

## The relationship between cholesteryl ester transfer protein Taq I genotype and plasma level of HDL subgroups in coronary heart disease

SADEGH HASANNIA, SHIRIN JALILI and REYHANEH SARIRI\*

Department of Biology, The University of Guilan, Rasht (Iran)

(Received: April 04, 2008; Accepted: May 30, 2008)

### ABSTRACT

Cholesteryl ester transfer protein (CETP) catalysis the exchange of triglycerides (TG) and cholesterol esters among plasma lipoproteins. It has been shown that variations at CETP locus is important in the levels and activity of CETP and high density lipoprotein (HDL) plasma concentration. In this research, we assessed the relationship between TaqIB CETP polymorphism and high density lipoprotein-cholesterol (HDL-C) concentration in a study sample of 128 Iranian residents.

Based on our investigations, it was shown that presence of B<sub>1</sub>B<sub>1</sub> genotype related to TaqIB polymorphism was negatively correlated with HDL-C and HDL<sub>2</sub>-C, but positively correlated with LDL-C. On the other hand, no correlation with HDL<sub>3</sub>-C was observed. Analysis of HDL-C subfraction in CHD and control subjects revealed that variation of the CETP TaqI B locus was significantly associated with concentration of HDL<sub>2</sub>-C subclass. Homozygous and heterozygous carried the B<sub>2</sub> alleles, compared with B<sub>1</sub> homozygote had significantly elevated concentration of cholesterol distribution in HDL. We concluded that variation at the CETP gene locus is a significant determinant of HDL-C levels and CETP activity. Moreover, these effects appear to translate into a lower CHD risk among patients with B<sub>2</sub> allele.

**Key words:** Cholesteryl ester transfer protein, Gene Polymorphisms, HDL-C, Taq1B, RFLP.

### INTRODUCTION

CETP catalysis the exchange of triglycerides (TG) and cholesterol esters among plasma lipoproteins, a key step in reverse cholesterol transport (RCT) in humans. Although the antiatherogenic properties of HDL have not been fully elucidated, it has been suggested that HDL exerts its cardioprotective function through RCT<sup>1</sup>. RCT is a metabolic pathway initiated by HDL-mediated efflux from peripheral tissues and subsequent delivery to the liver<sup>2</sup>. In human, CETP mRNA encodes a polypeptide of M<sub>r</sub> 53000, which is N-glycosylated at 4 sites, giving rise to the mature form of CETP of M<sub>r</sub> 74000 [1]. CETP is a hydrophilic glycoprotein that is secreted mainly from the liver and that circulate in plasma, bound to HDL. Some lower levels of the protein is also secreted from spleen, and adipose tissues, and lower levels have been detected in the small intestine, adrenal gland, heart, kidney, and skeletal muscle<sup>2</sup>. The CETP gene

encompasses 16 exons, and it is located on chromosome 16q21 adjacent to the lecithin-cholesterol acyltransferase gene. CETP net transfer of cholesterol from HDL to very low density lipoprotein (VLDL), low density lipoprotein (LDL) and chylomicron in exchange for triglycerides (TG)<sup>3</sup>. The importance of CETP activity in the metabolism and composition of HDL particles is clearly demonstrated in individuals with genetic CETP deficiency<sup>4-6</sup>. The restriction site, TaqI B polymorphism of CETP, is an important regulatory factor of lipid metabolism especially in plasma HDL-C and LDL-C levels in patient with CHD<sup>7,8</sup>. HDL-C levels have been shown to be intensively and independently correlated with the risk of CHD. It has been shown that high HDL-C equal to or greater than 60 mg/dL as a negative risk factor for CHD.

In this study, we assessed the relationship between TaqIB CETP polymorphism and high density lipoprotein-cholesterol (HDL-C) concentration

in a study sample of 128 patients with CHD from Heshmat heart hospital in Rasht.

## MATERIAL AND METHODS

### Subjects

The study population consisted of 142 patients selected based on the result of their angiography from heart center of Heshmat hospital in Rasht. A total of 14 patients were excluded from the study due to having one or more of the following factors:

1. Diabetes melitus (fast blood sugar FBS>140 mg/dL)
2. History of using insulin or oral antibiotics
3. Miocardic heart attack within the last 6 weeks
4. The use of lipid lowering drugs
5. History of cronary surgery
6. History of major sugeries within the last 6 weeks
7. History of surgery, lack of activities and being in hospital during the last 4 weeks.

After these exclusions, 128 subjects remained eligible for this study. Informations on smoking, eating habits and exercise were obtained by interview.

### Plasma lipid and lipoproteins

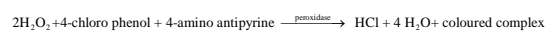
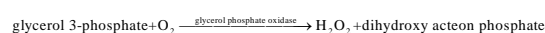
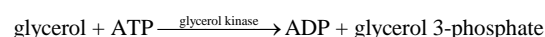
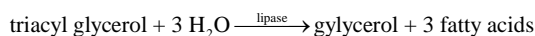
Blood samples were obtained by venipuncture into vacum tubes containing EDTA after an overnight (14 hours) fast. Plasma was immediately centrifuged at 2000 rpm for 15 minutes and the samples were delivered immediately (4°C) to our laboratory and stored at -20°C until use for lipid measurement and genotyping. Plasma total cholestrol (TC) and TG levels were measured by enzymatic methods, while the lipoprotein subgroups were measured using polyanion precipitation method.

