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An Exploratory Study on the *CHRNA3-CHRNA5-CHRNB4* Cluster, Smoking, and Parkinson's Disease

Jianjun Gao[a] Hong Xu[b] Clarice Weinberg[c] Xuemei Huang[d] Yikyung Park[e] Albert Hollenbeck[g] Aaron Blair[f] Arthur Schatzkin[e] Lauranell Burch[b] Honglei Chen[a]

[a]Epidemiology Branch, [b]Molecular Genetics Core Facility, and [c]Biostatistics Branch, National Institute of Environmental Health Sciences, Research Triangle Park, N.C., [d]Department of Neurology, Pennsylvania State University/Milton S. Hershey Medical Center, Hershey, Pa., [e]Nutritional Epidemiology Branch, and [f]Occupational and Environmental Epidemiology Branch, National Cancer Institute, Rockville, Md., and [g]AARP, Washington, D.C., USA

Key Words
Parkinson's disease · Smoking · *CHRNA3-CHRNA5-CHRNB4* cluster · Nicotine dependence

Abstract
Background: Smokers have a lower risk of Parkinson's disease (PD). Recent genome-wide association studies (GWAS) have consistently linked several single nucleotide polymorphisms (SNPs) in the *CHRNA3-CHRNA5-CHRNB4* cluster on chromosome 15.q25 to smoking behaviors and nicotine dependence. Investigations into these SNPs may help explain the nature and mechanisms of the smoking-PD relationship. ***Objective:*** To examine whether the genetic variations that were consistently associated with smoking or nicotine dependence in recent GWAS also predict the risk of PD. ***Methods:*** This is a population-based case-control study of 788 physician-diagnosed PD patients and 911 controls, all non-Hispanic Whites. Seven SNPs were selected based on findings from recent GWAS on smoking and nicotine dependence, all from the nicotinic acetylcholine receptor subunits *(CHRN)* A3-A5-B4. Odds ratios (ORs) and 95% confidence intervals were derived from logistic regression models under the assumption of logit-additive allelic effects. ***Results:*** Four SNPs in linkage disequilibrium from the *CHRNA3-CHRNA5-CHRNB4* cluster were associated with smoking duration (OR >1.3, $p < 0.05$). However, none of the SNPs from this cluster was associated with PD risk in the overall analysis or after stratifying on smoking status. ***Conclusion:*** This preliminary analysis does not support a relationship between these smoking-related GWAS SNPs and PD.

Introduction

Both genetic and environmental factors are involved in the development of Parkinson's disease (PD). Despite its well-known adverse health effects, cigarette smoking is the strongest environmental link to PD with a lower risk among smokers [1]. The explanation for this observation, however, has been a subject of debate. Nicotinic acetylcholine receptors *(CHRN)* are widely expressed in the brain and have been the target of drug development for

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Honglei Chen, MD, PhD
Epidemiology Branch, National Institute of Environmental Health Sciences
111 T.W. Alexander Dr. PO Box 12233, Mail drop A3–05
Research Triangle Park, NC 27709 (USA)
Tel. +1 919 541 3782, Fax +1 919 541 2511, E-Mail chenh2@niehs.nih.gov

smoking cessation and neuropsychological disorders, including Alzheimer's disease and PD [2]. Investigations into nicotinic receptor genetics may help us understand the nature and mechanisms of the smoking-PD relationship. Recently, a few large genome-wide association studies (GWAS) have consistently found associations of single nucleotide polymorphisms (SNPs) in the *CHRNA3-CHRNA5-CHRNB4* cluster on chromosome 15q25 with smoking behaviors or nicotine dependence [3–5]. We therefore examined these genetic variations in relation to PD in the large population-based Parkinson's Genes and Environment Study.

Subjects and Methods

Study Population and PD Case Identification

The Parkinson's Genes and Environment Study was built based on the large prospective cohort of the NIH-AARP Diet and Health Study that was initiated in 1995–1996 to investigate the etiology of cancers. We identified potential PD patients from self-reports in the cohort's follow-up survey in 2004–2006 and then tried to confirm the diagnosis by obtaining diagnostic information from their treating physicians [6]. A detailed description of the study population and diagnostic confirmation process was published previously [6]. Briefly, after obtaining permission from self-reported patients, we contacted the patients' treating physicians (86.7% movement disorder specialists or neurologists), and asked them to fill out a short diagnostic questionnaire, and also send a copy of relevant medical records. The diagnoses were either confirmed by their treating neurologists or by medical record review showing a final PD diagnosis or at least 2 cardinal signs with 1 being resting tremor or bradykinesia, a progressive course, and the absence of unresponsiveness to levodopa or other features suggesting an alternative diagnosis. Only confirmed PD patients were included in the current study. Controls were randomly selected from cohort participants who reported no PD on the follow-up questionnaire, and frequency matched to cases by sex, ethnicity, and year of birth in 5-year groups. The ethnicity was self-reported on the NIH-AARP cohort's baseline questionnaire as non-Hispanic White; non-Hispanic Black; Hispanic; Asian; Pacific Islander; American Indian or Alaska natives. To avoid population stratification, the current analysis was limited to 788 cases and 911 controls, all non-Hispanic Whites.

