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Functional vascular endothelial growth factor gene polymorphisms and diabetes: Effect on coronary collaterals in patients with significant coronary artery disease

Tsung-Hsien Lin [a,b], Chiao-Ling Wang [c], Ho-Ming Su [a,b], Po-Chao Hsu [a], Suh-Hang Hank Juo [d], Wen-Chol Voon [a,b], Shyi-Jang Shin [d,e], Wen-Ter Lai [a,b], Sheng-Hsiung Sheu [a,b,*]

[a] Division of Cardiology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Taiwan
[b] Department of Internal Medicine, Faculty of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[c] Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[d] Medical Genetics, Kaohsiung Medical University, Kaohsiung, Taiwan
[e] Division of Endocrinology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Taiwan

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ABSTRACT

Background: Vascular endothelial growth factor (*VEGF*) plays a pivotal role in angiogenesis. This study tested the association between functional VEGF +405 C>G (rs2010963), −2578C>A (rs699947) polymorphisms, and coronary collaterals in patients with coronary artery disease (CAD).
Method: The collateral scoring system developed by Rentrop was used to classify 393 patients according to their collaterals as either "poor" (grades 0 and 1) or "good" (grades 2 and 3). Gene polymorphisms were analyzed by TaqMan assay.
Results: The frequency of +405C and −2578A alleles was higher in the good collaterals group ($p=0.007$ and 0.005, respectively). For the +405C>G allele, the odds ratio (OR) of good collaterals for CC to GG genotype was 2.54 ($p=0.003$). For the −2578A allele, the OR of good collaterals for AA to CC genotype was 2.31 ($p=0.038$). Univariate and logistic regression analysis found 2 polymorphisms in the additive model for associations with collateral development: +405C>G ($p=0.005$ and 0.010) and −2578C>A ($p=0.006$ and 0.006). The *VEGF* +405C>G polymorphism and DM revealed an interactive effect on collateral development ($p=0.027$).
Conclusions: The *VEGF* +405C>G and −2578C>A polymorphisms might be novel genetic factors affecting collateral development in Chinese patients.

1. Introduction

Collateral circulation can protect and preserve myocardium from episodes of ischemia, enhance residual myocardial contractility, and reduce angina symptoms and cardiovascular events [1–3]. Unfortunately, some patients with significant coronary artery disease (CAD) may not have well-developed collateral vessels. The reasons for the differing individual ability to develop collateral circulation are still unclear.

Angiogenesis plays a role in collateral vessel formation, and vascular endothelial growth factor (VEGF), a major mediator of vascular angiogenesis, may play a pivotal role in the development of coronary collaterals [2]. We previously demonstrated that cardiac VEGF concentration is associated with coronary collateral development [4]. Other trial studies have also demonstrated the use of VEGF as a therapeutic angiogenesis agent in severe coronary disease patients who cannot undergo standard revascularization [5,6].

The gene encoding VEGF is located on chromosome 6 and comprises a 14 kb coding region with 8 exons and 7 introns [7]. The VEGF polymorphisms at positions +405 and −2578 are known to have functional significance [8–10]. Functional studies indicate that the +405 C allele of VEGF genetic polymorphism increases VEGF production when peripheral blood mononuclear cells are stimulated by lipopolysaccharide [8]. The VEGF mRNA expression is significantly higher in patients carrying the A allele genotype at the −2578 position [10]. Therefore, these two functional VEGF polymorphisms may have important modulating roles in the collateral development. Given the above findings, we examined two single-nucleotide polymorphisms (SNPs) of the *VEGF* gene, +405 C>G (rs2010963) and −2578C>A (rs699947), to assess their possible relationships to coronary collaterals in patients with significant CAD.

Abbreviations: VEGF, vascular endothelial growth factor; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; HIF-1, hypoxia-inducible factor 1; eNOS, endothelium-derived nitric oxide synthase; CFI, collateral flow index.

* Corresponding author. Division of Cardiology, Department of Internal Medicine, Kaohsiung Medical University Hospital, No. 100, Tzyou 1st Road Kaohsiung 807, Taiwan. Tel.: +886 73121101x7738; fax: +886 73234845.
E-mail address: Sheush@kmu.edu.tw (S.-H. Sheu).

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2. Materials and methods

2.1. Study subjects

From February, 2002 to October, 2006, we evaluated 832 consecutive patients who had been scheduled to undergo diagnostic coronary angiography at Kaohsiung Medical University Hospital (KMUH) in Taiwan. The analysis excluded patients with coronary artery lumen diameter stenosis <70%, history of coronary artery bypass surgery (CABG), history of percutaneous coronary intervention (PCI), inconclusive restriction digest results, or inadequate angiograms for collateral evaluation. None of the study subjects had major autoimmune or hematological diseases. Other analyzed demographic and baseline data included gender, age, and any history of the following: diabetes mellitus (DM), hypertension, hypercholesterolemia, cigarette smoking, and medications. The research protocol was approved and registered by the ethics committee (KMUH-IRB-940253) at our institution, and informed consent was obtained from all patients.

2.2. Coronary angiography and collateral scoring

The coronary artery angiography films were reviewed by 2 experienced cardiologists blinded to the clinical and genotype data for all patients. Any differences in interpretation were resolved by a third reviewer who was blinded to the readings of the first two reviewers. Coronary artery stenosis was determined by quantitative coronary angiography. The recorded data also included the number of diseased vessels, the vessel to which the collaterals were connected, and the grade of coronary collateral circulation. Vessels exhibiting a 70% or greater reduction in lumen diameter were classified as "significant". In subjects with more than one significant CAD vessel, the vessel with the highest collateral grade was chosen for analysis.

