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Association study of the thrombomodulin −33G>A polymorphism with coronary artery disease and myocardial infarction in Chinese Han population

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Abstract

Background: Thrombomodulin (TM) is the anticoagulant endothelial cell membrane-bound protein cofactor in the thrombin-mediated activation of protein C. Recently, conflicting data have been reported regarding the possible contribution of the TM −33G>A polymorphism to coronary artery disease (CAD) or myocardial infarction (MI) in some Asian populations. We investigated this polymorphism in northern Han Chinese.

Methods: We performed a case-control study, including 808 patients with angiographically verified CAD or a history of an acute MI and 813 age- and sex-matched controls. The TM −33G>A polymorphism was determined by polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP) analysis.

Results: We did not find a significant difference in the frequency of the A allele between CAD patients (11%) and controls (9.8%; P=0.249), between MI patients (11.5%) and controls (P=0.163), or between premature MI patients (11.7%) and controls (P=0.265). Similarly, the difference of the genotypic distributions could be neglected across the groups: GG: (GA/AA) was 81.4%:18.6% in controls, 79.7%:20.3% in patients with CAD, 78.8%:21.2% in patients with MI, and 77.7%:22.3% in patients with premature MI, respectively (vs. controls, all P>0.05). The lack of association also persisted after adjusting for other conventional risk factors.

Conclusions: Our results seemed not to support a significant association of the TM −33G>A polymorphism with CAD, MI or premature MI in our population.

Keywords: Coronary artery disease; Myocardial infarction; Thrombomodulin; Polymorphism; Association study

1. Introduction

Over the past few years, studies have focused on the role of haemostatic markers that reflect the inherited or acquired propensity to coronary artery disease (CAD) or myocardial infarction (MI), and several genetic mutations affecting coagulation proteins have been suggested as prothrombotic risk factors [1,2]. Among these, a potential candidate is the thrombomodulin (TM) gene.

TM, the product of the TM gene, is an endothelial cell surface glycoprotein receptor that forms a high-affinity complex with thrombin. The thrombin-TM 1:1 complex rapidly activates protein C, which in turn, degrades the clotting cofactors activated factors (F) V and VIII. Moreover, thrombin bound to TM loses all its procoagulant activities. Thus, TM plays an important role in converting

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thrombin from a procoagulant to a physiological anticoagulant factor [3].

The −33G>A polymorphism in the 5′ -promoter region of the TM gene was firstly identified by Ireland et al. [4]. Interestingly, the prevalence of the mutation A allele was more frequent in some Asians (8–10%) than in Caucasians (<1%) [4–9]. Moreover, the GA/AA genotypes have been reported to be associated with decreased promoter activity, and increased risk for CAD or MI in both Taiwanese and Korean populations [6–8]. To our knowledge, a more recent study performed in Japanese did not replicate the above positive findings [9].

In view of these contradictory findings and the smaller sample sizes of the previously positive studies, we further investigated a possible association of the −33G >A polymorphism with CAD and MI in a relatively large Chinese Han population.

2. Materials and methods

2.1. Subjects

A total of 1621 unrelated Chinese Han subjects were included in this study. Eight hundred eight patients with CAD were recruited from hospitalized patients of Fu Wai Hospital and Cardiovascular Institute (Beijing, P.R. China) between October 1997 and December 2001. Eligible patients included those who survived an acute MI or documented by a coronary angiography with evidence of at least a 70% stenosis in a major epicardial artery. Subjects with congenital heart disease, cardiomyopathy, valvular disease, and renal or hepatic disease were excluded from the study. Eight hundred thirteen age- and sex-matched control subjects were randomly selected from individuals participating in a community-based survey of cardiovascular risk factors. The control subjects were judged to be free of CAD by history, clinical examination, electrocardiography and the Rose questionnaire [10]. This study was approved by the local Research Ethics Committee and all subjects gave written informed consent.

A set of questionnaires was completed that included details of medical history, family history of CAD, drug intake, and cigarette smoking. Blood pressure, weight, height, waistline and hip circumference were recorded, and the body mass index (BMI) and waist-to-hip ratio were calculated. Diagnosis of diabetes mellitus was based on an actively antidiabetic treatment and/or fasting blood glucose ≥7.0 mmol/l on two occasions; diagnosis of hypertension was based on an elevated systolic (≥140 mm Hg) and/or diastolic (≥90 mm Hg) blood pressure on three occasions and/or the current use of antihypertensive drugs. Premature MI is characterized by age at MI presentation of 50 years or less for men and 55 years for women.

Venous blood was drawn from all subjects after an overnight fast. Blood, serum and plasma were separated immediately and stored at −70 °C.

2.2. Genotyping

Genomic DNA was isolated from white blood cells by the standard salt precipitation method [11]. Genotyping for the TM −33G>A polymorphism was carried out by polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP). A 259-bp DNA fragment, containing the −33G>A polymorphism site for TM, was amplified by PCR using the following primers: forward, 5′ -GGC CAG GGC TCG AGT TTA TAA AGG C-3′ ; and reverse, 5′ -CGG GGA CAG TCG TCT GTT ACA G-3′ . Samples were subjected to denaturizing at 94 °C for 5 min, followed by 32 cycles of 94 °C for 40 s, annealing at 64 °C for 30 s, then extension at 72 °C for 40 s, and a final step at 72 °C for 8 min. Each 10-μl reaction contained approximately 50 ng DNA, 2.0 mM $MgCl_2$, 5 pmol each primer, 0.2 mM of each deoxynucleoside triphosphate, and 1.0 U *Taq* polymerase (TaKaRa) in a corresponding buffer. Digests (10 μl) containing five units of restriction enzyme (*Stu*I, from MBI Fermentas) were incubated for 12 h at 37 °C for the PCR products. The restriction site for *StuI* is 5′ -AGG*CCT-3′ (where * indicates the cleavage site), which represents the mutant-type A allele and produces two fragments for the AA genotype (24 and 235 bp). The wild-type G allele is not recognized by the StuI enzyme, and only shows one 259 bp band (for the GG genotype). The GA heterozygote has three bands, with sizes of 259, 235 and 24 bp. The digests were then separated on a 3% agarose gel and visualized by ethidium bromide staining.

2.3. Statistical analysis

Statistical analysis was carried out using SPSS 10.0 version for Windows. All measured variables were presented as mean±S.D. and compared between cases and controls using the unpaired Student's *t*-test. Hardy-Weinberg equilibrium was assessed by the χ^2 test. Subjects either heterozygous or homozygous for the TM −33A allele were taken together into one group, because the mutant homozygotes were too small for separate analysis. Univariate analysis, used to measure the association of the −33G>A polymorphism with CAD or MI, was tested by the χ^2 test. Multivariate analysis, applied to investigate the independent role of the −33G>A polymorphism, was done by multiple logistic regression. A two-tailed probability value of <0.05 was considered significant. To detect a difference in allele frequencies between CAD and control group which would be of a similar magnitude as previously reported (13% vs. 8%) [6], we estimated that counting about 797 alleles for each group would be enough with a conservative approach (90% power, 5% significance level, two-sided test) with the