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Original Article

The association of PD-L1 gene polymorphisms with non-small-cell lung cancer susceptibility and clinical outcomes in a Chinese population

Xiaolong Zhao, Yu Peng, Xuemei Li, Shiheng Zhang, Yang Peng, Jinlu Shan, Mengxia Li, Nan Dai, Yan Feng, Chengxiong Xu, Dong Wang

Cancer Center, Daping Hospital, Army Medical University (Third Military Medical University), Chongqing, China

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Abstract: Objective: To investigate the influence of PD-L1 polymorphisms on the susceptibility of non-small-cell lung cancer (NSCLC) and the prognosis of NSCLC patients who undergo platinum-based chemotherapy. Materials and methods: 9 single nucleotide polymorphisms (SNPs) in the PD-L1 gene, including rs822336 (G>C), rs822337 (T>A), rs10815225 (G>C), rs7866740 (C>G), rs866066 (C>T), rs822338 (C>T), rs2890657 (C>G), rs2890658 (C>A), and rs229136 (C>G) were selected for this study. Genotyping was performed in 281 advanced NSCLC patients and 251 healthy volunteers using the matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) method. Results: The G allele of PD-L1 rs7866740 was significantly related to the risk of NSCLC. Compared with the C allele, the G allele an increase the risk of NSCLC (OR=3.532, 95% CI: 1.232-10.129, *P*=0.001). In terms of the clinical outcomes, PD-L1 rs2890658 C>A was significantly associated with both a worse progression-free survival (adjusted HR=1.367, 95% CI=1.0-1.8, *P*=0.038) and a worse overall survival (adjusted HR=1.402, 95% CI=1.0-1.9, *P*=0.026) of NSCLC patients. PD-L1 rs822336 G>C was significantly related to a worse overall survival (adjusted HR=1.393, 95% CI=1.1-1.8, *P*=0.021). Conclusions: PD-L1 rs7866740 C>G may play a role in the pathogenesis of NSCLC. PD-L1 rs2890658 C>A and rs822336 G>C are related to the prognoses of patients receiving platinum-based chemotherapy.

Keywords: Non-small-cell lung cancer, PD-L1, polymorphism, susceptibility, prognosis

Introduction

Lung cancer, specifically non-small-cell lung cancer (NSCLC) [1], is the most common malignant tumor in the world, and its morbidity and mortality rank first among various cancers [2]. It is generally known that NSCLC can be caused by the interaction of a variety of genetic and environmental factors [3]. The mutations in some genes are related to a susceptibility for NSCLC, while some mutations can predict the curative effect and guide individualized treatment, such as P53, ALK, KRAS, and EGFR. Programmed death ligand-1 (PD-L1), the third member of the costimulatory molecule B7 superfamily [4], acts as the negative part of immune response regulation and has a wide range of expressions in non-small-cell lung cancer cells [5]. The interaction of PD-1/PD-L1 can down-regulate the T cell immune response and plays a critical role in the process of tumor cells evading host immune surveillance [6]. The latest findings indicate that the anomalous expression of PD-L1 can not only affect the immunological reaction, leading to autoimmune diseases and tumors [7], it can also affect the clinical prognosis of various tumors [8].

Consistently, single nucleotide polymorphisms (SNP) in some specific loci of PD-L1 play a potent role in affecting the susceptibility and prognosis of NSCLC [9, 10]. However, some results are paradoxical and need to be further verified. Therefore, we examined 9 SNPs (rs822336 G>C, rs822337 T>A, rs10815225 G>C, rs7866740 C>G, rs866066 C>T, rs822338 C>T, rs2890657 C>G, rs2890658 C>A, and rs2297136 A>G), which are located in the wide-ranging regions of the PD-L1 gene, to get a better understanding of the PD-L1 gene

SNPs' effect on susceptibility and prognosis in NSCLC.

Materials and methods

Study population

From July 2007 to July 2012, a total of 281 patients diagnosed with NSCLC at Daping Hospital, Army Medical University (Third Military Medical University), were included in the study. None of the patients had received any treatment prior to this study, such as chemotherapy or radiotherapy, and none had any other types of malignancy. All the cases were in stages IIIA, IIIB, or IV and they underwent standardized platinum-based chemotherapy. The control group consisted of 251 volunteers who received health check-ups at the same time and did not suffer from cancer or any other serious diseases. The study was approved by the Ethics Committees of Daping Hospital and was conducted in accordance with the Declaration of Helsinki.

