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Lipoprotein Lipase Gene Polymorphism and Lipid Profile in Patients with Hypertriglyceridemia

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Aim. To assess lipid profile and the genotype distribution of lipoprotein lipase gene polymorphism at Pvu II polymorphic site within the intron between exons 6 and 7 in patients with hypertriglyceridemia.

Methods. Pvu II polymorphism was determined in 116 hypertriglyceridemic patients and 50 normolipidemic controls from Zagreb, Croatia. DNA was extracted from peripheral blood mononuclear cells. Polymerase chain reaction was used for amplification of 6th intron, which was then restricted with Pvu II-restriction endonuclease. Serum lipid and lipoprotein fractions were determined by standard enzymatic methods. Cholesterol concentrations in HDL subfractions, HDL_2 and HDL_3, were determined after precipitation with polyethyleneglycol. Apolipoproteins (apo) A-I and B were determined by immunonephelometry.

Results. Triglycerides showed a positive correlation with total cholesterol (r = 0.222, 95% CI = 0.041-0.389, p = 0.017) and inverse correlation with HDL-cholesterol (r = -0.278, 95% CI = -0.449 to -0.088, p = 0.005), especially with HDL_3-cholesterol (r = -0.333, 95% CI = -0.497 to -0.147, p = 0.001). The respective frequencies for genotypes –/–, +/–, and +/+ were 22, 58, and 36 in the patient group, and 17, 17, and 16 in the control group. Serum triglycerides in the patient group, expressed as median in mmol/L, were 3.30 (range, 2.60-10.90), 3.60 (range, 2.50-21.50), and 3.99 (range, 2.50-15.56), respectively. Serum concentration of triglycerides differed significantly between the +/+ and –/– genotype (p = 0.043).

Conclusion. There is an association between genetic variation at the locus for lipoprotein lipase and high serum triglyceride levels. This might prove useful in the detection of individuals susceptible to the development of hypertriglyceridemia, as well as a marker in the analysis of this genetic defect in patient families.

Key words: *apolipoprotein A-I; apolipoproteins B; Croatia; lipoproteins, HDL cholesterol; lipoprotein lipase; polymorphism, restriction fragment length; triglycerides*

Lipoprotein lipase (LPL) plays a major role in lipoprotein metabolism by hydrolyzing core triglycerides of circulating chylomicrons and VLDL. The action of lipoprotein lipase on triglyceride-rich lipoproteins promotes the exchange of lipids and apolipoproteins between lipoproteins. Therefore, LPL is indirectly involved in the maturation of the majority of plasma lipoproteins (1). As LPL has a contributory role in the manifestation of many pathological conditions related to the metabolism of triglyceride-rich lipoproteins, dysfunction and deficiency of LPL activity has been associated with the pathogenesis of various dyslipoproteinemias and with the production of atherogenic particles, such as intermediate-density lipoproteins and low-density lipoproteins (LDL) (2).

LPL gene, mapped to the short arm of human chromosome 8 (3), contains 10 exons, separated by 9 introns (4). Gene analysis showed many mutations involving the noncoding and coding sequences of the human LPL gene (2,5). Eighty-eight variable sites were identified across the 9.7 kb region of 3'-end of the LPL-gene in three different populations: African-American, European, and European-American (6,7). Although intron nucleotides do not code for amino acids, at least some parts of their sequence play a critical role in the processing of the messenger RNA (mRNA) precursor and in correct splicing of coding exons (1). The occurrence of alternate types of nucleotides at the same position in the nucleic acid sequence, with no concomitant apparent phenotypic differences, is generally referred to as polymorphism and it can be easily detected if it leads to restriction site alterations. The most extensively investigated of these are the Pvu II (8) and Hind III (9) sites. Pvu II polymorphism is the result of change in the restriction site of the LPL gene 6th intron, 1.57 kb from SA-site (10). The region containing Pvu II site resembles the

splicing site in its homology to the consensus sequence required for 3′-splicing and the formation of the lariat structure, suggesting that $C^{497} \rightarrow T$ (CAG CTG $\Rightarrow$ TAG CTG) change may interfere with correct splicing of mRNA.

DNA polymorphism is a useful marker to analyze disorders with genetic backgrounds, even when the genetic cause of the disease has not been elucidated (11). A number of DNA polymorphisms have been investigated for their possible linkage with a hereditary predisposition to common polygenic disorders, such as dyslipidemia (2,12,13). Several trials 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 (14-18), but others failed to find significant association (19-21).

