C deletion of the SLC39A8 gene polymorphism (rs74650330) increases the risk of coronary artery disease in individuals with low LDL cholesterol levels

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Research

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Abstract

Background: The genetic variant of *SLC39A8* is associated with several cardiovascular disease risk factors, including body mass index, systolic blood pressure (SBP), diastolic blood pressure (DBP), N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-density lipoprotein cholesterol (HDL-C) levels. The present study aimed to investigate the association between the *SLC39A8* SNPs rs13107325 and rs74650330 and CAD in the Han population in Jiangsu (China).

Methods: Genotyping of these SNPs was performed in 258 patients with CAD and 170 healthy controls using the base-quenched probe technique. The association between rs74650330 and blood lipid and glucose profiles was investigated.

Results: The rs13107325 polymorphism was not found in the 428 Chinese individuals enrolled in the current study. For rs74650330, individuals harboring the C allele had significantly higher HDL levels than those without this allele in the control group (P=0.039), while the opposite was true for LDL-C levels (P=0.046). Further analysis indicated that when LDL-C levels were lower than 2.365 mmol/L, subjects with C/del and del/del had a 7.293-fold increased risk of CAD compared with that of controls without the mutation (odds ratio: 7.293; 95% confidence interval: 0.953-55.79).

Conclusions: The results obtained in the current study suggested that the rs74650330 polymorphism is associated with reduced HDL-C and elevated LDL-C levels in healthy individuals. When LDL-C levels were lower than 2.365 mmol/L, the C/del genotype significantly increased the risk of CAD in the Chinese Han population.

Background

Globally, coronary artery disease (CAD) is a common cardiovascular disease that is the main cause of morbidity and mortality, particularly in developing countries [1]. CAD is a complex disease affected by a combination of environmental and genetic factors. A familial predisposition for CAD has been identified, with the heritability of CAD was estimated to be 40-60% [2]. Genome-wide association studies have revealed various genetic polymorphisms that contribute to disease susceptibility [3].

SLC39A8 (solute carrier family 39 member 8) is a member of the ZIP family of transporters located in the plasma membrane, which play a role in the transport of divalent metal ions such as zinc, manganese, cadmium and iron [4]. Several epidemiological studies have suggested that exposure to metals, including cadmium and lead, is associated with the development of high blood pressure and other cardiovascular diseases [5, 6]. Furthermore, previous studies have revealed that genetic mutations in the *SLC39A8* gene have an impact on human health [7, 8]. The single nucleotide polymorphism (SNP) rs13107325 located in exon 8 of the *SLC39A8* gene results in an amino acid change from alanine to threonine at position 391 (NC_000004.11:g.103188709C>T). Rs13107325 is one of the most pleiotropic variants in the human genome and is associated with several cardiovascular disease-associated risk factors, such as body mass index, systolic blood pressure (SBP), diastolic blood pressure (DBP), and N-terminal pro-B-type

natriuretic peptide (NT-proBNP), high-density lipoprotein cholesterol (HDL-C) and manganese levels [9-14]. Rs74650330 is an intronic SNP in the *SLC39A8* gene, which results in a deletion of the C allele, and is in close proximity to rs13107325. To date, the association between *SLC39A8* polymorphisms and CAD in the Chinese Han population has not been explored. The current study therefore investigated the association between *SLC39A8* SNPs and CAD in the Han population in Jiangsu (China) using molecular biology techniques.

Materials And Methods

Study subjects

A total of 258 patients (191 males and 67 females) with a mean age of 62.5±10.1 years who underwent coronary angiography and had at least one coronary artery with a diameter stenosis ≥ 50% were included. All subjects met the 1979 WHO diagnostic criteria for CAD [15]. A total of 170 healthy examinees (133 males and 37 females) with a mean age of 61.4±9.3 years for preventive check-ups, matched for sex and age with CAD patients, were enrolled as the control group. The control subjects had no prior history of CAD. For all participants, the exclusion criteria included cerebrovascular disease, severe hepatic and renal dysfunction, infections, malignant tumors and autoimmune diseases. The participants were recruited from the Medical Examination Center and Department of Cardiology of the Third Affiliated Hospital of Soochow University (Changzhou, China) from January 2006 to March 2007 and from June to September 2014. Written informed consent was obtained from the patients and healthy controls.

Collection of clinical data

A total of 2 mL of cubital venous blood was drawn from all the subjects after 12 h of fasting. The fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), HDL-C and low-density lipoprotein cholesterol (LDL-C) levels were subsequently measured using an automatic biochemical analyzer.

