C-Reactive Protein 1059G/C Gene Polymorphism in Type 2 Diabetic Patients

Tip 2 Diabetik Hastalarda C-Reaktif Protein 1059G/C Gen Polimorfizmi

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Abstract

Objective: C-reactive protein (CRP) is considered to be a cardiovascular risk marker and changes in its level have been attributed to genetic factors. The aim of the study was to determine CRP 1059G/C gene polymorphism frequency and its relationship with CRP levels and carotid artery intima-media thickness (CIMT) in type 2 diabetic patients (DM).

Materials and Methods: One hundred and sixty-four type 2 diabetic patients (mean age: 57±7 years; F/M: 80/84) and 151 controls (mean age: 53±7 years; F/M: 81/70) were recruited. CIMT was assessed by carotid ultrasonography. CRP 1059G/C polymorphism was determined by polymerase chain reaction and restriction fragment length polymorphism analyses.

Results: The CRP 1059G/C polymorphism distribution in diabetic group and controls were similar (1059GG in 92% vs. 88%, 1059GC in 2% vs. 5%; 1059CC in 6% vs. 7%). CRP levels (4.3±6.6 mg/L vs. 2.5±2.3 mg/L; p=0.02) and CIMT (0.67±0.18mm vs. 0.56±0.19mm; p<0.0001) were increased in diabetics compared to controls. No association of CRP and CIMT with CRP 1059G/C polymorphism was found.

Conclusions: Increased CRP levels and CIMT seem to be independent of CRP 1059G/C gene polymorphism in our group of type 2 diabetic patients. *Turk Jem 2010; 14: 85-8*

Key words: Type 2 diabetes mellitus, CRP gene polymorphisms, CRP, carotid intima-media thickness

Özet

Amaç: C reaktif protein (CRP) kardiyovasküler bir risk göstergesi olarak kabul edilmektedir. CRP düzeylerinde meydana gelen değişikliklerin genetik faktörlerden etkilendiği düşünülmektedir. Bu çalışmanın amacı Tip 2 diyabetik hastalarda CRP 1059G/C gen polimorfizmi dağılımının ve bunun CRP düzeyleri ve karotis intima media kalınlığı (CIMT) ile ilişkisinin belirlenmesidir.

Gereç ve Yöntemler: Çalışmaya 164 tip 2 diyabetik hasta (yaş:57±7; K/E:80/84) ve 151 kontrol (53±7; K/E:81/70) dahil edilmiştir. CIMT ölçümü Doppler ekokardiyografi yöntemiyle yapılmıştır. Çalışma gruplarının periferik kanlarından izole edilen DNA'dan CRP 1059G/C gen polimorfizmi belirlenmiştir.

Bulgular: Diyabetik grupta ve kontrollerde CRP 1059G/C gen polimorfizmi dağılımı benzerdir (1059GG %92 vs %88, 1059GC %2 vs %5; 1059CC %6 vs %7). CRP düzeyleri (4,3±6,6 mg/L vs 2,5±2,3 mg/L; p=0,02) ve CIMT ölçümleri (0,67±0,18mm vs 0,56±0,19mm; p<0,0001) diyabetiklerde kontrollere göre daha yüksek bulunmuştur. CRP 1059 C/G polimorfizm taşıyıcılığıyla serum CRP ve CIMT ölçümleri arasında ilişki görülmemiştir.

Sonuç: Sonuç olarak Tip 2 diyabetik hastalarda kontrollere göre artmış CRP ve CIMT düzeylerinin CRP'nin 1059 C/G polimorfizm taşıyıcılığından bağımsız olduğu gözlenmektedir. *Türk Jem 2010; 14: 85-8*

Anahtar kelimeler: Tip 2 diabetes mellitus, CRP gen polimorfizmleri, CRP, karotid intima medya kalınlığı

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Turkish Journal of Endocrinology and Metabolism, published by Galenos Publishing.