We investigated the frequency of Pvu II RFLP polymorphism in the Croatian population and its association with hypertriglyceridemia. We also investigated correlations of different lipid fractions in sera of patients with hypertriglyceridemia and changes of lipid profile in different RFLP-Pvu II genotypes.

Methods

Study Population

The study included 116 patients with hypertriglyceridemia (74 men, median age 45.5, range, 23-61; and 42 women, median age 46.0, range 13-61): 37 patients treated at the Vuk Vrhovac Institute for hyperlipoproteinemia and/or diabetes mellitus type II, four patients treated at the Department of Dermatology and Venereology for psoriasis, and 75 patients who came for routine examination at the Zagreb University Hospital Center. Triglyceride concetration over 2.5 mmol/L was the selection criteria. Patients with terminal renal and hepatic diseases, diabetes mellitus type I, hypothyroidism, and those on hormone therapy were excluded from the study. The control group consisted of 50 subjects (33 men, median age 31.0; range 22-91; and 17 women, median age 70.0, range 21-97): 28 young persons involved in recreational sports, and 22 unrelated elderly people without hypertriglyceridemia. Serum was used for lipid profile analysis, whereas whole blood was used for DNA extraction.

Plasma Measurements

Total serum cholesterol and triglyceride concentrations were determined by standard enzymatic methods (22) on an Olympus AU 800 autoanalyzer (Tokyo, Japan). HDL-cholesterol and its HDL_2 and HDL_3 fractions were also determined enzymatically after precipitation with polyethylene glycol solutions at different concentrations (Quantolip Immuno AG, Wien, Austria) (22). Apo A-I and apo B were determined by immunonephelometry on the Behring Nephelometer II (Marburg, Germany).

DNA Analysis

Blood was drawn into tubes containing ethylenediethylaminetetraacetic acid (EDTA) as an anticoagulant (Becton Dickinson VACUTAINER Systems, Plymouth, UK). Human DNA was isolated from white blood cells of hypertriglyceridemic patients by standard phenol-chloroform extraction (23). Polymerase chain reaction (PCR) analyses of 6th intron were performed in a DNA thermocycler (Eppendorf-Mastercycler 3550, Hamburg, Germany) using 50-μL reactions with commercially avaliable buffer composed of $MgCl_2$, 300 μmol/L of deoxynucleotide triphosphates (dNTPs), 5.6 μmol/L of forward and reverse primers, and 1.25 units of thermostabile DNA polymerase from thermus aquaticus (Boehringer, Mannheim, Germany). The oligonucleotide primers were ordered from MWG Biotech (Ebersberg, Germany). The selected sequences for 5′ and 3′ oligomers were SB-75: 5′-ATG GCA CCC ATG TGT AAG GTG-3′, and SB-76: 5′GTG AAC TTC TGA TAA CAA TCT C-3′ (16). Quality analysis of the PCR products was performed by 1.5% agarose gel electrophoresis (90 V/1 h) with a 50 bp marker (Pharmacia Biotech, Uppsala, Sweden). Samples of 430 bp-long PCR products (8 μL) were then incubated with Pvu II restriction endonuclease (Boehringer) overnight at 37 °C. The digested DNA was run on a 2% agarose gel (90 V/1 h). The 430 bp-long product was digested to 320 and 110 bp-long products if there was a Pvu II restriction site.

Statistics

MedCalc 4.10 (Frank Schoonjans, Mariakerke, Belgium) and Excel 97 SR-1 (Microsoft, Redmond, Washington, D.C., USA) PC programs were used for statistical analysis. Hardy-Weinberg equilibrium was tested by chi-square test. The levels of quantitative variables were presented as mean, standard deviation (SD), median, range, and 95% confidence interval (CI) for mean or median. Kolmogorov-Smirnov test was used to test the normality of distribution for quantitative variables. Lipid and apolipoprotein concentrations of different genotypes were compared by Student t-test or Mann-Whitney-U test, depending on distribution normality. Hypertriglyceridemia and control group frequencies were compared by chi-square test. Relationships between triglycerides and other variables were presented as Spearman's rank correlation coefficients.

