Research Article

Tumor Necrosis Factor-Alpha Gene Promoter Region Polymorphism and the Risk of Coronary Heart Disease

Gul Zareen Asifa,1 Afrose Liaquat,1 Iram Murtaza,1
Syed Ali Raza Kazmi,2 and Qamar Javed1

1 Department of Biochemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan
2 Institute of Biomedical and Genetic Engineering, Islamabad 2891, Pakistan

Correspondence should be addressed to Qamar Javed; qjaved6@gmail.com

Received 31 August 2013; Accepted 29 September 2013

Academic Editors: S. Mastana and P. Quax

Copyright © 2013 Gul Zareen Asifa et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Tumor necrosis factor-alpha (TNF-𝛼) gene polymorphisms have been implicated in the manifestation of atherosclerosis. Controversy exists regarding the link between the cytokine’s variant genotype and CHD among different ethnic groups. There have been fewer studies on the TNF-𝛼 gene −1031T>C and −863C>A polymorphisms in relation to CHD. Therefore, the current study was designed to investigate the association of the TNF-𝛼 gene −1031T>C and −863C>A polymorphisms with CHD in a Pakistani population.

Methods. Patients with CHD (𝑛=310) and healthy individuals (𝑛=310) were enrolled in this study. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results. A significant difference was observed in the −863C>A polymorphism between patients with CHD and control subjects (𝑃<0.0001). CHD risk was positively associated with the variant allele −863A (𝑃<0.0001) in the study subjects. There was no significant link between the −1031T>C polymorphism and CHD risk in the study population. Haplotypes A-T and A-C of the TNF-alpha gene loci at −863 and −1031 showed higher frequency in the patient group compared with controls (𝑃<0.05). Conclusion. The TNF-𝛼 −863C>A gene polymorphism was associated with the pathogenesis of CHD while the −1031T>C polymorphism did not show any link with the disease in a Pakistani population.

1. Introduction

Coronary heart disease (CHD) is a leading health problem around the world. Atherosclerosis is a severe clinical manifestation of the disease [1]. Inflammation is a hallmark of CHD and other outcomes of atherosclerosis [1–3]. Inflammatory cytokines appear to stimulate the expression of mediators involved in the development of atherosclerosis and thromboembolic complications [4, 5]. Release of proinflammatory cytokines including the gene encoding TNF-𝛼 is regulated by several genes, which are thought to be involved in the pathogenesis of CHD. TNF-𝛼 gene is located on the human chromosome 6p21.3 and is arranged within class III region between MHC class II (HLA-II) and MHC class I antigens (HLA-I) [6, 7]. TNF-𝛼 gene induction is regulated by the p38 mitogen-activated protein kinase of the signaling cascade pathway [8].

TNF-𝛼 is one of the primary proinflammatory cytokines, mainly produced and secreted by inflammatory cells (i.e., monocytes and macrophages) [9, 10]. Evidence shows that TNF-𝛼 is a key contributor in the development, progression, and complications of atherosclerosis [11]. TNF-𝛼 is involved in reduced expression of endothelial nitric oxide synthase (eNOS) and thus impaired nitric oxide (NO) production leading to endothelial dysfunction [11, 12]. It has a profound effect on lipid metabolism and has been implicated in insulin resistance which produces changes in lipid and glucose associated with the cardiovascular disease risk [12, 13].

Genetic variants in the TNF-𝛼 promoter region are reported to be associated with the TNF-𝛼 serum levels [14–17]. These promoter polymorphisms regulate the transcriptional activity of TNF-𝛼 gene [18, 19]. Based upon these observations, the present study was designed to investigate the association of the −1031T>C (rs1799964) and −863C>A
polymorphisms of the promoter region of TNF-α gene with CHD in a Pakistani population. Prior to this study the said polymorphisms have not been investigated in the study population.

