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Original Article

Polymorphisms of *VEGF* and congenital heart disease in a Chinese population

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Abstract: Spatiotemporal expression pattern of vascular endothelial growth factor (VEGF) are required for proper heart morphogenesis. Genetic variants of *VEGF* contribute to the production and/or biological activity may determine the risk of congenital heart disease (CHD). To explore the impact of *VEGF* functional SNPs -2578 C>A, -1498 T>C, -634 G>C, +936 C>T on the susceptibility of CHD, we genotyped four functional polymorphisms of *VEGF* (-2578 C>A, -1498 T>C, -634 G>C, +936 C>T) in a hospital based case-control study of 476 CHD cases and 557 non-CHD controls in a Chinese population. We did not find any significant associations between the four *VEGF* polymorphisms and the risk of CHD or a certain kind of CHD such as ventricular septal defect (VSD) or tetralogy of Fallot (TOF). However, $C_{-2578}T_{-1498}G_{-634}C_{+936}$, $C_{-2578}T_{-1498}C_{-634}T_{+936}$ and $G_{-2578}G_{-1498}G_{-634}T_{+936}$ haplotypes were correlated with a significantly increased susceptibility of CHD. The $C_{-2578}T_{-1498}G_{-634}T_{+936}$ haplotypes were correlated with a significantly decreased risk of CHD.

Keywords: *VEGF,* polymorphisms, congenital heart disease, single nucleotide polymorphisms, molecular epidemiology

Introduction

Congenital heart disease (CHD) is the leading non-infectious cause of death in children and the incidence appears to be about 100 per 10,000 [1]. In China, the prevalence of CHD was 73.2 per 10,000 births in high-prevalence areas [2]. Known chromosomal defects and environment teratogens only cause a small portion of the disease. Most CHD is sporadic and considered to be multi-factorial in origin, with various genes interacting with each other or with other risk factors to determine the disease etiology [3-5]. Therefore, genetic variants contribute to the production and/or biological activity of heart modifier genes may contribute to the susceptibility of CHD [6].

The heart is the first organ to form and function during development. In the process, endocardial cushion (EC) formation is a critical step and EC malformation may result in CHD [7, 8]. EC divides the heart tube into atria and ventricle, and the common outflow tract (OFT) into dorsal aorta and pulmonary artery [8], which calls for the process of endocardium transform to mesenchyme (EMT), occurring on the inner walls of the atrioventricular canal and OFT [9]. Numerous signaling pathways contributed to the formation of EC, such as vascular endothelial growth factor (VEGF), NFATc1, Notch, Wnt/β-catenin, BMP/TGF-β, ErbB, and NF1/Ras [7]. Spatiotemporal expression pattern of VEGF was the first symbol of a specific role for VEGF in EC formation. It is reported that VEGF was required for proper heart morphogenesis at stages where the heart is still avascular [10], and VEGF-expressing endothelial cells of the cushion-forming region may be a unique subunit of endothelial cells predetermined to undergo EMT [11]. Evidence also showed that

increased VEGF levels during right ventricular outflow tract development can lead to abnormal development of both cushion and myocardial structures. Furthermore, the importance of appropriate timing and dosage of VEGF during heart upgrowth was demonstrated by shared cardiovascular developmental defects in animal models, such as transgenic mice and mice heterozygous for *VEGF* allele, which have a 2- to 3-fold increased VEGF [12, 13]. *VEGF* gene polymorphisms may play a role in susceptibility of congenital valvuloseptal heart defects [8]. However, it is reported that *VEGF* variants may also be correlated with congenital heart disease, such as tetralogy of Fallot (TOF) and pulmonary stenosis [14].

