Original Article

Interleukin-18 Gene Polymorphism in Patients with and without Atherosclerotic Coronary Artery Disease

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Background: Several studies have revealed that inflammation plays an important role in development of Coronary Artery Disease (CAD) and its other manifestations. IL-18 is a pleiotropic cytokine that enhances Th1(T helper 1) or Th2(T helper 2) immune response depending on its cytokine milieu and genetic background. It strongly induces formation of plaques in patients with CAD. Variations in the IL-18 gene found to influence both levels of IL-18 and clinical outcomes in individuals with history of heart disease. To investigate the association of two IL-18 promoter gene polymorphisms at -607C/A and -137G/C positions with CAD, and some CAD risk factors such as diabetes, arterial hypertension, hypercholesterolemia, cigarette smoking and obesity.

Methods: Genomic DNA was extracted by the salting out method from the peripheral arterial blood of 280 patients with CAD documented by coronary angiography (143 with a documented history of myocardial infarction termed positive MI and 137 without myocardial infarction designated negative MI) and 140 age- sex matched persons with a normal coronary angiography (control group).The genotype of both CAD and control groups were assessed by ASP-PCR method. Arlequin program was used for gametic phase estimation and haplotype analysis.

Results: There was no significant difference between patient and control groups either allelic, genotypic, and haplotypic for both variants ($p>0.05$). Furthermore, no significant correlation was found between IL-18 genotypes and CAD risk factors in the patient group ($P>0.05$).

Conclusion: These results suggest that the investigated IL-18 gene promoter polymorphisms at -607C/A and -137G/C positions are not associated with genetic susceptibility to CAD in southern Iran.

Key words: IL-18, Haplotype, Atherosclerotic Coronary Artery Disease

Introduction

Inflammation and immune system activation are two main reasons for initiation and development of atherosclerotic plaques.[1] IL-18 as a pleiotropic pro-inflammatory cytokine involved in both innate and adaptive immune responses is known as a strong predictor of atherosclerosis and its complications.[2-4]

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Recent evidence suggests that imbalance between Th1/Th2 response exists in patients with Chronic Heart Failure (CHF).[5] Cytokines control balance between Th1 and Th2 response.[4] IL-18 is a unique cytokine that induces both Th1/Th2 responses.[4]

Increased IL-18 concentration and Th1-related cytokine (IL-12, IFNγ) is seen in patients with CHF.[5] IL-18 also conducts development of atherosclerosis by over expression of IL-6, Intracellular Adhesion Molecule-1(ICAM-1), Vascular Cell Adhesion Molecule (VACAM-1) and

Matrix metalloproteinase (MMP) from related cells as atherogenesis factors.[6,7] High levels of IL-18 expression induce formation of plaques in both mouse and human models.[3, 8-10] Over expression of IL-18 binding protein (its natural inhibitor) confirms the role of IL-18 in plaques progression.[11]

A variation in IL-18 gene promoter influence its production and/or activity[12] and this may lead to genetic susceptibility to cardiovascular diseases.

This study was aimed at investigating the association of two IL-18 promoter gene polymorphisms at positions -607 C/A and -137 G/C with coronary artery disease (CAD) and CAD risk factors including diabetes, arterial hypertension, hypercholesterolemia, cigarette smoking and obesity.

Patients and Methods

The present study comprised a total of 280 patients with CAD confirmed using criteria of at least one involved coronary artery with more than 50% stenosis in coronary angiography, and 140 age-sex matched individuals (68 males and 72 females, mean age 57.5±8.83 years) with normal coronary angiography. Demographic details of CAD subgroups were: 143 with a documented history of myocardial infarction termed positive MI (75 males and 68 females, mean age 55.4±8.97 years) and 137 without myocardial infarction designated negative MI (74 males and 63 females, and mean age 57.6±9.44years). Control group was selected among patients without a history of chest pain who undervent coronary angiography for preoperative evaluation of coronary arteries before a noncardiac or a noncoronary cardiac surgery, according to American College of Cardiology/ American Heart Association (ACC/AHA) guidelines for coronary angiography.

There was neither personal nor family history of autoimmune, inflammatory and metabolic disease or malignancy in both control and patient groups.

The patient and control groups were matched according to age, sex, and presence of some CAD risk factors such as arterial hypertension (defined as taking antihypertensive medications or a blood pressure of 140/90 or more in at least two separate measurements at the time of study), diabetes(defined as a FBS of 126mg/dl or more at the time of angiography), hypercholesterolemia (defined as a serum LDL level of 100mg/dl or more in patient subgroups and based on National Cholesterol Education Program Adult Treatment Panel III "NCEP ATP III " guideline for control group), cigarette smoking (defined as current cigarette smoking), and body mass index (BMI).

