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Single nucleotide polymorphisms in the non-coding region of *STIM1* gene are associated with Parkinson disease risk in Chinese Han population

Danning Lou, MD[a], Jun Wang, MD[b], Xiaohang Wang, MD[c,*]

Abstract

The stromal interaction molecule 1 (*STIM1*) gene contributes essentially to Ca^{2+} transport, thus it is functionally related to neurodegenerative disorders. The objective of this study was to investigate the correlation between single nucleotide polymorphisms (SNP) in the non-coding region of *STIM1* gene and the risk for Parkinson disease (PD) in a Chinese Han population.

In a cohort composed of 300 PD patients and 300 healthy individuals from a Chinese Han population, we analyzed genotypes for five novel SNPs, rs7934581, rs3794050, rs1561876, rs3750994 and rs3750996 in the non-coding region of *STIM1* gene. The levels of STIM1 protein in plasma of these subjects were also assessed by enzyme-linked immunosorbent assay (ELISA).

We found that the SNPs of *STIM1* gene rs7934581, rs3794050, rs1561876, and rs3750996 were associated with increased PD risk, while rs3750994 SNP was not. An increased risk of PD was observed in subjects with the TAAG and TGAG haplotypes of rs7934581, rs3794050, rs1561876, rs3750996. Moreover, PD risk was significantly elevated only in subjects with age ≥60 years or females who carry the *STIM1* rs3794050 minor allele. There was a significant difference in plasma STIM1 protein levels between subjects with different genotypes of *STIM1* rs7934581, rs3794050, rs1561876, and rs3750996.

STIM1 gene rs7934581, rs3794050, rs1561876, rs3750996 SNPs are associated with increased PD risk, and its mechanism may be related to abnormal *STIM1* gene expression.

Abbreviations: 3'UTR = 3' untranslated region, AD = Alzheimer's disease, CRAC = calcium-release-activated calcium, CRP = C-reactive protein, ELISA = Enzyme-linked immunosorbent assay, ESR = erythrocyte sedimentation rate, LD = linkage disequilibrium, MAF = minor allele frequency, OR = odds ratio, PD = Parkinson disease, qRT-PCR = real-time quantitative PCR, SNPs = single nucleotide polymorphisms, STIM1 = stromal interaction molecule 1.

Keywords: haploid, Parkinson disease, single nucleotide polymorphism, stromal interaction molecule 1

1. Introduction

Parkinson disease (PD) is a common neurological disease that mainly occurs in elders. It seriously affects not only the social functions and life quality of patients, but also tremendously increases the social burden.[1,2] With the aging population in China, the number of PD patients is gradually increased, which has brought a heavy burden to the society.[3]

STIM1 is a type I transmembrane protein that mainly located to the endoplasmic reticulum, and around 20% extended to the plasma membrane.[4] It was reported that STIM1 functions as a calcium sensor in the endoplasmic reticulum, via its near N-terminus EF hand domain which is sensitive to Ca^{2+}.[5,6] Previous studies have shown that the expression of EF-hand mutants of *STIM1* can constitutively activate the calcium-release-activated calcium (CRAC) channel, while the CRAC channel is the only Ca^{2+} entry pathway in non-excitable cells.[5] It has been well documented that Ca^{2+} imbalance is involved in the development of Alzheimer disease (AD).[7–10] Importantly, STIM1 contributes essentially to Ca^{2+} transport, thus it is functionally related to neurodegenerative disorders. For instance, a significant reduced level of STIM1 protein was found in brain tissue of patients with AD.[11] The *STIM1* deficit is associated with AD and triggers SH-SY5Y cell death by up-regulating L-type voltage-operated Ca^{2+} entry.[11] In the PD patients, a complex formed by STIM1 and transient receptor potential channel 1 (TRPC1) inhibits CaV1.3 channel, which leads to disruption of neuronal Ca^{2+} homeostasis, and eventually causes the development of PD symptoms.[12,13]

The gene coding for STIM1 protein in humans (*STIM1* gene) is located on 11p15.4. In the present study, we analyzed the correlation between single nucleotide polymorphisms (SNP) in *STIM1* gene and the risk for PD. Five novel SNPs, namely rs7934581, rs3794050, rs1561876, rs3750994, rs3750996

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[a] Department of Neurology, The Affiliated Hospital of Hangzhou Normal University, [b] Binjiang clinic, Zhejiang Chinese Medical University, [c] Department of Neurology, First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China.

[*] Correspondence: Xiaohang Wang, Department of Neurology, First Affiliated Hospital of Zhejiang Chinese Medical University, 54 Youdian Road, Hangzhou, Zhejiang, China (e-mail: wangxiaohang1978@163.com).

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Table 1

Characteristics of *STIM1* gene SNPs.

SNP	Chromosome	Variation	MAF*	Location
rs7934581[22]	11:4090803	C > T	0.1714	intron
rs3794050[22,24]	11:4090670	G > A	0.2095	intron
rs1561876[23]	11:4092165	A > G	0.2718	3'UTR
rs3750994[23]	11:4092240	T > G	0.1714	3'UTR
rs3750996[23]	11:4091970	A > G	0.2048	3'UTR

MAF = Minor Allele Frequency in Southern Han Chinese, SNP = single nucleotide polymorphism, UTR = Untranslated Region.

(Table 1), that locate in non-coding regions and with a minor allele frequency (MAF) >0.05 were selected to investigate the regulation of *STIM1* gene expression by these SNPs and its correlation with PD susceptibility.

2. Materials and methods

2.1. Ethical aspects

This study was approved by the corresponding ethics committees of the Affiliated Hospital of Hangzhou Normal University and the First Affiliated Hospital of Zhejiang Chinese Medical University. This study was carried out in accordance with the World Medical Association *Declaration of Helsinki*. All the participants signed their written informed consent after full explanation of the purpose and procedure of the study.

