Interleukin-1 Receptor Antagonist Gene VNTR Polymorphism is Associated with Coronary Artery Disease

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Summary

Background: Coronary Artery Disease (CAD) is the atherosclerosis of coronary arteries that carry blood to the heart muscle. Atherosclerosis is an inflammatory disease. Cytokine gene variations such as those associated with the IL1 family are involved in the pathogenesis of atherosclerosis.

Objective: The purpose of this study was to determine the relationship between IL1 family polymorphisms (IL1RN VNTR, IL1B positions -511 and +3953) and CAD in Turkish population.

Methods: 427 individuals were submitted to coronary angiography and were grouped as 170 control subjects and 257 CAD patients. The CAD subjects were divided into two subgroups: 91 Single Vessel Disease (SVD) and 166 Multiple Vessel Disease (MVD) subjects. The genotypes of IL1RN and of IL1B (-511, +3953) were determined by polymerase chain reaction (PCR) followed by restriction digestion analysis.

Results: No significant difference was found in IL1RN and IL1B (-511 and +3953) genotype distributions between CAD and control subjects or MVD and control subjects. However, significant association was seen in IL1RN 2/2 genotype between SVD and control subjects (P= 0.016, x2: 10.289, OR: 2.94, 95% CI: 1.183-7.229). Similarly, no statistically significant difference was found in IL1RN and IL1B (-511 and +3953) allele frequencies between CAD and control subjects, MVD and control subjects or SVD and control subjects.

Conclusion: No association was found in either allele frequency or genotype distribution of IL1RN and IL1B polymorphisms between CAD and the control groups. However; IL1RN 2/2 genotype may be a risk factor for SVD in the Turkish population. (Arq Bras Cardiol 2008; 91(5) : 268-273)

Key words: Interleukin 1; mini-satellite repeats; coronary arteriosclerosis; population; Turkey / epidemiology.

Introduction

Atherosclerosis is an inflammatory disease that affects medium or large arteries causing gangrene, stroke and heart diseases. Coronary Artery Disease (CAD) is a multifactorial heart disease caused by atherosclerosis of coronary arteries. Growth and clotting factors, cytokines, adhesion molecules and their effects on vascular Endothelial Cells (EC) and Smooth Muscle Cells (SMC) were studied for insight into atherosclerotic processes. Inflammation in the artery wall triggers the production of primary proinflammatory cytokines such as Interleukin-1β (IL-1β), Tissue Necrosis Factor α (TNFα) which can induce the secondary proinflammatory cytokine (IL-6), the chemokine (IL-8), and adhesion molecule (E-selectin) production.

The IL-1 family has three well-studied members, two agonists, IL-1α and IL-1β, and the antagonist IL-1Ra. IL-1Ra inhibits IL-1-induced inflammation action by blocking the binding of IL-1 to IL-1 Type I Receptor (IL-1RI). IL-1Ra is expressing from IL1RN gene which has a length variation within intron 2 caused by 86 bp Variable Number of Tandem Repeats (VNTR). According to the number of 86 bp repeats, there are six alleles corresponding to 1, 2, 3, 4, 5, 6 repeats. Single nucleotide polymorphisms (SNPs) have been determined at promoter position -511 C/T and in exon 5 at position +3953 C/T of IL1B gene. These polymorphisms in IL1RN and IL1B genes are thought to influence gene expression.

There are various reports showing a relationship between VNTR and SNPs on the IL1 gene family and diseases. IL1RN genotype 2/2 has been found to be significantly associated with Single Vessel Disease (SVD) in Sheffield English population. Significant relevance was found between IL1RN allele 2 and CAD with Type 2 diabetes. Also IL1RN allele 2 has a protective effect on restenosis after Percutaneous Transluminal
Coronary Angioplasty (PTCA)\textsuperscript{12}. But no association was found between IL1B SNPs and CAD\textsuperscript{10,11}.

The purpose of this study was to investigate whether polymorphisms in IL1RN (VNTR) and IL1B (-511, +3953) genes are associated with CAD in Turkey. Our results showed that neither IL1B (+3953) nor IL1B (-511) SNP genotype distributions and allele frequencies are susceptible to CAD, but people carrying IL1RN 2/2 genotype may be susceptible to SVD in the Turkish population.

