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Original Article

Association of variants in *CELSR2-PSRC1-SORT1* with risk of serum lipid traits, coronary artery disease and ischemic stroke

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Abstract: Recent genome-wide association studies (GWAS) have identified genetic variants associated with coronary artery disease (CAD), ischemic stroke (IS) and serum lipid traits in different ethnic groups. Some loci were found to affect the risk of CAD and IS. However, there were no data in the southern Chinese populations. Our study was to assess the association of *CELSR2-PSRC1-SORT1* rs599839, rs464218 and rs6698443 SNPs and serum lipid levels and the risk of CAD and IS. The genotypes of 3 SNPs were detected in 561 CAD and 527 IS patients, and in 590 healthy controls. The genotypic and allelic frequencies of the rs599839 SNP were different between the controls and IS patients ($P < 0.05$). The minor G alleles of rs599839 and rs464218 SNPs were associated with higher high-density lipoprotein cholesterol concentrations in CAD and IS patients ($P < 0.05$); respectively. No association was found between the SNPs of rs599839, rs464218 and rs6698843 at the *CELSR2-PSRC1-SORT1* and the risk of CAD or IS. These results will be replicated in the other Chinese populations.

Keywords: CELSR2: cadherin EGF LAG seven-pass G-type receptor 2, PSRC1: proline/serine-rich coiled-coil 1, SORT1: sortilin, coronary artery disease, ischemic stroke, serum lipid traits

Introduction

Cardiovascular disease (CVD) includes coronary artery disease (CAD), cerebrovascular disease, hypertension, and other CVDs, while leading cause of death in the world, about 13.9 million (representing 22.9% of all deaths) died from them. In addition, the CVDs remain among the top six causes of burden of disease (DALYS) in 2004 [1]. Ischemic stroke (IS) has some common risk factors with CAD, such as hypertension, dyslipidemia, and genetic variants [2]. Concentrations of low- and high-density lipoprotein cholesterol (LDL-C and HDL-C) and triglyceride (TG) are each positively (or, in the case of HDL-C, negatively) associated with the risk of CAD [3, 4]. It is confirmed that genetic and environmental factors modulated serum lipid levels [5]. Understanding the genes involved blood lipoprotein or lipid traits and the association between common variants and CAD or IS may inform therapy or preventive methods.

Over the last 10 years, genome-wide association studies (GWAS) have identified *CELSR2-PSRC1-SORT1* variants on chromosome 1p13.3 associated with CAD and plasma lipoproteins based on populations of European [6, 7], eastern Asia [8, 9], southern Asia [10], Middle-Eastern Asia [11], and Africa Americans [12]. These SNPs encodes cadherin EGF LAG seven-pass G-type receptor 2, proline/serine-rich coiled-coil 1, and sortilin; respectively. Sortilin, encoded by *SORT1*, is a receptor for apolipoprotein (Apo) B100. It facilitates the formation and hepatic export of ApoB100-containing lipoproteins, regulating plasma LDL-C [13]. However, little is known about such association in the southern Chinese people, especially the association of *CELSR2-PSRC1-SORT1* variants and IS. Therefore, the present study was to assess

Table 1. Characteristics of all participants

Characteristic	Controls (n = 590)	CAD (n = 561)	IS (n = 527)	$P_{vs. Controls}$	
				CAD	IS
Male/female	431/159	417/144	383/144	0.335	0.471
Age, years	61.33±9.72	62.19±10.57	62.74±12.37	0.153	0.051
Body mass index, kg/m^2	22.42±2.86	23.81±3.35	24.79±2.25	0.000	0.017
Systolic blood pressure, mmHg	130.77±19.99	132.79±23.20	148.01±21.91	0.115	0.000
Diastolic blood pressure, mmHg	83.06±13.44	79.18±14.24	83.98±12.89	0.000	0.245
Pulse pressure, mmHg	49.77±14.75	53.60±17.43	64.11±17.79	0.000	0.000
Cigarette smoking, n (%)	156 (26.4)	92 (16.3)	88 (42.0)	0.000	0.000
Alcohol consumption, n (%)	273 (46.3)	141 (25.1)	186 (35.3)	0.000	0.000
Total cholesterol, mmol/L	4.93±1.11	4.52±1.20	4.53±1.15	0.000	0.000
Triglyceride, mmol/L	1.40±1.86	1.64±1.10	1.66±1.28	0.007	0.007
HDL-C, mmol/L	1.90±0.50	1.14±0.34	1.22±0.40	0.000	0.000
LDL-C, mmol/L	2.75±0.79	2.71±1.00	2.69±0.90	0.493	0.276
Apolipoprotein (Apo) AI, g/L	1.40±0.26	1.03±0.52	1.02±0.22	0.000	0.000
ApoB, g/L	0.91±0.22	0.90±0.27	0.89±0.25	0.874	0.359
ApoAI/ApoB	1.63±0.48	1.37±2.48	1.26±0.60	0.017	0.000

CAD, coronary artery disease; IS, ischemic stroke; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

the association of *CELSR2-PSRC1-SORT1* rs599839, rs464218 and rs6698443 SNPs and serum lipid levels and the risk of CAD and IS.

