Vascular endothelial growth factor-A (VEGFA) gene polymorphisms may serve as genetic marker for Coronary Heart Disease (CHD)

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Research

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Abstract

Purpose: Coronary heart disease (CHD) is a common cardiovascular disease resulting from interaction of multiple environmental and genetic factors. This study aimed to confirm whether single nucleotide polymorphisms (SNPs) in *VEGFA* gene were associated with CHD in the Han Chinese population.

Materials and Methods: Blood samples were collected from 501 CHD patients and 496 healthy individuals. Genotyping of five SNPs within VEGFA was performed using Agena MassARRAY platform. Odd ratios (ORs) and 95% confidence intervals (95%CIs) were calculated to evaluate the association between SNPs and CHD risk.

Results: The genotype "C/T" of rs3025021 in *VEGFA* was found to be associated with CHD susceptibility (OR = 1.35, 95% CI = 1.02- 1.80, p = 0.038) in the overall. Rs833068 was observed to be associated with the reduced risk of CHD at age > 61 years and ≤ 61 years, respectively. And three loci (rs833068, rs3025021 and rs6905288) were related to the CHD risk in males. In addition, rs3025021 was associated with increased risk of CHD in patients with hypertension or diabetes. Conversely, three loci (rs833068, rs3025030 and rs6905288) were related to the CHD risk in patients without hypertension. The rs833068 was associated with reduced risk of CHD in patients without diabetes. Finally, we found a strong LD between rs833068

and rs833070.

Conclusion: Gene polymorphisms in *VEGFA* were notably correlated with altered CHD risk in the Han Chinese population. Large sample size and well - designed studies are needed to further clarify the potential mechanisms underlying the CHD.

1. Introduction

Coronary artery disease (CAD) continues to be one of the most common cardiovascular disease with high morbidity and mortality (1), which represents a public health challenge in both industrialized and developing countries (2). Coronary heart disease (CHD) is the most common and severe manifestation of CAD (3). CHD, the myocardial functional or organic lesion, is caused by coronary artery stenosis or occlusion, or shortage of blood and oxygen supply (4). According to the data from the American Heart Association in 2009, cardiovascular disease has become the leading cause of death in the world (5). As China gradually enters an aging society and the number of the elderly gradually increases, CHD is the second leading cause of death after malignant tumors (6).

Numerous studies of its etiology and pathogenesis indicated that many factors have been proved to be associated with the occurrence of CHD, such as age, gender, diabetes, diet, and genetic factors (7, 8). Despite the specific factors influencing susceptibility of CHD is still unknown, recent molecular epidemiological studies have pointed to the potential and significant role of gene variants associated CHD, such as *CCDC92* (9), *AdipoQ* (10). The vascular permeability factor (*VEGF*) gene, consisting of *VEGFA*, *VEGFB*, *VEGFC*, *VEGFD*, *VEGFE*, *VEGFF* and placental growth factor, is located on chromosome 6p21.3 and could express different isoforms of proteins (11). Usually, *VEGFA* is referred as *VAGF*. The human *VEGFA* gene is very polymorphic. Doxing Liu et al (12) showed that 3 SNPs (rs699947, rs3025039 and rs1570360) of *VEGFA* were remarkably associated with the susceptibility to CHD in a Han Chinese population. In addition, a systematic review and meta-analysis showed that *VEGFA* rs699947 polymorphism was not associated with CHD (13). However, there are few studies which are able to clarify the potential relationship between *VEGFA* genetic polymorphisms and the susceptibility to CHD in a Han Chinese population.

Therefore, a case-control study was carried out to evaluate the influence of *VEGFA* polymorphisms at allele, genotype, and haplotype interface with development of CHD among Han Chinese population.

2. Materials And Methods

2.1. Study participants

Using a case-control study, 501 CHD patients and 496 unrelated healthy controls in a large cohort of Han Chinese population were recruited from Central South University Xiangya School of Medicine Affiliated Haikou Hospital to investigate whether the *VEGFA* variants have influence on CHD. All CHD patients were diagnosed including classic ischemic symptoms, plus one or more electrocardiographic (ECG) changes (ST-segment depression or elevation of ≥ 0.5 mm, T-wave inversion of ≥ 3 mm in ≥ 3 leads, or left bundle branch block), in addition to increased cardiac markers such as creatinine kinase-MB and troponin T (9). Patients with other diseases, such as complex hematologic, autoimmune, congenital cardiac structural or functional abnormalities, or tumors were excluded. At the same time, the 496 healthy controls were randomly selected from the same hospital in the same period and were diagnosed without heart disease or other diseases. All subjects were unrelated individuals and at least three generations of Han ancestors.