Results

Genotype distribution of Pvu II RFLP polymorphism and relation of hypertriglyceridemia with other serum lipid parameters (cholesterol, HDL-cholesterol, HDL_2-cholesterol, HDL_3-cholesterol, apo A-I, and apo B) were investigated in a total of 116 unrelated subjects with hypertriglyceridemia. All parameters, except triglycerides, showed normal distribution. The median of serum triglycerides was 3.60 mmol/L (95% CI = 3.30-3.80). Statistical analysis of lipid profile in sera of both patient and control groups is given in Table 1. Mean cholesterol concentration was higher than it was recommended by the Croatian Medical Biochemists Society (22), whereas the mean values of other serum parameters were within the recommended ranges.

Total cholesterol showed a significant positive correlation with triglyceride ($p = 0.017$), whereas HDL-cholesterol, especially HDL_3, showed a strong inverse correlation with triglyceride ($p = 0.005$ and $p = 0.001$, respectively) (Table 2). The concentration of HDL_2-cholesterol also showed an inverse but nonsignificant correlation with triglyceride ($p = 0.205$).

After PCR analysis, 430 bp-long fragments were obtained by agarose gel electrophoresis. They could be spliced to 320 and 110 bp-long fragments in the presence of the respective restriction sites (Fig. 1). Determination of Pvu II polymorphisms by PCR and RFLP showed the respective frequencies for –/–, +/– and +/+ genotypes to be 22, 58, and 36 in the subjects with hypertriglyceridemia, and 17, 17, and 16 in the control group. The chi-square test showed no significant differences between the genotypes (chi-square = 5.35, $p = 0.069$). Since there was no significant difference between genotype frequencies, Hardy-Weinberg equilibrium was tested on a whole study population (N = 166). According to Hardy-Weinberg proportion, the various genotypes are in equilibrium (chi-square = 0.69, $p = 0.710$) and the proportions might remain constant over generations. Serum triglycerides for –/–, +/–, and +/+ genotypes in subjects with hypertriglyceridemia, expressed as median

in mmol/L, were 3.30, 3.60, and 3.99, respectively (Table 3, Fig. 2). There was also significant difference in serum triglyceride concentrations between –/– and +/+ genotypes (p = 0.043). Apo B concentrations showed significant differences between –/– and two other genotypes (+/–, p<0.001 and +/+, p = 0.016). Other parameters did not show any significant differences.

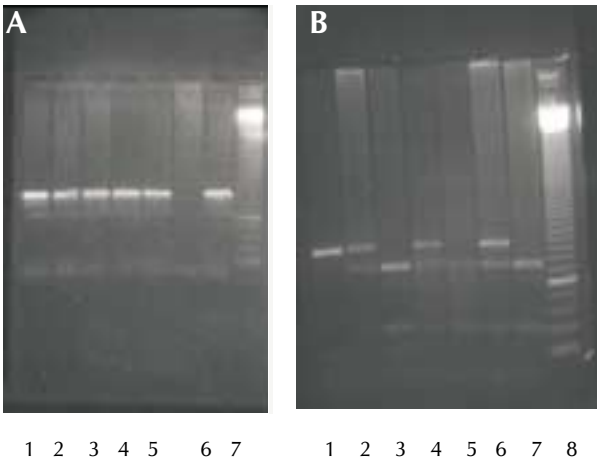

Figure 1. Restriction fragment length polymorphism of the lipoprotein lipase gene. **A.** Electrophoresis of PCR-amplified fragments of the 6th intron in 1.5% agarose gel (1-6 – samples of PCR-amplified fragments, 430 bp long; 7 – 50 bp marker). **B.** Electrophoresis of the same PCR-amplified fragments after incubation with Pvu II-restriction enzyme (1 – no restriction, genotype –/–; 2, 4, and 6 – genotype +/–; 3, 5, and 7 – genotype +/+; 8 – 50 bp marker).

Discussion

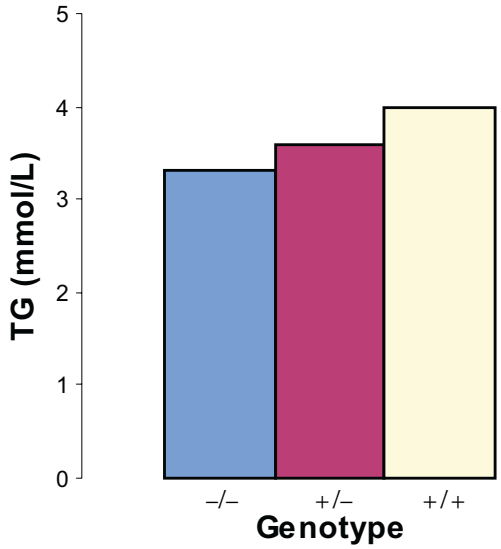

Figure 2. Concentrations (median) of different triglyceride (TG) genotypes in subjects with hypertriglyceridemia. Respective frequencies for genotypes are 22, 58, and 36.

