Impact of ESR1 Polymorphisms on Risk of Breast Cancer in the Chinese Han Population

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Abstract

Background The Estrogen receptor-1 (ESR1) gene encodes estrogen receptor-α which is a major biomarker in the development of breast cancer. This research aimed to investigate the effect of ESR1 polymorphisms on breast cancer in Chinese Han women.

Methods Four candidate single nucleotide polymorphisms (SNPs) in ESR1 gene among 503 breast cancer patients and 503 healthy people were genotyped using Agena MassARRAY platform. The association between ESR1 polymorphisms and breast cancer risk was evaluated using odds ratio (OR) and 95% confidence intervals (95% CIs) in four genetic models. The HaploReg v4.1 and GEPIA database were used for SNP functional annotation and ESR1 expression analysis respectively.

Results The allele T of rs9383938 in ESR1 was significantly associated with an increased breast cancer risk (OR = 1.26, 95% CI = 1.05 – 1.50, p = 0.013). In genetic models, rs9383938 increased breast cancer risk in codominant model (OR = 1.54, 95% CI = 1.07 – 2.22, p = 0.021), dominant model (OR = 1.31, 95% CI = 1.01 – 1.68, p = 0.040), and additive model (OR = 1.24, 95% CI = 1.04 – 1.48, p = 0.017). Stratification analysis showed that rs9383938 and rs2228480 raised the breast cancer susceptibility at age < 50 years. Rs1801132 of ESR1 was also associated with the status of ER, PR, and Her-2 in allele model and genetic models significantly.

Conclusion This study demonstrated that ESR1 polymorphisms might influence breast cancer susceptibility in Chinese Han population. Further mechanisms studies are needed to confirm the contribution of ESR1.

Background

Breast cancer is a lethal malignancy and a leading cause of morbidity and mortality among females globally. [1, 2] According to incomplete statistics, there were 272,400 new cases and 70,700 deaths of breast cancer in Chinese women in 2015. [3] Although the exact cause of breast cancer is unknown, it is widely considered that hereditary factors play a critical role in the occurrence of breast cancer. Approximately 5%—10% of all breast cancer cases mainly attributed to the genetic variation in susceptible genes. [4] The genome-wide association studies (GWASs) have identified that numerous single nucleotide polymorphisms (SNPs) involve in the occurrence and development of breast cancer. Many genes have been identified with important roles in the etiology of breast cancer such as *ACYP2, BRCA, BACH1, CHEK2, PALB2, PTEN,* and *ESR1.*[5, 6]

The *Estrogen receptor–1* (*ESR1)* gene encodes estrogen receptor-α (ERα) which expresses in most breast cancer. ERα was recognized as a major biomarker presence in tumors [7, 8]. ERα expression can regulate breast cancer risk by affecting estrogen metabolic pathways [9]. Because of the importance of ERα in the pathogenesis of breast cancer, *ESR1* gene has been targeted by many researchers. Previous studies have shown that the specific SNP of *ESR1* gene is associated with the occurrence of serious diseases in women such as breast, endometrial and ovarian cancer [10–12], as well as type 2 diabetes mellitus [13], osteoporosis [14], and metabolic syndrome depressive disorders [15]. GWAS have revealed that many SNPs of *ESR1* gene are linked with breast cancer. However, results of these studies are not consistent due to the differences in sample size and ethnic genetic backgrounds. [16, 17]. Therefore, the impact of *ESR1* polymorphisms in Chinese women with breast cancer need to be further demonstrated.

In this work, we carried out a case-control study of 503 breast cancer patients and 503 healthy individuals aim to discover some new SNPs of *ESR1* associated with breast cancer risk among Chinese Han population.

Materials And Methods

*Subjects.*A case-control research was implemented with 1006 participants randomly selected from the Shaanxi Province Cancer Hospital, including 503 breast cancer patients and 503 age-, sex-and ethnic- matched healthy people. None of the cases had received chemotherapy or radiotherapy before sample collection. Patients with other types of cancer were excluded. All patients were pathologically confirmed sporadic breast cancer, and clinical pathological data were collected, including, clinical stages, tumor size, lymph node metastasis (LNM), estrogen receptor (ER) status, progesterone receptor (PR) status, and human epidermal growth factor receptor type–2 (HER–2) status.