Introduction

C-reactive protein (CRP), a systemic acute-phase reactant, has been shown in vitro to play a role in all stages of atherosclerosis (1). CRP levels are elevated in people with type 2 diabetes mellitus (2,3). Since 40% heritability has been suggested for CRP levels (4), genetic polymorphisms of CRP have become a matter of interest in prediction of cardiovascular events. 1059G/C polymorphism, the focus of our study, was reported to be associated with arterial pulse wave velocity (5) and future arterial thrombosis (6), but no relationship was observed with restenosis risk in a post-angioplasty series (7).

Scarce data exist concerning CRP polymorphisms in diabetic patients. A recent investigation has contradicted the effect of rs3093059, rs2794521, rs3091244, rs1417938, rs1800947 (1059 G/C), rs1130864, and rs1205 haplotypes on diabetes risk (8). Similarly, three polymorphisms of CRP (rs1130864, rs1205, rs3093077) were found to be unrelated to the development of insulin resistance or diabetes in the Whitehall II study cohort (9).

There is conflicting evidence as to the relationship of CIMT, an early marker of atherosclerosis, with serum CRP levels in type 2 diabetic patients (2,3). Moreover, an association between CIMT and several single nucleotide polymorphism (SNP) of CRP, including 1059G/C, has been evaluated by only one group of investigators and no relationship has been observed (10).

In this study, we aimed to determine the frequency of 1059G/C gene polymorphism and investigate the relationship of this polymorphism with serum CRP levels and CIMT as a cardiovascular marker in a group of Turkish type 2 diabetic patients.

Materials and Methods

Subjects

One hundred and sixty-four type 2 diabetics followed at the Endocrinology and Metabolism Outpatient Clinic of Marmara University School of Medicine and 151 healthy controls were included in the study. The diagnosis of type 2 diabetes mellitus was based on the American Diabetes Association guidelines (11) and all patients were being followed up with the diagnosis of type 2 diabetes mellitus for at least one year. Exclusion criteria were smoking, evidence of any systemic inflammatory disease, acute infection in the past 2 weeks, history of coronary artery disease or any other atherosclerotic diseases, use of steroids or systemic anti-inflammatory agents, and unwillingness to participate in the study. All subjects gave informed consent for participation. The study was approved by the local ethics committee and was carried out in accordance with the declaration of Helsinki.

After obtaining a medical history, physical examination of the subjects was done. In order to determine high-sensitive (hs) CRP, glycosylated hemoglobin, serum creatinine, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride levels, all subjects had blood drawn after a fast of 12 hours.

DNA Extraction

2 cc of plasma sample was used for DNA extraction from peripheral blood leucocytes with a PCR template preparation kit (Roche, Pensberg, Germany).

Genotype Determination

1059G/C (dbSNP rs1800947) polymorphism was determined as previously described (12). Primers for the polymerase chain reaction (PCR) were:

CRP-1059F 5'-GATCTGTGTGATCTGAGAAACCTCT-3'

CRP-1059R 5'-GAGGTACCAGAGACAGAGACGTG-3'

Mae III restriction enzyme was used for restriction fragment length polymorphism (RFLP). Genotype scoring was carried out by two independent observers. Genotyping was repeated in case of a disagreement.

Carotid Artery İntima-Media Thickness (CIMT) Measurement

CIMT measurements were done by a blinded experienced investigator using General Electronics Vingmed System Five Echocardiography device provided with a 10MHz Broadband linear probe, as described previously (13).

Each subject was examined in the supine position, with head turned 45° away from the ultrasonographer, in a semi-dark room. The carotid artery was investigated bilaterally and scanned at the level of the bifurcation of the common carotid artery. The image was focused on the far wall of the artery. CIMT was measured as the distance from the leading edge of the first echogenic line to the leading edge of the second echogenic line. Intima-media thickness was measured on the longitudinal views of the far wall of the bilateral distal common carotid arteries (1 to 3 cm proximal to the carotid bifurcation) at the diastolic phase. CIMT was expressed as the mean of six measurements (three on each side). No measurement was made on the side where a plaque existed.

