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Original Article

Two *DOCK7* polymorphisms and their haplotypes are associated with the risk of coronary artery disease and ischemic stroke

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Abstract: The association between the dedicator of cytokinesis 7 (*DOCK7*) rs10889353 and rs10889335 polymorphisms and the risk of coronary artery disease (CAD) and ischemic stroke (IS) has not been reported previously. The present study was undertaken to examine the association between the *DOCK7* rs10889353 and rs10889335 polymorphisms and their haplotypes and the risk of CAD and IS in the Han Chinese population. Genotypes of the two polymorphisms were determined by the Snapshot technology platform in 1,139 unrelated patients (CAD, 584 and IS, 555) and 627 healthy controls. The frequency of the rs10889353 alleles and genotypes was different between controls and CAD ($P < 0.01$) or IS ($P < 0.05$). The frequency of the 10889335 alleles and genotypes was also different between controls and CAD ($P < 0.01$). The rs10889353C allele and CC genotype were associated with an increased risk of CAD and IS, and the rs10889335G allele, GG genotype and rs10889353C-rs10889335G (21.0%) haplotype were also associated with an increased risk of CAD. Both rs10889353CC and rs10889335GG genotypes were associated with the angiographic severity of CAD. The rs10889353C and rs10889335G allele carriers in the healthy controls had higher serum total cholesterol (TC) and triglyceride (TG) levels than the rs10889353C and rs10889335G allele non-carriers. Stratified analyses showed that the two polymorphisms may interact with gender, age, body mass index, smoking, drinking, hypertension and hyperlipidemia to influence the risk of CAD and IS. This study shows that the *DOCK7* rs10889353 and rs10889335 polymorphisms and their haplotypes in the Han Chinese population are associated with the risk of CAD and IS, and with serum TC and TG levels in the healthy controls.

Keywords: Dedicator of cytokinesis 7 gene, single nucleotide polymorphism, coronary artery disease, ischemic stroke, lipids

Introduction

Cardiovascular disease is the leading cause of morbidity and mortality in most industrialized nations and is of growing concern in developing countries [1]. Both coronary artery disease (CAD) and ischemic stroke (IS) are atherosclerotic diseases that may share many common aspects of their underlying pathogenesis, as well as risk factors, including dyslipidemia, hypertension, diabetes, chronic kidney disease and cigarette smoking [1, 2]. Previous twin and family studies have shown that CAD [3] and IS [4] are highly heritable, with evidence of a shared heritability for the two conditions [5]. Previous genomewide association studies (GWASs) and meta-analyses of such studies have identified various genes and loci in the predisposition to CAD [6] or to IS [7] in Caucasian populations. Furthermore, certain genetic variants originally shown to influence the risk of CAD were also subsequently found to be associated with IS [8, 9], suggestive of a shared genetic architecture for these conditions. Although CAD and IS may share genetic factors in Caucasian populations, the genes that confer susceptibility to the two diseases in the Chinese individuals are very few [10-14].

The dedicator of cytokinesis 7 protein (DOCK7) belongs to the DOCK family, which consists of eleven guanine nucleotide exchange factors (GEFs) [15]. Although the molecular structures of DOCK proteins are similar, the small guanine nucleotide triphosphatases (GTPases), including Rac and Cdc42, are regulated by specific

DOCK proteins [15]. DOCK1 (OR DOCK180), DOCK2 and DOCK3 are Rac-specific GEFs. DOCK4 and DOCK5 are structurally deduced GEFs for Rac. DOCK6, DOCK7 and DOCK8 are GEFs for Rac and Cdc42. DOCK9, DOCK10 and DOCK11 are Cdc42-specific GEFs. It is also known that each DOCK protein is differentially expressed in different cell types [15, 16]. DOCK7 is composed of two DOCK homology region (DHR) domains: the N-terminal (DHR-1) domain mediates a specific interaction with PIP2 and PIP3, and the C-terminal (DHR-2) domain is involved in binding to, and revealing GEF activity towards, Rac1, Rac3, and/or Cdc42 [17, 18]. DOCK7 is expressed mainly in neuronal cells [19]. Cellular functions of DOCK7 remain poorly defined; it is postulated it might be involved in neurogenesis [20, 21]. Perisic et al. [22] have demonstrated that the DOCK7 was upregulated in symptomatic carotid plaques, suggesting that the DOCK7 may be associated with the development of carotid atherosclerosis. DOCK7 gene (*DOCK7*; gene ID: 85440, MedGen: CN189147, OMIM: 615859) is located on chromosome 1p31.3 (Exon count: 53). Several transcript variants encoding different isoforms have been found for this gene [23]. Recent GWASs in different populations have showed that several *DOCK7* single nucleotide polymorphisms (SNPs; rs1168013, rs10889353, rs10889335, rs1167998 and rs11207995) were associated with total cholesterol (TC) [24-27], triglyceride (TG) [26-29] and low-density lipoprotein cholesterol (LDL-C) [30] levels. However, it is still unclear whether these loci identified in the general population and/or healthy subjects also exert the same effect on lipid metabolism in the patients with cardiovascular disease. Therefore, the purpose of the present study was to detect the association of the *DOCK7* rs10889353 and rs10889335 SNPs and serum lipid traits and the risk of CAD and IS in the Han Chinese population.