Genotyping assay. Limosis venous blood (5 ml) was collected into EDTA-coated blood collection tubes and stored at −80°C. Genomic DNA was extracted from using the GoldMag-Mini Purifcation Kit (GoldMag Co. Ltd, Xi'an, China). DNA concentrations were detected using the NanoDrop2000 (Thermo Scientific, Waltham, MA, USA). Four tag-SNPs (rs9383938, rs2234693, rs1801132, and rs2228480) were selected from the 1,000 Genomes Project database (*http://www.internationalgenome.org/),,* whose minor allele frequency (MAF) greater than 0.05 in global population. Agena MassARRAY Assay Design Software (version 3.0, Agena Bioscience, San Diego, CA, USA) was used to design multiplexed SNP Mass EXTEND assay. Agena MassARRAY RS1000 was used to test SNP genotypes. Finally, data management was carried out with Agena Typer Software (version 4.0, Agena Bioscience, San Diego, CA, USA). [6, 18, 19]

Bioinformatics analysis and expression analysis. HaploReg v4.1 (*https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php)* was employed to predict the latent functions of the candidate SNPs. The GEPIA database (*http://gepia.cancer-pku.cn/)* was used to analyze the *ESR1* expression differences between breast cancer and normal tissues.

Statistical analysis. SPSS software (version 22.0, SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The Hardy–Weinberg Equilibrium (HWE) *p* value was examined using the Fisher exact test. Difference between cases and controls in the age was compared using independent sample *t*-test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to estimate the relationships between *ESR1* polymorphisms and breast cancer risk using logistic regression analysis. Multiple inheritance models (codominant, dominant, recessive and additive) assessment were performed in the PLINK software (version 1.07). The two-sided *p*-value of less than 0.05 was considered statistically significant.

Results

Population characteristics. A total of 503 breast cancer patients and 503 healthy people were recruited in this study. The demographic and clinical characteristics of breast cancer cases and control subjects were listed in Table 1. The mean age were 51.90 ± 9.55 years in cases and 52.04 ± 9.52 years in controls. No significant difference in the distribution of age was found between the case and control groups ($p = 0.702$).

Basic information and predictive functions of the selected ESR1 SNPs. The basic information of the four SNPs in *ERS1* was demonstrated in Table 2, including gene, chromosome, alleles, and MAF in cases and controls. All these SNPs complied with Hardy-Weinberg equilibrium in the control group. Additionally, we used HaploRegv4.1 to predictive the latent functions of the candidate SNPs. The results showed that the SNPs of *ESR1* were involved in the DNAase related regulation, motifs change, GRASP QTL hits, histones enhance, and proteins bound, hinting they might exert biology functions in this way in patients.

Association of ESR1 polymorphisms with breast cancer risk. The differences in allele frequency between patients and control people were compared by χ^2 test (Table 2). The minor allele of each SNP was considered as a risk factor compared to wild-type alleles. The frequency of the allele T of rs9383938 in *ESR1* was higher in breast cancer cases than in controls (41.6% vs 36.5%), and the allele T of rs9383938 was a risk allele for breast cancer among Chinese Han women (OR = 1.26, 95% CI = 1.05—1.50, $p = 0.013$). Then, four inheritance models (codominant model, dominant model, recessive model, and additive model) were implemented for analyzing the relationship between each SNP and breast cancer susceptibility by adjusted for age (Table 3). The result indicated that TT genotype and GT-TT genotype in rs9383938 increased the risk of breast cancer in codominant model (OR = 1.54, 95% CI = 1.07—2.22, $p = 0.021$) and dominant model (OR = 1.31, 95% CI = 1.01—1.68, $p = 0.040$), respectively. Furthermore, rs9383938 was also associated with the susceptibility of breast cancer in additive model (OR = 1.24, 95% CI = 1.04—1.48, $p = 0.017$).

