Parkinsonism and Related Disorders 21 (2015) 292–296

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Head injury, potential interaction with genes, and risk for Parkinson's disease

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ARTICLE INFO

Article history:
Received 31 July 2014
Received in revised form
25 November 2014
Accepted 31 December 2014

Keywords:
Parkinson's disease
Head injury
Gene-environment interactions
Genome-wide association study

ABSTRACT

Introduction: To evaluate the association between head injury and Parkinson's disease (PD), focusing on the timing of head injury, and to explore potential interactions between head injury and genetic factors in PD etiology.
Methods: The analysis included 507 PD cases and 1330 controls, all non-Hispanic Whites. Head injury was retrospectively asked, and genotyping was performed mainly as part of a previous GWAS.
Results: We found a positive association between head injury and PD risk. Compared with no previous head injury, the odds ratio (OR) was 1.39 (95% confidence interval [CI]: 1.00, 1.94) for one and 2.33 (95% CI: 1.25, 4.35) for two or more head injuries (P for trend = 0.0016). We further found that the higher risk was largely attributed to head injuries before age 30. Compared with no previous head injury, the OR was 2.04 (95% CI: 1.33, 3.14) for head injury that occurred before age 18, 1.39 (95% CI: 0.81, 2.36) for head injury between ages 18-<30, and 1.04 (95% CI: 0.58, 1.87) for head injury that occurred at age 30 or older (P for trend = 0.001). Exploratory interaction analyses showed a significant interaction between head injury and a SNP at the *RBMS3* locus (rs10510622, uncorrected P = 0.0001). No interaction was found with GWAS tag SNPs at or near the *MAPT, SNCA, LRRK2,* and *HLA* loci.
Conclusion: Our study suggests that head injury early in life may be an important risk factor for PD. The potential interaction with *RBMS3* needs confirmation.

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disease, affecting approximately one million Americans. The causes of PD are complex and may involve both genetic and environmental factors with interactions among risk factors. Head injury has been hypothesized to increase the risk of PD. Although not entirely consistent [1], the overall epidemiological evidence appears to be supportive for a positive association [2]. Compared to adults, children and adolescents are much more likely to have head injuries and suffer from long-term consequences [3]. However, few studies have investigated the timing of head injury in relation to PD, although a long lag time has been suggested [4,5]. We therefore examined the hypothesis that early-life head injury was associated with a higher risk for PD in the Parkinson's Genes and Environment (PAGE) study. Further, we conducted an exploratory analysis to examine potential interactions of head injury in PD with genetic variants, using SNPs from the confirmation genotyping of a recent GWAS [6].

1. Methods

1.1. Study population and PD case recruitment

The PAGE is a case–control study nested in the large prospective NIH-AARP Diet and Health Study. Details of study design have been published elsewhere [7,8]. Briefly, the cohort was assembled in 1995–1996 with detailed baseline information on dietary habit and lifestyle [9]. We first identified self-reported PD cases from the cohort's follow-up survey in 2004–2006, then contacted surviving self-reported cases in 2008–2009 to verify diagnosis and to collect saliva samples for genetic analysis. After obtaining patients' permission, we contacted their treating physicians, mostly neurologists, and asked them to complete a diagnostic questionnaire and to send a copy of the patient's medical records. A PD case was confirmed if the diagnosis was considered clinically definitive or probable by the treating neurologist, or if the medical record included a final PD diagnosis or evidence of 2 or more cardinal signs (with one being rest tremor or bradykinesia), a progressive course, responsiveness to dopaminergic treatments, and absence of features that suggest an

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http://dx.doi.org/10.1016/j.parkreldis.2014.12.033
1353-8020/Published by Elsevier Ltd.

