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Genetic variants in nicotinic receptors and smoking cessation in Parkinson's disease

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ABSTRACT

Background: Negative associations between smoking and Parkinson's disease (PD) are well documented. While common biases may not explain this association, some studies have suggested reverse causality and ease of quitting might be an early sign of PD, possibly related to a reduced nicotinic response. We investigated nicotinic receptor (nAChR) genetics to add to our understanding of possible biologic mechanisms underlying the smoking-PD relationship.

Methods: We relied on 612 patients and 691 controls enrolled in the PEG (Parkinson's Environment and Gene) study for whom we obtained information on smoking and quitting ease through interviews. Genotyping in the nAChR genes, i.e. *CHRNA5-A3-B4* and *CHRNB3-A6* gene regions that have been linked to smoking or quitting behaviors, were based on blood and saliva DNA samples. We assessed associations with logistic regression assuming logit-additive allelic effects and used product terms for genetic allele status and smoking or quitting assessing interactions.

Results: As expected, we observed negative associations between smoking and PD that were strongest for current followed by former smokers. In former smokers, high quitting difficulty was negatively associated with PD risk (extremely hard vs. easy: OR = 0.62 [0.39–0.99], p = 0.05), meaning those who developed PD were able to quit smoking with less difficulty than controls. The *CHRNA3* rs578776-A allele predicted quitting difficulty in smoking controls (OR = 0.53 [0.32–0.91], p = 0.02), but not in smoking PD patients (OR = 1.09 [0.61–1.95], p = 0.77).

Conclusion: Our study further corroborates previous findings that ease of quitting may be an early sign of PD onset related to a loss of nicotinic response in prodromal stages.

1. Introduction

It is a well-documented fact that Parkinson's disease (PD) patients smoke less [1,2], and negative associations with PD are stronger in current than former smokers. A negative dose-response has also been reported with increasing pack-years of smoking complemented by a positive dose response with increasing years since quitting [2,3]. However, since smoking is a strong risk factor for many adverse health outcomes, these findings remain subject of debate. While, common biases including confounding and survival bias in epidemiologic studies have not been able to explain the negative associations, we recently suggested reverse causation as a potential alternative explanation. Previously in a large population-based Danish study, we showed that ease of quitting might be an early sign of PD onset and suggested that PD patients may lose their nicotinic response during the long prodromal disease stage making it easier for them to quit [4].

Nicotinic acetylcholine receptors (nAChR), composed of α and β subunits, are widely expressed in the brain and have been shown to regulate dopamine release in animal studies [5]. The α subunits express the acetylcholine recognition sites, while the β subunits modulate

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binding of acetylcholine to the α subunit. Recent genetic studies have consistently linked single nucleotide polymorphisms (SNPs) in the *CHRNA5-A3-B4* gene region and the *CHRNB3-A6* gene region to smoking behavior, nicotine dependence, and successful quitting attempts [6–9]. For instance, in a genome-wide meta-analysis the T allele of rs1051730 in *CHRNA3* showed the strongest association with heavy smoking [6]. Additionally, risk alleles for heavy smoking, *CHRNA3* rs1051730-T and *CHRNA5* rs588765-G, were also found to be strongly associated with quitting difficulty among those who were administered a placebo in a smoking cessation trial and with continued abstinence among those who after quitting received nicotine replacement therapy [10]. A study that genotyped eight *CHRNB3-A6* region SNPs found *CHRNA6* rs2304297-G to be associated with nicotine dependence in 1051 subjects of European ancestry, and *CHRNB3* rs4950-A with the number of unsuccessful quitting attempts [11]. Thus, nicotinic receptor genetics may provide a way to assess nicotinic response and point to potential pathomechanisms that could explain the smoking-PD relationship.

In the present case-control study, we rely on data from the PEG (Parkinson's Environment and Gene) study to examine whether PD status is related to self-reported quitting difficulty in smokers. In addition, we assess the influence of suspected functional nAChR genetic variants on smoking duration and intensity, as well as quitting difficulty in PD patients compared with population controls.