Stratification analysis of SNPs in ESR1 and breast cancer risk. Stratified analysis by age revealed the relationships of *ESR1* polymorphisms with breast cancer risk and the results were exhibited in Table 4. The results showed that rs9383938 was associated with an increased risk of breast cancer in individuals under 50 years old in codominant model (TT vs GG: OR = 1.82, 95% CI = 1.07—3.10, $p = 0.028$) and additive model (OR = 1.35, 95% CI = 1.04—1.74, $p = 0.024$). In addition, rs2228480 of *ESR1* raised the breast cancer susceptibility in additive model (OR = 1.39, 95% CI = 1.02—1.88, $p = 0.036$).

We also explored the association of *ESR1* SNPs with clinical and pathological information of breast cancer, including the status of ER, PR, Her−2, and tumor size, LNM, and pathological stage (Table 5). Multiple inheritance models analysis showed that rs1801132 of *ESR1* was significantly associated with the status of ER, PR, and Her−2. We observed that the C allele was related with the status of ER (OR = 1.52, $p = 0.003$), the status of PR (OR = 1.56, $p = 0.001$), and the status of Her−2 (OR = 1.48, $p = 0.034$). The CC and CG genotype were more common in ER-negative breast cancer, PR-negative breast cancer and Her−2-negative breast cancer patients (All p values were less than 0.05). In addition, the rs1801132 was related to the status of ER and PR in dominant model, recessive model, and additive model ($p < 0.05$). And, a relation between rs1801132 and Her−2 status was also observed in dominant model ($p = 0.006$), and additive model ($p = 0.035$). Moreover, the CC genotype of rs1801132 was predominant in the patients with larger tumor size (CC vs.GG-GC: $p = 0.011$). There were no significant correlation between the other three polymorphisms (rs9383938, rs2234693, and rs2228480) and breast cancer clinical parameters.

Discussion

ER is a hormone-regulated transcription factor, which affects the expression of multiple genes by combining estrogen to promote the proliferation and survival of breast cancer cells. [20, 21]. So far, many studies have shown a strong connection between the ER and the breast cancer pathogenesis. GEPIA analysis showed a significant difference in *ESR1* expression levels between breast cancer tissues and normal tissues as well (Supplementary Figure 1, $p < 0.001$). Our statistical results revealed the connection between the *ESR1* SNPs (rs9383938, rs1801132, and rs2228480) and the breast cancer risk in Chinese Han population, and confirmed the important role of *ESR1* in breast cancer.

The rs9383938 is located in the 5' untranslated region of *ESR1.* Previous work reported the evidence of rs9383938 associations with Mammographic density (MD) which is one of the primary known risk factors of breast cancer and is independent of other risk factors. [22] It seems likely that rs9383938 influence the risk of breast cancer through impact the MD. Another research has shown that rs9383938 variants were bound up with breast cancer in the northern European population ($p = 1.41 \times 10^{-7}$). [17] In a subsequent study, the rs9383938 showed a statistically important association with male breast cancer in England and Wales ($p = 0.004$). [23] In the present study, we proved

that rs9383938 was a susceptibility site increasing the breast cancer susceptibility among Chinese women. The predicted results of the database indicated that rs9383938 might be related to the enhancer histone marks, and therefore influence *ESR1* gene expression, which in turn affects breast cancer susceptibility.

The rs2234693 is located in the first intron of *ESR1* and has been demonstrated to be associated with several gynecological tumor such as breast cancer, and ovarian cancer. Early studies have indicated that the rs2234693-C provides a functional binding site for transcription factor B-Myb, to increasing transcriptional activity in downstream [24], which uncovers its role in breast cancer development. The relationship between rs2234693 and breast cancer risk is attract more attention, however, the correlation strength varied by ethnicity. [25] In this study, the relation of rs2234693 and breast cancer was not detected among Chinese Han subjects, which may be due to the ethnical genetic background of our studied samples.

In the published researches, rs2228480 was identified to be linked with the risk of regional lymph node metastasis (Iran), [26] distant metastases (Romania), and the breast cancer risk in menopausal women (India). [27, 28] In our study, rs2228480 was a susceptibility locus to breast cancer in patients diagnosed before age 50 years. Thus, the identification of rs2228480 genotype is supposed to be used in breast cancer risk prediction among individuals aged younger than 50 years.