The collateral scoring system developed by Rentrop and Cohen was used [11]. In subjects with more than one collateral supplying the distal aspect of the diseased artery, the highest collateral grade was recorded. Patients were then classified according to their collateral grades as either "poor" (grade 0 or grade 1 collateral) or "good" (grade 2 or grade 3 collateral). The 2 readers obtained a 96% agreement in their patient classifications.

2.3. Extraction and amplification of genomic DNA

Blood samples were collected during angiography. After isolating the buffy coat, which is leukocyte-enriched fraction of whole blood, genomic DNA was extracted from peripheral blood by using a DNA extraction kit (Puregene Gentra System, Minneapolis MN) according to manufacturer instructions. The extracted genomic DNA was suspended in 10 mmol/l Tris–HCl, 1 mmol/l EDTA pH 8.0, and DNA concentrations were measured by spectrophotometry.

Genotypes were determined by TaqMan allelic discrimination assay using a commercial kit (Applied Biosystems; ABI, Foster City, CA) according to manufacturer instructions. Genotyping for VEGF +405 C>G and −2578C>A (rs2010963 and rs699947) was performed using TaqMan-MGB probes and primers and the Applied Biosystems Assay-on-Demand service. Allele VEGF +405G and −2578C probes were labeled with 6-carboxy-fluorescein (FAM), and allele VEGF +405C and −2578A probes were labeled with VIC at their 5′ ends. The thermal cycling conditions were 50 °C for 2 min and 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s and 60 °C for 90 s. After completing PCR amplification, allelic discrimination was performed using the Applied Biosystems 7900 real-time PCR system.

Approximately 5% of the replicate samples were sequenced for quality control, and a 100% concordance rate was noted. The genotyping call rate was 97% for *VEGF* +405 C>G polymorphisms and 95% for −2578C>A polymorphisms. Laboratory personnel were blinded to the disease status of all subjects. The linkage disequilibrium correlation coefficient was 0. The D′ value was 0.028.

2.4. Statistical analysis

All data were expressed as means ± standard deviation. If the minor allele frequency (MAF) for VEGF +405 C>G polymorphism among the good collateral was estimated to be 0.486, the study should enroll at least 132 patients with good collaterals to have more than 80% statistical power to detect an association with ORs ≥ 2.5. If the MAF for VEGF −2578C>A polymorphism among the good collateral was 0.314, the study should enroll at least 110 patients with good collaterals to have more than 80% statistical power to detect an association with ORs ≥ 2.3. The genotypic distribution was tested by Hardy–Weinberg equilibrium. Independent *t* test or Mann–Whitney rank sum test was used to compare continuous variables between the two groups. Chi-square test was used to compare categorical data. Univariate logistic regression for collateral development as a binary outcome (poor or good) was initially performed using each genotype as a dummy variable and without assuming a specific genetic model. A mode of inheritance was then assumed after this initial result. Multivariate analysis was performed using an appropriate genetic model that included significant or relevant covariates. Interaction terms between SNP and significant covariates were also tested in the multivariate regression model. All *p* values were two-sided with a significance level of $p<.05$. The Statistical Package for the Social Sciences 11.0 for Windows (SPSS Inc., Chicago, IL) was used for statistical analysis.

3. Results

3.1. Clinical characteristics

Of the 832 subjects initially enrolled, 439 patients were excluded for the following reasons: coronary artery lumen diameter stenosis <70%, history of CABG or PCI, genotyping failure, or inadequate angiograms for collateral evaluation. The final study population of 393 subjects (307 males, 86 females; average age, 63.3 ± 12.2 years) included only those with genotyping data for both SNPs.

Of the 393 patients enrolled, 182 (46.3%) patients had no coronary collaterals. Of the remaining 211 patients with coronary collaterals, the received vessel was left anterior descending artery (LAD) in 82 (38.9%) patients, left circumflex artery (LCX) in 35 (16.6%), and right coronary artery (RCA) in ninety-four (44.5%). In subjects with collaterals, the grading was distributed as follows: 63 (29.9%) with grade 1, 99 (46.9%) with grade 2 and 49 (23.2%) with grade 3.

Table 1 compares the demographic data between the poor group (n = 245) and the good group (n = 148). The poor group had a lower incidence of family history of CAD (2% vs 6.1%, p = 0.036) and fewer diseased vessels (2.0 ± 0.8 vs 2.5 ± 0.7, p<0.001) than the good group did. All the diabetics are type II diabetes.

3.2. +405 C > G and −2578C > A gene polymorphisms

Among the 393 study subjects, 71 (18.1%) had VEGF +405 CC, 213 (54.2%) had CG, and 109 (27.7%) had GG genotype. Twenty-seven (6.9%) had VEGF −2578 AA, 149 (37.9%) had CA, and 217 (55.2%) had CC genotype. The genotypic distribution was in Hardy–Weinberg equilibrium in both genes. The VEGF +405 genotype was associated with number of diseased vessels (p = 0.021) and use of angiotensin receptor blocker (p = 0.038). The VEGF −2578 genotype was associated with diabetes (p = 0.040) and use of β-blocker (p = 0.002) and diuretic (p = 0.032).

Given a reference group of patients with VEGF +405GG genotype, the OR for the good group was 1.22 (95% CI: 0.75–2.00, p = 0.422) in the CG genotype and 2.54 (95% CI: 1.37–4.71, p = 0.003) in the CC