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

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Abstract
The stromal interaction molecule 1 (STIM1) gene contributes essentially to Ca²⁺ 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 immunosorobent 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 Ca²⁺.[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 Ca²⁺ entry pathway in non-excitable cells.[5]

It has been well documented that Ca²⁺ imbalance is involved in the development of Alzheimer disease (AD).[7-10] Importantly, STIM1 contributes essentially to Ca²⁺ 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 Ca²⁺ entry.[11] In the PD patients, a complex formed by STIM1 and transient receptor potential channel I (TRPC1) inhibits CaV1.3 channel, which leads to disruption of neuronal Ca²⁺ 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
(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) 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.

2.3. Genetic analyses

The 10 ml of venous blood was collected from patients with Parkinson disease (staging, 2) 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 Mg2+ (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, Valenca, 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 (PBMC) from peripheral blood was performed according to Lou et al. 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’-GCCAAGGCGCCTAATGGCAAT-3’, Rev., 5’-GGCCTTCTGCAGTCTCAGT-3’, and β-actin For., 5’-TGGCACACACCTTACATATT-3’, Rev., 5’-AGGCAGATTAGGAGAATG-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 1

| SNP     | Chromosome | Variation | MAF*  | Location |
|---------|------------|-----------|-------|----------|
| rs7934581[20] | 11:4090603 | C>T       | 0.1714 | Intron   |
| rs3794050[22,24] | 11:4090670 | G>A       | 0.2005 | Intron   |
| rs1561876 | 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 2

| SNP     | Primer sequence |
|---------|-----------------|
| rs7934581 | Forward primer: 5’-GTGTGAGGCTGTGCACTTCCAAGA-3’; Reverse primer: 5’-CACGGCGTGGACACACAAAT-3’ |
| rs3794050 | Forward primer: 5’-CTGGCCACAGATGTTAGAAT-3’; Reverse primer: 5’-GAACCTCAGACCTGCTCCG-3’ |
| rs1561876 | Forward primer: 5’-CTCCTGGGGTCTACGCTTCGC-3’; Reverse primer: 5’-CTGTAGGGCTGGTGGGACAG-3’ |
| rs3750994 | Forward primer: 5’-GCTGATGCTGTCTCAGC-3’; Reverse primer: 5’-GCTGGGCTGCGCTGACCTG-3’ |
| rs3750996 | Forward primer: 5’-AGCTGAGGAAAACGCACT-3’ |

Table 3

| Variation          | PD (n=300) | Control (n=300) | P     |
|--------------------|------------|-----------------|-------|
| Age (year, mean±SD)| 65.5±10.5  | 67.0±9.5        | 0.067 |
| Gender [n%]         |            |                 |       |
| Men                 | 158 (52.67%)| 152 (50.67%)    | 0.624 |
| 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   | 22.1±8.1        |       |

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. ABIN524174, 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 χ² test. For testing Hardy-Weinberg equilibrium in controls was performed using the χ² 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 Haplovie 4.2 software. All statistical analyses were two-tailed, with $P < .05$ 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 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 STIM1 gene 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 Haplovie 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 STIM1 gene 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. 6D).

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).
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 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%) | 0.000 | 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 |          |                  |       |                   |       |
| rs3794050 |          |                  |       |                   |       |
| GG      | 165 (55.00%) | 198 (66.00%) | 0.098 | 1.024 (0.843–1.239) | .874  |
| GA      | 105 (35.00%) | 86 (28.67%) | 1.368 | 1.090–1.651        | .007  |
| AA      | 30 (10.00%)  | 16 (5.33%) | 0.000 | 1.000 (REFERENCE) |       |
| Additive |          |                  |       |                   |       |
| rs1561876 |          |                  |       |                   |       |
| GG      | 53 (17.67%)  | 29 (9.67%) | 0.000 | 1.000 (REFERENCE) |       |
| GA      | 102 (34.00%) | 109 (36.33%) | 0.098 | 1.024 (0.843–1.239) | .874  |
| AA      | 145 (48.33%) | 162 (54.00%) | 1.000 | 1.000 (REFERENCE) |       |
| Additive |          |                  |       |                   |       |
| rs3750996 |          |                  |       |                   |       |
| GG      | 15 (5.00%)   | 14 (4.67%) | 0.000 | 1.000 (REFERENCE) |       |
| GA      | 94 (31.33%)  | 89 (29.67%) | 0.000 | 1.000 (REFERENCE) |       |
| AA      | 208 (67.67%) | 207 (69.00%) | 1.000 | 1.000 (REFERENCE) |       |
| Additive |          |                  |       |                   |       |