**Methods**

**Subjects**

The study population was selected from patients undergoing coronary angiography at Dr. Siyami Ersek Hospital between 2003-2006. Indications for coronary arteriography in our clinic are based on guidelines for coronary angiography\textsuperscript{14}. According to coronary angiography results\textsuperscript{11,15} participants who presented $\geq 70\%$ stenoses in at least one of the major coronary arteries were considered as CAD (n= 257) and the remains who presented $\leq 30\%$ stenosis were accepted in the normal group (n= 170). CAD group was divided into two subgroups: SVD (only one coronary artery stenosis; n= 91) and Multiple Vessel Disease (MVD; more than one coronary artery stenosis; n= 166).

**Risk factor assessment**

Age, gender, family history of CAD, smoking habit, history of hypertension were obtained by filling a questionnaire form. Weight, height, Systolic (SBP) and Diastolic Blood Pressures (DBP) were measured. Body Mass Index (BMI) was calculated from height and weight measurements. The presence of Diabetes Mellitus (DM) was defined by repeated fasting glucose level $>$ glucose 126 mg/dl, the use of antidiabetic drugs or both. Total Cholesterol (TC) and High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) cholesterol levels were determined enzymatically, and also measured enzymatically after dextrane sulfate magnesium precipitation\textsuperscript{16}. Information about the study was given to all patients and control group, the informed consent was received from each of them.

**Genotyping**

Blood samples were taken from 427 participants and kept in tubes with EDTA. Genomic DNA isolation was done using GENTRA DNA isolation kit. We have amplified VNTR region in intron 2 of IL1RN gene, IL1B (-511) and IL1B (+3953) exon 5 regions as described previously\textsuperscript{17}. Genotypes of IL1RN gene were determined by repeating unit size of PCR products. IL1B (-511) genotype was done based on the size of digested PCR products with Aval, and IL1B (+3953) genotype was determined based on the digested PCR products with Taq I.

**Statistical analysis**

Mann-Whitney U and $x^2$ tests were used for the comparison of two groups of individuals according to CAD risk factors. Allele and genotype frequencies among cases and controls were compared with Hardy-Weinberg predictions using $x^2$-analysis. Multivariate logistic regression analysis was done to assess the distribution of IL1RN, IL1B genotypes in the control groups and cases. The results were expressed as odds ratio (OR) and 95% Confidence Interval (CI). The final model was adjusted for age, gender, family history of CAD, smoking habit, history of hypertension. Allele frequencies and genotype distribution among cases and controls were compared and values were calculated by $x^2$ test. Probability for $p<0.05$ values (2-sided) were considered statistically significant. SPSS 11.5 software program was used for all statistical analysis.

**Ethics approval**

This research was approved by the ethics committee of Marmara University in Turkey. All human subjects’ rights in this research are protected and any necessary approval was secured from the ethics committee.

**Results**

Baseline characteristics of control and CAD groups can be found in Table I. Significant difference between CAD and control subjects for gender ($P= <0.001$), family history of CAD ($P= 0.014$), Diabetes ($P<0.001$) History of Hypertension ($P= <0.001$) and current smoking ($P= <0.001$) can be found in Table 1.

Genotype distribution and allele frequency of IL1RN VNTR are shown in Table 2. Four of six genotypes at IL1RN gene were observed in CAD and control subjects. Among all these genotypes, only IL1RN 2/2 is significantly different.
between SVD and healthy controls \((P = 0.016, \chi^2: 10.289, 3 \text{ df}, \text{OR}: 2.94, 95\% \text{ CI: } 1.183-7.229)\). However, no significant difference was found between both controls and CAD \((P = 0.262)\) and MVD vs control subjects \((P = 0.257)\). Also, the genotype distribution between SVD and MVD was found to be statistically significant \((P = 0.003, \chi^2: 13.806, 3 \text{ df}, \text{OR}: 5.22, 95\% \text{ CI: } 1.995-14.195)\). For IL1RN allele frequencies, no association was detected between control and cases: CAD \((P = 0.211)\), MVD \((P = 0.247)\), SVD \((P = 0.423)\) (Table 2).

