**JMD**

## ORIGINAL ARTICLE

# Association of *AXIN1* With Parkinson's Disease in a Taiwanese Population

Hwa-Shin Fang,[1] Chih-Ying Chao,[2] Chun-Chieh Wang,[2] Wen-Lang Fan,[3]
Po-Jung Huang,[3,4] Hon-Chung Fung,[5] Yih-Ru Wu[2,6]

[1]Division of General Medicine, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan
[2]Department of Neurology and [3]Genomic Medicine Core Laboratory, Chang Gung Memorial Hospital, Linkou Medical Center, Taoyuan, Taiwan
[4]Department of Biomedical Sciences, Chang Gung University, Taoyuan, Taiwan
[5]Fu Jen Faculty of Theology of St. Robert Bellarmine, Fu Jen University Clinic Taiwan, New Taipei, Taiwan
[6]Department of Neurology, College of Medicine, Chang Gung University, Taoyuan, Taiwan

## ABSTRACT

**Objective**  A meta-analysis of locus-based genome-wide association studies recently identified a relationship between *AXIN1* and Parkinson's disease (PD). Few studies of Asian populations, however, have reported such a genetic association. The influences of rs13337493, rs758033, and rs2361988, three PD-associated genetic variants of *AXIN1*, were investigated in the present study because AXIN1 is related to Wnt/β-catenin signaling.

**Methods**  A total of 2,418 individuals were enrolled in our Taiwanese cohort for analysis of the genotypic and allelic frequency. Polymerase chain reaction–restriction fragment length polymorphism analysis was employed for rs13337493 genotyping, and the Agena MassARRAY platform (Agena Bioscience, San Diego, CA, USA) was used for rs758033 and rs2361988 genotyping in 672 patients with PD and 392 controls. Taiwan Biobank data of another 1,354 healthy controls were subjected to whole-genome sequencing performed using Illumina platforms at approximately 30× average depth.

**Results**  Our results revealed that rs758033 {odds ratios [OR] (95% confidence interval [CI]) = 0.267 [0.064, 0.795], $p = 0.014$} was associated with the risk of PD, and there was a trend toward a protective effect of rs2361988 (OR [95% CI] = 0.296 [0.071, 0.884], $p = 0.026$) under the recessive model. The TT genotype of rs758033 (OR [95% CI] = 0.271 [0.065, 0.805], $p = 0.015$) and the CC genotype of rs2361988 (OR [95% CI] = 0.305 [0.073, 0.913], $p = 0.031$) were less common in the PD group than in the non-PD group.

**Conclusion**  Our findings indicate that the rs758033 and rs2361988 polymorphisms of *AXIN1* may affect the risk of PD in the Taiwanese population.

**Keywords**  *AXIN1*; Disease association; Neuroinflammation; Parkinson's disease; Polymorphism.

After Alzheimer's disease (AD), Parkinson's disease (PD) is the most prevalent neurodegenerative disorder.[1] Evidence increasingly shows that genetic factors play major roles in PD. However, few of the heritable components have been identified.[2] Pathologically, the hallmarks of PD are abnormal α-synuclein aggregation and dopaminergic neuron loss in the substantia nigra (SN);[3] these factors lead to tremors, rigidity, bradykinesia, and stooped posture, among other major symptoms of PD.[4] Although the detailed cause of dopaminergic neuron loss remains unclear, accumulating evidence indicates the important role of neuroinflammation in neurodegenerative diseases such as PD.[5] In one study, activated astrocytes and microglia were used for chronic release of pro-

inflammatory cytokines, contributing to the degeneration of dopaminergic neurons in the SN.[6]

Several signaling pathways, such as that of Wnt, are believed to be involved in neuroinflammation in AD and PD.[7-9] The Wnt signaling pathway is commonly considered to have canonical and noncanonical components, with the canonical pathway believed to promote anti-inflammatory activity.[7] AXIN1 promotes the GSK-3β phosphorylation of β-catenin and subsequent degradation of β-catenin in the canonical Wnt pathway, resulting in reduced Wnt expression.[10] A study also discovered a regulatory role of the canonical Wnt pathway in neuroinflammation.[11] Because AXIN1 negatively regulates this pathway, it may affect the pathogenesis of PD by promoting brain inflammation and impairing adult neurogenesis.[8,12]