2. Methods

2.1. Study population

Our study included 826 idiopathic PD patients from three rural California counties (Kern, Tulare, Fresno) enrolled in the PEG study. Of these, 360 were recruited within 3 years of first diagnosis through neurologists, large medical groups, or public service announcements (wave 1 known as PEG1: 2000–2007) [12,13] and 476 patients, recruited on average 4 years from diagnosis, identified through the California PD Registry (PEG2: 2010-ongoing) [14,15]. Our UCLA movement disorder specialists (JB, YB) confirmed PD diagnoses using UK Brain Bank and Gelb diagnostic criteria and we collected bio-samples [16–18]. Furthermore, 403 PEG1 and 618 PEG2 population controls were recruited during the same timeframe after being randomly selected from the tri-county areas using Medicare or residential parcels records with marginal matching on age, sex, and race (see Refs. [12,15] for more recruitment details). While 826 patients (360 PEG1, 466 PE2) and 832 controls (403 PEG1, 429 PEG2) provided smoking and quitting information in interviews, we excluded subjects with missing information for all 5 nicotinic receptor SNPs we decided to investigate, leaving 612 (74%) patients and 691 controls (83%) for analysis.

All study participants provided written consent and the study protocol was approved by the Institutional Review Board of the UCLA.

2.2. Data collection and smoking assessment

We obtained information on demographics and lifelong smoking history in a structured interview. The questions on cigarette smoking included smoking status, age at quitting smoking, years and quantity smoked for multiple life periods. “Ever smokers” reported having smoked regularly for at least one year, and “former smokers” reported having smoked but were not smoking at the index date (age at PD diagnosis for cases and age at interview for controls). In addition, we asked former smokers to report how much difficulty they experienced when they attempted quitting (remarkably easy, medium, and extremely hard).

2.3. Genotyping

Three SNPs located in the *CHRNA5-A3-B4* gene region on chromosome 15.q25 (rs578776, rs1051730) and 2 SNPs in the *CHRNB3-A6* gene region on chromosome 8 (rs4950, rs2304297) were selected based on reported association with smoking, nicotine dependence, and quitting attempts in human studies or their potential role in protein function (Table S1) [6–8,11]. DNA was extracted from blood (PEG1) and saliva (PEG2) samples, and genotyping was conducted using multiplex Taqman allelic discrimination assays (Applied Biosystems) according to the manufacturer's protocol. The call rates of SNPs were greater than 95%.

2.4. Statistical analysis

Hardy-Weinberg equilibrium in controls was examined with Pearson's chi-square test for all SNPs ($\alpha = 0.05/4$ SNPs ~ 0.01). Odds ratio (OR) and 95% confidence intervals (CIs) were obtained from logistic regression under the assumption of logit-additive allelic effects. We also conducted sensitivity analyses using codominant and dominant models. Since results were similar for all models, we mainly present results from an additive genetic model, which assumes each copy of the minor allele increases the risk by the same amount, when assessing the influence of presumed functional nAChR SNPs on smoking or quitting difficulty and their interactions separately for PD patients and controls. We also assessed associations of smoking with PD or quitting difficulty among former smokers with PD. In all regression models, we adjusted for index age, sex, and European ancestry. We conducted sensitivity analysis restricting to subjects of European ancestry to account for confounding by population structure. Quitting among former smokers was analyzed as a binary [19] and categorical [4] variable and in sensitivity analysis we stratified according to quitting age (< 50 vs. ≥ 50 years of age) and we added current smokers to those who reported finding it “extremely hard to quit”. Trend tests for categorical variables were conducted. All analyses were performed using SAS v9.4 (SAS Institute, Cary, NC, USA).

