The Association of Lipoprotein Lipase Genes, HindIII and S447X Polymorphisms With Coronary Artery Disease in Shiraz City

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Introduction
Lipoprotein lipase (LPL) found in 1943. This enzyme plays a central role in lipid metabolism by hydrolyzing triglyceride-rich particles in the muscles. Free fatty acids and glycerol have been produced by adipose tissue and macrophages for energy utilization and storage. The LPL gene is located on chromosome 8p22.100 mutations have been described in this gene. Several polymorphisms at the LPL locus are associated with variations in LPL activity serum lipid concentrations and the risk of coronary artery disease (CAD).

The aim of this study was to investigate the role of the LPL S447X and HindIII polymorphism in a sample of subjects with CAD and compare them with healthy subjects.

Methods: The study enrolled 115 patients and 89 healthy subjects who were recruited from Namazi hospital in 2010-2012. The presence of two common polymorphisms of the LPL gene (HindIII and S447X) was determined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis using genomic DNA. SPSS 16.0 was used for statistical analysis.

Results: S447X was significantly different between the patients with CAD and the healthy subjects (P<0.001). But HindIII was not significantly different between the patients with CAD and the healthy subjects (P=0.741). Risk factors such as smoking, hypertension, hyperlipidemia, triglyceride (TG) and high-density lipoprotein (HDL) levels had a significant association with CAD.

Conclusion: In our study, the presence of G allele S447X polymorphism increases the TG level and decrease HDL level, so it increases the susceptibility CAD. Moreover, HindIII polymorphism did not have any significant association with CAD.
Sample size was estimated upon primary study by MedCalc software with α=0.05 and 1−β=%80.

**DNA Isolation and Polymorphism Analysis**

Genomic DNA for polymerase chain reaction (PCR) was isolated from the whole blood by using salting out methods. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) determined the presence of two common polymorphisms of the LPL gene. The primer sets and procedures were selected from the published information as follows:

- HindIII forward primer: 5’-TGAAGCTCAAATGGAGT-3’, and reverse primer: 5’-TACAAGCAAATGAGCTAA-3’;
- S447X forward primer: 5’-TACACTAGCAATGTCTAGCTGAAGGCAGA-3’, and reverse primer: 5’-TCAGCTTTAGCCCAGAATGCTCACC-3’.

The MnLI restriction endonuclease (Fermentase) was used to detect Ser447Stop polymorphism. Homozygote of the mutation was designated (GG), that of the wild type was designated (CC), and heterozygote was also designated (CG). Also HindIII polymorphism was detected by digestion of PCR products with HindIII restriction endonuclease (Fermentase). Homozygote of the mutation was designated H+H+, that of the wild type was designated H−H−, and heterozygote was designated H+H−.

**Statistical Analysis**

Gene-Counting was used to calculate the frequencies of genotypes and alleles. SPSS 16.0 was used for statistical analysis. Comparisons of groups were performed using chi-square test. The binary logistic test was also used to further estimate the association between the polymorphism and the developing risk of CAD. P-value less than 0.05 was considered significant.

**Results**

The distribution of the HindIII genotype in the whole population was compatible with Hardy-Weinberg proportion (P=0.47), with allele frequencies of 77% and 23% for the H+ and H, respectively (Table 1). Also the distribution of the S447X genotype in the whole population was not compatible with Hardy-Weinberg proportion (P<0.001), with allele frequencies of 73% and 27% for the C and G, respectively (Table 1). Likewise as Table 2 shows the genotype distribution and allele frequencies for S447X and HindIII gene between CAD patients and control subjects.

### Table 1. Hardy-Weinberg Equilibrium Test of HindIII and S447x of LPL Gene

|        | Observed count | Expected count | Genotype frequency (%) | Allele | Observed count | Allele frequencies | \(X^2\) | \(P\) |
|--------|----------------|----------------|------------------------|--------|----------------|-------------------|--------|------|
| HindIII|                |                |                        |        |                |                   |        |      |
| H+H+   | 114            | 116.80         | 57.87                  | H+     | 302            | 0.77              | 0.524  | 0.47 |
| H+H−   | 74             | 69.78          | 37.56                  | H−     | 92             | 0.23              |        |      |
| H−H−   | 9              | 10.42          | 4.57                   |        |                |                   |        |      |
| Total  | 197            | 197            | 100.00                 |        | 394            |                   |        |      |
| S447X  |                |                |                        |        |                |                   |        |      |
| CC     | 133            | 108.71         | 65.20                  | C      | 296            | 0.73              | 82.96  | <0.001|
| CG     | 30             | 80.42          | 14.70                  | G      | 112            | 0.23              |        |      |
| GG     | 41             | 14.87          | 20.10                  |        |                |                   |        |      |
| Total  | 204            | 204            | 100.00                 |        | 408            |                   |        |      |

