

**Research Article****Investigation of I405V Polymorphism in the CETP gene and association with metabolic syndrome parameters in an Iranian population**

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[Received 21 Nov-2021, Accepted 20 June-2022, Published 25 June-2022]

**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 I405V polymorphisms in CETP gene with metabolic syndrome parameters in 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 I405V polymorphism. Statistical analyses were applied using SPSS software. **Results:** lipid pattern of plasma and other parameters of metabolic syndrome had significant difference 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 mutations in CETP gene are accompanied with change in lipids profile and other metabolic

syndrome parameters. 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 showed I405V 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 an 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 the CETP gene from individual with metabolic syndrome in an Iranian population. (21-24).

The relationship between I405V polymorphism and parameters of metabolic syndrome may 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 groups (120 individual with metabolic syndrome as cases and 200 healthy subjects as controls) from Iranian population performed. These subjects with metabolic syndrome had at least three of the following 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 after an overnight fast of 12 h. A serum sample was analyzed for fasting serum glucose, lipid profile (triglycerides, total cholesterol, HDL-C and LDL-C). The plasma level of triglyceride, total cholesterol, HDL-C and fasting blood sugar, LDL-C was determined using spectrophotometry and commercially available kits (Pars Azmoun, Iran). Genomic DNA was isolated from leukocytes after lysis 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 electrophoresis techniques determined. The CETP I405V genotype was replicated the 553 bp

sequence of I405V in the exon 14 of CETP gene. The PCR reaction was carried out in a total volume of 50  $\mu$ l determined by PCR amplification 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 to 0.5  $\mu$ g genomic DNA, 5  $\mu$ l Buffer 10x, pH 8.4, 1.2  $\mu$ l MgCl<sub>2</sub>, 150  $\mu$ mol dNTPs, 500 nmol of each primer and 0.4 U Taq DNA polymerase. Then follows 15  $\mu$ l of products were incubated with 2 U of RsaI restriction enzyme at 37°C for 4 h. Finally the products of the I405V digestion were electrophoresed on 1.5% agarose gel. The thermo cycler conditions after optimizing the technique were: initial denaturation at 95°C for 10 min followed by 35 cycles of amplification, each cycle consisting of 60 s at 95°C, 60 s at 60°C and 90 s at 72°C. The reaction ended with an additional 10 min of extension at 72°C. The PCR resulted in an amplified product of 553 bp and was digested using the restriction enzyme RsaI. The presence of the I405V restriction site produces the V allele pattern in electrophoresis with 2 DNA fragments at 520 and 33 bp. The lack

of I405V restriction site results in the I pattern with only one band at 553 bp. The IV genotypes resulted in three bands at 553, 520 and 33 bp. Data were shown as mean  $\pm$  standard deviation (SD). Student's 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. To analyze experimental findings in different genotypes groups multiple 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 was considered significant.

## RESULTS

The study was performed during a period of one year. A total of 220 healthy subjects mean age 46.8  $\pm$  9.3 years and metabolic syndrome patients mean age 49.1  $\pm$  10.9 years were included for the genotyping studies. Informed consent has been taken from study subjects. Demographic and biochemical characteristics of the studied subjects are presented in Table 1. Age and sex were similar in two groups.

**Table 1.** Demographic and biochemical characteristics of the studied subjects in two groups.

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

Data are expressed as mean  $\pm$  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.

p-value	MS N=120	controls N=220	specificity
P<0.05	134 ± 33.2	97.4 ± 28.9	CETP activity (pmol/μl.h)
Data are expressed as mean ± standard deviation MS: metabolic syndrome CETP activity: Cholesteryl ester transfer protein activity. P<0.05 considered as significant level.			

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

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.

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

**Table 3.** Clinical Parameters of CETPI405V genotype, in metabolic syndrome patients 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 genotypes between controls and patients groups (P <0.05). The patient group showed a significantly higher VI genotype and lower II genotype compared to the controls. The prevalence of the different I405V genotypes in both controls and 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 the two groups were

stratified according to the genotype, serum triglyceride, systolic blood pressure, total cholesterol, FBS and LDL-c was significantly higher in all subjects with the VV genotype than those with II and VI genotypes in patients included in the study. Although the level of HDL-C was higher in patients with VV genotype but it did not reach statistical significance. (Table 3). In the control group, triglyceride level were significantly higher in VV genotype. The other parameters did not show significant differences between the different genotypes. In our study there was significant effect of this polymorphism on increased prevalence of metabolic syndrome (Table 4). The odds ratio for metabolic

syndrome was 1.7 for the V allele. The frequency of the alleles, was not significantly different between the two groups ( $p$  value  $>0.05$ ).

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

% CI 95	Odds ratio	P value variable
0.9-1.62	1.7	$0.05 < p / IIIV + VV$

$P < 0.05$  considered as significant level.

CI: confidence interval

## 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 HDL to the chylomicrons and VLDL remnants, also CETP can have a significant role in balancing the quantity of plasma lipids and lipoproteins (1-3, 7-17). It is important to determine if the CETP protein is associated with the I405V polymorphisms in CETP gene. The present survey attempts to show the association of CETP polymorphisms with parameters involved in metabolic syndrome in CETP gene in an Iranian population. Our study showed, The biochemical markers and metabolic syndrome parameters include waist circumference, 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 of the different genotypes of the I405V polymorphism between controls and patients with metabolic syndrome ( $P < 0.05$ ). There was no significant difference in the distribution of the alleles

between controls and patients. Metabolic syndrome group had higher frequency of I allele (53.1%) as compared to the V allele (46.9%) and a decreasing percentage frequency of the genotypes in 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 syndrome observed ( $p < 0.05$ ). Data also showed in subjects without metabolic syndrome with VV genotype higher levels of serum triglyceride observed ( $p < 0.05$ ). In this study there was no significant correlation was observed between various I405V 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 showed I405V polymorphism are associated 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 total cholesterol 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 between genotypes 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 showed I405V polymorphism 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, total cholesterol, 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.

**Acknowledge:** None stated.

**Conflict of Interest:** No potential conflict of interest declared.

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