3. Results

Demographics and smoking- or quitting-related characteristics of PD patients and controls are shown in Table 1. As expected, PD patients were less likely to ever smoke cigarettes compared with control subjects (48% vs. 53%, Table 1). Among ever smokers, 91% of PD patients and 79% of controls quit smoking before the index date. Most former smokers quit before the age of 40 and on average smoked for < 20 years. Shorter duration of smoking was associated with gender (e.g. 54% of women vs. 40% of men smoked < 20 years). Among controls the average quitting age was 41 years for men and 40 years for women; among PD patients it was 39 years for men and 41 years for women. Negative associations between smoking and PD were stronger for current than former smokers (OR = 0.34 [95% CI = 0.21–0.54] vs. OR = 0.81 [95% CI = 0.64–1.02]) (Table 2), and we observed a negative dose-response between every 10 pack-years of cigarette smoking and PD (OR = 0.92 [95% CI = 0.85–0.98], p = 0.01). Moreover, among former smokers there was some suggestion for a positive dose-response with PD risk and younger age at quitting. We also found that the risk of developing PD was 40% lower among former smokers who reported that it had been extremely hard to quit compared with those who reported quitting had been easy (OR = 0.62 [0.39–0.99], p = 0.05).

All nAChR SNPs met Hardy-Weinberg equilibrium in European ancestry controls (Table S1). We found a significant inverse association between the A allele of rs578776 (*CHRNA3*) and quitting difficulty in controls in the expected direction (OR = 0.63 [0.41–0.97], p = 0.03; Table 3 and Table S2). This association was attenuated and not statistically significant among PD patients (OR = 0.81 [0.52–1.28], p = 0.37). The A allele of rs578776 also showed an inverse association with pack-years of smoking only in controls who had been smokers (β-value = −0.38, p = 0.05). Results were similar in sensitivity analyses

Table 1
Characteristics of the study population with genotyping data by Parkinson disease (PD) status (n = 1303).

	PD patients (n = 612)	Controls (n = 691)
Index age[a,b], yr, mean (range)	68.3 (34–89)	66.5 (35–99)
Sex, male	382 (62.4)	333 (48.2)
European ancestry[b]	466 (76.1)	519 (75.2)
Family history of PD[b]	99 (16.2)	59 (8.6)
Smoking history		
Never smoked	321 (52.5)	325 (47.0)
Former smoker	265 (43.3)	288 (41.7)
Current smoker	26 (4.2)	78 (11.3)
For ever cigarette smokers only		
Current/ever smoker, %	8.9	21.3
Pack-years, per 10 units, mean (range)	2.0 (0–17.5)	2.4 (0–12.5)
Quitting-related characteristics (for former cigarette smokers only)		
Years of smoking[b], yr		
< 20	143 (54.0)	131 (45.8)
20-29	51 (19.2)	68 (23.8)
30-39	40 (15.1)	53 (18.5)
≥40	31 (11.7)	34 (11.9)
Age when quit smoking[b], yr		
< 40	137 (51.8)	140 (49.0)
40-49	58 (21.9)	59 (20.6)
50-59	42 (15.9)	51 (17.8)
≥60	27 (10.2)	36 (12.6)
Difficulty of quitting smoking[b]		
Remarkably easy	103 (46.0)	100 (42.7)
Medium	76 (34.0)	61 (26.1)
Extremely hard	45 (20.0)	73 (31.2)

Data represented as n (%) unless otherwise indicated.

[a] Index age is the age at PD diagnosis for patients and the age at interview for controls.

[b] Missing: index age (n = 1), European ancestry (n = 1), family history of PD (n = 3), years of smoking (n = 2), age when quit smoking (n = 3), and difficulty of quitting smoking (n = 95).

Table 2
Associations between smoking- and quitting and PD status
The odds ratio compares the odds of exposure (e.g. ever smoking) among PD patients, which is the numerator, and the odds of exposure (e.g. ever smoking) among population controls, which is the denominator.