2.2. Study participants

A total of 300 neurological PD patients who fulfilled the standardized diagnostic criteria for PD (MDS clinical diagnostic criteria for Parkinson disease[14]) were recruited from October 2015 to October 2018 in the present study. Another total of 300 "age- and gender-matched" healthy Chinese Han individuals were also recruited as control. All control subjects were examined and confirmed without any neurodegenerative disorders by neurologists from the Affiliated Hospital of Hangzhou Normal University and the First Affiliated Hospital of Zhejiang Chinese Medical University. Demographic information for all participants was recorded, and the exclusion criteria include:

(1) patients with gouty arthritis;
(2) patients with severe liver, kidney, and other organ damage;
(3) patients with former cerebrovascular disease, encephalitis, taking antipsychotic drugs, family history of Parkinsonism, and secondary forms of Parkinsonism. General clinical data of PD group and control group are shown in Table 3.

Table 2

Primer sequence for *STIM1*gene SNPs.

SNP	Primer sequence
rs7934581	Forward primer: 5'-GTGTTAGCTTGAGTCCCAGGAA-3'; Reverse primer: 5'-CACAGGCGGCATCCTCAATA-3'.
rs3794050	Forward primer: 5'-CCTGGCCCAGACTGGATAAT-3'; Reverse primer: 5'-GAACCTACAGCCTGCTCCG-3'.
rs1561876	Forward primer: 5'-CCTCTGGGGTTCAGCTTCTG-3'; Reverse primer: 5'-CTGTGAGTGGGGTGGAACAG-3'.
rs3750994	Forward primer: 5'-GCCTCAGATCTGTTCCACCC-3'; Reverse primer: 5'-GGCTCCTTCTGACCCACATC-3'.
rs3750996	Forward primer: 5'-ACTGTACATACCTGCCCCCT-3'; Reverse primer: 5'-GAGCTGAGGGAACAGCAACT-3'.

2.3. Genetic analyses

The 10 ml of venous blood was collected from all subjects with fasting, 2 ml for genotyping and 3 ml for plasma. QIAamp blood DNA isolation kit (Qiagen, Crawley, UK) was used to extract genomic DNA from peripheral blood. Polymerase Chain Reaction (PCR) was performed using the nucleotide sequence of primers for *STIM1* gene SNPs as shown in Table 2. PCR was conducted in a total volume of 25 μl, containing 100 ng genomic DNA, 2.5 μl 10 × buffer, 1.5 μl Mg^{2+} (25 mM), 0.5 μl dNTP (10 mM), 0.5 μl Taq (5U/μl), 0.5 μl of each primer (10 μM), and added sterile water to a final volume of 25 μl. PCR conditions were: initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 30 seconds, finally followed by an extension at 72°C for 7 minutes. The amplified target fragment was electrophoresed in a 1% agarose gel with a voltage of 90 V for 30 minutes, extracted by QiaQuick gel extraction kit (Qiagen, Valencia, CA), and then genotyped by Sanger sequencing. SNPs genotype were finally verified based on the sequencing result.

2.4. Real-time Quantitative PCR (qRT-PCR)

For both cases and controls, 3 ml of whole blood was centrifuged at 3000 rpm for 15 minutes, and the isolated plasma was stored at −20°C until further use. Isolation of peripheral blood mononuclear leukocytes (PMBC) from peripheral blood was performed according to Lou et al.[15] Total RNA was extracted from PBMC using the RNeasy mini kit (Qiagen, Valencia, CA), and cDNA was reverse transcribed using the RevertAid First-Strand cDNA Synthesis kit (Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. The following primers were used for the amplification of *STIM1* mRNA: For., 5'-CCC CAA CCC TGC TCA CTT C-3'; Rev., 5'-GCT GGC GGT CAC TCA TGT G-3', and *β-actin* For., 5'-TGG CAC CAC ACC TTC TAC AAT-3'; Rev., 5'-AGA GGC GTA CAG GGA TAG AGC A-3'. The qRT-PCR was conducted using standard SYBR Green RT-PCR kit (Takara, Dalian, China), according to the manufacturer's instructions. An Applied Biosystems 7500 real-time PCR system (Applied Biosystems) was adopted under the following cycling conditions: initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. The expression of *STIM1*

Table 3

Clinical characteristics of PD patients and healthy controls.

Variation	PD (n = 300)	Control (n = 300)	*P*
Age (year, mean±SD)	65.5 ± 10.5	67.0 ± 9.5	.067
Gender [n(%)]			
Men	158 (52.67%)	152 (50.67%)	.624
Women	142 (47.33%)	148 (49.33%)	
Course of disease (year, mean±SD)	6.9 ± 3.8		
Hoehn and Yahr score	2.4 ± 1.2		
UPDRS-III	22.1 ± 8.1		

PD = Parkinson Disease, SD = standard deviation, UPDRS = Unified Parkinson Disease Rating Scale.

mRNA was normalized to *β-actin* using the comparative $2^{-\Delta\Delta Ct}$ method, each sample was repeated measured for three times.

2.5. Enzyme-linked immunosorbent assay (ELISA)

The remaining 5 ml of whole blood was centrifuged at 3000 rpm for 30 minutes to isolate the plasma and then stored at −80°C for further studies. The STIM1 protein concentration in the plasma was assayed using double antibody sandwich method by STIM1 ELISA kit (Cat. No. ABIN824174, Cusabio Biotech, China) according to the manufacturer's kit instructions. Each blood sample was repeated 3 times, and the absorbance was measured at a wavelength of 450 nm to calculate the STIM1 protein concentration using a standard curve.

2.6. Statistical analysis

In the current study, all statistical analyses were conducted using SPSS 21.0 (IBM, Chicago, IL). The categorical variables were expressed as a percentage [n(%)], and the statistical analysis was carried out using the χ^2 test. Testing for Hardy-Weinberg equilibrium in controls was performed using the χ^2 test. Normally distributed continuous variables were expressed as mean ± SD, and the correlation between *STIM1* SNPs and PD risk was determined based on the distribution of allele frequencies and genetic models (additive model, dominant model and recessive model). Unconditional logistic regression analysis was used to calculate odds ratio (OR) and 95% confidence interval (CI), with adjustment to age and gender factors. Linkage disequilibrium (LD) among *STIM1* SNPs was analyzed using Haploview 4.2 software. All statistical analyses were two-tailed, with $P < .05$ was considered statistically significant.