Biochemical Measurements

High-sensitive (hs) CRP was determined using immunoturbidimetric assay (Roche Diagnostics GmbH) with an intra-assay variability of 3.1-4.4% and inter-assay variability of 2.5-5.7%. Hemoglobin A1c (HbA1c) levels were measured by high-pressure liquid chromatography (HPLC) with a Thermo system.

Statistical Analysis

SPSS 15.0 version for Windows was used for statistical analyses. Categorical variables were expressed as number, while continuous variables were expressed as mean ± standard deviation. The Pearson's chi-square (χ^2) test was used to compare groups regarding categorical variables. Continuous variables were compared with the Student t-test (for comparison of parametric variables between diabetic patients and controls) or the Mann-Whitney U test (for comparison of nonparametric variables between diabetic patients and controls). ANOVA was used to compare the continuous variables with normal distribution within the three groups of the different genotypes of GG, GC and CC among diabetic patients. The Tukey-Kramer test was applied for multiple comparisons. Correlation analysis was performed using either the Pearson's or Spearman's test according to the distribution of the data. Logistic regression analysis was used to find out the determinants of CRP

1059G/C polymorphism regarding age and measurements of fasting blood glucose, HbA1c, BMI, total cholesterol, triglyceride, LDL, HDL, CRP and CIMT. A linear regression analysis model was created to determine whether age, fasting blood glucose, HbA1c, BMI, total cholesterol, triglyceride, LDL, HDL, CIMT and CRP 1059G/C polymorphism had any influence on serum CRP levels. Another linear regression model was used to find out the relationship of age, fasting blood glucose, HbA1c, BMI, total cholesterol, triglyceride, LDL, HDL, serum CRP levels and CRP 1059G/C polymorphism with CIMT measurements. Levels of statistical significance were set at a p value <0.05.

Results

The mean age of the patients and controls was 57±7 years and 53±7 years (p<0.0001), respectively. The female/male ratio in diabetics and controls was similar (80/84 vs. 81/70). CRP levels were higher in the diabetic group (4.3±6.6 mg/L) compared to controls (2.5±2.3 mg/L, p=0.02). Similarly, CIMT of the diabetic patients (0.67±0.18 mm) was increased compared to controls (0.56±0.19 mm, p<0.0001).

There was no difference in the frequency of genotypes in both patients and controls, with CRP 1059GG carriers being the most frequent. The genotype and allele frequencies are shown in Table 1.

No difference was observed in terms of demographics, biochemical findings, lipid levels and CIMT measurements among the three subgroups divided as genotypes GG, GC and CC in the diabetic group.

CRP correlated positively with HbA1c (r=0.17, p=0.04) and triglyceride levels (r=0.18, p=0.03) and negatively with HDL cholesterol (r=-0.23, p=0.005) in the diabetic group.

In a logistic regression model, none of the variables among age, measurements of fasting blood glucose, HbA1c, BMI, total cholesterol, triglyceride, LDL, HDL, CRP and CIMT had any association with CRP 1059G/C polymorphism. In a linear regression analysis model, none of the variables among age, fasting blood glucose, HbA1c, BMI, total cholesterol, triglyceride, LDL, HDL, CIMT and CRP 1059G/C polymorphism had any influence on CRP levels. Age, fasting blood glucose, HbA1c, triglyceride and HDL cholesterol were found to be significantly affecting CIMT among the above variables (r^2 of the model=0.26).

Table 1. Genotype and allele distribution in the study groups

	CONTROLS (n=151)	TYPE 2 DM (n=164)	p
1059 G/C			
GG (%)	133 (88%)	151 (92%)	
GC (%)	8 (5%)	4 (2%)	0.36
CC (%)	10 (7%)	9 (6%)	
Allele			
G (%)	137 (91%)	153 (93%)	0.72
C (%)	14 (9%)	11 (7%)	

DM= diabetes mellitus

Discussion

1059GG is the most common CRP 1059G/C polymorphism in our cohort of type 2 diabetic patients. No significant relationship was observed either between CRP 1059G/C polymorphism and serum CRP levels or between CRP 1059G/C polymorphism and CIMT in the diabetic group.

