C771G (His241Gln) Polymorphism of MLXIPL Gene, TG levels and coronary artery disease: A case control study

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Abstract

Objective: It is suggested that C771G (His241Gln) polymorphism of MLXIPL gene might be a genetic risk factor for coronary artery disease (CAD); therefore, the aim of the present study was to investigate the association between C771G polymorphism of MLXIPL gene and the pathogenesis of CAD in Iranian patients with coronary artery stenosis and control subjects.

Methods: Two hundred and five patients with coronary artery stenosis and 195 healthy control subjects were included in this study. MLXIPL genotypes were determined by polymerase chain reaction and restriction fragment length polymorphism (RFLP).

Results: There was an association between the MLXIPL polymorphism and quantitative lipid traits in patient group. Distribution of the CC genotype of MLXIPL was more frequent in patients, (χ^2=5.13; p<0.005) and after adjustment for classical CAD risk factors, the MLXIPL CC genotype was independently associated with CAD (OR=1.98, 95% CI, 1.12-4.11; p=0.02). Distribution of MLXIPL genotypes were significantly different as compared with the severity of stenosis (χ^2=6.34; p<0.05).

Conclusion: These results suggest that C771G polymorphism of MLXIPL gene is associated with stenosis and its severity.

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Key words: coronary artery disease, MLXIPL, polymorphism

Introduction

Coronary artery disease (CAD) is the leading cause of death in industrialized countries. Apart from some rare Mendelian forms of CAD, most CAD is believed to have a multifactorial genetic basis involving a number of genes and environmental factors that interact to determine whether a person will develop the disease (1, 2). Despite extensive efforts using the candidate gene approach or genome-wide linkage studies, the responsible molecular and genetic determinants remain largely unidentified (3, 4). Genome-wide association studies provided more convincing evidence for CAD-associated genomic loci, generating cautious optimism for disentangling the disease pathophysiology and defining novel targets for treatment (5).

Genetic variability has been identified in humans for all the known lipid-related genes, and some of those variants have been studied for the past two decades, resulting in a plethora of reports and associations with abnormal lipid metabolism and plasma lipoprotein profiles. A detailed description of the biological role of each of the products has been studied in other publications (6, 7).

MLX-interacting-protein-like (MLXIPL; or carbohydrate response element binding protein, ChREBP) is located in the Williams-Beuren Syndrome critical region gene 14 (WBSCR14) deletion region, at chromosome 7q11.23. The gene encodes a transcriptional factor protein that is composed of 852 amino acids (8). Recently, SNPs localized within the MLXIPL loci have been associated with plasma triglycerides (9, 10). The most significant association was described for the rs3812316 SNP (C771G, His241Gln); a non synonomous polymorphism with G and C alleles as a rare and common allele respectively. The identified SNP is located at evolutionary conserved domain responsible for glucose dependent activation of MLXIPL. Glucose flux into hepatocytes results in the nuclear translocation of MLXIPL. Nuclear MLXIPL dimerizes with MLX and the complex increases the transcription of genes involved in lipogenesis and triglyceride synthesis (11).

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The present study was designed as a case-control study in a sample of Iranian subjects undergoing diagnostic coronary angiography with the objective of addressing the question of whether C771G polymorphism of MLXIPL gene influences the risk of coronary atherosclerosis and the number of affected vessels.

Methods

Subjects

Four hundred subjects who were taken to Shariati Hospital were studied in this study from 2010-2012. The study population were all blinded Iranian that consisted of 195 controls (99 male, 96 female; mean age: 53.35±8.41) and 205 cases (105 male, 100 female; mean age: 55.44±2.32) who underwent coronary angiography to evaluate coronary artery disease. All angiograms were evaluated by cardiologist and in this regard CAD was defined as the presence of one or more stenosis >50% in at least one major coronary artery (12). The classification was based on the visual assessment of 15 coronary segments following the criteria defined by an ad hoc committee of the American Heart Association (12). In patients severity of CAD was determined by the number of coronary arteries as patient with single vessel disease (SVD), double vessel disease (2VD), and triple vessel disease (3VD). Participants with hepatic or renal disease, cardiomyopathy, congestive heart failure and acute myocardial infarction within the last three months were excluded from the study. Medical history, drug intake and demographic data of the study population were collected on a questionnaire. Blood pressure, weight and height were also recorded. Controls which suspected to have CAD, attended a routine health check and served as a part of study population that was defined as the <10% of coronary artery disease. The data collected included age, sex, BMI, systolic and diastolic blood pressures (SBP and DBP). Information on medical history, conventional stenosis risk factors such as hypertension, diabetes, and hyperlipidemia was obtained by a questionnaire and a blood sample for laboratory testing and genotyping was collected. Subjects with a history of diabetes mellitus, liver and renal disease, hypertension, hyperlipidemia were excluded.. This study was approved by the University Hospital Ethics Committee and written informed consent was obtained from all patients and control subjects.