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

| Table 5 | Linkage disequilibrium and haplotype analysis for alleles of STIM1 gene rs7934581, rs3794050, rs1561876, rs3750996 SNPs. |
|---------|---------------------------------------------------------------|
| Haplotype | PD (n = 300) | Control (n = 300) | x² | OR (95% CI) | P     |
| rs7934581 |            |                  |    |             |       |
| CGAA     | 159 (53.00%) | 190 (63.33%) | 0.159 | 1.000 (REFERENCE) |       |
| TAAG     | 74 (24.67%)  | 45 (15.00%) | 3.895 | 1.089 (0.900–1.304) | .390  |
| TGAG     | 54 (18.00%)  | 35 (11.67%) | 0.373 | 1.041 (0.883–1.239) | .687  |
| rs3794050 |            |                  |    |             |       |
| CGGA     | 142 (47.33%) | 147 (49.00%) | 0.824 | 1.000 (REFERENCE) |       |
| CGAG     | 158 (52.67%) | 153 (51.00%) | 0.373 | 1.041 (0.883–1.239) | .687  |

CI = confidence interval, OR = odds ratio, PD = Parkinson disease.

* rs7934581, rs3794050, rs1561876, rs3750996.
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. In addition, STIM1 mediated disruption of Ca\(^{2+}\) signaling is also associated with the development of human cancers. Knockdown of STIM1 gene by siRNA affects Ca\(^{2+}\) influx, prevents transport of transcription factors and activates inflammatory COX-2 gene.

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, rs3750994 SNPs influence the binding site of transcription factors 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). 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 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) | .908 |
| ≥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 | 96 (60.13%) | 108 (71.05%) | 1.000 (REFERENCE) |
| GA+AA | 63 (39.87%) | 49 (28.95%) | 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.

| Age (Year) | PD (n=300) | Control (n=300) | Adjusted OR (95%) | P  |
|------------|------------|-----------------|-------------------|----|
| <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.

| Age (Year) | PD (n=300) | Control (n=300) | Adjusted OR (95%) | P  |
|------------|------------|-----------------|-------------------|----|
| <60        |            |                 |                   |    |
| AA         | 62 (54.39%)| 59 (67.3%)      | 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).
transcription and translation of the STIM1 gene.\cite{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.

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**References**

\[1\] Hagell P, Alvariza A, Westergren A, et al. Assessment of burden among family caregivers of people with Parkinson’s Disease using the Zarit Burden interview. J Pain Symptom Manage 2017;53:272–8.

\[2\] Bartolomei L, Pastore A, Meligrana L, et al. Relevance of sleep quality on caregiver burden in Parkinson’s disease. Neurol Sci 2018;39:835–9.

\[3\] Tian YY, Tang CJ, Wu J, et al. Parkinson’s disease in China. Neurol Sci 2011;32:23–30.

\[4\] Pascual-Caro C, Espinosa-Bermejo N, Pozo-Guisado E, et al. Role of STIM1 in neurodegeneration. World J Biol Chem 2018;9:16–24.

\[5\] Zhang SL, Yu Y, Roos J, et al. STIM1 is a Ca2+ sensor that activates CRAC channels and migrates from the Ca2+ store to the plasma membrane. Nature 2005;437:902–5.

