THE EFFECTS OF RS1801157G>A AND RS1746048C>T POLYMORPHISMS IN THE CXCL12 GENE ON CORONARY ARTERY DISEASE

MOHAMMAD PIRYAEI[1], SAYYED MOHAMMAD HOSSEIN GHADERIAN[1], REZA MIRFAKHRAIE[1], NASTARAN MOHAMMADI GHAHHARI[2], MARYAM AHANI[3], MARYAM MAFI GOLCHIN[4]

[1]Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran - [2]Department of Biochemistry, Pasteur Institute of Iran, Tehran - [3]Department of Genetics, Tehran Medical Branch, Islamic Azad University, Tehran - [4]Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran

ABSTRACT

Several studies have shown that a risk of coronary artery disease (CAD) is strongly associated with 10q11 chromosomal locus. Two rs1801157G>A and rs1746048C>T polymorphisms have been mapped to chemokine CXCL12 gene. These genetic variants have been revealed to affect atherosclerosis, CAD, and myocardial infarction (MI). This study attempts to explore the association between these two polymorphisms with the risk of CAD and its variables in Iranian population. 117 healthy controls and 105 CAD patients, who underwent angiography, were recruited from Iranian population. After genomic DNA extraction, TaqMan Probe Real Time PCR presented the genotypes from each sample. Statistical analyses were performed by SPSS 19 software to calculate genotype and allele distributions and compare the values with the clinical variables. Most of the variables significantly differed in CAD and control groups. Genotype and allele distributions of CAD and control groups showed no significant association for the rs1801157 and rs1746048 polymorphisms. However, frequency of the TT genotype was significantly higher in healthy group (P = 0.04, Odds Ratio = 0.20, 95% CI = 0.04-0.96). None of the variants for either polymorphism were associated with the clinical parameters. These findings suggested that the expression of rs1801157 and rs1746048 polymorphisms in CXCL12 are neither associated with CAD. Functional analysis may help to assess the exact correlation between the implications of 10q11 polymorphisms on CXCL12 expression in incidence of CAD, which benefits the designation of effective and personalized medicine against CAD complications.

***Key words**: CXCL12, polymorphism, rs1801157, rs1746048, Real Time PCR.*

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Introduction

Genetic predisposition, environmental risk factors, and inflammation are major contributors of atherosclerosis, coronary artery disease (CAD), and myocardial infarction (MI)[1,2,3]. Two classes of genes, including disease susceptibility and disease modifying genes belong to genetic factors in multifactorial disorders such as CAD. They can either influence the procedure by which a disease is initially developed or effect its development and prognosis[4]. Atherosclerosis is the primary cause of CAD, and it is induced by a process of chronic inflammation and formation of cholesterol plaques in the arterial walls[5].

Innate and adaptive immune responses are stimulated in reaction to specific molecules released by plaques. Subsequently, inflammatory cells such as macrophages and T cells are recruited to the atherosclerotic lesions. Inflammatory leukocytes release proteolytic enzymes, leading to the degeneration of the inner layer of arteries and thrombosis. A thrombus blocks the normal flow of oxygenated blood to the heart and consequently leads to MI[6].

There is accumulating evidence from genome-wide association studies (GWAS), describing an association between many novel chromosomal loci and gene variants with CAD and MI. Moreover, based on these studies, single nucleotide

polymorphisms (SNPs) that influence an individual's genetic predisposition for various CAD have been globally investigated in different ethnic groups[7,8,9,10]. As inflammatory responses are involved in the pathophysiology of atherosclerosis, genes that contribute to the inflammation pathways are suitable candidates to be studied in association with CAD[11]. The 10q11.21 is one of the proposed loci, which encompasses an inflammatory chemokine gene with an important role in atherosclerosis and CAD[12,13]. C–X–C motif chemokine 12 (CXCL12) or stromal cell-derived factor 1 (SDF-1) is encoded by the CXCL12 gene with ~88kb length and four exons[14,15]. Only two of the six coding isoforms related to CXCL12 (α and β) are predominantly expressed in human[16]. Atherosclerotic plaques and smooth muscle cells highly express CXCL12 and α isoform is mostly released by platelets as a platelet agonist[17,18,19]. The receptor for CXCL12 is CXCR4, which is a G-protein coupled receptor with important functions in hematopoiesis and synchronization of immune system[20]. CXCR4 is present on macrophages and vascular smooth muscle cells. Its increased co-expression with CXCL12 has been shown in heart failure[19,21]. When compared with healthy individuals, patients with angina showed decreased level of free circulating CXCL12[22]. Recent studies have shown that platelets, atherosclerotic plaques, and cardiac tissue express SDF-1/CXCR4 and, therefore; the risk for MI was increased[20]. Production of SDF-1/CXCR4 by platelets induces bone marrow-derived stem cells (CD34+) to differentiate into pre-endothelial cells in peripheral vessels[23]. Another study in rats suggested that SDF1/CXCL12 translocation to blocked coronary vessels activated heart precursor cells to stimulate neovascularization and reconstruction of cardiac muscle. These findings may confirm the critical role of CXCL12 in coronary artery regeneration process and cellular treatment of CAD/MI[24].

