Polymorphisms of KDR Gene Are Associated With Coronary Heart Disease

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Objectives

Our purpose was to determine whether the common polymorphisms (SNP-604, SNP1192, and SNP1719) in KDR are associated with risk of coronary heart disease.

Background

Vascular endothelial growth factor (VEGF) and its receptor KDR (kinase insert domain-containing receptor/fetal liver kinase-1, also called VEGFR2) play critical roles in angiogenesis and vascular repair, which are involved in the progress of coronary heart disease.

Methods

The association of the 3 polymorphisms with risk of coronary heart disease was determined in 2 independent case-control studies: one comprised of 665 patients with coronary heart disease and 1,015 control subjects, and the other comprised of 369 patients and 625 control subjects. The SNP functions of KDR gene were studied by using luciferase reporter assays, determination of serum levels of KDR, and ligand-binding assays.

Results

The 2 independent population studies showed that the 3 polymorphisms were associated with risk of coronary heart disease with odds ratios of 1.37 for SNP-604 (p = 0.006), 1.41 for SNP1192 (p = 0.011), and 1.37 for SNP1719 (p = 0.007) in the first population, and 1.40 for SNP-604 (p = 0.015), 1.75 for SNP1192 (p = 0.003), and 1.50 for SNP1719 (p = 0.010) in the second population. The SNP-604C–bearing KDR promoter exhibited 68% of lower transcription activity than the SNP-604T–bearing promoter. The SNP1192 and SNP1719 could obviously influence the efficiency of VEGF binding to KDR.

Conclusions

The KDR polymorphisms may serve as novel genetic markers for the risk of coronary heart disease. (J Am Coll Cardiol 2007;50:760–7) © 2007 by the American College of Cardiology Foundation.
vitro, including angiogenesis, endothelial survival, proliferation, migration, and increased production of nitric oxide and prostaglandin I\_2 (9–11). Dysregulated vessel growth is implicated in the pathogenesis of a wide variety of diseases, including proliferative retinopathies, tumors, rheumatoid arthritis, atherosclerosis, as well as CHD (12,13). Recent evidence suggests that vascular function depends not only on cells that reside within the vessel wall but also on circulating EPCs (14–17). The circulating EPCs can be incorporated into the sites of neovascularization and accumulated in the sites of endothelial denudation to contribute to vascular repair (14,15,18,19). The enhancement of the number of circulating EPCs improves the replenishment of the endothelial monolayer after vascular injury due to enhanced restoration of the endothelial monolayer and diminishes neointima formation (20–23).

The variation of KDR gene may change the biological function of KDR. By bioinformatic analysis, we found that the single nucleotide polymorphism (SNP)-604T/C (rs2071559) leads to structural alteration of the binding site for transcriptional factor E2F (involved in cell cycle regulation, interacting with Rb p107 protein) in KDR gene promoter region, which may alter KDR expression. Exonic polymorphisms SNP1192G/A (rs2305948, in exon 7) and SNP1719A/T (rs1870377, in exon 11) are located in the third and fifth NH\_2-terminal Ig-like domains within the extracellular region, which are important for ligand binding, and result in nonsynonymous amino acid changes at residue 297V\_1 and 472H\_Q, respectively. Therefore, we hypothesized that these mutations would contribute to susceptibility to CHD.

**Methods**

**Subjects for 2 independent case-control studies.** The subjects of the first study were from FuWai Hospital, Beijing, Chinese Academy of Medical Sciences. Patients were unrelated subjects ages 19 to 83 years old. Inclusion criteria were ≥50% narrowing of the lumina of at least 1 of the major coronary arteries by coronary angiography. A detailed history of angina or myocardial infarction was obtained. Of the patients, 79.2% had myocardial infarction diagnosed by typical electrocardiogram (ECG) change (Minnesota Code 1.1 or 1.2 in ECG) and by changes in serum enzymes (troponin T, troponin I, creatine kinase-MB, aspartate aminotransferase, and glutamic pyruvic transaminase). The control subjects were selected from the subjects admitted to the hospital for excluding CHD, whose major coronary artery had no more than 20% stenosis, and did not have any vascular disease.