Materials and methods

Study population

A total of 1,139 unrelated patients with CAD (*n* = 584) and IS (*n* = 555) were recruited from hospitalized patients in the First Affiliated Hospital, Guangxi Medical University. The diagnosis of CAD was based on typical clinical symptoms and electrocardiographic changes, as well as increases in the serum markers including creatinine kinase-MB and troponin T. Coronary angiography was performed in patients with CAD. The selected CAD patients were subject to significant coronary stenosis (≥ 50%) in at least either one of the three main coronary arteries or their major branches (branch diameter ≥ 2 mm). Additionally, angiographic severity of disease was defined as single or multi-vessel disease based on the number of involved artery (luminal narrowing ≥ 50%) in the three major coronary arteries [31, 32]. The classification of IS was made according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria [33]. The selected IS patients in the study included individuals who were eligible for one of the two subtypes of TOAST criteria: Large-artery atherosclerosis and small-vessel occlusion. Subjects with a history of hematologic, neoplastic, renal, liver, thyroid, autoimmune diseases and type 1 diabetes were excluded. The selected IS patients who had a past history of CAD were excluded, while the selected CAD patients who had a past history of IS were excluded from the study.

A total of 627 control subjects matched by age, gender, and ethnic group were consecutively recruited from Physical Examination Center of the First Affiliated Hospital, Guangxi Medical University during the same period when IS and CAD patients were recruited. The controls were free of IS and CAD by questionnaires, history taking and clinical examination. All enrolled individuals were Han Chinese from Guangxi, the People's Republic of China. A standard questionnaire was used to ascertain the general information and medical history for 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 they received a full explanation of the study.

Biochemical measurements

Venous blood specimens were obtained from all subjects after at least 12 hours of fasting. The levels of serum TC, TG, high-density lipoprotein cholesterol (HDL-C), and LDL-C in samples were determined by enzymatic methods with commercially available kits. Serum apolipoprotein (Apo) A1 and ApoB levels were detected by the immunoturbidimetric immunoassay. The normal values of serum TC, TG, HDL-

C, LDL-C, ApoA1, ApoB levels, and the ratio of ApoA1 to ApoB in our Clinical Science Experiment Center 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 [12, 13]. Type 2 diabetes was diagnosed according to the WHO diagnostic criteria for diabetes: (1) fasting glucose (FPG) ≥ 7.0 mmol/L; (2) 2 h postprandial glucose ≥ 11.1 mmol/L; or (3) self-reported diagnosis of diabetes or use of anti-diabetic medications [34]. The individuals with TC > 5.17 mmol/L, and/or TG > 1.70 mmol/L were defined as hyperlipidemic [35]. Hypertension was defined according to the criteria outlined by the 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension [36]. Uncontrolled hypertension was defined as a systolic blood pressure of 140 mmHg or higher and a diastolic blood pressure of 90 mmHg or higher. The subjects with systolic blood pressure of only 140 mmHg or higher but a diastolic blood pressure of < 90 mmHg were diagnosed as isolated systolic hypertension. Normal weight, overweight and obesity were defined as a body mass index (BMI) < 24, 24-28, and > 28 kg/m^2; respectively [37].

SNP selection and genotyping

The SNPs of rs10889353 and rs10889335 were selected as genetic markers. The two SNPs were selected on the basis of the following assumptions: (1) Selected SNPs were established by Haploview (Broad Institute of MIT and Harvard, USA, version 4.2); (2) SNPs information was obtained from NCBI dbSNP Build 132 (http://www.Ncbi.nlm.nih.gov/SNP/); (3) SNPs were restricted to minor allele frequency (MAF) > 1%; (4) SNPs might be associated with the serum or plasma lipid levels in a recent GWAS [24-30].

Genomic DNA was extracted from leucocytes of venous blood using the phenol-chloroform method, and then sent to the Center for Human Genetics Research, Shanghai Genesky Bio-Tech Co. Ltd. Genotyping of the two SNPs were performed by the Snapshot technology platform [13]. The restriction enzymes for rs10889353 and rs10889335 SNPs were SAP and Exonuclease I (Promega, Epicentre), respectively. The sense and antisense primers were: rs10889353F: 5'-CTCTGAGCCTGAGCCACCTTATCT-3', rs10889353R: 5'-TGTTAACCTTGGTTTAGGCAAGAGGA-3', rs10889335F: 5'-CTGTGCAGCTTCAGCATGATTG-3', rs10889335R: 5'-CTCCCAGCTTGGGAAGCACATA-3'.