3. Result

3.1. Clinical characteristics of PD patients and control subjects

A total of 600 Chinese Han participants were recruited in the current study, including 300 PD patients and 300 control participants. The basic clinical characteristics of both cases and controls are shown in Table 3. For PD patients, the course of disease ranges from 1 to 20 years, with a mean of 6.9 ± 3.8 years; Hoehn and Yahr score 1 to 5 points, with an average of 2.4 ± 1.2 points; and UPDRS-III 2 to 54 points, with an average of 22.1 ± 8.1 points. No significant difference in age and gender were observed between cases and controls (both $P > .05$).

3.2. Correlation analysis between STIM1gene SNPs and PD risk

The genotype frequencies of *STIM1* gene SNPs rs7934581, rs3794050, rs1561876, rs3750994, and rs3750996 were in Hardy-Weinberg equilibrium ($P > .05$) for both groups. We found that the MAF of the rs7934581 was increased to 21.67% in PD patients compared to 17.00% in the control (adjusted OR = 1.154, 95% CI: 1.001–1.313, $P = .048$). And the MAF of the rs3794050 was significantly increased to 27.50% in PD patients compared to 19.67% in the control (adjusted OR = 1.229, 95% CI: 1.081–1.383, $P = .002$). Further, PD risk was significantly elevated when carrying the G allele at the rs1561876 (adjusted OR = 1.167, 95% CI: 1.033–1.311, $P = .013$). The MAF of rs3750994 was not statistically differ between PD patients and control group (adjusted OR = 1.039, 95% CI: 0.890–1.196, $P = .655$). We also found that subjects carrying the G allele of the rs3750996 had a 1.180-fold higher risk of PD than the A allele carrier (95% CI: 1.034–1.332, $P = .014$) (Table 4).

3.3. STIM1 gene haplotype analysis

The linkage disequilibrium (LD) and haplotype analysis (Table 5, Fig. 1) of *STIM1* gene rs7934581, rs3794050, rs1561876, rs3750996 SNPs were analyzed by Haploview 4.2 software. The results showed that there are 5 haplotypes in the *STIM1* gene rs7934581, rs3794050, rs1561876, and rs3750996 SNPs. Further analysis revealed that subjects with rs7934581, rs3794050, rs1561876, rs3750996 SNP TAAG haplotype and TGAG haplotype had an increased risk of PD (OR = 1.365, 95% CI: 1.118–1.627, $P = .002$; OR = 1.332, 95% CI: 1.058–1.617, $P = .015$), however, other haplotypes were not associated with PD risk ($P > .05$).

3.4. Correlation between STIM1gene SNPs and the course of PD patients

Further, we analyzed the association of *STIM1* gene rs7934581, rs3794050, rs1561876, rs3750994, rs3750996 SNPs with the course of PD patients, Hoehn and Yahr score, UPDRS-III, and did not observe any correlation between *STIM1* gene SNPs and the course of disease, Hoehn and Yahr score, UPDRS-III ($P > .05$) (Figs. 2–4).

3.5. Multiple comparisons for correlations between STIM1 gene SNPs and PD risk

We then included different ages (<60 or ≥60 years old), gender (men or women) in the comparisons as confounding factors, and found that there were no significance between *STIM1* rs7934581, rs1561876, rs3750994, rs3750996 minor allele carriers and PD risk no matter different ages (<60 or ≥60 years old) and gender (men or women) of the subjects ($P > .05$) (Table 6, and Tables 8–10). However, the risk of PD was significantly increased only in subjects aged ≥ 60 years or females who carries the *STIM1* rs3794050 minor allele carriers (OR = 1.411, 95% CI: 1.135–1.742, $P = .002$; OR = 1.358, 95% CI: 1.060–1.725, $P = .015$) (Table 7).

3.6. Association of STIM1 gene SNPs with STIM1 mRNA level

To clarify effects of *STIM1* gene SNPs on *STIM1* gene transcription, we detected the *STIM1* mRNA relative to β-actin levels in PMBC isolated from all participants by RT-PCR. We found that the differences in *STIM1* mRNA levels were significant in subjects with rs7934581, rs3794050, rs1561876, and rs3750996 variants ($P < .05$; Fig. 5A, B, C, E), but not in subjects with *STIM1* rs3750994 ($P = .940$; Fig. 5D).

3.7. Association of STIM1 gene SNP with plasma STIM1 protein level

To further analyze the *STIM1* gene expression, we used ELISA to measure STIM1 protein levels in plasma samples. We found that the differences in plasma STIM1 protein level were significant in subjects with *STIM1* rs7934581, rs3794050, rs1561876, and rs3750996 genotypes ($P < .05$; Fig. 6A, B, C, E), but not in subjects with STIM1 rs3750994 genotype ($P = .488$; Fig. 6D).

