The rs1892818 polymorphism of ADRB3 is associated with coronary artery disease in a Han population

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Abstract

Background: Coronary artery disease (CAD) is the most common type of heart disease and causes high morbidity worldwide. The β_3-adrenergic receptor gene (*ADRB3*) is potentially linked to obesity, insulin resistance, diabetes, and hypertension based on its role in energy homeostasis and lipolysis in human adipose tissue. However, the relationship between *ADRB3* gene polymorphisms and CAD remains unclear. we sought to assess this association in the Han and Uygur populations of China.

Methods: We used the following two case-control studies: a Han population (308 CAD patients and 294 control subjects) and an Uygur population (259 CAD patients and 161 control subjects). All 1022 participants were genotyped for the two single nucleotide polymorphisms (SNPs) (rs1892818, rs9693898) of *ADRB3* using real-time polymerase chain reaction (TaqMan).

Results: We found that the rs1892818 CT genotype (8.5% compared with 3.9%, $P = 0.019$) and T allele (4.3% compared with 1.9%, $P = 0.021$) were more frequent in control subjects than in CAD patients of the Han, but not Uygur population. No significant differences in rs9693898 of *ADRB3* were found between CAD patients and control subjects for both populations. After adjusting for other confounders, logistic regression analysis suggested that the difference remained significant between the two groups in the Han population (CT compared with CC, $P = 0.036$, OR = 0.410, 95% CI: 0.178–0.944).

Conclusion: Our results suggest that *ADRB3* rs1892818 is associated with CAD in a Han population and that the CT genotype of *ADRB3* rs1892818 might be a protective factor for CAD in Han individuals.

Introduction

Coronary artery disease (CAD) is one of the major cardiovascular diseases resulting from interactions between genetics, the environment, and an unhealthy lifestyle, and it is the leading cause of death globally [1]. Advances in genome-wide association studies (GWASs) have provided insights into many different genetic factors that contribute to CAD. From such studies, more than 50 independent CAD-associated genetic loci have been found to be firmly associated with this disease, most of which reside in non-coding regions of the genome [2]. Many studies have demonstrated that different gene

variations are associated with CAD. Elnaggar et al. found that there are significant differences in 584C/T polymorphisms of the endothelial lipase-encoding gene with respect to CAD and high-density lipoprotein cholesterol (HDL-C) levels. Specifically, T-allele carriers with a higher HDL-C level are protected from CAD and this allele was found to be significantly associated with a decreased risk of CAD, independent of plasma HDL-C levels [3]. Xiao et al. performed a meta-analysis and found that the leptin rs7799039 variant might affect individual susceptibility to CAD [4]. Liu et al. identified five genes (encoding signal-induced proliferation-associated 1, transcription factor 21, SMAD family member 3, FES proto-oncogene, and platelet-derived growth factor receptor alpha) that might modulate CAD risk through human coronary artery smooth muscle cells, with all genes having relevant functional roles in vascular remodeling [5]. Furthermore, Li et al. provided additional evidence that a genetic variation in the platelet endothelial cell adhesion molecule 1-encoding gene, namely rs1867624, and hypoxia-inducible factor 1 subunit alpha gene, namely rs2057482, can modulate lipid levels in myocardial infarction patients [6].

The β_3 adrenergic receptor (β_3AR) belongs to the G protein-coupled receptor superfamily and has seven α-helix transmembrane regions that form six rings, with three each inside and outside of the cell, and comprises 402 amino acids [7]. β_3AR is mainly distributed in visceral adipose tissue, with low levels also distributed in other tissues such as myocardial tissue, gallbladder, the gastrointestinal tract, and prostate [8]. It participates in a series of lipolysis and energy regulatory processes mediated by adenylate cyclase and mediates fat metabolism-related reactions, catabolizing these molecules to produce heat [9]. A single nucleotide polymorphism (SNP) of the β_3 adrenergic receptor gene (ADRB3), namely Trp64Arg, has been deeply studied. Many studies have shown that this gene is associated with risk factors of CAD such as hypertension, insulin resistance, diabetes, atherosclerosis, abnormal lipid metabolism, and obesity [10–16]. In view of the important role of ADRB3 in these diseases, we hypothesized that it is an important factor for CAD and might also be a key candidate gene underlying the onset of this disease. However, this relationship had not been previously studied. The aim of the present study was thus to assess the association between two tag SNPs, rs1892818

and rs9693898, of ADRB3 and CAD using a case-control design with the Han and Uygur populations of China, to provide a scientific basis for CAD pathogenesis, intervention, and gene-targeted therapy.