\[6\] LaFerla FM. Calcium dyshomeostasis and intracellular signalling in Alzheimer’s disease. Nat Rev Neurosci 2002;3:862–72.

\[7\] Berridge MJ. Calcium regulation of neural rhythms, memory and Alzheimer’s disease. J Physiol 2014;592:281–93.

\[8\] Raza M, Deshpande LS, Blair RE, et al. Aging is associated with elevated intracellular calcium levels and altered calcium homeostatic mechanisms in hippocampal neurons. Neurosci Lett 2007;418:77–81.
[10] Popugaeva E, Pchitskaya E, Bezprouzvanny I. Dysregulation of neuronal calcium homeostasis in Alzheimer’s disease - A therapeutic opportunity? Biochem Biophys Res Commun 2017;483:998–1004.

[11] Pascual-Caro C, Berrocal M, Lopez-Guerrero AM, et al. STIM1 deficiency is linked to Alzheimer’s disease and triggers cell death in SH-SY5Y cells by upregulation of L-type voltage-operated Ca(2+) entry. J Mol Med (Berl) 2018;96:1061–79.

[12] Liu Y, Harding M, Pittman A, et al. Cav1.2 and Cav1.3 L-type calcium channels regulate dopaminergic firing activity in the mouse ventral tegmental area. J Neurophysiol 2014;112:1119–30.

[13] Sun Y, Zhang H, Selvaraj S, et al. Inhibition of L-Type Ca(2+) Channels by TRPC1-STIM1 Complex Is Essential for the Protection of Dopaminergic Neurons. J Neurosci 2017;37:3364–77.

[14] Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson’s disease. Mov Disord 2015;30:1591–601.

[15] Lou O, Alcaide P, Luscinskas FW, et al. CD99 is a key mediator of the transendothelial migration of neutrophils. J Immunol 2007;178:1136–43.

[16] Won SJ, Kim DY, Gwag BJ. Cellular and molecular pathways of ischemic neuronal death. J Biochem Mol Biol 2002;35:67–86.

[17] Calvo M, Villalobos C, Nunez L. Calcium imaging in neuron cell death. Methods Mol Biol 2015;1254:73–85.

[18] Fedida-Metula S, Feldman B, Koshelev V, et al. Lipid rafts couple store-operated Ca2+ entry to constitutive activation of PKB/Akt in a Ca2+/calmodulin-, Src- and PP2A-mediated pathway and promote melanoma tumor growth. Carcinogenesis 2012;33:740–50.

[19] Chen YF, Chiu WT, Chen YT, et al. Calcium store sensor stromal-interaction molecule 1-dependent signaling plays an important role in cervical cancer growth, migration, and angiogenesis. Proc Natl Acad Sci U S A 2011;108:15223–30.

[20] Huang WC, Chai CY, Chen WC, et al. Histamine regulates cyclooxygenase 2 gene activation through Orai1-mediated NFkappaB activation in lung cancer cells. Cell Calcium 2011;50:27–35.

[21] Wang JY, Chen BK, Wang YS, et al. Involvement of store-operated calcium signaling in EGF-mediated COX-2 gene activation in cancer cells. Cell Signal 2012;24:162–9.

[22] Wang J, Wang X, Zhao M, et al. Potentially functional SNPs (pfSNPs) as novel genomic predictors of 5-FU response in metastatic colorectal cancer patients. PLoS One 2014;9:e111694.

[23] Wei JC, Hung KS, Hsu YW, et al. Genetic polymorphisms of stromal interaction molecule 1 associated with the erythrocyte sedimentation rate and C-reactive protein in HLA-B27 positive ankylosing spondylitis patients. PLoS One 2012;7:e49698.

[24] Vachon CM, Li J, Scott CG, et al. No evidence for association of inherited variation in genes involved in mitosis and percent mammographic density. Breast Cancer Res 2012;14:R7.