2.2 Data collection

The protocol of the present study was approved by the clinical investigative ethical committee of Central South University Xiangya School of Medicine Affiliated Haikou Hospital, and all procedures were performed in compliance with medical research involving humans of the World Medical Association Declaration of Helsinki. Informed consent forms were obtained from all participants after a full explanation. Blood samples from each individual were harvested respectively at the time of initial diagnosis. Subsequently, about 5 ml venous blood samples were collected from each participant into tubes containing ethylenediamine tetraacetic acid (EDTA) for anticoagulation. A Whole Blood Genomic DNA Extraction Kit (Tiangen Biotech, Beijing, China) was used to extract genomic DNA from peripheral blood samples according to the manufacturer's instructions. And the purity and concentration of the DNA samples were evaluated with the NanoDrop 2000C (Thermo Scientifc, Waltham, Massachusetts, USA) (14). The isolated DNA samples were stored at − 80 °C until analysis.

2.3 SNP selection and genotyping

Five candidate SNPs (rs833068, rs833070, rs3025021, rs3025030, rs6905288) in the *VEGFA* gene were selected with the minor allele frequency (MAF) > 0.05 in Han Chinese from the 1000 Genomes Project (http://www.1000genomes.org/). The primers for amplification and extension reactions were designed with Agena MassARRAY Assay Design 3.0 Software (Table 1) (15). The SNP genotyping was performed with Agena MassARRAY RS1000 according to the manufacturer's instruction. The data management and analysis were carried using Agena Typer 4.0 software (15, 16).

Table 1
PCR primer for this study.

SNP-ID	Foward primer (5'-3') for PCR	Reverse primer (5'-3') for PCR	UEP_DIR	UEP SEQ
rs833068	ACGTTGGATGGGGAGAGTGGACATTTAGTG	ACGTTGGATGGAGATCCCATTAGGCTGAG	R	GCTCACACAGGAAGGGT
rs833070	ACGTTGGATGGCTCAGCCTAATGGGATCTC	ACGTTGGATGAGTTCACAGCACCCGAACAT	R	cattaACAGCACCCGAACATAGTCAA
rs3025021	ACGTTGGATGCACAGAGGCCTCCTTGCAG	ACGTTGGATGGGTGTGATGGGAGGCTAAG	R	CACAGCCTCCGACCC
rs3025030	ACGTTGGATGTGGCTGTTCGTTTAGGATGG	ACGTTGGATGGACTGGTGGAGGATTAAAGG	R	GGAGGATTAAAGGTATCTAGTATT
rs6905288	ACGTTGGATGTGAGGAATAATAACCACCCC	ACGTTGGATGAAGTAGAGAGAAGCCATCCC	F	GCCATCCCTGCCCCA

SNP, Single-nucleotide polymorphism; UEP SEQ, Unextended mini-sequencing primer; DIR: direction.

2.4 Statistical analysis

Statistical analyses were analyzed using SPSS 19.0 (SPSS, Chicago, IL, USA) and Microsoft Excel. The two-sided Chi-square tests and independent sample Student's t-test were applied to assess the differences in the distribution of gender and age between cases and controls, respectively. The genotype frequencies of the control group were tested for the Hardy-Weinberg equilibrium (HWE) using Chi-square test. Chi-squared test was used to calculate the allele and genotype frequencies of each SNP between cases and controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the association between *VEGFA* gene polymorphisms and the CHD risk using logistic regression analysis with or without adjustment for age and gender (17). The wild type allele was used as a reference. The four genetic models analyses were applied using PLINK software (Version 1.07) to evaluate the associations between SNPs and the CHD risk (18). Then, we conducted stratification analysis on age, gender, CHD-associated diseases. Finally, we performed linkage disequilibrium (LD) and haplotype analysis using the Haploview software package (version 4.2). All p values of statistical tests in this study were two-sided, and $p < 0.05$ indicated statistical significance.