Materials and methods

Study subjects

A total of 561 patients with CAD and 527 patients with IS were recruited from hospitalized patients in the First Affiliated Hospital, Guangxi Medical University. All of the enrolled CAD patients were evaluated by coronary angiography due to suspected CAD or unrelated conditions requiring angiographic evaluation; the coronary angiograms were analyzed by two experienced interventional cardiologists. CAD was defined as significant coronary stenosis (≥ 50%) in at least one of the three main coronary arteries or their major branches (branch diameter ≥ 2 mm). Subjects with congenital heart disease and type I diabetes mellitus were excluded. All of the enrolled IS patients received a strict neurological examination and brain magnetic resonance imaging. The diagnosis of IS was according to the International Classification of Diseases (9th Revision). Patients with a transient ischemic attack, embolic brain infarction, stroke caused by inflammatory disease, cardio-embolic stroke, autoimmune disease, or serious chronic diseases were excluded from this study. Subjects with a past history of CAD were also excluded from the study [14].

A total of 590 healthy controls matched by age, gender, and geographical area were included. The controls were judged to be free of CAD and IS by questionnaires, medical history, and clinical examination. All individuals enrolled were from the Han population in Guangxi, China. A standard questionnaire was used to ascertain general information and medical history from all participants. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Informed consent was obtained from all subjects after receiving a full explanation of the study.

Genotyping and biochemical analysis

All of the biochemical assays and genotyping in CAD and IS patients were performed after hospitalization, and all of the venous blood samples were obtained from the patients and controls after at least 12 h of fasting. Genomic DNA was isolated from peripheral blood leukocytes using the phenol-chloroform method. We selected single nucleotide polymorphisms (SNPs) from NCBI dbSNP Build 132 (http://www.Ncbi.nlm.nih.gov/SNP/). Genotyping of the three SNPs was performed by the Snapshot technology platform. All experimental manipulations were completed in the Center for Human Genetics Research, Shanghai Genesky Bio-Tech Co. Ltd. The primers were as follows: rs599839F: 5'-CCCAGATCGCGCCATTAAAC-3';

Table 2. Genotype distribution and allele frequencies of rs599839 and rs464218 and rs6698843 in cases and control

SNP	Control (%)	Case (%)		*P*	
		CAD	IS	CAD	IS
Rs599839					
AA	527 (89.3)	511 (90.6)	449 (85.2)		
AG	63 (10.7)	53 (9.4)	75 (14.2)		
GG	0	0	3 (0.6)	0.469	0.034
A	1117 (94.7)	1075 (95.3)	973 (92.3)		
G	63 (5.3)	53 (4.7)	81 (7.7)	0.481	0.024
HWE(*P*)	0.171	0.241	0.945		
Rs464218					
AA	207 (35.1)	191 (33.9)	182 (34.5)		
AG	276 (46.8)	282 (50)	91 (50.1)		
GG	107 (18.1)	264 (16.1)	81 (15.4)	0.493	0.383
A	690 (58.5)	664 (58.9)	628 (59.6)		
G	490 (41.5)	464 (41.1)	426 (40.4)	0.849	0.595
HWE(*P*)	0.372	0.441	0.357		
Rs6698843					
CC	207 (35.1)	174 (30.9)	172 (32.6)		
CT	266 (45.1)	275 (48.8)	255 (48.4)		
TT	117 (19.8)	115 (20.3)	100 (19.0)	0.295	0.536
C	680 (57.6)	623 (55.2)	599 (56.8)		
T	500 (42.4)	505 (44.8)	455 (43.2)	0.246	0.704
HWE(*P*)	0.062	0.739	0.751		

