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Research Article

Investigation of I405V Polymorphism in the CETP gene and association with metabolic syndrome parametersin an Iranian population

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ABSTRACT

Introduction: Metabolic Syndrome is a potential threatening factor for cardiovascular disorders and atherosclerosis which is accompanied by increase in plasma Triglyceride,cholesterol, LDL-c, Fasting blood sugar (FBS) and low level of High density lipoproteins (HDL-c). Cholesteryl ester transfer protein (CETP) catalyzed transfer natural lipids and phospholipids between lipoproteins. CETP can have a significant role in balancing the quantity of plasma lipids and lipoproteins. The present survey attempts to show the association of I405Vpolymorphisms in CETP gene with metabolicsyndrome parametersan Iranian populations. Methods: In order to identify the association between the I405V polymorphisms of this gene and the lipid pattern of plasma and other parameters of metabolic syndrome, the quantity of Lipids in metabolic Syndrome(N=120) and Normal individuals(N=200) were measured. The abundance of alleles and genotypic distribution of the I405V polymorphisms were defined along with comparison between two control and patient groups.. Blood samples were collected then followed by routine biochemical analysis, DNA extraction performed. Polymerase chain reaction-restriction fragment length polymorphism was applied to identify I405Vpolymorphism. Statistical analyses were applied using SPSS software. Results: lipid pattern of plasma and other parameters of metabolic syndrome had significant different between the patient and control groups. Also the abundance of alleles and genotypic distribution of this polymorphism showed a significant difference between two groups. I405V polymorphism, chosen in the samples, was accompanied with metabolic syndrome. Conclusion: The results confirm that in metabolic syndrome patients.

These genetic mutation in CEPT gene are accompanied with change in lipids profile and other metabolic syndrome parameters.Our study suggest that the promoting effect of I405V polymorphism in process of metabolic syndrome disorder. We obtained that this polymorphism can increase occurrence of metabolic syndrome. Our results showedI405V polymorphism are associated with some MS-associated variables in our population

Key words: Metabolic Syndrome, polymorphism, Iran

INTRODUCTION

Metabolic syndrome (MS) is a potential threatening factor for cardiovascular disorders and atherosclerosis which is accompanied by some risk factors such as: central obesity (waist circumference >102 cm in men and >88 cm in women), low high density lipoprotein (HDL) cholesterol (<40 mg/dl in men and<50 mg/dl in women), hypertriglyceridemia (triglycerides>150 mg/dl), elevated blood pressure (>130/85 mmHg or taking anti-hypertensive therapy for control of blood pressure), and impaired blood glucose (fasting blood glucose >110 mg/dl) (1-4) various other abnormalities of uric acid, inflammation, hemostasis, and fibrinolysis are often considered part of this syndrome. Hypertriglyceridemia and low HDL-C are related with small dense–low-density lipoprotein (LDL) (5-8). CETP is an enzyme that plays a important role in HDL-C metabolism by shuttling cholesteryl esters (CEs) from HDL particles to apolipoprotein B (Apo B) containing particles in exchange for triglycerides (9-12). It collects triglycerides from very-low-density (VLDL) or low-density lipoproteins(LDL) and exchanges them for cholesteryl esters from high-density lipoproteins (HDL) (13-16). Most of the time, however, CETP does a heteroexchange, trading a triglyceride for a cholesteryl ester or a cholesteryl ester for a triglyceride, in fact Plasma lipoproteins are continuously remodeled through the actions of this enzymes.(17-20) Evidence suggests that certain CETP polymorphic sites may be linked to coronary artery disease, as well as hypertension.

The present survey attempts to show the association of I405V polymorphism in in CETP gene from individual with metabolic syndrome in an Iranian populations. (21-24).

The relationship between I405V polymorphism and parameters of metabolic syndrome may be exist or not (25-29). We have therefore investigated the effects of I405V polymorphism on plasma lipid profile and metabolic syndrome parameters this study was carried out in a population of patients with and without metabolic syndrome.

MATERIALS AND METHODS

In order to identify the association between the I405V polymorphism of CETP gene and the metabolic syndrome parameters a case–control study included two group(120 individual with metabolic syndrome as cases and 200 healthy subjects as controls) from Iranian population performed. Thesubjects with metabolic syndrome had at least three of thefollowing five components: central obesity (waist circumference >102 cm in men and >88 cm in women), low high density lipoprotein (HDL) cholesterol (<40 mg/dl in men and<50 mg/dl in women), hypertriglyceridemia (triglycerides>150 mg/dl), elevated blood pressure (>130/85 mmHg) (1-4). Patient demographic parameters such as sex, age, systolic and diastolic blood pressure, waist circumference, body weight, and BMI were recorded. Venous blood samples were extracted from each subject afteran overnight fast of 12 h. A serum sample was analyzed for fastingserum glucose, lipid profile (triglycerides, totalcholesterol, HDL-C and LDL-C). The plasma levelof triglyceride, total cholesterol, HDL-C and fasting bloodsugar,LDL-Cwas determined using spectrophotometry and commerciallyavailable kits (Pars Azmoun, Iran). Genomic DNA was isolated from leukocytes afterlysis of the erythrocytes from the blood cell,DNA was extracted using standard DNA extraction kits(Cinagen, Iran) and the quality and quantity of the extracted DNA with spectrophotometric assay and electrophoresistechniques determined .The CETP I405V genotype was replicate the 553 bp sequence of I405V in the exon 14 of CETP

gene. The PCR reaction was carried out ina total volume of 50uldetermined by PCRamplification using following Mismatch primers:

