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Polymorphisms of TGFβ-1 and TGFBR2 in relation to coronary artery disease in a Chinese population

Minjun Yang [a,b], Min Zhu [c], Lijiang Tang [a,d], Huanhuan Zhu [a,b], Yifei Lu [a,b], Baohui Xu [e], Jianjun Jiang [a,b,*], Xiaofeng Chen [a,b,c,*]

[a] Laboratory of Cardiovascular Disease, China
[b] Department of Cardiology, Taizhou Hospital, Wenzhou Medical University, Zhejiang Province 317000, China
[c] Enze Medical Research Center, Taizhou, Zhejiang Province 317000, China
[d] Zhejiang Hospital, Zhejiang Province 317000, China
[e] Vascular Center, Stanford University School of Medicine, Stanford, CA 94305, USA

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ABSTRACT

Background and aim: TGF-β1 has been previously reported to be involved in the pathogenesis of atherosclerosis. The aim of the present study was to assess whether functional gene polymorphisms of TGF-β1 and its key receptor TGF-β receptor type II (TGFBR2) contribute as risk factors to the onset and severity of atherosclerotic coronary artery disease (CAD).

Design and methods: A total of 605 patients who underwent angiography for suspected CAD were prospectively recruited to this study. Coronary stenosis severity was assessed by the number of narrowed coronary vessels and the Gensini score. Among them, 502 patients had documented CAD, and 103 patients without documented CAD served as non-CAD controls. All patients were genotyped for one TGF-β1 polymorphism (rs1800470 (+T29C)) and two TGFBR2 polymorphisms (rs6785385 (−3779A/G), rs764522 (−1444C/G)) by polymerase chain reaction–restriction fragment length polymorphism and confirmed by direct sequencing.

Results: No significant difference in the frequency for either polymorphism was found between CAD and control patients. Neither TGFBR2 rs6785385 (−3779A/G) nor rs764522 (−1444C/G) gene polymorphisms were associated with the severity of CAD ($P > 0.05$). In male CAD patients, polymorphisms at TGF-β1 rs1800470 (+T29C) were, however, associated with the severity of CAD. The T allele frequency was significantly and positively correlated with the number of narrowed coronary arteries (three or more vessels: 49.3%, two vessels: 44.1%, one vessel: 36.9%) ($P = 0.039$). Gensini scores in patients with the TT, CT, and CC genotype were 34.33 ± 2.23, 32.06 ± 4.79, and 26.90 ± 3.83, respectively ($P < 0.05$). In multiple linear regression analysis, the T allele of TGF-β1 polymorphism was independently correlated with the Gensini score ($\beta = 0.131$).

Conclusion: TGF-β1 T29C gene polymorphism may be associated with severity of CAD in male patients. TGFBR2 polymorphisms may not determine the genetic susceptibility to CAD.

1. Introduction

Coronary artery disease (CAD) is one of the leading causes of mortality and morbidity. The cytokine transforming growth factor-beta (TGF-β) regulates cellular functions and thus may play important roles in the pathogenesis of atherosclerosis [1,2]. As the most abundant isoform of the TGF-β family, TGF-β1 is a multifunctional cytokine and is expressed by a variety of cells, including hematopoietic, connective and endothelial tissue cells. It regulates cell growth, cell differentiation and matrix production [1–3]. TGF-β receptor (TGFBR), including TGFBR1, TGFBR2 and TGFBR3, is a serine/threonine protein kinase, present on the cell surface. TGF-β1 signals across the plasma membrane by binding to TGF-β receptor type II (TGFBR2), the key component of TGF-β signaling pathways; it controls the activation of the TGF-β signaling pathway [3–6].

Increasing evidence implicates the dysregulated TGF-β1 pathway in the development of CAD. In particular, TGF-β1 has been reported to be involved in the pathogenesis of atherosclerosis. However, whether TGF-β1 is pro- or anti-atherogenic remains controversial. TGF-β1 is generally regarded as an anti-inflammatory cytokine. However, TGF-β1 signaling has also been shown to increase the expression of proatherogenic genes [3,4,6]. Although environmental factors play an important role in atherosclerosis development, the genetic impact on atherosclerotic process has been increasingly realized. The dysregulation of TGF-β1 signaling resulting from genetic mutations within the

* Corresponding authors at: Department of Cardiology, Taizhou Hospital, Wenzhou Medical University, Zhejiang Province 317000, China.
E-mail addresses: Jiangjj@enzemed.com (J. Jiang), zjxiaofengchen@hotmail.com, chenxf@enzemed.com (X. Chen).

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components of the TGF-β1 pathway have been reported in many medical conditions such as cancer and vascular diseases [7–10]. Several genetic variants in TGF-β1 genes, which affect TGF- β1 protein expression, have been identified. One of the most commonly studied TGF-β1 genetic variant is T29C polymorphism (rs1800470), a change from proline (CCG) to leucine (CTG) at codon 10 (Pro10Leu) of the protein [7]. Some, but not all, studies suggest that TGF-β1 C29T polymorphism was associated with coronary atherosclerosis [10–13]. Thus, in published studies, the role of TGF-β1 C29T polymorphism in CAD pathogenesis remains unclear. In addition, studies in the Chinese Han population were very limited.

The TGFBR2 gene was mapped on human chromosome 3p22. Either quantitative or qualitative changes in TGFBR2 would affect the TGF-β signaling pathway and particularly the cellular responses to TGF-β1. Genetic variations in TGFBR2 have been found to be related to cancer, sudden cardiac arrests in patients with CAD, and other vascular diseases, including hypertension, Kawasaki disease, Marfan syndrome, Loeys–Dietz syndrome, and intracerebral hemorrhage [14–18]. Furthermore, polymorphisms in the promoter region of TGFBR2 (rs6785358, −3779A/G; rs764522, −1444C/G) were also associated with an increased risk of congenital heart defects in the Chinese Han population [19]. However, few studies have been conducted to examine whether genetic variation of the TGFBR2 gene is associated with the onset and severity of CADs in the Chinese Han population.