Smoking Data Collection

Smoking data were collected primarily from the baseline survey in 1995–1996. We have previously reported lower PD risk with longer smoking, higher smoking intensity, and fewer years since cessation in this cohort with strong dose-response relationships [6].

SNPs Selection and Genotyping

Seven SNPs located in the *CHRNA3-CHRNA5-CHRNB4* cluster on chromosome 15.q25 were selected based on findings from recent GWAS on smoking and nicotine dependence [3–5]. DNA was extracted from saliva samples collected with the Oragene™ collection kits. The genotyping was conducted using the Illumina BeadXpress platform. We added approximately 10% duplicates and the reproducibility error was less than 1% with a call rate of 99.9%.

Statistical Analyses

Hardy-Weinberg equilibrium among controls was confirmed with χ^2 statistics for all SNPs ($p > 0.05$). Odds ratios (ORs) and 95% confidence intervals (CIs) were derived from logistic regression models under the assumption of logit-additive allelic effects. The analysis was first conducted in all participants and then stratified by smoking to evaluate potential effect modification. Analyses were conducted with and without adjusting for year of birth and sex. The results were similar, and therefore, we presented results without the adjustment. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, N.C., USA) and Plink version 1.06. Two-sided $p < 0.05$ was considered statistically significant. Haplotypes and D'/R^2 values were calculated with Haploview 4.1. Power calculation was performed with Quanto version 1.2.4.

Standard Protocol Approvals, Registrations and Patient Consent

All study participants provided written consent and the study protocol was approved by the Institutional Review Board of the National Institute of Environmental Health Sciences and the Special Studies Institutional Review Board of the National Cancer Institute.

Results

Population characteristics of PD cases and controls are provided in the online supplementary table 1 (www.karger.com/doi/10.1159/000323190). These SNPs in the *CHRNA3-CHRNA5-CHRNB4* cluster represent 2 haplotype blocks. The first included rs8034191, rs17486278, rs16969968 and rs1051730 with $R^2 \geq 0.87$ and the second included rs569207, rs578776 and rs6495308 with $R^2 \geq 0.76$. The first 4 SNPs were associated with smoking duration in the expected direction (table 1). However, none of the SNPs was associated with ever/never smoking status or cigarettes smoked per day.

We did not find a significant association between these SNPs and PD in the overall or stratified analysis by smoking status (table 2). The haplotype analysis confirmed the null associations (data not shown).

Discussion

In this study, we did not observe any associations with PD for SNPs in the *CHRNA3-CHRNA5-CHRNB4* cluster that have been consistently linked to nicotine depen-

Table 1. Selected SNPs in the *CHRNA3-CHRNA5-CHRNB4* cluster in relation to smoking variables among controls[1]

SNPs	Ever smokers					Among ever smokers									
						>20 cigarettes/day					≥20 years of smoking				
	AA[2] %	Aa %	aa %	OR[3] (95% CI)	p	AA %	Aa %	aa %	OR[4] (95% CI)	p	AA %	Aa %	aa %	OR[5] (95% CI)	p
rs8034191	65.3	64.0	67.4	1.01 (0.82–1.24)	0.9	39.9	37.5	48.4	1.09 (0.85–1.39)	0.5	47.6	56.1	60.3	1.33 (1.03–1.71)	0.03
rs17486278	65.9	63.9	66.0	0.97 (0.79–1.19)	0.8	38.8	40.0	45.2	1.11 (0.87–1.42)	0.4	47.5	55.7	63.3	1.39 (1.07–1.79)	0.01
rs569207	64.5	65.5	66.1	1.04 (0.83–1.30)	0.7	44.1	31.0	52.8	0.83 (0.63–1.10)	0.2	54.0	50.8	52.9	0.93 (0.71–1.22)	0.6
rs16969968	65.9	63.5	67.0	0.98 (0.80–1.20)	0.8	39.7	39.0	44.4	1.06 (0.82–1.35)	0.7	48.1	55.9	60.7	1.32 (1.02–1.70)	0.03
rs578776	65.6	64.7	63.8	0.96 (0.78–1.19)	0.7	44.4	34.2	40.0	0.79 (0.61–1.03)	0.1	53.2	53.3	47.8	0.94 (0.73–1.22)	0.7
rs1051730	66.0	63.2	66.3	0.96 (0.78–1.18)	0.7	39.5	39.1	44.3	1.06 (0.83–1.36)	0.6	48.3	55.3	62.7	1.34 (1.04–1.72)	0.03
rs6495308	64.6	65.6	66.1	1.04 (0.83–1.30)	0.7	43.9	31.5	52.8	0.85 (0.64–1.11)	0.2	53.6	51.0	55.9	0.97 (0.74–1.27)	0.8

[1] Due to missing values on smoking variables, 908 controls were included in the ever/never smoking analysis, and among ever smokers, 584 in the smoking amount analysis, and 557 in the smoking duration analysis. [2] A denotes the major allele. [3] OR for being a smoker per risk allele under the additive model. [4] OR for smoking >20 cigarettes/day per risk allele under the additive model. [5] OR for smoking ≥20 years per risk allele under the additive model.

