TNF-α polymorphisms and coronary artery disease: Association study in the Korean population

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ABSTRACT

Coronary artery disease (CAD) results from atherosclerosis, a chronic inflammatory disease mediated in part by proinflammatory cytokines, particularly tumor necrosis factor-α (TNF-α), which is expressed by atherosclerotic plaques. In this study, we investigated whether TNF-α gene promoter polymorphisms affect the incidence of CAD in Koreans by genotyping. 404 Control subjects and 197 patients who previously received a coronary artery stent for the G/A, C/T, and C/A polymorphisms at position –238, –863 and –863, respectively. The G/G, G/A and A/A genotypes at position –238 occurred in 85.8%, 14.2% and 0% CAD patients and 91.8%, 7.9% and 0.3% control subjects, respectively. The G/A polymorphisms at position –238 were significantly associated with CAD when assuming a dominant model of inheritance (OR = 1.87; 95% CI = 1.10–3.20; P = 0.02), and A allele carriers had a significantly increased risk of developing CAD relative to the G allele (OR = 1.74; 95% CI = 1.04–2.92; P = 0.03). However, the polymorphisms at positions –857 and –863 were not associated with CAD. Haplotype-based analysis revealed the CAD and control groups differed significantly in the frequencies of haplotype ACC at positions –238, –857 and –863 (OR = 1.77; 95% CI = 1.05–2.98; P = 0.03). This was confirmed by multivariate analysis after adjusting body mass index and the presence of diabetes and hypertension (OR = 2.06; 95% CI = 1.15–3.68; P = 0.015). Thus, the –238A allele of TNF-α is associated with an increased risk of CAD and could be used as predictor for CAD in Koreans. Further studies are needed to elucidate the clinical implications of these findings.

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1. Introduction

Coronary artery disease (CAD) is multifactorial and polygenic in nature. The development and progression of this disease is mediated in part by inflammatory processes in which proinflammatory cytokines play a role [1–3]. One of these is the pleiotropic cytokine tumor necrosis factor-α (TNF-α), which is associated with the pathophysiology of various conditions including inflammatory and autoimmune diseases [4,5]. It is recently suggested that TNF-α may play an important role in apoptosis, obesity-related metabolic disorders such as insulin resistance, disturbance of lipid metabolism, and hypercoagulability, and may be one of the mediators of atherosclerosis [6–8]. TNF-α therefore may play an important role in the pathophysiology of cardiovascular diseases [9–11].

In particular, TNF-α may not only promote the initiation and evolution of atheroma [12,13] but it may also precipitate thrombotic events and plaque ruptures that characterizes CAD [14–17], in part by affecting endothelial function and vascular remodeling [15,18,19]. Thus, TNF-α is believed to be directly involved in the development of atherosclerosis and its progression to CAD [12,13].

The gene encoding TNF-α is located within the major histocompatibility complex class III region between HLA-B (class I) and HLA-DR (class II). There is a strong relation of these genes and different linkage disequilibrium phenomenon [20,21]. Some studies have reported that a specific TNF-α gene polymorphism wherein G is substituted to A in the promoter region at position –308 is associated with several infectious, autoimmune, and immune-mediated diseases [22,23]. Moreover, the polymorphism wherein G is substituted to A in the promoter region at position –238 was reported to be associated with chronic hepatitis B and insulin resistance [24,25]. Other studies have shown that the TNF-α gene promoter region bears other polymorphisms, including –857 (C/T), –863 (C/A) and –1031 (T/C) [26,27]. Several studies have been performed to determine whether these polymorphisms are associated with CAD [1,28,29]. To date, however, CAD-associated TNF-α gene polymorphism in Koreans has not been definitively investigated.
Therefore, we investigated the relationship between polymorphisms at positions –238, –857 and –863 in the promoter region of the TNF-α gene and the prevalence of CAD in a Korean population.

2. Materials and methods

2.1. Study population

We carried out a case-control study to investigate the association between TNF-α polymorphisms and CAD in Koreans. We recruited 197 unrelated Korean patients (130 men, 67 women; mean age, 61.40 ± 9.73; 55 with myocardial infarction and 142 with angina), all of whom had received a coronary artery stent in an epicardial coronary artery at Keimyung University Dongсан Medical Center. As controls, 404 subjects (263 men, 141 women; mean age, 62.01 ± 11.25) were recruited. All studies were carried out according to Declaration of Helsinki guidelines. The study protocol was approved by the ethics review committee of the Institutional Review Board, College of Medicine, Keimyung University.