Prognosis evaluation

205 of the NSCLC patients had follow-up records and were used for the prognosis evaluation. The time from the initiation of chemotherapy to the disease progression (death before disease progression was also considered as disease progression) was defined as progression-free survival (PFS). The time from the initiation of chemotherapy to death was defined as overall survival (OS). The follow-up deadline for the survival data was May 2013. The patients' disease progression was assessed using CT scans according to Response Evaluation Criteria in Solid Tumor (RECIST, version 1.1) [11].

Genotyping

Genomic DNA was isolated from venous blood samples (2 ml each) using SE Blood DNA Kits (Omega Bio-Tek, Norcross, GA, USA) according to manufacturer's instructions. DNA is amplified using the following steps: denaturation at 94°C for 15 min, then denaturation for 20 s at 94°C for each cycle (45 cycles), hybridization at 56°C for 30 s, and 1 min of elongation at 72°C, 3 min of a final extension at 72°C (the sequences of the primers are shown in Table S1). The SNPs were genotyped using MALDI-TOF MS in a MassARRAY system (Sequenom, San Diego, California, USA) as previously described [12].

Statistical analysis

Social Package for Statistical Studies (SPSS 19.0) software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis of the data. The heredity equilibrium and the allele frequencies of the PD-L1 SNPs were evaluated using Hardy-Weinberg tests and chi-square tests, respectively. The demographic data of the patients and the controls were compared using Student's t-tests and chi-square tests. A logistic regression analysis was utilized to analyze the odds ratios (OR) and the 95% confidence intervals (95% CIs). The Kaplan-Meier method was performed during the survival analysis and s log-rank test was calculated. Survival curves for the NSCLC patients were calculated using a Kaplan-Meier analysis, and the statistical significance was evaluated using a log-rank test. The hazard ratios (HRs) and 95% CIs of the potential prognostic factors were assessed using univariate and multivariate Cox regression analyses. Because of the small number of patients with homozygous mutant genotypes in rs822336, rs822337, rs10815225, rs7866740, rs866066, rs890658, and rs2297136, we combined the mutant and heterozygous genotype groups in the analysis, as suggested in other studies [13]. Two-sided *P* values less than 0.05 were considered statistically significant.

Results

Demographic characteristics

The demographic data of the enrolled population are shown in **Table 1**. The mean age of the individuals was 58.4 years-old (range: 27 to 84 years old) in the case group and 57.2 years-old (range: 33 to 83 years old) in the control group. 226 (80.4%) of the cases and 205 (81.7%) of the controls were male. Individuals with a history of smoking accounted for 61.6% and 57.8% in the case and control groups, respectively. There were no significant differences in age, sex, or smoking status between the two groups (P>0.05).

Associations of PD-L1 gene polymorphisms with susceptibility to NSCLC

The genotype distributions of the 9 SNPs in the two groups are shown in Table S2. All the SNP

Table 1. Characteristics of the NSCLC patients and controls

Characteristic	Cases (n=281)	Controls (n=251)	*P*
Age, years (means ± SD)	58.4±9.9	57.2±10.3	0.160
Sex			
Male	226 (80.4%)	205 (81.7%)	0.714
Female	55 (19.6%)	46 (18.3%)	
Smoking status			
Never smokers	108 (38.4%)	106 (42.2%)	0.377
Ever smokers	173 (61.6%)	145 (57.8%)	
Histological cell type			
Adenocarcinoma	158 (56.2%)		
Squamous cell	96 (34.2%)		
Others*	27 (9.6%)		
Tumor stage			
III	107 (38.1%)		
IV	174 (61.9%)		

*Others include mixed cell, neuroendocrine carcinoma, and undifferentiated carcinoma.

genotypes followed the Hardy-Weinberg equilibrium law (*P*>0.05). For PD-L1 rs7866740, there are 7 patients with homozygous GG, but there was no individual with GG in the control group. The frequencies of the C allele and the G allele among the NSCLC patients were 96.6% and 3.4%, and they were 99.4% and 0.6% in the control group (**Table 2**). The frequency of the G allele in the NSCLC patients was significantly higher than C allele's frequency (*P*=0.001). The risk for NSCLC in individuals carrying the G allele was significantly higher than it was in those carrying the C allele (OR=3.532, 95% CI=1.232-10.129).