Statistical analyses

The statistical analyses were carried out using the statistical software package SPSS 21.0 (SPSS Inc., Chicago, Illinois). Quantitative variables were expressed as mean ± standard deviation (serum TG levels were presented as medians and interquartile ranges). Qualitative variables were expressed as percentages. Allele frequency was determined via direct counting, and the standard goodness-of-fit test was used to test the Handy-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 patient and control groups were tested by the Student's unpaired *t*-test. The association of genotypes and serum lipid parameters was tested by analysis of covariance (ANCOVA). Any variants associated with the serum lipid parameter at a value of $P < 0.025$ (corresponding to $P < 0.05$ after adjusting for two independent tests by the Bonferroni correction) were considered statistically significant. Unconditional logistic regression was used to assess the correlation between the risk of CAD and IS and genotypes. Age, gender, BMI, smoking and alcohol consumption were adjusted for the statistical analysis. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated using unconditional logistic regression. Results were considered to be statistically significant if bilateral *P*-values were less than 0.05. The pattern of pair-wise linkage disequilibrium (LD) between the selected SNPs was measured by *D'* and r^2 using the SHEsis software [38]. Haplotype frequency was determined by means of the algorithms implemented in the PHASE program.

Results

General characteristics of the subjects

The general characteristics of the patients and healthy controls are provided in **Table 1**. As compared with the control group, both CAD and IS groups had higher height, weight, BMI, blood pressure, TG, percentages of subjects who smoked cigarettes, and prevalence of type 2 diabetes, hypertension and hyperlipidemia;

Table 1. General characteristics and serum lipid levels between the controls and patients

Characteristic	Control (n = 627)	CAD (n = 584)	IS (n = 555)	P_1	P_2	P_3
Male/Female	466/161	432/152	401/154	0.890	0.422	0.513
Age, years	62.22±6.75	62.38±10.64	62.85±12.32	0.759	0.286	0.492
Height	155.05±7.87	164.14±6.83	163.80±7.16	0.000	0.000	0.412
Weight	54.51±9.04	64.46±10.70	63.00±11.14	0.000	0.000	0.024
Body mass index, kg/m^2	22.65±3.19	23.82±3.39	23.41±3.49	0.000	0.000	0.045
Systolic blood pressure, mmHg	127.43±19.76	133.02±23.23	147.68±22.11	0.000	0.000	0.000
Diastolic blood pressure, mmHg	81.28±13.13	79.06±14.20	83.71±12.95	0.000	0.001	0.000
Pulse pressure, mmHg	46.27±18.19	53.96±17.50	63.97±17.87	0.000	0.000	0.000
Cigarette smoking, n (%)						
Nonsmoker	383 (61.0)	332 (56.8)	324 (58.4)			
< 20 cigarettes/day	122 (19.5)	57 (9.8)	162 (29.2)			
≥ 20 cigarettes/day	122 (19.5)	195 (33.4)	69 (12.4)	0.000	0.000	0.000
Alcohol consumption, n (%)						
Nondrinker	358 (57.1)	451 (77.2)	398 (71.7)			
< 25 g/day	150 (23.9)	56 (9.6)	124 (22.3)			
≥ 25 g/day	119 (19.0)	77 (13.2)	33 (5.9)	0.000	0.000	0.000
Total cholesterol, mmol/L	4.89±1.06	4.53±1.19	4.52±1.15	0.000	0.000	0.886
Triglyceride, mmol/L	1.37±1.78	1.66±1.11	1.67±1.37	0.001	0.001	0.893
HDL-C, mmol/L	1.90±0.48	1.14±0.34	1.22±0.40	0.000	0.000	0.000
LDL-C, mmol/L	2.73±0.78	2.71±1.00	2.68±0.90	0.713	0.321	0.594
Apolipoprotein (Apo) A1, g/L	1.41±0.27	1.04±0.52	1.03±0.22	0.000	0.000	0.670
ApoB, g/L	0.90±0.21	0.91±0.27	0.89±0.25	0.506	0.473	0.195
ApoA1/ApoB	1.65±0.52	1.37±2.45	1.26±0.60	0.008	0.000	0.293
Type 2 diabetes, n (%)	25 (4.0)	95 (16.3)	124 (22.3)	0.000	0.000	0.009
Hypertension, n (%)	180 (28.7)	298 (51.0)	272 (49.0)	0.000	0.000	0.496
Hyperlipidemia, n (%)	198 (31.6)	267 (45.7)	254 (45.8)	0.000	0.000	0.987

CAD, coronary artery disease; IS, ischemic stroke; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. The value of triglyceride was presented as median (interquartile range), the difference between CAD/IS patients and controls was determined by the Wilcoxon-Mann-Whitney test. P_1: CAD *vs*. contro ls; P_2: IS *vs*. controls; P_3: IS *vs*. CAD.

and lower TC, HDL-C, ApoA1, ApoA1/ApoB ratio, and percentages of subjects who consumed alcohol ($P < 0.01$ for all). The IS patients had higher blood pressure, HDL-C, and prevalence of type 2 diabetes than the CAD patients ($P < 0.01$ for all). The constituent ratio of cigarette smoking and alcohol consumption was also different between the CAD and IS groups, the percentages of subjects who smoked < 20 cigarettes/day and consumed < 25 g/day alcohol were higher in IS than in CAD groups ($P < 0.001$). There were no differences in the age, gender, serum LDL-C and ApoB levels between the control and patient groups ($P > 0.05$).