Rs1801132 is in codon 325 (325Pro) of exon 4 of *ESR1,* which is a hormone binding domain. Moreover, Rs1801132 is found to affect mRNA stability and translation efficiency and is predicted to be an exon splicing enhancer. [29] In previous literature, rs1801132 was associated with an increased risk of breast cancer in European and African American women. [30] But there were some inconsistent evidences in other studies, which might because of the differences in sample size and ethnical genetic backgrounds. Xu Hu et al. indicated that they have not observe statistically significant association between the rs1801132 and breast cancer as Wang et al. did [25, 31]. In this study, we found that rs1801132 showed stronger associations with the status of ER, PR and Her–2 in the Chinese Han population. It means that the polymorphism of this locus may increase the susceptibility to triple-negative breast cancer (TNBC), which accounts for approximately 10% of all cases and has the worst prognosis and survival rates. This founding implied that rs1801132 might play a crucial role in the pathogenesis of TNBC. However, the underlying mechanism of this finding is unclear by now. Therefore, further experiments should be prepared and conducted to support our findings.

Conclusion

Taken together, our case-control study indicates that the polymorphismsin *ESR1* may enhance the susceptibility of breast cancer in Chinese Han women. In-depth functional studies and large sample size studies are still required to elucidate the impact of *ESR1* polymorphisms on breast cancer.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the First Affiliated Hospital of Xi'an Jiaotong University School of Medicine committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication

All participants were informed in writing and verbally of the procedures and purpose of this study, and signed written informed consent forms.

Availability of data and materials

The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest

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Not applicable.

Authors' contributions

YW and XLW carried out the studies, data analyses and drafted the manuscript. ZH and CTZ participated in the design of the study and performed the statistical analysis. YWC conceived of the study and participated in its design and coordination and helped draft the manuscript. XHZ and ZQB designed, coordinated, and supervised the study and critically reviewed and discussed the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Characteristics of breast cancer cases and healthy controls

Characteristics	Cases, n (%)	Controls, n (%)	*p* value [a]
Total participants	503	503	
Age (mean ± SD)	51.90 ± 9.55	52.04 ± 9.52	0.702
ER status			
Negative	154 (31.2%)		
Positive	340 (67.6%)		
Unavailable	9 (1.2%)		
PR status			
Negative	201 (40.0%)		
Positive	292 (58.0%)		
Unavailable	10 (2.0%)		
Her-2 status			
Negative	85 (16.9%)		
Positive	260 (51.7%)		
Unavailable	158 (31.4%)		
LN metastasis			
Negative	115 (43.1%)		
Positive	114 (42.7%)		
Unavailable	38 (14.2%)		
Stage			
0 ~ II	259 (51.5%)		
III ~ IV	215 (42.7%)		
Unavailable	29 (5.8%)		
Tumor size			
< 2 cm	98 (19.5%)		
≥ 2 cm	295 (58.6%)		
Unavailable	110 (21.9%)		

ER: Estrogen receptor; PR: Progesterone receptor; Her-2: human epidermal growth factor receptor 2; LN: Axillary lymph node;

[a] *p* values were calculated with independent sample *t*-test.

Table 2 Basic information of candidate SNPs in the study

SNP ID	Genes	Chromosome	Position	Alleles A/B	MAF Case	MAF Control	$p^{\dagger}$	OR (95%CI)	HaploReg
rs9383938	ESR1	Chr6	151666222	T/G	0.413	0.359	**0.013***	1.26 (1.05 – 1.50)	Enhancer histone marks, NHGRI/EBI GWAS hits
rs2234693	ESR1	Chr6	151842200	C/T	0.384	0.379	0.837	1.02 (0.85 – 1.22)	Motifs changed
rs1801132	ESR1	Chr6	151944387	C/G	0.494	0.496	0.929	0.99 (0.83 – 1.18)	SiPhy cons, Proteins bound, Motifs changed, GRASP QTL hits
rs2228480	ESR1	Chr6	152098960	A/G	0.230	0.225	0.790	1.03 (0.84 – 1.27)	Enhancer histone marks, DNAase, Proteins bound

SNP = single nucleotide polymorphism, MAF = minor allele frequency, OR = odds ratio, 95%CI = 95%confidence interval.

$^{\dagger}p$ values were calculated from two-sided χ^2 test.

