

Analysis of the Association Hind III Polymorphism of Lipoprotein Lipase Gene on the Risk of Coronary Artery Disease

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Abstract

Background: Coronary artery disease (CAD) is one of the leading causes of death and disability around the world. Interaction between genetic and environmental factors determines susceptibility of an individual to develop coronary artery disease. Lipoprotein lipase (LPL) plays an important role in the metabolism of HDL-C (High Density Lipoprotein Cholesterol), LDL-C (Low Density Lipoprotein Cholesterol) and triglycerides (TG). Dysfunction of LPL as a result of genetic variants of lipoprotein lipase gene is associated with increased risk of CAD. The aim of the present study was to investigate the relationship between the risk of coronary artery disease and LDL-C, HDL-C and TG (triglycerides) levels by lipoprotein lipase gene *Hind III* polymorphism.

Materials and Methods: A total of 202 subjects including 114 patients with coronary artery disease and 88 controls participated in this study. The *Hind III* polymorphism of the lipoprotein lipase gene was determined by PCR- RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism). In the presence and absence of restriction site, the genotypes are described H+/+, H-/- respectively.

Results: In this survey, a significant association between the frequent H+/+ genotype and unfavorable TG levels was observed in our population. For the *Hind III* genotypes, within the healthy subjects (n=88), the H+/+ genotype was found in 67 individuals (58.8%), H-/+ genotype in 38 individuals (33.3%), and 9 individuals (7.8%) carried the H-/- genotype. Within the CAD group (n=114), 47 individuals (53.4%) with H+/+ genotype, 36 (41%) with H-/+ genotype, and 5 (5.6%) carried the H-/- genotype.

Conclusion: There was no significant difference between the distribution of LPL-*Hind III* genotypes and the healthy subjects and the patients with CAD (P<0.05, 0.645). The study of LPL genotypes confirms the existence of interrelations between TG levels (P<0.05), but this polymorphisms were not detected as independent risk factors for CAD (P<0.05).

Keywords: Coronary artery disease (CAD); Lipoprotein lipase (LPL); Hind III Polymorphism; RFLP; Dyslipidemia

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Introduction

Coronary artery disease (CAD) and clinical manifestation of myocardial infarction (MI) is the

major cause of death in the world (1). It is a complex disease which is influenced by environmental factors

as well as genetic factors (2). Interaction between genetic and environmental factors, are determined to develop the CAD (3, 4). Among different groups, in particular, mutations and genomic polymorphisms of genes involved in lipid metabolism, causing changes in plasma lipoprotein levels, have specific role on the risk of coronary artery disease (5).

One of the most important gene is lipoprotein lipase (LPL) gene on chromosome 8P22, the enzyme with a major role in lipoprotein metabolism (5-7). One of the most important polymorphisms in LPL gene results from replacement of a thymine (T) with a guanine (G) in intron 8 is *Hind III* polymorphism (7, 8). Allele H + (presence of cutting site, T) is associated with decreased LPL activity compared with allele H- (absence of cutting site, G) (7) and thus individuals carrying the allele H+ has been shown to increase the levels of triglycerides (TG), high LDL-C (Low Density Lipoprotein Cholesterol) and low HDL-C (High Density Lipoprotein Cholesterol) levels and increased risk of coronary artery disease (5, 9, 10). Some studies have shown a relationship between this polymorphism and coronary artery disease, lipoproteins level. In this study we investigated the association of *Hind III* polymorphism in LPL gene with CAD and dyslipidemia in the Iranian population.

Materials and Methods

We enrolled 202 participants (114 cases and 88 controls), who undergone a coronary angiography examination from Shahid Rajaie referance Hospital, Tehran, Iran.

Inclusion criteria for the cases were: 1) Age at diagnosis of CAD in patients, 55 years or younger in men and 65 or younger in women, 2) At least 50% of stenosis in one of major coronary artery, or one of their branches which have been confirmed by angiography, and also absence of other diseases.

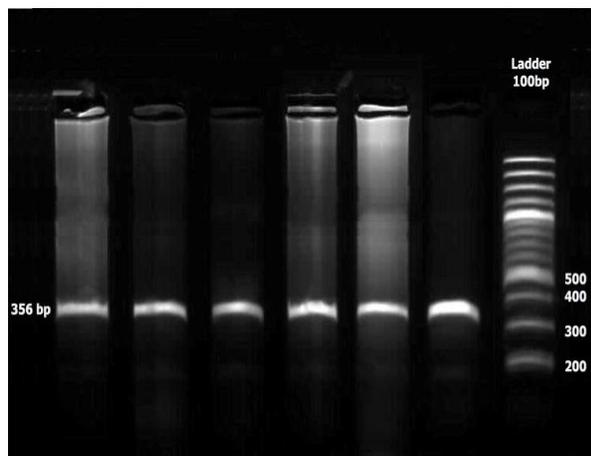


Figure 1A. PCR products *Hind III* polymorphism on agarose gel 2 %, amplified product is 356 bp

Our control samples were selected among those who had undergone angiography for reasons other than CAD and have normal coronary arteries.