Preparation of DNA from peripheral blood samples

Blood (2 mL) was drawn from the cubital vein and stored in a microtainer tube containing EDTA-K_2 anticoagulant. Using a TIANamp Blood DNA Kit (TIANGEN Biotech, Co., Ltd., Beijing, China) to extract genomic DNA, DNA was dissolved in Tris-EDTA (TE) buffer and stored at -20 °C.

Vector construction

A vector representing the *SLC39A8* rs13107325 T homozygote genotype was constructed and used as an amplification template for validating the base-quenched probe technique. A 460-bp fragment of the *SLC39A8* gene was synthesized and cloned into the PUC57 vector by Sangon Biotech (Shanghai, China). The plasmid was subsequently extracted and used as a template for amplification.

Genotype identification of the *SLC39A8* SNPs rs13107325 and rs74650330

SNPs were identified using the base-quenched probe technique [16]. Primers and probes were designed according to the *SLC39A8* sequence in NCBI (NC_000004.12) on the Primer 5.0 platform (Table 1) and then synthesized by Sangon Biotech (Shanghai, China). Polymerase chain reaction (PCR) was carried out in a 25-μL reaction system containing 2 μL of DNA template, 2.5 μL of 10x buffer, 2.5 μL of magnesium chloride (25 mM), 0.5 μL of 4x dNTPs, 0.25 μL of TaqDNA polymerase (5 U/μL), 0.1 μL of each primer (100 μM) and 0.3 μL of the probe (10 μM). Thermal cycling was performed on a LightCycler (version 480II, Roche). The conditions were as follows: 2 min of denaturation at 95 °C, followed by 40 cycles at 95 °C for 10 s and 60 °C for 90 s. An analytical melting procedure involved incubation at 95 °C for 30 s and 30 °C for 4 min, which was increased to 80 °C (temperature transition rate: 0.1 °C/s) with continuous acquisition of fluorescence data. Forty-eight samples from the CAD group (n=24) and the control group (n=24) were randomly selected, and a 460-bp fragment from the PCR amplification product was sequenced on an automatic sequencer to verify the accuracy of the base-quenched probe technique (model 3730; Applied Biosystems; Shanghai; China).

Coronary angiography and Gensini score

All CAD patients underwent coronary angiography according to the Judkins method [17]. Two experienced cardiologists examined the angiographic findings, and the Gensini integral and number of coronary lesion branches were used to evaluate the degree of coronary stenosis [18].

Statistical analysis

Data analyses were performed using GraphPad Prism software (version 5.0). The Kolmogorov-Smirnov normality test was applied to assess the distribution of the data, and skewed data are shown as the median and interquartile range. The Mann-Whitney U test was used to compare differences in the general characteristics between the two groups. The deviation in the allele and genotype frequencies from the expected numbers was analyzed using Hardy-Weinberg equilibrium. Allele and genotype frequencies, odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by Chi-squared analyses or Fisher's exact test. Receiver operating characteristic (ROC) curve analysis was used to quantify the optimal thresholds for lipid and FBG levels. ROC and logistic regression analyses were performed by SPSS 25. $P<0.05$ was considered statistically significant.

Results

Comparison of clinical characteristics between CAD patients and controls

The comparison of general characteristics, serum lipid levels and FBG levels between CAD patients and controls is summarized in Table 2. There were no significant differences in the age and sex distributions between the two groups (all $P>0.05$). The levels of TG and FBG in the CAD group were significantly higher than those in the control group (all $P<0.0001$), while the levels of TC, HDL-C and LDL-C were significantly lower than those in the control group ($P<0.0001$).

Melting curve analyses

As shown in Fig. 1, PCR products of rs13107325 (C/C) and rs74650330 (C/C) melted at ~50 and 55 ℃, respectively. For homozygous mutant types of the two SNPs, PCR products melted at ~37 and 48 °C. The two inverted peaks at both temperatures for each SNP represent heterozygotes.

Accuracy analysis

A total of 48 DNA samples from the CAD and control groups were amplified, and these amplicons were sent to Sangon Company for further validation by sequencing (Fig 2). DNA sequencing results were completely consistent with the aforementioned base-quenched probe method by Kappa test (k=1; P=0.001).

Genotype and allele frequency distribution of the *SLC39A8* SNPs rs13107325 and rs74650330

The mutation of rs13107325 was not observed in the Chinese Han population from Jiangsu Province. The genotype distribution of rs74650330 in the CAD and control groups conformed to Hardy-Weinberg equilibrium (P=0.632 and P=0.223, respectively), indicating that the study population was representative of the general population. The genotype and allele frequencies of the rs74650330 SNP are summarized in Table 3. There was no significant difference in the genotype and allele frequencies of rs74650330 between the CAD group and the control group (all P>0.05).

