Original Article

Association Analysis of Proteasome Subunits and Transporter Associated with Antigen Processing on Chinese Patients with Parkinson's Disease

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Abstract

Background: Proteasome subunits (*PSMB*) and transporter associated with antigen processing (*TAP*) loci are located in the human leukocyte antigen (*HLA*) Class II region play important roles in immune response and protein degradation in neurodegenerative diseases. This study aimed to explore the association between single nucleotide polymorphisms (*SNPs*) of *PSMB* and *TAP* and Parkinson's disease (PD).
Methods: A case–control study was conducted by genotyping SNPs in *PSMB8, PSMB9, TAP1*, and *TAP2* genes in the Chinese population. Subjects included 542 sporadic patients with PD and 674 healthy controls. Nine identified SNPs in *PSMB8, PSMB9, TAP1*, and *TAP2* were genotyped through SNaPshot testing.
Results: The stratified analysis of rs17587 was specially performed on gender. Data revealed that female patients carry a higher frequency of rs17587-G/G versus (A/A + G/A) compared with controls. But there was no significant difference with respect to the genotypic frequencies of the SNPs in *PSMB8, TAP1,* and *TAP2* loci in PD patients.
Conclusion: Chinese females carrying the rs17587-G/G genotype in *PSMB9* may increase a higher risk for PD, but no linkage was found between other SNPs in *HLA* Class II region and PD.

Key words: Han Chinese; Proteasome Subunit Beta Type; Sporadic Parkinson's Disease; *TAP*

Introduction

Parkinson's disease (PD) is characterized by the dopaminergic neuron degeneration accompanying the aggregation of α-synuclein and neuroinflammation in the midbrain.[1] The elevated major histocompatibility complex (MHC) Class I antigens, which are associated with T-cells infiltration, were found in the substantial nigra of PD patients.[2,3] It was reported the MHC-I is expressed in catecholaminergic neurons and makes the neurons more susceptible to cytotoxic attack.[4,5] Much evidence has showed that immune dysregulation, which was associated with MHC Class I pathway, play an important role in the development of PD.[6,7]

The proteasome subunits (*PSMB*) and transporter associated with antigen processing (*TAP*) genes are responsible for immune activity and protein degradation in MHC Class I pathway.[8,9] It was reported that the classical MHC class molecules, human leukocyte antigen (*HLA*)-*DRB,* represent the antigens for immune effector cells associated with PD.[10] The *PSMB* and *TAP* genes are adjacent to *HLA-DR* within the *HLA* Class II region [Figure 1a].[11] The *PSMB9* and *PSMB8* encode β1i (low molecular weight protein 2) and β2i (low

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Received: 04-11-2015 **Edited by:** Yi Cui
How to cite this article: Mo MS, Huang W, Sun CC, Zhang LM, Cen L, Xiao YS, Li GF, Yang XL, Qu SG, Xu PY. Association Analysis of Proteasome Subunits and Transporter Associated with Antigen Processing on Chinese Patients with Parkinson's Disease. Chin Med J 2016;129:1053-8.

Access this article online
Quick Response Code:
Website: www.cmj.org
DOI: 10.4103/0366-6999.180513

molecular weight protein 7) subunits of immunoproteasome,[8] replace the proteasome subunits in ubiquitin-proteasome system as "immune" subunits.[12,13] The immunoproteasome enhances the degradation of *Aβ* amyloid in Alzheimer's disease (AD), keep intracellular protein homeostasis as well as provide antigen peptides to *TAP*.[5,8] *TAP* delivers the antigenic peptides from cytoplasm to endoplasmic reticulum by loading on MHC-I molecules.[14] Based on the similar pathogenesis of protein degradation on PD,[15] we hypotheses that *PSMB* and *TAP* gene may be involved in the α-synuclein degradation with more genetic susceptibility to PD by the regulation of immunoproteasome in dopaminergic neurons.