Methods

Ethics approval of study protocol

Written informed consent was obtained from each participant after a full explanation of this study. The study protocol was conducted according to the standards of the Declaration of Helsinki, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University.

Study population

All 1022 participations were selected from Xinjiang, the northwestern part of China. From January 2016 to December 2018, 567 CAD patients were recruited from the First Affiliated Hospital of Xinjiang Medical University. This study population consisted of 308 Han patients and 259 Uygur patients with CAD. CAD patients with typical chest pain, electrocardiographic changes (new pathologic Q waves, at least 1 mm ST segment elevation in any two or more contiguous limb leads or a new left bundle branch block, or new persistent ST-T wave changes indicative of a non-Q wave myocardial infarction) and serum creatine kinase-MB isoenzyme (CK-MB) elevations (more than 3-fold higher than the upper reference limit) were examined by coronary angiography according to the guidelines [17]. The diagnostic criteria of CAD were defined as the presence of at least one significant coronary artery stenosis of more than 50% luminal diameter based on coronary angiography. CAD patients who had a congenital hypercoagulable status with proven disease-limiting life expectancy, malignancy, connective tissue disease, impaired renal function, or chronic inflammatory disease were excluded from the study.

The control (non-CAD) participants of 294 Han and 161 Uygur Chinese individuals were selected from the Cardiovascular Risk Survey [18,19]. This study comprised 14,618 subjects and is a multiple-ethnic, community-based, cross-sectional study designed to investigate the prevalence, incidence, and risk factors for cardiovascular diseases in the Han, Uygur, and Kazakh populations of Xinjiang, in the northwestern part of China. This study consisted of interviews, physical examinations, and data

from blood sample analyses. These subjects did not have any of the following conditions related to CAD: a positive family history, stable and unstable angina, myocardial infarction, evidence of CAD by electrocardiography and angiography, abnormality of regional wall motion, or relevant valvular abnormalities based on echocardiography. Both CAD patients and control subjects were matched for age and sex.

Data collection

Clinical data and information about the presence of traditional CAD risk factors including essential hypertension, diabetes mellitus (DM), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), smoking, drinking, height, and weight were obtained from all study participants by reviewing the patients' medical records. Essential hypertension was defined as a history of hypertension and/or average systolic blood pressure (SBP) ≥ 140 mmHg and/or an average diastolic blood pressure (DBP) ≥ 90 mmHg on at least two separate occasions according to medical examination and history. DM was defined as fasting plasma glucose levels ≥ 7.0 mmol/L (126 mg/dL), glucose levels ≥ 11.1 mmol/L (200 mg/dL) 2h after the administration of a 75g oral glucose load, a history of diabetes, or patients with a history of anti-diabetic medication use. Participants were considered smokers when consuming more than five cigarettes per day or nonsmokers when they had never smoked or had stopped smoking at least 1 year before sample collection. Patients who consumed 20ml or more of alcohol per day in the previous 6 months were considered alcohol users. The height and weight of each individual were recorded to calculate the body mass index (BMI) and determine the risk of obesity. BMI was calculated as weight divided by height squared (kg/m^2).

Blood collection and DNA extraction

Blood samples were collected from all subjects using a standard venipuncture technique with ethylene diamine tetraacetic acid (EDTA)-containing tubes and centrifuged at 4000 ´ *g* for 5 min to separate the plasma for a range of biochemical assays. DNA was extracted from the peripheral vein blood leukocytes using a whole-blood genome extraction kit (Beijing Bioteke Corporation, Beijing, China). DNA samples were stored at −80 ℃ for genotyping.