Association between clinical characteristics and the rs74650330 SNP in CAD patients and controls

Table 4 shows the relationship between the levels of lipids and FBG and the rs74650330 polymorphism in the CAD and control groups. There were no statistically significant differences in age, lipid levels or FBG levels between different genotypes in the CAD group. However, compared with C/C homozygotes, carriers of the mutant allele had significantly lower HDL-C levels in the control group (P=0.039), whereas the LDL-C levels were higher in those with the mutant allele (P=0.046). Compared with the controls, CAD patients with the CC genotype had higher TG levels and lower HDL-C and LDL-C levels. The levels of TC and LDL-C in CAD patients with C allele deletions were lower than those in controls. Determining an optimal threshold of lipid and FBG levels based on the ROC curve (Fig 3), the case-control cohort populations were divided into two groups. When the LDL-C level was lower than 2.365 mmol/L, subjects with C/del had a 7.293-fold increased risk of CAD (OR: 7.293; 95% CI: 0.953-55.79) compared with that of controls without the mutation (Table 5). Logistic regression analysis indicated that the genotype in the rs74650330 SNP was not significantly associated with CAD (OR=1.075, 95% CI: 0.564-2.047; P=0.827). However, sex and TG, TC and HDL-C levels were considered risk factors for CAD (Table 6).

Discussion

The present study investigated the genetic polymorphisms of rs74650330 in patients with CAD and controls. The results obtained in the current study suggested that the rs74650330 SNPs exhibited no significantly different genotype or allele frequencies between patients with CAD and controls.

From the literature [12], we knew that rs13107325 of the *SLC39A8* gene was associated with NT-proBNP levels in patients with acute coronary syndrome. We therefore wanted to investigate whether this SNP was associated with CAD in the Chinese population. Our data showed that the rs13107325 polymorphism was not present in the Han Chinese participants enrolled in the current study. However, there were several reports in the literature about a missense mutation in the *SLC39A8* gene (rs13107325) that was associated with schizophrenia, Crohn's disease and severe idiopathic scoliosis in European populations [19-21]. Exploratory analyses suggested that the rs13107325 SNP is monomorphic in individuals of Asian and African descent, while it is prevalent in patients of European descent, which is related to the selective pressure of colder climate in Europe, leading to an increase in the frequency of the T allele so that humans can adapt to the environment [22].

In the analysis of PCR product sequencing results, a single base deletion SNP (subsequently confirmed as rs74650330 in NCBI's SNP database) was observed at 261 bp for rs13107325. rs74650330 is an intron mutation, and further investigation is required to elucidate its effects. From the Regulome DB (https://www.regulomedb.org/regulome-search/) and HaploReg (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) databases, we could not obtain annotation information for the rs74650330 SNP. Most of the risk SNPs detected by GWAS are located in the noncoding regions of the genome, indicating that these SNPs play their functional roles mainly by regulating gene expression [23]. Introns may regulate gene expression through splicing and "exon shuffling" during evolution. If a mutation occurs in splice junctions, the splicing process is usually disrupted, as the splicing machinery is unable to recognize the sequence. Furthermore, Pagani *et al* revealed that mutations in the middle of intron sequences may result in changes to splicing patterns [24]. Sometimes, lethality is attributed to intron mutations rather than missense mutations [25].

To explore the potential effect on CAD, we further compared clinical features in the case and control groups according to the rs74650330 genotype. The data showed that statistically significant differences in the serum HDL-C and LDL-C levels were present between the C/C and C/del genotypes in the control group ($P<0.05$). The mutant allele carriers had lower HDL-C (1.167-fold decrease) and higher LDL-C levels (1.126-fold increase) than the C/C homozygotes, which suggested that the CC genotype could be a protective genotype in healthy people. However, no statistically significant differences in the lipid profiles of C allele and mutation allele carriers in the CAD group were observed ($P>0.05$). Comparing the lipid levels between the two groups, for the C/C genotype, CAD patients had higher TG levels and lower HDL-C and LDL-C levels, and for the C/del and del/del genotypes, CAD patients had lower TC and LDL-C levels.

CAD has a complex pathogenesis that may be caused by risk factors other than cholesterol levels, including smoking, hypertension, glucose intolerance, dyslipidemia and obesity [26]. This meaningful result was not observed in the CAD population, which may be related to the possibility of CAD patients taking statin lipid-lowering drugs. Statins are comprehensive lipid-regulating drugs that not only strongly reduce TC and LDL-C levels but also lower TG levels to some extent. The effect of the drugs may mask the protective effect of the CC genotype. In addition, according to our results, we speculate that *SLC39A8* with the C mutation could regulate the expression of genes that promote the development of

atherosclerosis and CAD or inhibit the expression of cardiovascular-protective genes. These negative effects might outweigh the blood fat-lowering benefits of statins, which may explain why the C deletion of rs74650330 increases the risk of CAD in patients with low LDL-C levels. However, how it regulates the expression of genes associated with CAD needs further study.