In this study, we assessed whether polymorphisms of TGF- β1 and TGFBR2 gene were associated with the susceptibility and severity of atherosclerotic CAD in the Chinese Han population.

2. Materials and methods

2.1. Study population

A total of 605 patients who underwent angiography for suspected CAD were prospectively recruited to this study, including 374 men and 128 women (average age: 65 years). CAD was diagnosed according to the World Health Organization guidelines [20]. We excluded patients diagnosed with secondary hypertension, congestive heart failure, congenital heart disease, cardiomyopathy, infective endocarditis, rheumatic heart disease, active cancer, syphilis, and connective tissue disease. Among them, 502 patients had documented CAD, and 103 patients without documented CAD served as non-CAD controls for analysis. Peripheral venous blood (2 ml) was drawn into an ethylene diamine tetraacetic acid (EDTA) tube and stored at −20 °C prior to angiography. Information on other medical conditions, including the patients' ages, gender, body mass index (BMI), blood pressure, diabetes history, any history of smoking and/or alcoholism, and medications, were collected from hospital medical records. Written informed consent was obtained from all participants. The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Review Committee of Taizhou Hospital, Wenzhou Medical University.

2.2. Assessment of coronary artery stenosis severity

Coronary stenosis severity was assessed by the number of narrowed coronary vessels and the Gensini score [21]. A stenosis was defined as >50% luminal narrowing in a coronary artery. The lesions involving the left anterior descending branch, left circumflex, or right coronary artery were scored as one branch, whereas those involving left main coronary artery was noted as two. The patients were categorized into single-branch, two-branch, and multiple-branch groups according to the number of coronary arteries involved. The Gensini scores for assessing coronary stenosis severity are coronary angiography < 25% (1),25% ≤ ~ < 50% (2),50% ≤ ~ < 75% (4),75% ≤ ~ < 90% (8),90% ≤ ~ < 99% (16), and ≥99% (32). The total Gensini score was the sum of all lesion branches.

2.3. Analysis of TGF-β1 and TGFBR2 gene polymorphisms

Genomic DNA was extracted from the peripheral blood leukocytes and dissolved in DNase-free water. Only samples with an A_{260}/A_{280} ratio of 1.7–2.0 were used for analysis. The primers were designed to flank the region of the single-nucleotide polymorphisms (SNPs) including TGF-β1 gene T29C and TGFBR2 gene −3779A/G and −1444C/G (Table 1). Each 25-μl PCR reaction contained 0.1 μg DNA,2.5 μl of PCR buffer, 0.5 μl of dinucleotide triphosphate (dNTP) (10 mM each), 0.25 μl each primer, and two units of TaqDNA polymerase. The PCR conditions were as follows: 94 °C for 5 min followed by 35 cycles at 94 °C for 45 s, 60 °C for 1 min, and 72 °C for 1 min and an additional 1 min at 72 °C. The PCR products were purified, and the TGF-β1 gene rs1800470 (+T29C), and TGFBR2 gene rs6785385 (−3779A/G) and rs764522 (−1444C/G) polymorphisms were determined by direct sequencing at the Shanghai SemiBioTechnology Co. (Fig. 1).

2.4. Biochemical analyses

The serum levels of total cholesterol, triglycerides, glucose, creatinine, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured at the hospital's clinical chemistry department using standard enzymatic methods. The levels of Apo lipoprotein A1 (apoA-I) and apoB were measured using immune turbidimetry kits (Beijing Leadman biochemical company). The immune colloidal gold technique was used to measure the serum levels of C-reactive protein.

2.5. Statistical analysis

Gene frequencies of polymorphisms were obtained by direct counting. Hardy–Weinberg equilibrium was evaluated by the chi-squared test. All statistical analyses were performed using SPSS version 18.0 statistical package (manufacture information for software). All data are given as either mean ± standard deviation (SD) or the frequencies of baseline characteristics. Chi-squared tests were used to compare frequencies among the groups. Analysis of variance (ANOVA), Student's *t*-test, or non-parametric Mann–Whitney test were used to test the difference for continuous variables between groups. A multiple linear regression was performed to evaluate the effects of the various variables, gene T29C, diabetes, LDL-C, systolic blood pressure (SBP), and smoking on the severity of CAD (the Gensini score). Pairwise linkage disequilibrium (LD, D′) estimations between polymorphisms and haplotype reconstruction were performed with SHEsis on line software (http://analysis.bio-x.cn). A P-value < 0.05 was considered statistically significant.

3. Results

3.1. Clinical characteristics of the study population

On coronary angiography, 17% of patients had normal coronary arteries (non-CAD group), and 83.0% of patients had minimal CAD (CAD group). One-vessel, two-vessel, and three-vessel or more coronaries arteries were involved in 33.3%, 26.1%, and 40.6%, respectively, of CAD patients. The baseline characteristics of the participants with and without CAD are presented in Table 2. The distributions in gender and BMI did not differ between patients with and without CAD. CAD patients were older with a high prevalence of diabetes, history of cigarette smoking, and high levels of serum C-reactive protein compared with control patients. No significant difference was noted in hypertension prevalence, systolic and diastolic blood pressure, fasting glucose, and plasma lipid levels between two groups. In addition, cardiovascular medications, including antiplatelet drugs, statin, angiotensin-converting inhibitors, angiotensin receptor blockers, calcium channel blockers, and nitrates, did not differ between two groups.