Laboratory measurements

The samples were collected after 12 hour fasting. Plasma total cholesterol and triglycerides were measured by routine enzymatic methods (Parsazmun instruments, Tehran, Iran). HDL cholesterol was determined after precipitation of the apoB-containing lipoproteins (parsazmun instruments, Tehran, Iran). LDL-cholesterol was calculated using Friedewald formula.

Genotype determinations

Genomic DNA was extracted from peripheral blood leucocytes by the salting-out method (13). 174bp fragment was amplified by the polymerase chain reaction (PCR) method with using primers 5'- ATCCTCAGGCGGCAGCTGCAGGGGA -3' (forward) and 5'- AATGGTGCAAACAGCTCTTCTCCA -3'(reverse). Genomic DNA was amplified in 50 µL mixture containing 300 ng DNA template, 0.5 µmol/L each primers, 200 µmol/L dNTPs, 5 µL of 10x reaction buffer and 1.0 unites Taq DNA polymerase. After the DNA was denatured for 1 minute at 94°C, the reaction mixture was subjected to 35 cycles of denaturation for 50 seconds of 94°C, 1 minute of annealing at 59°C, and 1 minute of extension at 72°C. PCR product was digested with 1 units Alu1 (Fermentas) and RFLP fragments were separated by 3% agarose gel electrophoresis and identified by ethidium bromide staining. C allele corresponded to a 96+78 bp and G allele 174 bp fragment.

Statistical analysis

Student's t-test was used for comparison of age, BMI, and lipid profile in controls and cases. Genotype frequencies were compared by the chi-square test. Allele frequencies were determined by the gene counting method, and Hardy-Weinberg equilibrium was tested by the χ^2 test. The odds ratios (ORs) and 95% CIs were calculated as a measure of the association of the MLXIPL genotypes with CAD, and severity of this disease. The relationship between MLXIPL genotypes and severity of disease in cases was evaluated by the χ^2 test. The analysis was also carried out by means of a logistic linear regression analysis to assess the independent role of the MLXIPL genotype and other CAD risk factors. All calculations were performed using the SPSS 16 program (SPSS for Windows, Chicago, IL).

Results

Clinical and laboratory measurements in the population study

Table 1 represents clinical and laboratory measurement data in the population study. There is no statistical differences in classical coronary risk factors and lipid profile in cases and controls except triglyceride (p=0.03). Patients were taking aspirin, cholesterol and blood pressure lowering medications.

MLXIPL genotypes and plasma lipids

Table 2 represents certain classical coronary risk factors and lipid profile of patients with different genotypes. When laboratory values were compared among different genotypes of CAD patients, no significant difference was detected with regard to age, BMI, cholesterol, HDL, LDL, VLDL, SBP and DBP but there was a significant association between CC genotype and TGs levels.

Genotype and allele frequencies

Table 3 shows the genotype distribution and allele frequencies of the MLXIPL polymorphism in CAD cases and controls. Genotype distribution was consistent with the Hardy-Weinberg equilibrium (χ^2=0.02, p=0.96). Allele frequencies were C=0.69, and, G=0.31 in cases and C=0.46, G=0.54 in controls.

Table 1. Classical coronary risk factors and lipid profile in patient and control subjects

Variable	Control group (n=195)	Patient group (n=205)	*P*
Age, year	53.35±8.41	55.44±2.32	NS
Sex, Male/Female	(99/96)	(105/100)	NS
BMI, kg/m^2	27.24±4.06	26.14±4.52	NS
SBP, mm Hg	12.35±1.33	12.75±1.32	NS
DBP, mm Hg	7.67±0.54	7.73±0.34	NS
Cholesterol, mg/dL	147.12±38.65	179.43±36.76	NS
Triglyceride, mg/dL	136.23±64.34	179.31±61.54	0.03
HDL-C, mg/dL	38.85±9.19	35.44±9.04	NS
LDL-C, mg/dL	85.13±23.13	94.14±29.53	NS
VLDL-C, mg/dL	38/01±21.17	45.12±20.40	NS