rs599839R: 5'-TGCCCTCTGAGGAGCCATCTT-3'; rs464218F: 5'-GTGAGGAGCTGGTGTGCAGTGT-3'; rs464218R: 5'-TGGAATTCGAAGGGACCTT-TTCA-3'; rs6698843F: 5'-ACAGGGCTTCAGGCC-TCCTCT-3'; rs6698843R: 5'-GGCCCAGGTTGC-CCTCTG-3'. The levels of total cholesterol (TC), TG, HDL-C, and LDL-C in the samples were determined by enzymatic methods with commercially available kits. Serum Apo AI and ApoB levels were detected by an immunoturbidimetric immunoassay using a commercial kit (RANDOX Laboratories Ltd.) [15]. The normal values for serum TC, TG, HDL-C, LDL-C, ApoAI, ApoB and ApoAI to ApoB ratio at our Clinical Science Experiment Center (Nanning, China) were 3.10-5.17, 0.56-1.70, 0.91-1.81, 2.70-3.20 mmol/L, 1.00-1.78, 0.63-1.14 g/L, and 1.00-2.50; respectively.

Statistical analyses

All statistical analyses were performed using the statistical software package SPSS 13.0 (SPSS Inc., Chicago, IL, USA). A standard goodness-of-fit test was used to test the Hardy-Weinberg equilibrium. A chi-square analysis was used to evaluate the difference in genotype distribution and sex ratio between the groups. The general characteristics between the cases and controls were tested using Student's unpaired *t*-test. The association between genotypes and serum lipid parameters was tested by analysis of covariance (ANCOVA). Sex, age, body mass index (BMI), blood pressure, alcohol consumption, and cigarette smoking were adjusted for in the statistical analysis. ORs and 95% CIs were calculated using conditional logistic regression. A two-tailed *P* value less than 0.05 was considered to be statistically significant. The pattern of pair-wise linkage disequilibrium (LD) between the selected SNPs was measured by D' and r^2 using the SHEsis software.

Results

Characteristics of the studied subjects

Table 1 compares the general characteristics and serum lipid levels between the healthy controls and CAD or IS patients. The mean age, gender distribution, serum LDL-C and ApoB levels were not different between controls and CAD or IS patients ($P > 0.05$ for all). The CAD patients had higher BMI, pulse pressure and serum TG levels, but lower diastolic blood pressure, serum TC, HDL-C, ApoAI levels, ApoAI/ApoB ratio, and the percentages of subjects who consumed alcohol or smoked cigarettes than the controls ($P < 0.05$-0.001). The IS patients had higher BMI, systolic blood pressure, pulse pressure, serum TG levels, and the percentage of subjects who smoked cigarettes; and lower serum TC, HDL-C, ApoAI levels, ApoAI/ApoB ratio, and the percentage of subjects who consumed alcohol than the controls ($P < 0.05$-0.001).

Genotypic and allele frequencies

Table 2 describes the genotype and allele frequencies of the *CELSR2-PSRC1-SORT1* SNPs. The genotype distribution of all 3 SNPs agreed with Hardy–Weinberg equilibrium ($P > 0.05$ for all). The genotype and allele frequencies of the

Table 3. Effect of the genotypes on serum lipid levels in the control and cases

Genotype	control				CAD				IS			
	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
rs599839												
AA	4.9198393	1.41±.419	1.89±0.51	2.73±0.80	4.51±1.21	1.66±1.13	1.13±0.32	2.70±1.02	4.55±1.15	1.66±1.32	1.22±0.41	2.70±0.90
AG/GG	5.11G8394	1.37±.37G	1.94±0.42	2.86±0.73	4.61±1.04	1.4748391	1.23±0.46	2.80±0.88	4.40±1.11	1.70±.701	1.21±0.33	2.60±0.90
F	2.056	0.004	0.432	1.792	0.209	1.727	4.700	0.373	1.788	0.002	0.045	1.604
P	0.152	0.952	0.511	0.181	0.647	0.189	0.031	0.542	0.182	0.960	0.832	0.206
rs464218												
AA	4.93±.934	1.41±1.57	1.92±0.48	2.72±0.77	4.49±1.06	1.72±1.17	1.12±0.32	2.67±0.91	4.49±1.08	1.60±1.15	1.23±0.33	2.67±0.91
GA	4.92±1.17	1.31±.317	1.90±0.53	2.78±0.84	4.53±1.32	1.57±1.03	1.14±0.32	2.73±1.06	4.58±.586	1.77±.773	1.18±0.35	2.72±0.91
GG	4.95±1.12	1.65±2.71	1.86±0.48	2.71±0.72	4.55±1.08	1.71±1.16	1.18±0.42	2.74±1.01	4.48±0.97	1.47±.471	1.33±.331	2.64±.641
F	0.079	1.651	1.101	0.307	0.218	1.144	0.367	0.195	0.475	2.245	5.718	0.517
P	0.924	0.193	0.333	0.736	0.804	0.319	0.693	0.823	0.622	0.107	0.003	0.597
Rs6698843												
CC	5.02±0.98	1.44±2.10	1.91±0.49	2.79±0.75	4.49±1.12	1.56±1.06	1.17±0.37	2.69±0.95	4.66±1.28	1.78±1.48	1.23±0.50	2.75±0.89
CT	4.89±1.28	1.45±1.98	1.87±0.51	2.72±0.81	4.59±1.31	1.66±1.05	1.13±0.35	2.76±1.09	4.41±1.07	1.60±1.17	1.21±0.36	2.61±0.89
TT	4.88±0.91	1.17±0.65	1.95±0.51	2.75±0.70	4.39±1.05	1.731.33	1.13±0.29	2.60±0.85	4.60±1.11	1.59±1.16	1.24±0.31	2.79±0.93
F	1.159	1.872	1.034	0.590	1.246	1.356	0.926	1.198	2.354	0.994	0.300	2.094
P	0.314	0.155	0.356	0.555	0.289	0.259	0.397	0.303	0.096	0.371	0.741	0.124