alternative diagnosis [10]. The present analysis included only PD cases with diagnoses confirmed by this process. Controls were randomly selected from cohort participants who did not report PD on the follow-up questionnaire, and were frequency matched to cases by gender, ethnicity, and year of birth. We also requested dates of diagnosis and first symptoms for PD cases and family history of PD defined as having at least one first-degree blood relative with physician-diagnosed PD. For both PD cases and controls, we further retrospectively collected additional exposures of interest in 2009–2010 that had not been collected at the cohort's baseline survey, including head injury. The response rate for this retrospective data collection was 75.0% for cases and 82.2% for controls. A total of 611 PD cases and 1389 controls provided data on head injury. To avoid population stratification in the genetic analysis, we excluded 27 cases and 59 controls who were not Non-Hispanic Whites or did not report ethnicity. We further excluded 77 patients whose PD diagnoses were before 1995 to reduce the potential impact of reverse causality on the analysis. The final analysis included a total of 507 PD cases and 1330 controls.

1.2. Assessments of head injury and other environmental exposures

In the retrospective case–control data collection via structured questionnaire by mail, we asked if the participant had ever had a head injury prior to 1995 that resulted in unconsciousness, concussion or hospitalization (see Appendix). For those who answered yes, we further asked the number of head injuries, and the year of first and last head injuries before 1995. Information on other covariates such as cigarette smoking and consumption of caffeinated drinks was obtained from the cohort's baseline survey in 1995–1996 [9]. Participants were asked whether they had ever smoked more than 100 cigarettes. For ever smokers, we further asked the typical amount of smoking, smoking status at baseline; and for past smokers, years since last smoking [10]. Caffeine intake was calculated from consumption of coffee and other caffeine containing drinks and foods in the past year as part of a food frequency questionnaire [11]. We previously reported that both smoking and caffeine intake were inversely associated with the risk for PD in this study population [10,11]. In addition, the baseline survey also collected information on date of birth, sex, and ethnicity.

1.3. Genotyping

Genotyping data were from a previous GWAS [6] which were primarily performed by our collaborators at the National Institute on Aging (NIA). A total of 384 SNPs showing the lowest *P* values at the discovery screening were further selected for the confirmation genotyping. PAGE samples were the primary replication samples in that previous GWAS [6]. The selected 384 SNPs were genotyped with customized GoldenGate assays (Illumina, San Diego, CA) with an overall call rate of 97% [6], including tag SNPs at or near the known PD loci (*SNCA*, *MAPT*, and *LRRK2*) that showed GWAS associations with PD. Of these, 357 SNPs passed strict quality control procedures and were included in the current analysis. More recently, our NIA collaborators further genotyped PAGE cases and controls using the NeuroX array that covers >240,000 exonic variants on the Illumina Infinium HumanExome BeadChip and an additional ~24,000 variants proven or hypothesized to be relevant in neurodegenerative disease [12]. Details of this effort were published as part of large scale GWAS meta-analysis that confirmed a total of 28 PD GWAS SNPs [12], of which 22 are available for the current analysis. In addition to these GWAS SNPs, we further genotyped a SNP at the HLA locus (rs3129882) that showed genome-wide association with PD in another GWAS study [13]. This SNP was genotyped by BioServe Biotechnologies, Ltd. (Beltsville, MD) using MassARRAY iPLEXTM platform with a call rate of 97%.

1.4. Statistical analyses

We calculated odds ratios (OR) and 95% confidence intervals (CI) from multivariate logistic regression models, adjusting for year of birth, sex, smoking status (never, past and current smokers), daily caffeine intake (≤or > median), and family history of PD (yes or no). In the analysis, we first analyzed head injury as ever or never and then according to the number of head injuries before 1995. In the analysis, we combined participants with two or more head injuries as few reported more than two head injuries (n = 44). Most (66.8%) head injuries occurred before age 30, and therefore in the temporal relationship analysis, we defined head injuries as before age 18, age 18–30, and after age 30 for sample size considerations. In the analysis, we tested the statistical significance for a linear trend by assigning a value to each category of the head injury variable and including it as a continuous variable in the regression model. In our survey, participants were instructed to recall head injuries occurred in the distant past. To indirectly assess the potential impact of recall bias, we further conducted stratified analyses by median age at the cohort's baseline survey.