NS - not significant
All data were indicated the mean±SD. p<0.05 was considered to be statistically significant.
BMI - body mass index; DBP - diastolic blood pressure; HDL-C - high density lipoprotein-cholesterol; LDL-C - low density lipoprotein- cholesterol; SBP - systolic blood pressure; VLDL-C - very low density lipoprotein- cholesterol

Table 2. Classical coronary risk factors and lipid profile of patients with different genotypes

Characteristics	CC	CG	GG	*P**
Age, year	56.33±7.42	58.91±4.81	59.45±8.91	NS**
BMI, kg/m^2	25.27±3.25	24.51±4.71	26.55±4.31	NS
TC, mg/dL	174.31±21.77	170.12±31.53	170.11±28.54	NS
TG, mg/dL	170±42.30	150.51±50.55	145.32±40.33	0.03
HDL-C, mg/dL	41.55±8.32	38.45±7.62	37.52±9.01	NS
LDL-C, mg/dL	94.62±18.21	89.92±11.32	96.91±16.21	NS
VLDL-C, mg/dL	39.61±12.21	38.61±15.21	38.02±12.23	NS
SBP, mm Hg	11.93±1.01	12.35±1.00	12.01±1.11	NS
DBP, mm Hg	8.02±.032	7.51±.041	7.98±0.51	NS

Calculated by ANOVA.
NS - not significant; BMI - body mass index; DBP - diastolic blood pressure; HDL-C - high density lipoprotein-cholesterol; LDL-C - low density lipoprotein- cholesterol; SBP - systolic blood pressure; TC-total cholesterol; TG- Triglycerides; VLDL-C - very low density lipoprotein- cholesterol

The association of MLXIPL genotypes with the severity of disease

When we compared the distribution of genotypes and allele frequencies by the number of diseased vessels, there was a significant difference between the CC genotype and the extent of CAD (Table 4). The findings from the univariate analyses were further investigated in a conditional multiple logistic regression model incorporating the C771G polymorphism of MLXIPL gene, age, sex, and classical coronary artery disease risk factors. The results of this analysis are shown in Table 5. MLXIPL CC genotype independently associated with the incidence of CAD and the number of diseased vessels.

Table 3. The genotypes and allele distributions of the MLXIPL polymorphisms among controls and patients

Genotype	Patient group (n=205)	Control group (n=195)	*P*
CC	113 (55.12%)	58 (29.74%)	<0.005
CG	60 (29.26%)	62 (31.76%)	NS
GG	32 (16.60%)	75 (38.46%)	< 0.005
Allele			
C	143 (69.75%)	89 (45.65%)	<0.01
G	62 (30.25%)	106 (54.35%)	NS

NS - not significant; CC - classical coronary; CG - control group; GG - gene group

Table 4. The association of C771G MLXIPL genotypes with the severity of disease

Genotype	One vessels n (%)	Two vessels n (%)	Three vessels n (%)
CC	15 (22.05%)	22 (31.42%)	36 (53.73%)
CG	28 (41.17%)	28 (40.00%)	21 (31.34%)
GG	25 (36.76%)	20 (28.57%)	10 (14.92)*
Allele			
C	58 (43%)	72 (51%)	93 (69%)
G	78 (57%)	68 (49%)	41 (31%)

*p<0.05, the frequency of the GG genotype of MLXIPL was lower in patients with 3 diseased vessels than it was in patients with only 1 diseased vessel; CC - classical coronary; CG - control group; GG - gene group

Discussion

In this study we evaluated the distribution of MLXIPL genotype in angiographically diagnosed cases and controls and found a significant association of the SNP with TG levels (p<0.05) and this polymorphism as a risk factor for atherosclerosis (p<0.005). Moreover, this study demonstrated that with considering of other risk factors, C771G polymorphism is related to severity of coronary artery stenosis.