In the second study, the population was from Qingdao FuWai Hospital, Shandong Provincial Hospital, and Tianjin Chest Hospital comprised of 369 cases with CHD (50.1% had myocardial infarction) and 625 control subjects. They were recruited with the same criteria as the first study. All samples were coded to protect donor identity. The study was approved by the ethics committee of FuWai Hospital, Peking Union Medical College, and the participating hospitals. All subjects who participated in the study provided their written informed consents and were self-reported as Han Chinese.

**Risk factors for CHD.** A complete clinical history was obtained from all subjects. The following vascular risk factors were also recorded including smoking, body mass index (BMI), high-density lipoprotein cholesterol (HDL-C), non-HDL-C lipids, total plasma cholesterol (TC), and triglycerides (TG). Plasma biochemical variables were determined by an automatic analyzer (Hitachi 7060, Hitachi, Japan). Non–HDL-C was calculated by the Friedewald formula.

Hypertension was defined as a mean of 3 independent blood pressures, systolic blood pressure/diastolic blood pressure >140/90 mm Hg or the use of antihypertensive drugs (24). Diabetes mellitus (DM) was diagnosed when the subject had a fasting glucose >7.8 mmol/l, or >11.1 mmol/l at 2 h after oral glucose challenge, or both. Smoking was defined as a history of smoking >2 pack-years and/or smoking in the last year.

**Genotyping of SNP-604, SNP1192, and SNP1719.** The numbering of polymorphisms in this article is based on the reference sequence X89776 for the polymorphism in the promoter—rs2071559 and NM_002253 for the 2 exonic polymorphisms—rs2305948 and rs1870377. The transcriptional initiation site is +1 and the nucleotide 5’ to +1 is numbered −1.

Single nucleotide polymorphism-604 was analyzed by amplification of a 290-base pair (bp) sequence with primers: 5’-CACAAGTTTTCATAGGGCTTCGT-3’ and 5’-AGCCACAAGGGAGAACCGGATA-3’. The polymerase chain reaction (PCR) products were digested with BanI (New England Biolabs, Beverly, Massachusetts); 2 DNA fragments of 174 bp and 116 bp were yielded for the C allele on 3% agarose gel and only 1 band for the T allele.

The primers for SNP1192 were 5’-TGAGGTTTA-AAGTTCGTGTTGCCCTGTGTT-3’ and 5’-AAGTCTACCATCCTTGGAACAGACCGGATA-3’. The 262-bp PCR products were digested with BstZ17I (New England Biolabs); 2 DNA fragments of 30 bp and 232 bp were yielded for the A allele on 4% agarose gel and only 1 band for the G allele.

The primers for SNP1719 were 5’-GCCATCAT-TCCGTAAATGAATCT-3’ and 5’-GCTCACAT-ATTATTGTACCATTCC-3’. The 404-bp PCR products were digested with BstZ17I and HinfI (New England Biolabs); 4 DNA fragments of 13, 66, 131, and 84 bp were yielded for the C allele on 3% agarose gel and only 1 band for the T allele.
were digested with AluI (New England Biolabs); 2 DNA fragments of 191 bp and 213 bp were yielded for the A allele on 3% agarose gel and only 1 band for the T allele.

Reproducibility of genotyping was confirmed by bidirectional sequencing in 100 randomly selected samples, and the reproducibility was 100%.

Luciferase reporter assays. To determine the KDR promoter activity, the KDR promoter encompassing SNP-604 (from −887 to +413) from patients carrying SNP-604 TT and SNP-604 CC genotypes was first cloned into the pGEM-T Easy vector (Promega, Madison, Wisconsin) with the forward primer: 5′-TAGCGAGCTCTGCCCACAGGAAAGTCACACACA (contained Sacl restriction site) and the reverse primer: 5′-CACCAGACCTGTCGCTTCC. The fragment containing the KDR promoter (from −887 to +295) was released from the pGEM-T Easy vector by digesting the vector with Sacl and XhoI restriction enzymes and was then subcloned into the multiple cloning sites (Sacl and XhoI) of pGL3-basic vector (Promega). The pGL3-3 vector contains the cDNA encoding firefly luciferase. When it was fused with a promoter and transfected into mammalian cells, the construct can be used to analyze the inserted promoter activity. The vector containing the SNP-604 C/C genotype was designated as pGL3-C and the vector containing the SNP-604 T/T genotype as pGL3-T. The KDR promoter sequences in both vectors were confirmed by direct sequencing in 100 randomly selected samples, and the reproducibility was 100%.