I405V*:*

F: 5'-CTG TTT CCA ACT TGA CTG AG-3'

R: 5'- CAG CGG TGA TCA TTG ACT GCA GGA AGC TCT GTA-3

PCR technique was used to0.5µg genomic DNA, 5µlBuffer 10x, pH 8.4,1.2 µl MgCl2, 150µmoldNTPs, 500nmol of each primer and 0.4 UTaq DNA polymerase. Then follows 15ul of products were incubated with 2U of Rsa1 restrictionenzyme at 37°C for 4 h.Finally the products of the I405V digestion were electrophoresedon 1.5% agarose gel.The thermo cycler conditions after optimizing the techniquewere: initial denaturation at 95°C for 10 min followedby 35 cycles of amplification, each cycle consistingof 60 s at 95°C, 60s at 60°C and 90 s at 72 °C, The reaction endedwith an additional 10 min of extension at 72° C.The PCR resulted in an amplified product of553bp and was digested using the restriction enzyme Rsa1,The presence of the I405V restriction site produces the V allelepattern in electrophoresis with 2 DNA fragments at 520 and33bp. The lack of I405V restriction site results in the IIpattern with only one band at 553bp. The IV genotypes resultedin three bands at 553, 520 and 33 bp.data were shown as mean ± standard deviation (SD). Students T-test was used for the comparison of lipid parameters between two studied groups. Correlations between CETP activity and lipid parameters were determined using Pearson test.for analyze experimental findings in different genotypes groupsmultiple Tukey tests and analysis of variance between cases and controls were performed. All statistical analysis was performed using SPSS software (Version 11, USA) and P<0.05 wasconsidered significant.

RESULTS

The study wasperformed during a period of one years. A total of 220 healthysubjects mean age 46.8 ± 9.3years and metabolic syndrome patients mean age49.1±10.9years were included for the genotyping studies.Informed consent has been taken from study subjects. Demographic and biochemical characteristics of the studied subjectsare presented in Table 1. Age and sex were similar in two groups.

Table 1. Demographic and biochemical characteristics of the studied subjectsin two groups.

Parameters	MS	Controls	P Value
Number of participants	120	220	_
Age (year)	49.1±10.9	46.8 ± 9.3	P>0.05
WC (cm)	114.1±8.9	84.1±7.3	P<0.05
SBP (mmHg)	135.6±13	109±13	P<0.05
DBP (mmHg)	90.2±6.1	73.9±4.2	P<0.05
FBS (mg/dl	122.1±40.3	90.4±10.1	P<0.05
TG (mg/dl)	310±117.9	139.6±49	P<0.05
HDL-C	46±5.1	57.1±11.9	P<0.05
T C	225±31.2	162±23.9	P<0.05
LDL-C	112±29.8	86.2±18.1	P<0.05

Data are expressed as mean ± standard deviation. WC: Waist circumference; sBP: systolic blood pressure; dBP: diastolic blood pressure; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride,FBS:fasting blood sugar. P<0.05 considered as significant level.

CETP activity in two groups of participants (metabolic syndrome and controls) presented in table 2.

Table 2.CETP activity in two groups of participants (metabolic syndrome and controls)

p-value	MS N=120	controls N=220	specificity
P<0.05	134 ± 33.2	97.4 ±28.9	CETP activity (pmol/µl.h)

Parameters	II	VI	VV	Pvalue
Number of participants	30	67	23	_
SBP (mmHg)	120.4 ± 12.9	128.4± 9.7	134.6 ± 14.2	P<0.05
DBP (mmHg)	84.2± 3.6	85.7 ± 8.9	89.2 ±6.2	P>0.05
FBS (mg/dl)	89.3± 22.6	112.4 ± 8.2	120.0 ± 37.3	P<0.05
TG (mg/dl)	280 ± 87.7	301.2 ± 105.7	317.5 ± 120.3	P<0.05
TC (mg/dl)	186.2 ± 12.9	212.6 ± 20.9	236.9 ± 17.8	P<0.05
HDL-C	45.8 ± 5.3	44.2 ± 4.1	46.1 ± 9.2	P>0.05
LDL-c	88.6 ± 29.4	97.2 ± 37.2	119.7 ± 30.1	P<0.05

Data are expressed as mean ± standard deviation
MS: metabolic syndrome
CETP activity:Cholesteryl ester transfer protein activity. P<0.05 considered as significant level.

CETP activity levels were significantly higher in patients compared to controls. Also there was significant high levels of total cholesterol, LDL-c, and, fasting blood sugar (FBS), systolic and diastolic blood pressure, and low level of HDL-c in patients group compared to controls. Clinical parameters, in two patient group in three genotypes VV, VI, and II of I405V polymorphism in CETP presented in Table 3.