To date, the association of single nucleotide polymorphisms (SNPs) in *PSMB* and *TAP* genes with PD is not clear yet. In this case–control study, we made a linkage analysis on PD population by choosing 15 SNPs of *PSMB* and *TAP* genes which location in coding exons and the average minor allele frequency ≥0.05 [Figure 1b]. The 15 SNPs include rs116076690, rs2071543 in *PSMB8*, rs17587 in *PSMB9*, rs1135216 in *TAP1*, rs2228391, rs2228396, rs241447, rs241448, and rs4148876 in *TAP2*, respectively [Figure 1a]. Among them, several SNPs have been proved to be associated with the immune dysregulation. For example, rs2071543 and rs2228396 were found to be associated to rheumatoid arthritis,[16] rs1135216 and rs2228396 associated to leprosy,[17] rs241447 and rs4148876 associated to spondyloarthritis,[18] rs241448 associated to type 1 diabetes,[19,20] but no information was reported on the association between SNPs of rs17587 and rs116076690 and PD. Thus, using SNPs genotyping analyses, we tried to investigate the PD-susceptible SNPs of *PSMB8*, *PSMB9*, *TAP1*, and *TAP2* genes in the Han Chinese population.

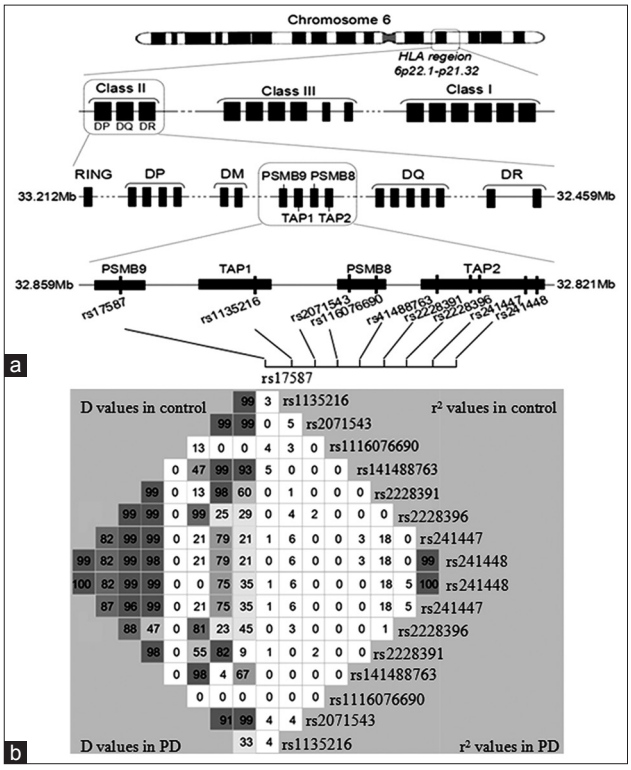

Figure 1: Single nucleotide polymorphisms in proteasome subunit beta type and *TAP* loci. (a) Map of candidate single nucleotide polymorphisms in proteasome subunit beta type and *TAP* genes. (b) Linkage disequilibrium of identified single nucleotide polymorphisms. The *D* or r^2 value was indicated as numbers in each square, and the diminishing was shown as the color from dark to light.

Methods

Study population

Subjects included 542 sporadic PD patients and 674 healthy controls, which were recruited from PD Research Center and Healthcare Center of the First Affiliated Hospital of Sun Yat-Sen University since 2009–2014. All patients were diagnosed by the United Kingdom Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria for PD.[21] The mean age of PD patients was 59.6 ± 17.2 years, of which 320 were male and 222 were female. The mean age of healthy controls was 55.4 ± 12.5 years, of which 462 volunteers were male and 212 volunteers were female. Informed consent was obtained from all subjects who specified self-claimed membership in the Han ethnic population, residency in the Guangdong Province of China with no family migration of four generations. This study followed the ethnic guidelines and was approved by the hospital ethnic committee.

