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Research paper

Genetic analysis of *TREM2* variants in Chinese Han patients with sporadic Parkinson's disease

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HIGHLIGHTS

- We assessed the association between *TREM2* variants and sporadic Parkinson's disease.
- 512 Chinese Han patients with sporadic Parkinson's disease were genotyped.
- Rs75932628 and rs2234253 are not related to Chinese sporadic Parkinson's disease.

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ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease and is characterized by the degeneration of dopaminergic neurons in substantia nigra. Recently, rs75932628 (p.R47H) of the triggering receptor expressed on myeloid cells 2 gene (*TREM2*) was identified to be associated with PD in American, Spanish, Irish, and Polish population. To explore whether *TREM2* variants are related to susceptibility of sporadic PD in Chinese Han population, we designed a case-control comparison study and studied two variants rs75932628 (p.R47H) and rs2234253 (p.T96K) of the *TREM2* gene in 512 Chinese Han patients with sporadic PD and 512 age, gender and ethnicity matched normal controls from Mainland China. No variant for either rs75932628 or rs2234253 was found in both PD and control cohorts. Our data suggest that neither variant rs75932628 nor rs2234253 be a major susceptibility factor of sporadic PD in Chinese Han population from Mainland China.

1. Introduction

Parkinson's disease (PD, OMIM 168600) is the second most common progressive neurodegenerative disorder after Alzheimer's disease (AD, OMIM 104300). Its prevalence increases with age [1]. It is characterized by progressive loss of dopaminergic neurons in the substantia nigra, as well as in other brain areas, and sometimes is accompanied by the appearance of Lewy bodies [2,3]. These neuropathologic features lead to a broad spectrum of motor and non-motor features, including four cardinal features (rest tremor, bradykinesia, rigidity and loss of postural reflexes), many secondary motor symptoms (hypomimia, dysarthria, dysphagia, sialorrhoea, micrographia, shuffling gait, festination, freezing, dystonia, glabellar reflexes, etc.) and various non-motor features (autonomic nervous dysfunction, cognitive neurobehavioral abnormalities, sleep disorders, sensory abnormalities, such as anosmia,

Abbreviations: PD, Parkinson's disease; AD, Alzheimer's disease; *TREM2*, the triggering receptor expressed on myeloid cells 2 gene; *VPS35*, the vacuolar protein sorting 35 gene; *EIF4G1*, the eukaryotic translation initiation factor 4-gamma 1 gene; *S100B*, the S100 calcium binding protein B gene; *FBXO48*, the F-box protein 48 gene; *RAB39B*, the RAB39B, member RAS oncogene family gene; *TCEANC2*, the transcription elongation factor A (SII) N-terminal and central domain containing 2 gene; *MC1R*, the melanocortin 1 receptor gene; SIFT, Sorting Intolerant from Tolerant; PolyPhen-2, Polymorphism Phenotyping v2; SNP, single nucleotide polymorphism; MAF, minor allele frequency; PCR, polymerase chain reaction; FTD, frontotemporal dementia; FTLD, frontotemporal lobar degeneration; ALS, amyotrophic lateral sclerosis.

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Table 1
Primers for detecting two variants of the *TREM2* gene.

dbSNP ID	rs75932628	rs2234253
Prediction[a]	T/P/DC	D/P/Po
Forward primer (5′ → 3′)	ACGTTGGATGCACAAGTTGTGCGTGCTGAC	ACGTTGGATGACAGCCATCACAGACGATAC
Reverse primer (5′ → 3′)	ACGTTGGATGCCTATGACTCCATGAAGCAC	ACGTTGGATGACTGGTAGAGACCCGCATCA
Product size (bp)	100	85
Extended primer (5′ → 3′)	GCACCAGGCCTTG	GAATTGGTGGCACTCTCACCATTA

T: tolerated; P: probably damaging; DC: disease causing; D: damaging; Po: polymorphism.
[a] Sorting Intolerant from Tolerant/Polymorphisms Phenotyping v2/MutationTaster.