	PD patients/ Controls	OR[a] (95% CI)	p-value
Smoking history			
Non-smoker	321/325	1	
Former smoker	265/288	0.81 (0.64–1.02)	0.08
Current smoker	26/78	0.34 (0.21–0.54)	< 0.001
		p for trend =	< 0.001
Ever smoker	291/366	0.71 (0.57–0.89)	0.003
For ever cigarette smokers only			
Current vs. former smoker, %	8.9/21.3	0.38 (0.23–0.62)	< 0.001
Pack-years, per 10 units, mean	2.0/2.4	0.92 (0.85–0.98)	0.01
Quitting-related characteristics (for former cigarette smokers only)			
Years of smoking, yr			
< 20	143/131	1	
20-29	51/68	0.62 (0.39–0.96)	0.03
30-39	40/53	0.67 (0.41–1.09)	0.11
≥40	31/34	0.78 (0.45–1.36)	0.38
		p for trend =	0.13
Age when quit smoking, yr			
< 40	137/140	1	
40-49	58/59	0.98 (0.63–1.52)	0.92
50-59	42/51	0.82 (0.51–1.32)	0.41
≥60	2736	0.76 (0.43–1.34)	0.34
		p for trend =	0.26
Difficulty of quitting smoking			
Remarkably easy	103/100	1	
Medium	76/61	1.24 (0.80–1.93)	0.34
Extremely hard	45/73	0.62 (0.39–0.99)	0.05
		p for trend =	0.09

OR: odds ratios; CI: confidence interval.

[a] Unconditional logistic model adjusted for index age (continuous), sex, and European ancestry.

after adding current smokers to the “extremely hard to quit” category. When restricting to subjects of European ancestry, although the associations were not statistically significant likely due to reduced sample size, direction of associations with quitting difficulty were preserved (OR = 0.68, 95%CI = 0.42–1.09 for controls; OR = 0.78, 95%CI = 0.47–1.28 for PD patients). Moreover, when restricting to those who quit before age 50, the associations were much stronger in controls (OR = 0.44; 95%CI = 0.23–0.85) and for PD patients the associations remained similar with confidence intervals including the null (OR = 0.76; 95% CI 0.41–1.40). Among those who reported quitting at or after age 50, the OR in controls was 0.92 (0.49–1.75) and in PD patients it was 1.00 (0.49–2.04). None of the other SNPs were associated with smoking duration and intensity, or quitting difficulty in either patients or controls.

For rs578776, we also explored quitting difficulty and again found associations only in former smokers from the control group and not for patients (Table 4). Specifically, among controls, former smokers who reported that it had been extremely hard to quit were only half as likely to be carrying the A allele rs578776 (extremely hard vs. easy: OR = 0.53 [0.32–0.91], p = 0.02) and there was a trend for quitting difficulty with the number of A alleles (trend p = 0.02). The frequencies of nAChR SNPs by smoking or quitting in patients and controls can be found in Table S3.

We did not find statistically significant interactions between other nAChR SNPs and other smoking- or quitting-variables and risk of PD (data not shown).

4. Discussion

Our study replicates the inverse association between ‘difficulty to quit smoking’ and PD first reported in a Danish case-control study [4]. According to the literature, the *CHRNA3* rs578776 A allele influences quitting difficulty, and we observed this association in our population controls especially strong among those who quit smoking at a younger age (< 50 years). On the other hand, this genetic association was not observed among those who later developed PD. That is, among former smokers, only controls but not patients who were carriers of the A allele of rs578776 found it easier to quit smoking.

A large study conducted using the registry of Danish citizens reported for former smokers who found it extremely hard to quit an OR for PD of 0.69 (95% CI = 0.48–0.99) - the same effect size we estimated in the current study for California residents of unspecified European ancestry. Danish PD patients compared with controls were also less likely to use nicotine substitutes when quitting, possibly suggesting less response to nicotine stimulation among PD patients [4]. Thus, our California study corroborates the finding that smoking cessation difficulty is negatively associated with PD risk. Postmortem studies have shown a substantial loss of nicotinic receptors in the striatum and substantia nigra of PD patients, possibly explaining some Parkinson's symptoms related to cognition and behavior [20,21]. Nicotine, the main addictive substance in cigarette smoke, is absorbed by epithelial cells of the lung, circulates in the bloodstream and - after crossing the blood-brain barrier - binds to nAChRs on dopaminergic terminals in the striatum modulating reward-related dopamine signals [22].