Table 4. Effect of the genotypes on serum lipid levels in the combined population

Genotype	n	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ApoAI (g/L)	ApoB (g/L)
rs599839							
AA	1486	4.6798398	1.57±1.50	1.43±0.55	2.71±0.91	1.16±0.41	0.89±0.24
AG/GG	194	4.69±1.08	1.53±1.17	1.45±0.52	2.74±0.85	1.17±0.30	0.92±0.24
F		0.009	0.732	0.798	0.006	0.359	0.636
P		0.925	0.392	0.372	0.940	0.549	0.425
rs464218							
AA	576	4.65±1.06	1.57±1.33	1.44±0.53	2.69±0.86	1.17±0.31	0.90±0.25
GA	820	4.68±1.26	1.54±1.42	1.41±0.54	2.74±0.94	1.14±0.47	0.90±0.24
GG	273	4.68±0.24	1.62±1.86	1.48±0.59	2.70±0.59	1.18±0.32	0.90±0.32
F		0.253	0.019	2.844	1.255	1.199	0.632
P		0.776	0.981	0.058	0.285	0.302	0.532
Rs6698843							
CC	517	4.7598843	1.5998843	1.4798843	2.7598843	1.18±0.31	0.91±0.31
CT	795	4.63±0.31	1.57±0.31	1.40±0.31	2.70±0.31	1.15±0.31	0.89±0.31
TT	332	4.63±0.31	1.49±0.31	1.45±0.31	2.71±0.31	1.15±0.31	0.89±0.31
F		1.087	0.315	2.859	0.175	0.908	0.631
P		0.338	0.730	0.058	0.839	0.404	0.532

rs599839, rs464218 and rs6698843 SNPs were no differences between the controls and CAD patients ($P > 0.05$). However, the genotype and allele frequencies of the rs599839 SNP were different between the controls and IS patients ($P < 0.05$).

Genotypes and serum lipid levels

As shown in **Table 3**, the minor G alleles of rs599839 and rs464218 were associated with high HDL-C concentrations in CAD and IS patients ($P < 0.05$); respectively. We found that the rs599839, rs464218 and rs6698843 SNPs were not associated with lipoprotein or lipid-related traits in the total population ($P > 0.05$); respectively (**Table 4**). A weak linkage disequilibrium was found among the rs599839, rs464218 and rs6698843 SNPs ($r^2 < 0.1$).

CELSR2-PSRC1-SORT1 SNPs and the risk of CAD and IS

Table 5 shows no association of the rs599839, rs464218 and rs6698843 SNPs and the risk of CAD or IS in different genetic models.

Discussion

In this case-control genetic study, we first reported that the G allele of rs599839 and rs464218 was associated with high HDL-C concentrations in CAD and IS patients. These findings are inconsistent with previous research results which were associated with LDL-C concentrations in the GWAS [16] or in the recent pooled analysis. The minor G allele of rs599839 was associated with low LDL-C levels in Austrians [17], Indians [18], Japanese population [19], central Chinese population [20] and Pakistanis [21], and with high TC in Netherland population [22] Angelakopoulou *et al.* [23] found that rs599839 SNP was associated with TC, LDL-C and ApoB in seven prospective studies. Kathiresan *et al*. [24] showed that the SNPs of rs599839 and rs646776 were associated with LDL-C in 18,554 independent participants. In addition, rs12740374 SNP was strongly associated with LDL-C in African Americans [12]. We conjectured that the reason for these differences may include: (i) the minor allele frequency of rs599839 SNP in the controls (3%) was much lower than that in the International HapMap Utah residents with ancestry from northern and western Europe (32%; http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap24_B36/); (ii) possible gene-gene interaction; *SORT1* encodes sortilin, which is one of five members of the Vps10p domain receptor family, a group of multifunctional proteins typically found in intracellular compartments of the *trans*-Golgi *net* work (TGN) and early endosomes [25]. At the 1q42 locus near *GALNT2* for HDL-C, encodes polypeptide N-acetyl galactos-