### Table 2. Allelic and Genotypic Frequencies as Well as Association of S447X Polymorphism and CAD

| S447X polymorphism | Control (n=89) | Case (n=115) | \(P\) | OR | 95% CI |
|--------------------|---------------|--------------|-------|----|--------|
| CC                 | 75 (84.3%)    | 58 (50.4%)   | <0.001|    |        |
| CG                 | 7 (7.9%)      | 23 (20.0%)   |       |    |        |
| GG                 | 7 (7.9%)      | 34 (29.6%)   |       |    |        |
| HindIII polymorphism |              |              |       |    |        |
| H+H+               | 53 (59.6%)    | 61 (56.5%)   | 0.741 |    |        |
| H+H−               | 33 (37.1%)    | 41 (38.0%)   |       |    |        |
| H−H−               | 3 (3.4%)      | 6 (5.6%)     |       |    |        |
| Allele frequency   |               |              |       |    |        |
| C                  | 157 (88.2%)   | 139 (60.4%)  | <0.001|    |        |
| G                  | 21 (11.8%)    | 91 (39.6%)   |       |    |        |
| H+                 | 139 (78.1%)   | 163 (75.5%)  | 0.540 |    |        |
| H−                 | 39 (21.9%)    | 53 (24.5%)   |       |    |        |
| S447X polymorphism |              |              |       |    |        |
| CC                 | 75 (84.3%)    | 58 (50.4%)   | <0.001|    |        |
| CG + GG            | 14 (15.7%)    | 57 (49.6%)   |       |    | 5.265  | 2.673-10.368 |
summarized in Table 3. The results presented in Table 3 indicate that these risk factors had a significant association with CAD (smoking: OR=3.256, P<0.001; hypertension: OR=1.952, P=0.025; hyperlipidemia: OR=2.347, P=0.004; TG>200 mg/dl: OR=2.345, P=0.004; HDL<40 mg/dl: OR=1.917, P=0.024). As shown in Table 2, S447X polymorphism had a significant association with CAD. Moreover, CG+GG genotype was an additive risk factor for CAD (OR=5.265, P<0.001). The interaction between S447X polymorphism and TG and HDL-C with CAD is shown in Table 4. The interaction between polymorphism S447X and demographic risk factors (hyperlipidemia, hypertension, and smoking) separately with CAD is shown in Table 5. The results indicate that there was a significant association with CAD.

### Discussion

CAD is a complex disease with genetic and environmental components. The results of most genetic studies of CAD are different, perhaps because the genetic risk of CAD is not based on a single gene but is based on interactions among multiple genes and environmental risk factors. LPL is a potential target for treatment of CAD because LPL gene variants are involved in multiple genes and environmental risk factors associated with CAD. One of the most desirable candidate genes is LPL gene that may explain some of the lipid and lipoprotein abnormalities faced in numerous cases of CAD. In this study, we presented the results on polymorphisms of the LPL gene, HindIII and S447X and their association with CAD and environmental risk factors including smoking, hypertension, hyperlipidemia, TG and HDL-C levels. To the best of our knowledge, it is the first study on the population of the south of Iran.

The frequency of H allele HindIII polymorphism is 22%. Sayad et al. reported that the frequency of H allele HindIII polymorphism was 30%. In a recent study, there was not any significant association between CAD and the control groups (P=0.540). Moreover, the frequency of G allele S447X polymorphism was 12% and there was an association between CAD and the control group (P<0.001). Therefore, we can conclude that S447X polymorphism is associated with CAD (P<0.001, OR=5.265, 95% CI=2.673-10.368). The presence of G allele is due to increased susceptibility to CAD. In this study, we investigated the associations of environmental risk factors with CAD. We could observe that as previous studies, in this study, there were associations between demographic risk factors (smoking, hypertension, hyperlipidemia, TG>200 mg/dl and HDL<40 mg/dl) and CAD. These risk factors were due to the increase in the susceptibility to CAD. Smoking had the most influence on this disease (smoking: OR=3.256, 95% CI=1.781-5.953; hypertension: OR=1.952, 95% CI=1.086-3.508; hyperlipidemia: OR=2.347, 95% CI=1.315-4.189; TG>200 mg/dl and HDL<40 mg/dl and CAD). These risk factors were due to the increase in the susceptibility to CAD.

### Table 3. Risk Factors for CAD in Patients and Controls

| Risk Factor | Control (%) | Case (%) | P | OR | 95% CI |
|-------------|-------------|----------|---|----|-------|
| Smoking     |             |          |   |    |       |
| No          | 66 (74.2)   | 52 (46.8)| <0.001 | 3.256 | 1.781-5.953 |
| Yes         | 23 (25.8)   | 59 (53.2)|           |       |       |
| Hypertension|             |          |   |    |       |
| No          | 62 (69.7)   | 60 (54.1)| 0.025  | 1.952 | 1.086-3.508 |
| Yes         | 27 (30.3)   | 51 (45.9)|           |       |       |
| Hyperlipidemia|           |          |   |    |       |
| No          | 60 (67.4)   | 52 (46.8)| 0.004  | 2.347 | 1.315-4.189 |
| Yes         | 29 (32.6)   | 59 (53.2)|           |       |       |
| TG <200 mg/dl | 11 (44.9)  | 17 (14.9)| <0.001 | 3.256 | 1.781-5.953 |
| >200 mg/dl  | 11 (44.9)   | 17 (14.9)|           |       |       |
| HDL >40 mg/dl | 11 (44.9)  | 17 (14.9)|           |       |       |
| <40 mg/dl   | 56 (62.9)   | 54 (47.0)| 0.024  | 1.917 | 1.090-3.372 |
| >40 mg/dl   | 33 (37.1)   | 61 (53.0)|           |       |       |