3. Results

3.1 Participant characteristics.

We recruited 501 CHD patients (mean age 61.32 ± 11.70 years old) consisting of 320 males and 181 females and 496 unrelated healthy individuals (mean age 60.69 ± 6.42 years old) containing of 318 healthy males and 178 females in this study. There were no statistically significant differences on the distribution of gender and age between the cases and controls ($p = 0.885$, $p = 0.289$, respectively). In the case group, there were 296 patients with hypertension and 101 patients with diabetes, while there were 205 patients without hypertension and 400 patients without diabetes, respectively. The basic characteristics of all subjects were shown in Table 2.

Table 2
Characteristics of the cases and controls.

Variables	Cases (n = 501)	Controls (n = 496)	*p*
Gender			0.885[a]
Male	320(64%)	318(64%)	
Female	181(36%)	178(36%)	
Age	61.32 ± 11.70	60.69 ± 6.42	0.289[b]
> 61	250(50%)	233(47%)	
≤ 61	251(50%)	263(53%)	
Hypertension			
yes	296(60%)		
no	205(40%)		
Diabetes			
yes	101(20%)		
no	400(80%)		
[a]*p* value was calculated from two-sided Chi-squared tests; [b]*p* value was calculated from Student's t test.			
$p < 0.05$ indicates statistical significance.			

3.2. Association between VEGFA variations and CHD risk.

In our study, the basic information about the selected SNPs in *VEGFA* gene was listed in Table 3. The genotype distribution of all candidate SNPs in control group were in accordance with HWE (*p* value > 0.05). We had not found that any SNPs were significantly associated with the risk of CHD in the allele model (all $p > 0.05$). Four genetic models, including the codominant model, the dominant model, the recessive model, and the Log-additive model were carried out to analyze the relationship between SNPs genotypes and CHD risk. The results were showed in Table 4. The significantly positive association was found between the "C/T" of rs3025021 in *VEGFA* and CHD susceptibility in the codominant model (OR = 1.35, 95% CI = 1.02–1.80, $p = 0.038$) after adjustment for gender and age, which showed that the rs3025021 was a risk factor in the development of CHD. However, we observed no significant correlation between other SNPs and CHD risk.

Table 3
Basic information of the 5 SNPs in this study.

SNP-ID	Chr.	Position	Allele	MAF		p^a-HWE	Allele model	
			(A/B)	Cases	Controls		OR(95%CI)	p^b
rs833068	6	43774790	A/G	0.411	0.440	0.412	0.89(0.74–1.06)	0.185
rs833070	6	43774889	C/T	0.238	0.229	0.252	1.05(0.85–1.30)	0.631
rs3025021	6	43781426	C/T	0.168	0.145	0.204	1.19(0.93–1.51)	0.167
rs3025030	6	43782850	C/G	0.178	0.156	0.393	1.17(0.92–1.48)	0.200
rs6905288	6	43791136	A/G	0.262	0.268	0.210	0.97(0.8–1.19)	0.774
SNP, single-nucleotide polymorphism; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval.								
$p < 0.05$ indicates statistical significance.								

Table 4
SNPs associated with the CHD with adjustments for gender and age.

