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Research paper

Genetic analysis of *MC1R* variants in Chinese Han patients with sporadic Parkinson's disease

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HIGHLIGHTS

- We evaluated the association between *MC1R* variants and sporadic Parkinson's disease.
- 512 Chinese Han patients with sporadic Parkinson's disease were genotyped.
- Rs3212366, rs33932559, and rs34090186 are not related to Chinese Parkinson's disease.

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ABSTRACT

Parkinson's disease (PD, OMIM 168600) is a neurodegenerative disorder featured by degeneration of melanin-positive dopaminergic neurons. Epidemiologic studies have suggested that PD and malignant melanoma (MM) might share common genetic components. Recently, the p.R160W variant in the melanocortin 1 receptor gene (*MC1R*, OMIM 155555), a risk factor for MM, has been identified to be associated with PD in Spanish population. To explore whether the *MC1R* variants are associated with sporadic PD in Chinese population, we designed a case-control comparison study and studied three variants, including rs3212366 (p.F196L), rs33932559 (p.I120T) and rs34090186 (p.R67Q), in the *MC1R* gene in 512 Chinese Han patients with sporadic PD and 512 age, gender and ethnicity matched normal controls. For rs3212366, only the TT genotype was identified in both PD and control cohorts. For variants rs33932559 and rs34090186, we did not identify any statistically significant difference in either genotypic distribution or allelic distribution between the PD cohort and control cohort, and in addition, we did not identify any related haplotype that would either increase the risk for PD or play a protective role against PD. Our data suggest that none of the three variants of the *MC1R* gene and related haplotypes be associated with sporadic form of PD in Chinese Han population from Mainland China.

Abbreviations: PD, Parkinson's disease; MM, malignant melanoma; *MC1R*, the melanocortin 1 receptor gene; GPCR, G-protein-coupled receptor; MCRs, melanocortin receptors; α-MSH, α-melanocyte stimulating hormone; cAMP, the cyclic AMP; *VPS35*, the vacuolar protein sorting 35 gene; *FBXO48*, the F-box only protein 48 gene; *EIF4G1*, the eukaryotic translation initiation factor 4-gamma 1 gene; *S100B*, the S100 calcium binding protein B gene; *RAB39B*, the RAB39B, member RAS oncogene family gene; *TCEANC2*, the transcription elongation factor A (SII) N-terminal and central domain containing 2 gene; SIFT, Sorting intolerant from tolerant; PolyPhen-2, Polymorphism Phenotyping v2; SNP, single nucleotide polymorphism; MAF, minor allele frequency; PCR, polymerase chain reaction; SPSS, Statistical Program for Social Sciences; HWE, Hardy–Weinberg equilibrium; OR, odds ratio; CI, confidence interval; RR, relative risk.

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1. Introduction

Parkinson's disease (PD, OMIM 168600) is a common neurodegenerative disorder featured by degeneration and death of melanin-positive dopaminergic neurons in the nigrostriatum [1,2]. There is epidemiological evidence suggesting an inverse association between cancer and neurodegenerative disease, i.e., patients with PD having a decreased risk for almost all types of cancer [3,4]. Intriguingly, malignant melanoma (MM), a melanocyte-derived neoplasm, is a notable exception with a higher-than-expected incidence among patients with PD and vice versa based on several epidemiologic studies [1,5–9]. Furthermore, people with lighter hair color was reported to be associated with higher risks for both PD and MM [10], suggesting that PD and MM may share some common pathogenic pathways, though the direct evidence of this hypothesis is still lacking [7,11].

The melanocortin 1 receptor gene (*MC1R*, OMIM 155555), a susceptibility gene associated with increased risk for MM and a key regulator of skin pigmentation, may play a role for the co-occurrence of PD and MM [12]. *MC1R* is located on chromosome 16q24.3 spanning over 3.0 kb and contains one exon encoding a 317-amino acid seven-pass transmembrane G-protein coupled-receptor (GPCR) [13,14]. MC1R belongs to the melanocortin receptors (MCRs), a five-member subfamily of GPCRs. MC1R is expressed in melanocytes and melanoma cells, and is activated by adrenocorticotrophin or α-melanocyte stimulating hormone (α-MSH) coupled to the Gs-protein-dependent activation of the cyclic AMP (cAMP) signaling pathway, stimulating the production of melanogenesis and eumelanin [13,15]. More recently, the *MC1R* gene variant, p.R160W, was identified to be associated with PD in Spanish population [16], but was reported not to be associated with PD in a non-Hispanic white cohort [5]. To explore whether *MC1R* variants are associated with sporadic PD in Chinese Han population, we performed a case-control comparison study in 512 Chinese Han patients with sporadic PD and 512 age, gender and ethnicity matched normal controls.