Furthermore, the results of this study indicated that the C/del genotype did not affect the risk of CAD when the LDL-C level was equal to or greater than 2.365 mmol/L. The possible reason is that atherogenic dyslipidemia, characterized by increased levels of LDL particles, is considered to be a major factor in CAD risk [27]. Compared with the strong effect of LDL on CAD, the pathogenic effect size of the rs74650330 C→del mutation may be smaller. However, since there is a risk, and it is more than seven times higher, this mutation should not be clinically ignored.

Study strengths and limitations

In this study, we introduced a method for detecting the SNPs (rs13107325 and rs74650330) of *SLC39A8* in one tube by base-quenched probe technique. And, the potential relationship between rs74650330 and lipid levels in CAD patients from Han population in Jiangsu (China) had been further revealed. However, the present study had a number of limitations. Certain patients may receive lipid-lowering therapy prior to diagnosis with CAD, suggesting why the TC levels were lower in patients with CAD than in the control group subjects in the current study, which differed from previously published results [28]. We had a limited sample size to detect SNPs, and weak effects can be observed when considering multiple corrections. In addition, our sample was limited to the Han population. The control group may contain some volunteers whose coronary arteries have stenosis but no symptoms. Further research is warranted on patients with large sample sizes and other ethnic groups as well as patients enrolled from multiple sources to confirm our findings.

Conclusions

To the best of the authors' knowledge, the present study was the first to investigate the genetic SLC39A8 susceptibility to CAD in a Chinese Han population, revealing that rs74650330 was associated with lower HDL-C and higher LDL-C levels in healthy individuals. When LDL-C levels were lower than 2.365 mmol/L, the C/del genotype significantly increased the risk of CAD in the Chinese Han population. However, this study was only preliminary, and further research is needed to confirm our results. We hoped that this polymorphic locus could be a predictor of CAD risk, especially in normolipidemic populations.

Abbreviations

SLC39A8: solute carrier family 39 member 8; SNP: single nucleotide polymorphisms; CAD: coronary artery disease; NT-proBNP: N-terminal pro-B-type natriuretic peptide; TG: triglycerides; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; FBG: fasting blood

glucose; SBP: systolic blood pressure; DBP: diastolic blood pressure; HWE: hardy-Weinberg equilibrium; CI: confidence interval; OR: odds ratio; ROC: receiver operating characteristic; MAF: minor allele frequency.

Declarations

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Authors' contributions

GH L conceived and designed the study and made critical revision of the manuscript. J Z interpreted the results, finished the data analysis and wrote the draft manuscript. Y Y, LL P and TH Y participated in the laboratory tests and data collection, helped interpret the results. All authors read and approved the final manuscript.

Availability of data and materials

The data analyzed during the current study are not available because of the agreement between the authors and participants on their privacy.

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Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The Third Affiliated Hospital of Soochow University (Jiangsu, China) and performed in accordance with the institution's guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interests.

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Tables

Table 1. Sequences of the primers and probes used to identify rs13107325 and rs74650330.

SNPs	Primer/Probe	Sequence (5'→3')
	Sense primer	AGGGATGAGCACTCGACAAGC
rs13107325	Antisense primer	GATGTACCAACCACAAGGGGAAT
	Probe	TTGGAGCGAAATTGTT-FAM
	Sense primer	AATCCCATTTCAACAGATCATTCTAC
rs74650330	Antisense primer	TCTCGGCTCACAGCAACCTC
	Probe	HEX-CAAATCATTTCACCTTCAAACA-P

The underlined nucleotides represent polymorphisms.

Table 2. Clinical characteristics of 428 participants with CAD patients and the control group.

Parameter	CAD group	Control Group	*P* value
Age	63(57,69)	61(54,67)	0.242
Gender(Male/Female)	191/67	133/37	0.321
TG(mmol/L)	**1.95(1.38,2.78)*** **	1.50(1.09,2.00)	<0.0001
TC(mmol/L)	**4.41(3.74,5.15) *** **	5.07(4.52,5.94)	<0.0001
HDL-C(mmol/L)	**1.05(0.91,1.22) *** **	1.31(1.08,1.56)	<0.0001
LDL-C(mmol/L)	**2.25(1.74,2.81)*** **	2.76(2.37,3.20)	<0.0001
FBG(mmol/L)	**6.00(5.10,7.85) *** **	5.84(5.38,6.49)	<0.0001

Data were presented as median (interquartile range). ****P*<0.0001 vs. the control group.