The fragment containing the KDR promoter (from −887 to +295) was released from the pGEM-T Easy vector by digesting the vector with Sacl and XhoI restriction enzymes and was then subcloned into the multiple cloning sites (Sacl and XhoI) of pGL3-basic vector (Promega). The pGL3-3 vector contains the cDNA encoding firefly luciferase. When it was fused with a promoter and transfected into mammalian cells, the construct can be used to analyze the inserted promoter activity. The vector containing the SNP-604 C/C genotype was designated as pGL3-C and the vector containing the SNP-604 T/T genotype as pGL3-T. The KDR promoter sequences in both vectors were confirmed by direct sequencing.

Twenty-four hours before transfection, 1.5×10⁵ human umbilical vein endothelial cells were seeded in each well of a 12-well plate. On the day of transfection, each well was cotransfected with 1.5 µg of the pGL3 vector and 50 ng of the pRL-TK vector (Promega) using LIPOFECTAMINE 2000 reagents (Invitrogen Corp., Carlsbad, California). The pRL-TK vector, encoding the Renilla luciferase transcribed by a HSV-TK promoter, was used as an internal control to normalize firefly luciferase expression. Forty-eight hours after transfection, the cells were lysed in passive lysis buffer (Promega). Cell lysate was added to the luciferase substrate (dual luciferase reporter system, Promega), and firefly and Renilla luciferase activity was measured with a luminometer (SIRIUS, Pforzheim, Germany).

Construction of KDR plasmids for expression of pcDNA3.1-KDR-VH, pcDNA3.1KDR-IQ, pcDNA3.1KDR-IH, and pcDNA3.1-KDR-VQ. The plasmid pcDNA3.1-KDR-VQ (1192-G, 1719-A) was constructed in our laboratory. The mutated plasmids pcDNA3.1-KDR-VH (1192-G, 1719-T), pcDNA3.1-KDR-IQ (1192-A, 1719-A), and pcDNA3.1-KDR-IH (1192-A, 1719-T) were obtained by introducing mutation with QuikChange II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, California). The primers for introducing mutation from KDR-VQ to KDR-VH were 5′-AAGATGTTAATACCCGGAGTGCAAGGATTT-3′ and 5′-CAATCCTTTGTCCTACCTCCGGTATTACCATCTATAGTT-3′, and the primers for introducing mutation from KDR-VQ to KDR-VH and from KDR-IQ to KDR-IH were 5′-CCAACCGAGCCACGACCATGC-TGTCTCAGTGA-3′ and 5′-TCACTGAGACAGCAGTGCGTTGCGTTG-3′.

Determination of the binding efficiency of VEGF to KDR. The efficiency of VEGF binding to KDR was determined according to Dr. Anat Weisz’s method with minor modification (25). Briefly, HEK293s cells were plated at 30% to 50% confluency in 60-mm dishes 1 day before transfection with 8 µg of pcDNA3.1-KDR (KDR-VH, KDR-IH, KDR-VQ, or KDR-IQ) using LIPOFECTAMINE 2000 reagents (Invitrogen Corp.) according to the manufacturer’s protocol. After 36 h, the cells were rinsed 3 times with cold phosphate-buffered saline. The binding of VEGF165 (10 ng/ml, R&D Systems, Minneapolis, Minnesota) to KDR was carried out in the buffer containing DMEM, 25 mM HEPES (pH 7.4), 1 µg/ml heparin, and 0.1% gelatin and incubated at 4°C for 2 h. Then the cells were washed 5 times with cold phosphate-buffered saline, lysed with cell lysis buffer (10 mM HEPES, pH 7.5, 10 mM KCl, 5 mM MgCl₂, 1 mM DTT, 5% glycrol, 1 mM EDTA, 2 mM PMSF, 1 mM Na₃VO₄, 10 mM NaF, 3 µg/ml aprotinin, 3 µg/ml leupeptin, 2 µg/ml pepstatin), and followed by enzyme-linked immunoadsorbent assay with commercially available kits (R&D Systems). The binding efficiency of VEGF to KDR was expressed as the ratio of VEGF to KDR. The experiments were repeated 3 times and each was with 2 replicates.

