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Polymorphisms in the Lipoprotein Lipase and Hepatic Lipase Genes and Plasma Lipid Values in the Czech Population

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Summary

We have determined the genotypes of two common polymorphisms in the lipoprotein lipase (S447X) and hepatic lipase (-480C/T) genes in a cohort of 285 representative selected Czech probands (131 male and 154 female), examined in 1988 and reinvestigated in 1996. The genotype distributions of both polymorphisms were in Hardy-Weinberg equilibrium and did not differ between male and female subjects. The rare allele frequency of the lipoprotein lipase polymorphism did not differ significantly from the other European populations. Compared to the German populations, the frequency of the hepatic lipase -480T allele was significantly higher in the Czech group (20% vs. 36%, $p<0.0001$). There were no significant associations between the lipoprotein lipase gene variants and lipid parameters measured either in 1988, or in 1996 or with changes of lipid parameters over the 8-year period. The carriers of the T-480 allele of the hepatic lipase polymorphism were found to have higher HDL cholesterol levels ($p=0.02$). However, this difference was confined to female subjects only. The male carriers of the –480T allele had higher concentrations of total cholesterol ($p=0.03$) as compared to CC-480 subjects. Both associations were observed in 1996 only. In the Slavic Czech population, a common polymorphism in the hepatic lipase gene (-480C/T), but not in the lipoprotein lipase gene (S447X), is a significant determinant of plasma HDL cholesterol in females and plasma total cholesterol in males and indicates the importance of gender-associated effects in the genetic determinations of plasma lipids.

Key words

Hepatic lipase • Lipoprotein lipase • Lipids • Polymorphism

Introduction

Cardiovascular diseases are the most common cause of death in all industrialized countries. One of the important risk factors in the development of cardiovascular diseases is the high level of lipids (cholesterol and triglycerides) in the plasma. Although environmental factors, for example dietary fat intake, physical activity and smoking, influence the plasma lipids, genetic factors also contribute to the determination of plasma lipid levels.

In this present study we have examined the impact of common variation in the lipoprotein lipase and hepatic lipase, both of which play important roles in

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triglyceride metabolism. These two enzymes have considerable nucleotide and amino acid sequence homology (Datta *et al.* 1988), and are members of the large triacylglycerol lipase family.

Lipoprotein lipase (LPL) is localized on the endothelium of vessels in the adipose and muscle tissues, where it hydrolyses the triglycerides (TG) in the triglyceride-rich particles (chylomicrons – CH, very low density lipoproteins – VLDL). A number of polymorphisms have been reported to date. A common variant is the Serine447Stop (S447X), which results in a premature stop codon truncating LPL by two carboxyterminal amino acids (Hata *et al.* 1990). The 447X allele has been found to be associated with a favorable lipid profile (lower plasma cholesterol, lower TG, higher HDL cholesterol) (Mattu *et al.* 1994, Kuivenhoven *et al.* 1997).

Hepatic lipase (HL) hydrolyses TG in the intermediate density lipoproteins (IDL) and high-density lipoproteins (HDL) as well as phospholipids in HDL particles (for review see Applebaum-Bowden 1995). Hydrolysis of the HDL phospholipids promotes the cholesterol uptake by liver (Bamberger *et al.* 1985) and thus HL could be an important element in reverse cholesterol transport. Several polymorphisms have been identified in HL, amongst them -480C/T polymorphism in the HL gene promoter. It was shown that in males the T allele is associated with lower HL activity and with increased concentration of HDL and total cholesterol (Jansen *et al.* 1997).

We evaluated the impact of common polymorphisms in the lipoprotein lipase (S447X), and hepatic lipase (-480C/T) genes in a representative Czech population sample (131 male and 154 female), and investigated their influence on plasma lipids.