Recently, the relationship between *AXIN1* and PD was reported in a meta-analysis of locus-based genome-wide association studies investigating genomic convergence and linking PD with inflammation.[12] AXIN1 expression was upregulated in the SN of patients with PD, and its expression in the SN was discovered to be significantly modulated by the intronic single nucleotide polymorphism (SNP) rs13337493.[12] Additionally, rs13337493 is in linkage disequilibrium with rs758033 and rs2361988. Data on quantitative trait loci revealed that in the basal ganglia, AXIN1 expression was modulated by the SNPs rs13337493, rs758033, and rs2361988.[12] The present research is a case-control study in which the genotypic and allelic frequencies of *AXIN1* rs13337493, rs758033, and rs2361988 were examined in 672 and 1,746 Taiwanese individuals with and without PD, respectively, to determine whether these novel genetic loci are relevant to the incidence of PD among the population of Taiwan.

## MATERIALS & METHODS

### Ethics statement

The Institutional Review Board of Chang Gung Memorial Hospital (CGMH; ethical license No: 201701458B0C601 and 201800893B0C102) approved the protocol employed in this study, which was conducted in accordance with the 1975 (revised in 2013) Declaration of Helsinki. Every participant provided informed consent in writing.

### Patient population

In total, 2,418 Taiwanese participants were included in the study. Neurological clinics at CGMH were the source of the 672 patients with PD. PD was diagnosed by an experienced movement disorder specialist (Yih-Ru Wu) using the UK Parkinson's Disease Society Brain Bank criteria.[13]

Additionally, 392 healthy controls were recruited from the same neurological clinics. We also included Taiwan Biobank data on 1,354 additional healthy controls.

### Genetic analysis

The three SNPs investigated, which may modulate *AXIN1* expression in the SN, were rs13337493, rs758033, and rs2361988.

Polymerase chain reaction (PCR)−restriction fragment length polymorphism analysis was used to confirm rs13337493. The following primer sequences were used: 5'-TGCGGCATATTGT-GTCCTAA-3' (forward primer) and 5'-GCGACAAGAGGA-CAAGAGTG-3' (reverse primer). The Hpy166II (New England Biolabs, Ipswich, MA, USA) enzyme recognition site was GTN-NAC (the polymorphic site is underlined). The material for and process of PRC amplification are described in Supplementary Material 1 (in the online-only Data Supplement). Hpy166II was employed to digest the amplified PCR fragments, after which they were separated on a 1.0% agarose gel (allele A, 454-bp fragment; allele G, 274- and 180-bp fragments).

The Agena MassARRAY platform with iPLEX Gold chemistry (Agena Bioscience, San Diego, CA, USA) was employed to genotype rs758033 and rs2361988. Assay Designer version 4.0 was used (in accordance with the manufacturer's guide) to determine the specific PCR primer and extension primer sequences (Supplementary Table 1 in the online-only Data Supplement). Multiplex PCR was conducted and is described in Supplementary Material 2 (in the online-only Data Supplement). Residual salt was removed by adding a cation exchange resin. Subsequently, the matrix pad from a SpectroCHIP array (Agena Bioscience, San Diego, CA, USA) was loaded with 7 nL of the purified primer extension reaction mix. A MassARRAY Analyzer 4 (Agena Bioscience) was employed for SpectroCHIP evaluation, and TYPER 4.0 software (Sequenom, San Diego, CA, USA) was employed to perform clustering analysis of the calling.

### Statistical analysis

We evaluated the Hardy−Weinberg equilibrium of the genotypic frequencies in the PD and non-PD groups. The allelic and genotypic distributions of the two groups were compared with the chi-square test. For analysis of the PD−SNP associations, odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. As this study involved three independent genetic loci, we made a modest correction using the Bonferroni method for multiple comparisons with statistical significance defined at $p < 0.017$. The power of our study was calculated by using G*power 3.1 software.