SNP	Model	Genotype	Case	Control	Before adjusted		After adjusted	
					OR(95%CI)	*p*-value	OR(95%CI)	*p*-value
rs833068	Co-dominant	G/G	175(35.00%)	150(30.31%)	1[Ref]		1[Ref]	
		A/G	239(47.80%)	254(51.31%)	0.81(0.61–1.07)	0.133	0.80(0.61–1.07)	0.128
		A/A	86(17.20%)	91(18.38%)	0.81(0.56–1.17)	0.260	0.81(0.56–1.17)	0.254
	Dominant	G/G	175(35.00%)	150(30.31%)	1[Ref]		1[Ref]	
		A/G-A/A	325(65.00%)	345(69.69)	0.81(0.62–1.05)	0.114	0.80(0.62–1.05)	0.110
	Recessive	G/G-A/G	414(82.80%)	404(81.62%)	1[Ref]		1[Ref]	
		A/A	86(17.20%)	91(18.38%)	0.92(0.67–1.28)	0.625	0.92(0.67–1.28)	0.623
	Log-additive	-	-	-	0.89(0.74–1.06)	0.182	0.88(0.74–1.06)	0.177
rs833070	Co-dominant	T/T	285(57.34%)	289(58.38%)	1[Ref]		1[Ref]	
		C/T	187(37.63%)	185(37.38%)	1.03(0.79–1.33)	0.853	1.02(0.79–1.33)	0.881
		C/C	25(5.03%)	21(4.24%)	1.21(0.66–2.21)	0.540	1.20(0.66–2.19)	0.558
	Dominant	T/T	285(57.34%)	289(58.38%)	1[Ref]		1[Ref]	
		C/T-C/C	212(42.66%)	206(41.62%)	1.04(0.81–1.34)	0.740	1.04(0.81–1.34)	0.770
	Recessive	T/T-C/T	472(94.97)	474(95.76%)	1[Ref]		1[Ref]	
		C/C	25(5.03%)	21(4.24%)	1.20(0.66–2.17)	0.556	1.19(0.66–2.15)	0.569
	Log-additive	-	-	-	1.06(0.85–1.31)	0.623	1.05(0.85–1.3)	0.651
rs3025021	Co-dominant	T/T	344(68.66%)	366(73.79%)	1[Ref]		1[Ref]	
		C/T	146(29.14%)	116(23.39%)	**1.34(1.01–1.78)**	**0.044***	**1.35(1.02–1.80)**	**0.038***
		C/C	11(2.20%)	14(2.82%)	0.84(0.37–1.87)	0.662	0.85(0.38–1.89)	0.687
	Dominant	T/T	344(68.66%)	366(73.79%)	1[Ref]		1[Ref]	
		C/T-C/C	157(31.34%)	130(26.21%)	1.29(0.98–1.69)	0.074	1.30(0.99–1.71)	0.063
	Recessive	T/T-C/T	490(97.80%)	482(97.18%)	1[Ref]		1[Ref]	
		C/C	11(2.20%)	14(2.82%)	0.77(0.35–1.72)	0.528	0.78(0.35–1.74)	0.544
	Log-additive	-	-	-	1.19(0.93–1.51)	0.168	1.20(0.94–1.53)	0.147
rs3025030	Co-dominant	G/G	339(67.66%)	350(70.56%)	1[Ref]		1[Ref]	
		C/G	146(29.14%)	137(27.62%)	1.10(0.83–1.45)	0.499	1.10(0.83–1.45)	0.507
		C/C	16(3.19%)	9(1.81%)	1.84(0.80–4.21)	0.152	1.81(0.79–4.15)	0.164
	Dominant	G/G	339(67.66%)	350(70.56%)	1[Ref]		1[Ref]	
		C/G-C/C	162(32.34%)	146(29.43%)	1.15(0.88–1.50)	0.322	1.14(0.87–1.50)	0.333
	Recessive	G/G-A/G	485(96.81%)	487(98.19%)	1[Ref]		1[Ref]	
		C/C	16(3.19%)	9(1.81%)	1.79(0.78–4.08)	0.169	1.76(0.77–4.02)	0.182
	Log-additive	-	-	-	1.17(0.92–1.49)	0.196	1.17(0.92–1.48)	0.208
rs6905288	Co-dominant	G/G	280(55.89%)	260(52.42%)	1[Ref]		1[Ref]	
		A/G	179(35.73%)	206(41.53%)	0.81(0.62–1.05)	0.108	0.81(0.62–1.05)	0.105
		A/A	42(8.38%)	30(6.05%)	1.30(0.79–2.14)	0.302	1.30(0.79–2.15)	0.296
	Dominant	G/G	280(55.89%)	260(52.42%)	1[Ref]		1[Ref]	
		A/G-A/A	221(44.11%)	236(47.58%)	0.87(0.68–1.12)	0.272	0.87(0.68–1.12)	0.268
	Recessive	G/G-A/G	459(91.62%)	466(93.95%)	1[Ref]		1[Ref]	
		A/A	42(8.38%)	30(6.05%)	1.42(0.87–2.31)	0.156	1.43(0.88–2.32)	0.152

SNP: single-nucleotide polymorphism; EC: Esophageal cancer; OR, odds ratio; CI, confidence interval. $p < 0.05$ indicates statistical significance.