paresthesias and pain, etc.) [4]. Although the exact pathogenesis underlying the selective degeneration of dopamine-producing cells in PD is still unclear [1], numerous evidences indicate that genetic abnormalities play an important role in the pathogenic mechanism of PD [5]. Genetic research in PD has been productive over the last 18 years, with the identification of more than 20 loci and 15 pathogenic genes involved in familial and sporadic forms of PD [6]. However, mutations in these genes account for only a small part of PD cases. In 90% of PD cases, non- genetic factors may play a main role, probably with susceptibility genes [1]. Recent researches indicate that rs75932628 (p.R47H) of the triggering receptor expressed on myeloid cells 2 gene (*TREM2*, OMIM 605086) is related to PD in American, Spanish, Irish, and Polish population [7,8]. Additionally, the role of *TREM2* in PD susceptibility was highlighted by convergent genetic and expression datasets [9]. To explore whether *TREM2* variants are associated with risk of sporadic PD in Chinese Han population, we performed a case-control comparison study in 512 Chinese Han patients with sporadic PD and 512 gender, age and ethnicity matched normal controls from Mainland China.

2. Material and methods

2.1. Subjects

The case-control comparison study consists of 512 Chinese Han patients with sporadic PD (male/female: 308/204; age: 65.81 ± 10.32 years; age at onset: 62.43 ± 7.81 years), and 512 gender, age and ethnicity matched normal controls (male/female: 308/204; age: 65.92 ± 10.51 years) from Mainland China. The diagnosis of PD was established according to the common diagnostic criteria [4], and all controls were healthy without any neurological diseases. All participants signed informed consent. We also obtained appropriate ethical approval from the Institutional Review Board of the Third Xiangya Hospital, Central South University, China. Some patients had previously been analyzed and were negative for causal mutations in several known genes potentially associated with PD: 25.39% (130/512) PD patients were screened and had no evidence of mutation in the vacuolar protein sorting 35 gene (*VPS35*) [10], 59.77% (306/512) were negative for either p.A502V or p.R1205H point mutations in the eukaryotic translation initiation factor 4-gamma 1 gene (*EIF4G1*) [11], 74.80% (383/512) were negative for any mutation in the S100 calcium binding protein B gene (*S100B*) [12], 66.21% (339/512) had no evidence of mutation in the F-box protein 48 gene (*FBXO48*) [13], 74.80% (383/512) were negative for any mutation in the RAB39B, member RAS oncogene family gene (*RAB39B*) [14], 97.66% (500/512) were tested for variants rs10788972 and rs12046178 in the transcription elongation factor A (SII) N-terminal and central domain containing 2 gene (*TCEANC2*) [15], 100% (512/512) were tested for variants rs3212366, rs33932559, and rs34090186 in the melanocortin 1 receptor gene (*MC1R*) [16].

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 [17,18]. Two SNPs (rs75932628 and rs2234253) of the *TREM2* gene were predicted as damaging/disease causing. In addition, rs75932628 (p.R47H) was selected since it has been reported to be a PD-associated variant with various frequencies across different populations [7,8]. We chose rs2234253 (p.T96K) because its minor allele frequency (MAF) 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 using standard phenol–chloroform extraction method [15]. Genotyping of the selected SNPs was performed by using Sequenom MassARRAY iPLEX Gold platform (Sequenom, San Diego, California) according to the manufacturer's instructions [19]. Primers for polymerase chain reaction (PCR) amplification were designed using the MassARRAY Assay Design 3.1 software and the sequences of the primers are 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 control 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 by using the MassARRAY Typer 4.0 software. All genotyping results were produced and checked by a masked investigator who was unaware of participants' clinical status.

3. Results

In this study, no variant for either rs75932628 or rs2234253 was found in both PD and control cohorts. Our data suggest that neither variant rs75932628 nor rs2234253 be a major susceptibility factor of sporadic PD in Chinese Han population from Mainland China.

4. Discussion

PD is a complex age-related neurodegenerative disease, affecting 1–2% people over 60 years of age and up to 4% people over 80 years of age. Although the exact cause remains unknown, it is likely multifactorial. There is abundant evidence suggesting that genetic factors, including pathogenic mutations and susceptibility variants, play an important role in the pathogenesis of PD [1,20–22]. To date, at least 20 loci and 15 pathogenic genes have been identified to be associated with PD [6]. However, these loci and pathogenic genes