Table 3. The allele frequencies and genotype distributions of the rs74650330 SNP in the CAD and control groups.

Group	n	Allele		Genotype		
		C (%)	del (%)	C/C (%)	C / del (%)	del/del (%)
CAD	258	476 (92.2)	40 (7.8)	219 (84.9)	38 (14.7)	1 (0.4)
Control	170	315 (92.6)	25 (7.4)	147 (86.5)	21 (12.3)	2 (1.2)
P		0.83	0.83	0.65	0.49	0.57[a]
OR (95% CI)		0.94 (0.56~1.59)	1.06 (0.63~1.78)	0.88 (0.50~1.53)	1.22 (0.69~2.17)	0.33 (0.03~3.64)

[a] Fisher's exact test

Table 4. Rs74650330 SNP genotype and clinical characteristics of patients with CAD and controls.

	Control group		CAD patients	
	C/C	C/ del + del / del	C/C	C/ del + del / del
Age	61(54,67)	59(53,66)	63(57,69)	62(57,71)
TG(mmol/L)	1.50(1.09,1.90)	1.54(1.15,2.33)	1.92(1.39,2.79)[c]	2.34(1.34,3.13)
TC(mmol/L)	5.00(4.51,5.93)	5.50(4.56,6.10)	4.42(3.69,5.15)	4.29(3.02,4.98)[f]
HDL-C(mmol/L)	1.33(1.10,1.59)	1.14(0.95,1.42) [a]	1.05(0.92,1.22)[d]	1.05(0.86,1.24)
LDL-C(mmol/L)	2.69(2.3,3.17)	3.03(2.6,3.38)[b]	2.28(1.63,2.82)[e]	2.16(1.86,2.81)[g]
FBG(mmol/L)	5.89(5.45,6.50)	5.70(5.11,6.32)	5.90(5.10,7.90)	6.25(5.23,7.35)

Genotype C/C vs genotype C/ del and del / del in control group: [a]*P*=0.039 for HDL-C, [b]*P*=0.046 for LDL-C; genotype C/C in control group vs genotype C/C in CAD patients: [c]*P*<0.0001 for TG, [d]*P*<0.0001 for HDL-C and [e]*P*<0.0001 for LDL-C; genotype C/ del and del / del in control group vs genotype C/ del and del / del in CAD patients: [f]*P*=0.0006 for TC and [g]*P*=0.0002 for LDL-C. Data were presented as median (interquartile range).

Table 5. Risk of CAD in subjects with elevated serum lipids and FBG assessed by rs74650330 allele C status in a case-control study

Variables	Range	Genotype	N of CAD (%)	N of Control (%)	OR	(95%CI)	*P*
TG (mmol/L)	>=2.005	C/del	22 (8.5)	7 (4.1)	0.928	(0.362-2.376)	0.876
		C/C	105 (40.7)	31 (18.2)			
	<2.005	C/del	17 (6.6)	16 (9.4)	1.081	(0.521-2.244)	0.834
		C/C	114 (44.2)	116 (68.2)			
TC (mmol/L)	>=4.325	C/del	19 (7.4)	20 (11.8)	1	(0.507-1.971)	1
		C/C	114 (44.2)	120 (70.6)			
	<4.325	C/del	20 (7.8)	3 (1.8)	1.714	(0.474-6.199)	0.57
		C/C	105 (40.7)	27 (15.9)			
HDL-C (mmol/L)	>=1.285	C/del	8 (3.1)	9 (5.3)	2.018	(0.722-5.642)	0.175
		C/C	37 (14.3)	84 (49.4)			
	<1.285	C/del	31 (12.0)	14 (8.2)	0.766	(0.383-1.533)	0.451
		C/C	182 (70.5)	63 (37.1)			
LDL –C (mmol/L)	>=2.365	C/del	16 (6.2)	22 (12.9)	0.818	(0.406-1.648)	0.574
		C/C	96 (37.2)	108 (63.5)			
	<2.365	C/del	23 (8.9)	1 (0.6)	7.293	(0.953-55.79)	**0.031***
		C/C	123 (47.7)	39 (22.9)			
FBG (mmol/L)	>=5.245	C/del	29 (11.2)	17 (10.0)	1.456	(0.765-2.771)	0.251
		C/C	150 (58.1)	128 (75.3)			
	<5.245	C/del	10 (3.9)	6 (3.5)	0.459	(0.148-1.424)	0.171
		C/C	69 (26.7)	19 (11.2)			
Sex	Male	C/del	28 (10.9)	20 (11.8)	0.971	(0.521-1.808)	0.925
		C/C	163 (63.2)	113 (66.5)			
	Female	C/del	11 (4.3)	3 (1.8)	2.226	(0.579-8.555)	0.234
		C/C	56 (21.7)	34 (20.0)			

N, number of coronary heart disease or controls classified by genotype and baseline characteristics.
*P<0.05.