The *VEGF* gene is located on the chromosome 6p21.3 and consists of eight exons, exhibiting alternate splicing to form a family of proteins [15]. Recently, a number of single nuclear polymorphisms (SNPs) have been identified to be correlated with differential VEGF expression *in vitro* [16]. Two of these SNPs (-2578 C>A, rs699947 and -1498 T>C, also assigned as -460 T>C, rs833061) are located in *VEGF* promoter region, one (-634 G>C, rs2010963, also named G+405C) in exon 1 and one (+936 C>T, rs3025039) in exon 8, corresponding to the 3' untranslated (UTR) region [17]. These polymorphisms have been extensively investigated both in their function and in their associations with different diseases, including CHDs. For example, Vannay *et al* found that *VEGF*-634C (+405C) presented increased risk for CHD [8], whereas Lambrecht *et al* reported that *VEGF* haplotype -2578A/-1154A/-634G (AAG) was associated with decreased risk for TOF [18]. Xie *et al* found that *VEGF*-634C allele was significantly protective against ventricular septal defect (VSD) [19]. To evaluate the impact of the four functional SNPs on CHD susceptibility, we performed genotyping analyses for the four SNPs in a hospital based case-control study with 476 CHD cases and 557 controls in a Chinese population.

Materials and methods

Study population

This study included 476 CHD patients and 557 non-CHD controls. Patients were consecutively recruited from the First Affiliated Hospital and the Affiliated Nanjing Children's Hospital, Nanjing Medical University (Nanjing city, China), between March 2006 and June 2007. The patients were Ultrasonic diagnosed non-syndromic CHDs and confirmed in the operations. The controls were non-CHD out-patients, frequency-matched to the cases on age (±3 years) and sex, recruited from the above two hospitals during the same time period and most of them were with trauma or infectious diseases. All subjects were genetically unrelated ethnic Han Chinese. After informed consent was obtained from their parents, each subject and her/his parents were personally interviewed by trained interviewers using a structured questionnaire to obtain information about teratogenic contact during pregnancy, maternal diabetes and family history of CHD in first degree relatives (parents, siblings, and children). Information about regular multivitamin supplements (including folic acid) intake, influenza, rubella and any febrile illnesses during pregnancy were also collected.

The major excluded criterion was: cases with structural malformations involving known chromosomal or other organ system abnormalities. Exclusion criteria also included a positive family history of CHD in a first-degree relative (parents, siblings, and children), maternal diabetes mellitus, phenyl ketonuria, maternal teratogen exposures (e.g., pesticides and organic solvents), and influenza, rubella and any febrile illnesses during pregnancy. Controls with congenital anomalies were excluded.

A 2-ml venous blood sample was collected from each subject. Institutional Ethical committee approval was obtained Nanjing Medical University (Nanjing city, China).

Genotyping of -2578 C>A, -1498 T>C, -634 G>C, and +936 C>T polymorphisms

Genomic DNA was extracted from the peripheral blood leukocyte pellet by the standard method with proteinase K digestion and followed by phenol-chloroform extraction and ethanol precipitation. The PCR-restriction fragment length polymorphism (RFLP) assay was used to detect *VEGF*-2578 C>A with the primers as sense-5'-CATACCGATGGAACTGG-3' and antisense-5'-GTTTCTGACCTGGCTATTT-3'. The PCR amplification products were digested all night with the restriction enzymes *Bgl*II (New England BioLabs, Beverly, MA) and then resolved on 3%