All patients provided information about coronary risk factors such as diabetes mellitus, hypertension, hypercholesterolemia and cigarette smoking; Triglycerides, total cholesterol, high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) levels were measured by conventional methods of clinical chemistry. Arterial hypertension was defined as systolic blood pressure equal to or greater than 140 mm Hg and/or diastolic blood pressure equal to or greater than 90 mm Hg on more than one occasion. Patients with a history of diabetes or basal glycemia higher than 120 mg/dl were defined as diabetes.

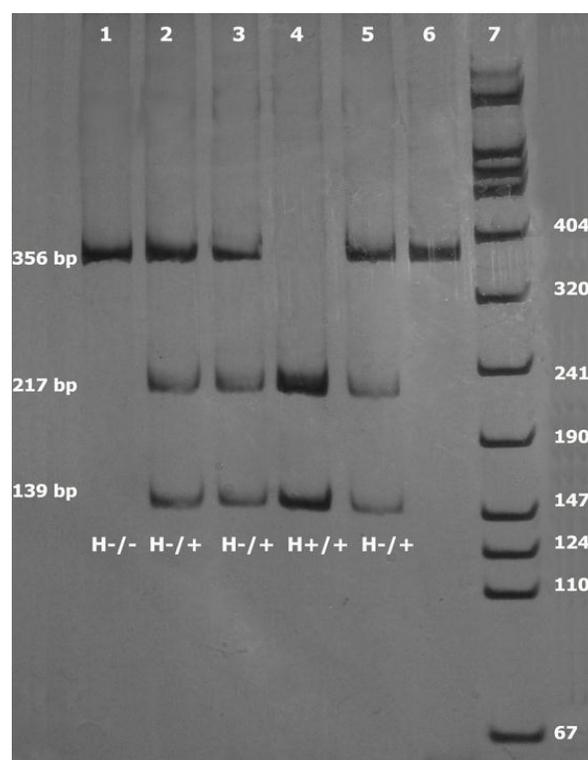


Figure 1B. RFLP on polyacrylamide gel 8 %: Lane 1.genoype H-/- (356 bp), Lane 2, 3, 5. Genotype H-/+ (356, 217, 139 bp), Lane 4.genotype H+/+ (217, 139 bp), Lane 6. Control, Lane 7. Marker VIII

Blood samples were taken using sample tubes containing EDTA (Ethylene dinitrotetra acetic acid, disodium salt dehydrate), and DNA extraction was performed using salting out.

Polymerase chain reaction–restriction fragment length polymorphism analysis

The 356 bp fragment which containing the *Hind III* polymorphism was amplified using the following primers: the primer sequences for *Hind III* polymorphism were forward primer 5' GATGTCTAC-

CTGGATAATCAAAG3' and reverse primer 5' CTT-CAGCTAGACATTGCTAGTGT3'(9).

DNA is amplified for 40 cycles, each cycle comprising predenaturation at 96 °C for 5 min, denaturation at 94 °C for 1 min, annealing at 57 °C for 1 min, extension at 72 °C for 1 min with final extension time of 5 min at 72 °C.

The PCR products were separated on a 2% agarose gel (Figure 1A). PCR products were digested with the fast digest restriction enzyme *Hind III* at 37 °C for 2

hour. In the presentation of restriction site for the enzyme (H+/+ genotype), PCR product is cleaved in to two fragments of 217 and 139 bp , whereas the absence of restriction site (H-/- genotype) shows a band of 365 bp (Fig 1B).

The validity of this PCR-RFLP analyses was confirmed by direct sequencing of several PCR samples with each genotype (Figure 1C).