Table 6. Logistic regression analysis estimating the risk factors for CAD

Variables	Estimate	Std. Error	*P*	OR(95%CI)
Age	0.015	0.012	0.222	1.015(0.991-1.041)
Gender	0.720	0.300	**0.016***	2.055(1.141-3.701)
TG	0.485	0.121	**<0.0001*****	1.624(1.281-2.058)
TC	-0.629	0.191	**0.001*****	0.533(0.366-0.776)
HDL-C	-2.189	0.457	**<0.0001*****	0.112(0.046-0.274)
LDL-C	0.056	0.251	0.823	1.058(0.647-1.728)
FBG	0.155	0.068	0.023	1.167(1.022-1.333)
c_del	0.072	0.329	0.827	1.075(0.564-2.047)

*P<0.05, ***P≤0.001

Figures

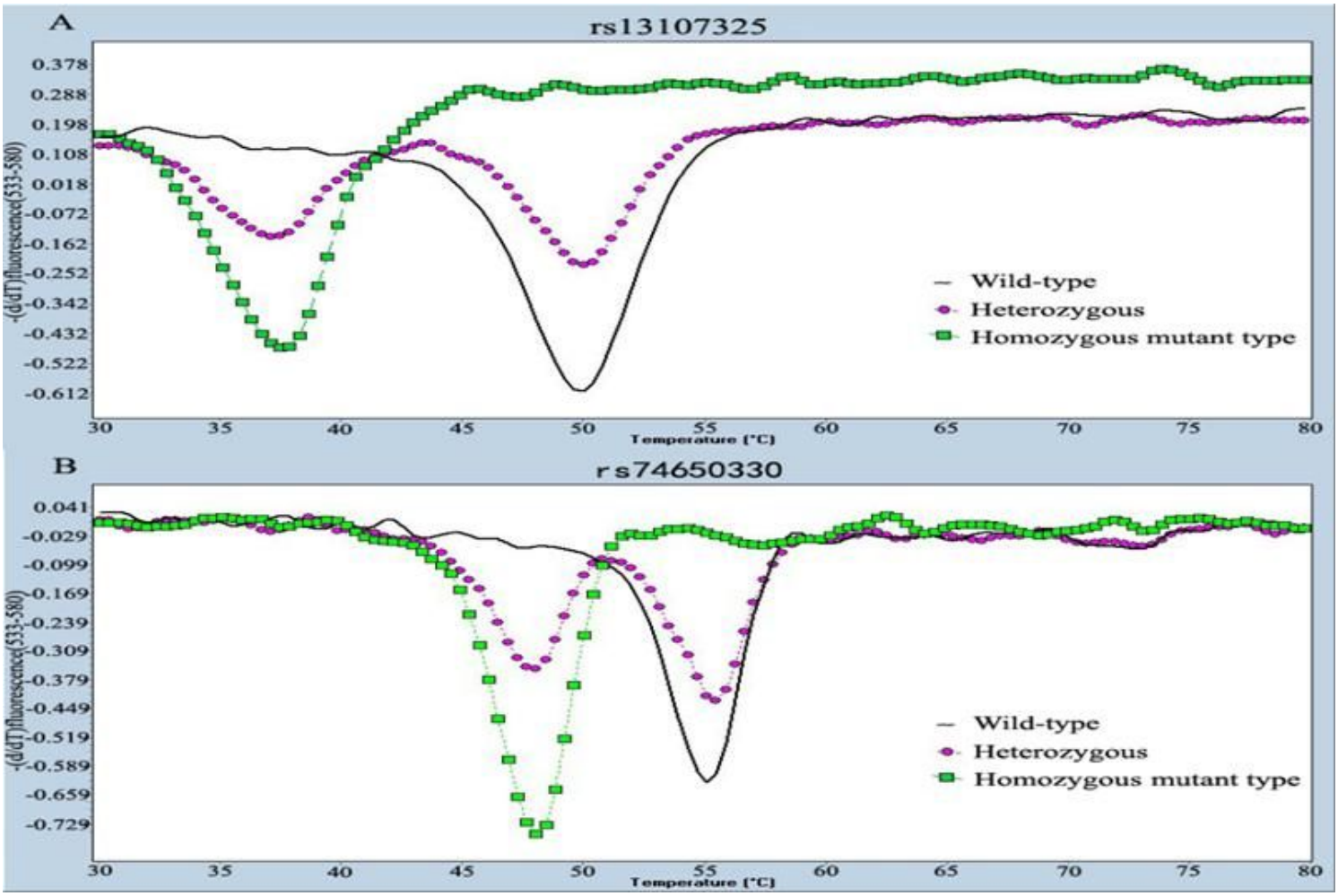

Figure 1

Melting curve analyses. Fig. 1 shows the melting curves (-dF/dT vs. T) that depict the three genotypes. All of the derivative melting valleys are oriented in negative scale and afford easier visualization of Tms. (A) The melting