Genotypic and allelic frequencies

The genotypic and allelic frequencies of *DOCK7* rs10889353 and rs10889335 SNPs are presented in **Table 2**. The genotype distribution of the two SNPs was concordant with the Hardy-Weinberg proportions in both cases and controls. For the rs10889353 SNP, the frequency of the A and C alleles was 82.62% and 17.38% in the controls, 77.65% and 22.35% in the CAD patients ($P < 0.01$ *vs*. controls), and 79.19% and 20.81% in the IS patients ($P < 0.05$ for IS *vs*. controls; $P > 0.05$ for IS *vs*. CAD); respectively. The frequency of the AA, AC and CC genotypes was 67.63%, 29.98% and 2.39% in the controls; 61.30%, 32.71% and 5.99% in the CAD patients ($P < 0.01$ *vs*. controls); and 63.42%, 31.53% and 5.05% in the IS patients ($P < 0.05$ for IS *vs*. controls; $P > 0.05$ for IS *vs*. CAD); respectively.

For the rs10889335 SNP, the frequency of the A and G alleles was 82.78% and 17.22% in the controls; 77.91% and 22.09% in the CAD patients ($P < 0.01$ *vs*. controls); and 81.62%

Table 2. Genotypic and allelic frequencies and the risk of coronary artery disease (CAD) and ischemic stroke (IS)

Genotype/allele	Control, n (%)	CAD, n (%)	IS, n (%)	CAD		IS	
	N = 627	N = 584	N = 555	OR (95% CI)	*P*	OR (95% CI)	*P*
rs10889353							
AA	424 (67.63)	358 (61.30)	352 (63.42)	1		1	
AC	188 (29.98)	191 (32.71)	175 (31.53)	1.20 (0.94-1.54)	0.079	1.12 (0.87-1.44)	0.202
CC	15 (2.39)	35 (5.99)	28 (5.05)	2.76 (1.49-5.14)	0.001	2.25 (1.18-4.28)	0.009
X^2		12.082	6.715				
P		0.002	0.035				
AA	424 (67.63)	358 (61.30)	352 (63.42)	1		1	
AC+CC	203 (22.37)	226 (38.70)	203 (36.58)	1.32 (1.04-1.67)	0.013	1.21 (0.95-1.53)	0.073
X^2		5.283	2.303				
P		0.022	0.129				
A	1036 (82.62)	907 (77.65)	879 (79.19)	1		1	
C	218 (17.38)	261 (22.35)	231 (20.81)	1.37 (1.12-1.67)	0.001	1.25 (1.02-1.53)	0.019
X^2		9.383	4.493				
P		0.002	0.034				
HWE (*P*)	0.272	0.164	0.307				
rs10889335							
AA	428 (68.26)	361 (61.82)	376 (67.75)	1		1	
AG	182 (29.03)	188 (32.19)	154 (27.75)	1.23 (0.96-1.57)	0.061	0.96 (0.75-1.24)	0.412
GG	17 (2.71)	35 (5.99)	25 (4.50)	2.44 (1.35-4.43)	0.002	1.67 (0.89-3.15)	0.073
X^2		10.504	2.845				
P		0.005	0.241				
AA	428 (68.26)	361 (61.82)	376 (67.75)	1		1	
AG+GG	199 (31.74)	223 (38.18)	179 (32.25)	1.33 (1.05-1.68)	0.011	1.02 (0.80-1.31)	0.449
X^2		5.535	0.036				
P		0.019	0.850				
A	1038 (82.78)	910 (77.91)	906 (81.62)	1		1	
G	216 (17.22)	258 (22.09)	204 (18.38)	1.36 (1.11-1.67)	0.002	1.08 (0.88-1.34)	0.249
X^2		9.090	0.536				
P		0.003	0.464				
HWE (*P*)	0.653	0.118	0.077				

Adjusted for sex, age, smoking, drinking, BMI, diabetes, hypertension, hyperlipidemia. CAD, coronary artery disease; IS, ischemic stroke. HWE, Hardy-Weinberg equilibrium.

and 18.38% in the IS patients ($P > 0.05$ for IS *vs*. controls; $P > 0.05$ for IS *vs*. CAD); respectively. The frequency of the AA, AG and GG genotypes was 68.26%, 29.03% and 2.71% in the controls; 61.82%, 32.19% and 5.99% in the CAD patients ($P < 0.01$ *vs*. controls); and 67.75%, 27.75% and 4.50% in the IS patients ($P > 0.05$ for IS *vs*. controls; $P < 0.05$ for IS *vs*. CAD); respectively.