*Bold values indicate statistical significance ($p < 0.05$).

Table 3 Analysis of association between *ESR1*polymorphisms and risk of breast cancer

SNP ID	Model	Genotype	Case	Control	Crude		Adjusted[a]	
					OR (95%CI)	$p^{\dagger}$	OR (95%CI)	$p^{\dagger}$
rs9383938	Codominant	GG	182	214	1			
		GT	227	217	1.23 (0.94 - 1.61)	0.135	1.23 (0.94 - 1.61)	0.139
		TT	94	72	1.54 (1.07 - 2.21)	**0.021***	1.54 (1.07 - 2.22)	**0.021***
	Dominant	GG	182	214	1		1	
		GT-TT	321	289	1.31 (1.01 - 1.68)	**0.039***	1.31 (1.01 - 1.68)	**0.040***
	Recessive	GG-GT	409	431	1		1	
		TT	94	72	1.38 (0.98 - 1.92)	0.062	1.38 (0.99 - 1.93)	0.059
	Additive	—	—	—	1.24 (1.04 - 1.47)	**0.017***	1.24 (1.04 - 1.48)	**0.017***
rs2234693	Codominant	TT	196	205	1			
		TC	228	212	1.13 (0.86 - 1.48)	0.394	1.13 (0.86 - 1.48)	0.395
		CC	79	84	0.98 (0.68 - 1.42)	0.929	0.91 (0.67 - 1.40)	0.869
	Dominant	TT	196	205	1		1	
		TC-CC	307	296	1.09 (0.84 - 1.40)	0.528	1.08 (0.84 - 1.39)	0.547
	Recessive	TT-TC	424	417	1		1	
		TT	79	84	0.92 (0.66 - 1.29)	0.649	0.91 (0.65 - 1.28)	0.591
	Additive	—	—	—	1.02 (0.86 - 1.21)	0.843	1.01 (0.85 - 1.21)	0.891
rs1801132	Codominant	GG	131	128	1			
		GC	247	251	0.96 (0.71 - 1.30)	0.798	0.96 (0.71 - 1.29)	0.771
		CC	125	124	0.91 (0.70 - 1.40)	0.932	0.99 (0.70 - 1.40)	0.933
	Dominant	GG	131	128	1		1	
		GC-CC	372	375	0.97 (0.73 - 1.29)	0.829	0.97 (0.73 - 1.28)	0.810
	Recessive	GG-GC	378	379	1		1	
		CC	125	124	1.01 (0.76 - 1.35)	0.942	1.01 (0.76 - 1.35)	0.922
	Additive	—	—	—	0.99 (0.83 - 1.18)	0.929	0.99 (0.83 - 1.18)	0.930
rs2228480	Codominant	GG	301	303	1			
		GA	173	174	1.00 (0.77 - 1.30)	0.995	1.00 (0.77 - 1.31)	0.981
		AA	29	26	1.12 (0.65 - 1.95)	0.681	1.13 (0.65 - 1.96)	0.670
	Dominant	GG	301	303	1		1	
		GA-AA	202	200	1.02 (0.79 - 1.31)	0.898	1.02 (0.79 - 1.31)	0.881
	Recessive	GG-GA	474	477	1		1	
		AA	29	26	1.12 (0.65 - 1.94)	0.678	1.12 (0.65 - 1.94)	0.668
	Additive	—	—	—	1.03 (0.84 - 1.26)	0.792	1.03 (0.64 - 1.27)	0.775

[a]Adjusted for age and sex in a logistic regression model.

$^{\dagger}p$ values were calculated from wald test.

*Bold values indicate statistical significance ($p < 0.05$).