curves of rs13107325. The Tms of the wild-type and homozygous mutant type are ~50 and 37℃, respectively. (B) The melting curves of rs74650330. The Tms of the wild-type and homozygous mutant type are ~55 and 48℃, respectively. The heterozygote has two inverted peaks at both temperatures.

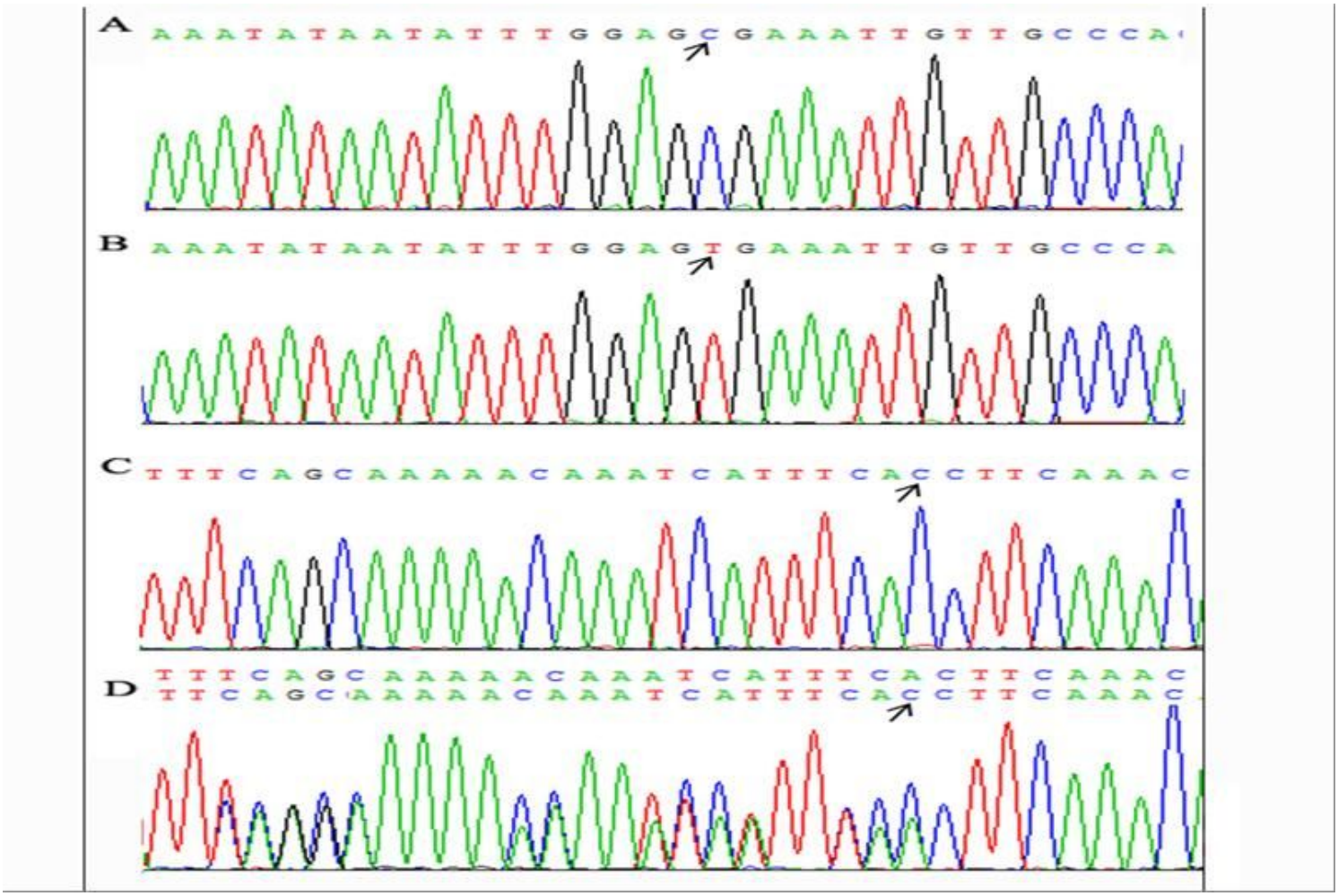

Figure 2

represents a stretch of nucleotide sequences of the SLC39A8 rs13107325 and rs74650330 SNPs obtained by sequencing. (A) Wild-type rs13107325 sequence. (B) Homozygous mutant type rs13107325 sequence. (C) Wild-type rs74650330 sequence. (D) A compound heterozygote for the rs74650330 mutation. In the heterozygote, the deletion mutation causes the appearance of a series of double peaks (presented in the grey box). The arrows indicate the mutant alleles.