DNA collections and single nucleotide polymorphism genotyping

Venous blood samples (5 ml) were collected from all subjects. DNA was extracted by TRIZOL method (Life Technologies Inc., Grand Island, NY, USA). The reference genomic DNA sequences were obtained from the Genebank database (http://www.ncbi.nlm.nih.gov/Genbank/). Primers for SNPs in *PSMB* and *TAP* were designed using the Primer Premier V5.0 software (Premier Biosoft International Inc., Palo Alto, CA, USA), then synthesized by Invitrogen (Thermo Fisher Scientific Inc., Waltham, MA, USA). The SNaPshot genotyping (Applied Biosystems Co., Foster City, CA, USA) of rs116076690, rs2071543 in *PSMB8*, rs17587 in *PSMB9*, rs1135216 in *TAP1*, and rs2228391, rs2228396, rs241447, rs241448, and rs4148876 in *TAP2* were shown in Supplement Table 1. Capillary electrophoresis was conducted through ABI 3730XL DNA Analyzer (Applied Biosystems Co., Foster City, CA, USA). DNA sequences were analyzed by Mutation Surveyor v2.2 software (SoftGenetics Inc. LLC, State College, PA, USA) and GeneMapper 4.1 software (Applied Biosystems Co., Foster City, CA, USA).[22,23]

Statistical analysis

All the groups were tested for deviation from Hardy–Weinberg equilibrium (HWE) by statistical software SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA). The haplotype analysis was performed by Arlequin 3.5 software program (http://cmpg.unibe.ch/software/arlequin3). The power analysis was calculated to predict the sample size of patient and control groups or evaluate the test by Power and Sample Size Calculation software version 3.0 (http://biostat.mc.vanderbilt.edu/, Vanderbilt University, TN, USA).

Table 1: Information of SNPs in *PSMB9, PSMB8, TAP1,* and *TAP2* on Chinese population

Gene	SNP	Position	Genotype counts (AA/GG/AG)		MA	MAF		*P*	*P* for HWE
			Cases	Controls		Cases	Controls		
PSMB9	rs17587	6:32857313	20/150/372	30/214/430	A	0.175	0.203	0.090	0.608
TAP1	rs1135216	6:32847198	16/170/356	15/210/449	A	0.186	0.178	0.635	0.094
PSMB8	rs116076690	6:32842238	542	674	G	–	–	–	–
	rs2071543	6:32843852	23/146/373	30/152/492	C	0.177	0.157	0.210	0.0001
TAP2	rs2228391	6:32829996	8/106/428	6/124/544	A	0.113	0.101	0.389	0.715
	rs2228396	6:32830030	14/102/426	8/144/520	G	0.120	0.119	0.997	0.575
	rs241447	6:32828974	58/248/236	68/324/282	A	0.336	0.341	0.811	0.072
	rs241448	6:32828908	61/247/234	80/315/279	T	0.340	0.352	0.566	0.534
	rs4148876	6:32829016	1/52/490	3/80/590	C	0.050	0.064	0.160	0.871

MA: Minor alleles; MAF: Minor allele frequencies; *P* values: MAF comparisons in the case and control groups. HWE *P* values were the *P* values of HWE test in the control group. SNPs: Single nucleotide polymorphisms; HWE: Hardy–Weinberg equilibrium; –: Not available.

Data processing and statistical analysis were performed with SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Allele frequency was calculated following the formula (2 × no. of homozygous + heterozygous)/(2 × No. of samples). Differences in genotypic and allelic frequencies between PD patients and healthy controls for each SNP were determined by the Pearson's Chi-square test or Yates' Chi-square with continuity correction. The Cochran–Mantel–Haenszel test and Breslow–Day test were also conducted on SAS to examine the stratified and heterogeneous association between males and females. Logistic regression models were constructed to further assess the association between genotypes and case–control status with adjustments for the covariates.