Table 4

***STIM1*gene SNPs genotypes and allele frequency.**

	PD (n = 300)	Control (n = 300)	HWE p	Adjusted OR (95%)	*P*
rs7934581					
CC	201 (67.00%)	211 (70.33%)		1.000 (REFERENCE)	
CT	68 (22.67%)	76 (25.33%)	0.170	0.968 (0.778–1.181)	.821
TT	31 (10.33%)	13 (4.33%)		1.444 (1.098–1.737)	**.010**
Additive				1.025 (0.900–1.171)	.752
Dominant				1.079 (0.901–1.278)	.428
Recessive				1.456 (1.112–1.741)	**.008**
C	470 (78.33%)	498 (83.00%)		1.000 (REFERENCE)	
T	130 (21.67%)	102 (17.00%)		1.154 (1.001–1.313)	**.048**
rs3794050					
GG	165 (55.00%)	198 (66.00%)		1.000 (REFERENCE)	
GA	105 (35.00%)	86 (28.67%)	0.108	1.209 (1.007–1.437)	**.041**
AA	30 (10.00%)	16 (5.33%)		1.435 (1.068–1.774)	**.018**
Additive				1.100 (0.956–1.272)	.193
Dominant				1.253 (1.061–1.471)	**.008**
Recessive				1.338 (1.005–1.635)	**.046**
G	435 (72.50%)	482 (80.33%)		1.000 (REFERENCE)	
A	165 (27.50%)	118 (19.67%)		1.229 (1.081–1.383)	**.002**
rs1561876					
AA	145 (48.33%)	162 (54.00%)		1.000 (REFERENCE)	
AG	102 (34.00%)	109 (36.33%)	0.098	1.024 (0.843–1.235)	.874
GG	53 (17.67%)	29 (9.67%)		1.368 (1.090–1.651)	.007
Additive				1.059 (0.916–1.231)	.472
Dominant				1.120 (0.948–1.322)	.191
Recessive				1.355 (1.094–1.607)	**.006**
A	392 (65.33%)	433 (72.17%)		1.000 (REFERENCE)	
G	208 (34.67%)	167 (27.83%)		1.167 (1.033–1.311)	**.013**
rs3750994					
TT	201 (67.00%)	207 (69.00%)		1.000 (REFERENCE)	
TG	84 (28.00%)	79 (26.33%)	0.079	1.046 (0.861–1.251)	.691
GG	15 (5.00%)	14 (4.67%)		1.050 (0.660–1.452)	.949
Additive				1.015 (0.892–1.159)	.869
Dominant				1.047 (0.873–1.241)	.662
Recessive					
T	486 (81.00%)	493 (82.17%)		1.000 (REFERENCE)	
G	114 (19.00%)	107 (17.83%)		1.039 (0.890–1.196)	.655
rs3750996					
AA	174 (58.00%)	195 (65.00%)		1.000 (REFERENCE)	
AG	94 (31.33%)	89 (29.67%)	0.173	1.089 (0.900–1.304)	.400
GG	32 (10.67%)	16 (5.33%)		1.414 (1.068–1.729)	**.017**
Additive				1.060 (0.925–1.221)	.427
Dominant				1.157 (0.977–1.361)	.093
Recessive				1.373 (1.045–1.662)	**.024**
A	442 (73.67%)	479 (79.83%)		1.000 (REFERENCE)	
G	158 (26.33%)	121 (20.17%)		1.180 (1.034–1.332)	**.014**

CI = confidence interval, HWE = Hardy-Weinberg equilibrium, OR = odds ratio, PD = Parkinson disease.

4. Discussion

Here, we reported that PD risk was significantly higher in carriers with *STIM1* gene rs7934581, rs3794050, rs1561876, rs3750996 SNPs. Further, s7934581, rs3794050, rs1561876, rs3750996 SNPs were associated with *STIM1* gene expression level, both *STIM1* mRNA in the PMBC and plasma STIM1 protein were decreased in subjects carrying these minor alleles compared to those carrying major allele homozygous. It was likely that *STIM1* gene rs7934581, rs3794050, rs1561876, rs3750996 SNPs cause abnormal *STIM1* gene expression, which leads to elevated risk for PD.

Table 5

Linkage disequilibrium and haplotype analysis for alleles of *STIM1* gene rs7934581, rs3794050, rs1561876, rs3750996 SNP.

Haplotype*	PD (n = 300)	Control (n = 300)	χ^2	OR (95%CI)	*P*
CGAA	159 (53.00%)	190 (63.33%)		1.000 (REFERENCE)	
TAAG	74 (24.67%)	45 (15.00%)	9.159	1.365 (1.118–1.627)	.002
TGAG	54 (18.00%)	35 (11.67%)	5.895	1.332 (1.058–1.617)	.015
CGGA	9 (3.00%)	18 (6.00%)	1.061	0.732 (0.373–1.211)	.303
CGAG	4 (1.33%)	12 (4.00%)	1.851	0.549 (0.181–1.175)	.174

CI = confidence interval, OR = odds ratio, PD = Parkinson disease.
* rs7934581, rs3794050, rs1561876, rs3750996.

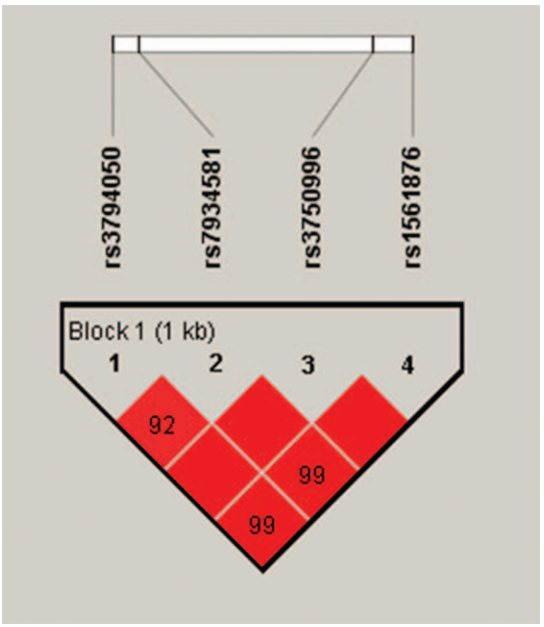

Figure 1. Linkage disequilibrium of *STIM1* gene rs7934581, rs3794050, rs1561876, rs3750996 SNP.

PD is a neurodegenerative disease. It has been well documented that dysfunction of dopaminergic (DA) neurons in the substantia nigra is the basis of main motor symptoms of the disease, but the mechanism is uncertain.[13] More recently, a growing body of evidence suggests that Ca^{2+} plays a critical role in the pathogenesis of PD. It has been recognized that Ca^{2+}-induced excitotoxicity is one of importantly underlying mechanisms, which causes cell death of DA neurons.[16,17] In addition, STIM1 mediated disruption of Ca^{2+} signaling is also associated with the development of human cancers.[18,19] Knockdown of STIM1 gene by siRNA affects Ca^{2+} influx, prevents transport of transcription factors and activates inflammatory COX-2 gene.[20,21]

In the present study, we selected 5 SNPs in the non-coding region of *STIM1* gene, in which rs7934581 and rs3794050 are located in the intron region, rs1561876, rs3750994, and rs3750996 are located in the 3'UTR region of the STIM1 gene. The rs7934581, rs3794050, rs1561876, rs3750996 SNPs influence the binding site of transcription factors,[22] while the G-C haplotype formed by rs3750996/ rs3750994 was significantly associated with higher levels of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).[23] Furthermore, these SNPs are prevalent in the Han Chinese of southern China (Table 1). Therefore, it is of great clinical significance to analyze the correlation between these SNPs and the risk of PD. In the current study, we found that *STIM1* gene rs7934581, rs3794050, rs1561876, rs3750996 SNPs were associated with increased risk of PD. We further analyzed *STIM1* mRNA levels in PMBC and STIM1 protein levels in plasma, and found that *STIM1* gene SNPs of rs7934581, rs3794050, rs1561876, and rs3750996 were significantly associated with *STIM1* mRNA and STIM1 protein levels, and *STIM1* mRNA and STIM1 protein levels were lower in a subjects carrying minor alleles, whose PD risk was higher. Based on these observations, we speculate that the rs7934581 and rs3794050 loci are located in the intron region of the *STIM1* gene, and the rs1561876 and rs3750996 loci are located in the 3'UTR region of the *STIM1* gene. It has been shown that these four SNPs alter the binding site of the transcription factors, thereby, affect the efficiency of