Material and Methods

The 285 unrelated individuals (131 male and 154 female, mean age 55.4±11.6 and 55.5±11.2 years in 1996, respectively) included in the study represented an 8-year cohort of the 1 % representative selected Czech population sample (region Benešov) according the protocol of the MONICA study (Multinational monitoring of trends and determinants in cardiovascular diseases: "MONICA Project". Manual of operations WHO/MNC 82.2, Nov. 1983). The basic lipid parameters have been evaluated in the same subjects in both 1988 and 1996. Written informed consent was obtained from the study participants and the design of the study was approved by the local ethics committee.

Blood for isolation of the genetic material was diluted with sterile water at ratio 1:1 and stored at –20 °C. DNA was isolated by standard methods (Miller *et al.* 1988). Analysis of genetic polymorphisms in the lipoprotein lipase (Humphries *et al.* 1998) and hepatic lipase genes (Jansen *et al.* 1999) was performed using PCR on an MJ Research thermal cycler PTC-220 with subsequent restriction of the PCR product by the appropriate restriction enzyme as described previously. The PCR products were after restriction analysis analyzed by 7.5 % polyacrylamide gel with microtitre array diagonal gel electrophoresis (Day and Humphries 1994). From the whole population sample, 278 individuals for LPL and 271 individuals for HL have been successfully genotyped.

Table 1. Lipid parameters of the 285 probands (131 males, 154 females) in 1988 and 1996.

Parameters	**Population 88**	**Population 96**	**P<**
TC	6.04 ± 1.21	5.52 ± 1.12	0.001
LDL-C	3.72 ± 1.03	3.30 ± 0.95	0.01
HDL-C	1.44 ± 0.32	1.33 ± 0.36	n.s.
TG	2.00 ± 2.44	1.99 ± 1.50	n.s.
apo B	1.21 ± 0.34	1.17 ± 0.28	n.s.
Parameters	**Male 88**	**Male 96**	**P<**
TC	6.22 ± 1.31	5.43 ± 1.06	0.001
LDL-C	3.78 ± 1.10	3.27 ± 0.88	0.01
HDL-C	1.31 ± 0.28	1.21 ± 0.31	n.s.
TG	2.36 ± 3.38	2.16 ± 1.75	n.s.
apo B	1.28 ± 0.36	1.19 ± 0.26	n.s.
Parameters	**Female 88**	**Female 96**	**P<**
TC	5.88 ± 1.10	5.60 ± 1.16	0.01
LDL-C	3.67 ± 0.96	3.32 ± 1.01	0.01
HDL-C	1.54 ± 0.32	1.44 ± 0.38	n.s.
TG	1.47 ± 0.84	1.85 ± 1.24	0.01
apo B	1.16 ± 0.31	1.16 ± 0.29	n.s.

Data are given as mean ± S.D., total, LDL, HDL cholesterol and triglycerides are in mmol /l, apo B in g/l.

The lipoprotein parameters were measured enzymatically on the Roche COBAS MIRA autoanalyser (Hoffmann-

La Roche, Switzerland), using reagents from Boehringer Mannheim Diagnostics and Hoffmann-La Roche.

The statistical analyses have been performed using the SPSS statistical software package and ANOVA. Observed numbers of each genotype were compared to the expected frequencies (Hardy-Weinberg equilibrium) by the chi-square test. Triglycerides have been logarithmically transformed before the analysis to obtain normal distribution of data. For HL as well as for LPL polymorphisms, the heterozygous and homozygous carriers of the rare allele have been pooled and analyzed together.

Table 2. The genotype frequencies of the lipoprotein lipase and hepatic lipase polymorphisms in the Czech population sample.

Polymorphism		Population		Male		Female	
		N	%	N	%	N	%
LPL	*SS*	227	81.7	104	80.6	123	82.6
S447X	*SX*	50	18.0	25	19.4	25	16.8
	XX	1	0.3	0	0	1	0.6
HL	*CC*	174	64.2	83	64.3	91	62.8
-480C/T	*CT*	82	30.3	38	29.5	44	30.3
	TT	15	5.5	5	3.9	10	6.9

Table 3. Lipid parameters in 1996 according to lipoprotein lipase and hepatic lipase polymorphisms in males.