Table 3. Clinical Parameters of CETPI405V genotype, inmetabolic syndrome patientss group
Data are expressed as mean ± standard deviation, FBS:fasting blood sugar; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride.P<0.05 considered as significant level.

Analysis of the I405V gene polymorphism revealed a significant difference in the distribution of the different genotypesbetween controls and patients groups (P <0.05). The patientgroup showed a significantly higher VI genotype and lower IIgenotype compared to the controls. The prevalence of the different I405V genotypes in both controlsand patients groups was in agreement with Hardy–Weinberg equilibrium. The percentage of the different I405V genotypes in the studied groups were: VV (19.1%% and 13.6%), VI (55.8% and 48.1%) and II
(25.1% and 38.3%) in the patients and controls respectively. When the levels of the different biochemical parameters in thetwo groups were stratified according to the genotype, serum triglyceride,systolic blood presure ,totallcholesterolFBSandLDL-c was significantly higher in all subjects with the VV genotypethan those with IIand VI genotypes in patients included in the study.Although the level of HDL-C was higher in patientswith VVgenotype but it did not reach statistical significance.(Table3). In the control group, triglyceride level were significantly higher in VV genotypeThe other parameters did notshow significant differences between the different genotypes.in our study there was significanteffect of this poly morphism on increased prevalence of metabolic syndrome (Table 4). The odds ratio for metabolic syndrome was 1.7 for the V allele. The frequencyof the alleles, was not significantly differentbetween the two groups (p value>0.05).

Table 4. Effect of I405V polymorphism genotype on prevalence of Metabolic Syndrome

P<0.05 considered as significant level.
CI:confidence interval

%CI 95	Odds ratio	Pvaluevariable
0.9-1.62	1.7	0.05<p/IIIV+VV

DISCUSSION

It is well established that Cholesteryl ester transfer protein(CETP) catalyzed transfer natural lipids and phospholipids between lipoproteins .(1-3).CETP involved in transfer of cholesterol esters from HDLCto the chylomicrons and VLDL remnants, also CETP can have a significant role in balancing the quantity of plasma lipids and lipoproteines(1-3,7-17).it is important to determine if the CETPprotein is associated with the I405V polymorphisms inCETP gene. The present survey attempts to show the association of CETP polymorphisms with parameters involved in metabolic syndrome in CETP gene in an Iranian populations. our study showed, The biochemical markers and metabolic syndrome parameters include wait circumstance, systolic blood pressure ,diastolic blood pressure ,serum total cholesterol,FBS, serum triglyceride,LDL-c,HDL-c Fasting blood sugar had significant difference In patient with metabolic syndrome compared to healthy controls.Indeed Patients with high plasma CETP activity had significantly higher total cholesterol, LDL cholesterol and triglycerides compared to those with lower CETP levels (controls). Patients with high CETP levels had also higher systolic and diastolic blood pressure, whereas HDL cholesterol levels were inversely correlated to CETP activity. We found a significant difference in the distribution ofthe different genotypes of the I405Vpolymorphism between controls and patients with metabolic syndrome.(P<0.05).There was no significant difference in the distribution of the allels between controls and patients.metabolic syndrome grouphadhigher frequency of I allele (53.1%) as compared to the V allele (46.9%) anda decreasing percentage frequency of the genotypesin the order VI>II >VV. there was positive effect between VV and high serum triglyceride,systolic blood pressure,LDL-c and total cholesterol in subjects with metabolic syndromeobserved(p<0.05). data also showed in subjects without metabolic syndrome with VV genotype higher levels of serum triglycerid observed. (p<0.05). In this study there was no significant correlation was observed between variousI405V genotypes and other parameters of metabolic syndrome.I405V polymorphism have promoting effect in process of metabolic syndrome disorder and can increase 1.7 fold the occurrence of metabolic syndrome.for later result our odd ratio were 1.7 and so there was significant difference between presence or absence of this polymorphism in metabolic syndrome patients. Our results showedI405Vpolymorphism areassociated with some MS-associated variables in our population. In fact I405V polymorphism may be an additional stigma of MS. Increased triglyceride,LDL-c,FBS,Systolic blood pressure and totalcholesterol observed in MS with this polymorphism was associated with emphasize the view of a proatherogenic role of I405V polymorphism in this patients.On the other hand, a recent study revealed significant differences in some component of lipid parameters betweengenotypes of I405V polymorphisms in an Iranian population.On the other hand, our study confirms the promoting effect of I405V polymorphism in process of metabolic syndrome disorder.

CONCLUSION

Our study suggests that the promoting effect of I405V polymorphism in process of metabolic syndrome disorder. We obtained that this polymorphism can increase occurrence of metabolic syndrome. Our results showedI405Vpolymorphism are associated with some MS-associated variables in our population. In fact I405V polymorphism may be an additional stigma of MS. Increased CETP activity , plasma triglyceride and LDL-c, totalcholesterol,systolic blood pressure, FBS observed in MS with this polymorphism was associated with emphasize the view of a proatherogenic role of I405V polymorphism in this patients.

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