### Table 4. Association of S447X Polymorphism and TG and HDL Levels With CAD

| Polymorphism S447X | TG (mg/dl) | HDL (mg/dl) | Control (%) | Case (%) | P-value | OR | 95% CI |
|--------------------|------------|-------------|-------------|----------|---------|----|-------|
| CC                 | <200       | >40         | 40 (44.9)   | 17 (14.9)| <0.001 | -  | -     |
|                    | <40        | <40         | 11 (12.4)   | 8 (7.0)  | 0.326  | 1.711 | 0.585-5.004 |
|                    | >200       | >40         | 9 (10.1)    | 12 (10.5)| 0.030  | 3.137 | 1.116-8.822 |
|                    | <40        | <40         | 15 (16.9)   | 20 (17.6)| 0.011  | 3.137 | 1.304-7.545 |
| CG + GG            | <200       | >40         | 6 (6.7)     | 20 (17.6)| <0.001 | 7.843 | 2.678-22.966 |
|                    | <40        | <40         | 2 (2.3)     | 7 (6.1)  | 0.013  | 8.235 | 1.549-43.782 |
|                    | >200       | >40         | 1 (1.1)     | 4 (3.5)  | 0.052  | 9.412 | 0.979-90.518 |
|                    | <40        | <40         | 5 (5.6)     | 26 (22.8)| <0.001 | 12.235 | 4.021-37.226 |
Table 5. Association of S447X Polymorphism and Demographic Risk Factors With CAD

| Polymorphism | Risk factors | Control (%) | Case (%) | P       | OR      | 95% CI     |
|--------------|-------------|-------------|----------|---------|---------|------------|
| S447X        | Hyperlipidemia |             |          |         |         |            |
| CC           | No          | 52 (58.4)   | 26 (23.4) | <0.001  | -       | -          |
|              | Yes         | 23 (25.8)   | 30 (27.0) | 0.009   | 2.609   | 1.271-5.353|
| CG + GG      | No          | 8 (9.0)     | 26 (23.4) | <0.001  | 6.500   | 2.586-16.338|
|              | Yes         | 6 (6.8)     | 29 (26.2) | <0.001  | 9.667   | 3.566-26.202|
| S447X        | Hypertension |             |          |         |         |            |
| CC           | No          | 53 (59.6)   | 26 (24.1) | <0.001  | -       | -          |
|              | Yes         | 22 (24.7)   | 27 (25.0) | 0.014   | 2.502   | 1.202-5.206|
| CG + GG      | No          | 9 (10.1)    | 31 (28.7) | <0.001  | 7.021   | 2.918-16.896|
|              | Yes         | 5 (5.6)     | 24 (22.2) | <0.001  | 9.785   | 3.350-28.575|
| S447X        | Smoking     |             |          |         |         |            |
| CC           | No          | 55 (61.8)   | 31 (27.9) | <0.001  | -       | -          |
|              | Yes         | 20 (22.5)   | 25 (22.5) | 0.034   | 2.218   | 1.064-4.623|
| CG + GG      | No          | 11 (12.3)   | 21 (18.9) | 0.005   | 3.387   | 1.445-7.941|
|              | Yes         | 3 (3.4)     | 34 (30.6) | <0.001  | 20.108  | 5.704-70.877|

Conflict of Interests

There is no conflict of interest on this article.

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dl: P=0.024, OR=1.917, 95% CI=1.090-3.372).
Another analysis done in this study is the impact of each risk factor with S447X polymorphism. In all analyses, we could observe that the presence of G allele and environmental risk factor is due to the increased susceptibility to CAD.
About TG and HDL-C level, LPL gene has changed the TG and HDL level measurement. We could study the influence of both risk factors. The result is G allele, increasing TG level and decreasing HDL level in the case group. Therefore, we conclude that S447X polymorphism influences the plasma lipid levels. In several studies, S447X polymorphism has played a protective role in CAD although in a recent study the result is different.1,12,14,18 Perhaps, S447X polymorphism has affected another function in our population that increases the risk for disease and did not have any protection effect.
In conclusion, in our study, the presence of G allele S447X polymorphism increases the TG level and decrease HDL level, so it increases the susceptibility CAD. Moreover, HindIII polymorphism doesn’t have any significant association with CAD.
The limitation of this study is the number of our groups. It is recommended that in future studies, the number of samples should be more than our study.

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Ethical Issues
All patients gave written informed consents and the study was approved by our local ethic committee.
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