2. Methods

2.1. Study Population. This study was reviewed and approved by the Institutional Review Board (IRB), Quaid-i-Azam University, Islamabad. Informed consent was obtained from all participants of the study according to the Helsinki Declaration of 1975 as revised in 1997. A questionnaire was designed to characterize the study subjects.

We investigated 310 unrelated CHD patients (219 males and 91 females, mean age: 54.3 ± 10.15 years) attending hospitals in different regions of the country. The study participants were evaluated by clinical history, physical examination, 12-lead electrocardiography (ECG), and coronary angiography. ECG and coronary angiogram were analyzed by two cardiologists, who were unaware of the proposed study. Angiographic evidence showing ≥50% stenosis of a major coronary artery was defined as CHD [20]. 310 unrelated control subjects (222 males and 88 females, mean age: 53.2 ± 10.46 years) from the same geographic location were included in this study. The control subjects were clinically healthy with normal ECG and angiography. According to the revised criteria for Asian population, the category of overweight was defined as body mass index (BMI) of ≥23 Kg/m² [21].

2.2. Sample Collection. Peripheral venous blood samples were obtained from the study population. For biochemical analysis, the blood samples were transferred into sterile plain serum tubes and the blood was allowed to clot. Then the samples were centrifuged to separate serum and the serum aliquots were stored at −80°C. For molecular analysis, blood samples were immediately transferred into ethylene-diaminetetraacetic acid (EDTA) vacutainer tubes to prevent coagulation. Whole blood samples were used to extract genomic DNA using standard phenol-chloroform extraction method.

2.3. Biochemical Analyses. Biochemical analyses were carried out using AMP Diagnostics kits (Austria) for the determination of serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C) on an automated analyzer (Vitalab Selectra E Chemistry Analyzer, The Netherlands).

2.4. DNA Extraction and Genotyping of the TNF-α Gene Promoter Polymorphism. The TNF-α gene −1031T>C polymorphism was investigated by the polymerase chain reaction (PCR) using forward primer 5'-TAT GTG ATG GAC TCA CCA GGT-3' and reverse primer 5'-CCT CTA CAT GGC CCT GTC TT-3'. For the −863C>A polymorphism of the cytokine gene, forward primer 5'-GGC TCT GAG GAA TGG GTT AC-3' and reverse primer 5'-CTA CAT GGC CCT GTC TTC GTT ACG-3' were used for amplification. PCR amplification was carried out in 0.2 mL tubes (Axxygen, CA, USA) in a total volume of 50 μL. The reaction mixture contained 3 μL of genomic DNA, 2.5 μL of each forward and reverse primer (20 μM stock), 5 μL of 10X PCR buffer (200 mM of (NH₄)₂SO₄, 750 mM of Tris-HCl (pH 8.8), and 0.1% Tween 20), 4 μL of 25 mM MgCl₂ (MBI-Fermentas, England), 1 μL of 10 mM dNTPs (MBI-Fermentas, England), and 0.5 μL (5 U/μL) of Taq DNA polymerase (MBI-Fermentas, England) in 31.5 μL of PCR water. PCR reactions were performed by means of GeneAmp PCR System 9700 (Applied Biosystems Inc, Foster City, CA, USA). PCR was carried out with the following thermal cycling conditions: an initial denaturation step at 94°C for 12 min, followed by amplification for 35 cycles at 94°C for 30 s, 59°C for 1 min, and 72°C for 2 min, followed by a final extension step at 72°C for 2 min. The PCR products were analyzed by 2% agarose gel electrophoresis.

BbsI (MBI-Fermentas, England) restriction endonuclease was used for the detection of the TNF-α gene −1031T>C polymorphism. RFLP was performed in 0.2 mL tubes (Axxygen, CA, USA) in a total volume of 20 μL containing 12 μL of amplified products, 2 μL of 10X G buffer (10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 50 mM NaCl, and 0.1 mg/mL BSA), 0.3 μL of BbsI enzyme, and 5.7 μL of PCR water. Digested products were analyzed by 3% agarose gel electrophoresis and visualized under ultraviolet light. Similarly, TaqI (MBI-Fermentas, England) enzyme was used to investigate the TNF-α gene −863C>A polymorphism using 10X R buffer (10 mM Tris-HCl (pH 8.5), 10 mM MgCl₂, 100 mM KCl, and 0.1 mg/mL BSA). The digested products were subjected to 4% agarose gel electrophoresis and visualized under ultraviolet light.