Table 1. Descriptive statistics of lipid profile in serum of patients with hypertriglyceridemia compared with control group

Parameter	Group[a]		No. of samples	Mean value	Standard deviation	Median	95% CI for the mean or *median	Range
Triglycerides (mmol/L)	patients	T	116	4.62	3.10	3.60	*3.30-3.80	2.50-21.50
		M	74	4.79	3.57	3.60	*3.30-4.14	2.50-21.50
		W	42	4.33	2.74	3.45	*2.80-3.98	2.50-13.40
	controls	T	50	1.09	0.30	1.04	*0.92-1.12	0.57-1.89
		M	33	1.10	0.30	1.06	*0.92-1.18	0.57-1.89
		W	17	1.06	0.31	0.97	*0.80-1.19	0.69-1.74
Cholesterol (mmol/L)	patients	T	116	7.08	1.36	7.00	6.84-7.33	4.30-12.30
		M	74	6.96	1.36	6.80	6.65-7.28	4.30-11.90
		W	42	7.30	0.18	7.35	6.88-7.72	5.10-12.30
	controls	T	50	4.64	0.60	4.85	4.47-4.81	2.70-5-20
		M	33	4.49	0.63	4.60	4.27-4.72	2.70-5.20
		W	17	4.92	0.40	5.00	4.72-5.13	3.70-5.20
HDL-cholesterol (mmol/L)	patients	T	101	1.01	0.26	0.97	0.96-1.06	0.46-1.90
		M	64	0.93	0.22	0.91	0.87-0.98	0.46-1.62
		W	37	1.14	0.27	1.15	1.05-1.23	0.68-1.90
HDL_2-cholesterol (mmol/L)	patients	T	100	0.30	0.13	0.28	0.28-0.33	0.09-0.74
		M	63	0.26	0.09	0.26	0.24-0.28	0.09-0.50
		W	37	0.37	0.15	0.33	0.32-0.42	0.10-0.74
HDL_3-cholesterol (mmol/L)	patients	T	100	0.71	0.18	0.69	0.67-0.74	0.33-1.22
		M	63	0.67	0.17	0.66	0.63-0.71	0.33-1.12
		W	37	0.77	0.17	0.76	0.71-0.83	0.51-1.22
Apo A-I (g/L)	patients	T	108	1.34	0.28	1.31	1.29-1.40	0.81-2.43
		M	68	1.28	0.23	1.27	1.22-1.33	0.81-1.94
		W	40	1.46	0.32	1.45	1.35-1.56	0.90-2.43
Apo B (g/L)	patients	T	108	1.43	0.31	1.40	1.38-1.49	0.79-2.37
		M	68	1.42	0.31	1.39	1.35-1.50	0.79-2.31
		W	40	1.45	0.31	1.44	1.35-1.55	0.97-2.37
Apo B/A-I	patients	T	108	1.10	0.32	1.06	1.03-1.15	0.12-1.83
		M	68	1.13	0.32	1.14	1.05-1.20	0.12-1.83
		W	40	1.04	0.32	0.98	0.94-1.14	0.50-1.83

[a]T – all samples, M – men, W – women.

The genotype distribution of LPL polymorphism at Pvu II polymorphic site and lipid profile of different genotypes for the Croatian population were investigated at the gene locus in patients with triglyceride concentrations over 2.5 mmol/L. Positive correlation of total cholesterol and strong inverse correlation of HDL-cholesterol, especially HDL_3-cholesterol (Table 2), with triglycerides could be explained by the pivotal role of LPL in triglyceride hydrolysis in plasma lipoproteins. This process initiates the conversion of chylomicrons and VLDLs to their remnant particles, and also has a major modulating effect on the levels and lipid composition of HDLs and LDLs (24). An inverse correlation between HDL levels and coronary artery disease has been demonstrated previously in the Framingham study (25). Also, the levels of HDL are in positive correlation with lipoprotein lipase activity (26), possibly indicating a protective role of LPL in coronary artery disease. A study of French-Canadian heterozygotes for LPL gene mutations showed that they have reduced HDL-cholesterol (27-29). The amount of HDL-cholesterol exchanged for triglycerides is modulated by the amount of VLDL in the circulation. Therefore, by decreasing plasma triglycerides, LPL limits the cholesteryl ester transfer protein-mediated HDL-cholesterol reduction (24). If HDL is triglyceride-enriched, triglycerides can be more rapidly removed (30). Thus, HDLs are reduced in the circulation.