All participants were from Shiraz hospitals and informed consent was taken from each of them according to the local Ethic Committee recommendation.

Genotyping

DNA was extracted from arterial blood samples (7ml of arterial blood which was obtained from femoral artery after coronary angiography and preserved in the tubes containing EDTA 0.5 ml, in +4 degree of Celsius temperature) by the salting out method described by Miller, et al.[13] The -607C/A and -137G/C polymorphisms were assessed by allele specific primer PCR method according to original protocol used previously by Giedraitis et al.[12]

Amplification products of 196 bp and 261bp were detected at positions -607C/A and -137G/C respectively. For each sample two sepa-

Table 1. Comparison of genotype and allele frequency of IL-18 promoter gene polymorphism between control group and patient subgroups (MI positive and MI negative)

Loci			MI postive subgroup (n=143)	Control group(n=140)	P value	MI negative subgroup (n=137)	P value
-607	Genotype	AA	18 (13.8%)	14 (11.0%)	0.60	12 (9.9%)	0.36
		CC	53 (40.7%)	48 (37.7%)		44 (36.4%)	
		AC	59 (45.5%)	65 (51.3%)		65 (53.7%)	
	Allele	A	95 (36.5%)	93 (36.6%)	0.98	89 (36.8%)	0.95
		C	165 (63.5%)	61 (63.4%)		153 (63.2%)	
-137	Genotype	GG	64 (47.0%)	60 (42.8%)	0.49	71 (53.8%)	0.49
		CC	11 (8.2%)	17 (12.2%)		11 (8.3%)	
		GC	61(44.8%)	63 (45%)		50 (37.9%)	
	Allele	G	83 (30.5%)	97 (34.6%)	0.30	72 (27.3%)	0.40
		C	89 (69.5%)	83 (65.4%)		192 (72.7%)	

rate reactions were carried out. For the −137 SNP(Single Nucleotide Polymorphism), PCR was performed using a common reverse primer, 5′-AGG AGG GCA AAA TGC ACT GG-3′, and two sequence-specific forward primers, 5′-CCC CAA CTT TTA CGG AAG AAA AAC-3′ and 5′-CCC CAA CTT TTA CGG AAG AAA AAG-3′. A control forward primer, 5′-CCA ATA GGA CTG ATT ATT CCG CA-3′, was used to amplify a 446-bp fragment covering the polymorphic site to serve as an internal positive amplification control. PCR for the polymorphism at −607 was performed using a common reverse primer, 5′-TAA CCT CAT TCA GCA CTT CC-3′, and two sequence-specific forward primers, 5′-GTT GCA GAA AGT GTA AAA ATT ATT AC-3′ and 5′-GTT GCA GAA AGT GTA AAA ATT ATT AG-3′. A control forward primer, 5′-CTT TGC TAT CAT TCC ACG AA-3′, was used to amplify a 301-bp fragment covering the polymorphic site as an internal positive amplification control.

Statistical Analysis

All statistical analysis were performed using the SPSS software (version 13; SPSS Inc,Chicago,IL,USA). The haplotype frequencies, and also the consistency of the genotype frequencies with the Hardy-Weinberg equilibrium were examined on the Arlequin program (http//anthropologie.unige.ch/arlequin). P values less than 0.05 were considered statistically significant.

Result

The allele and genotype frequencies of -607C/A and -137G/C in both subgroups of patients (MI positive and MI negative) were similar to those observed in control subjects and

Table 2. Comparison of haplotype frequency of IL-18 promoter gene polymorphism between control group and patient subgroups (MI positive and MI negative)

Haplotype		Control group (n=140)	MI positive subgroup (n=143)	P value	MI negative subgroup (n=137)	P value
-137	-137					
A	C	63(25%)	63(25.4%)	0.91	49(20.2%)	0.20
A	G	30(11.8%)	28(11.4%)	0.83	39(16.2%)	0.17
C	C	13(5.2%)	21(8.4%)	0.14	15(6.2%)	0.61
C	G	146(58%)	136(54.8%)	0.48	139(57.4%)	0.91

Table 3. Characteristics of study population

Variable	Control (n=140)	CAD with MI (n=143)	CAD without MI (n=137)	P vlaue
Age (year)	57.59±8.83	55.48±8.97	57.60±9.44	>0.05
Sex (F / M)	72 / 68	68 / 75	63 / 74	>0.05
BMI (Kg/m2)	23.05±3.15	23.12±2.74	23.15±2.60	>0.05
Diabetes mellitus (%)	15	20.2	20.8	>0.05
Hypertension (%)	40	44	47.7	>0.05
Hypercholesterolemia (%)	32.8	36.3	39.5	>0.05
Cigarette smoking (%)	29.2	39.8	31.3	>0.05

The age and BMI values are presented as mean ± SD BMI, body mass index; CAD, coronary artery disease; MI, myocardial infarction.

difference did not reach statistical significance (P>0.05)(Table 1).