Plasma levels of TGs are influenced by dietary composition, smoking, body weight and genetic factors. Similarly to the other risk factors, it is estimated that the contribution of genetic and environmental factors on plasma levels of TG is roughly the same. The genetic predisposition to a high level of plasma TG levels has been intensively analyzed in last 15 years. There are polymorphisms in different genes that could have some effect on plasma TG levels (14, 15). Our study confirmed previous findings indicating an association between the C771G polymorphism of MLXIPL gene and plasma triglycerides (9, 10). MLXIPL is a basic helix-loop helix/leucine zipper transcription factor involved in mediating glucose-responsive gene activation (16). Mice with a disruption of the MLXIPL gene or hepatocytes treated with siRNA to reduce MLXIPL expression cannot induce lipogenic gene expression in response to carbohydrate (17, 18). In hepatocytes prepared from MLXIPL null mice, the induction can be

Table 5. ORs for presence of CAD and three-vessel disease, according to MLXIPL CC genotype and other variables by multiple logistic regression analysis

Variable	Presence of CAD OR (95% CI)	*P*	Three vessel disease OR (95% CI)	*P*
Age	1.04 (0.84-1.27)	NS	1.06 (0.94-1.12)	NS
Sex	1.23 (0.50-2.38)	NS	1.18 (0.62-2.55)	NS
Cholesterol	1.87 (0.82-4.34)	NS	1.95 (1.04-3.88)	NS
Triglyceride	2.90 (0.1.25-5.65)	0.03	2.85 (1.35-5.52)	0.02
HDL	2.38 (0.62-9.21)	NS	1.15 (0.54-2.21)	NS
LDL	1.09 (0.60-2.10)	NS	1.39 (0.40-3.93)	NS
MLXIPL CC genotype	1.98 (1.12-4.11)	0.02	3.25 (1.45-4.95)	0.04

HDL - high density lipoprotein; LDL - low density lipoproteinl; NS - not significant;

restored by the addition of a MLXIPL expression vector (19). Importantly, animal model supports the role of MLXIPL in TG metabolism, since knockout mice have low TG levels (20). Thus, MLXIPL is essential for regulating lipogenic gene expression. Previous study have investigated the association between C771G polymorphism of MLXIPL and blood lipids. Pan et al. (21) found a significant association of this polymorphism with plasma concentrations of triglycerides, however, no significant association was found between the GG genotype and TC, HDL or LDL. The authors observed that homozygotes for the C allele have a less atherogenic lipid profile than heterozygotes and homozygotes for the G allele. Conflicting observation has been reported in a study (22), in whom C allele carriers didnt show higher concentrations of TGs. Our data consistent with Pan et al. (21) demonstrated that C771G (His241Gln) polymorphism of MLXIPL gene influences plasma TGs, but confirming the C allele (not G allele) to be associated with high TG levels and failed to demonstrate any significant association between this polymorphism and HDL, LDL, TC levels.

We examined whether the disease-associated genotype was related to the severity of coronary atherosclerosis and found an association between CC genotype and the number of diseased vessels. However, Pan et al. (21) failed to demonstrate any significant association.

In present study, we found a significant difference in genotype and allele frequencies for C771G MLXIPL polymorphisms between cases and controls. Minor allele frequencies differed significantly by different studies. The rs3812316 G allele frequency was lower in Han population of Hubei province (0.07 in cases and 0.14 in controls) (23) than in Chines (0.88 in cases and 0.36 in controls). The rs662 G allele was a minor allele in cases in present study (0.31 in cases and 0.54 in controls) and in Han population but had higher frequency in Chinese. Different genetic and environmental factors might lead to variable levels of associations in different populations. Exposure to different lifestyles and environments may modulate the effect of genetic variation CAD risk. Even the same population with different clinical characteristics, environmental exposures, or, different living styles may be particularly relevant for the association study. So researchers always got different results on the association studies. In present study, we were able to demonstrate a correlation between rs3812316 and the risk of coronary heart disease. The frequency of rare allele G at the rs3812316 site was significantly lower in the CAD group than the control group, indicating that the rare allele might have protective effects in the development of early atherosclerosis in which gene-risk factor interactions played an important role. The biological plausibility of C (His241) allele association with higher stenosis risk is not well established.. Since in present study the CC genotype of MLXIPL gene was associated with elevated TGs and elevated plasma triglycerides (TG) are an independent risk factor for cardiovascular disease development (24) it is reasonable that CC genetype has a high frequency in the case group and might be a risk factor for atherosclerosis.

Study limitations

The limitation of this study is the small sample size of the population and lack of other atheorgenic profiles as small dense lipoprotein particles. Another limitation is even though association studies are very important in special populations, CAD and hyperlipidemia are complex traits. Novel bioinformatic methods are in need to assess individual risk.

Conclusion

In summary, there is an association between the CC genotypes of MLXIPL gene and increased risk of stenosis and its severity. However, further studies are needed to ascertain the role of this gene in the pathogenesis of coronary artery disease.

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