Association of the PD-L1 gene polymorphisms with the clinical outcomes

The 205 NSCLC patients who had follow-up records were used for the prognosis evaluation. All the patients had been given platinum-based chemotherapy. For the tumor stages, stage III and stage IV disease accounted for 36.6% and 63.4% respectively. Histopathologically, 118 (57.6%) of the patients were diagnosed with adenocarcinoma, 73 (35.6%) patients were diagnosed with squamous cell carcinoma, and 14 (6.8%) patients had some other histology (**Table 3**).

The relevance of the PD-L1 polymorphisms to progression-free survival (PFS) and overall survival (OS) is shown in **Table 4**. PD-L1 rs822336 and rs2890658 are significantly related to the clinical outcomes after chemotherapy. The rs2890658 CA+AA was significantly associated with both a worse PFS (adjusted HR=1.367, 95% CI=1.0-1.8, *P*=0.038) and a worse OS (adjusted HR=1.402, 95% CI=1.0-1.9, *P*=0.026) of NSCLC patients (**Figure 1A** and **1B**). PD-L1 rs822336 GC+CC was significantly related to a worse OS (adjusted HR=1.393, 95% CI=1.1-1.8, *P*=0.021), but it was not significantly related to PFS (**Figure 1C**).

Discussion

One of the most important strategies that cancer cells use to evade immune attack is achieved by up-regulating the PD-L1 expression level. Once the immune function is reduced, the incidence rate of cancer increases greatly. In addition, changes in the immune microenvironment are closely related to the clinical prognoses of tumor patients [14]. For these reasons, the correlation between the PD-L1 genetic polymorphism and oncogenesis has been widely reported recently [15-18].

In our study, 9 SNPs in different regions of the PD-L1 gene were selected for genotyping to explore their relationship with susceptibility and clinical outcomes. First, our analysis revealed that the SNP genotype of PD-L1 rs7866740 is associated with the susceptibility of NSCLC, and the G allele plays a risk-related role in NSCLC. Notably, compared to the healthy controls, PD-L1 rs7866740 GG only appears in NSCLC patients, indicating that the GG genotype may be highly correlated with oncogenesis. PD-L1 rs7866740 is located in the 5' untranslated region (5'UTR), and whether this region can inhibit protein translation depends on its secondary structure or its binding to certain proteins [19]. It can be inferred that PD-L1 gene translation inhibition is weakened after some mutations in 5'UTR, which lead to an elevated PD-L1 protein expression, so the risk of developing lung cancer is increased.

Chemotherapy regimens based on platinum have always been the main treatment for ad-

Table 2. The association between the rs7866740 genotypes and the susceptibility to NSCLC

Genotype	Location	Cases	Control	*P* (HWE)[1]	OR (95% CI)	*P*
		N (%)	N (%)			
rs7866740	5'UTR			0.924		
CC		266 (95.7)	248 (98.8)		1	
CG		5 (1.8)	3 (1.2)		1.287 (0.524-3.162)	0.726[2]
GG		7 (2.5)	0 (0)		-	-
C-allele		537 (96.6)	499 (99.4)		1	
G-allele		19 (3.4)	3 (0.6)		3.532 (1.232-10.129)	0.001[2]

[1]Hardy-Weinberg equilibrium based on genotype frequency of the control group. [2]Fisher's exact test. Genotype failure in 3 cases for rs7866740.

Table 3. The characteristics of the NSCLC patients in the prognosis evaluation group

Characteristic	Cases (n=205)
Age, years (means ± SD)	58.2±9.8
Sex	
Male	159 (77.6%)
Female	46 (22.4%)
Smoking status	
Never smokers	82 (40.0%)
Ever smokers	123 (60.0%)
Histological cell type	
Adenocarcinoma	118 (57.6%)
Squamous cell	73 (35.6%)
Others[1]	14 (6.8%)
Tumor stage	
III	75 (36.6%)
IV	130 (63.4%)
ECOG PS	
0-1	189 (92.2%)
≥2	16 (7.8%)
Chemotherapy regimens	
Platinum-paclitaxel	154 (75.1%)
Platinum-gemcitabine	26 (12.7%)
Platinum-pemetrexed	19 (9.3%)
Other platinum combinations[2]	6 (2.9%)

[1]Others include mixed cell, neuroendocrine carcinoma, and undifferentiated carcinoma. [2]Other platinum combinations: vinorelbine plus cisplatin or etoposide plus cisplatin. Abbreviations: ECOG PS: Eastern Cooperative Oncology Group performance status.