However, Multinomial Logistic Regression method showed that in MI negative CAD patients, risk of disease was twice among G/G patients compared to those of G/C cases (P=0.041).

As demonstrated in Table 2, there was no significant difference in the haplotype frequencies between patient subgroups (MI positive and MI negative) and control group (P>0.05). Table 3 shows the baseline characteristics of study participants, there is not any significant difference among groups (P>0.05).

Discussion

We investigated the relationship between -137/-607 SNP polymorphisms in IL-18 gene promoter and CAD. Our data showed that there was no significant difference in the -137G/C and -607C/A between both subgroups of patients and control group.

In an attempt to confirm or refute these findings, the haplotype frequencies were used to determine the genetic correlation between these SNPs. This analysis showed that -137/-607 haplotype frequencies were also similar in patients and control. The association between different IL-18 gene SNPs with CAD has been extensively studied by several research groups. Tiret et al [14] estimated the causal impact of the IL-18 gene polymorphisms in CAD patients, demonstrating that IL-18 haplotypes caused variation in IL-18 serum levels and were correlated with CAD complications. A more recent study by Fang et al[15] reported that the SNP polymorphism at position -607 C/A may be associated with risk of acute myocardial infarction in northern Chinese Han population.

Hernesniemi et al[16] demonstrated that the SNP polymorphism at position -137 G/C was associated with the occurrence of sudden cardiac death among Caucasian males. In support of this finding, Liu et al [17] suggested that IL-18 promoter -137 G/C polymorphism influenced the development of atherosclerosis in the Chinese Han population. The latter study was inconsistent with the similar study described by Fang et al [15] in northern Chinese Han population. Different ethnic groups and different environmental factors might be probable reasons for differences in results among various populations.

It was reported that H4TF-1 nuclear fac-

tor binding site for an unknown factor found in the GM-CSF promoter will be transformed by a change from G to C at position -137.[12] After gene cloning of different IL-18 single nucleotide polymorphism (SNPs), Giedraitis et al [12] indicated that individuals with G allele at position -137 has somewhat high level of IL-18 mRNA expression compared to other individuals. Therefore, G/G homozygous patients for IL-18 promoter -137 G/C associated with an increase in the mRNA expression of atherogenic IFNγ, whereas reduced expression of such mRNA was observed in C/C homozygous patients.[12]

IL-18 expression usually associates with inflammation and Th-1 responses.[2] The results of previous studies and our findings about the decline in frequency of CAD among MI negative patients with G/C genotype (compared with G/G genotype) at -137 position, are suggestive of the possible effect of -137 G/C genotype on Th2 rather than Th1 responses.

We also investigated IL-18 genotypes with some risk factors for CAD. Our results did not show an association between any genotypes and CAD risk factors. Recently, Juliet Evans et al [18] highlighted the GC genotype of the IL-18 at position -137 G/C and circulating IL-18 levels which indirectly linked with elevated blood pressure(BP).Cytokines induced by IL-18 (IFNγ,TNFα,IL-6 and IL-1) may act on BP via up-regulation angiotensin gene expression and deregulation in the inflammatory response.[7,19] Furthermore, Simon et al 20 also demonstrated that a common IL-18 haplotype is associated with BMI in patients with CAD. A recent investigation has noted that some circulating inflammatory mediators such as IL-18 are released from fat tissues.[21,22] There is evidence to suggest that low-grade inflammation causing obesity [21] and high serum IL-18 levels associate with BMI.[23]

In conclusion, our results suggest that the investigated IL-18 promoter gene polymorphisms are not associated with genetic susceptibility to CAD or CAD risk factors in southern Iranian population. Genetic background of individuals from different ethnic groups as well as the environmental factors which usually affect this genetic background in different populations might be the reason for controversial results in different studies.

Moreover, linkage between the selected SNPs and their contiguous variants can influence the result of the study and might further explain different results among various studies.

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