Table 2. Selected SNPs in the *CHRNA3-CHRNA5-CHRNB4* cluster in relation to PD[1]

SNPs	Minor allele frequency		All participants		Never smokers		Ever smokers	
	of controls	of cases	OR (95% CI)[2]	p	OR (95% CI)[2]	p	OR (95% CI)[2]	p
rs8034191	0.33	0.34	1.08 (0.93–1.25)	0.3	1.18 (0.93–1.49)	0.2	1.01 (0.84–1.22)	0.9
rs17486278	0.33	0.34	1.06 (0.92–1.23)	0.4	1.14 (0.90–1.43)	0.3	1.01 (0.83–1.22)	0.9
rs569207	0.24	0.25	1.05 (0.90–1.22)	0.6	0.91 (0.71–1.17)	0.5	1.18 (0.97–1.44)	0.1
rs16969968	0.33	0.34	1.07 (0.92–1.23)	0.4	1.16 (0.92–1.46)	0.2	1.00 (0.83–1.21)	0.98
rs578776	0.29	0.29	1.00 (0.86–1.16)	0.96	0.85 (0.67–1.08)	0.2	1.12 (0.93–1.35)	0.3
rs1051730	0.32	0.34	1.07 (0.93–1.24)	0.3	1.16 (0.92–1.46)	0.2	1.02 (0.84–1.23)	0.9
rs6495308	0.24	0.25	1.03 (0.89–1.21)	0.7	0.89 (0.69–1.15)	0.4	1.17 (0.96–1.43)	0.1

[1] The numbers of cases/controls for each analysis were: 788/911 for all participants, 363/318 for never smokers, 418/590 for ever smokers. [2] ORs (95% CI) for PD per risk allele under the additive model.

dence in several large GWAS. Our study is among the largest population-based studies on PD with both genetic and environmental data. With 788 cases and 911 controls, we estimated that we had 80% power to identify an OR of higher than 1.25 or lower than 0.79 for a minor allele frequency of 25%.

The nature of the robust epidemiological observation that smokers have lower PD risk has been controversial since its first report. Some believe that nicotine or other component(s) of cigarette protect against PD via unknown mechanisms, while others argue that this association is spurious due to some unmeasured third factor(s) such as personality or gene that is associated with both smoking and PD. This controversy may continue despite several lines of novel epidemiological evidence indicating biological possibility [7, 8]. If this association is indeed biological, the mechanism(s) remain(s) elusive. Investigations into smoking genetics may help clarify this observation and elucidate the underlying mechanisms, for example, through Mendelian randomization [9]. Such research efforts had been hampered by the lack of consistent findings on smoking genetics.

The several smoking-related SNPs in the *CHRNA3-CHRNA5-CHRNB4* cluster predict the risk of lung cancer, although studies disagree on whether this effect is direct or mediated by smoking [4, 5]. Interestingly, these SNPs were also associated with peripheral artery disease [5], whose association with smoking is not as strong as that for

lung cancer. These GWAS findings thus provided a good opportunity to understand the smoking-PD relationship from a genetic perspective, as nicotine has been hypothesized to protect against PD by stimulating nicotinic acetylcholine receptors [10]. To the best of our knowledge, the current study is the first to examine the relationship between *CHRNA* SNPs identified from smoking GWAS and PD risk. Although we confirmed that some of these SNPs may be associated with smoking duration, none of them were associated with the risk of PD.

Several considerations need to be taken into account in interpreting our results. The diagnosis of PD was made by the patients' individual treating physician, rather than by a uniformed examination, or by a movement disorder specialist. Therefore, a few misdiagnoses were possible. However, recent clinicopathological studies showed that 90% of the clinical PD diagnoses by neurologists were accurate [11]. Despite the robustness of the GWAS finding, these SNPs explain only a small part of the variation in smoking behavior. Therefore, their potential effect on PD through smoking, if any, is expected to be small. Further, although our analysis was based on the most consistent smoking genetic findings, smoking genetics is complex and most genetic determinants are yet to be identified. Therefore, our study should be considered exploratory and does not necessarily exclude roles of genes related to nicotine dependency or metabolism in the etiology of PD.

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