vanced NSCLC for more than thirty years. However, up to now, there are still a considerable number of patients suffering from chemotherapy resistance. Some researchers found that the immune system plays an indispensable role in killing tumor cells after chemotherapy [20-22]. With the death of cancer cells, chemotherapy can increase the immunogenicity of tumors to further stimulate the tumor-specific immune response, eventually leading to the further killing of tumor cells [23, 24]. It is important to assess the expression of PD-1/PD-L1 in tumor tissues and their surrounding environment before and after chemotherapy [25]. Our study found that patients with rs2890658 C>A had a poor response to chemotherapy and a worse prognosis based on the shorter PFS and OS respectively. PD-L1 rs2890658 seems to be a meaningful loci, and many studies have found that its mutation is related to tumorigenesis [16, 26]. The function of the SNP in rs2890658 is still unclear. One possible explanation is that rs2890658 is located in or close to the transcription factor binding site, and the rs2890658 mutation may affect the binding and the function of the transcription factors, ultimately leading to the synthesis of proteins with incomplete functional domains. In addition, we found that the PD-L1 rs822336 GG genotype indicated a better OS in NSCLC patients, which coincided with the findings of Yeo et al. [10]. PD-L1 rs822336 is located in the promoter region, so a mutation of rs822336 may affect the transcription and the expression of PD-L1.

There is some debate on the relationship between PD-L1 expression and the prognosis of NSCLC. Some studies have found that a high expression of PD-L1 is related to a poor prognosis of NSCLC [27]. Conversely, some studies have found that patients who have high PD-L1 expressions have a better prognosis [28, 29]. Spranger et al. [30] showed that the expression of PD-L1 can reflect the state of the anti-tumor immune response and is positively correlated with the production of $CD8^+$ TILs as well as OS.

Table 4. PD-L1 polymorphisms and NSCLC clinical outcomes

Genotype	N (%)	Progression-free survival				Overall survival			
		MST (month)	Log-Rank *P*	HR (95% CI)*	*P**	MST (month)	Log-Rank *P*	HR (95% CI)*	*P**
rs822336									
GG	106 (51.7)	6		1		15		1	
GC+CC	99 (48.3)	6	0.344	1.114 (0.8-1.5)	0.455	12	0.044	1.393 (1.1-1.8)	0.021
rs822337									
TT	86 (58.3)	6		1		14		1	
TA+AA	119 (41.7)	6	0.459	1.041 (0.8-1.4)	0.782	12	0.142	1.241 (0.9-1.7)	0.147
rs10815225									
GG	168 (83.2)	6		1		12		1	
GC+CC	34 (16.7)	5	0.851	0.936 (0.6-1.4)	0.735	12	0.793	0.985 (0.7-1.5)	0.939
rs7866740									
CC	195 (95.6)	6		1		12		1	
CG+GG	9 (4.4)	6	0.571	0.813 (0.4-1.6)	0.553	16	0.507	0.824 (0.4-1.6)	0.575
rs866066									
CC	175 (85.4)	6		1		12		1	
CT+TT	30 (14.6)	4	0.932	1.085 (0.7-1.6)	0.686	15	0.527	1.172 (0.8-1.7)	0.434
rs822338									
CC	62 (30.8)	6		1		15		1	
CT	101 (50.2)	6		1.065 (0.8-1.5)	0.711	12		1.073 (0.8-1.5)	0.677
TT	38 (18.9)	6	0.364	1.021 (0.7-1.6)	0.924	12	0.429	1.167 (0.8-1.8)	0.486
rs2890657									
CC	50 (24.4)	6		1		14		1	
CG	98 (47.8)	7		0.972 (0.7-1.4)	0.872	13		0.907 (0.6-1.3)	0.583
GG	57 (27.8)	6	0.89	1.106 (0.7-1.6)	0.616	11	0.552	1.197 (0.8-1.8)	0.376
rs2890658									
CC	137 (66.8)	7		1		14		1	
CA+AA	68 (33.2)	6	0.033	1.367 (1.0-1.8)	0.038	11	0.016	1.402 (1.0-1.9)	0.026
rs2297136									
AA	129 (62.9)	6		1		12		1	
AG+GG	76 (37.1)	7	0.831	1.047 (0.8-1.4)	0.576	15	0.493	0.895 (0.7-1.2)	0.456

*Adjusted for age, gender, disease stage, histology, chemotherapy regimens, smoking status, performance status. Genotype failure in 3 cases for rs10815225, 1 case for rs7866740 and 4 cases for rs822338. Abbreviations: MST: median survival time; HR: hazard ratio.