Measurements of serum KDR level. The method has been described previously (26). In order to investigate whether SNP-604 influences the expression of KDR, the serum concentration of KDR antigen was determined in 43 subjects (16 women) age 45 to 65 years. To avoid potential confounding effects of risk factors on KDR serum levels, the subjects without conventional risk factors were randomly selected from the control group, including 19 with homozygote, 14 with heterozygote for the T allele, and 10 with homozygote for the C allele of the SNP-604. All of them had normal blood pressure (<140/90 mm Hg and no use of antihypertensive drugs), and had no DM (fasting glucose <7.8 mmol/l). The levels of TC, TG, HDL-C, non-HDL-C were normal. All samples were analyzed twice, and mean values were used for further statistical analysis.

Statistical analysis. The distribution of quantitative variables was tested for normality by use of 1-sample Kolmogorov-Smirnov test. Because TG level was highly skewed, the difference between cases and control subjects was compared with Mann-Whitney nonparametric test. Other quantitative variables were tested with Student t test. A chi-square test was applied to compare qualitative variables, genotype/allele frequencies, and the Hardy-Weinberg equilibrium of the polymorphisms. Association of SNPs with CHD was analyzed by multivariate logistic regression adjusted for age, gender, BMI, smoking, hypertension, DM, HDL-C, non-HDL-C, TC, and TG. Multiple testing was adjusted using Bonferroni correction. Spearman correlation test was used to assess the correlation between the serum levels of KDR and SNP-604 genotypes. The EH
The association remained after adjustment for age, gender, TG, and the presence of hypertension and DM. *M Crude odds ratio (OR) was calculated with 2-tailed probability value of 0.05.

Table 1. Clinical Characteristics of Cases and Control Subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>The First Population</th>
<th>The Second Population</th>
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<tbody>
<tr>
<td></td>
<td>Control Subjects</td>
<td>Cases</td>
</tr>
<tr>
<td>n</td>
<td>1,015</td>
<td>665</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>57.9 (9.5)</td>
<td>57.7 (8.7)</td>
</tr>
<tr>
<td>Male gender, %</td>
<td>84.4</td>
<td>82.6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.2 (3.3)</td>
<td>25.2 (3.1)*</td>
</tr>
<tr>
<td>HDL-C, mmol/l</td>
<td>1.02 (0.30)</td>
<td>1.07 (0.28)*</td>
</tr>
<tr>
<td>Non–HDL-C, mmol/l</td>
<td>2.95 (0.92)</td>
<td>3.20 (1.08)*</td>
</tr>
<tr>
<td>TC, mmol/l</td>
<td>4.79 (0.95)</td>
<td>5.11 (1.13)*</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>1.45 (15.10)</td>
<td>1.60 (13.11)*</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>37.4</td>
<td>58.0*</td>
</tr>
<tr>
<td>Hypertension history, %</td>
<td>24.9</td>
<td>47.4*</td>
</tr>
<tr>
<td>DM history, %</td>
<td>4.6</td>
<td>14.9*</td>
</tr>
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</table>

Age, body mass index (BMI); high-density lipoprotein cholesterol (HDL-C), non–HDL-C, and total plasma cholesterol (TC) values are given as mean (standard deviation); triglycerides (TG) values as median (range); and other values as number of individuals (n) with percentage (n/N) in parentheses. *p < 0.01 versus control group. DM = diabetes mellitus.