SNP	Model	Genotype	Case	Control	Before adjusted		After adjusted	
					OR(95%CI)	*p*-value	OR(95%CI)	*p*-value
	Log-additive	-	-	-	0.97(0.80–1.18)	0.775	0.97(0.80–1.18)	0.775

SNP: single-nucleotide polymorphism; EC: Esophageal cancer; OR, odds ratio; CI, confidence interval. $p < 0.05$ indicates statistical significance.

3.3 Stratification analysis by age and gender.

According to gender and age parameters, stratified analysis regarding the effects of SNPs on CHD risk was summarized in Table 5. The stratification analysis by age adjusted for age and gender showed that rs833068 in VEGFA was observed to be associated with the reduced risk of CHD at age > 61 years in the codominant model (OR = 0.64, 95% CI = 0.42–0.98, $p = 0.042$ for "A/G" genotype). Meanwhile, the rs833068 was also associated with the reduced risk of CHD at age ≤ 61 years in the recessive model (OR = 0.58, 95% CI = 0.34–0.98, $p = 0.043$). In addition, the stratification analysis by gender adjusted for age found that three loci (rs833068, rs3025021 and rs6905288) in *VEGFA* were related to the CHD risk in males. The rs833068 was associated with reduced risk of CHD in the allele model (adjusted, OR = 0.75, 95% CI = 0.01–0.75, $p = 0.010$), the codominant model (adjusted, OR = 0.58, 95% CI = 0.37–0.92, $p = 0.020$ for "A/A" genotype; OR = 0.65, 95% CI = 0.45–0.92, $p = 0.016$ for "A/G" genotype), the dominant model (OR = 0.63, 95% CI = 0.45–0.88, $p = 0.006$), the log-additive model (OR = 0.74, 95% CI = 0.60–0.93, $p = 0.010$). The rs3025021 was associated with increased risk of CHD in the codominant model (adjusted, OR = 1.49, 95% CI = 1.04–2.13, $p = 0.029$ for "C/T" genotype), the dominant model (OR = 1.43, 95% CI = 1.01–2.02, $p = 0.041$). The rs6905288 was associated with increased risk of CHD in the recessive model (OR = 2.03, 95% CI = 1.08–3.78, $p = 0.027$). However, we found no any genotypes of the selected SNPs were associated with CHD in females in any genetic model.

Table 5
Stratified analysis on association between selected SNPs and CHD risk.

