relevant for and helpful in explaining the inverse smoking-PD association and elucidating whether this association is causal. Further research in animal models and humans is required to confirm our findings.

Author contributions

Pei-Chen Lee and Ismaïl Ahmed: conception and design of the study, acquisition and analysis of data, statistical analysis, and drafting a significant portion of the manuscript or figure. Marie-Anne Loriot, Claire Mulot, Kimberly C. Paul, and Jeff M. Bronstein: acquisition and analysis of data. Beate Ritz: conception and design of the study, acquisition and analysis of data. Alexis Elbaz: conception and design of the study, acquisition and analysis of data, and drafting a significant portion of the manuscript or figure.

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Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

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References

1. Wirdefeldt K, Adami HO, Cole P, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. Eur J Epidemiol 2011;26 (suppl 1):S1–S58.
2. Tanner CM, Ottman R, Goldman SM, et al. Parkinson disease in twins: an etiologic study. JAMA 1999;281:341–346.
3. Goldwurm S, Tunesi S, Tesei S, et al. Kin-cohort analysis of LRRK2-G2019S penetrance in Parkinson's disease. Mov Disord 2011;26:2144–2145.
4. Nalls MA, Pankratz N, Lill CM, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nat Genet 2014;46: 989–993.
5. Noyce AJ, Bestwick JP, Silveira-Moriyama L, et al. Meta-analysis of early nonmotor features and risk factors for Parkinson disease. Ann Neurol 2012;72:893–901.
6. Ritz B, Rhodes SL. After half a century of research on smoking and PD, where do we go now? Neurology 2010;74:870–871.
7. Dutheil F, Beaune P, Tzourio C, Loriot MA, Elbaz A. Interaction between ABCB1 and professional exposure to organochlorine insecticides in Parkinson disease. Arch Neurol 2010;67:739–745.
8. Galanaud JP, Elbaz A, Clavel J, et al. Cigarette smoking and Parkinson's disease: a case-control study in a population characterized by a high prevalence of pesticide exposure. Mov Disord 2005;20:181–189.
9. Moisan F, Spinosi J, Delabre L, et al. Association of Parkinson's disease and its subtypes with agricultural pesticide exposures in men: a case-control study in France. Environ Health Perspect 2015;123:1123–1129.
10. Fayard C, Bonaventure A, Benatru I, et al. Impact of recommendations on the initial therapy of Parkinson's disease: a population-based study in France. Parkinsonism Relat Disord 2011;17:543–546.
11. Bower JH, Maraganore DM, McDonnell SK, Rocca WA. Incidence and distribution of parkinsonism in Olmsted County, Minnesota, 1976-1990. Neurology 1999;52: 1214–1220.
12. Rylander-Rudqvist T, Hakansson N, Tybring G, Wolk A. Quality and quantity of saliva DNA obtained from the self-administrated oragene method: a pilot study on the cohort of Swedish men. Cancer Epidemiol Biomarkers Prev 2006;15:1742–1745.
13. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81: 559–575.
14. Tchetgen Tchetgen EJ, Kraft P. On the robustness of tests of genetic associations incorporating gene-environment interaction when the environmental exposure is misspecified. Epidemiology 2011;22:257–261.
15. Mukherjee B, Ahn J, Gruber SB, Rennert G, Moreno V, Chatterjee N. Tests for gene-environment interaction from case-control data: a novel study of type I error, power and designs. Genet Epidemiol 2008;32:615–626.
16. Thomas DC, Lewinger JP, Murcray CE, Gauderman WJ. Invited commentary: GE-Whiz! Ratcheting gene-environment studies up to the whole genome and the whole exposome. Am J Epidemiol 2012;175:203–207.
17. Yang Q, Khoury MJ, Flanders WD. Sample size requirements in case-only designs to detect gene-environment interaction. Am J Epidemiol 1997;146:713–720.
18. Gao X, Starmer J, Martin ER. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. Genet Epidemiol 2008;32:361–369.