### Determination of total cholestrol (TC)

In this enzymatic method, total plasma cholestrol was converted to free cholestrol by the action of cholestrol esterase. The free cholestrol was then oxidized to cholest-4-ox-3-one and the librated  $H_2O_2$  produced a coloured complex from phenolic substrate in the presence of peroxidase. The intensity of the colour was related to the total plasma cholestrol which was measured at 570 nm using a known cholestrol solution as standard.

### Determination of plasma triglyceide ( TG)

The following enzymatic reaction were the basis of the procedure used for determination of plasma triacyl glycerols. The intensity of the coloured complex was measured spectrophotomerically at 500 nm in the presence of a standard triglyceride solution.



### Isolation of HDL from other lipoproteins

100 ml DS-MgCl<sub>2</sub> (dextran sulphate 10g/lit and MgCl<sub>2</sub> 500mmol/lit) was added to 1 ml plasma. The test tube was left at room tempreature for 10 minutes after mixing for 3 seconds. The tube was kept at 4°C for 30 minutes,centrifuged at 1500 rpm and the concentration of cholestrol was then measured in the supernatant. Another portion of the supernatant was used for separation of HDL subunits.

### Measurement of HDL<sub>2</sub> and HDL<sub>3</sub>

50 ml of SD-MgCl<sub>2</sub> solution was added to 0.5 ml of the supernatant and the mixed for 3 seconds. The tube was centrifuged at 4°C after remaining at room temperature for 10 minutes. The supernatant contaning HDL<sub>3</sub> was used for appropriate measurement. The quantity of HDL<sub>2</sub> was then obtained by substracting this amount from HDL-C.

### TaqIB polymorphism of CETP gene

Genomic DNA was extracted from blood leukocytes by a simple salting out method described by Muller<sup>9</sup>. A fragment of 535 bp in intron 1 of the CETP gene was amplified with polymerase chain reaction (PCR) in a DNA Thermal Cycler (PTC-100, MJ Research, Inc) using ologonucleotide primers (forward 5'-CACTAGCCCAGAGAGAGGAGTGCC-3', reverse 5'-CTGAGCCCAGCCGCACACTAAC-3'). Each amplification was performed by using 2 µl of DNA template in a volume of 25 ml containing 1 ml of each primer, 0.5 ml of DNTP, 1.1 µl MgCl<sub>2</sub>, 0.5 µl Taq polymerase and 16.5 µl deionized water. DNA templates were denatured at 95°C for 10 minutes,

and then each PCR was subjected to 30 cycles with a temperature cycle consisting of 95°C for 3 minutes, 62 °C for 35 seconds, 72°C for 45 seconds, and finally, an extension at 72°C for 10 minutes. The PCR products were subjected to restriction enzyme analysis by digestion with 1.5 µl of the restriction endonuclease TaqI for 10 µl of PCR sample at 65°C for 16 hours in the buffer recommended by the manufacturer, and the fragments were separated by electrophoresis on an 1.5% agarose gel. Gel-duct instrument was used for visualization of DNA fragments at the end of electrophoresis run. The resulting fragments were 174 and 361 bp for the B1 allele and 535 bp for the uncut B2 allele.

**Statistical analyses**

Statistical analysis was carried out using one of the most common and reliable softwares i.e. statistical analysis system (SAS) software. A sensitivity analysis was carried out to estimate the validity and precision of the regression coefficients for the CETP genotype variables when additional independent terms were included into the model.

**RESULTS AND DISCUSSION**

**Subject characteristics**

We analyzed a total of 142 subjects, 43 controls and 85 patients who were registered in Heshmat heart hospital of Rasht and diagnosed with CHD according to the results of their angiography. The frequency and phenotype association of the TaqIB-CETP polymorphism was investigated at the population level. Table 1 represents a summary of

**Table 1. Biochemical characteristics of CHD and control group**

Concentration (mg/dl)	Control n=143	CHD n=85	P value control vs CHD
TC	169.349	216/765	0.0001
TG	142.14	190.18	0.0001
LDL	111	146.835	0.0001
HDL	41.95	34.23	0.0018
HDL <sub>2</sub>	13.48	6.81	0.0001
HDL <sub>3</sub>	28.43	27.52	0.9500