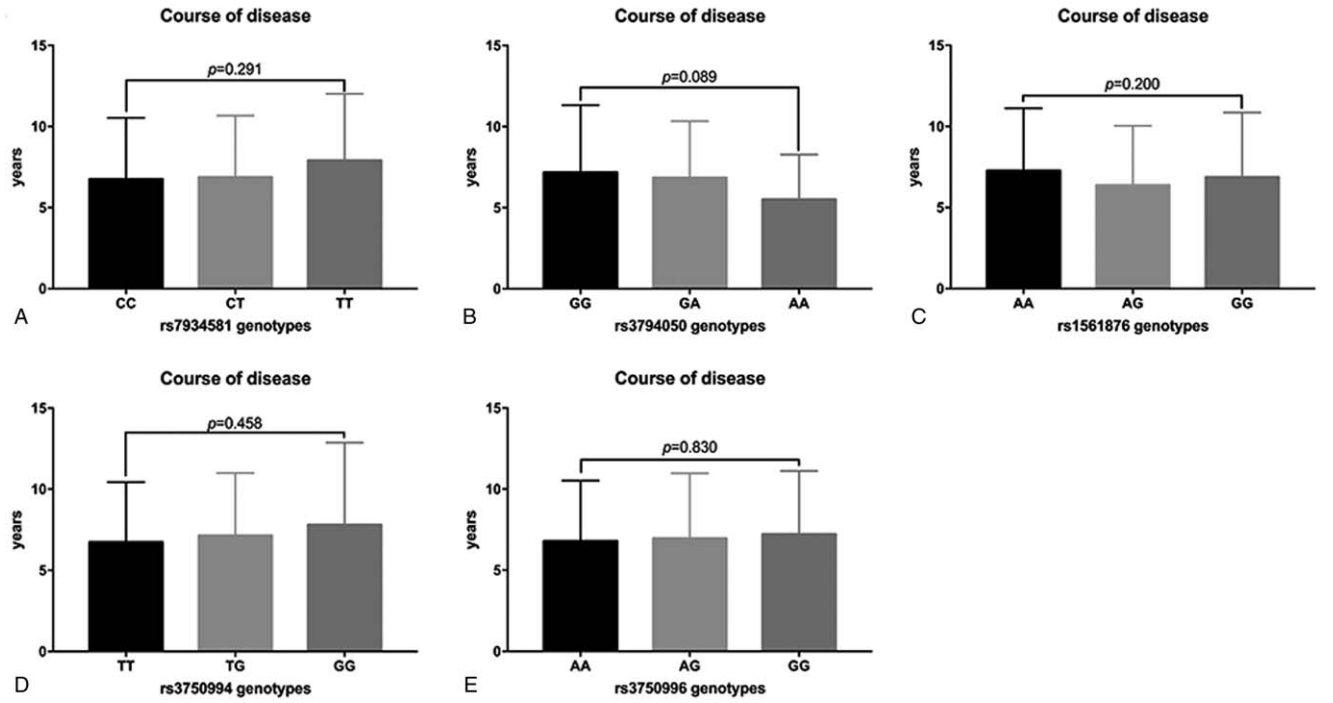

Figure 2. Association of *STIM1*gene SNPs with the course of PD patients. There was no correlation between different genotypes of *STIM1* gene rs7934581 (A), rs3794050 (B), rs1561876 (C), rs3750994 (D), rs3750996 (E) and the course of PD patients.