Table 4 Analysis of association between *ESR1* polymorphisms and risk of breast cancer with stratified by age

SNP ID	Model	Genotype	< 52 years				≥ 52 years			
			Case	Control	OR(95%CI)	*p*†	Case	Control	OR(95%CI)	*p*†
rs9383938	Codominant	GG	80	99	1		102	115	1	
		GT	109	103	1.33 (0.89 - 1.98)	0.168	118	114	1.17 (0.81 - 1.70)	0.408
		TT	48	33	1.82 (1.07 - 3.10)	**0.028***	46	39	1.33 (0.81 - 2.21)	0.262
	Dominant	GG	80	99	1		102	115	1	
		GT-TT	157	136	1.45 (0.99 - 2.10)	0.054	164	153	1.21 (0.86 - 1.71)	0.278
	Recessive	GG-GT	189	202	1		220	229	1	
		TT	48	33	1.56 (0.96 - 2.54)	0.073	46	39	1.23 (0.77 - 1.96)	0.384
	Additive	—	—	—	1.35 (1.04 - 1.74)	**0.024***	—	—	1.16 (0.91 - 1.47)	0.231
rs2228480	Codominant	GG	134	153	1		167	150	1	
		GA	86	72	1.36 (0.92 - 2.01)	0.122	87	102	0.77 (0.53 - 1.10)	0.146
		AA	17	10	2.01 (0.88 - 4.56)	0.096	12	16	0.67 (0.31 - 1.47)	0.317
	Dominant	GG	134	153	1		167	150	1	
		GA-AA	103	82	1.44 (0.99 - 2.08)	0.056	99	118	0.75 (0.53 - 1.06)	0.108
	Recessive	GG-GA	220	225	1		254	252	1	
		AA	17	10	1.80 (0.80 - 4.04)	0.154	12	16	0.74 (0.34 - 1.60)	0.447
	Additive	—	—	—	1.39 (1.02 - 1.88)	**0.036***	—	—	0.79 (0.59 - 1.05)	0.107

†*p* values were calculated from wald test.

***Bold values indicate statistical significance ($p < 0.05$).

Table 5 The association between rs1801132 and clinical and histological features of BC

Variables		$p^{\dagger}$, OR (95% CI)					
		Allele	Homozygote (CC)	Heterozygote (GC)	Dominant	Recessive	Additive
ER	(+)	1.00					
	(−)	**0.003**, 1.52(1.16-1.99)	**0.003**, 2.34(1.34-4.08)	**0.040**, 1.69(1.02-2.78)	**0.008**, 1.89(1.18-3.03)	**0.023**, 1.64(1.07-2.51)	**0.003**, 1.52(1.16-2.00)
PR	(+)	1.00					
	(−)	**0.001**, 1.56(1.21-2.01)	**0.0005**, 2.55(1.51-4.31)	**0.009**, 1.85(1.17-2.94)	**0.001**, 2.06(1.33-3.20)	**0.013**, 1.69(1.12-2.55)	**0.001**, 1.59(1.23-2.06)
Her-2	(+)	1.00					
	(−)	**0.034**, 1.48(1.04-2.10)	**0.028**, 2.31(1.09-4.91)	**0.006**, 2.59(1.32-5.09)	**0.006**, 2.49(1.30-4.76)	0.522, 1.19(0.69-2.08)	**0.035**, 1.45(1.03-2.04)
Tumor size	< 2 cm	1.00					
	≥ 2 cm	0.201, 1.24(0.89-1.71)	0.120, 1.77(0.86-3.62)	0.302, 0.74(0.43-1.31)	0.876, 0.96(0.56-1.65)	**0.011**, 2.17(1.19-3.94)	0.133, 1.29(0.93-1.79)
LN metastasis	(−)	1.00					
	(+)	0.301, 1.20(0.85-1.68)	0.368, 1.37(0.69-2.69)	0.620, 1.17(0.64-2.14)	0.475, 1.23(0.70-2.18)	0.447, 1.23(0.72-2.11)	0.367, 1.17(0.83-1.64)
Stage	0~II	1.00					
	III~IV	0.380, 1.12(0.87-1.45)	0.340, 1.28(0.77-2.13)	0.664, 1.10(0.71-1.73)	0.483, 1.16(0.76-1.77)	0.388, 1.20(0.79-1.81)	0.339, 1.13(0.88-1.46)

ER: Estrogen receptor; PR: Progesterone receptor; Her-2: human epidermal growth factor receptor 2; LN: Axillary lymph node.

Allele: G > C; Homozygote: CC; Heterozygote: GC; Dominant: GG vs GC-CC; Recessive: GG-GC vs CC.

$^{\dagger}p$ values were calculated from wald test.

Bold values indicate statistical significance ($p < 0.05$).

Supplementary Files

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