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Research article

Association between a heme oxygenase-2 genetic variant and risk of Parkinson's disease in Han Chinese

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HIGHLIGHTS

- We assessed the association between three heme oxygenase genetic variants and sporadic Parkinson's disease.
- 583 Chinese Han patients with sporadic Parkinson's disease were genotyped.
- Our data suggest that rs1051308 is related to the risk of Parkinson's disease in Han Chinese.

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ABSTRACT

Studies have reported conflicting results about possible associations between variants in heme oxygenase (*HMOX*) genes and risk of Parkinson's disease (PD) in Caucasians, and little is known about these associations in Asians. We genotyped the single-nucleotide polymorphisms (SNPs) rs2071746 and rs2071747 in *HMOX1* and rs1051308 in *HMOX2* in 583 Han Chinese with PD and 627 healthy controls using a customized 2 × 48-Plex SNP Scan™ kit. Frequencies of genotypes and minor alleles were similar between patients and controls for rs2071746 and rs2071747, but different for rs1051308($P = 0.004$, OR 1.705, 95%CI 1.191–2.442 for genotypes; $P = 0.009$, OR 1.249, 95%CI 1.037–1.476 for alleles). Our results suggest that rs1051308 is associated with risk of developing PD in Han Chinese, and further studies involving various ethnicities are needed to validate the association.

1. Introduction

Parkinson's disease (PD), one of the most common neurodegenerative diseases worldwide, is associated with rigidity, rest tremor, bradykinesia and imbalance caused by decreased dopamine levels [1]. The etiology of PD remains elusive, but is generally thought to involve interaction of environmental and genetic factors [2–4]. Numerous studies in cellular and animal models have implicated several processes in PD pathogenesis, including oxidative stress, mitochondrial dysfunction and iron deposition [5].

Heme oxygenase (*HMOX*) converts heme into iron, carbon monoxide and biliverdin, which is catabolized into bilirubin by biliverdin reductase [6]. *HMOX* exerts both pro- and anti-oxidant effects, which may influence the pathogenesis or progression of PD as well as Alzheimer's disease [7,8]. Studies have provided indirect evidence that *HMOX* is up-regulated in PD, perhaps as a compensatory response to oxidative stress. Post-mortem and in vivo studies have reported iron deposition in PD patients [9,10], which may result from *HMOX* up-regulation as well as age-related disturbance of iron homeostasis [11,12]. This iron deposition may contribute to the characteristic loss of dopaminergic neurons in PD. A longitudinal case-control study reported higher levels of bilirubin in PD patients than healthy controls, and a correlation between higher bilirubin and worse motor symptoms [13].

Two *HMOX* isoforms have been identified in the mammalian central nervous system: *HMOX1* is expressed in neurons and neuroglia, and its activity is strongly influenced by changes in the cellular microenvironment [14,15]; while *HMOX2* is expressed at constant levels predominantly in the substantia nigra, septum and hippocampus [16,17]. Studies have reported sometimes conflicting conclusions about whether associations exist between polymorphisms in *HMOX* genes and risk of PD in Caucasians [18–22]. Whether these possible associations exist in Asians remains unclear.

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Here we investigated possible relationships of the variants rs2071746 and rs2071747 in *HMOX1* and the variant rs1051308 in *HOMX2* with risk of PD in Han Chinese.

2. Subjects and methods

2.1. Patients and controls

The study protocol was approved by the Human Research Ethics Committee of Sichuan University, and all subjects provided informed consent. PD patients were consecutively recruited from two movement disorder units: West China Hospital of Sichuan University, located in southwest China; and the First Affiliated Hospital of Sun Yat-sen University, located in southeast China. All patients were diagnosed independently by two specialists in movement disorders based on the UK Parkinson's Disease Society Brain Bank criteria. Patients were excluded if they had at least one relative with PD. Patients were classified as having early-onset PD if they were younger than 50 years old at disease onset; other patients were classified as having late-onset PD [23]. A total of 583 PD patients and 627 healthy controls were involved, and both groups were ethnically matched.

Controls without neurodegenerative disease and unrelated to patients were recruited from among healthy individuals undergoing medical examinations at the two study sites.