2.2. Clinical data and laboratory measurements

All of subjects completed a standard questionnaire and were interviewed about their personal history including age, gender, smoking status, presence of hypertension and diabetes. We measured height and weight to calculate body mass index (BMI), and performed ECG and treadmill tests. We measured the laboratory data of each patient including blood pressure, blood sugar, cholesterol, triglycerides, and C-reactive protein (CRP). DNA was also isolated from the blood samples.

2.3. Genomic DNA extraction and polymerase chain reaction (PCR)

Human genomic DNA was extracted from blood samples collected in ethylenediaminetetraacetic acid (EDTA) tubes by using the NucleoSpin 96 Blood kit (Macherey Nagel, Germany). The isolated DNA was stored at -20 °C. PCR was performed using single nucleotide polymorphisms (SNPs) of TNF-α gene at positions –238, –857 and –863 using Gene-Amp PCR System 9600 (Perkin-Elmer, Foster City, CA). The forward and reverse primers were designed on the basis of the DNA sequence of the TNF-α gene (GenBank accession number, NM_000584). The forward primer was biotinylated at the 5’-end.

2.4. Pyrosequencing reactions

The reverse primer was biotinylated at the 5’-end to allow immobilization onto streptavidin Sepharose beads (Streptavidin Sepharose HP; Amersham Pharmacia Biotech, Uppsala, Sweden). Pyrosequencing of 20 μl of the PCR product immobilized onto the beads was performed using the sequencing primers and SNP Reagent kits (Pyrosequencing Advanced Biotechnologies, Uppsala, Sweden) according to the manufacturer’s instructions. SNP genotyping analysis was performed by using the SNP software in a PSQ 96 system (Pyrosequencing Advanced Biotechnologies, Uppsala, Sweden).

2.5. Statistical analysis

The association among TNF-α polymorphisms including genotype distributions and allele frequencies, and the clinical characteristics of patients and control subjects was analyzed by using the chi-square (χ²) test. The Hardy–Weinberg equilibrium (HWE) of all SNPs and logistic regression analysis of genetic data in both patients and controls was assessed by using SNP stat [30]. Haplotype analysis was performed by using HapAnalyzer version 1.0 [31]. Logistic regression analysis was performed to adjust age, gender, and clinical characteristics results. Logistic regression analysis was performed to adjust BMI, diabetes and hypertension parameters which differed significantly between the case and control groups in univariate analysis. The mean different analysis of laboratory data in all SNPs according to CAD and control subjects and according to genotype were compared using the Independent T-test. A P-value of <0.05 was considered to be as statistically significant. Statistical analysis was carried out using the SPSS 18.0 for Windows.

3. Results

3.1. Clinical characteristics of the subjects

As shown in Table 1, the 197 patients and 404 controls were well matched in age and sex. Univariate analysis for the major risk factors for CAD revealed that the patients had a significantly higher BMI and greater prevalence of diabetes and hypertension than the control group (Table 1). The lipid profiles could not be directly compared because of possible concomitant medication taken by the patients. The CAD patients and controls were then genotyped for the TNF-α polymorphism at position –238 (rs361525), –857 (rs1799724), and –863 (rs1800630). The CAD patients did not differ significantly in terms of the major CAD risk factors and other clinical characteristics when they were grouped according to the allele present (Table 2).

3.2. TNF-α polymorphism genotypes and alleles

The distributions of the TNF-α genotypes at positions –238 (rs361525), –857 (rs1799724), and –863 (rs1800630) is in the control and patient groups are shown in Table 3. The frequencies of the TNF-α polymorphisms did not deviate from the Hardy–Weinberg equilibrium (P > 0.05). When calculating the Odds Ratios (ORs) for each genotype, we also tested the genotype as a 3-class variable such as dominant, codominant, recessive models. We found that the A allele of the –238 (G/A) polymorphism was associated with an increased risk of CAD in dominant (OR = 1.87; 95% CI = 1.130–3.32; P = 0.02) and co-dominant (OR = 1.93; 95% CI = 1.10–3.20; P = 0.02) and co-dominant (OR = 1.93; 95% CI = 1.130–3.32; P = 0.04). However, the other polymorphisms, namely, –857 (C/T) and –863 (C/A) were not associated with a changed risk of CAD. Moreover, as shown in Table 4, the A allele at position –238 was more prevalent than the G allele in the patient group than in the control group (OR = 1.74; 95% CI = 1.04–2.92; P = 0.03). However, allelic frequencies at positions –857 and –863 did not differ.