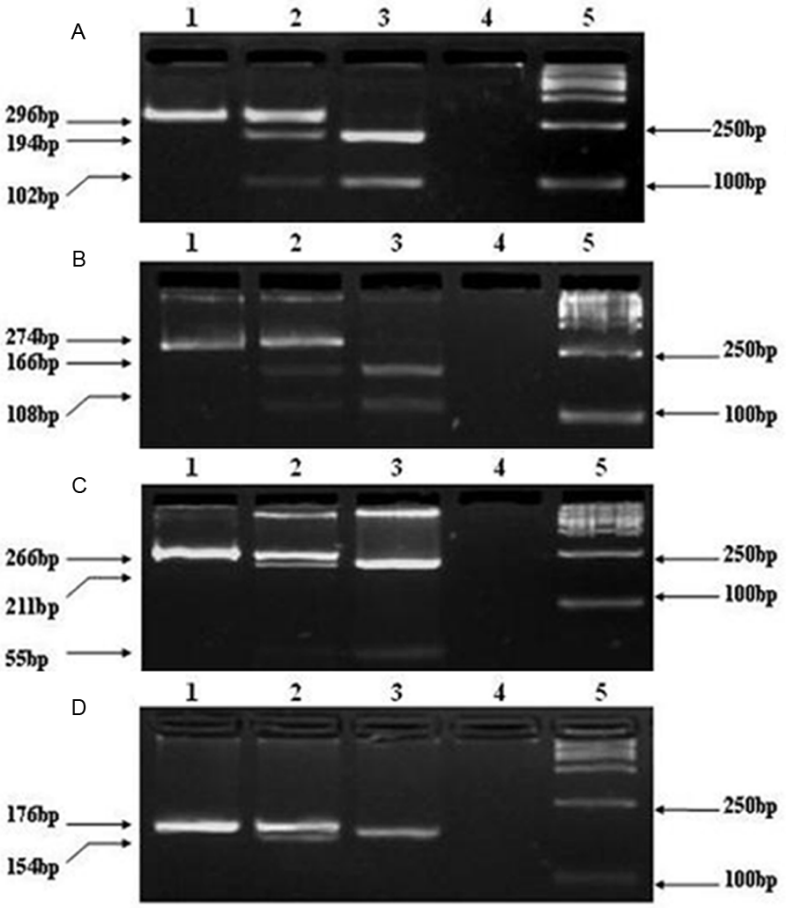

Figure 1. *VEGF* genotypes were performed using the PCR-based assays (Electrophoresis in 3% agarose gel). A: *VEGF*-2578 C>A genotype patterns: -2578 CC (296 bp) (lane 1), -2578 CA (296 bp + 194 bp + 102 bp) (lane 2) and -2578 AA (194 bp + 102 bp) (lane 3). Lane 4: water control; Lane 5: DNA marker. B: *VEGF*-634 G>C genotype patterns: -634 CC (274 bp) (lane 1), -634 GC (274 bp + 166 bp + 108 bp) (lane 2) and -634 GG (166 bp + 108 bp) (lane 3). Lane 4: water control; Lane 5: DNA marker. C: *VEGF*+936 C>T genotype patterns: +936 CC (266 bp) (lane 1), +936 CT (266 bp + 211 bp + 55 bp) (lane 2) and +936 TT (211 bp + 55 bp) (lane 3). Lane 4: water control; Lane 5: DNA marker. D: *VEGF*-1498 T>C genotype patterns: -1498 TT (176 bp) (lane 1), -1498 TC (176 bp + 154 bp + 22 bp) (lane 2) and -1498 CC (154 bp + 22 bp) (lane 3). Lane 4: water control; Lane 5: DNA marker.

agarose gel (**Figure 1A**). The polymorphic (-2578 A) allele produces two fragments of 194- and 102-bp and the wild-type (-2578 C) results in a single 296-bp fragment. Similarly, for *VEGF*-634 G>C and +936 C>T, the primers are sense-5'-CGACGGCTTGGGGAGA-TTGC-3', antisense-5'-GGGCGGTGTCTGTCTGT-CTG-3' for *VEGF*-634 G>C and sense-5'-AGGGTTTCGG-GAACCAGATC-3', antisense-5'-CTCGGTGATTTA-GCAGCAAG-3' for *VEGF*+936 C>T. The two fragments (274-bp for -634 G>C and 266-bp for +936 C>T) were then digested, respectively, by *Bsm*FI and *Nla*III (New England BioLabs, Beverly, MA) and separated on a 3% agarose gel (**Figure 1B** and **1C**, respectively). For the -634 G>C of *VEGF*, the -634 G allele results in two fragments of 166- and 108-bp and the -634 C allele produces one fragment of 274-bp. The variant allele *VEGF*+936 T produces two fragments of 211- and 55-bp and the wild-type allele +936 C produces only one fragment of 266-bp. *VEGF*-1498 T>C was detected using a primer-introduced restriction analysis (PIRA)-PCR assay [10]. For the *VEGF*-1498 T>C polymorphism, the anti-sense-primer was introduced a mismatched G to replace A at +3 bp from the polymorphic site to create a *Bsa*HI restriction site (sense-5'-CCTCTTTAGCCAGAGCCG-GGG-3', antisense-5'-TGGC CTTCTCCCCGCTCCGAC-3'). The 176-bp PCR products were then digested by *Bsa*HI (New England BioLabs, Beverly, MA) and separated on a 3% agarose gel (**Figure 1D**). The polymorphic (-1498 C) allele produces two fragments of 154- and 22-bp and the wild-type (-1498 T) results in a single 176-bp fragment.Genotypingwasdone with unaware of the subject's status. If there is any conflicting result, samples were repeated by two research assistants independently until reached 100% concordance. All the samples (476 CHD cases and 557 controls) were successfully genotyped for the four SNPs.