## RESULTS

This study recruited 2,418 individuals: 672 with PD and 1,746 controls. The genotypic frequency distributions of three SNPs

**Table 1.** Genotypic and allele frequency distribution of 3 SNPs in PD patients and controls

| | PD (%); *n* = 672 | Control (%); *n* = 1,746 | OR (95% CI) | *p*-value | Power |
|---|---|---|---|---|---|
| rs13337493 | | | | | |
| Genotype | | | | | |
| AA | 22 (3.3) | 71 (4.1) | 0.833 (0.501, 1.347) | 0.474 | 0.741 |
| GA | 210 (31.7) | 515 (29.5) | 1.096 (0.902, 1.332) | 0.354 | |
| GG | 431 (65) | 1,159 (66.4) | | | |
| Dominant model | | | | | |
| AA + GA | 232 (35) | 586 (33.6) | 1.065 (0.882, 1.284) | 0.513 | 0.307 |
| GG | 431 (65) | 1,159 (66.4) | | | |
| Recessive model | | | | | |
| AA | 22 (3.3) | 71 (4.1) | 0.809 (0.488, 1.303) | 0.399 | 0.508 |
| GA + GG | 641 (96.7) | 1,674 (95.9) | | | |
| Minor allele | | | | | |
| A | 254 (19.2) | 657 (18.8) | 1.022 (0.869, 1.199) | 0.791 | 0.110 |
| Major allele | | | | | |
| G | 1,072 (80.8) | 2,833 (81.2) | | | |
| rs758033 | | | | | |
| Genotype | | | | | |
| TT | 3 (0.4) | 29 (1.7) | 0.271 (0.065, 0.805) | 0.015 | 0.997 |
| GT | 150 (22.5) | 372 (21.3) | 1.054 (0.849, 1.306) | 0.628 | |
| GG | 514 (77.1) | 1,344 (77) | | | |
| Dominant model | | | | | |
| TT + GT | 153 (22.9) | 401 (23) | 0.998 (0.806, 1.232) | 0.986 | 0.052 |
| GG | 514 (77.1) | 1,344 (77) | | | |
| Recessive model | | | | | |
| TT | 3 (0.4) | 29 (1.7) | 0.267 (0.064, 0.795) | 0.014 | 0.999 |
| GT + GG | 664 (99.6) | 1,716 (98.3) | | | |
| Minor allele | | | | | |
| T | 156 (11.7) | 430 (12.3) | 0.942 (0.774, 1.144) | 0.554 | 0.245 |
| Major allele | | | | | |
| G | 1,178 (88.3) | 3,060 (87.7) | | | |
| rs2361988 | | | | | |
| Genotype | | | | | |
| CC | 3 (0.5) | 27 (1.6) | 0.305 (0.073, 0.913) | 0.031 | 0.998 |
| TC | 150 (23.3) | 357 (20.6) | 1.153 (0.927, 1.431) | 0.200 | |
| TT | 492 (76.3) | 1,350 (77.9) | | | |
| Dominant model | | | | | |
| CC + TC | 153 (23.7) | 384 (22.1) | 1.093 (0.881, 1.353) | 0.414 | 0.469 |
| TT | 492 (76.3) | 1,350 (77.9) | | | |
| Recessive model | | | | | |
| CC | 3 (0.5) | 27 (1.6) | 0.296 (0.071, 0.884) | 0.026 | 0.990 |
| TC + TT | 642 (99.5) | 1,707 (98.4) | | | |
| Minor allele | | | | | |
| C | 156 (12.1) | 411 (11.9) | 1.023 (0.839, 1.244) | 0.815 | 0.071 |
| Major allele | | | | | |
| T | 1,134 (87.9) | 3,057 (88.1) | | | |

Values are presented as *n* (%) unless otherwise indicated. SNP, single nucleotide polymorphism; PD, Parkinson's disease; OR, odds ratio; CI, confidence interval.

in the two groups were compared (Table 1). The rs758033 TT genotype was less frequent in the PD patients than the controls (OR [95% CI] = 0.271 [0.065, 0.805], *p* = 0.015) and had a lower risk of PD compared with that of the genotypes GT + GG in the recessive model (OR [95% CI] = 0.267 [0.064, 0.795], *p* = 0.014). The rs2361988 CC genotype was also less frequent in the patients with PD than the controls (OR [95% CI] = 0.305 [0.073, 0.913], *p* = 0.031), and in the recessive model, the individuals with this CC genotype had a lower risk of PD than did those with the genotypes TC + TT [OR (95% CI) = 0.296 (0.071, 0.884), *p* = 0.026]. The two groups had similar rs13337493 genotypic frequencies.