LPL	N	year	TC	LDL-C	TG	apo B	HDL-C
SS	104	1988	6.18±1.37	3.74±1.12	2.68±3.70	1.27±0.38	1.31±0.28
+ allele X	25	1988	6.23±1.04	3.96±1.04	2.61±1.11	1.28±0.32	1.36±0.31
SS	104	1996	5.51±1.39	3.31±0.90	2.31±1.91	1.22±0.28	1.19±0.29
+ allele X	25	1996	5.50±1.10	3.32±0.86	1.88±1.22	1.20±0.22	1.30±0.39
HL	**N**	**year**	**TC**	**LDL-C**	**TG**	**apo B**	**HDL-C**
CC	83	1988	6.18±1.39	3.78±1.12	2.58±3.95	1.27±0.37	1.32±0.29
+ allele T	43	1988	6.31±1.23	3.81±1.05	2.61±2.29	1.30±0.38	1.31±0.28
CC	83	1996	5.32±1.00	3.24±0.88	2.00±1.44	1.19±0.28	1.21±0.32
+ allele T	43	1996	5.78±1.17	3.45±0.82	2.57±2.27	1.25±0.24	1.20±0.30

Data are given as mean ± S.D., total, LDL, HDL cholesterol and triglycerides are in mmol /l, apo B in g/l.

Results

Lipid parameters and population frequency of the HL and LPL alleles

The basic characteristics of the individuals studied in 1988 and 1996 follow-up are summarized in Table 1. In 1996, no differences between males and females in the levels of TG, apo B, total and LDL cholesterol were detected. Females had significantly higher levels of HDL cholesterol ($p<0.0001$). As most of the females had reached the menopause by 1996, this was

reflected in the increase in the follow-up plasma triglyceride levels.

The frequencies of genotypes of the polymorphisms studied are summarized in the Table 2. Genotype frequencies were in Hardy-Weinberg equilibrium and did not differ significantly between males and females.

Lipoprotein lipase polymorphism

There was no statistically significant association between the lipoprotein lipase S447X polymorphism and any of the lipid parameters in either the whole population sample or for females and males separately. This was true for both baseline (1988) and follow-up (1996) (Tables 3 and 4). The difference in lipid parameters over the 8-year period were not significantly different for the LPL genotype.

Hepatic lipase polymorphism

No associations between the hepatic lipase C-480T variant and the lipid parameters of the whole population sample, or for females and males separately, were detected in 1988. In 1996 male carriers of the allele T-480 had higher total cholesterol levels (Table 3, p=0.03) than CC-480 subjects, but no such association was observed for females (Table 4). In the same year, female carriers of the –480T allele had higher HDL cholesterol levels than CC-480 subjects (p=0.02), an effect which was additionally modified by menopausal status (p=0.01, Fig. 1). The change in lipid parameters over the 8 years was not significantly different between the subjects according to HL genotypes.

Table 4. Lipid parameters in 1996 according to lipoprotein lipase and hepatic lipase polymorphisms in females.

LPL	**N**	**year**	**TC**	**LDL-C**	**TG**	**apo B**	**HDL-C**
SS	123	1988	5.79±1.11	3.58±0.99	1.45±0.75	1.15±0.31	1.56±0.32
+ allele X	26	1988	5.98±0.88	3.85±0.77	1.28±0.59	1.17±0.27	1.55±0.31
SS	123	1996	5.54±1.16	3.41±1.00	1.82±1.24	1.15±0.31	1.46±0.38
+ allele X	26	1996	5.57±0.98	3.56±0.96	1.93±1.25	1.17±0.21	1.41±0.36
HL	**N**	**year**	**TC**	**LDL-C**	**TG**	**apo B**	**HDL-C**
CC	91	1988	5.84±1.08	3.65±0.95	1.50±0.82	1.15±0.32	1.50±0.30
+ allele T	54	1988	5.76±1.08	3.56±1.00	1.31±0.55	1.14±0.29	1.60±0.30
CC	91	1996	5.61±1.23	3.36±1.06	1.92±1.25	1.17±0.31	1.37±0.32*
+ allele T	54	1996	5.50±1.05	3.19±0.94	1.70±1.15	1.14±0.28	1.54±0.42