Results

SNaPshot analysis on *PSMB8, PSMB9, TAP1*, and *TAP2*

Healthy controls were subjected to HWE for rs116076690, rs2071543 in *PSMB8*, rs17587 in *PSMB9*, rs1135216 in *TAP1*, and rs2228391, rs2228396, rs241447, rs241448, and rs4148876 in *TAP2* (df = 1, $P > 0.05$). The standardized measure of linkage disequilibrium (LD) was calculated for all identified SNPs on the patients and controls. There was only one significant LD value between rs241448 and rs241447 in *TAP2* in both patients and controls [$D > 0.8$ and $r^2 > 0.8$, Figure 1b]. The power analysis suggested that the sample size was enough for the susceptibility analysis under the conditions: relative risk (odds ratio [*OR*]) ≥1.5, disorder related gene frequency = 0.30, $\alpha = 0.05$ and $1-\beta = 80\%$. There was no significant difference in genotype or allele frequency between PD patients and healthy controls at all SNPs [Table 1]. The haplotype analysis was performed with the SNPs across *PSMB8*, *PSMB9*, *TAP1*, and *TAP2* genes. The *PSMB9-TAP1-PSMB8-TAP2* haplotype (AA-G-C-AGATC) showed an obvious difference in PD group compared with control group (33.5% vs. 39%; $P < 0.049$), but the difference lost significance after the Bonferroni correction (corrected $P > 0.05$).

Stratified association analysis for rs17587

The sequence result of rs17587 locus was presented in Figure 2. It showed that no significant association was found between genotype frequencies of rs17587 and PD [Table 1]. After stratified analysis based on gender, the frequencies of rs17587 polymorphism were still distributed in HWE. We observed a significant association in females, but nonsignificant association in males. The Breslow–Day test for heterogeneous association between males and females was significant ($P = 0.013$). Base on the dominant model, the female PD population showed a significant ($P = 0.002$) lower odds of A/A + A/G to GG compared with female controls, with $OR = 0.535$ (95% confidence interval = 0.358–0.798), after age adjustment [Table 2]. Furthermore, stratified analysis on age of disease onset did not show any significant difference between PD patients and healthy controls.

Discussion

The main finding of this study was that only rs17587-G/G at *PSMB9* may increase a risk of PD for Chinese female besides other identified SNPs variants in *PSMB8, PSMB9, TAP1,* and *TAP2* genes. *PSMB9* containing rs17587 at exon 4 encodes the immunoproteasome β1 subunit possessing glutamyl peptide hydrolyzing activity in protein degradation and participates in the immune response to MHC Class I molecules.[24] It was reported that AD patients carrying the G/G of rs17587 presented a higher immunoproteasome activity in the brain than that carrying G/A.[25] The immunoproteasome failed to promote protein degradation in *PSMB9* knockout mice.[26,27] Similarly, α-synuclein accumulation accompanying microglial inflammation was found in PD with MHC-II expression, although their relationship between *PSMB9* and PD is still unclear.[2] Here, we found that PD female patients carried a higher frequency of rs17587-G/G versus (A/A + G/A), suggesting rs17587-G/G may be a potential risk loci for female PD. It is worth noting that the female PD patients are usually older more than 50 years and accompanied a decreased estrogen level in menopause.[28] Thus, we inferred that estrogen may be involved in the regulation of *PSMB9* in female PD patients. It was reported that estrogen suppresses microglia and astrocyte activation and modulate the inflammation process by inhibiting the MHC pathways in central nervous system, play a role in the anti-inflammatory to prevent