## DISCUSSION

The associations between three *AXIN1* SNPs (rs13337493, rs758033, and rs2361988) and susceptibility to PD were investigated in the present study. Our results revealed that the *AXIN1* SNP rs758033 was associated with the risk of PD under the recessive model, and there was a trend toward a protective effect of rs2361988.

*AXIN1*, known as a tumor suppressor gene, inhibits Wnt signaling and is implicated in several cancers, such as colorectal adenocarcinoma,[14] non-small-cell lung carcinoma,[15] breast cancer,[16] hepatocellular carcinomas,[17] medulloblastomas,[18] and melanoma.[19] Melanoma has also been found to have a positive association with PD.[20,21] which may be due to *LRRK2* mutation and its linkage in the Wnt signaling pathway.[22,23]

Vertebrates have two alternative AXIN splicing products, AXIN1 and AXIN2, which are almost functionally equivalent in the canonical Wnt pathway.[24,25] There are two types of Wnt signaling pathways: canonical and noncanonical Wnt signaling pathways. Canonical Wnt signaling promotes anti-inflammatory activity, whereas noncanonical Wnt signaling serves as a proinflammatory mechanism.[7]

Binding of Wnt ligands to Frizzled family receptors and low-density lipoprotein receptor-related protein 5/6 leads to activation of the scaffold protein Disheveled and AXIN1 recruitment, further resulting in disassembly of the β-catenin destruction complex. Disrupting this complex—originally composed of adenomatous polyposis coli, AXIN1, GSK3β, and CK1—impairs the degradation and phosphorylation of β-catenin. Thereafter, this protein accumulates in the cytoplasm and is subsequently translocated to nuclei, where it binds to the transcription factor T-cell factor/lymphoid enhancer factor, in turn inducing *Wnt* gene expression.[24,26,27] This pathway may eventually promote anti-inflammatory activity.[7] Impaired canonical Wnt/β-catenin signaling was found to predispose midbrain dopaminergic neurons to death in PD and in other major neurodegenerative disorders.[28] In a mu-

rine model of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, neuroprotective and neurorestorative effects in the PD-injured brain were activated by canonical Wnt/β-catenin signaling.[28]

Although the direct mechanism through which AXIN1 is related to PD is uncertain, various studies have implicated the dysregulation of Wnt/β-catenin signaling in the pathogenesis of neuroinflammatory and neurodegenerative disorders such as AD[7,29] and PD.[9] Furthermore, Song et al.[25] reported that AXIN1 crucially affects the regulation of Wnt/β-catenin signaling. *AXIN1* gene expression was recently found to be upregulated in a murine PD model.[8] In addition, knockdown of AXIN2, a functionally interchangeable isoform of AXIN1, was reported to increase Wnt/β-catenin signaling pathway activity and thus enhance mitochondrial biogenesis and dopaminergic neurogenesis in rats with PD.[30]

AXIN1 was identified in the gene list of the top pathways related to neurodegeneration in the study by Foo et al.,[31] but it was not proven to be significant. Thus, the three single nucleotide variants (SNVs) were not analyzed in this genome-wide association study of a large Han Chinese cohort. The significant association between the two SNVs (rs758033 and rs2361988) and PD could be attributed to their altered gene expression level of AXIN1, which has been revealed in Mohammad Saeed's study.[12]

We revealed for the first time that in a Taiwanese population, the recessive model and TT genotype of *AXIN1* rs758033 were associated with lower risks of developing PD, and the recessive model and CC genotype of rs2601988 tended to be associated with a lower risk of PD. More case-control studies are needed to confirm the *AXIN1*—PD association and the associations of *AXIN1* with other neurodegenerative diseases; such research could aid in the design of novel therapies.

## Author Contributions

Conceptualization: Yih-Ru Wu. Data curation: Hwa-Shin Fang, Yih-Ru Wu. Formal analysis: Chih-Ying Chao, Hwa-Shin Fang, Wen-Lang Fan,

## ORCID iDs

Hwa-Shin Fang     https://orcid.org/0000-0002-3729-9806
Chih-Ying Chao     https://orcid.org/0000-0002-2655-5237
Chun-Chieh Wang     https://orcid.org/0000-0003-0177-6663
Wen-Lang Fan     https://orcid.org/0000-0003-0633-3933
Po-Jung Huang     https://orcid.org/0000-0002-8728-8881
Hon-Chung Fung     https://orcid.org/0000-0002-4829-500X
Yih-Ru Wu     https://orcid.org/0000-0003-1191-2542