	SNP ID	$p^{\dagger}$, OR (95% CI)					
		Allele	Homozygote	Heterozygote	Dominant	Recessive	Additive
Age							
> 61	rs833068	0.219,0.86(0.67–1.10)	0.875,0.96(0.56–1.64)	**0.042*,0.64(0.42–0.98)**	0.105,0.72(0.48–1.07)	0.343,1.26(0.78–2.02)	0.586,0.93(0.71–1.21)
≤ 61	rs833068	0.551,0.92(0.72–1.20)	0.150,0.65(0.36–1.17)	0.375,1.22(0.79–1.88)	0.851,1.04(0.69–1.57)	**0.043*,0.58(0.34–0.98)**	0.335,0.87(0.66–1.15)
Gender							
Male	rs833068	**0.010*,0.75(0.01–0.75)**	**0.020*,0.58(0.37–0.92)**	**0.016*,0.65(0.45–0.92)**	**0.006*,0.63(0.45–0.88)**	0.172,0.76(0.50–1.13)	**0.010*,0.74(0.60–0.93)**
	rs3025021	0.087,1.30(0.09–1.30)	0.966,0.98(0.37–2.58)	**0.029*,1.49(1.04–2.13)**	**0.041*,1.43(1.01–2.02)**	0.796,0.88(0.34–2.31)	0.089,1.3(0.96–1.76)
	rs6905288	0.449,1.10(0.45–1.10)	0.05,1.89(1.00-3.59)	0.332,0.85(0.61–1.18)	0.817,0.96(0.71–1.32)	**0.027*,2.03(1.08–3.78)**	0.453,1.1(0.86–1.41)
Female	rs833068	0.213,1.21(0.90–1.62)	0.227,1.48(0.79–2.77)	0.506,1.17(0.73–1.87)	0.344,1.24(0.79–1.94)	0.309,1.34(0.76–2.35)	0.228,1.21(0.89–1.64)
	rs3025021	0.993,1.00(0.67–1.51)	0.622,0.69(0.16–2.99)	0.63,1.13(0.70–1.81)	0.734,1.08(0.68–1.72)	0.591,0.67(0.16–2.88)	0.885,1.03(0.68–1.56)
	rs6905288	0.137,0.78(0.56–1.08)	0.341,0.66(0.29–1.54)	0.161,0.73(0.47–1.13)	0.125,0.72(0.47–1.10)	0.508,0.76(0.33–1.72)	0.135,0.77(0.55–1.08)
Hypertension							
yes	rs3025021	0.088,1.27(0.96–1.67)	0.824,1.11(0.45–2.69)	**0.029*,1.44(1.04-2.00)**	**0.035*,1.41(1.02–1.93)**	0.999,1.00(0.41–2.42)	0.067,1.29(0.98–1.70)
no	rs833068	0.046,0.79(0.62-1.00)	**0.037*,0.58(0.35–0.97)**	0.212,0.79(0.55–1.14)	0.084,0.74(0.52–1.04)	0.087,0.67(0.42–1.06)	**0.034*,0.77(0.60–0.98)**
	rs3025030	0.047,1.35(1.00-1.82)	**0.041*,2.7(1.04–6.99)**	0.206,1.26(0.88–1.81)	0.090,1.35(0.95–1.91)	0.055,2.52(0.98–6.47)	**0.037*,1.38(1.02–1.87)**
	rs6905288	0.930,0.99(0.76–1.28)	0.102,1.64(0.91–2.97)	0.051,0.7(0.49-1.00)	0.241,0.82(0.59–1.14)	**0.031*,1.89(1.06–3.37)**	0.983,1.00(0.77–1.29)
Diabetes							
yes	rs3025021	**0.025*,1.55(1.05–2.27)**	0.928,0.93(0.20–4.25)	**0.002*,2.06(1.30–3.27)**	**0.004*,1.94(1.24–3.05)**	0.704,0.75(0.17–3.37)	0.016,1.60(1.09–2.37)
no	rs833068	0.130,0.86(0.72–1.04)	0.246,0.80(0.54–1.17)	**0.035*,0.73(0.54–0.98)**	**0.039*,0.75(0.56–0.99)**	0.821,0.96(0.68–1.36)	0.130,0.86(0.72–1.04)

SNP: single-nucleotide polymorphism; OR: odds ratio; CI: confidence interval; CHD: coronary heart disease.

$p < 0.05$ indicates statistical significance.

3.4 Stratification analysis with/without hypertension or diabetes.

We next analyzed the relationship between selected SNPs and CHD-associated diseases, which include hypertension and diabetes. In CHD patients with hypertension, we found that rs3025021 was associated with increased risk of CHD in the codominant model (adjusted, OR = 1.44, 95% CI = 1.04–2.00, p = 0.029 for "C/T" genotype), the dominant model (OR = 1.41, 95% CI = 1.012–1.93, p = 0.035). Conversely, in the CHD patients without hypertension, three loci (rs833068, rs3025030 and rs6905288) in *VEGFA* were related to the CHD risk. The rs833068 was associated with reduced risk of CHD in the codominant model (adjusted, OR = 0.58, 95% CI = 0.35–0.97, p = 0.037 for "A/A" genotype), the log-additive model (OR = 0.77, 95% CI = 0.60–0.98, p = 0.034). The rs3025030 was associated with increased risk of CHD in the codominant model (adjusted, OR = 2.70, 95% CI = 1.04–6.99, p = 0.041 for "C/C" genotype), the log-additive model (OR = 1.38, 95% CI = 1.02–1.87, p = 0.037). The rs6905288 was associated with increased risk of CHD in the recessive model (adjusted, OR = 1.89, 95% CI = 1.06–3.37, p = 0.031).

In CHD patients with diabetes, we found that rs3025021 was associated with increased risk of CHD in the allele model (adjusted, OR = 1.55, 95% CI = 1.05–2.27, p = 0.025), the codominant model (adjusted, OR = 2.06, 95% CI = 1.30–3.27, p = 0.002 for "C/T" genotype), the dominant model (OR = 1.94, 95% CI = 1.24–3.05, p = 0.004). In CHD patients without diabetes, the rs833068 was associated with reduced risk of CHD in the codominant model (adjusted, OR = 0.73, 95% CI = 0.54–0.98, p = 0.035 for "A/G" genotype), the dominant model (OR = 0.75, 95% CI = 0.56–0.99, p = 0.039).