Biochemical measurements and genotyping

Serum and plasma collected for measurements were immediately frozen at −80 ℃ until use. Plasma concentrations of TG, TC, HDL-C, and LDL-C were measured using standard methods in the Central Laboratory of the First Affiliated Hospital of Xinjiang Medical University. Using the Haploview 4.2 software and the 1000 Genomes database, we obtained two tagging SNPs (rs1892818, rs9693898) for Chinese individuals using a minor allele frequency ≥ 0.05 and linkage disequilibrium patterns with r^2 ≥ 0.8 as a cutoff (Figure 1). We titrated the DNA concentration at 50 ng/mL. The rs1892818 and rs9693898 polymorphisms of *ADRB3* were detected by TaqMan single nucleotide polymorphism genotyping assays in a 7900 HT Standard real-time polymerase chain reaction (PCR) system, according to the manufacturer's recommendations (Applied Biosystems). Primers and probes used in the TaqMan SNP Genotyping Assays (Applied Biosystems) were chosen based on information available at the ABI website (www3.appliedbiosys tems.com/AB_Home/index.htm). PCR amplification was performed in a total reaction volume of 6 μL containing 3 μL of TaqMan Universal Master Mix, 0.15 μL probes, 1.85 μL ddH2O, and 1 μL DNA with the following amplification protocol: 95 ℃ for 10 min, 40 cycles of 95 ℃ for 15 s and 60 ℃ for 1 min. All 96-well plates were read using the Sequence Detection Systems (SDS) automation controller software v2.4 (ABI). The SDSv2.4 endpoint typing software package was used for automatic genotyping. The results are shown in Figure 2, and the three colors represent different genotypes. Variants in the SNP (rs1892818) of the *ADRB3* gene were classified into two genotypes, namely CC and CT genotypes, whereas rs9693898 of the *ADRB3* gene was classified into three genotypes, AA, AG, and GG (Figure 2).

Statistical analyses

The normality of parameters was assessed by a Shapiro–Wilk test. Continuous statistics that met the normality assumption are shown as the mean and standard deviation (mean ± SD), and differences in continuous variables between the CAD patients and control subjects were analyzed using an independent-sample t-test. As some measurement data in this study did not meet the normality assumption, they were described as medians (interquartile range) and compared with the Mann–Whitney U test. The Hardy–Weinberg equilibrium was assessed by Chi-square analysis. Differences in

enumeration data between CAD patients and control subjects were analyzed using the Chi-square test, as were differences in distributions of genotypes and alleles between CAD patients and control subjects. Logistic regression analyses with effect ratios (odds ratio [OR] and 95% confidence interval [CI]) were used to assess the contribution of the major risk factors. All statistical analyses were performed using SPSS 22.0 for Windows (SPSS Institute). A two-tailed value of $p < 0.05$ was considered statistically significant.

Results

Characteristics of study participants

The distribution of demographic characteristics of the study participants is shown in Table 1. Overall, a total of 567 CAD patients (mean age 57.87 ± 9.08 and 58.2% men) and 455 controls (mean age 56.96 ± 8.54 and 52.53% men) were recruited in the present study. No significant differences were observed in age, sex, BMI, TC, and LDL-C between CAD patients and control subjects. The following variables were significantly different between CAD patients and control subjects: plasma levels of TG and HDL-C and the prevalence of hypertension, diabetes, smoking, and drinking ($p < 0.05$ for all). In the Han population, no significant differences were observed in age, sex, BMI, TC, and LDL-C between CAD patients and control subjects. The following variables were significantly different between CAD patients and control subjects: plasma levels of TG and HDL-C and the prevalence of hypertension, diabetes, smoking, and drinking ($p < 0.05$ for all). In the Uygur population, no significant differences were observed in age, sex, BMI, TC, LDL-C, diabetes, and smoking between CAD patients and control subjects. The following variables were significantly different between CAD patients and control subjects: plasma levels of TG and HDL-C and the prevalence of hypertension and drinking ($p < 0.05$ for all).

ADRB3 genotype and allele frequencies in the two groups

Rs1892818 and rs9693898 were in Hardy–Weinberg equilibrium in both the case and control groups (all P > 0.05, Table 2). Table 3 shows the frequency of genotypes and alleles for the tested SNP (rs1892818) of the *ADRB3* gene. Overall, the results showed that the CT genotype (6.8% compared with 3.9%, p = 0.036) and T allele (3.4% compared with 1.9%, p = 0.038) were more frequent in the

control subjects than in the CAD patients. Further, we also found that the CT genotype (8.5% compared with 3.9%, p = 0.019) and T allele (4.3% compared with 1.9%, p = 0.021) were more frequent in the control subjects than in the CAD patients of the Han, but not Uygur, population. Table 4 shows the frequency of genotypes and alleles for the SNP (rs9693898) of the *ADRB3* gene. The distribution of rs9693898 genotypes and alleles showed no significant difference between CAD patients and control subjects both in Han and Uygur populations. To further confirm the relationship between the frequency of the CT variant of the rs1892818 SNP in the *ADRB3* gene and CAD risk in the Han population, multiple logistic regression analyses were performed by adjusting for confounding factors such as age, sex, hypertension, diabetes, smoking, drinking, and serum levels of TG and HDL-C and the difference remained significant (CT compared with CC, p = 0.036, OR = 0.410, 95%CI: 0.178–0.944; Table 5).