Results

All 3 SNPs were associated with risk of CHD. The characteristics of patients and control subjects are shown in Table 1. The distribution of the genotypes of the 3 KDR SNPs is shown in Tables 2 and 3. All genotypes conformed to Hardy-Weinberg equilibrium expectation in each group. In all 3 SNPs, the estimated risk of the subjects carrying 1 copy of the risk allele was significantly higher than those with no copy of the risk allele, but comparable to homozygote of risk allele, which indicates a dominant effect of the risk allele; thus, we analyzed the association of the 3 SNPs with CHD using a dominant model. In all 3 SNPs, the genotype frequencies of risk allele homozygote and heterozygote were significantly higher in patients with CHD than in control subjects, and the risk allelic frequencies were also higher in patients than in control subjects (Tables 2 and 3). The association remained after adjustment for age, gender, BMI, smoking, HDL, non–HDL, TC, TG, hypertension, and DM with multivariate logistic regression analysis, supporting that all 3 SNPs were risk factors for CHD. After Bonferroni correction, SNP1719 was still significantly associated with CHD in the first population, where a p value of 0.017 (0.05/3) was considered significant. Since p values for SNP–604 (0.033) and for SNP1192 (0.025) were <0.05, we tested all 3 SNPs in the second case-control study. The association of the 3 SNPs with CHD remained significant even after Bonferroni correction in the second population (Table 3).

SNP–604C–bearing KDR promoter had lower transcription activity. The SNP–604C–bearing KDR promoter had 68% lower luciferase activity than did SNP–604T–bearing promoter. The pGL-3 empty vector had a negligible amount of the luciferase activity (Fig. 1). The results supported that C allele has lower transcription activity than T allele.

Polymorphisms in coding region influenced the binding efficiency of VEGF to KDR. In order to test whether KDR variants could change the binding efficiency of VEGF, we studied the in vitro binding of VEGF to the different KDR variants by measuring the ratio of binding VEGF...
to KDR in cultured cells that were transfected with wild-type KDR and mutated KDR (KDR-VH, KDR-IH, KDR-VQ, or KDR-IQ). The highest ratio was found in the cells carrying wild-type KDR-VH, then KDR-VQ, KDR-IH; the lowest ratio was found in the cells carrying KDR-IQ (Fig. 2).

Serum KDR antigen levels were correlated with genotypes of SNP-604. Serum KDR antigen levels were positively correlated to the genotype of SNP-604. Accordingly, Spearman coefficient for the existing correlation was rs = 0.374, \(p = 0.013\) (Fig. 3). Serum KDR antigen levels were significantly lower in the CC genotype carriers than that in the TT genotype carriers of SNP-604 (\(p = 0.011\)). This change was in line with our results of promoter activity study.

**Discussion**

The results of the present study revealed that the 3 variants of KDR are associated with risk of CHD. We also examined whether the disease-associated alleles were related to specific vascular risk factors, such as hypertension, diabetes, age, and gender. After adjusting for those conventional vascular risk factors, the association remained significant, suggesting that the contribution of the SNPs to the risk of CHD is independent of conventional vascular risk factors. To control the potential false-positive associations, we used 2 independent populations to perform the case-control studies and Bonferroni correction for multiple testing. In the second population, the association of the 3 SNPs with CHD was statistically significant at the Bonferroni-corrected alpha level. Our results also indicate that the variation in the promoter could lead to a reduction of KDR promoter activity and consequently downregulation of the expression of the gene; SNP1192 and SNP1719 are located in exon 7 and exon 11, which are the key elements of KDR binding domain for VEGF. Mutations at these exons could