Statistical analyses

Differences in the distributions of selected variables, the variant alleles and genotypes of *VEGF* between the cases and controls were evaluated using the student t test (for continuous variables) and χ^2 tests (for categorical vari-

Table 1. Logistic regression analysis of associations between *VEGF* polymorphisms and risk of CHDs

Genotype	Cases (n = 476)		Controls (n = 557)		Crude OR (95% CI)	*P*	Adjusted OR[a] (95% CI)	*P*
	n	%	n	%				
VEGF-2578 C>A								
CC	272	57.1	305	54.8	1.00		1.00	
CA	182	38.2	225	40.4	0.91 (0.70-1.17)	0.453	0.91 (0.70-1.17)	0.458
AA	22	4.6	27	4.9	0.91 (0.51-1.64)	0.763	0.91 (0.51-1.64)	0.753
CA+AA	204	42.9	252	45.2	0.91 (0.71-1.16)	0.442	0.91 (0.71-1.16)	0.444
A allele	226	23.7	279	25.0				
VEGF-1498 T>C								
TT	276	58.0	321	57.6	1.00		1.00	
TC	165	34.7	193	34.7	0.99 (0.77-1.29)	0.966	1.00 (0.77-1.30)	0.990
CC	35	7.4	43	7.7	0.95 (0.59-1.52)	0.821	0.94 (0.59-1.52)	0.808
TC+CC	200	42.0	236	42.4	0.99 (0.77-1.26)	0.909	0.99 (0.77-1.27)	0.925
C allele	235	24.7	279	25.0				
VEGF-634 G>C								
GG	153	32.1	183	32.9	1.00		1.00	
GC	248	52.1	283	50.8	1.05 (0.80-1.38)	0.737	1.06 (0.81-1.40)	0.661
CC	75	15.8	91	16.3	0.99 (0.68-1.43)	0.940	1.00 (0.68-1.45)	0.978
GC+CC	323	67.9	374	67.1	1.03 (0.80-1.34)	0.808	1.05 (0.81-1.36)	0.733
C allele	398	41.8	465	41.7				
VEGF+936 C>T								
CC	328	68.9	388	69.7	1.00		1.00	
CT	135	28.4	147	26.4	1.09 (0.82-1.43)	0.556	1.10 (0.83-1.45)	0.501
TT	13	2.7	22	3.9	0.70 (0.35-1.41)	0.317	0.71 (0.35-1.44)	0.344
CT+TT	148	31.1	169	30.3	1.04 (0.80-1.35)	0.794	1.05 (0.80-1.37)	0.722
T allele	161	16.9	191	17.1				

[a]Adjusted for age and sex.

ables). The associations between *VEGF* genotypes and risk of CHD were estimated by computing the ORs and their 95% CIs from logistic regression analyses. Haplotype frequencies were obtained from the PHASE 2.0 programs based on the observed *VEGF* genotypes. All of the statistical analyses were performed with Statistical Analysis System software (v.9.1.3e; SAS Institute, Cary, North Carolina, United States of America).

Results

Characteristics of the study population

The age were 3.70 yrs (±4.27 yrs) for the cases and 3.74 yrs (±3.70 yrs) for the controls (P = 0.870), and 53.6% of the cases and 56.2% of the controls were males and the difference was not significant (P = 0.398), indicating that the frequency-matching by age and sex was adequate. Of the 476 CHD patients, 244 (51.3%) were VSD, 42 (8.8%) were atrial septal defect, 11 (2.3%) were pulmonary stenosis, 13 (2.7%) were complete atrioventricular canal defects, 72 (15.1%) were complicated CHDs which combined at least two kinds of single CHD, 72 (15.1%) were TOF, 8 (1.7%) were characterized as transposition of the great arteries, and 14 (2.9%) were double outlet right ventricle.