Discussion

CAD is a complex disease and its etiology and pathogenesis are likely polyfactorial due to the inheritance of several susceptibility genes, as well as multiple environmental factors [20, 21].Recently, there has been an increase in the in-depth research on the role of SNPs in the pathogenesis of CAD [22]. Ultimately, studying genetic backgrounds might provide new insights to explore diagnostic and therapeutic approaches for CAD [23]. In the present study, we found that rs1892818 variations in the ADRB3 gene are associated with CAD in a Han population. This was the first study to investigate common allelic variants (rs1892818, rs9693898) in ADRB3 and its association with this disease in Chinese populations.

β_3AR is an important component of the sympathetic nervous system. In cardiomyocytes, activation of endothelial nitric oxide synthase or neuronal nitric oxide synthase induced by activation of the β_3 receptor induces nitric oxide production and the activation of soluble guanylate cyclase, resulting in cGMP production and the activation of cGMP-dependent protein kinase G [24]. Previous studies have shown that β3AR is present in the cardiovascular system, mainly in the myocardium and endothelium, where it has a prominent role in regulating metabolism, angiogenesis, vasodilation, and relaxation for cardiac contractility [25–28]. Thus, this receptor is of high interest especially for new potential

therapeutic approaches for heart disease. ADRB3 is expressed in white and brown adipose tissues and in peripheral lymphocytes and is also involved in lipolysis and thermogenesis regulation [29]. The ADRB3 Trp64Arg polymorphism has been deeply studied with respect to its association with CAD by numerous researchers. As early as 1997, Higashi et al [30]. analyzed 83 Japanese patients with CAD and 107 healthy controls and found that the ADRB3 Trp64Arg variation was significantly correlated with this disease. Further, Xia et al [31]. recently studied Chinese patients with myocardial infarction, including 717 patients with myocardial infarction and 612 healthy controls, and found that the ADRB3 Trp64Arg mutation increased the risk of myocardial infarction. A possible reason for this is that when the ADRB3 gene is mutated, it might cause β3AR structural and functional changes, affecting lipid metabolism and leading to an increased risk of hypertension, diabetes, and obesity [32, 33]. Previously, we found that the rs2298423 polymorphism of ADRB3 in an Uygur population was correlated with serum TC and LDL-C levels, and that the G allele might be a risk factor for elevated levels of both [34]. However, the research conclusions were not consistent, as a number of studies in Germany [35], India [36], Saudi Arabia [37], and China [38] did not find a significant correlation between the ADRB3 Trp64Arg polymorphism and CAD.

Because the ADRB3 gene is polymorphic in the human beings, the rs1892818 and rs9693898 SNPs were selected in this study. We performed a case-control study to observe the relationship between genetic polymorphisms in this gene and CAD. Univariate analysis showed that the age and sex of the case and control groups were matched. Further, rs1892818 and rs9693898 were in Hardy–Weinberg equilibrium both in the case and control groups. Variants in the rs1892818 SNP of ADRB3 were classified into two genotypes, CC and CT, whereas those of rs9693898 were classified into three genotypes, namely AA, AG, and GG. We found that the CT genotype and T allele of rs1892818 were more frequent in the control subjects than in the CAD patients of the Han, but not Uygur population. Further, after adjusting for some confounders, the association remained significant, which indicated that the rs1892818 CT genotype might be considered a protective factor for CAD in Han individuals. In our study, we found that the distributions of rs1892818 genotypes and alleles did not show any significant difference between CAD patients and control subjects in the Uygur population. The reason

for these distinct results might be attributed to differences in ethnicity, lifestyle, diet, and sources of test samples. Considering domestic and foreign studies, there has been almost no research on these two SNPs. Therefore, additional larger-scale studies based on different populations with more detailed data on environmental exposure are required to verify these findings.

