**Effects of lipoprotein lipase Pvu II gene polymorphism on serum lipoprotein levels in Turkish patients with type 2 diabetes mellitus**

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**Objectives:** Lipoprotein lipase (LPL) is an enzyme that plays a key role in lipid metabolism. As there are no existing data for the Turkish population, we investigated the effect of LPL Pvu II gene polymorphism on serum lipid profile in 52 patients with type 2 diabetes mellitus (T2DM) and 51 healthy controls in Turkish subjects.

**Methods:** Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphism (RFLP) and agarose gel electrophoresis techniques were used to determine the LPL Pvu II genotypes. Serum lipid levels were measured enzymatically.

**Results:** The genotype and allele frequencies of LPL Pvu II were not significantly different in type 2 diabetic and control groups (p>0.05). We found that serum levels of triacylglycerol (p<0.05) and VLDL-cholesterol (p<0.01) were significantly higher in the group of patients with type 2 diabetes than controls. In patient group, P(-/-) genotype had higher LDL-cholesterol levels than P(+/+) and P(+/-) genotypes (p=0.03). Also in control group, P(-/-) genotype had higher LDL-cholesterol levels compared with P(+/+) and P(+/-) genotypes, but not significantly (p=0.181).

**Conclusions:** In conclusion, our results suggest that the LPL Pvu II gene polymorphism is associated with high LDL cholesterol levels and atherosclerotic cardiovascular diseases in patients with T2DM in Turkish population.

**Key words:** Type 2 DM, lipoprotein lipase, polymorphism, LDL


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**Introduction**

The pathogenesis of atherosclerotic cardiovascular disease in patients with diabetes is complex and clearly multifactorial. The most common alterations in lipid and lipoprotein metabolism in the type 2 diabetes involve an elevation in both plasma triglyceride and VLDL-cholesterol concentrations, a dense LDL phenotype and low levels of HDL cholesterol.1-4

Because of LPL’s intimate involvement in the lipoprotein metabolism, the LPL gene is considered to be an important candidate gene in determining the risk factors levels in metabolic disorders, such as diabetes and atherosclerosis. Functional variants of genes coding for lipoproteins are responsible, in part, for the interindividual variation in the plasma levels of lipoproteins and thus, variation in the risk for atherosclerosis.5

One of the attractive candidates is the gene that codes for the lipoprotein lipase (LPL) enzyme, which is essen-
tial for clearance of triglyceride-rich lipoproteins such as chylomicrons and very low density lipoprotein-cholesterol (VLDL-C) from the plasma. LPL also plays a major role in exchange of lipids between VLDL and high density lipoprotein-cholesterol (HDL-C). The LPL activity is regulated by nutrients and hormones, for example, glucose and insulin. In diabetes mellitus, because of the lack of insulin or the resistance to the effect of insulin, the activity of LPL reduces.

The gene coding for LPL is located on chromosome 8p22, is comprised of 10 exons, and has a number of gene variants. Lipoprotein lipase gene variants have been found to correlate with lipid/lipoprotein concentrations, especially hypertriglyceridemia. Hypertriglyceridemia has been extensively associated with hypertension. However, the mechanism behind it is poorly understood. Patients with NIDDM are not only insulin resistant, but also have an increased prevalence of hypertension. In addition, insulin resistance is a common finding in patients with high blood pressure.

The most intensively investigated of LPL gene variants are the Pvu II and Hind III sites. The LPL Pvu II polymorphism is caused by a C-T transition within a Pvu II site of intron 6.

Several studies explored associations between LPL gene polymorphisms and lipoprotein phenotypes. Some studies provided evidence for an association between genotypes identified by the Pvu II restriction fragment length polymorphism (RFLP) and plasma triglyceride levels and blood pressures, but others failed to find significant association.

We therefore investigated the effects of Pvu II RFLP polymorphism on serum lipoprotein concentrations and blood pressures in patients with type 2 diabetes mellitus.

