Meta Gene 16 (2018) 1–4

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

Association of *interleukin-18* gene promoter polymorphisms with coronary artery disease in northern Iranian population

Afsaneh Bazgir[a], Touraj Farazmandfar[a], Aref Salehi[b], Reza Salehi Manzari[a,c], Marzieh Attar[a], Majid Shahbazi[a,d,*]

[a] Medical Cellular and Molecular Research Center, Golestan University of Medical Sciences, Gorgan, Iran
[b] Ischemic Disorders Research Center, Golestan University of Medical Sciences, Gorgan, Iran
[c] Resident of Internal Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, IR, Iran
[d] Arya Tina Gene, Biopharmaceutical Company, Gorgan, Iran

ARTICLE INFO

Keywords:
Atherosclerosis
Coronary artery disease
Interleukin 18
Polymorphism
Real-time PCR

ABSTRACT

Background: Coronary artery disease (CAD) is the leading cause of disability and mortality in the world. CAD includes a group of cardiovascular diseases such as angina, heart failure and myocardial infarction. Interleukin-18 (IL-18) is a pleiotropic proinflammatory cytokine and an inflammation inducer, which is encoded by the *IL-18* gene located on chromosome 11. Different studies have shown the involvement of IL-18 in the pathogenesis of diseases such as atherosclerosis.

Objective: This study investigated the association of the *IL-18* gene promoter polymorphisms (− 137 and − 607) with CAD in northern Iranian population.

Methods: Genomic DNA was extracted from peripheral blood of 314 patients with CAD and 364 healthy controls by a standard protocol. Sequence-Specific Primer-PCR was used for genotyping. Total RNA was extracted from peripheral blood leukocytes from 80 patients with CAD and 80 healthy individuals using Trizol reagent. Real-time PCR was used to measure the level of *IL-18* mRNA in the patients and controls.

Results: Analysis of genotypes revealed that the frequencies of genotype G/C and allele C in *IL-18*(− 137) polymorphism were significantly lower in patients group than control group ($p = 0.019$ and $p = 0.028$ respectively). No significant difference was found in the *IL-18*(− 607) between the patients and controls. Moreover, the level of *IL-18* expression was significantly higher in CAD patients than in controls ($p < 0.001$).

Conclusion: The results of the present study suggested a protective effect for genotype G/C of *IL-18*(− 137) in CAD. Moreover, due to an increased expression level of *IL-18* in CAD patients, it could be considered as a prognostic and protective factor in this disease.

1. Introduction

Coronary artery disease (CAD) is a chronic inflammatory disorder in the arterial wall, and the most prevalent heart disease in the world (Cassar et al., 2009). CAD includes a group of diseases such as angina, heart attack, and myocardial infarction. Atherosclerotic plaques have the main role in the progression of CAD, which are also associated with both innate and adaptive immune responses (Gistera and Hansson, 2017). Although the mechanism of atherosclerotic plaque formation is not determined completely, inflammation plays an important role in this process (Amirlatifi et al., 2018; Libby et al., 2009; Rezayani et al., 2017; Shateri et al., 2017; Zahra Mousavi et al., 2017). Many cytokines are involved in induction and development of inflammation process in CAD (Kampits et al., 2016). Interleukin 18 (IL-18) is a member of the IL-1 family of cytokines. It is an important pleiotropic and proinflammatory cytokine that can induce both innate and acquired immune responses. This cytokine is produced from Kupffer, dendritic, adrenal cortex and many other cells (Arango Duque and Descoteaux, 2014). IL-18 is mainly known as an interferon-gamma-inducing factor in T lymphocytes and natural killer cells, which are essential in the progression and stability of atherosclerotic plaque. High levels of IL-18 in inflammatory diseases demonstrated the key role of IL-18 in the inflammation cascade (Jefferis et al., 2011a, 2011b). Some studies reported an association between two the single nucleotide polymorphisms (SNP) in the *IL-18* gene promoter and development of cardiovascular disease. SNP of rs187238 (G > C) at the position − 137 from start site, that change nuclear factor-binding site of histone 4 transcription factor-1 (H4TF-1), and SNP of rs1946518 (C > A) at the