Conclusion

In brief, our study demonstrated that the ADRB3 rs1892818 polymorphism is significantly correlated with CAD, especially in Han populations. Additionally, the CT genotype of this polymorphism might be considered a protective factor for CAD in these individuals. This conclusion could be helpful to develop novel personalized CAD treatment approaches. Considering the sample size was relatively small, more research, based on large samples and multi-ethnic cohorts, on the association between ADRB3 gene polymorphisms and CAD is needed to further confirm our conclusions.

Declarations

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Authors' contributions

Jin-Yu Zhang and Qian Zhao contributed equally to this work. Jin-Yu Zhang and Qian Zhao performed all the experiments, analyzed all the data and prepared the manuscript, Yi-Ning Yang participated in the study design, supervised and assisted the statistical analysis and revised the manuscript. Other authors supervised the molecular genetic analysis and sample collection, assisted with input of clinical data. All the authors have read and approved the final manuscript.

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Availability of data and materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Written informed consent was obtained from each participant after a full explanation of this study. The study protocol was conducted according to the standards of the Declaration of Helsinki, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University.

Consent for publication

All authors have read and approved the manuscript for publication.

Competing interests

The authors declare that they have no conflict of interest.

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Abbreviations

ADRB3:β3-adrenergic receptor gene; BMI:body mass index; β3AR:β3 adrenergic receptor; CAD:Coronary artery disease; CK-MB:creatine kinase-MB isoenzyme; CI:confidence interval; DM:diabetes mellitus; DBP:diastolic blood pressure; EDTA:ethylene diamine tetraacetic acid; GWASs:genome-wide association studies; HDL-C:high-density lipoprotein cholesterol; LDL-C:low-density lipoprotein cholesterol; OR:odds ratio; PCR:polymerase chain reaction; SNP:single nucleotide polymorphism; SBP:systolic blood pressure; SDS:Sequence Detection Systems; TaqMan:real-time polymerase chain reaction; TC:total cholesterol; TG:triglycerides

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patients with coronary heart disease. Metab Clin Exp. 1999; 48(5):651-54.

Tables

Table 1. General characteristics of study participants

	Total				
	CAD patients	Control subjects	*p* value	CAD patients	C
Number (n)	567	455		308	
Age (years)	57.87 ± 9.08	56.96 ± 8.54	0.101	58.67 ± 9.39	
Male sex, n (%)	330 (58.20)	239 (52.53)	0.070	177 (57.50)	
BMI (kg/m^2)	26.21 ± 3.71	26.30 ± 3.85	0.710	25.60 ± 3.49	
TG (mmol/L)	1.57 (1.17–2.13)	1.38 (1.00–2.07)	< 0.001*	1.56 (1.17–2.09)	1.
TC (mmol/L)	4.20 ± 1.09	4.19 ± 0.94	0.977	4.20 ± 1.11	
HDL-C (mmol/L)	1.03 (0.84–1.23)	1.09 (0.94–1.34)	< 0.001*	1.09 (0.90–1.30)	1.
LDL-C (mmol/L)	2.52 (2.00–3.06)	2.58 (1.931–3.04)	0.621	2.52 (1.99–3.11)	2.
Hypertension, n (%)	298 (54.6)	188 (43.8)	0.001*	174 (58.6)	
Diabetes, n (%)	151 (27.7)	65 (15.2)	< 0.001*	82 (27.6)	
Smoking, n (%)	204 (36.1)	122 (26.8)	0.002*	124 (40.4)	
Drinking, n (%)	295 (52.0)	178 (39.1)	< 0.001*	186 (60.4)	

Continuous variables were expressed as the mean ± SD and median (interquartile range). Categorical variables are expressed as numbers and percentages. The figures outside the parentheses are the numbers of cases and inside the parentheses are the percentages. * indicates significance ($p < 0.05$)

CAD, coronary artery disease; BMI, body mass index; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 2. Hardy–Weinberg equilibrium test of rs1892818 and rs9693898 genotype distribution in Han and Uygur patients v

		rs1892818			*p* value
		CC	CT	TT	
Han	CAD patients				
	Number of experimenters	296	12	0	0.727
	Number expected	296.1	11.8	0.1	
	Control subjects				
	Number of experimenters	269	25	0	0.446
	Number expected	269.5	23.9	0.5	
Uygur	CAD patients				
	Number of experimenters	249	10	0	0.751
	Number expected	249.1	9.8	0.1	
	Control subjects				
	Number of experimenters	155	6	0	0.810
	Number expected	155.1	5.9	0.1	

CAD, coronary artery disease.