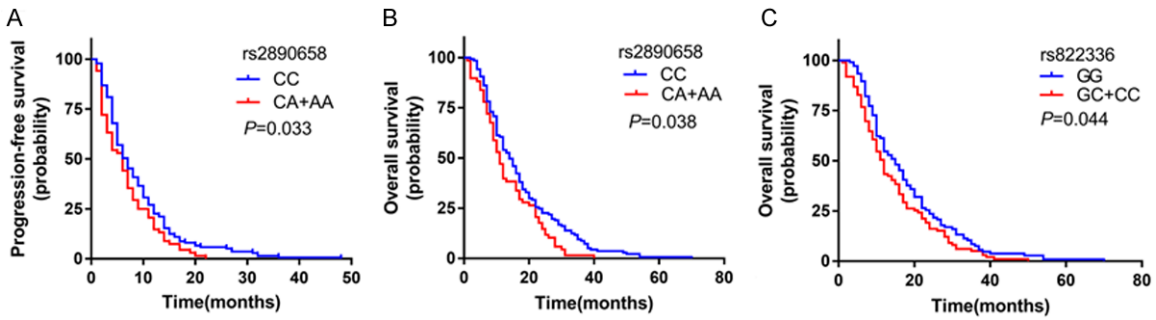

Figure 1. (A) Kaplan-Meier plots of the progression-free survival curve of PD-L1 rs 2890658 (CC vs. CA+AA). (B) Overall survival curves of rs2890658 (CC vs. CA+AA) and (C) rs822336 (GG vs. GC+CC).

So, it may be that the equilibrium of the capability between human immunity and tumor evasion decides the natural course and prognosis of the disease. According to our findings, we are more inclined to conclude that PD-L1 rs822336 G>C and rs2890658 C>A may lead to decreas-

ing expression or a dysfunction of PD-L1 and can lead to a worse prognosis for NSCLC patients. However, there are still a few studies suggesting that the expression of PD-L1 is not related to the prognosis of NSCLC [31, 32]. One possible assumption is that PD-L1 SNP might mainly affect the function of PD-L1 rather than its expression. Therefore, it is best to wait before making a final conclusion pending further studies.

In conclusion, the PD-L1 gene polymorphism is a potential biomarker of susceptibility and prognosis in NSCLC patients. The PD-L1 SNPs examination is conducive to predicting the prognosis of NSCLC patients and assists in treatment decision-making. There are still some limitations that might affect the results of this study. First, in view of the limited number of some genotypes, a further enlargement of the sample size is required. In addition, since there is no consensus on the role of the expression of PD-L1 in the development of NSCLC, further studies should confirm the effect of PD-L1 SNP on the protein expression as well as the function of PD-L1.

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Disclosure of conflict of interest

None.

Address correspondence to: Dong Wang, Cancer Center, Daping Hospital, Army Medical University (Third Military Medical University), Chongqing 400038, China. Tel: +86-236-875-7151; Fax: +86-236-889-4062; E-mail: dongwang64@hotmail.com

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Table S1. The sequence of the PCR primers

SNP	PCR primer 1	PCR primer 2
rs822336	ACGTTGGATGAACAGGTGGGAAAGATGAAC	ACGTTGGATGGTTGCTGATGGGAATTGAGG
rs822337	ACGTTGGATGACTTTGGTGACTGTGACCTC	ACGTTGGATGCATGTGTGTGTCATACACAG
rs10815225	ACGTTGGATGATTTGCTGCCTTGGGCAGAG	ACGTTGGATGTCGGGAAGCTGCGCAGAACT
rs7866740	ACGTTGGATGATTTGCTGCCTTGGGCAGAG	ACGTTGGATGTCGGGAAGCTGCGCAGAACT
rs866066	ACGTTGGATGTGATGCTAGGCTGGAGGTCT	ACGTTGGATGGCAGCTGGGATAACTTACAC
rs822338	ACGTTGGATGTCAGAGTATCATAGTTCTCC	ACGTTGGATGAATGCTTTCCAGAAAGGATG
rs2890657	ACGTTGGATGTCTAAACCTCATATCAGGGC	ACGTTGGATGTGTCTCCATTCGGATATGGG
rs2890658	ACGTTGGATGGGAGTGGCTGCTCACTATTA	ACGTTGGATGCAACAGAGAAAGACTCTGCC
rs2297136	ACGTTGGATGTCTCAACCTGTGGTTTAGGG	ACGTTGGATGTTAAGTCCCACATTGCCTGC

Table S2. Association between PD-L1 gene SNPs and susceptibility to NSCLC.