3.3. Haplotype frequency distribution

Haplotype analysis with regard to the polymorphisms at positions –238 (rs361525), –857 (rs1799724), and –863 (rs1800630) revealed that one haplotype was associated with an increased risk of CAD, namely, the ACC haplotype (Table 5). ACC was associated with the presence of CAD in both dominant (OR = 1.86; 95% CI = 1.09–3.18; P = 0.02) and co-dominant (OR = 1.77; 95% CI = 1.05–2.98; P = 0.03) models. The solid spine of linkage disequi-
library (LD) in haplview version 4.2 was performed to evaluate LD block (Fig. 1). The LD block between rs1800630, rs1799724 and rs361525 was made in control group (rs1800630, rs1799724: \( D^r = 1 \) and \( r^2 \)-squared = 0.039, rs1799724, rs361525: \( D^r = 1 \) and \( r^2 \)-squared = 0.012, rs1800630, rs361525: \( D^r = 1 \) and \( r^2 \)-squared = 0.01).

### 3.4. Cardiovascular risk factors and TNF-α genotypes

To estimate the disease risk associated with the various TNF-α genotypes, the data were further examined by multiple logistic regression analysis (Table 6). This analysis included BMI, diabetes and hypertension, the case and control groups differed significantly (Table 1). This analysis revealed that BMI, diabetes and hypertension were independent risk factors for CAD. Furthermore, −238 (G/A) TNF-α polymorphism was an independent risk factor, as the A allele conferred an increased risk of developing CAD. Thus, carrying the −238A allele is significantly associated with the presence of CAD in Koreans.

#### 4. Discussion

TNF-α is a marker of systemic inflammation known to be a part of the pathophysiologic mechanism in cardiovascular disorders and metabolic syndrome [32]. The most studied SNPs about TNF-α are those at position −238, −308 and −376, all of which include a G allele substituted to A [33].
and atherogenic dyslipidemia actions [11,44,45] and modulate directly block insulin actions, thereby inducing insulin resistance (PAI-1) and von Willebrand Factor (vWF) production while sup-endothelial cell hemostatic properties by enhancing their procoag-

eral lines of evidence support the notion that the primarily produced by monocytes and macrophages[38–40]. Sev-

compatibility complex and is a proinflammatory cytokine that is

Table 4
Allelic frequencies with regard to polymorphisms in TNF-α at positions –238, –857 and –863.

<table>
<thead>
<tr>
<th>Loci</th>
<th>Allele</th>
<th>Control (n = 404)</th>
<th>Case (n = 197)</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>–238</td>
<td>G</td>
<td>374 (95.8)</td>
<td>366 (92.9)</td>
<td>1.74 (1.04–2.92)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>34 (4.2)</td>
<td>28 (7.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HWE P-value</td>
<td>0.53</td>
<td>0.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>–857</td>
<td>C</td>
<td>672 (83.2)</td>
<td>320 (81.2)</td>
<td>1.14 (0.84–1.56)</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>136 (16.8)</td>
<td>74 (18.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HWE P-value</td>
<td>0.39</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>–863</td>
<td>C</td>
<td>677 (83.8)</td>
<td>336 (85.3)</td>
<td>0.89 (0.64–1.25)</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>131 (16.2)</td>
<td>58 (14.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HWE P-value</td>
<td>0.85</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n, Number of subjects; OR, Odds Ratio; CI, confidence intervals; HWE, Hardy–Weinberg equilibrium. * P < 0.05.

In this study, the TNF-α –238 (G/A) polymorphism was found to be an independent risk factor for CAD in a Korean population. The (G/A) TNF-α gene polymorphisms at position –238 has been re-
ported to be associated with several clinical disorders including non-alcoholic fatty liver disease [34,35] and lupus erythematosus [36]. It is also associated with an increased risk of new intracranial hemorrhage [37]. However, previous studies failed to show an association between CAD and this polymorphism [1,25]. This may be due to ethnic, phenotypic differences and disparate environ-
mental effects among countries.

The TNF-α gene lies within the class III region of the major histo-
compatibility complex and is a proinflammatory cytokine that is
primarily produced by monocytes and macrophages [38–40]. Sev-
eral lines of evidence support the notion that the TNF-α gene partic-
ipates in the development of cardiovascular disorders and that polymorphisms in this gene may be independent risk factors for CAD. TNF-α may affect major lipid metabolism, leading to hypertri-
glyceridemia [41–43] and attenuate insulin receptor signaling and
directly block insulin actions, thereby inducing insulin resistance and atherogenic dyslipidemia actions [11,44,45] and modulate endothelial cell hemostatic properties by enhancing their procoag-
ulant activity through elevation of plasminogen activator inhibitor (PAI-1) and von Willebrand Factor (vWF) production while sup-
pressing their antithrombotic protein C pathway [15]. TNF-α also
controls vascular endothelial cell function by inducing the production and/or activation of growth factors, chemotaxants, and ad-
hesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin [46,47]. These molecules and changes may play an important role in the pathogenesis of atherosclerosis or CAD. Finally, a recent study reported that CAD patients showed increased secretion of

Table 5
TNF-α haplotypes in patients and controls.