DOCK7 SNPs and the risk of CAD and IS

As shown in **Table 2**, the C allele and CC genotype of rs10889353 SNP were associated with an increased risk of CAD (adjusted OR = 1.37, 95% CI = 1.12-1.67, $P = 0.001$ and adjusted OR = 2.76, 95% CI = 1.49-5.14, $P = 0.001$; respectively) and IS (adjusted OR = 1.25, 95% CI = 1.02-1.53, $P = 0.019$ and adjusted OR = 2.25, 95% CI = 1.18-4.28, $P = 0.009$; respectively).

The G allele and GG genotype of rs10889335 SNP were associated with an increased risk of CAD (adjusted OR = 1.36, 95% CI = 1.11-1.67, $P = 0.002$ and adjusted OR = 2.44, 95% CI = 1.35-4.43, $P = 0.002$; respectively) but not with IS (adjusted OR = 1.08, 95% CI = 0.88-1.34, $P = 0.249$ and adjusted OR = 1.67, 95% CI = 0.89-3.15, $P = 0.073$; respectively).

Table 3. Haplotype and the risk of CAD and IS

Haplotype	Frequency			CAD		IS	
	Control	CAD	IS	OR (95% CI)	*P*	OR (95% CI)	*P*
rs10889353A-rs10889335A	0.819	0.765	0.788	0.721 (0.592-0.878)	0.001	0.823 (0.671-1.009)	0.060
rs10889353A-rs10889335G	0.007	0.011	0.004	1.563 (0.669-3.654)	0.299	0.509 (0.158-1.638)	0.249
rs10889353C-rs10889335A	0.009	0.014	0.028	1.574 (0.730-3.395)	0.243	3.234 (1.621-6.450)	0.000
rs10889353C-rs10889335G	0.165	0.210	0.180	1.342 (1.093-1.648)	0.005	1.111 (0.898-1.376)	0.332

Adjusted for age, gender, BMI, smoking status, alcohol consumption, hypertension, hyperlipidemia.

Table 4. Association of the rs10889353 and rs10889335 SNPs and angiographic severity of CAD

SNP	Genotype	Angiographic severity of CAD	
		OR (95% CI)	*P*
rs10889353			
	AA	1	
	AC	1.118 (0.764-1.637)	0.565
	CC	2.876 (1.088-7.604)	0.027
rs10889335			
	AA	1	
	AG	1.066 (0.729-1.560)	0.740
	GG	5.115 (1.535-17.046)	0.003

Adjust for age, gender, BMI, smoking status, alcohol consumption, hypertension, hyperlipidemia and type 2 diabetes.

Haplotype and the risk of CAD and IS

The rs10889353 SNP was in high LD with the rs10889335 SNP in controls and CAD (D' = 0.942, r^2 = 0.875), controls and IS (D' = 0.961, r^2 = 0.851), and CAD and IS (D' = 0.952, r^2 = 0.837). Thus, haplotype analyses of the two SNPs and the associations of their haplotypes and the risk of CAD and IS were also performed. The haplotype of rs10889353A-rs10889335A (76.5% frequency) was associated with a decreased risk for CAD (adjusted OR = 0.721, 95% CI = 0.592-0.878, *P* = 0.001). By contrast, the haplotype of rs10889353C-rs10889335G (21.0%) was associated with an increased risk for CAD (adjusted OR = 1.342, 95% CI = 1.093-1.648, *P* = 0.005). The haplotype of rs10889353C-rs10889335A (2.8%) was also associated with an increased risk for IS (adjusted OR = 3.234, 95% CI = 1.621-6.450, *P* < 0.001; **Table 3**).

DOCK7 SNPs and the angiographic severity of CAD

As shown in **Table 4**, the rs10889353CC and rs10889335GG genotypes were associated with the angiographic severity of CAD (*P* < 0.05 and *P* < 0.01; respectively).

DOCK7 SNPs and serum lipid levels

The association of the rs10889353 and rs10889335 SNPs and serum lipid levels in the controls is summarized in **Table 5**. Serum TC and TG levels were different among the three genotypes of the rs10889353 and rs10889335 SNPs (*P* = 0.012-0.001), the rs10889353C and rs10889335G allele carriers had higher TC and TG levels than the rs10889353C and rs10889335G allele non-carriers. There was no difference in serum HDL-C, LDL-C, ApoA1, ApoB levels and the ApoA1/ApoB ratio among the three genotypes of both SNPs.

Stratified analyses of the DOCK7 SNPs and the risk of CAD or IS

Table 6 lists the results of stratified analyses according to gender, age, BMI, smoking, drinking, hypertension, and hyperlipidemia. The *DOCK7* rs10889353CC and rs10889335GG genotypes were associated with an increased risk of CAD in patients with age > 60 years, BMI ≤ 24 kg/m^2, smoking, non-drinking, normotensive, non-hyperlipidemia (*P* < 0.05 for all). The *DOCK7* rs10889353CC genotype was associated with an increased risk of IS in patients with age > 60 years, BMI ≤ 24 kg/m^2, non-smoking, non-drinking, and normotensive (*P* < 0.05 for all). The *DOCK7* rs10889335GG genotype was also associated with an increased risk of IS in patients with age > 60 years (*P* < 0.05).