3.5 *Haplotype association*

Finally, the LD block and haplotype analyses of the selected SNPs in all subjects were further studied. And we found a strong LD between rs833068 and rs833070 with D' = 1.00 (Fig. 1). The results of the association between haplotypes and the CHD risk were shown in Table 6. However, we found no haplotypes were associated with the risk of CHD even if after logistic regression analysis adjustment for age and gender.

Table 6
Haplotype analysis results in this study.

Haplotype	Frequency		Before adjusted		After adjusted	
	Case	Control	OR(95%CI)	*p*-value	OR(95%CI)	p^a-value
rs833068|rs833070						
GT	0.239	0.230	1.06(0.85–1.31)	0.622	1.05(0.85–1.30)	0.651
AC	0.412	0.439	0.89(0.75–1.07)	0.221	0.89(0.75–1.07)	0.215
GC	0.651	0.669	0.92(0.77–1.11)	0.407	0.92(0.76–1.11)	0.378
SNP: single-nucleotide polymorphism; OR: odds ratio; CI: confidence interval.						
P^a: Adjusted by gender and age.						

4. Discussion

In this case-control study, allele, genotype and haplotype frequencies of five SNPs in the *VEGFA* gene between CHD patients and healthy controls were compared and stratification analyses were conducted. The genotype"C/T"of rs3025021 in *VEGFA* was found to be associated with CHD susceptibility in the codominant model (adjusted, OR = 1.35, 95% CI = 1.02–1.80, $p = 0.038$) in the overall. The stratification analysis by age showed that rs833068 in *VEGFA* were observed to be associated with the reduced risk of CHD at age > 61 years and age ≤ 61 years, respectively. And the stratification analysis by gender found that three loci (rs833068, rs3025021 and rs6905288) in *VEGFA* were related to the CHD risk in males. In addition, we found that rs3025021 was associated with increased risk of CHD in patients with hypertension. Conversely, three loci (rs833068, rs3025030 and rs6905288) in *VEGFA* were related to the CHD risk in patients without hypertension. Furthermore, rs3025021 was associated with increased risk of CHD in patients with diabetes. The rs833068 was associated with reduced risk of CHD in patients without diabetes. Finally, we found a strong LD between rs833068 and rs833070. To our knowledge, this is the first study that evaluate and show an association of *VEGFA* genetic variants with risk of developing CHD in Han Chinese population.

Coronary heart disease (CHD) is a common and complicated cardiovascular disease in the worldwide, and caused by narrowing of the coronary arteries and a lack of blood supply (12). However, the etiological factors for CHD are not fully understood. Like lung cancer (19) and glioma (20), it's clear that genetic factors, such as single nucleotide polymorphism (SNPs) may also play a pivotal role in determining susceptibility of CHD (21). *VEGF* can not only promote the vascular recanalization and establishment of collateral circulation, but also enhance the dependent vasodilatation of endothelial cells which are closely related to coronary heart disease (22). Several studies have shown that appropriate timing and dose of *VEGF* is essential to avoid cardiovascular defects during heart development (23, 24). *VEGFA*, as a member of *VEGF*, has been proved to promote the differentiation, proliferation and migration of microvascular endothelial cells by binding to their receptors, thus improving the formation and development (24). In addition, the study has shown that *VEGFA* may play an important role in the process of epithelial-mesenchymal transformation (EMT) and regulate the formation of endocardial cushion (22).

Recently, several studies have explored the association between the SNPs in *VEGFA* and the susceptibility to CHD (12, 25, 26). Han et al (25) showed that rs3025039 in *VEGFA* gene was remarkably associated with CHD risk in Han Chinese population. On the contrary, Griffin et al (26) conducted a meta-analysis which demonstrated VEGFA polymorphisms may not be associated with CHD. Furthermore, Dong et al (12) found three SNPs (rs699947, rs3025039, and rs1570360) were remarkably correlated with the susceptibility to CHD. To further clarify the relationship between SNPs within *VEGFA* and CHD,we genotyped five SNPs (rs833068, rs833070, rs3025021, rs3025030, rs6905288) in this study and discovered that all the five SNPs were significantly with the risk of CHD.