<table>
<thead>
<tr>
<th>Type</th>
<th>Haplotype</th>
<th>Control (n = 404)</th>
<th>Case (n = 197)</th>
<th>Model</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAP1 (GCC)</td>
<td>ht1/h1</td>
<td>154 (38.1%)</td>
<td>70 (35.5%)</td>
<td>Dominant</td>
<td>0.72 (0.45–1.16)</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>ht1/–</td>
<td>199 (49.3%)</td>
<td>94 (47.7%)</td>
<td>Co-dominant</td>
<td>0.86 (0.67–1.11)</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51 (12.6%)</td>
<td>33 (16.8%)</td>
<td>Recessive</td>
<td>0.89 (0.63–1.28)</td>
<td>0.54</td>
</tr>
<tr>
<td>HAP2 (CTC)</td>
<td>ht2/h2</td>
<td>9 (2.2%)</td>
<td>10 (5.1%)</td>
<td>Dominant</td>
<td>1.05 (0.73–1.51)</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>ht2/–</td>
<td>118 (68.6%)</td>
<td>54 (27.4%)</td>
<td>Co-dominant</td>
<td>1.14 (0.84–1.56)</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>277 (43.8%)</td>
<td>133 (67.5%)</td>
<td>Recessive</td>
<td>2.35 (0.94–5.87)</td>
<td>0.07</td>
</tr>
<tr>
<td>HAP3 (GCA)</td>
<td>ht3/h3</td>
<td>11 (2.7%)</td>
<td>2 (1.0%)</td>
<td>Dominant</td>
<td>0.94 (0.65–1.37)</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>ht3/–</td>
<td>109 (27.0%)</td>
<td>54 (27.4%)</td>
<td>Co-dominant</td>
<td>0.89 (0.63–1.25)</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>284 (70.3%)</td>
<td>141 (71.6%)</td>
<td>Recessive</td>
<td>0.37 (0.18–0.76)</td>
<td>0.19</td>
</tr>
<tr>
<td>HAP4 (ACC)</td>
<td>ht4/h4</td>
<td>1 (0.3%)</td>
<td>0 (0.0%)</td>
<td>Dominant</td>
<td>1.86 (1.09–3.18)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>ht4/–</td>
<td>32 (7.9%)</td>
<td>28 (14.2%)</td>
<td>Co-dominant</td>
<td>1.77 (1.05–2.98)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>371 (91.8%)</td>
<td>169 (85.8%)</td>
<td>Recessive</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

n, Number of subjects; OR, Odds Ratio; CI, confidence intervals; HAP, haplotype. * P < 0.05.

In the present study, we found that, compared to the control subjects, CAD patients had significantly higher genotype and allele frequencies with regard to the A allele at position –238. However, the –857 (C/T) and –863 (C/A) were not associated with CAD. In haplotype-based analysis, ACC at positions –238, –857 and –863 was found to be associated with an increased risk of CAD in co-
dominant (P = 0.03) and dominant (P = 0.02) models. Thus, carrying the –238 TNF-α A allele is significantly associated with the presence of CAD in the Korean population. This was confirmed by multiple logistic regression analysis. Notably, several lines of evidences have shown that the –238 and –308 variants are associated with high TNF-α production, higher TNF-α activity, and more severe dis-
ease in some conditions [53–56].

There were several limitations in the present study. First, we did not investigate the effects of medications in patients. Second, CAD
Table 6

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>1.12 (1.05–1.19)</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3.20 (1.96–5.26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.61 (1.76–3.88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNF-a-238&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.06 (1.15–3.68)</td>
<td>0.015</td>
</tr>
</tbody>
</table>

BMI, body mass index; OR, Odds Ratio; CI, confidence intervals.

<sup>a</sup> Assuming a dominant model of inheritance.

patients are somewhat small group in this study. Third, we investi-
gated TNF-α polymorphism at only three positions including
−238, −857, and −863. In future study, we are planning to inves-
tigate TNF-α polymorphism at positions −308 because TNF-a
−308G > A (rs1800629) has been reported as meaningful genetic
variant in previous studies. Finally, we did not investigate the rela-
tion at position −238. In future study, we are planning to inves-
tigate TNF-α polymorphism, with septic shock susceptibility and

In conclusion, the present study suggested that genetic varia-
tion at position −238 in the promoter region of the TNF-α gene
may be useful as a predictive factor for CAD. This supports previous
observations showing that TNF-α may play important roles in the
atherosclerotic process and CAD. The clinical implications of these
results should be tested in future studies.

Acknowledgment

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