A SNP in 10q11 locus, 80kb downstream of the CXCL12 gene has been recently shown to be associated with the transcription and plasma level of CXCL12[12]. The locus in the CXCL12 region is located in a genomic region, which is unlikely to encompass casual genes. Such loci may include genes for non-coding RNAs or be in linkage disequilibrium (LD) with causal genes for the disease[22,25]. However, some of these regions may be placed to thousands of nucleotides away from 3 and 5 ends, but hold the specific regulatory sites related to disease causing genes[12]. rs1801157 G>A polymorphism is located at the 3'-untranslated region (3'-UTR) and its mutant allele induces the overexpression of CXCL12[13]. The rs1746048 10q11.21 variant has been also replicated in several independent studies[1,12,13]. Recently, a study reported that rs1746048 was associated with increased carotid intimal-medial thickness[26]. Therefore, these data propose a crucial role for CXCL12 in human atherosclerosis leading to CAD and MI; however, it should be cleared whether its actions are detrimental or advantageous in the disease. Since GWA studies revealed an association between rs1801157 and rs1746048 in CXCL12 with CAD, we focused on assessing different variants of the SNPs and their potential biological association with the CAD in Iranian population.

Methods

Subjects

222 Iranian subjects were recruited, of which 105 underwent coronary angiography for diagnosis of CAD. 105 patients had positive angiographically diagnosed CAD as showing stenosis of ≥ 50% in at least one coronary artery. On the other hand, 117 healthy participants were selected as controls. One consultant cardiologist assessed the demographic results of angiographies. The study was authorized by the ethics review committee of the Shahid Beheshti University of Medical Sciences, Tehran, Iran, and all subjects signed informed consents. Demographic and clinical data such as age, gender, weight, height, systolic and diastolic blood pressure (SBP and DBP), body mass index (BMI), levels of HDL, LDL, cholesterol, triglyceride, and fasting blood sugar (FBS) were recorded. 5 ml blood was obtained from each subject, and genomic DNA was extracted according to the manufacturer's instruction (High Pure PCR Template Preparation Kit (Roche, Germany)).

Genotyping

The quality and amount of isolated DNA were verified by gel electrophoresis and spectrophotometry (Nanodrop 1000, Thermo Fisher Scientific, Wilmington, DE, USA). Primer-probe sets were designed and manufactured by Applied Biosystem (ABI). Genotyping for the rs1801157 -801 G>A and rs1746048 C>T in CXCL12 gene was performed using TaqMan Probe Real Time PCR (LightCycler 96, Roche, Germany).

Statistical analysis

SPSS version 19 (SPSS Inc., Chicago, IL, USA) was used to analyze the statistical data. Clinical data and risk factors for CAD were interpreted by t-test. Chi-square test was used to test the genotype and allele frequencies for the presence of the Hardy-Weinberg equilibrium. Logistic regression analysis was directed to test the association between different genotypes of the SNP and CAD. A subsequent logistic regression analysis was completed with rs1801157 and rs1746048 genotypes to evaluate independent contributions of these genotypes to CAD development. To assess the effect of independent variables such as age on genotype frequencies and CAD occurrence we used stepwise multivariate regression. A p-value of less than 0.05 was considered statistically significant.

Results

Demographic and clinical features of the subjects

105 patients with CAD recorded angiographically as having stenosis of ≥ 50% in at least one coronary artery and 117 controls without the disease were recruited in this study. The demographic and clinical characteristics of the study population are shown in Table 1. The age, BMI, SBP, DBP, and the levels of, triglyceride, FBS, and LDL were significantly higher in the angio-positive group. HDL level was revealed to be higher in the controls when compared to patients. There was no statistically significant difference between the groups for sex and total cholesterol (Table 1).