Table 2. Comparision of different lipid and apolipoprotein concentrations with triglyceride concentrations in the patient group

Parameter	No. of samples	Correlation coefficient (r)	95% CI for r	Significance (p)
Cholesterol	116	0.222	0.041 to 0.389	0.017
HDL-cholesterol	101	-0.278	-0.449 to -0.088	0.005
HDL_2-cholesterol	100	-0.127	-0.316 to 0.071	0.205
HDL_3-cholesterol	100	-0.333	-0.497 to -0.147	0.001
Apolipoprotein A-I	108	-0.002	-0.191 to 0.187	0.980
Apolipoprotein B	108	0.050	-1.400 to 0.237	0.602
Apo B/A-I	108	0.032	-0.158 to 0.220	0.740

Apo A-I and apo B have been reported to yield different correlation results (2). Long-term studies of apo A-I concentrations in numerous hypertriglyceridemic LPL mutation carriers showed normal results in Austrian (31), US American (32), Dutch, English, Swedish, and Scottish (33) population, which is consistent with our findings (Table 2). We found only 17% of patients with lower apo A-I. In contrast, some authors reported on significantly lower concentrations of apo A-I in the French-Canadian population (34). Theoretically, it is possible because triglyceride-containing HDL are better substrates for hepatic lipase, so the lipid-poor apo A-I is more rapidly cleared from the circulation (24).

Many authors reported on positive correlation between apo B and triglyceride levels (31-34). Kinetic

Table 3. Lipid and lipoprotein concetrations in three (lipoprotein lipase PvuII) genotypes

Parameter	Group	Genotype	No. of samples	Mean value	Standard deviation	Median	95% CI for the mean or *median	Range
Triglycerides (mmol/L)	patients	–/–	22	3.74	1.87	3.30	*2.70-3.76	2.60-10.90
	controls		17	1.04	0.22	1.03	*0.86-1.18	0.69-1.58
Cholesterol (mmol/L)	patients		22	7.41	1.74	7.55	6.64-8.18	5.10-12.30
	controls		17	4.62	0.60	4.90	4.31-4.93	3.50-5.20
HDL-cholesterol (mmol/L)	patients		19	0.99	0.18	1.02	0.91-1.08	0.71-1.31
HDL_2-cholesterol (mmol/L)	patients		19	0.29	0.10	0.31	0.24-0.34	0.09-0.45
HDL_3-cholesterol (mmol/L)	patients		19	0.70	0.12	0.69	0.64-0.76	0.49-0.92
Apo A-I (g/L)	patients		20	1.28	0.22	1.29	1.18-1.39	0.92-1.84
Apo B (g/L)	patients		20	1.64	0.35	1.64	1.47-1.80	1.10-2.37
Apo B/A-I	patients		20	1.24	0.38	1.25	1.06-1.41	0.12-1.83
Triglycerides (mmol/L)	patients	+/–	58	4.61	3.23	3.60	*3.10-4.10	2.50-21.50
	controls		17	1.05	0.31	0.97	*0.82-1.20	0.57-1.74
Cholesterol (mmol/L)	patients		58	6.87	1.14	6.75	6.57-7.17	4.30-9.50
	controls		17	4.63	0.62	4.80	4.31-4.95	2.70-5.20
HDL-cholesterol (mmol/L)	patients		52	0.99	0.26	0.94	0.92-1.06	0.53-1.66
HDL_2-cholesterol (mmol/L)	patients		52	0.29	0.13	0.27	0.25-0.32	0.09-0.74
HDL_3-cholesterol (mmol/L)	patients		52	0.70	0.17	0.67	0.65-0.74	0.35-1.02
Apo A-I (g/L)	patients		57	1.37	0.30	1.33	1.30-1.45	0.90-2.43
Apo B (g/L)	patients		57	1.38	0.25	1.39	1.32-1.45	0.79-1.93
Apo B/A-I	patients		57	1.05	0.28	1.01	0.97-1-12	0.53-1.83
Triglycerides (mmol/L)	patients	+/+	36	5.17	3.41	3.99	*3.21-4.93	2.50-15.56
	controls		16	1.17	0.37	1.11	*0.80-1.39	0.77-1.83
Cholesterol (mmol/L)	patients		36	7.23	1.41	7.23	6.76-7.71	5.00-11.90
	controls		16	4.67	0.61	5.00	4.34-4.99	3.50-5.20
HDL-cholesterol (mmol/L)	patients		30	1.05	0.31	1.01	0.93-1.16	0.46-1.90
HDL_2-cholesterol (mmol/L)	patients		29	0.33	0.14	0.29	0.27-0.38	0.11-0.68
HDL_3-cholesterol (mmol/L)	patients		29	0.73	0.23	0.69	0.64-0.81	0.31-1.22
Apo A-I (g/L)	patients		31	1.32	0.29	1.31	1.21-1.43	0.81-2.15
Apo B (g/L)	patients		31	1.40	0.33	1.35	1.27-1.52	0.83-2.31
Apo B/A-I	patients		31	1.09	0.32	1.01	0.97-1.21	0.50-1.83