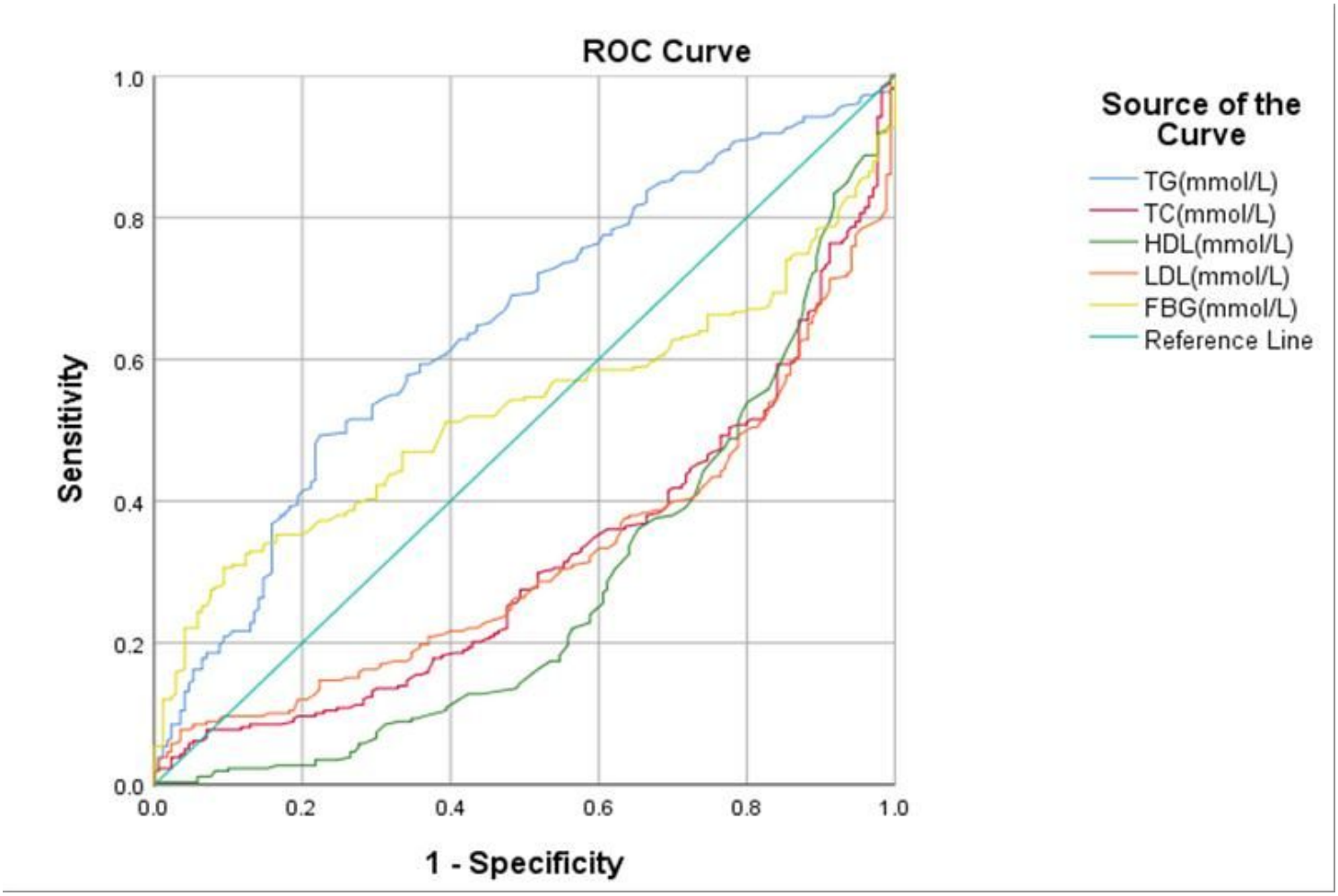

Figure 3

ROC curve of the predictive value of TG, TC, HDL-C, LDL-C and FBG to CAD. The maximum value of the Youden index was used as the cut-off value, which makes the result more objective and reliable.