The SNP rs578776 located in the 3’-untranslated region of *CHRNA3*, the α3 nicotinic receptor subunit gene, on chromosome 15 has been shown to be associated with nicotine dependence [7,8]. In a previous study, individuals who were homozygous for the major allele G were at a 1.4-fold risk of developing nicotine dependence compared with carriers of the minor allele A in subjects of European ancestry but not in

Table 3
Associations between nAChR genetic variants and smoking and quitting behavior in controls and PD patients separately.
The odds ratio compares the odds of exposure (e.g. ever smoking) among PD patients, which is the numerator, and the odds of exposure (e.g. ever smoking) among population controls, which is the denominator.

SNPs	MAF	Ever vs. never smoking		Among ever smokers Pack-years of smoking (per 10 unit)		Among former smokers (Ref. [19]) MAF	Hard to quit smoking	
		OR (95%CI)[a]	P	β-value (SE)[a]	P		OR (95%CI)[a]	P
Controls								
CHRNA5-A3-B4								
rs578776	0.34	0.87 (0.68–1.11)	0.27	−0.38 (0.19)	0.05	0.33	0.63 (0.41–0.97)	0.03
rs1051730	0.32	0.76 (0.57–1.02)	0.07	0.11 (0.24)	0.65	0.29	1.49 (0.83–2.65)	0.18
CHRNB3-A6								
rs4950	0.25	1.20 (0.91–1.59)	0.21	0.13 (0.21)	0.54	0.24	0.72 (0.45–1.16)	0.18
rs2304297	0.25	1.05 (0.80–1.38)	0.72	−0.02 (0.21)	0.94	0.23	0.77 (0.48–1.24)	0.28
PD patients								
CHRNA5-A3-B4								
rs578776	0.29	0.85 (0.64–1.12)	0.25	−0.17 (0.24)	0.49	0.29	0.81 (0.52–1.28)	0.37
rs1051730	0.27	0.71 (0.48–1.05)	0.08	0.28 (0.38)	0.46	0.23	1.04 (0.51–2.10)	0.91
CHRNB3-A6								
rs4950	0.25	1.24 (0.93–1.64)	0.14	−0.35 (0.24)	0.15	0.26	1.53 (0.92–2.54)	0.10
rs2304297	0.28	1.17 (0.89–1.54)	0.25	−0.15 (0.23)	0.51	0.28	1.25 (0.79–1.97)	0.35

Abbreviations: MAF: minor allele frequency; OR: odds ratios; CI: confidence interval.
Note: The numbers of cases/controls can be found in Supplementary Table 3.
[a] Unconditional logistic model adjusted for index age (continuous), sex, and European ancestry. ORs and β-values for smoking variables per risk allele under the additive model.

African Americans [23]. Moreover, a study of the brain reward system suggested that the protective effect of the rs578776-A allele against smoking addiction may be due to its association with normal reward sensitivity to intrinsically pleasant activity (e.g. food compared with drug-related stimuli) in smokers [24]. Thus, given the prior associations of the G allele of rs578776 with nicotine dependence, higher quitting difficulty is expected among G-allele carriers. In fact, we observed a statistically significant inverse association between the A allele of rs578776 and quitting difficulty in control subjects but not in PD patients. We interpreted this to suggest that the stronger nicotine dependence among rs578776 allele G carriers that may lead to craving after quitting was blunted or neutralized by Parkinson's disease processes, possibly due to an increasing malfunction of the nicotinic reward system in the prodromal stages of PD, and more strongly so among those who quit smoking at a younger age (< 50 years). Another SNP at the same locus *CHRNA3,* rs1051730, was found to be independently associated with smoking intensity and nicotine dependence in both European and African Americans [23,25]. The SNP rs1051730 has a weak correlation with rs578776 but is highly correlated with *CHRNA5* rs16969968, a missense mutation associated with reduced nicotinic receptor function [26]. Furthermore, genome-wide studies of lung cancer showed strong associations with rs16969968 and SNPs strongly correlated with it including rs578776 and rs1051730 [27–29]. For the T allele of rs1051730, the point estimate was on the expected positive side of the null. This suggests a stronger quitting difficulty in controls (OR = 1.49 [0.83–2.65] that we do not see in PD cases (Table 3); however, we lacked statistical power to draw firm conclusions about rs1051730.