2.5. Statistical Analysis. Statistical analysis was carried out using GraphPad Prism for Windows Version 5.00 (GraphPad Software, Inc., CA, USA). Basic and clinical variables were expressed as numbers and mean ± SD. Continuous variables were evaluated by Student’s t-test. Genotype and allele frequencies were compared by Chi-square analysis. P value < 0.05 was considered statistically significant.

3. Results

3.1. General Characteristics of the Study Population. The baseline and clinical characteristics of the study participants are shown in Table 1. Patients with CHD had significantly higher BMI (P = 0.002), systolic blood pressure (BP) (P < 0.0001), and lower HDL-C (P < 0.0001) than the control group. In this study, we did not find any significant difference in the levels of TC, TG, and LDL-C from patients with CHD and control subjects.

3.2. Genotype Analysis of the Study Population. The genotype frequencies of the TNF-α gene −1031T>C and −863C>A polymorphisms are shown in Table 2. The frequency of the TNF-α −1031 variant genotype (TC + CC) was 41.29% in control individuals and 42.58% in CHD patients (OR = 1.05; 95% CI = 0.76–1.45; P = 0.80). There was no significant
Table 1: Baseline and clinical characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (n = 310)</th>
<th>Patients (n = 310)</th>
<th>P valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.2 ± 10.46</td>
<td>54.3 ± 10.15</td>
<td>0.164</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>25.1 ± 3.21</td>
<td>25.9 ± 3.43</td>
<td>0.002*</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>120.6 ± 10.31</td>
<td>132.9 ± 26.20</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>83.2 ± 6.28</td>
<td>84.7 ± 13.43</td>
<td>0.07</td>
</tr>
<tr>
<td>Cholesterol (TC)(mg/dL)</td>
<td>167.57±29.08</td>
<td>161.48±51.85</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (TG)(mg/dL)</td>
<td>150.23±57.02</td>
<td>143.29±72.80</td>
<td></td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>97.41±33.21</td>
<td>95.96±36.11</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>40.07±9.68</td>
<td>35.73±13.38</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as means ± SD.

*P value calculated by Student’s t-test (unpaired).

n: number; SD: standard deviation.

Table 2: Genotype and allele frequencies of the TNF-α gene −1031T>C and −863C>A polymorphisms in the study population.

<table>
<thead>
<tr>
<th>TNF-α SNP</th>
<th>Controls (n = 310)</th>
<th>Patients (n = 310)</th>
<th>OR†</th>
<th>95% CI‡</th>
<th>P valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>−1031T/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>182 (58.71%)</td>
<td>178 (57.42%)</td>
<td>1.05</td>
<td>0.76–1.45</td>
<td>0.8</td>
</tr>
<tr>
<td>TC + CC</td>
<td>128 (41.29%)</td>
<td>132 (42.58%)</td>
<td>1.11</td>
<td>0.85–1.43</td>
<td>0.46</td>
</tr>
<tr>
<td>T</td>
<td>474 (76.45%)</td>
<td>462 (74.52%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>146 (23.55%)</td>
<td>158 (25.48%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−863C/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>201 (64.84%)</td>
<td>92 (29.68%)</td>
<td>4.37</td>
<td>3.11–6.12</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>CA + AA</td>
<td>109 (35.16%)</td>
<td>218 (70.32%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>503 (81.13%)</td>
<td>399 (64.35%)</td>
<td>2.38</td>
<td>1.83–3.08</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>A</td>
<td>117 (18.87%)</td>
<td>221 (35.65%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are given in numbers and percentage.