* Corresponding author at: Medical Cellular and Molecular Research Center, Golestan University of Medical Sciences, Shastkola Road, Falsafi Complex, 4934174611 Gorgan, Iran.
E-mail address: shahbazimajid@yahoo.co.uk (M. Shahbazi).

https://doi.org/10.1016/j.mgene.2018.01.001
Received 23 July 2017; Received in revised form 2 December 2017; Accepted 4 January 2018
Available online 04 January 2018

position − 607 that disrupts a cyclic adenosine monophosphate-responsive element protein binding site (Giedraitis et al., 2001; Hernesniemi et al., 2010; Liu et al., 2009; Lu et al., 2013; Pei et al., 2009).

This study was aimed to investigate the association of two *IL-18* gene promoter polymorphisms (separately and as haplotype) with CAD (as a group and as groups with a different number of affected arteries). In addition, the present study was aimed to compare *IL-18* mRNA expression between patients with CAD and normal individuals in Iranian population.

2. Material and methods

2.1. Study population

The association of the − 137 (G > C) polymorphism (rs187238) and the − 607 (C > A) polymorphism (rs1946518) in the *IL-18* gene promoter region of 314 patients with CAD and 364 healthy individuals were investigated. Artery stenosis was diagnosed by angiography in Amir Al-Momenin hospital, Kordkoy, Iran. Overall, 80 healthy individuals and 80 patients with CAD (age- and sex-matched) were chosen from the study group for real-time analysis. This work has been performed in accordance with the Declaration of Helsinki and approved by Ethics Committee of Golestan University of Medical Sciences (Ethnic code: 14791793061914). Written informed consent was obtained from all patients and healthy controls. None of the subjects refused to continue with the study.

2.2. DNA extraction and genotyping

Genomic DNA was extracted from10 ml peripheral whole blood by a standard phenol-chloroform protocol with some modifications (Rezayani et al., 2017). Polymorphisms of the *IL-18* gene at positions − 137 (G/C) and − 607 (C/A) were genotyped by sequence-specific primer-polymerase chain reaction (SSP-PCR) method as previously described (Shahbazi et al., 2017). The *IL18*(− 137) polymorphism was genotyped using a common reverse primer, 5′-AGGAGGGCAAAATGC ACTGG-3′ and two sequence-specific forward primers including 5′-CCCCAACTTTTACGGAAGAAAAG-3′ (for allele G) and 5′-CCCCAAC TTTTACGGAAGAAAAC-3′ (for allele C), which amplified a 261 bp fragment. The *IL18*(− 607) polymorphism was genotyped by a common reverse primer 5′-TAACCTCATTCAGGACTTCC-3′ and two sequence-specific forward primers including 5′-GTTGCAGAAAGTGTAAAAATTA TTAC-3′ (for allele C) and 5′-GTTGCAGAAAGTGTAAAAATTATTAA-3′ (for allele A), which amplified a 196 bp fragment. A conserved region of the Human Growth Hormone (hGH) gene was amplified to a 429 bp fragment as internal positive control using primers of forward 5′-GCC TTCCCAACCATTCCCTTA-3′ and reverse 5′-TCACGGATTTCTGTTGTGT TTC-3′. The PCR reaction was performed in a Thermal Cycler (Bio-Rad, Hercules, USA), with an optimized program that described previously (Farazmandfar et al., 2012). The PCR products were electrophoresed on agarose gel containing DNA safe stain and were visualized on a gel documentation system (UviTec, Warwickshire, UK).