**Materials and Methods**

**Patients selection and clinical investigation**

The study population comprised 52 unrelated Turkish patients with NIDDM (24 male and 28 women), aged 55.76±10.46 attending the Taksim State Hospital in Istanbul who volunteered for the study. During ascertainment the WHO definitions and criteria for diabetes were used. The patients received a Standard questionnaire containing questions regarding the age at T2DM diagnosis, family history, the treatment method and other medical issues. Only patients with a clinical diagnosis of T2DM and a history of at least 2 years of treatment without insulin use were recruited. The study individuals underwent a basic physical examination that included the measurement of height, weight, and blood pressure.

Blood pressure was measured as recommended by the American Association. The subject lay on his spine for 10 min after the preasure was measured with a mercury sphygmonanometer. The readings were taken from the left and the right arm and recorded to the nearest 2 mmHg and the mean was calculated. The weight and the height were recorded and the body mass index was calculated using the formula BMI: weight/height² (kg/m²). Mean BMI was 26.15±4.01 kg/m².

The control group (28 male, 23 female, mean age: 54.83±12.92) contained only individuals with normal fasting glucose and negative family history of T2DM among first degree relatives. This group constituted mainly the spouses of T2DM patients and volunteers.

**Biochemical analyses**

Blood samples were drawn in tubes both plain and with EDTA after the subjects had fasted overnight. The samples were centrifuged for 10 min at 1500 x g at room temperature and plasma was removed. Plasma glucose levels were determined using an automated glucose oxidase method. Serum total cholesterol (TC), high density lipoprotein (HDL-C) and triacylglycerol concentrations were measured with an enzymatic colormetric assay. Serum HDL-C was measured by a cholesterol method following precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic acid and magnesium ions. LDL-C concentrations were calculated by using the Friedewald formula.

DNA isolation. Blood specimens were collected in tubes containing EDTA, and DNA samples were extracted from whole blood with salting out procedure. LPL Pvu II genotype was performed as described by Anderson et al.

Statistical analyses, using SPSS version 11.0, included the χ² test for genotype and allele frequency comparison. Lipoprotein and the blood pressures of different genotypes and alleles were compared by Student's t-test. A p-value of <0.05 was regarded as being statistically significant.
Results

Demographic characteristics are summarized in Table 1. There was a significant difference in triacylglycerol (p<0.05), VLDL-cholesterol (p<0.01), glucose (p<0.001), systolic (p<0.05) and diastolic blood pressures (p<0.01) between patients with T2DM and the control subjects.

Table 1
Demographic characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Control n=51 (male/female)</th>
<th>T2DM n=52 (male/female)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>28/23</td>
<td>24/28</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.83±12.92</td>
<td>61.74±10.46</td>
</tr>
<tr>
<td>Smoking (%) (yes/no)</td>
<td>62.5% 37.5</td>
<td>65.86/34.14</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.76±2.49</td>
<td>26.15±4.01</td>
</tr>
<tr>
<td>Total-cholesterol (mg/dl)</td>
<td>177.38±70.24</td>
<td>191.04±51.59</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>111.31±46.86</td>
<td>153.81±79.03*</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>125.36±60.01</td>
<td>118.35±41.27</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>51.16±64.66</td>
<td>41.70±17.75</td>
</tr>
<tr>
<td>VLDL-cholesterol (mg/dl)</td>
<td>22.38±9.25</td>
<td>31.71±16.27**</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>122.60±18.39</td>
<td>134.00±31.87*</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>73.65±9.99</td>
<td>83.22±19.49**</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>96.83±22.79</td>
<td>230.77±134.72***</td>
</tr>
</tbody>
</table>

Data were compared between groups by chi-square test and Fisher's exact test.

The genotypes distribution of LPL gene of present study is given in Table 2. The genotype and allele frequencies of LPL Pvu II were not significantly different in type 2 diabetic and control groups (p>0.05).

When the distribution of lipoprotein concentrations were compared in the sample groups, we found that serum levels of triacylglycerol (p<0.05) and VLDL-cholesterol (p<0.01) were significantly higher in the group of patients with type 2 diabetes than controls.

The relationship between LPL genotypes and serum lipoprotein concentrations, BMI and blood pressure in T2DM and control groups are shown in Table 3 and 4.