Our results showed a predominance of 1059 GG genotype in both the patient and control groups. This was consistent with several previous reports. Morita et al. (5) in a healthy Japanese population, Zee et al. both in a Spanish population with coronary artery disease and controls (6) and in a healthy American cohort (7), de Maat et al. (14) in a healthy Danish population and Brull et al. (15) in an English cohort with coronary artery disease and controls have reported similar distribution, with predominance of the GG genotype. We observed a similar 1059 GG gene polymorphism distribution in our cohort of type 2 diabetic patients with other Caucasian populations (6,14,15).

The 1059G/C polymorphism has been evaluated in terms of several cardiovascular endpoints (5,6,7). The association of 1059G/C polymorphism with CIMT and clinical cardiovascular disease events has only been evaluated in Cardiovascular Health Study Cohort by Lange et al. (10) and the 1059 C allele was associated with lower plasma CRP concentration and decreased risk of cardiovascular mortality in white participants. No association was observed between CIMT and any of the CRP polymorphisms in this population. Similarly, Pessi et al. (16) investigated polymorphisms of Fcγ receptor 2A (FCGR2A) and CRP including -717A>G, -286C>T>A, +1059G>C, +1444C>T and +1846G>A and their effects on CIMT in 2260 young adults. They did not find any association between CIMT and several CRP polymorphisms, including 1059G/C. On the other hand, both CRP haplotype GCGCG (-717, -286,+1059, +1444, +1846) and CRP -717A polymorphism interacted with FCGR2A (Arg113His) on CIMT in men. They demonstrated that although CRP gene did not by itself seem to have an effect on CIMT, interaction with FCGR2A gene revealed an association with CIMT. Thus, they concluded that the effect of CRP genetics on early atherosclerotic changes is modulated by the FCGR2A genetics. Data evaluating the relationship of 1059G/C polymorphism with CIMT or any cardiovascular outcome does not exist in a diabetic population. Our study is the first in this aspect and our results suggest that this polymorphism is not related to CIMT in our population of type 2 diabetic patients. As Pessi et al. have pointed out in their population, the determination of polymorphisms of FCGR2A or other possible interacting genes in a diabetic population may reveal some relationship between CRP polymorphisms and CIMT. We did not study any other interacting genes and this might lead to absence of any relation between 1059G/C polymorphism and CIMT.

Many genetic studies have examined the relationship between serum CRP levels and CRP genotype. Increased serum CRP levels have been observed in association with the 1059GG genotype (5,6). On the other hand, other groups have not shown any correlation between this genotype and serum CRP levels (14,15). Our findings revealed no relationship of CRP 1059C/C polymorphism

with serum CRP levels in a diabetic population, confirming these latter studies. Although this finding has to be confirmed in larger and different populations, several explanations can be proposed for the time being. Genetic background for serum CRP levels may be related to enhancer factors or other genes that play a role in the inflammatory process (14). Moreover, since 1059G/C polymorphism is silent (10), it may be in linkage disequilibrium with an unidentified functional mutation in the CRP gene (6).

CIMT measurements have previously been found to be elevated in type 2 diabetic patients (2,3). Our findings are consistent with these findings. There are conflicting data as to the relationship between CRP levels and CIMT in type 2 diabetic patients (2,3,17). According to our analysis, there was no significant association between CRP levels and CIMT.

The main limitation of our study is the small sample size of our study population. This might have lead to the disassociation observed between CRP levels and the particular CRP1059G/C polymorphism.

In conclusion, serum CRP levels and CIMT are high in type 2 diabetic patients due to coexisting inflammation and atherosclerosis. Increased serum CRP levels in this population do not seem to be related to CRP 1059G/C polymorphism and the same polymorphism does not explain increased CIMT levels in our population of type 2 diabetic patients, with a limited sample size. Further work with this polymorphism and other CRP polymorphisms in larger diabetic populations are needed. Moreover, the relationship of CRP polymorphisms with cardiovascular outcomes needs to be determined to get a clue to their influence on the macrovascular complications of type 2 diabetes mellitus.

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