Table 3. Association analyses between genotypes and rs1892818 polymorphism alleles in control subjects and patients with coronary artery disease

SNP	Polymorphisms		Total			Ha	
			CAD	Control	*p* value	CAD	Cont
Rs1892818	Genotype	CC	545 (96.1)	424 (93.2)	0.036*	296 (96.1)	269 (9
	n (%)	CT	22 (3.9)	31 (6.8)		12 (3.9)	25 (8
		TT	0 (0)	0 (0)		0 (0)	0 ((
	Allele	C	1112 (98.1)	879 (96.6)	0.038*	604 (98.1)	563 (9
	n (%)	T	22 (1.9)	31 (3.4)		12 (1.9)	25 (4

* indicates significance ($p < 0.05$). CAD, coronary artery disease.

Table 4. Association analyses between genotypes and rs9693898 polymorphism alleles in control subjects and patients with coronary artery disease

SNP	Polymorphisms		Total			Ha	
			CAD	Control	*P* value	CAD	Control
Rs9693898	Genotype	AA	412 (72.7)	324 (71.2)	0.527	228 (74)	208 (70.8
	n (%)	AG	142 (25.0)	124 (27.3)		73 (23.7)	83 (28.2
		GG	13 (2.3)	7 (1.5)		7 (2.3)	3 (1.0)
	Allele	A	966 (85.2)	772 (84.8)	0.826	529 (85.8)	499 (84.8
	n (%)	G	168 (14.8)	138 (15.2)		87 (14.2)	89 (15.2

CAD, coronary artery disease.

Table 5. Multiple logistic regression analysis of coronary artery disease patients and control subjects in the Han population

Risk factor	OR	95% CI
Genotype (CT vs. CC)	0.410	0.178–0.944
drinking	5.050	3.471–7.349
diabetes	2.296	1.433–3.678
constant	1.191	0.484–2.933