Inevitably, several limitations should be addressed with regard to the case-control study. First, our sample was limited to the Han Chinese population, thus the investigation results might not be applicable to other Chinese populations or additional ethnic groups. Larger and more diversity sample is needed to identify the role of *VEGFA* genetic polymorphisms in CHD risk. Besides, it could not be ignored that the uniqueness of samples might exaggerate the correlation between the selected 5 SNPs and CHD risk, or neglect the role of some other vital polymorphisms in *VEGFA* in susceptibility to CHD. Therefore, large prospective cohort studies or combined meta-analyses should be designed to address these limitations.

5. Conclusion

In summary, the present study indicated that gene polymorphisms in *VEGFA* gene were notably correlated with altered CHD risk in the Han Chinese population. Thus, the *VEGFA* mutations might be used to clinical medicine as genetic marker.

Declarations

Acknowledgement

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Authors' contributions

Conceived and designed the experiments: Shijuan Lu. Performed the experiments: Kang Huang, Yilei Zhou. Analyzed the data: Dehong Lin, Zibin Chen. Contributed reagents/materials/analysis tools: Zanrui Zhong. Wrote the paper: Jianghua Zhong. Revised Shijuan Lu.

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Competing interests

None

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Figures

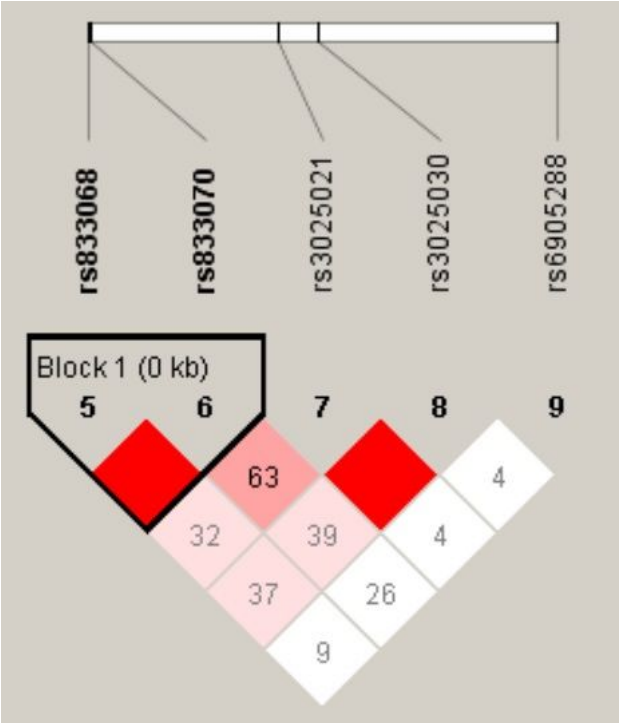
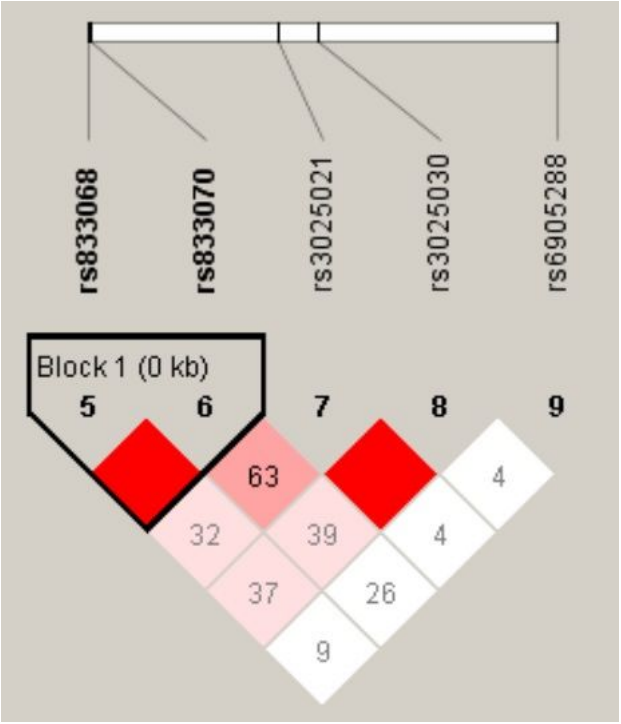


Figure 1

The LD block and haplotype analyses of the selected SNPs in all subjects were further studied. And we found a strong LD between rs833068 and rs833070 with D′ = 1.00 (Figure 1).



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