Variables	Control (n=105)	CAD (n=117)	*P* values
Age (years)	48.2 ± 7.0	58.5 ± 8.9	<0.00
Sex	0.4 ± 0.1	0.59 ± 0.9	0.2
Body mass index (kg/m2)	25.1 ± 3.8	27.5 ± 6.8	0.00
Systolic Blood Pressure (mm HG)	114.1 ± 10.8	135.9 ± 26.8	<0.00
Diastolic Blood Pressure (mm HG)	75.3 ± 6.8	83.6 ± 12.5	<0.00
Triglyceride (mg/dl)	114.0 ± 62.3	155.9 ± 68.2	<0.00
Total Cholesterol (mg/dl)	171.6 ± 18.6	173.7 ± 32.6	0.5
Fasting Blood Sugar (mg/dl)	85.1 ± 10.9	139.5 ± 62.7	<0.00
High Density Lipoprotein (mg/dl)	49.5 ± 1.8E+01	39.1 ± 8.1	<0.00
Low Density Lipoprotein (mg/dl)	87.2 ± 2.7E+01	102.0 ± 24.3	<0.00

Table 1: Comparison of clinical characteristics of healthy (control) and angio positive (CAD) groups.
Values are represented as mean ± SD for all the variables.

Genotype and allele frequencies for rs1801157 polymorphism

There was no significant difference between the distribution of rs1801157 G>A CXCL12 genotypes in patients and controls. Observed frequencies for CAD genotypes included 59% (GG), 36% (GA), and 5% (AA). On the other hand, frequencies for controls were, 54% (GG), 34% (GA), and 12% (AA) (Table 2). The association between the frequencies of two alleles and CAD occurrence remained insignificant (P = 0.19, Odds ratio = 0.75, 95% CI = 0.48-1.16, Table 2).

Genotype	CAD n (99)	Control n (117)	*p*-value	OR (95% CI)
GG	59 (59%)	64 (54%)	0.49	1.22 (0.68-2.17)
GA	35 (36%)	40 (34%)	0.88	1.02 (0.73-1.40)
AA	5 (5%)	13 (12%)	0.14	0.42 (0.12-1.34)
G allele	153 (77.3%)	168 (71.7%)		
A allele	45 (22.7%)	66 (28.3%)	0.19	0.75 (0.48-1.16)

Table 2: Distribution of genotypes and alleles for rs1801157 G>A CXCL12 patients with CAD and healthy subjects.

Genotype and allele frequencies for rs1746048 polymorphism

After $\chi 2$ analysis, there was a significant association between the distribution of TT genotype for the rs1746048 C>T polymorphism in CAD patients and healthy group where the frequency of the TT genotype increased in controls (Table 3). The TT genotype was associated with an odds ratio of 0.20 and 95%CI = 0.04-0.96 (P = 0.04), but the CT genotype associated with and odds ratio equal to 0.85 and 95%CI = 0.47-1.53 (P = 0.65). The association between the frequency of T non-risk allele remained significant in absence of CAD in healthy group (P = 0.0.4, Odds ratio = 0.61, 95%CI = 0.38-0.98, Table 3).

Genotype	CAD n (103)	Control n (88)	*p*-value	OR (95% CI)
CC	64 (62%)	45 (51%)	0.14	1.56 (0.84-2.91)
CT	37 (36%)	35 (39%)	0.65	0.85 (0.47-1.53)
TT	2 (2%)	8 (10%)	0.04	0.20 (0.04-0.96)
C allele	165 (80.1%)	125 (71.02%)		
T allele	41 (19.9%)	51 (28.98%)	0.04	0.61 (0.38-0.98)

Table 3: Distribution of genotypes and alleles for rs1746048 C>T CXCL12 in patients with CAD and healthy subjects.

CXCL12 genotypes and CAD variables

Both rs1801157 and rs1746048 polymorphisms showed similar genotype frequencies in male and females. There were also no notable differences in the mean age for neither polymorphism. None of the SNPs were associated with BMI, SBP, DBP, triglyceride, cholesterol, FBS, HDL, and LDL (Table 4, Table 5).

Characteristics	Control	CAD
Age (years)	0.16	0.64
Sex	0.74	0.54
Body mass index (kg/m2)	0.87	0.65
Systolic blood pressure (mm HG)	0.58	0.71
Diastolic blood pressure (mm HG)	0.56	0.80
Triglyceride (mg/dl)	0.34	0.93
Total cholesterol (mg/dl)	0.26	0.68
Fasting blood sugar(mg/dl)	0.80	0.96
High density lipoprotein (mg/dl)	0.75	0.52
Low density lipoprotein (mg/dl)	0.07	0.97

Table 4: One way ANOVA analysis of clinical parameters according to rs1801157 genotypes in control and CAD.

	P values	
Characteristics	Control	CAD
Age (years)	0.29	0.87
Sex	0.08	0.20
Body mass index (kg/m2)	0.43	0.32
Systolic blood pressure (mm HG)	0.68	0.09
Diastolic blood pressure (mm HG)	0.51	0.10
Triglyceride (mg/dl)	0.69	0.67
Total cholesterol (mg/dl)	0.58	0.92
Fasting blood sugar(mg/dl)	0.23	0.25
High density lipoprotein (mg/dl)	0.09	0.80
Low density lipoprotein (mg/dl)	0.43	0.87

Table 5: One way ANOVA analysis of clinical parameters according to rs1746048 genotypes in control and CAD.