## REFERENCES

1. Bertram L, Tanzi RE. The genetic epidemiology of neurodegenerative disease. J Clin Invest 2005;115:1449-1457.
2. Bonifati V. Genetics of Parkinson's disease--state of the art, 2013. Parkinsonism Relat Disord 2014;20 Suppl 1:S23-S28.
3. von Bohlen und Halbach O, Schober A, Krieglstein K. Genes, proteins, and neurotoxins involved in Parkinson's disease. Prog Neurobiol 2004;73:151-177.
4. Lang AE, Lozano AM. Parkinson's disease. Second of two parts. N Engl J Med 1998;339:1130-1143.
5. Lee JK, Tran T, Tansey MG. Neuroinflammation in Parkinson's disease. J Neuroimmune Pharmacol 2009;4:419-429.
6. Wang Q, Liu Y, Zhou J. Neuroinflammation in Parkinson's disease and its potential as therapeutic target. Transl Neurodegener 2015;4:19.
7. Zolezzi JM, Inestrosa NC. Wnt/TLR dialog in neuroinflammation, relevance in Alzheimer's disease. Front Immunol 2017;8:187.
8. Zhou L, Yang L, Li YJ, Mei R, Yu HL, Gong Y, et al. MicroRNA-128 protects dopamine neurons from apoptosis and upregulates the expression of excitatory amino acid transporter 4 in Parkinson's disease by binding to AXIN1. Cell Physiol Biochem 2018;51:2275-2289.
9. Berwick DC, Harvey K. The importance of Wnt signalling for neurodegeneration in Parkinson's disease. Biochem Soc Trans 2012;40:1123-1128.
10. Li VS, Ng SS, Boersema PJ, Low TY, Karthaus WR, Gerlach JP, et al. Wnt signaling through inhibition of β-catenin degradation in an intact Axin1 complex. Cell 2012;149:1245-1256.
11. Suryawanshi A, Manoharan I, Hong Y, Swafford D, Majumdar T, Taketo MM, et al. Canonical Wnt signaling in dendritic cells regulates Th1/Th17 responses and suppresses autoimmune neuroinflammation. J Immunol 2015;194:3295-3304.
12. Saeed M. Genomic convergence of locus-based GWAS meta-analysis identifies AXIN1 as a novel Parkinson's gene. Immunogenetics 2018;70:563-570.
13. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55:181-184.
14. Jin LH, Shao QJ, Luo W, Ye ZY, Li Q, Lin SC. Detection of point mutations of the Axin1 gene in colorectal cancers. Int J Cancer 2003;107:696-699.
15. Stewart DJ. Wnt signaling pathway in non-small cell lung cancer. J Natl Cancer Inst 2014;106:djt356.
16. Bao R, Christova T, Song S, Angers S, Yan X, Attisano L. Inhibition of tankyrases induces Axin stabilization and blocks Wnt signalling in breast cancer cells. PLoS One 2012;7:e48670.
17. Satoh S, Daigo Y, Furukawa Y, Kato T, Miwa N, Nishiwaki T, et al. AXIN1 mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXIN1. Nat Genet 2000;24:245-250.
18. Dahmen RP, Koch A, Denkhaus D, Tonn JC, Sörensen N, Berthold F, et al. Deletions of AXIN1, a component of the WNT/wingless pathway, in sporadic medulloblastomas. Cancer Res 2001;61:7039-7043.
19. Biechele TL, Kulikauskas RM, Toroni RA, Lucero OM, Swift RD, James RG, et al. Wnt/β-catenin signaling and AXIN1 regulate apoptosis triggered by inhibition of the mutant kinase BRAFV600E in human melanoma. Sci Signal 2012;5:ra3.
20. Bertoni JM, Arlette JP, Fernandez HH, Fitzer-Attas C, Frei K, Hassan MN, et al. Increased melanoma risk in Parkinson disease: a prospective clinicopathological study. Arch Neurol 2010;67:347-352.
21. Bose A, Petsko GA, Eliezer D. Parkinson's disease and melanoma: co-occurrence and mechanisms. J Parkinsons Dis 2018;8:385-398.
22. Berwick DC, Javaheri B, Wetzel A, Hopkinson M, Nixon-Abell J, Grannò S, et al. Pathogenic LRRK2 variants are gain-of-function mutations that enhance LRRK2-mediated repression of β-catenin signaling. Mol Neurodegener 2017;12:9.
23. Warø BJ, Aasly JO. Exploring cancer in LRRK2 mutation carriers and idiopathic Parkinson's disease. Brain Behav 2017;8:e00858.
24. Kikuchi A. Roles of Axin in the Wnt signalling pathway. Cell Signal 1999;11:777-788.
25. Song X, Wang S, Li L. New insights into the regulation of Axin function in canonical Wnt signaling pathway. Protein Cell 2014;5:186-193.
26. Clevers H, Nusse R. Wnt/β-catenin signaling and disease. Cell 2012;149:1192-1205.
27. Nusse R, Clevers H. Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. Cell 2017;169:985-999.
28. Marchetti B. Wnt/β-catenin signaling pathway governs a full program for dopaminergic neuron survival, neurorescue and regeneration in the MPTP mouse model of Parkinson's disease. Int J Mol Sci 2018;19:3743.
29. Palomer E, Buechler J, Salinas PC. Wnt signaling deregulation in the aging and Alzheimer's brain. Front Cell Neurosci 2019;13:227.
30. Singh S, Mishra A, Mohanbhai SJ, Tiwari V, Chaturvedi RK, Khurana S, et al. Axin-2 knockdown promote mitochondrial biogenesis and dopaminergic neurogenesis by regulating Wnt/β-catenin signaling in rat model of Parkinson's disease. Free Radic Biol Med 2018;129:73-87.
31. Foo JN, Tan LC, Irwan ID, Au WL, Low HQ, Prakash KM, et al. Genome-wide association study of Parkinson's disease in East Asians. Hum Mol Genet 2017;26:226-232.