2.3. Gene expression

Total RNA was extracted from 80 patients with CAD and 80 healthy individuals using Trizol reagent. Purified RNA samples were dissolved in water, and the concentration was measured by absorbance at 260 nm. RNA integrity was investigated by electrophoresis. cDNA synthesis was performed according to the manufacturer's protocol. The *IL-18* mRNA expression was determined by Real-time PCR using a forward primer 5′-TCTTCATTGACCAAGGAAATCGG-3′ and a reverse primer 5′-TCCGGGGTGCATTATCTCTAC-3′, to amplify a 75 bp fragment. The Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA was amplified as an internal control by a forward primer 5′-CCACCC ATGGCAAATTCC-3′ and a reverse primer 5′-GATGGGATTTCCATTGA TGACA-3′, to amplify a 101 bp fragment. Then, 8.5 µl DEPC water was added to 12.5 µl Power SYBR Green PCR Master Mix (Thermo Fisher Scientific, Waltham, USA), 10 mM primer, and 200 ng/ml cDNA. RT-PCR reaction was performed in Real-time system (Applied Biosystems, Foster City, USA) using the following program: 1 cycle of 2 min at 50 °C, 1 cycle of 10 min at 95 °C, 40 cycles of 15 s at 95 °C, 1 min at 60 °C, and 1 cycle of 15 s at 95 °C, 30 s at 60 °C, 15 s at 95 °C. Real-time PCR data was analyzed by comparative method ($2^{-\Delta ct}$) as previously described (Mohamadian et al., 2017).

2.4. Statistical analysis

Chi-square test was used to assess deviation from Hardy-Weinberg equilibrium (HWE) and the results showed no deviation from HWE. The risks associated with genotypes and alleles were calculated as the odds ratio with 95% confidence intervals. Data were recorded in the SPSS (Version 16.0) statistical software, and exact Fisher's analysis was performed by STATA (Version 8.0). p-Values < 0.05 were considered statistically significant.

3. Results

The present study was performed on 314 patients with CAD and 364 healthy individuals. Genotype and allelic frequencies of *IL-18*(− 137) and *IL-18*(− 607) polymorphisms are shown in Table 1. The genotype distribution in both groups was in the Hardy–Weinberg equilibrium.

As shown in Table 1, the frequencies of genotype G/C and allele C in *IL-18*(− 137) polymorphism were significantly lower in patients group than control group [OR (95% CI): 0.67 (0.48–0.94), p = 0.019 and OR (95% CI): 0.76 (0.60–0.97), p = 0.028 respectively]. There was no significant difference in genotypes and alleles of *IL-18*(− 607) polymorphisms between the patient group and control group (p > 0.05). To identify the inheritance models of two *IL-18* gene polymorphisms, *IL-18*(− 137) and *IL-18*(− 607), three models were considered, recessive, dominant and co-dominant (Table 1). Result of analysis showed that the distribution of *IL-18*(− 137) polymorphism is consistent with hereditary models of recessive and co-dominant [OR (95% CI): 0.68 (0.51–0.93), p = 0.013 and OR (95% CI): 1.39 (1.03–1.89), p = 0.035 respectively]. The distribution of *IL-18*(− 607) polymorphism was not in accordance with any of the inheritance models (p > 0.05). The genotype frequencies in CAD group according to a number of affected arteries are shown in Table 2. Results of this table show that genotype C/C of *IL-18*(− 137) polymorphism was significantly associated with the three vessel disease type in CAD patients (p = 0.004). The haplotype analysis of *IL-18*(− 137) and (− 607) polymorphisms showed no significant association between haplotypes and CAD (Table 3).

In order to compare the *IL-18* mRNA level in patient group and control group, we performed a relative Real-time PCR assay on *IL-18* cDNA samples. Our results showed, averages of *IL-18* cDNA level in control group (1.42 ± 0.27) was significantly higher than CAD patient group (0.71 ± 0.21) (p < 0.001) (Fig. 1).

4. Discussion

Atherothrombosis is a complex chronic disease that induces innate and adaptive immune responses (Gistera and Hansson, 2017). *IL-18* gene is the highly polymorphic that every alteration or mutation in its promoter can effect on mRNA expression and stability. The association between different *IL-18* gene SNPs and CAD has been extensively studied (Giedraitis et al., 2001; Hernesniemi et al., 2010; Liu et al., 2009; Lu et al., 2013; Pei et al., 2009). The genotype G/C at the − 137 position and allele C were found as protective factors for CAD (Table 1). However, no association was found between the − 607(C/A) polymorphism and CAD (Table 1). These results are in contrast with some previous reports that failed to show such association (Opstad et al.,