VEGF polymorphisms and CHD susceptibility

The *VEGF*-2578 C>A, -1498 T>C, -634 G>C, and +936 C>T genotype distributions in the cases and controls were shown in **Table 1**. The observed genotype frequencies for these polymorphisms were all in Hardy-Weinberg Equilibrium in the controls (data not shown). In the single locus analyses, no significant difference in the genotype distributions between the cases and the controls (P = 0.743, 0.975, 0.915 and 0.470 for -2578 C>A, -1498 T>C, -634 G>C, +936 C>T, respectively) was found. Multivariate logistic regression analyses revealed that none of the four SNPs was significantly associated

Table 2. Stratified analyses between *VEGF* polymorphisms and CHD by the CHD types

Genotypes	Controls (n = 557)		VSD (n = 244)			TOF (n = 72)			Others[a] (n = 160)		
	n	%	n	%	Adjusted OR (95% CI)[b]	n	%	Adjusted OR (95% CI)[b]	n	%	Adjusted OR (95% CI)[b]
VEGF-2578 C>A											
CC	305	54.8	138	56.6	1.00	46	63.9	1.00	88	55.0	1.00
CA	225	40.4	93	38.1	0.91 (0.67-1.25)	24	33.3	0.72 (0.43-1.21)	65	40.6	1.01 (0.70-1.46)
AA	27	4.9	13	5.3	1.08 (0.54-2.15)	2	2.8	0.48 (0.11-2.11)	7	4.4	0.88 (0.37-2.09)
CA+AA	252	45.2	106	43.4	0.93 (0.69-1.26)	26	36.1	0.69 (0.42-1.15)	72	45.0	1.00 (0.70-1.42)
A allele	279	25.0	119	24.4		28	19.4		79	24.7	
VEGF-1498 T>C											
TT	321	57.6	137	56.2	1.00	47	65.3	1.00	92	57.5	1.00
TC	193	34.7	91	37.3	1.11 (0.81-1.53)	20	27.8	0.73 (0.42-1.27)	54	33.8	0.99 (0.68-1.46)
CC	43	7.7	16	6.6	0.88 (0.48-1.62)	5	6.9	0.78 (0.29-2.07)	14	8.8	1.15 (0.60-2.21)
TC+CC	236	42.4	107	43.9	1.07 (0.79-1.45)	25	34.7	0.74 (0.44-1.24)	68	42.5	1.02 (0.72-1.46)
C allele	279	25.0	123	25.2		30	20.8		82	25.6	
VEGF-634 G>C											
GG	183	32.9	79	32.4	1.00	21	29.2	1.00	53	33.1	1.00
GC	283	50.8	131	53.7	1.09 (0.78-1.52)	37	51.4	1.15 (0.65-2.04)	80	50.0	0.98 (0.66-1.46)
CC	91	16.3	34	13.9	0.88 (0.55-1.42)	14	19.4	1.37 (0.66-2.83)	27	16.9	0.97 (0.57-1.65)
GC+CC	374	67.1	165	67.6	1.04 (0.75-1.43)	51	70.8	1.20 (0.70-2.07)	107	66.9	0.98 (0.67-1.43)
C allele	465	41.7	199	40.8		65	45.1		134	41.9	
VEGF+936 C>T											
CC	388	69.7	171	70.1	1.00	52	72.2	1.00	105	65.6	1.00
CT	147	26.4	65	26.6	1.02 (0.72-1.44)	18	25.0	0.96 (0.54-1.70)	52	32.5	1.33 (0.91-1.96)
TT	22	3.9	8	3.3	0.84 (0.37-1.92)	2	2.8	0.67 (0.15-2.96)	3	1.9	0.50 (0.15-1.71)
CT+TT	169	30.3	73	29.9	0.99 (0.72-1.38)	20	27.8	0.92 (0.53-1.60)	55	34.4	1.22 (0.84-1.78)
T allele	191	17.1	81	16.6		22	15.3		58	18.1	