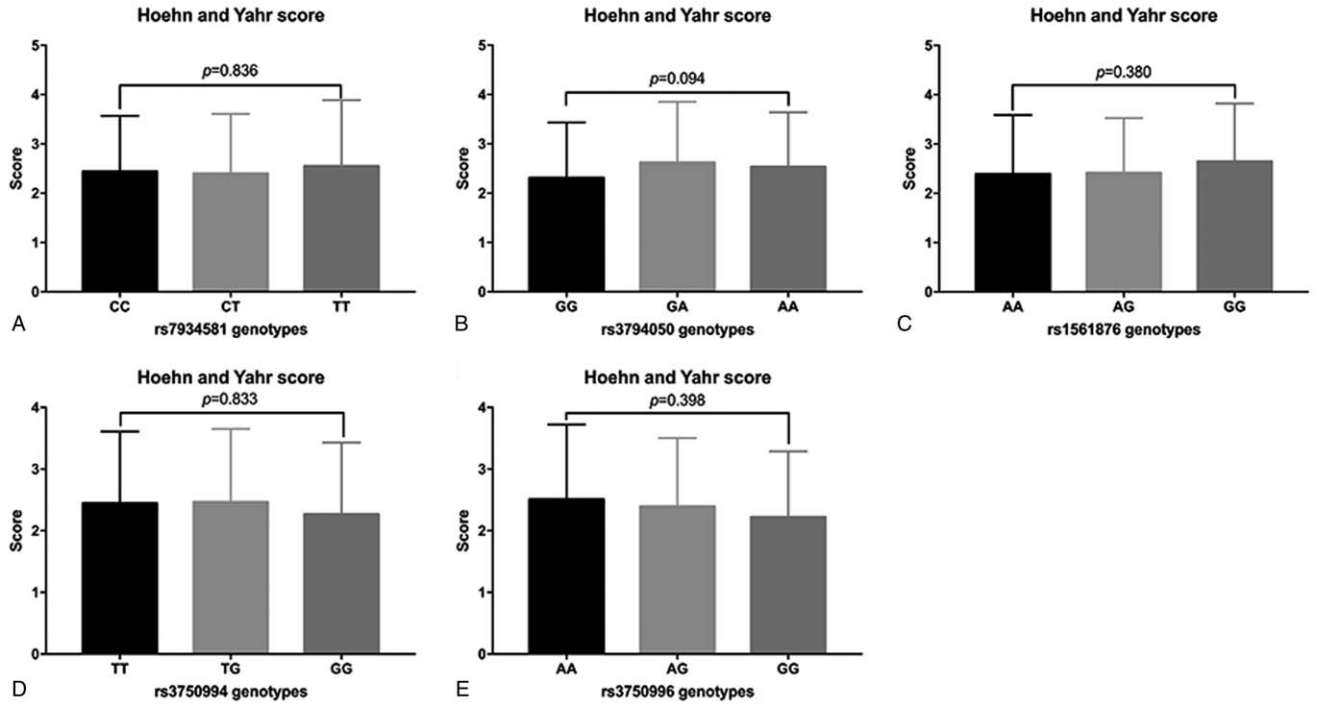

Figure 3. Association of *STIM1* gene SNPs with the Hoehn and Yahr score. There was no correlation between different genotypes of *STIM1* gene rs7934581 (A), rs3794050 (B), rs1561876 (C), rs3750994 (D), rs3750996 (E) and the Hoehn and Yahr score.

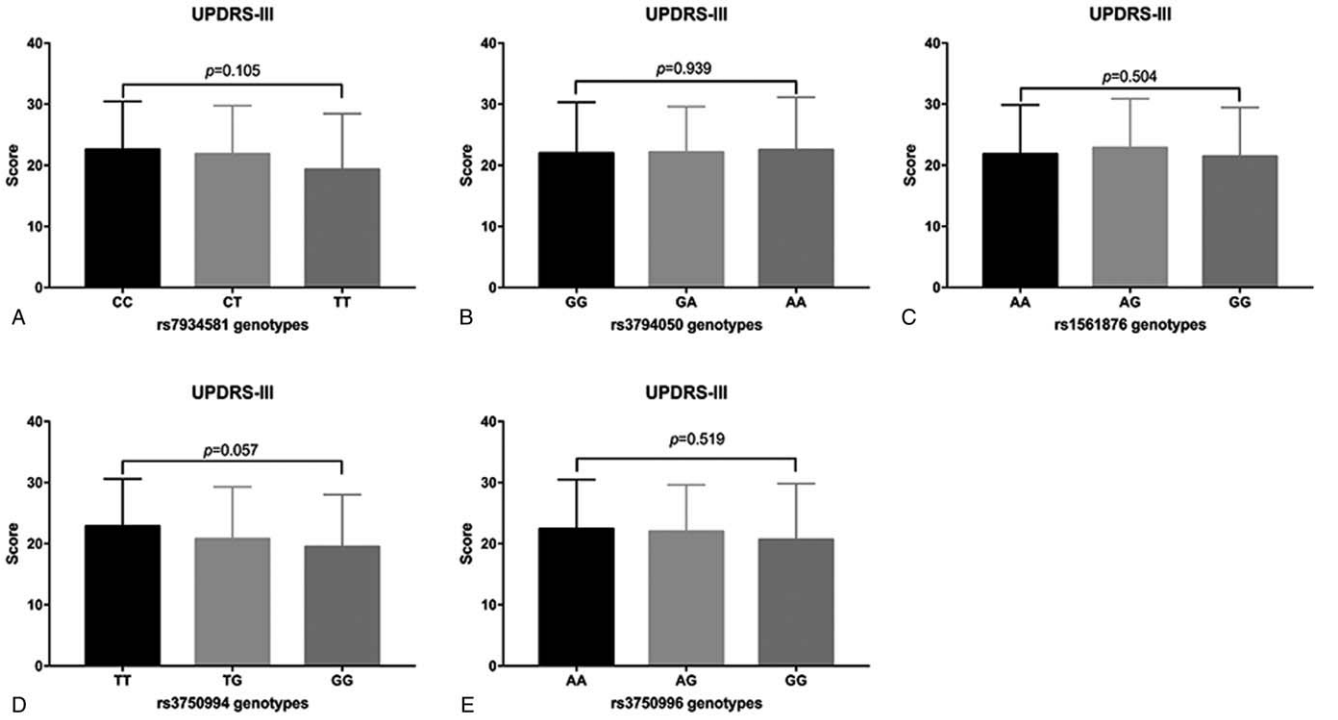

Figure 4. Association of *STIM1* gene SNPs with the UPDRS-III. There was no correlation between different genotypes of *STIM1* gene rs7934581 (A), rs3794050 (B), rs1561876 (C), rs3750994 (D), rs3750996 (E) and the UPDRS-III.

Table 6

Multiple comparisons for correlations between *STIM1* rs7934581 SNPs and PD risk.

	PD (n = 300)	Control (n = 300)	Adjusted OR (95%)	*P*
Age (year)				
<60				
CC	79 (69.30%)	61 (69.32%)	1.000 (REFERENCE)	
CT+TT	35 (30.70%)	27 (30.68%)	1.001 (0.524–1.913)	.998
≥60				
CC	122 (65.59%)	150 (70.75%)	1.000 (REFERENCE)	
CT+TT	64 (34.41%)	62 (29.25%)	1.132 (0.894–1.409)	.319
Gender				
Men				
CC	99 (62.66%)	108 (71.05%)	1.000 (REFERENCE)	
CT+TT	59 (37.34%)	44 (28.95%)	1.198 (0.941–1.493)	.148
Women				
CC	102 (71.83%)	103 (69.59%)	1.000 (REFERENCE)	
CT+TT	40 (28.17%)	45 (30.41%)	0.946 (0.704–1.233)	.772

CI = confidence interval, HWE = Hardy-Weinberg equilibrium, OR = odds ratio, PD = Parkinson disease.

Table 7

Multiple comparisons for correlations between *STIM1* rs3794050 SNPs and PD risk.