In patient group, P(-/-) genotype had higher LDL-cholesterol levels than P(+/+) and P(+/-) genotypes (p=0.03). Also in control group, P(-/-) genotype had higher LDL-cholesterol levels compared with P(+/+) and P(+/-) genotypes, but not significantly (p=0.181).

In diabetic group, frequency of hypertension (SBP>140mmHg and DBP>90mmHg) and BMI levels are not differed in LPL Pvu II genotypes (p>0.05). P(+) allele is more frequent among hypertensive patients compared with P(-) allele, but not statistically significant (p=0.305).

Discussion

Diabetes Mellitus is a multifactorial disease in which genetic and environmental factors play an important role. These factors may differ in each race and ethnic group.

Table 2
Distribution of LPL Pvu II genotypes and allele frequencies in study groups

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control n (%)</th>
<th>T2DM n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P (+/+)</td>
<td>18 (35.3)</td>
<td>19 (36.5)</td>
</tr>
<tr>
<td>P (-/-)</td>
<td>7 (13.7)</td>
<td>8 (15.4)</td>
</tr>
<tr>
<td>P (+/-)</td>
<td>26 (51.0)</td>
<td>25 (48.1)</td>
</tr>
</tbody>
</table>

Data were given X ± SD , *: p<0.05, **: p<0.01, ***: p<0.001.

Table 3
Effects of LPL polymorphism on serum lipid profile in study groups

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P (+/+)</td>
<td>174.62±70.16</td>
<td>118.62±49.77</td>
<td>157.56±77.66</td>
<td>118.62±51.56</td>
<td>51.16±64.66</td>
</tr>
<tr>
<td>P (-/-)</td>
<td>235.00±76.37</td>
<td>153.81±79.03*</td>
<td>158.35±41.27</td>
<td>118.35±41.27</td>
<td>31.71±16.27**</td>
</tr>
<tr>
<td>P (+/-)</td>
<td>170.18±63.7</td>
<td>122.60±18.39</td>
<td>107.63±46.86</td>
<td>118.35±41.27</td>
<td>31.71±16.27**</td>
</tr>
</tbody>
</table>

Data were given X ± SD , *: p<0.05, **: p<0.01, ***: p<0.001.
A number of previous studies have defined associations between the Pvu II polymorphism and plasma lipids and lipoproteins. It was observed that in our population, the distribution of LPL Pvu II genotypes is similar to the other populations (Table 5).

Anderson et al., investigated the Hind III and Pvu II polymorphisms in patients with coronary artery disease (CAD). They found associations with CAD for common LPL polymorphisms that were of moderate strength for carriage of the Hind III (+) allele (OR: 2.28) and of modest strength (trend only) for the Pvu II (-) allele (OR: 1.33).

In Finland population, Ukkola et al. studied the effect of variation at the lipoprotein lipase gene locus on the susceptibility of individuals with type 2 diabetes mellitus to atherosclerotic vascular disease in a population of 126 male and 114 female patients. They found that the prevalence of any evidence of coronary heart disease (CHD) (presence of ischaemic ECG changes or definite myocardial infarction) was low in the patients who were homozygous for the presence of the Pvu II restriction site -P(+/+) (%40.9) compared with those who were heterozygous-P(+/-) (%57.9; p=0.05) or homozygous for the absence of it -P(-/-) (%61.9; p<0.04).

### Table 4
**Effects of LPL polymorphism on body mass index and blood pressure in study groups**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>BMI (kg/m²)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control T2DM</td>
<td>Control T2DM</td>
<td>Control T2DM</td>
</tr>
<tr>
<td>P (+/+)</td>
<td>25.37±2.62</td>
<td>25.75±3.79</td>
<td>120.83±13.53</td>
</tr>
<tr>
<td>P (+/-)</td>
<td>23.87±2.20</td>
<td>27.83±5.82</td>
<td>136.00±47.75</td>
</tr>
<tr>
<td>P (-/-)</td>
<td>24.49±2.45</td>
<td>25.92±3.69</td>
<td>121.20±10.53</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alleles</th>
<th>BMI (kg/m²)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P (+)</td>
<td>24.83±2.52</td>
<td>25.85±3.66</td>
<td>121.04±11.73</td>
</tr>
<tr>
<td>P (-)</td>
<td>24.40±2.39</td>
<td>26.67±4.20</td>
<td>123.66±20.92</td>
</tr>
</tbody>
</table>