[a]Others include 42 atrial septal defect cases, 11 pulmonary stenosis cases, 13 complete atrioventricular canal defect cases, 72 complicated CHDs which combined at least two kinds of single CHD, 8 transpositions of the great arterie cases, and 14 double outlet right ventricle cases.
[b]Adjusted for age and sex.

with the risk of CHD (**Table 1**). In the stratified analyses, no associations were observed between -2578 C>A, -1498 T>C, -634 G>C, and +936 C>T genotypes and CHD risk for individuals categorized by CHD types (**Table 2**).

Haplotype analysis of VEGF polymorphisms and CHD susceptibility

As shown in **Table 3**, haplotype analysis was also performed and haplotypes were derived from the observed genotypes of these four *VEGF* polymorphisms. Among them, haplotypes $C_{-2578}T_{-1498}G_{-634}C_{+936}$, $C_{-2578}T_{-1498}C_{-634}T_{+936}$ and $G_{-2578}G_{-1498}G_{-634}T_{+936}$ were more common in the cases ($C_{-2578}T_{-1498}G_{-634}C_{+936}$: 0.305, $C_{-2578}T_{-1498}C_{-634}T_{+936}$: 0.067 and $G_{-2578}G_{-1498}G_{-634}T_{+936}$: 0.015) than in the controls (0.261, 0.030 and 0.003, respectively) (P = 0.017 for $C_{-2578}T_{-1498}G_{-634}C_{+936}$, P<0.001 for $C_{-2578}T_{-1498}C_{-634}T_{+936}$ and P = 0.005 for $G_{-2578}G_{-1498}G_{-634}T_{+936}$, respectively). Compared with the most common haplotype $C_{-2578}T_{-1498}C_{-634}C_{+936}$, the $C_{-2578}T_{-1498}G_{-634}C_{+936}$, $C_{-2578}T_{-1498}C_{-634}T_{+936}$ and $G_{-2578}G_{-1498}G_{-634}T_{+936}$ haplotypes were associated with a significantly increased risk of CHD (OR = 1.30, 95% CI = 1.05-1.62, P = 0.017, OR = 2.54, 95% CI = 1.63-3.96, P<0.001 and OR = 6.11, 95% CI = 1.74-21.43, P = 0.005 respectively).

Compared with the most common haplotype $C_{-2578}T_{-1498}C_{-634}C_{+936}$, the $C_{-2578}T_{-1498}G_{-634}T_{+936}$ haplotypes were associated with a significantly decreased risk of CHD (OR = 0.38, 95% CI = 0.23-0.65, P<0.001) (**Table 3**).

Discussion

In this case-control study, we invested the association of four *VEGF* SNPs, -2578 C>A, -1498 T>C, -634 G>C, +936 C>T, and risk of CHD in a Chinese population. We found that

Table 3. *VEGF* haplotype frequencies (%) in cases and controls and risk of CHDs

Haplotypes	Cases (n = 952)		Controls (n = 1114)		Crude OR (95% CI)	*P*
	n	%	n	%		
$C_{-2578}T_{-1498}C_{-634}C_{+936}$	321	33.72	420	37.70	1.00	
$C_{-2578}T_{-1498}G_{-634}C_{+936}$	290	30.46	291	26.12	**1.30 (1.05-1.62)**	**0.017**
$A_{-2578}C_{-1498}G_{-634}C_{+936}$	138	14.50	163	14.63	1.11 (0.85-1.45)	0.456
$A_{-2578}C_{-1498}G_{-634}T_{+936}$	62	6.51	86	7.72	0.94 (0.66-1.35)	0.749
$C_{-2578}T_{-1498}C_{-634}T_{+936}$	64	6.72	33	2.96	**2.54 (1.63-3.96)**	**<0.001**
$C_{-2578}T_{-1498}G_{-634}T_{+936}$	19	2.00	65	5.83	**0.38 (0.23-0.65)**	**<0.001**
$A_{-2578}T_{-1498}G_{-634}C_{+936}$	16	1.68	20	1.80	1.05 (0.53-2.05)	0.894
$C_{-2578}C_{-1498}G_{-634}C_{+936}$	15	1.58	20	1.80	0.98 (0.50-1.95)	0.957
$G_{-2578}G_{-1498}G_{-634}T_{+936}$	14	1.47	3	0.27	**6.11 (1.74-21.43)**	**0.005**
Others	13	1.37	13	1.17	1.31 (0.60-2.86)	0.501