# SUPPLEMENTARY MATERIAL 1

**PCR amplication of rs13337493 (restriction fragment length polymorphism analysis)**

In the polymerase chain reaction (PCR) amplication, 1 μL of genomic DNA (10 ng/μL) was applied to multiplex PCR reaction in 20 μL containing 0.5 μM of each primer and 10 μL of 2× Taq-Plus Master Mix (ACE Biosystems, Taoyuan, Taiwan). The following thermal cycling procedure was employed: 95°C for 5 min; 40 cycles of 95°C for 40 s, 52°C for 30 s, and 72°C for 40 s; extension at 72°C for 10 min; and finally, a hold cycle at 4°C.

# SUPPLEMENTARY MATERIAL 2

**Multiplex PCR of rs758033 and rs2361988 (Agena MassARRAY platform; Agena Bioscience, San Diego, CA, USA)**

Multiplex polymerase chain reaction (PCR) was conducted using 1 μL of a 10 ng/μL genomic DNA sample in 5 μL of a mixture comprising 1 unit of Taq polymerase, 500 nmol of each PCR primer mix, and each deoxynucleotide at 2.5 mM (PCR Accessory and Enzyme kit; Agena Bioscience, San Diego, CA, USA). The thermocycling procedure was as follows: 94°C for 4 min; 45 cycles of 94°C for 20 s, 56°C for 30 s, and 72°C for 60 s; and finally, 72°C for 3 min. A total of 0.3 units of shrimp alkaline phosphatase were used to deactivate the unincorporated dNTPs. The iPLEX enzyme, terminator mix, and extension primer mix (iPLEX Gold Kit; Agena Bioscience, San Diego, CA, USA) were employed for the single base extension reaction; the thermocycling proceeded as follows: 94°C for 30 s, 40 cycles of 94°C for 5 s with five nested inner cycles of 56°C for 5 s and 80°C for 5 s, and then 72°C for 3 min.

**Supplementary Table 1.** Sequences of primers used for genotyping in this study

| SNP | Forward primer | Reverse primer | Extension primer |
|---|---|---|---|
| rs758033 | ACGTTGGATGCTTTGGAGCTGTAAGTACTG | ACGTTGGATGTCCGAGGTCCAAGGGGAAA | CTTTTGCAGCGCTCATTGTTC |
| rs2361988 | ACGTTGGATGGAGTAGATAAGGGCTCCAAC | ACGTTGGATGTGTTTCAGTCACCTGCATGG | CACAGAACCCCTGCCA |

SNP, single nucleotide polymorphism.