Genotype distribution and allele frequency of IL1RN \((-511)\) in CAD, SVD, MVD and control groups are shown in Table 3. The IL1B \(-511\) genotype showed no significant difference between CAD and controls \((P = 0.242)\), SVD and control \((P = 0.712)\) and MVD and control \((P = 0.142)\). Similarly, no significant differences were observed in allelic distribution between controls and CAD \((P = 0.442)\), SVD \((P = 0.098)\), and MVD \((P = 0.142)(\text{Table } 3)\).

The allelic frequency and genotype distribution of IL1B \(+3953\) in CAD, SVD and MVD and control groups are shown in Table 4. When genotype distribution of IL1B \(+3953\) was compared among groups, no significant difference was observed between control groups and CAD \((P = 0.629)\), SVD \((P = 0.907)\), and MVD \((P = 0.472)\). Also, no significant difference was observed in allelic distribution between controls and CAD \((P = 0.569)\), SVD \((P = 0.750)\), and MVD \((P = 0.591)(\text{Table } 4)\).

Genotypes are expressed as number of patients (proportion in % within brackets), \(p\) values are from chi square test.

Homozygosity for IL1RN 2 allele was found significant between SVD and control groups \((\chi^2: 7.510, P = 0.010, \text{OR}: 3.253, 95\% \text{ CI: } 1.349-7.844)\). However, this association can not be seen in heterozygosity for IL1RN 2 allele \((P = 0.757)(\text{Table } 5)\). Also, significant association was found in homozygosity for IL1RN 2 allele between MVD and SVD groups \((\chi^2: 7.160, P = 0.011, \text{OR}: 2.838, 95\% \text{ CI: } 1.278-6.299)(\text{Table } 5)\). Additionally, the relative effect of the IL1 family polymorphism on the risk of CAD was not seen in a multiple logistic regression model including dominant, recessive and co-dominant effects (Table 6).

### Discussion

CAD is the major cause of mortality and morbidity in the United States, European countries and also in Turkey\(^{11}\). The
etiology and development processes of CAD are not well understood, but it has been shown that both inflammation and genetics play an important role in the pathogenesis of atherosclerosis. Many epidemiological studies have investigated the association between CAD and inflammatory cytokine gene polymorphisms. One of the candidate proinflammatory cytokines associated with CAD susceptibility is IL-1β, which affects the majority of cells and is involved in immunity, sepsis, infection and inflammation. In contrast, IL-1Ra acts as an anti-inflammatory agent that inhibits the action of IL-1.

Francis et al. found the association between IL-1RN 2/2 genotype and SVD in the UK Caucasian Sheffield population, but not in the London population. Also, no significant difference was found for MVD in either population group. Other reports did not find any statistical significance between IL-1RN 2 allele and SVD or IL1RN 2 allele and CAD. Furthermore, it has been reported that IL1RN 2 allele is significantly associated with CAD in the Type 2 diabetes patients. Also, IL1RN 2 allele has a protective effect on restenosis after PTCA and an association between IL1RN 2 and carotid atherosclerosis was reported. It was also reported that future Myocardial Infarction risk was increased with IL1RN 2 allele and high CRP levels in young patients. In this study, we found an association between SVD and IL1RN 2/2 genotype, but no statistical association between IL1RN alleles and CAD or MVD or SVD. People carrying homozygous IL1RN allele 2 are 3.25 times more likely to have SVD than people carrying the other genotypes. However, heterozygosity for IL1RN 2 carrier did not show this association. Furthermore, we could not find any statistically significant influence of IL1 family polymorphisms on the risk of CAD. Even though IL1 family polymorphisms did not show any direct relative effect on risks of CAD, those polymorphisms may modulate some of processes for the development of CAD.

There appears to be a balance between IL-1 and IL-1Ra protein, except in the case of autoimmune diseases.

### Table 5 - Gene dosage-dependent association of IL-1RN 2 allele and CAD

<table>
<thead>
<tr>
<th>IL1RN 2 Carrier</th>
<th>Homozygote</th>
<th>Heterozygote</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p % 95 CI</td>
<td>p % 95 CI</td>
</tr>
<tr>
<td>CAD vs CAD+</td>
<td>0.160 (0.793-3.999)</td>
<td>0.545 (0.775-1.713)</td>
</tr>
<tr>
<td>CAD vs MVD</td>
<td>0.959 (0.397-2.651)</td>
<td>0.268 (0.841-2.008)</td>
</tr>
<tr>
<td>CAD vs SVD</td>
<td>0.010 * (1.349-7.844)</td>
<td>0.757 (0.542-1.558)</td>
</tr>
<tr>
<td>MVD vs SVD</td>
<td>0.011 * (1.278-6.299)</td>
<td>0.234 (0.836-2.391)</td>
</tr>
</tbody>
</table>

*P < 0.05 is taken as statistically significant.