	PD (n = 300)	Control (n = 300)	Adjusted OR (95%)	*P*
Age (year)				
<60				
GG	71 (62.28%)	57 (64.77%)	1.000 (REFERENCE)	
GA+AA	43 (37.72%)	31 (35.23%)	1.048 (0.794–1.348)	.828
≥60				
GG	94 (50.54%)	141 (66.51%)	1.000 (REFERENCE)	
GA+AA	92 (49.46%)	71 (33.49%)	1.411 (1.135–1.742)	.002
Gender				
Men				
GG	95 (60.13%)	103 (67.76%)	1.000 (REFERENCE)	
GA+AA	63 (39.87%)	49 (32.24%)	1.172 (0.924–1.463)	.200
Women				
GG	70 (49.30%)	95 (64.19%)	1.000 (REFERENCE)	
GA+AA	72 (50.70%)	53 (35.81%)	1.358 (1.060–1.725)	.015

CI = confidence interval, HWE = Hardy-Weinberg equilibrium, OR = odds ratio, PD = Parkinson disease.

Table 8

Multiple comparisons for correlations between *STIM1* rs1561876 SNPs and PD risk.

	PD (n = 300)	Control (n = 300)	Adjusted OR (95%)	*P*
Age (year)				
<60				
AA	56 (49.12%)	45 (51.14%)	1.000 (REFERENCE)	
AG+GG	58 (50.88%)	43 (48.86%)	1.036 (0.800–1.341)	.887
≥60				
AA	89 (47.85%)	117 (55.19%)	1.000 (REFERENCE)	
AG+GG	97 (52.15%)	95 (44.81%)	1.169 (0.938–1.456)	.173
Gender				
Men				
AA	67 (42.41%)	82 (53.95%)	1.000 (REFERENCE)	
AG+GG	91 (57.59%)	70 (46.05%)	1.257 (0.996–1.591)	.055
Women				
AA	78 (54.93%)	80 (54.05%)	1.000 (REFERENCE)	
AG+GG	64 (45.07%)	68 (45.95%)	0.982 (0.763–1.258)	.975

CI = confidence interval, HWE = Hardy-Weinberg equilibrium, OR = odds ratio, PD = Parkinson disease.

Table 9

Multiple comparisons for correlations between *STIM1* rs3750994 SNPs and PD risk.

	PD (n = 300)	Control (n = 300)	Adjusted OR (95%)	*P*
Age (Year)				
<60				
TT	76 (66.67%)	63 (71.59%)	1.000 (REFERENCE)	
TG+GG	38 (33.33%)	25 (28.41%)	1.103 (0.827–1.415)	.551
≥60				
TT	125 (67.20%)	144 (67.92%)	1.000 (REFERENCE)	
TG+GG	61 (32.80%)	68 (32.08%)	1.018 (0.798–1.276)	.963
Gender				
Men				
TT	105 (66.46%)	108 (71.05%)	1.000 (REFERENCE)	
TG+GG	53 (33.54%)	44 (28.95%)	1.108 (0.862–1.392)	.453
Women				
TT	96 (67.61%)	99 (66.89%)	1.000 (REFERENCE)	
TG+GG	46 (32.39%)	49 (33.11%)	0.984 (0.744–1.270)	.997

CI = confidence interval, HWE = Hardy-Weinberg equilibrium, OR = odds ratio, PD = Parkinson disease.

Table 10

Multiple comparisons for correlations between *STIM1* rs3750996 SNPs and PD risk.

	PD (n = 300)	Control (n = 300)	Adjusted OR (95%)	*P*
Age (year)				
<60				
AA	62 (54.39%)	59 (67.%)	1.000 (REFERENCE)	
AG+GG	52 (45.61%)	29 (32.95%)	1.253 (0.964–1.594)	.094
≥60				
AA	112 (60.22%)	136 (64.15%)	1.000 (REFERENCE)	
AG+GG	74 (39.78%)	76 (35.85%)	1.092 (0.869–1.358)	.481
Gender				
Men				
AA	88 (55.70%)	98 (64.47%)	1.000 (REFERENCE)	
AG+GG	70 (44.30%)	54 (35.53%)	1.222 (0.973–1.518)	.086
Women				
AA	86 (60.56%)	97 (65.54%)	1.000 (REFERENCE)	
AG+GG	56 (39.44%)	51 (34.46%)	1.114 (0.859–1.420)	.449

CI = confidence interval, HWE = Hardy–Weinberg equilibrium, OR = odds ratio, PD = Parkinson disease.

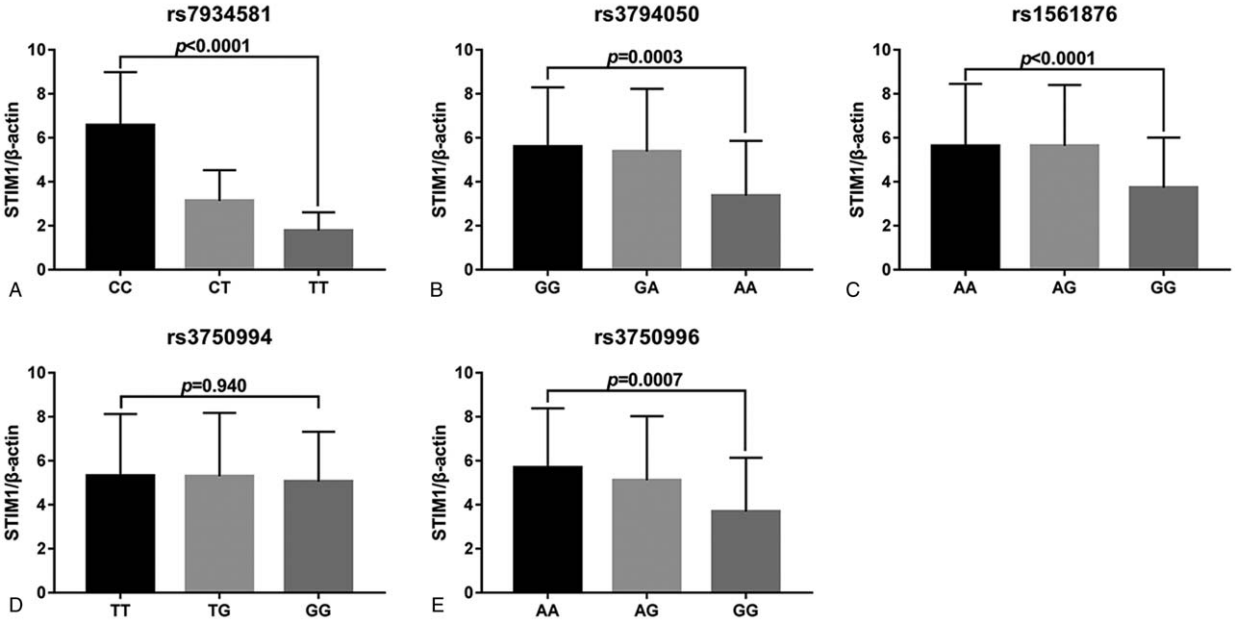

Figure 5. Effects of *STIM1* gene SNPs on *STIM1* mRNA level in PMBC. The *STIM1* mRNA level was significantly reduced in participants with *STIM1* gene rs7934581 (A), rs3794050 (B), rs1561876 (C), and rs3750996 genotypes (E), but no difference in subjects with rs3750994 genotype (D).