* indicates significance ($p < 0.05$). CI, confidence interval; OR, odds ratio

Figures

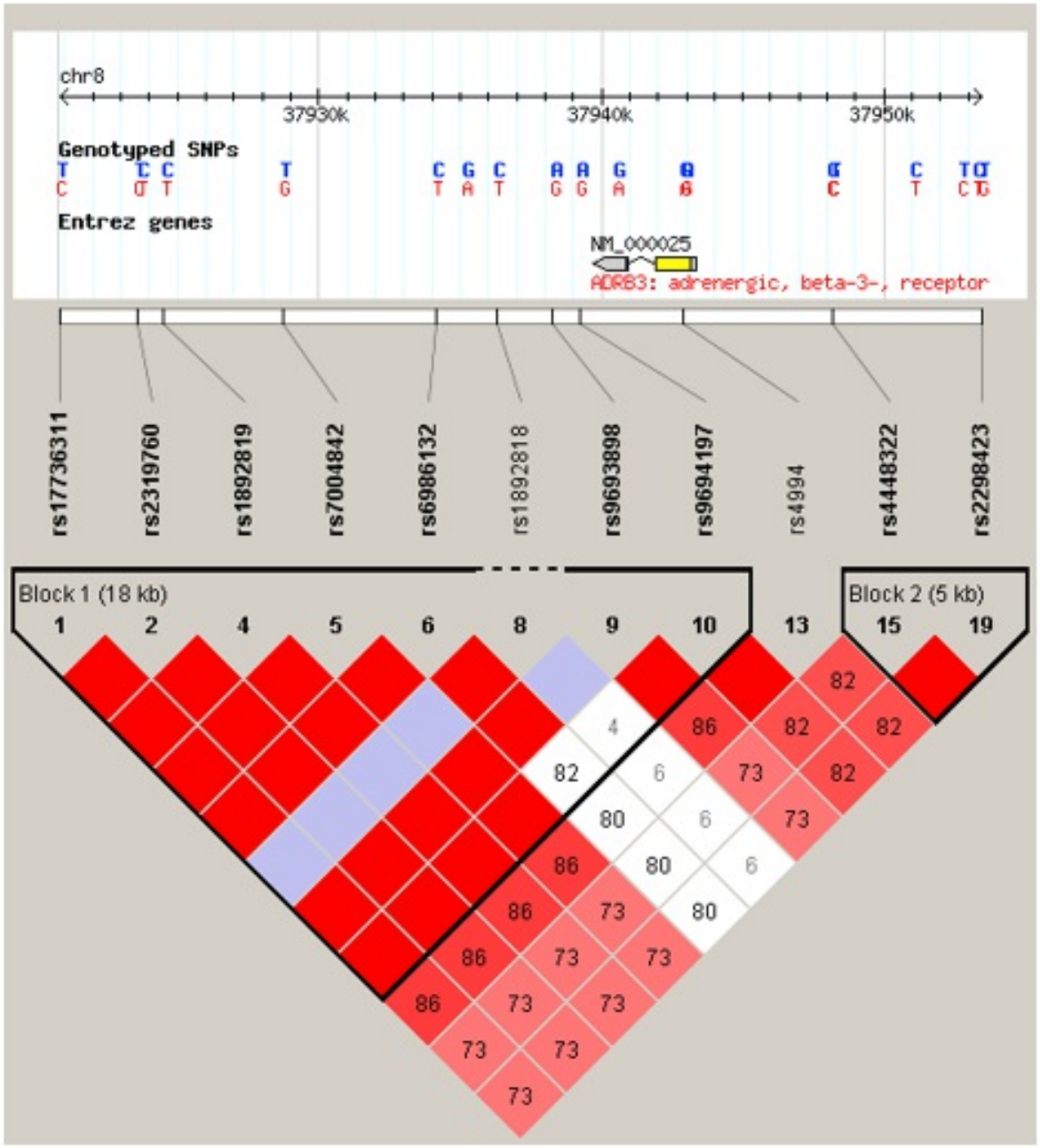
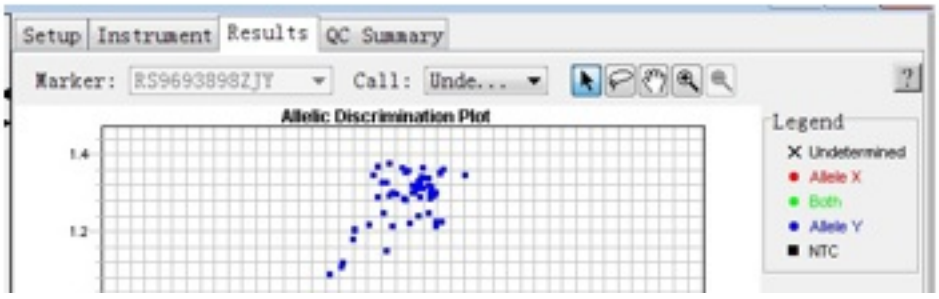
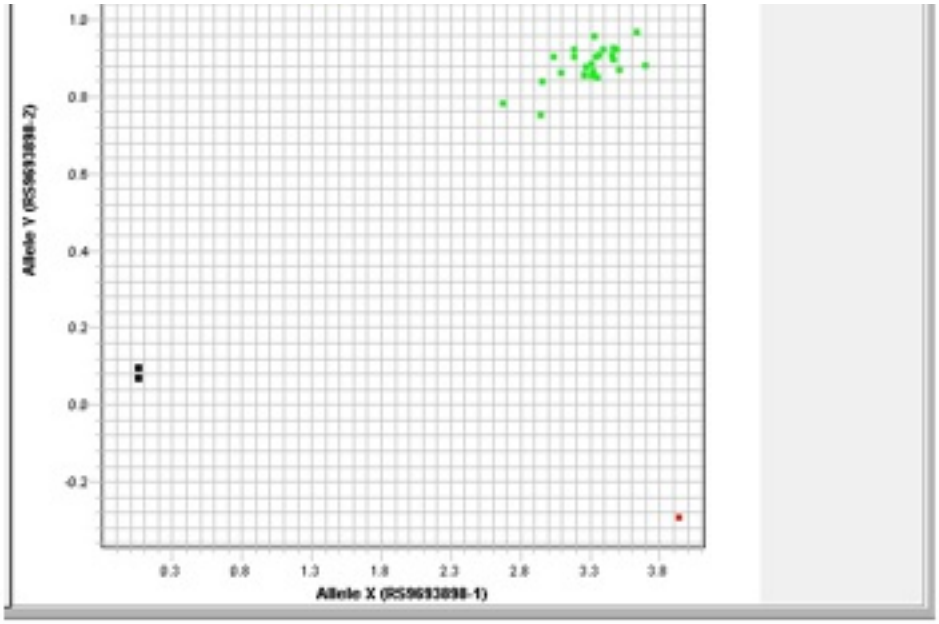