Discussion

In the present study, we detected the association between the *DOCK7* rs10889353 and rs10889335 SNPs and their haplotypes and

Table 5. Association of the rs10889353 and rs10889335 genotypes and serum lipid levels in controls

Genotype	n	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ApoA1 (g/L)	ApoB (g/L)	ApoA1/ApoB
rs10889353								
AA	424	4.80±0.99	0.97 (0.73)	1.92±0.55	2.70±0.76	1.41±0.28	0.89±0.20	1.66±0.52
AC	187	4.91±0.88	1.12 (0.83)	1.89±0.46	2.79±0.83	1.41±0.27	0.92±0.22	1.64±0.49
CC	15	5.78±1.67	1.55 (1.07)	1.86±0.29	2.82±0.69	1.41±0.22	0.92±0.26	1.61±0.53
F		7.254	4.451	0.424	0.776	0.008	1.595	0.718
P		0.001	0.012	0.654	0.461	0.992	0.204	0.488
rs10889335								
AA	428	4.81±1.00	0.97 (0.74)	1.93±0.53	2.70±0.77	1.42±0.27	0.90±0.21	1.68±0.49
AG	181	4.92±0.84	1.12 (0.80)	1.89±0.47	2.74±0.71	1.42±0.21	0.90±0.25	1.65±0.52
GG	17	5.56±1.70	1.55 (1.04)	1.86±0.27	2.79±0.80	1.41±0.28	0.92±0.21	1.63±0.52
F		4.834	4.772	0.673	0.447	0.092	1.073	0.218
P		0.008	0.009	0.511	0.640	0.912	0.342	0.804

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B. The value of triglyceride was presented as median (interquartile range), and the difference among the genotypes was determined by the Kruskal-Wallis test.

serum lipid levels and the risk of CAD and IS in the Han Chinese population. To the best of our knowledge, this is the first report to evaluate the association between the two SNPs and their haplotypes and the risk of CAD and IS. We showed that the MAF of the rs10889353C was higher in the CAD (22.35%, $P < 0.01$) and IS (20.81%, $P < 0.05$) patients than in the controls (17.38%). The frequency of the AA, AC and CC genotypes was different between the controls and CAD ($P < 0.01$) and IS patients ($P < 0.05$). The genotypic and allelic frequencies of rs10889335 SNP were also different between the controls and CAD patients ($P < 0.01$ and $P < 0.05$; respectively), but not between the controls and IS patients ($P > 0.05$). In several previous GWASs, the MAF of rs10889353C was 35% in Dutch [24] in Doetinchem, a town in a rural area in the east of the Netherlands; 32% in 16 European population cohorts [26]; 19% in the Chinese population [27]; 14% in a Japanese population [29]; and 38.2% in the Candidate gene Association Resource (CARe) African-American meta-analyses [30]. The MAF of rs10889335G was 39.4% in the CARe African-American meta-analyses [30]. Thus, our findings, coupled with reports in the several previous GWASs, provide evidence for the prevalence of the *DOCK7* rs10889353C and rs10889335G allele variation may have a racial/ethnic specificity. The frequencies of the *DOCK7* rs10889353C and rs10889335G alleles were higher in the European and African-American than in the Asian. These findings may also partly explain why the prevalence of cardiovascular disease is higher in the European and American than in the Asian.

The potential association between the *DOCK7* rs10889353 and rs10889335 SNPs and the risk of CAD and IS has not been previously explored. In a recent GWAS, Waterworth et al. [28] showed that the *DOCK7* rs1168013 SNP in the case-control studies of CAD derived from 9 studies comprising up to 9,633 cases and 38,684 controls of white European descent was potentially associated with the risk of CAD (OR = 0.96, 95% CI = 0.91 to 1.00, $P = 0.06$). The effect allele frequency of rs1168013G was 65%. In the current study, we showed that the C allele and CC genotype of rs10889353 SNP were associated with an increased risk of CAD and IS. The G allele and the GG genotype of rs10889335 SNP were associated with an increased risk of CAD. The haplotype of rs10889353C-rs10889335G (21.0%) was associated with an increased risk for CAD. The haplotype of rs10889353C-rs10889335A (2.8%) was also associated with an increased risk for IS. Thus, the results of the present study provide comprehensive and convincing evidence of the genetic determinants of CAD and IS in a Han Chinese population.