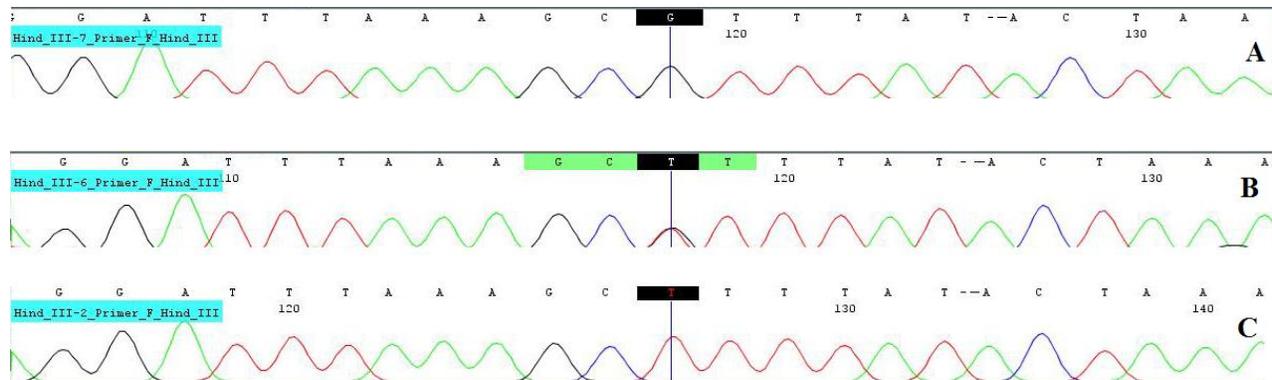


Figure 1C. A sample of sequencing result for confirming LPL genotyping], Arrow is the position of *Hind III* SNP [A: genotype GG or H-/- , B: genotype TG or H-/+ , C: genotype TT or H+/+

The statistical analyses

The R v.2.15.0 statistical program (for windows) was used to perform the analysis of variance, logistic regression analysis, and the Student T-test. Results were considered significant if

p-value was < 0.05 . Genotype distribution was investigated in relation to Hardy-Weinberg equilibrium.

Results

Table 1 shows the genotype *Hind III* polymorphism in patients with CAD and controls group. The characteristics of the subjects are summarized in Table 2.

Table 1. Comparison of the genotype frequencies *Hind III* polymorphism in Cases and Controls

H-/- : Absent restriction site (Homozygote genotype)
 H+/+ : Present two restriction site (Homozygote genotype)
 H+/- : Present one restriction site (Heterozygote genotype)

Genotype	Case	Control	Total	P- value
H+/+	67(58.8%)	47(53.4%)	114(56.4%)	0.43
H+/-	38(33.3%)	36(41%)	74(36.6%)	
H-/-	9(7.9%)	5(5.6%)	14(7%)	

P-value < 0.05

The patient group had a higher prevalence of hypertension, diabetes, smoking and family history of CAD compared with the controls. The patients also had higher BMI, total cholesterol, LDL-C and triglycerides levels.

According to our results; family history, hypertension, diabetes, smoking, obesity, high total cholesterol, LDL-C and triglycerides levels and low HDL-C were significantly increased risk Within the patients group (n=114), 67 individuals had genotype H+/+, 38 individuals with H-/+ and 9 individuals with H-/- , whereas in the controls group (n=88) 47 individuals had genotype H+/+, 36 individuals with H-/+ and 5 individuals with H-/- (Table 1)

There were no difference between the percentage (%) of the LPL-*HindIII* +/+, +/- and -/- genotypes among CAD patients and controls. For *HindIII* (+/+, +/- and -/-) LPL genotypes, the odd's ratio between CAD patients and normal controls was 0. 645 (95% CI: 0.2- 1.9) but the difference was not statistically significant.

Association analysis of systolic blood pressure , lipid levels and diabetes with *Hind III* polymorphism was performed by the analysis of variance (P<0.05).

Systolic blood pressure in the three genotypes are not statistically significant. This means that individuals with the corresponding genotypes had almost identical mean systolic blood pressure levels. However, the diastolic blood pressure in the three

groups was statistically significant, and the mean diastolic blood pressure of the H^{+/+} and H^{-/} - increases, so we expect people with H^{-/} - genotype, have slightly higher diastolic blood pressure compare with the other two genotypes (Table 2).

There is significant association between VLDL (Very Low Density Lipoprotein) & TG with LPL - *Hind III*

polymorphism. It means that individuals with H^{+/+} genotypes had high VLDL and TG compared with two other groups (Table 2). Also, there is a significant association between HDL (High Density Lipoprotein) with LPL-*Hind II* polymorphism.

Table 2. Different LPL genotypes in relation to clinical data of patients with Coronary Artery Diseases.