Discussion

Recent GWAS and replication studies have identified rs1801157 and rs1746048 polymorphisms on chromosome 10q11 to impact the risk of CAD[12,27,28]. GWAS reports have provided scientists with remarkable amount of data on the association of SNPs with CAD incidence; however, rs1801157 is one of the missing SNPs, which has not been included in such studies. On the other hand, rs1746048 has been investigated in several GWAS where Kathiresan and colleagues demonstrated that a C-risk allele in this intergenic SNP was associated with MI (OR = 1.17, P = 7×10-9)[29]. Therefore, for better acknowledgement of the pathogenesis of CAD as a multifactorial disease, it is beneficial to perform replicate studies of the most notably associated SNPs in populations with genetically diverse backgrounds and different lifestyles.

In our study, neither genotypes of rs1801157 were displayed a significant association with CAD. We also performed an association analysis for both G and An alleles, which was not indicative of a positive association with increased CAD risk (Table 2). In processing the data for CAD variables, we observed significant differences for most of the characteristics between CAD and controls (Table 1). However, in our subsequent comparison analysis of the most significant phenotypes of CAD with rs1801157 genotypes we could not detect a positive association (Table 4). A meta-analysis study of CXCL12 G801A polymorphism has been demonstrated its association with an increased risk of cancer development[30]. Feng et al. 2014 recently performed a single replication study in China on 84 patients and 253 healthy controls. The distribution of GG genotype was higher in the patient group and the AA genotype was rare in respect to the previous studies, which is in accord to our observations on patients homozygous for the A allele[13].

Although in our analysis of the rs1746048 C>T polymorphism we could not emerge a significant association between C risk allele with CAD, the T allele frequency was notably higher in healthy group (P = 0.04, Table 3). This implies that the T allele implies a protective role against CAD. Many replicated GWAS discoveries have not performed a follow-up functional evaluation of the causative genes in relation to CAD phenotypes. Mehta et al. 2011 found that rs1746048 genotype and allele frequencies were associated with high plasma levels of CXCL12 (P value for C risk allele = 0.007). It is thus possible that CAD C risk allele associates with increased mRNA expression levels of CXCL12[12]. In addition, this SNP produced a positive association with CAD in 2,521 American and European subjects with MI (OR = 1.09, P = 3×10-10)[31]. However, a Lebanese cohort on 2,002 subjects was unable to report an association between rs1746048 and CAD[32], which is in agreement with our results. The determination of functional impact of additional variants of CXCL12 may explain the reason for contradictory results. Conversely, Zhu and colleagues showed a protective role for the C allele in ischemic stroke (IS), and observed a significant association between the T allele and IS risk in males, which was strongly associated with IS risk factors (P = 0.001, OR = 1.52, 95% CI = 1.182-1.977)[27].

Among chemokines CXCL12 functions in a variety of cellular processes such as stem cell organization, haematopoiesis, angiogenesis, and cell signaling, as well as a direct implication in atherosclerosis. Moreover, different studies have reported either detrimental or beneficial effects for CXCL12, depending on tissue context. The indirect physiological responses to atherogenic provocations caused by other 10q11 SNPs in CAD should be included on CXCL12 stimulation[12]. An SNP in the CXCL12 gene may produce a mutated

protein, which induces the pathophysiologic mechanism of CAD initiation and progression. But, this requires functional assessment of the genotypes to clarify CXCL12-mediated CAD. Therefore, it appears that various biological mechanisms affect the 10q11 region. The inconsistency between our results and similar reports could be caused by differences in lifestyle habits, genetic predisposition, geographical ethnic groups, nutrition, age, sample size, and study design. Our study had some limitations as it was conducted on Iranian population, which suggests that these results may not apply to other ethnic groups. This study also did not investigate the underlying molecular mechanisms of how these SNP mutations improve CAD risk. Thus, it is necessary to discover the mechanisms that are regulated by CXCL12 SNPs during atherosclerotic plaque formation or CAD development. Such association studies assist scientists to receive reliable data for the designation of more effective therapeutic interventions and drugs for the treatment of cardiovascular disease and improving patients' lives.

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Corresponding author

SAYYED MOHAMMAD HOSSEIN GHADERIAN

Department of Biochemistry, Genetic and Nutrition, School of Medicine, Alborz University of Medical Sciences, Karaj, I.R. *(Iran)*

Tel/Fax: +98 21 2387 2572; Email: sghaderian@sbmu.ac.ir; sghaderian@yahoo.co.uk