Genotype	Location	Cases N (%)	Control N (%)	*P* (HWE)[a]	OR (95% CI)	*P*
rs822336	promoter			0.210		
GG		149 (53.2)	131 (52.2)		1	
GC		108 (38.6)	106 (42.2)		0.896 (0.627-1.279)	0.545
CC		23 (8.2)	14 (5.6)		1.444 (0.714-2.922)	0.306
G-allele		406 (72.5)	368 (73.3)		1	
C-allele		154 (27.5)	134 (26.7)		1.022 (0.885-1.180)	0.768
rs822337	promoter			0.331		
TT		118 (42.1)	105 (41.8)		1	
TA		129 (46.1)	120 (47.8)		0.966 (0.673-1.386)	0.850
AA		33 (11.8)	26 (10.4)		1.140 (0.640-2.030)	0.656
T-allele		365 (65.2)	330 (65.7)		1	
A-allele		195 (34.8)	172 (34.3)		1.013 (0.886-1.159)	0.848
rs10815225	TF binding site			0.132		
GG		227 (81.9)	216 (86.4)		1	
GC		42 (15.2)	31 (12.4)		1.289 (0.782-2.126)	0.319
CC		8 (2.9)	3 (1.2)		2.537 (0.664-9.690)	0.173
G-allele		496 (89.5)	463 (92.6)		1	
C-allele		58 (10.5)	37 (7.4)		1.240 (0.956-1.608)	0.082
rs7866740	5'UTR			0.924		
CC		266 (95.7)	248 (98.8)		1	
CG		5 (1.8)	3 (1.2)		1.287 (0.524-3.162)	0.726[b]
GG		7 (2.5)	0 (0)		-	-
C-allele		537 (96.6)	499 (99.4)		1	
G-allele		19 (3.4)	3 (0.6)		3.532 (1.232-10.129)	0.001[b]
rs866066	intron			0.594		
CC		235 (83.9)	200 (79.7)		1	
CT		41 (14.6)	49 (19.5)		0.712 (0.451-1.123)	0.144
TT		4 (1.4)	2 (0.8)		1.702 (0.309-9.391)	0.542
C-allele		511 (91.3)	449 (89.4)		1	
T-allele		49 (8.7)	53 (10.6)		0.900 (0.738-1.098)	0.318
rs822338	intron			0.217		
CC		82 (29.7)	70 (28.1)		1	
CT		138 (50.0)	133 (53.4)		0.869 (0.586-1.287)	0.483
TT		56 (20.3)	46 (18.5)		1.019 (0.618-1.681)	0.941
C-allele		302 (54.7)	273 (54.8)		1	
T-allele		250 (45.3)	225 (45.2)		1.002 (0.882-1.139)	0.972

rs2890657	intron			0.747		
CC		67 (23.9)	64 (25.5)		1	
CG		135 (48.2)	128(51.0)		0.798 (0.526-1.209)	0.287
GG		78 (27.9)	59 (23.5)		0.792 (0.489-1.281)	0.342
C-allele		269 (48.0)	256 (51.0)		1	
G-allele		291 (52.0)	246 (49.0)		1.064 (0.937-1.209)	0.335
rs2890658	intron			0.651		
CC		194 (69.3)	169 (67.9)		1	
CA		73 (26.1)	71 (28.5)		0.896 (0.609-1.318)	0.576
AA		13 (4.6)	9 (3.6)		1.258 (0.525-3.017)	0.607
C-allele		461 (82.3)	409 (82.1)		1	
A-allele		99 (17.7)	89 (17.9)		0.993 (0.841-1.173)	0.935
rs2297136	3'UTR			0.511		
AA		180 (64.3)	174 (69)		1	
AG		92 (32.9)	69 (27.4)		0.896 (0.627-1.279)	0.545
GG		8 (2.9)	9 (3.6)		1.444 (0.714-2.922)	0.306
A-allele		452 (80.7)	417 (82.7)		1	
G-allele		108 (19.3)	87 (17.3)		1.076 (0.906-1.276)	0.394

[a]Hardy-Weinberg equilibrium based on genotype frequency of control group; [b]Fisher's exact test.