BMI: Body mass index, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol, TC: Total Cholesterol, TG: Triglycerides, VLDL-C: Very Low Density Lipoprotein Cholesterol

Variable	H ^{+/+}	H ^{-/+}	H ^{-/-}	F-value	p-value
Number of people	114	74	14		
Systolic hypertension (mmHg)	12.34±1.66	12.42±1.68	11.67±4.22	0.568	0.567
Diastolic hypertension (mmHg)	7.39±0.83	7.45±0.99	8.58±3.14	3.723	0.025*
LDL-C (mg/dl)	96.05±39.47	95.17±35.99	105.64±40.17	0.189	0.828
HDL-C (mg/dl)	40.40±12.26	39.39±11.21	40.93±16.01	0.201	0.818
VLDL-C (mg/dl)	31.14±25.69	23.14±11.68	26.53±9.84	3.308	0.038*
TC (mg/dl)	153.11±38.04	159.17±44.01	181.14±26.44	1.57	0.21
TG (mg/dl)	174.77±99.45	145.65±62.11	152.5±70.03	2.54	0.08*
BMI (Kg/m ²)	26.77±4.25	28.10±4.54	0.27±3.75	1.04	0.132
Diabetes	0.24	0.44	0.93	5.20	0.007*

* P-value <0.05

It means that individuals with H^{+/+} genotypes have not low HDL compare with two other groups (Table 2). People with H^{-/} genotype had higher blood glucose compared the two other groups, also individuals with this genotype have higher diastolic hypertension. According to regression analysis there was no significant association between LPL-*Hind III* polymorphism and CAD (P<0.05).

Discussion

Cardiovascular disease is the major cause of death in the most countries (11) and the first cause of death due to illness in Iran (12) and its prevalence is increasing in developing countries, also decreased plasma LPL activity is associated with high triglycerides and low HDL levels, which is often observed in patients with cardiovascular disease. Several studies revealed that there were various mutations on LPL gene and these mutations might be

a risk factor for CAD, high TG and hypertension. They also found that these polymorphisms differed largely among races due to large differences in the linkage pattern of *Hind III*, *Pvu II*, and *S447X* polymorphisms of LPL among races.

In this study, we investigated the relationship between *Hind III* polymorphism of LPL gene and CAD for first time in the Iranian population. The changes in LPL activity associated with *Hind III* polymorphism which characterized by the H⁺ or H⁻ (5, 7, 9). There are several studies of the relationship between *Hind III* polymorphism of LPL gene and risk of coronary artery disease. In this study, we investigated the relation between the *Hind III* polymorphisms and risk of coronary artery disease, dyslipidemia conditions.

In the present study, the frequency of genotypes H^{+/+}, H^{+/-} and H^{-/-} were 56.4%, 36.63% and 6.93%, respectively. This indicated that our results for the

H+/+ genotype frequency are similar to Croatian population (54.3%), Brazil population (52.33%), Saudi Arab (51.8%), Spanish population (50.41%) and Danish population (50.1%) but higher than, Egypt (38%) and California (47.3%), also lower than Japan (64.4%) (8, 9, 14, 16-18, 22).

In many studies, the relationship between the *Hind III* polymorphism and CAD has been determined. Study by Mattu et al. have showed a significant relationship between H + allele *Hind III* polymorphism and coronary artery disease (10). Study in Brazilian and Croatian CAD patients suggested that the frequency of the H+ allele was greater in CAD group than in the control subjects (17, 22). Also, Amer et al demonstrated subjects with coronary artery disease there was an association of the H+ allele to CAD (9). Another study by Abu-Amero et al. on the Saudi population could not found any association (9, 14). Our results were similar to Saudi population which there is no significant association between *Hind III* polymorphism and severity of CAD.

H+ allele is associated with increased plasma TG concentrations. Ariza et al in Spain have reported that the H+/H+ genotype is associated with high levels of TG which is significantly more frequent in patients with CAD than in controls (16). Also, In Croatian, Asian Indians and European populations, the H+/H+ genotype is a significant with high TG level (17, 20, 19). In our study there is significant association between the H + /H+ genotype and TG level.

Javosky et al, in the Slovak population, found an association between the LPL-*Hind III* +/+ genotype and Reduction of HDL levels (21).

Radha et al and Gerdes et al have showed a significant relationship between H+allele *Hind III* polymorphism with reduced plasma HDL concentrations (18, 19). Our findings showed that the H + allele was not associated with HDL level.

Study by Hemimi et al in Egypt showed a significant association between H+ allele and increase risk of hypertension (7), but our study showed different results which individuals with genotype H+/+ have reduced diastolic hypertension .

Conclusions

In the present study, the LPL- *Hind III* polymorphism can not be used as a genetic risk marker for CAD. In addition, no significant associations between this polymorphism with lower concentration of HDL-C were found but we found a significant association of the *Hind III* polymorphism on high TG.

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