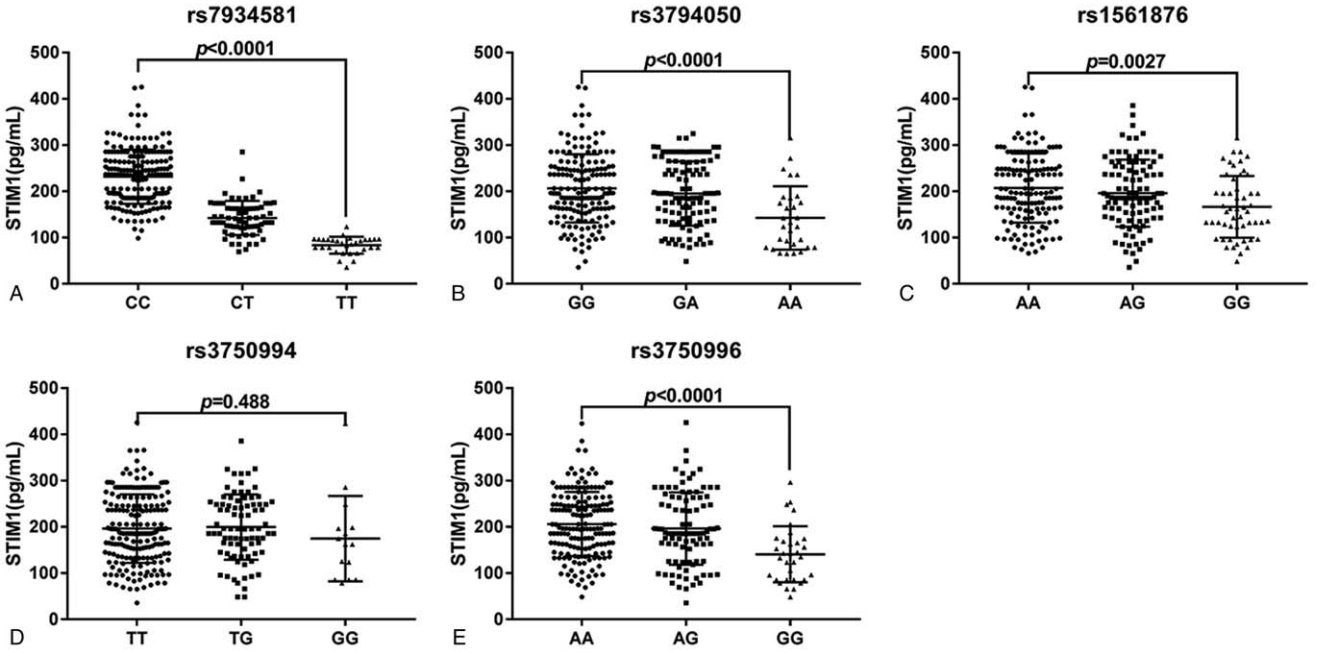

Figure 6. Effects of *STIM1* gene SNPs on plasma STIM1 protein level. The STIM1 protein level was significantly reduced in participants with *STIM1* gene rs7934581 (A), rs3794050 (B), rs1561876 (C), and rs3750996 genotypes (E), but no difference in subjects with rs3750994 genotype (D).

transcription and translation of the *STIM1* gene.[22] Since the 3'UTR region of *STIM1* gene is common binding sites of microRNA (miRNA), it is still unclear whether rs1561876 or rs3750996 SNP affects the regulation of *STIM1* gene expression by miRNA, and further studies are needed to verify this.

Moreover, we also analyzed the linkage disequilibrium of these SNPs, and found that there are 5 haplotypes in *STIM1* gene rs7934581, rs3794050, rs1561876, rs3750996 SNPs, in which subjects with rs7934581, rs3794050, rs1561876, rs3750996 TAAG haplotype and TGAG haplotype had an increased risk of PD compared to those with CGAA haplotype by 1.365-fold and 1.332-fold, respectively. We further analyzed the influences of age as well as gender factors, and found that participants with age ≥60 years or women carrying *STIM1* rs3794050 minor allele had a 1.411-fold and 1.358-fold higher risk of PD than major allele carriers, respectively. These results provided additional evidence that we should also consider the environmental factors when study genetic factors on PD etiology.

There were several limitations in the present study. First, the number of SNPs that we screened is fairly small; also they were limited to the non-coding region of the *STIM1* gene. In fact, there are a large number of SNPs in the *STIM1* gene that need to be further investigated seriously. In particularly, those located in the coding region as mutations of these SNPs may alter the amino acid sequence of the STIM1 protein. Second, limited by the sample size that we collected, the number of minor allele carriers is small, which might lead to large variations. In addition, more *in vitro* studies are necessary to support the conclusion of this study, aimed to enrich the precise mechanisms of PD etiology.

In conclusion, our results confirmed that *STIM1* gene rs7934581, rs3794050, rs1561876, rs3750996 SNPs are associated with elevated PD risk, *STIM1* SNPs are also associated with abnormal STIM1 protein expression. It is likely that the interactions between age or gender and SNPs are associated with the risk of PD.

Author contributions

Conceptualization: Xiaohang Wang.
Data curation: Danning Lou, Jun Wang.
Formal analysis: Danning Lou, Jun Wang.
Funding acquisition: Xiaohang Wang.
Investigation: Danning Lou, Jun Wang.
Methodology: Danning Lou.
Writing – original draft: Danning Lou, Xiaohang Wang.
Writing – review & editing: Xiaohang Wang.

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