Bold values are statistically significant ($P<0.05$).

none of the four polymorphisms was significantly associated with CHD risk. However, compared with $C_{-2578}T_{-1498}C_{-634}C_{+936}$, the $C_{-2578}T_{-1498}G_{-634}C_{+936}$, $C_{-2578}T_{-1498}C_{-634}T_{+936}$ and $G_{-2578}G_{-1498}G_{-634}T_{+936}$ haplotypes were associated with a significantly increased risk of CHD. The $C_{-2578}T_{-1498}G_{-634}T_{+936}$ haplotypes were associated with a significantly decreased risk of CHD.VEGF play a critical role during EMT processes and mediate EC formation. Spatiotemporal expression pattern of *VEGF* should be tightly regulated and even a tiny disturbing could cause CHD.

A few studies have been carried out to examing the vital role of *VEGF* polymorphisms in CHD risk and the focus was mainly on *VEGF*-634 G>C. A recent study by Xie *et al* reported that *VEGF*-634C allele was correlated with the decreased the risk of VSD in a case-control study of 222 CHD patients and 352 controls [19]. However, in a small case-control study in Hungary (102 CHD cases and 112 controls), Vannay *et al* found that *VEGF*-634C presented an increased risk for CHD [8]. In the present study, we genotyped four potentially functional SNPs in *VEGF* gene including 476 CHD cases and 557 controls and failed to find evidence for the significant associations of *VEGF* SNPs with CHD risk. The discrepancy could be resulted from the chance finding of small studies and/or ethnic difference in terms of genetic associations. Our study is in according with previous study and meta-analysis which supported the hypothesis that either common or rare genetic variation in VEGF may not predisposes to the risk of CHD [20].

Genetic polymorphisms often vary between ethnic groups. In this study with 557 non-CHD controls, we reported that the allele frequency of *VEGF*-634C was similar to those reported in Chinese, Korea and European populations [19, 21-23]. However, the mutant homozygote among controls was higher than those reported in Hungary controls (16.3% *vs.* 6%) [8].

Several limitations of the study need to be addressed. First, we did not obtain plasma samples from the mothers to evaluate the etiological role of VEGF in CHDs. Elevated plasma VEGF in CHDs could be a biomarker of CHD complications, such as PAH and hypoxia. Second, because our study was a hospital-based case-control study and selection bias could not be fully excluded, large population-based studies are warranted to further elucidate the role of *VEGF* polymorphisms in susceptibility of CHDs, especially in a certain type of CHD. Third, the sample size of this study was limited and not large enough to confirm the low penetrance effect of the SNPs. With the sample size of 476 cases and 557 controls, we have an 80% statistical power to test the lowest OR of 1.45 (0.67).

In conclusion, our findings suggest that *VEGF*-2578 C>A, -1498 T>C, -634 G>C and +936 C>T polymorphisms may not play a role in the susceptibility of CHD. $C_{-2578}T_{-1498}G_{-634}C_{+936}$, $C_{-2578}T_{-1498}C_{-634}T_{+936}$ and $G_{-2578}G_{-1498}G_{-634}T_{+936}$ haplotypes were associated with a significantly increased risk of CHD. The $C_{-2578}T_{-1498}G_{-634}T_{+936}$ haplotypes were associated with a significantly decreased risk of CHD.

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Disclosure of conflict of interest

None.

Abbreviations

CI, Confidential interval; CHD, congenital heart disease; EC, endocardial cushion; EMT, endocardium transform to mesenchyme; LD, linkage disequilibrium; OR, odds ratio; SNPs, single nucleotide polymorphisms; RFLP, restriction fragment length polymorphism; VEGF, vascular endothelial growth factor.

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