In the gene–environment interaction analysis, we first focused on SNPs at or near known PD loci: *SNCA* (n = 3), *MAPT* (n = 3), *LRRK2* (n = 3), and HLA (n = 1), and then we conducted exploratory analysis with other SNPs that were included in the GWAS confirmation. For each SNP, we first examined the main effect based on log-additive model, and then its interactions with head injury. Statistical significance for interaction was examined by including a multiplicative term between environmental exposure and each SNP. The main effects of SNPs were saturated in the model to ensure the validity of tests on gene–environment interactions. For the SNP that showed a statistical interaction after Bonferroni correction, we conducted stratified analyses by genotypes. In this analysis, we combined the heterozygous with homozygous variants to maintain reasonable sample sizes. Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, NC) and Plink v1.07.

1.5. Standard protocol approvals, registrations, and patient consents

Participants consented to the study by returning survey questionnaires. 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.

2. Results

Population characteristics of PD cases and controls are listed in Table 1. Cases and controls were matched by year of birth and gender and for cases the average age of PD diagnosis was 68.3 (SD, 5.8) years. Compared with controls, PD cases were less likely to smoke and had lower caffeine intake, they were however more likely to report a family history of PD and head injury. After adjusting for potential confounders, ever head injury was associated with a 59% higher risk for PD (OR = 1.59, 95% CI: 1.18, 2.13). Further, PD cases tended to report first head injury at a younger age than controls ($P = 0.04$). Among cases, the average lag time between first head injury and PD diagnosis was 45.4 (SD, 15.4) years.

Fig. 1 showed a positive association between the number of head injuries and PD risk. The OR was 1.39 (95% CI: 1.00, 1.94) for participants with one previous head injury and 2.33 (95% CI: 1.25, 4.35) for those with two or more head injuries, with a dose–response relationship (P for trend = 0.0016). Analysis on the timing of head injury showed the importance of head injury in early life as a risk factor for PD (Fig. 2). The OR was 2.04 (95% CI: 1.33, 3.14) for head injury before age 18 and 1.39 (95% CI: 0.81, 2.36) for head injury between ages 18 and 29. In contrast, head injury that occurred at age 30 or older was not related to PD risk (OR = 1.04, 95% CI: 0.58, 1.87). This analysis also showed a significant trend (P for trend = 0.001). Similar results were obtained in age-stratified analyses (Supplementary Figs. 1 and 2).

The main effects of 10 GWAS tag SNPs at or near the *MAPT, SNCA, LRRK2,* and *HLA* loci in the PAGE study were published previously [8]. We did not see any significant gene–environment interactions between head injury and these SNPs (Supplementary Table 1), nor did we observe any significant interactions with any of the 22 SNPs reported by the recent GWAS meta-analysis [12] (Supplementary Table 2).

In the exploratory analysis with other SNPs included in the previous GWAS confirmation genotyping (Supplementary Table 3),

Table 1
Population characteristics of PD cases and controls.[a]

	PD cases (n = 507)	Controls (n = 1330)
Men, %	75.5	79.0
Year of Birth	1932.6 (5.0)	1932.1 (4.8)
Smoking, %		
Never	45.6	35.8
Former	51.1	58.1
Current	2.2	5.3
Missing	1.2	0.9
Caffeine intake, mg/day	310.4 (327.0)	353.3 (357.4)
Family history of PD, %		
No	84.8	85.6
Yes	14.8	5.6
Missing	0.4	8.8
Age at PD diagnosis, years	68.3 (5.8)	
Head injury before 1995, %		
No	82.5	87.9
Yes	17.6	12.1
Age at first head injury, years	22.0 (14.6)	26.5 (16.7)
Years between first head injury and PD	45.4 (15.4)	N/A

Abbreviations: PD, Parkinson disease.
[a] Means (standard deviations) are presented for continuous variables and percentage for categorical variables.