[a]T-total; M-men; W.women

studies showed the apo B production rates to be increased in familial combined hyperlipoproteinemia in French-Canadians (27). Direct measurement of VLDL apo B production in the postabsorptive state showed a linear relation between triglyceride levels and VLDL apo B production rate in familial combined hypertriglyceridemia (35). In spite of this, some studies in French-Canadian (29,34,36) and different European (31,33) populations failed to show notable increase of apo B concentration. We did not find significant positive correlation of apo B and triglycerides, either (Table 2).

Different gene analysis showed the importance of investigating clinical characteristics for mutations of coding and noncoding nucleotide sequences. Intron gene polymorphisms do not affect phenotype characteristic of the mature protein, but they could affect the maturation and turnover of mRNA, its size, translatability, and the nature and number of the protein products formed (37). Such polymorphisms in nucleic acids can be readily detected if they lead to an alteration at the restriction sites. The relation of hypertriglyceridemia and Pvu II RFLP polymorphism of the LPL gene was investigated in different populations. An association between LPL Pvu II polymorphism and lipid disorders, recorded in a Japanese population, was found to be due to significantly higher triglyceride concentrations in +/+ than in –/– Pvu II-genotype (14). Similar results have been reported for Australian (15), French (16), and Japanese schoolchildren (17) as well as for Welsh (37) population. These authors have also shown a significant decrease of HDL-cholesterol concentrations. In contrast to these findings, –/– genotype was observed to be associated with higher triglyceride concentrations compared with +/+ genotype in Chinese Beijing area (18). Although Jamaa et al (21) failed to demonstrate any significant relationship between the lipid concentrations and Pvu II polymorphism in French population, their observations pointed to an association between Pvu II polymorphism and severity of coronary lesions. Similar results have been reported by Wang et al (15), whose study included 500 Australian cardiologic patients. Except for a significant relationship between Pvu II RFLP polymorphism and hypertriglyceridemia, they found a correlation between this polymorphism and coronary-artery disease and diabetes. Our results are in agreement with these findings in that there was a significant relationship between elevated triglyceride and Pvu II (+) allele, but there was no association between Pvu II and HDL-cholesterol. It is unclear why apo B concentrations were the highest, because it is opposite to what was mentioned above about apo B. This issue needs to be examined and calls for additional studies which would include larger groups of subjects from general patient population.

There was a difference in genotype relative frequencies between patient and control groups. However, it did not reach statistical significance. The result was consistent with the data reported for the Chinese from the Beijing area (18). On the other hand, a study carried out in Japanese schoolchildren showed a higher frequency of +/+ genotype in hypertriglyceridemic patients (17). The difference was also found in Hispanic and non-Hispanic white US-population between diabetics and non-diabetics (38).

Although the difference in genotype frequencies between the study and control group was not significant, it could prove useful because of the possibility that some young normolipemic subjects would develop hypertriglyceridemia later in life.

Association between the genetic variation at the LPL locus with high levels of serum triglycerides, as our study confirmed for the Croatian population, may be useful in the detection of individuals most likely to develop hypertriglyceridemia, and it may be a useful marker in the analysis of genetical defects underlying hypertriglyceridemia in various patient families.

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