### Table 3

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype, n (%)</th>
<th>M + m</th>
<th>Mm + mm</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>604</td>
<td>Control subjects</td>
<td>314 (50.2)</td>
<td>247 (39.5)</td>
<td>64 (10.2)</td>
<td>1.25 (1.03–1.52)</td>
</tr>
<tr>
<td>Cases</td>
<td>154 (41.7)</td>
<td>172 (46.6)</td>
<td>43 (11.7)</td>
<td>p = 0.022</td>
<td>1.41 (1.09–1.83)</td>
</tr>
<tr>
<td>1192</td>
<td>Control subjects</td>
<td>529 (83.7)</td>
<td>95 (15.2)</td>
<td>7 (1.1)</td>
<td>1.55 (1.155–2.07)</td>
</tr>
<tr>
<td>Cases</td>
<td>279 (75.6)</td>
<td>85 (23.0)</td>
<td>5 (1.4)</td>
<td>p = 0.003</td>
<td>1.65 (1.20–2.27)</td>
</tr>
<tr>
<td>1719</td>
<td>Control subjects</td>
<td>206 (33.0)</td>
<td>310 (49.6)</td>
<td>109 (17.4)</td>
<td>1.30 (1.09–1.56)</td>
</tr>
<tr>
<td>Cases</td>
<td>92 (24.9)</td>
<td>193 (52.3)</td>
<td>84 (22.8)</td>
<td>p = 0.004</td>
<td>1.48 (1.11–1.98)</td>
</tr>
</tbody>
</table>

Crude OR was calculated with 2 \(\times\) 2 cross tabulation, and adjusted OR was obtained on multivariate logistic regression after controlling for age, gender, BMI, smoking, HDL-C, non-HDL-C, TC, TG, and the presence of hypertension and DM. *M = T and m = C for SNP-604; M = G and m = A for SNP1192; M = T and m = A for SNP1719.

Abbreviations as in Tables 1 and 2.

**Figure 1**

SNP-604-C-Bearing KDR Promoter Had Lower Transcription Activity

The pGL3 luciferase reporter contained either the T (pGL3-T) or C allele (pGL3-C) at the promoter –604 locus. Values represent the average of 6 experiments and the bars represent the standard deviation. The pGL3-basic was used as a negative control without any promoter sequence, and pGL3-control as a positive control. KDR = kinase insert domain-containing receptor; SNP = single nucleotide polymorphism.

**Figure 2**

SNP1192 and SNP1719 Influenced the Binding Efficiency of VEGF to KDR

HEK293s cells were transfected with 8 \(\mu\)g of pcDNA3.1-KDR (KDR-VH, KDR-IH, KDR-VQ, or KDR-IQ). After 36 h, the cells were rinsed with cold phosphate-buffered saline 3 times, and the binding of vascular endothelial growth factor (VEGF)165 (10 ng/ml, R&D Systems, Minneapolis, Minnesota) was carried out in binding buffer containing DMEM, 25 mmol/l HEPES (pH 7.4), 1 \(\mu\)g/ml heparin, and 0.1% gelatin for 2 h at 4°C. Then the cells were rinsed with cold phosphate-buffered saline 5 times, lysed with cell lysis buffer, followed by enzyme-linked immunosorbent assay. The values were the ratio of VEGF to KDR. The experiments were repeated 3 times, and 2 replicates were performed for each experiment. The values were presented as means, and the T bars represented standard deviations. Abbreviations as in Figure 1.
lead to mutant KDR and, in turn, change the efficiency of VEGF binding to KDR. Our study showed that patients carrying the KDR mutations are more susceptible to CHD. The higher CHD risk could be due to downregulation of the VEGF/KDR signaling pathway.

Vascular endothelial growth factor stimulates proliferation, migration, and tube formation of endothelial cells primarily through KDR. Vascular endothelial growth factor binds to 2 tyrosine kinase receptors, VEGF receptor-1 (VEGFR1, Flt-1) and KDR, in endothelial cells. The mitogenic and chemotactic effects of VEGF are mediated mainly through KDR in endothelial cells (27), which is activated through autophosphorylation of tyrosine residues in the cytoplasmic kinase domain of KDR. This event is followed by activation of downstream signaling pathways such as mitogen-activated protein kinases, Akt and eNOS, which are essential for migration and proliferation of endothelial cells, thereby stimulating angiogenesis (27). Gene targeting experiments demonstrate that a functional KDR receptor is essential for the development of hematopoiesis and vasculogenesis (6). Vascular endothelial growth factor–dependent angiogenesis induces a complex array of biological responses, including increased vascular permeability, monocyte chemotaxis, vasodilatation, and hypotension. Therefore, it is still a hot debate whether VEGF-stimulated angiogenesis has therapeutic effects on ischemic heart and limb by stimulating collateral blood vessel formation through KDR, or the VEGF/KDR-induced neovascularization contributes to the growth of atherosclerotic lesions and the plaque destabilization leading to rupture.