Figure 1

Selection of ADRB3 gene tag SNPs (rs1892818, rs9693898) by Haploview 4.2




Allele Y (RS9693898-2)
Allele X (RS9693898-1)

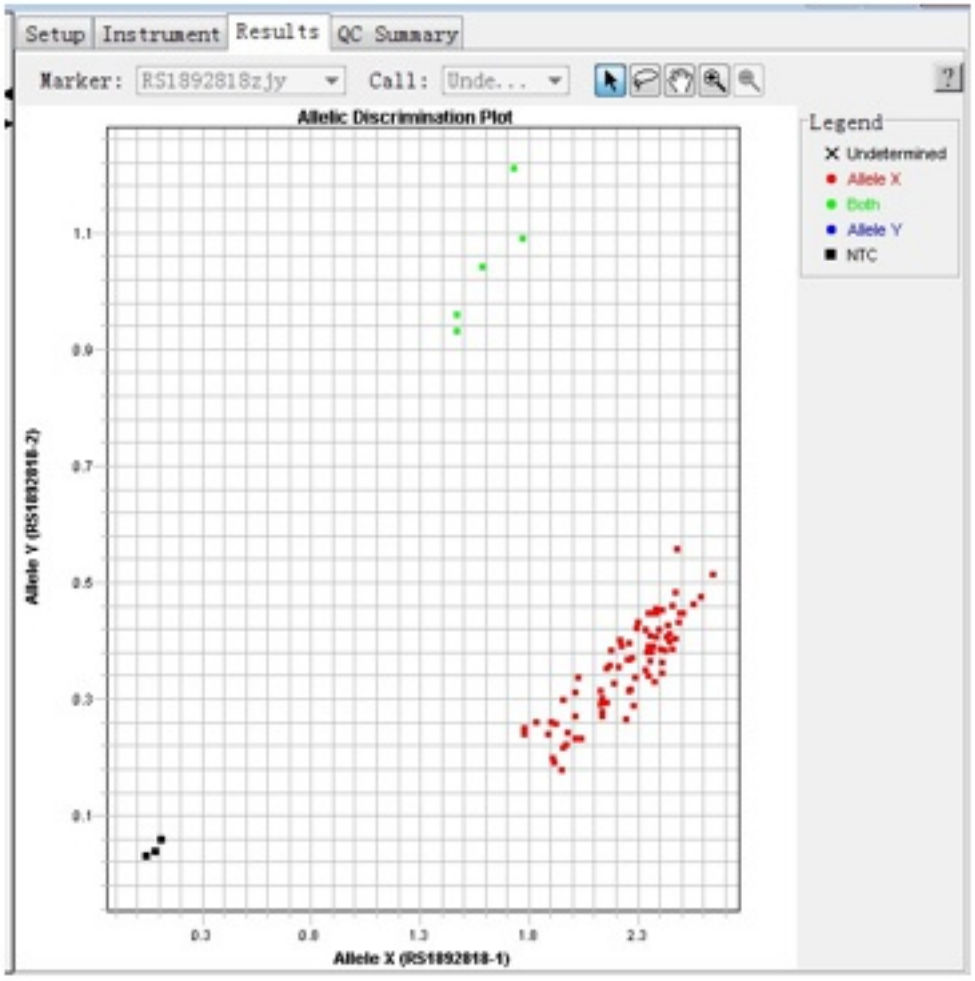

Setup
Instrument
Results
QC Summary
Marker: RS1892818zjy
Call: Unde...
Allelic Discrimination Plot
Legend
Undetermined
Allele X
Both
Allele Y
NTC
Allele Y (RS1892818-2)
Allele X (RS1892818-1)

Figure 2

Schematic diagram of Taqman genotyping results.