*P value calculated by Chi-square test.

†Odds ratio.

‡95% confidence intervals.

difference between the T and C alleles at −1031 from patients with CHD and controls (OR = 1.11; 95% CI = 0.85–1.43; P = 0.46).

At position −863 of TNF-α gene, the variant genotype (CA + AA) was more prevalent in the patient group (70.32%) compared with controls (35.16%) (OR = 4.37; 95% CI = 3.11–6.12; P < 0.0001). The frequency of the TNF-α −863A allele was significantly higher in patients with CHD (35.65%) than in control subjects (18.87%) (OR = 2.38; 95% CI = 1.83–3.08; P < 0.0001).

Haplotypes A-T and A-C of the TNF-α gene loci at −863C>A and −1031T>C showed higher frequency in the patient group compared with controls (P < 0.05; Table 3).

4. Discussion

Several genetic components are involved in the pathophysiology of CHD. Studies on the involvement of TNF-α gene's promoter polymorphisms in its regulation are of legitimate clinical importance because of the potentially damaging proinflammatory effects of the cytokine. Relatively fewer studies have been conducted on the TNF-α gene −1031T>C and −863C>A polymorphisms in relation to CHD. Therefore, in the present case-control study, we have investigated the association of single nucleotide polymorphisms (SNPs and TNF-α −1031T>C and −863C>A) with CHD in a Pakistani population.

Our findings suggest that the TNF-α gene −1031T>C polymorphism had no measurable influence on the occurrence of CHD in the study population. The TNF-α−1031C allele frequency was 23.55% in control individuals versus 25.48% in patients with CHD (P = 0.46), indicating that the −1031C allele is not a risk factor of CHD in our population. This is in accordance with the results of Ghazouani et al. who have demonstrated no link between the TNF-α−1031C promoter polymorphism and coronary artery disease (CAD) in a Tunisian population [22]. Oda et al. have reported that the TNF-α−1031C allele acts as a protective factor in atherosclerotic severity [23]. Our results are also in agreement with the findings of Bennett and associates in Swedish patients with myocardial infarction [15] and Liang et al. in Chinese patients with DCM [24]. Interestingly, Kamoun and colleagues have reported a link between the TNF-α−1031T>C polymorphisms with another inflammatory condition in a Tunisian population [25]. Genetic heterogeneity exists among
Table 3: Haplotype analysis of TNF-α −1031T>C and −863C>A polymorphisms in the study population.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Controls n (% age)</th>
<th>Patients n (% age)</th>
<th>χ²</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (100)</td>
<td>n (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-T</td>
<td>433 (70)</td>
<td>355 (57)</td>
<td>40.36</td>
<td>3.44(2.326–5.088)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>A-T</td>
<td>39 (6)</td>
<td>110 (18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-C</td>
<td>69 (11)</td>
<td>44 (7)</td>
<td>1.259</td>
<td>0.77(0.519–1.164)</td>
<td>0.226</td>
</tr>
<tr>
<td>A-C</td>
<td>79 (13)</td>
<td>111 (18)</td>
<td>10.44</td>
<td>1.71(1.243–2.362)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

P value calculated by Chi-square test (Yates corrected).
* P value (<0.05) indicates statistical significance.
n: number; OR: odds ratio; CI: confidence interval.

5. Conclusion

A significant association was observed between the TNF-α gene −863C>A promoter polymorphism and CHD; the cytokine’s SNP may have a role in the pathogenesis of the disease. In this study the TNF-α gene −1031T>C polymorphism was not linked with CHD.

Conflicts of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This research was funded by a research Grant from Pakistan Science Foundation (Ref: PSF/Res/C-QU/Med (272)).

References


[31] D. A. van Heel, I. A. Udalova, A. P. De Silva et al., "Inflammatory bowel disease is associated with a TNF polymorphism that affects an interaction between the OCTI and NF-KB transcription factors," Human Molecular Genetics, vol. 11, no. 11, pp. 1281–1289, 2002.