Recent studies have shown that VEGF promotes atherosclerosis in certain animal models and potentially destabilizes coronary plaque by promoting intraplaque angiogenesis. A growing body of evidences supports an association between intraplaque angiogenesis with atherosclerotic plaques that cause acute coronary syndrome (28). However, the angiogenesis preceded by VEGF/KDR signaling pathway could be decreased due to low KDR activity. Therefore, abnormal angiogenesis and endothelial damage/dysfunction would increase the risk of CHD.

Since resident endothelial cells infrequently proliferate (29), other sources of vascular replenishment have been postulated in response to continuous damage (30). Recent studies indicate that EPCs characterized by coexpression of CD133, CD34, and KDR may contribute to ongoing endothelial repair by providing a circulating pool of cells that can home to denuded parts of the artery after balloon injury or could replace dysfunctional endothelial cells (16,19,31). In the vascular repairing course, the mobilization and homing to injured sites of EPCs are very crucial. An important regulator of this mobilization is VEGF, which binds to KDR, thus mediating the further maturation of the cascade hemangioblast-angioblast–early EPC–late EPC (32).

Some studies have identified the KDR receptor and the PI3-kinase/Akt signal transduction pathway as crucial elements in the processes of endothelial cell survival induced by VEGF (9,14). Inhibition of apoptosis may represent a major aspect of the regulatory activity of VEGF on the vascular endothelium. Vascular endothelial growth factor also can prevent oxidized-LDL–induced endothelial cell damage via an intracellular glutathione–dependent mechanism through the KDR (33).

Vascular endothelial growth factor and KDR would have an impact on the progress of CHD in vivo by these mechanisms or more. The SNP–604T>C decreased mRNA levels of KDR. Both SNP1192G>A and SNP1719T>A resulted in slight but significant decrease in the VEGF binding efficiency to KDR. One can speculate that the decrease in KDR function is correlated with vascular dysfunction, including endothelial cell damage, impaired endothelial cell survival, decreased antiapoptotic effects of VEGF, and abnormal vascular repair. All of these can promote the progression of atherosclerotic disease. A previous study has suggested that −907T>C, +11903G>A, and +18487A>T (now called SNP–604, SNP1192, and SNP1719, respectively) have no significant association with the development of coronary artery lesion in Japanese subjects with Kawasaki disease (34). The difference between the Japanese study and ours could be due to the different pathologic mechanism between the coronary artery lesion with Kawasaki disease and CHD, different genetic background of the 2 populations (35), as well as the relatively small sample size in the Japanese study. Consistent with our results, the number of circulating EPCs in peripheral blood has been reported to be inversely correlated with vascular dysfunction (17,36–38), indicating that low levels of circulating EPCs may be associated with risk of CHD.

The data from the International HapMap project provide some information on the underlying genetic architecture of
the KDR gene (35). The genotype frequencies of the 3 SNPs from the HapMap data were similar to ours. The rs2071559 can capture rs7667298 (exon_1, untranslated). No linkage disequilibrium was found in rs2305948 with other SNPs in HapMap CHB data. The rs1870377 (exon_11) can capture rs10016064 (intron_13), rs17085265 (intron_21), rs3816584 (intron_16), rs6838752 (intron_17), rs1870379 (intron_15), rs2219471 (intron_20), rs1870378 (intron_15), rs13136007 (intron_13), and rs17085262 (intron_21). The results showed that 2 blocks were captured by SNP-604 (rs2071559) and SNP1719 (rs1870377), respectively, and SNP1192 (rs2305948) were associated with CHD.

Conclusions

Our observations suggest that the 3 SNPs in KDR gene are novel genetic risk markers for CHD. Our findings need to be replicated in additional studies, especially in prospective cohort studies.

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