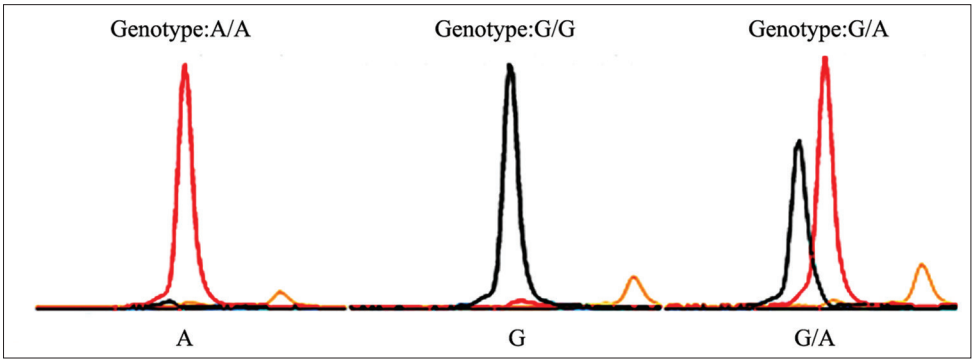

Figure 2: Genotypic analysis of rs17587 at PSMB9 in Chinese population. SNaPshot analysis showed that rs17587 at PSMB9 is heterozygous in Parkinson's disease and healthy control. The rs17587 genotypes contain A/A, G/G, and G/A.

Table 2: Association of the rs17587 variant with Chinese sporadic PD

Sex	Genotype	Subject counts (%)		*OR* (95% *CI*)*	Age adjustment	
		Cases	Controls		*OR* (95% *CI*)	*P*
Male	GG	212 (66.25)	307 (66.45)	–	–	0.952
	AG+AA	108 (33.75)	155 (33.55)	1.009 (0.746–1.364)	1.009 (0.746–1.365)	
Female	GG	160 (72.07)	123 (58.02)	–	–	0.002
	AG+AA	62 (27.93)	89 (41.98)	0.536 (0.359–0.799)	0.535 (0.358–0.798)	
All	GG	430 (0.638)	370 (0.685)	–	–	0.077
	AG+AA	244 (0.362)	170 (0.315)	0.805 (0.634–1.024)	0.805 (0.634–1.024)	

The *OR*s and *P* values were calculated using logistic regression with or without age adjustment in which the subjects with genotype GG served as the reference. 95% *CI* refers to the 95% *CI* of the *OR*. *The Breslow–Day test ($P = 0.013$) for homogeneity of the *OR*s, indicating a heterogeneous association between males and females. *OR*: Odds ratio; *CI*: Confidence interval; PD: Parkinson's disease; –: Not available.

lipopolysaccharide-induced inflammatory mediators released by microglia and also promotes the degradation of aggregated protein.[29,30] For example, the menopausal women face an increased risk of AD, which is closely related with cleaning of amyloid-β peptides aggregates.[31,32] Estrogen plays a role in the improvement of motor bradykinesia in postmenopausal women in a randomized pilot trial of PD.[33] It is interest that the G → A substitution of rs17587 in exon 4 of *PSMB9* may lead to alteration of arginine to histidine, potentially affecting the immunoproteasome activity.[25,34] Due to the female menopausal PD cases carrying higher frequency of rs17587-G/G, we here inferred that *PSMB9* and estrogen may play an important role on PD development under the modification of immunoproteasome and protein degradation.

PSMB8 encodes β2 subunit of immunoproteasome and shares a similar mechanism on the protein degradation and immune response like *PSMB9*.[35] Mutation of *PSMB8* was reported to be closely related to lipodystrophy and autoinflammatory disorder.[36] In our study, we found no significant differences of rs116076690 and rs2071543 polymorphisms in *PSMB8* between PD and controls. A larger sample for further investigation on *PSMB8* could help to disclose the right relationship with PD.

TAP1 and *TAP2* belong to an ATP-binding cassette family, transporting small antigenic peptides into the lumen of the endoplasmic reticulum.[5] In *TAP* knockout cells, the antigenic peptides were not effectively transported into the endoplasmic reticulum to abolish endogenous antigen presentation.[37,38] Bullido *et al*. reported that rs241448 in *TAP2* is associated with AD.[39] However, we found no significant differences in *TAP* polymorphisms between PD patients and healthy controls in the Han Chinese population after screening for the genotype and allele distribution of rs1135216, rs4148876, rs2228391, rs2228396, rs241447, and rs241448 in *TAP1* and *TAP2*. Stratified analysis based on gender or onset age did not reveal any significant variation between PD patients and control.