Our study did not find other associations between nAChR SNPs and smoking or quitting behaviors reported previously in the literature. However, a previous study of 1051 subjects that reported associations for SNPs in the *CHRNB3* and *CHRNA6* genes with nicotine dependence and quitting attempts was larger than our control sample size [11]. Therefore, we cannot rule out the possibility that our study lacked statistical power to show weaker associations.

Recall bias can occur if smoking or quitting is differently reported by PD patients and population controls. However, this does not seem likely. On the other hand, nondifferential exposure misclassification cannot be ruled out due to the age of our population, which may have impaired their recall overall. Besides, if we account for multiple comparisons, the formal p-value significance threshold of 0.05 would not be reached. However, the estimated effect for the rs578776 genetic variant and smoking cessation difficulty in controls is rather strong (0.63), and we also see a significant trend with cessation difficulty in controls but not cases (Table 4). A major strength of our study is the detailed information about lifetime smoking and quitting among former smokers.

Table 4
Associations for quitting difficulty (among former smokers only) according to rs578776 risk alleles in controls and PD patients separately[a]. The odds ratio compares the odds of smoking cessation difficulty among PD patients, which is the numerator, and the odds of smoking cessation difficulty among population controls, which is the denominator.

	Control			PD		P
How difficult was it to quit smoking?	N (easy/medium/hard)	OR (95%CI)[b]	P	N (easy/medium/hard)	OR (95%CI)[b]	
rs578776						
GG	31/26/34			42/39/21		
GA	50/24/25			39/25/15		
AA	14/6/4			10/3/5		
Medium vs. Easy		0.74 (0.44–1.25)	0.25		0.67 (0.40–1.13)	0.14
Extremely hard vs. Easy		0.53 (0.32–0.91)	0.02		1.09 (0.61–1.95)	0.77
		p for trend =	0.02		p for trend =	0.94

[a] ORs for smoking variables per risk allele under the additive model.
[b] Unconditional logistic model adjusted for index age (continuous), sex, and European ancestry.

PD patients in this study were interviewed early after disease diagnosis (within 3–4 years) and were identified in a population-based manner from neurologists and the California PD registry. To reduce disease misclassification, diagnosis has been confirmed in repeated exams conducted by movement disorder specialist.

Our findings that smoking cessation difficulty is associated with reduced PD risk suggest that reverse causation may contribute to the inverse smoking-PD association. Specifically, that similar to the loss of a sense of smell, REM sleep disorder, depression and constipation, ease of quitting might be acting as a marker of loss of the nicotinic response as part of a prodromal stage long before PD diagnosis [30]. Our study not only corroborates previous findings that ease of quitting may be an early sign of PD onset, but it also suggests that some biologic predictors of nicotinic response, such as the G allele of *CHRNA3* rs578776 that has been related to smoking cessation difficulty, are not relevant for the quitting behavior of PD patients. Finding an association with SNP rs578776 in controls (as expected from literature reports) but not cases is exactly what we would expect if PD patients are not responsive to nicotine in the manner that their genetic make-up would suggest; i.e. that the disease process overrides the genetically programmed nicotinic response. Our results are preliminary and need to be replicated in a larger study that investigates the hypothesis that nicotinic receptors play a role in the observed negative associations between smoking and PD.

Author contributions

Conception and design of the study: BR; acquisition of data: BR, JB, YB; analysis of data: YC; drafting the text and preparing the tables: YC. All co-authors contributed to study concept, design, and writing of the manuscript, as well as, approved the final manuscript.

Conflict of interest disclosures

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.01.031.

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