2. Material and methods

2.1. Subjects

A case-control comparison study in 512 Chinese Han patients with sporadic PD (male/female: 308/204; age: 65.8 ± 10.3 years; age at onset: 62.4 ± 7.8 years) and 512 gender, age and ethnicity matched normal controls (male/female: 308/204; age: 65.9 ± 10.5 years) from Mainland China was performed. The diagnosis of PD met the common diagnostic criteria [17]. All controls are healthy without any neurological, dermatologic or oncological conditions. All participants or their relatives provided written informed consents and our study obtained the approval from the local Ethics Committee. Some patients had previously been studied to be negative for causal mutations in several known genes potentially associated with PD: 25.4% (130/512) PD patients had no evidence of mutation in the vacuolar protein sorting 35 gene (*VPS35*) [18], 66.2% (339/512) were negative for any mutation in the F-box only protein 48 gene (*FBXO48*) [19], 59.8% (306/512) had no evidence of either p.A502 V or p.R1205H point mutations in the eukaryotic translation initiation factor 4-gamma 1 gene (*EIF4G1*) [20], 74.8% (383/512) had no evidence of mutation in the S100 calcium binding protein B gene (*S100B*) [21], 74.8% (383/512) were negative for any mutation in the RAB39B, member RAS oncogene family gene (*RAB39B*) [22], 97.7% (500/512) were negative for either rs10788972 or rs12046178 variant in the transcription elongation factor A (SII) N-terminal and central domain containing 2 gene (*TCEANC2*) [23].

2.2. Selection of variants

We used three prediction software programs, including Sorting intolerant from tolerant (SIFT) prediction (http://sift.jcvi.org/), HumVar-trained Polyphen-2 (Polymorphism Phenotyping v2, http://genetics.bwh.harvard.edu/pph2/), and MutationTaster prediction (http://www.mutationtaster.org/), to estimate whether a single nucleotide polymorphism (SNP) affects protein function [24,25]. The chosen SNPs of the *MC1R* gene should be predicted as damaging/disease causing. In addition, we chose rs34090186 because it was reported with an allele frequency of 1.26% in Chinese PD patients from Singapore [26]. The rs3212366 and rs33932559 were selected since the minor allele frequency (MAF) of both is above 0.05.

2.3. DNA preparation and SNP genotyping

Blood samples were collected from all participants and genomic DNA was extracted from peripheral lymphocytes by using standard phenol–chloroform extraction method [22]. Genotyping of the three selected SNPs was performed using Sequenom MassARRAY iPLEX Gold platform (Sequenom, San Diego, California) based on the manufacturer's instructions [27]. Primers for polymerase chain reaction (PCR) amplification were designed using the MassARRAY Assay Design 3.1 software, and were shown in Table 1. Briefly, multiplex PCR amplification was performed in standard 384-well plates using an ABI-9700 instrument (GeneAmp PCR system 9700, ABI, California) in the following running conditions: Taq polymerase activation at 95 °C for 2 min, followed by 45 cycles of degeneration at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 60 s. After removing of unreacted primers and dNTPs, single-base extension PCR was carried out by iPLEX assay. Then, the depurative extended reaction products were transferred to a 384-well Spectro-CHIP (Sequenom, Inc.) installed with 2 no-template controls and 4 duplicated samples as quality controls, and were analyzed in a MALDI-TOF-MS spectrometer (Sequenom, San Diego, CA). Direct sequencing of randomly selected samples was also performed as quality control. Finally, data collection and genotyping were carried out in a Compact Mass Spectrometer using the MassARRAY Typer 4.0 software. All genotyping results were verified by a masked investigator who was unaware of the clinical status of participants with random sampling.

2.4. Statistical analysis

Difference in frequencies of the alleles and genotypes between the PD and control cohorts was evaluated using Pearson's χ^2 test in Statistical Program for Social Sciences (SPSS) software 17.0 (SPSS Inc., Chicago, IL). Hardy–Weinberg equilibrium (HWE) was tested using Pearson's χ^2 test. The haplotype construction and association analysis, as well as the calculation of odds ratios (ORs) and 95% confidence intervals (CIs), were performed using SHEsis Online Version (http://analysis.bio-x.cn/SHEsisMain.htm) [28,29]. A value of $P < 0.05$ was considered as statistical significance.

3. Results

No evidence of deviation from HWE was detected in either the PD cohort or the control cohort ($P > 0.05$). For variant rs3212366, only the T allele was detected, and only the homozygous TT genotype was found in both PD and control cohorts. Genotypic frequencies and allelic frequencies of variants rs33932559 and rs34090186 in the two cohorts were shown in Table 2. Pearson's χ^2 test indicates that there is no statistically significant difference in either genotypic distribution or allelic distribution between the PD cohort and control cohort for the