The association between the *DOCK7* rs10889353 [24, 26, 27, 29, 30] and rs10889335 [30] SNPs and serum or plasma lipid phenotypes has been investigated in several previ-

Table 6. Stratified analyses of the *DOCK7* SNPs and the risk of CAD or IS

SNP/Factor	OR (95% CI)$_{CAD}$					OR (95% CI)$_{IS}$				
	AA	AC/AG	CC/GG	$P_{AC/AG}$	$P_{CC/GG}$	AA	AC/AG	CC/GG	$P_{AC/AG}$	$P_{CC/GG}$
rs10889353										
Gender/male	1	1.257 (0.943-1.675)	2.607 (1.290-5.265)	0.119	0.006	1	1.053 (0.782-1.418)	2.231 (1.084-4.592)	0.732	0.026
Gender/female	1	1.068 (0.664-1.716)	3.391 (0.891-12.905)	0.787	0.059	1	1.277 (0.802-2.033)	2.337 (0.568-9.616)	0.303	0.227
Age/≤ 60 years	1	1.008 (0.658-1.543)	1.261 (0.481-3.303)	0.971	0.637	1	1.402 (0.919-2.139)	1.368 (0.513-3.645)	0.116	0.530
Age > 60 years	1	1.333 (0.983-1.808)	4.390 (1.926-10.006)	0.064	0.000	1	0.921 (0.669-1.268)	2.981 (1.264-7.029)	0.615	0.009
BMI/≤ 24 kg/m^2	1	1.384 (1.008-1.901)	2.892 (1.345-6.214)	0.044	0.005	1	1.216 (0.890-1.662)	2.251 (1.033-4.905)	0.220	0.037
BMI/> 24 kg/m^2	1	0.964 (0.645-1.440)	2.734 (0.892-8.379)	0.857	0.068	1	0.950 (0.624-1.448)	2.311 (0.717-7.442)	0.813	0.150
Smoking/No	1	1.204 (0.876-1.656)	2.237 (0.969-5.167)	0.252	0.054	1	1.167 (0.846-1.609)	2.271 (0.983-5.245)	0.346	0.049
Smoking/Yes	1	1.206 (0.819-1.775)	3.436 (1.337-8.828)	0.343	0.007	1	1.061 (0.713-1.578)	2.213 (0.811-6.044)	0.772	0.113
Drinking/No	1	1.109 (0.821-1.499)	3.113 (1.396-6.940)	0.498	0.004	1	0.964 (0.705-1.316)	2.288 (0.989-5.292)	0.816	0.047
Drinking/Yes	1	1.368 (0.871-2.149)	1.955 (0.637-5.996)	0.173	0.234	1	1.501 (0.980-2.299)	2.298 (0.809-6.530)	0.061	0.110
Hypertension/No	1	1.222 (0.910-1.640)	3.308 (1.608-6.804)	0.183	0.001	1	1.207 (0.849-1.717)	1.743 (0.686-4.433)	0.295	0.238
Hypertension/Yes	1	1.030 (0.815-1.300)	1.054 (0.625-1.779)	0.807	0.843	1	1.015 (0.803-1.282)	1.119 (0.668-1.874)	0.902	0.668
Hyperlipidemia/No	1	1.155 (0.828-1.612)	4.955 (1.631-15.056)	0.395	0.002	1	1.246 (0.895-1.735)	3.811 (1.210-12.000)	0.196	0.014
Hyperlipidemia/Yes	1	1.054 (0.819-1.357)	0.949 (0.599-1.503)	0.683	0.823	1	0.988 (0.765-1.277)	0.902 (0.565-1.441)	0.929	0.667
rs10889335										
Gender/male	1	1.356 (1.014-1.814)	2.551 (1.291-5.042)	0.040	0.005	1	0.982 (0.723-1.332)	1.724 (0.837-3.554)	0.905	0.136
Gender/female	1	0.933 (0.582-1.497)	2.128 (0.620-7.300)	0.774	0.221	1	0.895 (0.559-1.434)	1.531 (0.419-5.591)	0.645	0.517
Age/≤ 60 years	1	0.935 (0.609-1.436)	1.340 (0.518-3.464)	0.760	0.545	1	0.905 (0.586-1.396)	0.952 (0.344-2.631)	0.651	0.924
Age > 60 years	1	1.426 (1.050-1.937)	3.403 (1.579-7.335)	0.023	0.001	1	0.972 (0.705-1.341)	2.263 (1.008-5.080)	0.864	0.043
BMI/≤ 24 kg/m^2	1	1.458 (1.060-2.004)	2.237 (1.035-4.834)	0.020	0.036	1	1.098 (0.799-1.510)	1.744 (0.801-3.800)	0.565	0.157
BMI/> 24 kg/m^2	1	0.929 (0.620-1.393)	2.583 (0.939-7.105)	0.722	0.058	1	0.750 (0.487-1.155)	1.551 (0.517-4.658)	0.191	0.431
Smoking/No	1	1.193 (0.867-1.642)	1.815 (0.824-3.995)	0.278	0.134	1	0.933 (0.671-1.296)	1.608 (0.723-3.573)	0.679	0.240
Smoking/Yes	1	1.284 (0.868-1.901)	3.494 (1.360-8.972)	0.211	0.006	1	1.018 (0.678-1.528)	1.804 (0.641-5.078)	0.933	0.258
Drinking/No	1	1.138 (0.842-1.538)	2.578 (1.189-5.589)	0.401	0.013	1	0.848 (0.618-1.164)	1.647 (0.721-3.763)	0.308	0.233
Drinking/Yes	1	1.306 (0.823-2.047)	2.262 (0.821-6.229)	0.257	0.106	1	1.195 (0.768-1.859)	1.845 (0.673-5.059)	0.429	0.228
Hypertension/No	1	1.280 (0.952-1.720)	2.948 (1.457-5.964)	0.101	0.002	1	1.023 (0.712-1.469)	1.510 (0.604-3.774)	0.901	0.376
Hypertension/Yes	1	1.017 (0.801-1.292)	1.050 (0.617-1.785)	0.889	0.858	1	1.002 (0.789-1.273)	1.117 (0.662-1.884)	0.988	0.678
Hyperlipidemia/No	1	1.235 (0.884-1.726)	4.032 (1.452-11.195)	0.217	0.004	1	1.063 (0.757-1.492)	2.389 (0.804-7.097)	0.724	0.107
Hyperlipidemia/Yes	1	1.041 (0.806-1.344)	0.944 (0.596-1.495)	0.758	0.806	1	0.933 (0.719-1.211)	0.838 (0.522-1.345)	0.603	0.464