### Table 6 - A multiple logistic regression model for evaluating the relative effects of the polymorphisms on the risk of CAD

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>β</th>
<th>SE</th>
<th>Exp (B)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1RN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant effect</td>
<td>0.156</td>
<td>0.236</td>
<td>1.168</td>
<td>0.509</td>
</tr>
<tr>
<td>Recessive effect</td>
<td>-0.069</td>
<td>0.469</td>
<td>0.933</td>
<td>0.883</td>
</tr>
<tr>
<td>Co-dominant effect</td>
<td>-0.063</td>
<td>0.483</td>
<td>0.939</td>
<td>0.896</td>
</tr>
<tr>
<td>IL1 B (-511)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant effect</td>
<td>0.126</td>
<td>0.249</td>
<td>1.134</td>
<td>0.615</td>
</tr>
<tr>
<td>Recessive effect</td>
<td>0.459</td>
<td>0.273</td>
<td>1.582</td>
<td>0.093</td>
</tr>
<tr>
<td>Co-dominant effect</td>
<td>0.469</td>
<td>0.292</td>
<td>1.599</td>
<td>0.109</td>
</tr>
<tr>
<td>IL1B (+3953)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant effect</td>
<td>-0.148</td>
<td>0.229</td>
<td>0.862</td>
<td>0.518</td>
</tr>
<tr>
<td>Recessive effect</td>
<td>-0.372</td>
<td>0.491</td>
<td>0.689</td>
<td>0.449</td>
</tr>
<tr>
<td>Co-dominant effect</td>
<td>0.290</td>
<td>0.500</td>
<td>1.337</td>
<td>0.561</td>
</tr>
</tbody>
</table>

*Model adjusted for age, gender, familial CAD, smoking habit, history of hypertension.
are controversial results for the function of IL1RN 2 allele in IL-1Ra expression. It has been reported that IL1RN 2 allele is associated with increased IL-1Ra levels in vitro25-27, decreased levels in Ulcerative colitis27 but at similar levels for South African patients with inflammatory bowel disease28. The results above imply that IL1RN 2 polymorphism function in affecting IL-1Ra protein expression depends on cell type and ethnic origin.

It has been suggested that IL-1β may play an important role in the pathogenesis of atherosclerosis by the stimulation of vascular SMC. Also, increased levels of IL-1β mRNA was detected in atherosclerotic plaques29. The polymorphisms in IL-1B gene can affect severity or susceptibility to different diseases. Two important SNPs at -511 C/T in the promoter and +3953 C/T in exon 5 of IL1B gene are shown respectively30,31.

Different controversial results have been reported for the effect of IL1B (-511) polymorphism on the production of IL-1Ra and IL-1B. It has been reported that individuals who have IL1B (-511) allele 2 show higher levels of IL-1Ra26. LPS-induced IL-1β production was increased 2-3 fold by a T allele at -511 position32. No significant relationship between -511 alleles of IL1B and CAD has been determined10,11. However, lacoviello et al32 indicated that the IL1B (-511) 1/1 (C/C) genotype was associated with MI at young age31. Similarly, Zhang et al12 reported that IL1B (-511) polymorphism was associated to the severity of coronary heart disease in the Chinese population13. However, we did not find any risk for CAD related to IL1B (-511) polymorphism in the Turkish population14. Thus, the present study has shown that the homozygote carrier of IL-1RN allele 2 may be a risk factor for SVD. Controversial results were obtained for the association between IL1RN 2 and CAD from the different studies and different locations. This showed that ethnic background and different geographical locations play an important role for that association.

**References**

Haematologica. 2003; 88: 54-60.


21. Hurme M, Santtila S. IL-1 receptor antagonist (IL-1Ra) plasma levels are coordinately regulated by both IL-1Ra and IL-1beta genes. Eur J Immunol. 1998; 28 (8): 2598-602.