Table 5. *CELSR2-PSRC1-SORT1* polymorphisms and association with CAD and IS in different genetic models

Locus	Genotype	CAD		IS	
		OR (95% CI)	*P*	OR (95% CI)	*P*
rs599839					
Dominant	AA	1		1	
	AG+GG	1.27 (0.70-2.32)	0.432	1.39 (0.81-2.39)	0.233
rs464218					
Codominant	AA	1		1	
	GA	1.13 (0.76-1.70)	0.539	1.12 (0.75-1.66)	0.591
	GG	1.07 (0.62-1.83)	0.817	1.05 (0.61-1.81)	0.863
Dominant	AA	1		1	
	GA+GG	1.11 (0.76-1.63)	0.576	1.10 (0.75-1.60)	0.625
Recessive	GG	1		1	
	GA+AA	1.01 (0.62-1.66)	0.957	1.02 (0.62-1.66)	0.954
Rs6698843					
Codominant	CC	1		1	
	CT	1.12 (0.73-1.70)	0.596	1.246 (0.918-1.69)	0.158
	TT	1.21 (0.72-2.03)	0.464	0.995 (0.68-1.46)	0.979
Dominant	CC	1		1	
	CT+TT	1.28 (0.97-1.68)	0.081	1.17 (0.88-1.55)	0.292
Recessive	TT	1		1	
	CC+CT	0.88 (0.56-1.39)	0.588	1.08 (0.68-1.72)	0.731

aminyl transferase 2, an enzyme involved in O-linked glycosylation and transfer of N-acetylgalactosamine to the serine or threonine residues on proteins. O-linked glycosylation has a regulatory role for many proteins [26]. Like this adjacent genes interactions and linkage disequilibrium on lipid metabolism also need a lot of molecular and cell biology experiments in the future. (iii) Diet and physical activity are strongly associated with serum lipid levels [27, 28]. (iv) The influence of drug treatment; CAD and IS patients take anti-atherosclerotic medicines, such as statins, fibrates. It is a potential confounder when evaluating the impact of some genes on lipids.

A large number of studies have found that *CELSR2-PSRC1-SORT1* polymorphism is associated with the risk of CAD [16, 20, 23, 24, 29-31], whereas the SNP of rs646776 showed no significant associations with CAD in Lebanese cohort [11]. In the MORGAM Prospective Cohorts from Finland, Sweden, France and Northern Ireland [32], the SNPs of rs599839 and rs4970834 were not strongly associated with incident CAD and stroke. No significant associations were observed between the rs599839 and rs646776 SNPs and CAD mortality a Norwegian case-cohort study [33]. In the current study, we found that the SNPs of rs599839, rs464218 and rs6698843 at *CELSR2-PSRC1-SORT1* were uncorrelated with the risk of CAD. This inconsistent results indicate that the possible effected mechanisms of these SNPs on CAD are yet unknown. Gender, race, age or sample size and possible gene-environment interactions on CAD risks may cause the reduction of significant associations. Moreover, after disease started, phenotype for diagnosis may turn up, but in a specific point in time, individuals with certain genes may show a series of alternative phenotypes, affected by possible environmental exposure. More research is needed.

Although the genotype and allele frequencies of the rs599839 SNP were higher in IS patients than in the controls, our findings showed that the SNPs of rs599839, rs464218 and rs6698843 were not associated with the risk of IS. *CELSR2-PSRC1-SORT1* was also not the candidate gene for IS in previous GWAS [34].

There are two limitations in the present study. First, the number of subjects for minor allele of SNPs was too small to interpret the associations of SNPs and the risk of diseases. Second, the interactions of gene-gene and gene-envi-

ronment on serum lipid levels remain to be determined. Therefore, larger sample size and multi-ethnic population studies are needed to confirm our results.

In conclusion, the present study shows that the minor G allele of rs599839 and rs464218 SNPs is associated with high HDL-C concentrations in CAD and IS patients, no associations were found between the SNPs of rs599839, rs464218 and rs6698843 at *CELSR2-PSRC1-SORT1* and the risk of CAD or IS.

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Disclosure of conflict of interest

None.

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