**Table 2: The relationship between genotyping and plasma lipids and lipoproteins in control and CHD groups**

Lipid lipoprotein (mg/dl)	TaqIB- CETP polymorphism					
	Control		CHD			
	B <sub>2</sub> B <sub>2</sub> n=19	B <sub>1</sub> B <sub>1</sub> n=7	B <sub>1</sub> B <sub>2</sub> n=17	B <sub>2</sub> B <sub>2</sub> n=12	B <sub>1</sub> B <sub>1</sub> n=47	B <sub>1</sub> B <sub>2</sub> n=26
TC	170.26±40.49	160.71±14.40	171.88±26.28	208.42±25.14	228.51±39.92	199.38±35.69
TG	144.37±53.02	129.86±2597	144.7±51.27	204.5±70	149.55±55.23	175.65±85.32
LDL-C	112.42±30.23	101.43±1578	113.35±19.45	136.8±38.40	161.42±38.23	125.42±40.66
HDL-C	40.84±11.13	37±7.07	45.23±15.35	35.58±5.67	32.70±6.84	36.38±7.55
HDL <sub>2</sub> -C	14.35±3.35	11.09±1.14	13.47±3.84	7.42±2.24	6.37±1.30	7.33±1.85
HDL <sub>3</sub> -C	26.38±8.15	25.90±6.21	31.75±11.64	28.15±4.08	26.48±5.66	29.13±6.38
frequency	42.2	16.3	39.5	14.1	55.3	30.6

the biochemical characteristics of the participants. The mean age of both control and CHD group was 51.5 and 51.6 respectively, all subjects were male and non-smokers. It is evident from the data in Table 1 that there is a noticeable difference in HDL<sub>2</sub>, HDL-C, LDL, TG and TC values between the two groups (CHD and control). On the other hand, the values of HDL<sub>3</sub>-C show negligible differences with a p-value of 0.95 compared to 0.0001 in other cases.

#### The effect of TaqIB polymorphism with plasma lipids and lipoproteins

The relationship between genotyping and biochemical characteristics in control and CHD groups was investigated using Logistic regression analysis with the GLM procedure and SAS software and the results are presented in Table 2. The results presented in Table 2 demonstrate that homozygotes for the B<sub>1</sub> allele had lower HDL-C than levels did B<sub>1</sub>B<sub>2</sub> and B<sub>2</sub>B<sub>2</sub>. It has been shown that a similar association exists for apo-A values and that the results are similar between male and female subjects in a similar age grouping<sup>6</sup>.

#### CONCLUSIONS

According to the results obtained from this study, the following conclusion remarks could be made:

1. Presence of B<sub>1</sub>B<sub>1</sub> genotype related to TaqIB polymorphism was negatively correlated with HDL-C and HDL<sub>2</sub>-C, and positively correlated with LDL-C.
2. There was no correlation between B<sub>1</sub>B<sub>1</sub> genotype and HDL<sub>3</sub>-C. Analysis of HDL-C subfraction in CHD and control subjects revealed that variation of the CETP TaqI B locus was significantly associated with concentration of HDL<sub>2</sub>-C subclass.
3. Homozygous and heterozygous carried the B<sub>2</sub> alleles, compared with B<sub>1</sub> homozygote had significantly elevated concentration of cholesterol distribution in HDL.
4. Variation at the CETP gene locus is a significant determinant of HDL-C levels and CETP activity.
5. Moreover, these effects appear to translate into a lower CHD risk among patients with B<sub>2</sub> allele.

#### REFERENCES

1. Ikewaki K., Mabuchi H., Teramoto T., et al. association of cholesteryl ester transfer protein activity and TaqIB polymorphism with lipoprotein variation in Japanese subjects. *Metabolism*. **52**(12): 1564-70 (2003).
2. Expert panel on detection, evaluation and treatment of high blood cholesterol in adults; Executive summary of the Third Report of the National Cholesterol Education Program (NECP). *JAMA*. **285**: 2486-97 (2001).
3. Tall A.R. Plasma cholesteryl ester transfer protein and high density lipoproteins: New insights from molecular genetics studies. *J. Intern. Med*. **237**: 5-12 (1995).
4. Yamashita S., Matsuzawa Y, Okazaki M., et al. Small polydisperse low density lipoproteins in familial hyperalphalipoproteinemia with complete deficiency of cholesteryl ester transfer activity. *Atherosclerosis*. **70**: 7-12 (1988).
5. Ikewaki K., Rader D.J., Sakamoto T., et al. Delayed catabolism of high density lipoprotein apolipoprotein A-I and A-II in human cholesteryl ester transfer protein deficiency. *J. Clin. invest*. **92**: 1650-58 (1993).
6. Ikewaki K., Nishiwaki M., Sakamoto T., et al. Increased catabolic rate of low density lipoproteins in human with cholesteryl ester transfer protein deficiency. *J. Clin. Invest*. **96**: 1573-81 (1995).
7. Kuivenhoven J.A., Jukema J.W., Zwinderman A.H., et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. The regression Growth Evaluation Statin Study Group. *New Eng. J. Med*. **338**: 86-93 (1998).
8. Ordovas J.M., Cupples L.A., Corella D., et al. Association of cholesteryl ester transfer protein-TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk. The Farmingham Study. *Arterioscler Thromb Vase Biol*. **20**: 1323-29 (2000).
9. Müller S.A., Dykes D.D., Polesky H.F. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Res*. **16**: 1215-19 (1989).