19. Nolan YM, Sullivan AM, Toulouse A. Parkinson's disease in the nuclear age of neuroinflammation. Trends Molecular Medicine 2013;19:187–196.
20. Yin LH, Shen H, Diaz-Ruiz O, et al. Early post-treatment with 9-cis retinoic acid reduces neurodegeneration of dopaminergic neurons in a rat model of Parkinson's disease. BMC Neurosci 2012;13:120.
21. Ulusoy GK, Celik T, Kayir H, Gursoy M, Isik AT, Uzbay TI. Effects of pioglitazone and retinoic acid in a rotenone model of Parkinson's disease. Brain Res Bull 2011;85: 380–384.
22. Samad TA, Krezel W, Chambon P, Borrelli E. Regulation of dopaminergic pathways by retinoids: activation of the D2 receptor promoter by members of the retinoic acid receptor-retinoid X receptor family. Proc Natl Acad Sci USA 1997;94: 14349–14354.
23. Krezel W, Ghyselinck N, Samad TA, et al. Impaired locomotion and dopamine signaling in retinoid receptor mutant mice. Science 1998;279:863–867.
24. Friling S, Bergsland M, Kjellander S. Activation of retinoid X receptor increases dopamine cell survival in models for Parkinson's disease. BMC Neurosci 2009; 10:146.
25. McFarland K, Spalding TA, Hubbard D, Ma JN, Olsson R, Burstein ES. Low dose bexarotene treatment rescues dopamine neurons and restores behavioral function in models of Parkinson's disease. ACS Chem Neurosci 2013;4: 1430–1438.
26. Wang J, Bi W, Zhao W, et al. Selective brain penetrable Nurr1 transactivator for treating Parkinson's disease. Oncotarget 2016;7:7469–7479.
27. Sanders M, Chandraratna R, Marek K, Jennings D. A phase 1 clinical study of the retinoid X receptor (RXR) selective agonist IRX4204 in patients with early Parkinson's disease (PD) (P2.342). Neurology 2016;86.
28. Claro da Silva T, Hiller C, Gai Z, Kullak-Ublick GA. Vitamin D3 transactivates the zinc and manganese transporter SLC30A10 via the vitamin D receptor. J Steroid Biochem Mol Biol 2016;163:77–87.
29. Shen L, Ji HF. Associations between vitamin D status, supplementation, outdoor work and risk of Parkinson's disease: a meta-analysis assessment. Nutrients 2015;7: 4817–4827.
30. Willson TM, Kliewer SA. PXR, CAR and drug metabolism. Nat Rev Drug Discov 2002;1:259–266.
31. Mernitz H, Wang X. Alcohol and tobacco smoke in retinoid metabolism and signaling: implications for carcinogenesis. In: Cho CH, Purohit V, editors. Alcohol, Tobacco and Cancer. Basel: Karger; 2006:20.
32. Martinet N, Alla F, Farre G, et al. Retinoic acid receptor and retinoid X receptor alterations in lung cancer precursor lesions. Cancer Res 2000;60:2869–2875.
33. Lee SM, Lee JY, Choi JE, Lee SY, Park JY, Kim DS. Epigenetic inactivation of retinoid X receptor genes in non-small cell lung cancer and the relationship with clinicopathologic features. Cancer Genet Cytogenet 2010;197:39–45.
34. Blandini F, Porter RH, Greenamyre JT. Glutamate and Parkinson's disease. Mol Neurobiol 1996;12:73–94.
35. Maragakis NJ, Rothstein JD. Glutamate transporters: animal models to neurologic disease. Neurobiol Dis 2004;15:461–473.
36. Kashani A, Betancur C, Giros B, Hirsch E, El Mestikawy S. Altered expression of vesicular glutamate transporters VGLUT1 and VGLUT2 in Parkinson disease. Neurobiol Aging 2007;28:568–578.
37. Litim N, Morissette M, Di Paolo T. Metabotropic glutamate receptors as therapeutic targets in Parkinson's disease: an update from the last 5 years of research. Neuropharmacology 2017;115:166–179.
38. Alsio J, Nordenankar K, Arvidsson E, et al. Enhanced sucrose and cocaine self-administration and cue-induced drug seeking after loss of VGLUT2 in midbrain dopamine neurons in mice. J Neurosci 2011;31:12593–12603.
39. Flatscher-Bader T, Zuvela N, Landis N, Wilce PA. Smoking and alcoholism target genes associated with plasticity and glutamate transmission in the human ventral tegmental area. Hum Mol Genet 2008;17:38–51.
40. Thomas D. Gene–environment-wide association studies: emerging approaches. Nat Rev Genet 2010;11:259–272.