The *PSMB8*, *PSMB9*, *TAP1*, and *TAP2* genes at chromosome 6 are located at an adjacent range from 32.859 Mb and 32.821 Mb and involved in the protein degradation and antigen presentation,[40] suggesting a possible LD of the above SNPs with PD patients. However, our result showed that a significant LD value was only calculated for rs241448 and rs241447 in *TAP2*, but no significant linkage to PD patients under the *TAP2* haplotypic analysis. The *PSMB9-TAP1-PSMB8-TAP2* haplotype (AA-G-C-AGATC) was also found to be a slight association with PD, but no significance after the Bonferroni correction. In conclusion, we found that Chinese females carrying rs17587-G/G of *PSMB9* may increase a risk for developing PD, but other SNPs and their haplotypes seem no risk effect on PD. Further investigation recruited more patients may need to uncover any association between SNPs in *HLA* Class II with PD population to explore its immune dysfunction on the pathogenesis of PD.

In summary, we evaluated the association of SNPs in *PSMB* and *TAP* with PD and found: (i) no linkage was revealed

between SNPs at *PSMB8*, *TAP1*, and *TAP2* and PD (ii) the rs17587-G at *PSMB9* may develop an increased risk of PD for Chinese female population.

Supplementary information is linked to the online version of the paper on the Chinese Medical Journal website.

Financial support and sponsorship

This work was supported by research grants from the National High Technology Research and Development Program of China (grant No. 2007AA02Z460), the State Key Development Program for Basic Research of China (grant No. 2011CB510000), the National Natural Science Foundation of China (grant No. 81271428, 81471292, U1503222, and 81430021) and the key point Science Foundation of Guangdong of China (No. 2015A030311021), a grant supported by technology project of Guangzhou (No. 20151260) and a grant supported by assisting research project of science and technology for Xinjiang (No. 201591160).

Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: Primers for direct DNA sequencing of SNPs covering 1000 kb flanking rs3129882 in *HLA-DRA* and primers for SNaPshot genotyping of rs116076690, rs2071543 in *PSMB8*, rs17587 in PSMB9, rs1135216 in TAP1, and rs2228391, rs2228396, rs241447, rs241448, and rs4148876 in TAP2

Type	SNPs	Primer sequence
PCR reaction	rs116076690F	CCACCTTCTTATCCCAGCCACAG
	rs116076690R	CCTTGTCCTCACCCAGGCTGTA
	rs17587F	GGTGACTGTTGACTCCCTCCTGAC
	rs17587R	CCAGCTCCTGGAACAGCACACT
	rs2071543F	TCCCTAGGGGCTTCCCTACTGC
	rs2071543R	TCGATCTGTGGCTTTCGCTTTC
	rs1135216F	AGGGCACTGGTGGCATCATC
	rs1135216R	CTCATCTTGGCCCTTTGCTCTG
	rs241448SR	TGCAGAAGCTTGCCCAGCTC
	rs241447SR	TTGGTGATTGCTCACAGGCTGCAG
	rs4148876SR	TTTTTTTCAGCTGCAGGACTGGAATTCC
	rs1135216SR	TTTTTTTTTGGCCCTTTGCTCTGCAGAGGTAG
	rs116076690SR	TTTTTTTTAGGCTGTACTATCTGCGAAATGGAGAA
	rs2228396SR	TTTTTTTTTTTTTTTCCGGTTCTGTGAGGAACAACATT
	rs2071543SF	TTTTTTTTTTTTTTTTTTTTTTTTGCTTCCCTACTGCCCCGACCT
	rs2228391SR	TTTTTTTTTTTTTTTTTTTTTTTTGCAGAGCTGCGAAG (G/A) TGATAAG GTG
Extension reaction	rs17587SR	TTTTTTTTTTTTTTTTTTTTTTTTTGCTGAACCAGAGAGTG (G/A) ACAGT AGATG

SNPs: Single nucleotide polymorphisms; *HLA*: Human leukocyte antigen; PCR: Polymerase chain reaction.