2.2. Genotyping

For all subjects, genomic DNA was obtained from peripheral leukocytes and purified using the classical phenol-chloroform DNA extraction method. Single-nucleotide polymorphisms (SNPs) were genotyped using a customized 2 × 48-Plex SNP Scan™ kit (cat. no. G0104, Genesky Biotechnologies, Shanghai, China) as previous described [24]. This kit is based on a patented SNP genotyping technology involving double ligation and multiplex fluorescence PCR. Briefly, 100–200 ng of DNA sample was first denatured at 98 °C for 5 min in a 10 μl reaction containing 1 × DNA lysis buffer and then mixed well with a 10 μl ligation premix composed of 2 μl 10 × ligase buffer, 0.5 μl ligase, 1 μl probe mix, and 7.5 μl Milli-Q water. The ligation reaction was carried out in an ABI2720 thermal cycler. Two 48-plex fluorescence PCR reactions were performed for each ligation product. PCR reactions were prepared in a 20 μl mixture containing 1 × PCR master mix, 1 μl primer mix set A or set B, and 1 μl ligation product. PCR products were separated and detected by capillary electrophoresis on an ABI3730XL sequencer. Raw data were analyzed using GeneMapper 4.0, and genotypes for each locus were determined based on labeling dye color and fragment size of specific ligation-PCR products.

Several measures were taken to ensure genotyping accuracy and reliability. First, the researchers performing the genotyping procedures were blinded to sample identity and samples from cases and controls were genotyped together. Second, 20% of samples were randomly selected and re-genotyped by a different researcher; a replication rate of 100% was obtained. Third, 10 samples showing each of the observed genotypes based on SNPscan were sequenced directly on an ABI Prism 3730; a reproducibility rate of 100% was obtained.

2.3. Statistical analysis

Age of all individuals was reported as mean ± standard deviation, while gender, allele, and genotype were reported as percentages. Inter-group differences in gender composition were assessed for significance using the chi-squared test, while differences in age were assessed using Student's *t* test. Logistic regression was carried out using PD as the dependent variable, while age, gender, and genotype served as independent variables. Concordance of genotype distribution with the predictions of Hardy-Weinberg equilibrium (HWE) was assessed. Statistical analyses were performed using SPSS 22.0 (IBM, Chicago, USA), using a significance threshold of $P < 0.05$. Haplotype analysis was performed using SHEsis 4.0 (http://analysis.bio-x.cn/myAnalysis.php). Power calculations were carried out using PS Power and Sample Size software.

Table 1
Demographic characteristics of Han Chinese patients with Parkinson's disease and healthy controls.

Characteristic	Patients	Controls	*P*
n	583	627	–
Mean age, yr	61.80 ± 12.15	60.85 ± 11.08	0.16
Age range, yr	20–86	24–95	–
Female	256 (43.9%)	263 (41.9%)	0.52
Mean age at disease onset, yr	56.91 ± 11.56	–	–
Early-onset	177 (30.4%)	–	–
Late-onset	406 (69.6%)	–	–

Results are shown as mean ± standard deviation, range, or n (%).

3. Results

The two groups were similar in terms of age and gender composition (Table 1). Frequencies of genotypes and minor alleles at rs1051308, rs2071746 and rs2071747 were in HWE in patients and controls.

The major allele for SNP rs1051308 was A; for rs2071746, T; and for rs2071747, G. The CC genotype was not detected in patients and in only one control. Frequencies of genotypes and minor alleles were similar between patients and controls for rs2071746 and rs2071747, but different for rs1051308 (Table 2). The G allele at rs1051308 occurred significantly more often among patients than controls ($P = 0.009$, OR 1.249, 95%CI 1.037–1.476; Table 2). A significant association was identified between rs1051308 and risk of PD according to a recessive model, even after adjusting for age and gender ($P = 0.04$, OR 1.630, 95%CI 1.165–2.281; Table 2). Given our sample size, our study had 92.2% power to detect an association between rs1051308 and risk of PD with OR 1.63 at the 0.05 significance level using a recessive model.

No association was found between rs2071746 and risk of PD using dominant or recessive models (Table 2).

Subgroup analysis was performed based on gender and age at PD onset. Among females, no significant differences were observed in frequencies of genotypes or minor alleles. Among male patients and controls, rs1051308 was associated with risk of PD in a recessive model ($P = 0.017$, OR 1.694, 95%CI 1.100–2.611; Table 2), but no individual allele appeared to be associated with disease. No significant differences were observed in frequencies of genotypes or minor alleles between patients with late-onset PD (n = 406, mean age at onset, 67.72 ± 7.88 yr) or those with early-onset disease (n = 177, mean age at onset, 49.32 ± 10.96 yr). Subgroup analysis showed no significant differences in frequencies of genotypes or minor alleles in both rs2071746 and rs2071747(Table 2).

Haplotype analysis showed that the SNPs rs2071746 and rs2071747 were not in linkage disequilibrium ($D' = 0.53$, $r^2 = 0.01$). After excluding haplotypes with frequencies lower than 0.03, three haplotypes were identified (A-G, T-C, and T-G), none of which showed a significant association with risk of PD (Table 3).

4. Discussion

Our case-control study suggests that the GG genotype at rs1051308 in *HMOX2* is associated with risk of PD in Han Chinese, while no such association was found for rs2071746 or rs2071747