OR and 95% CI were obtained from unconditional Logistic regression model after adjusted for age, gender, BMI, smoking status, alcohol consumption, hypertension, hyperlipidemia.

ous GWASs. The *DOCK7* rs10889353 SNP was associated with TC in European population cohorts [24, 26] and in the Chinese population [27], TG in a Japanese population [29], and LDL-C in the CARe African-American meta-analyses [30]. The *DOCK7* rs10889335 SNP was associated with LDL-C in 8,090 African Americans from five population-based cohorts [30]. In addition, 3 other GWASs also showed that the *DOCK7* rs11207995 SNP was associated with TC in the Han Chinese ethnicity [25], the *DOCK7* rs1167998 SNP with TC and TG in 16 European population cohorts [26], and the *DOCK7* rs1168013 SNP with TG in the white European descent [28]. In the present study, we showed that both *DOCK7* rs10889353 and rs10889335 SNPs were associated with serum TC and TG levels in the healthy controls, the rs10889353C and rs10889335G allele carriers had higher TC and TG levels than the rs10889353C and rs10889335G allele non-carriers. There was no difference in serum HDL-C, LDL-C, ApoA1, ApoB levels and the ApoA1/ApoB ratio among the three genotypes of the SNPs. These findings suggest that the *DOCK7* rs10889353 and rs10889335 SNPs associated with serum lipid traits in Europeans and African-Americans can also be replicated in a Han Chinese southern population.

The interactions of the two SNPs and some environmental factors on the risk of CAD and IS are not known. In the present study, stratified analyses according to gender, age, BMI, smoking, drinking, hypertension, and hyperlipidemia showed that the *DOCK7* rs10889353CC and rs10889335GG genotypes were associated with an increased risk of CAD in patients with age > 60 years, BMI ≤ 24 kg/m^2, smoking, non-drinking, normotensive, non-hyperlipidemia. The *DOCK7* rs10889353CC genotype was associated with an increased risk of IS in patients with age > 60 years, BMI ≤ 24 kg/m^2, non-smoking, non-drinking, and normotensive. The *DOCK7* rs10889335GG genotype was also associated with an increased risk of IS in patients with age > 60 years. These findings suggest that the two SNPs may interact with gender, age, BMI, smoking, drinking, type 2 diabetes, hypertension and hyperlipidemia to influence the risk of CAD and IS. But these interactions still need to be determined.

Limitations

There were several potential limitations in this study. Firstly, the sample size was relatively small compared to many GWASs and replication studies. Therefore, further studies with larger sample sizes are needed to confirm our results. Secondly, there were significant differences in the general characteristics between the control and patient groups, or between the CAD and IS patients. Although age, gender, BMI, cigarette smoking and alcohol consumption have been adjusted for the statistical analysis, we could not completely eliminate the potential effects of these factors on serum lipid levels and the risk of CAD and IS. Thirdly, the association of the two SNPs and serum lipid levels in the CAD and IS groups was not analyzed because of the interference of lipid-lowering drugs. Finally, it is well known that both CAD and IS are the complex multifactorial disorders that are believed to result from an interaction between the genetic background of an individual and various environmental factors. Although we have detected the association between the *DOCK7* rs10889353 and rs10889335 SNPs and their haplotypes and the risk of CAD and IS, there are still many unmeasured environmental and genetic factors and their interactions.

Conclusions

The results of the present study showed that the *DOCK7* rs10889353 and rs10889335 SNPs and their haplotypes in the Han Chinese population are associated with the risk of CAD and IS, the rs10889353C allele and CC genotype were associated with an increased risk of CAD and IS. The rs10889335G allele and the GG genotype and rs10889353C-rs10889335G (21.0%) haplotype were associated with an increased risk of CAD. The rs10889353C and rs10889335G allele carriers in the healthy controls had higher TC and TG levels than the rs10889353C and rs10889335G allele non-carriers. The genotypes of the two SNPs may interact with gender, age, body mass index, smoking, drinking, hypertension and hyperlipidemia to increase or decrease the risk of CAD and IS. However, large studies of populations with different ethnic origins are required to confirm these observations.

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Disclosure of conflict of interest

None.

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