T2DM: Type 2 diabetes mellitus, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure. Data were given X ± SD , *: p<0.05

### Table 5
**Genotype numbers and frequencies of the RFLPs in the LPL gene in the Turkish and the other population**

<table>
<thead>
<tr>
<th>Population</th>
<th>LPL Pvu II polymorphism</th>
<th>(%)</th>
<th>(%)</th>
<th>(%)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkish (Present study)</td>
<td>(+)/-</td>
<td>26</td>
<td>18</td>
<td>7</td>
<td>13.7</td>
</tr>
<tr>
<td>Caucasian (Mattu et al., 2002)</td>
<td>(+)/-</td>
<td>107</td>
<td>79</td>
<td>55</td>
<td>33</td>
</tr>
<tr>
<td>Northern European (Anglo-Scandinavian) (Anderson et al., 1999)</td>
<td>(+)/-</td>
<td>76</td>
<td>60</td>
<td>32</td>
<td>19</td>
</tr>
<tr>
<td>Mediterranean migrants (from Italy and Greece) (Mitchell et al., 1984)</td>
<td>(+)/-</td>
<td>8</td>
<td>11</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>Danish (Gerdes et al., 1995)</td>
<td>(+)/-</td>
<td>229</td>
<td>100</td>
<td>133</td>
<td>28</td>
</tr>
<tr>
<td>French-Canadian (Quebec Family Study) (Ukkola et al., 2001)</td>
<td>(+)/-</td>
<td>167</td>
<td>83</td>
<td>74</td>
<td>23</td>
</tr>
</tbody>
</table>

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In contrast, Wang et al. found a significant association between the Pvu II polymorphism and the number of significantly diseased vessels (p=0.0099) and coronary score (p=0.028) with the Pvu II (-) allele associated with less severe disease. They found a close relationship between the Pvu II (+/-) genotype and the presence of diabetes (p=0.0025) with an OR of 3.12 compared with the Pvu II (-/-) genotype. They also found a dosage-dependent relationship between the Pvu II polymorphism and levels of TAG. The Pvu II (-) allele was associated with low levels and variances of triglycerides. They suggested that the LPL Pvu II polymorphism is significantly associated with CAD severity and with type 2 diabetes mellitus in CAD patients, independent of changes in circulating lipid levels.

Wu et al. have identified a genetic locus at or near the LPL gene locus which contributes to the variation of systolic blood pressure levels in nondiabetic family members at high risk for insulin resistance and NIDDM. They obtained significant evidence for linkage of systolic blood pressure to a genetic region at or near the LPL locus on the short arm of chromosome 8 (p=0.002).

Also a positive linkage signal between LPL and young-onset hypertension has been identified by Chen et al. They concluded that LPL variants might play a casual role in the development of hypertension in Taiwan Han Chinese.

Hagberg et al. have found that systolic (p=0.08) and diastolic (p=0.10) blood pressure reductions tended to be greater in P(+/-) (n=4) than P(+) and P(-/-) individuals (n=14) with exercise training. We don’t found that single nucleotide polymorphism in intron 6 (Pvu II) in the LPL gene associate with the high blood pressure and body mass index (BMI) in type 2 diabetic patients.

We found that P(-/-) genotype had higher LDL-cholesterol levels than P(+/+) and P(+/-) genotypes in our type 2 DM (p<0.05) and control groups. So, P(+/+) genotype has lower risk for atherosclerotic vascular diseases.

In the present study, we demonstrate that Pvu II polymorphism of the LPL gene has an important effect on serum LDL-cholesterol level in patients with T2DM and healthy controls in Turkey.

Our findings add substantially to previous evidence for an association between P(-) allele and atherosclerotic cardiovascular diseases. LPL Pvu II P(-/-) genotype may cause dyslipidemia in patients with T2DM contributing atherogenic lipoprotein profile. Our observations suggest that genetic variation in the lipoprotein lipase gene could be important in the development and progression of lipoprotein disorders in patients with type 2 diabetes mellitus.

Acknowledgements

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References