*Data are given as mean ± S.D., total, LDL, HDL cholesterol and triglycerides are in mmol /l, apo B in g/l, * p = 0.02*

Discussion

Raised plasma triglyceride levels are now accepted as an independent risk factor in the development of cardiovascular diseases (Austin 1997). Thus lipoprotein lipase and hepatic lipase, both key players in the hydrolysis of lipoprotein triglycerides, are good candidate genes which could potentially influence plasma triglyceride and lipoprotein levels and individual risk of cardiovascular diseases.

Little is known about the genetic determinants of plasma lipid parameters in the Slavic populations (Kowalska *et al.* 1998, Hubáček *et al.* 1998, 2000). Our present study represents the first large investigation of common polymorphisms of the lipoprotein lipase and hepatic lipase genes in the Slavic (Czech) population and their influence on lipid parameters.

Our previous studies of the insertion/deletion polymorphism in the angiotensin converting enzyme gene (Hubáček *et al.* 1999) and apoCIII gene polymorphisms (C3238G and C-482T) (Waterworth *et al.* 2000), suggested genetic differences between Czech and German populations. These findings have not been replicated for the LPL polymorphism studied. The allele

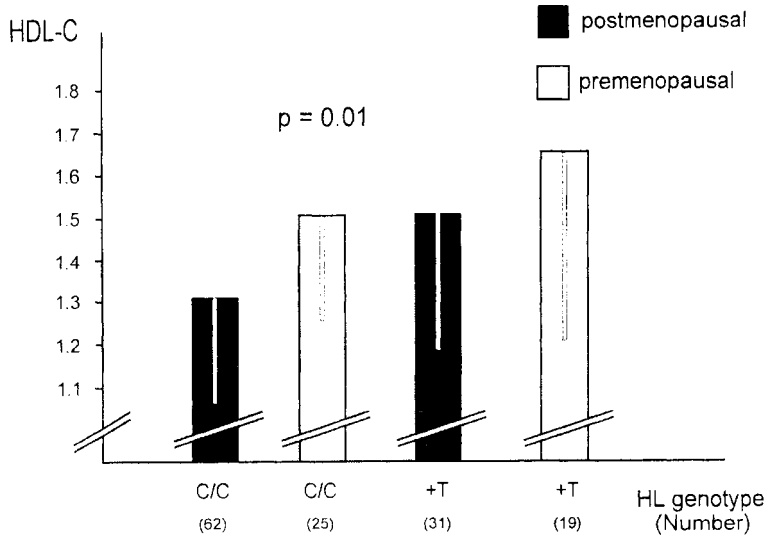

Fig. 1. *HDL cholesterol (mmol/l) in women according the -480C/T hepatic lipase polymorphism and menopausal status. Premenopausal carriers of the allele T have the highest and post-menopausal CC-480 homozygotes the lowest levels of the HDL cholesterol.*

and genotype frequency did not significantly differ from the frequencies described previously for other European populations (Fisher *et al*. 1997). However, the frequency of the HL -480T allele was significantly higher ($p<0.0001$) in the Czech population (35.8 %) compared to the non-Slavic populations (18.9 % and 20.7 %) (Jansen *et al.* 1997, 1999).

No association between the LPL polymorphism and the lipid parameters in any of the investigated years (1988 or 1996) were obtained either in the whole population or in female or male subjects analyzed separately. The change in the lipid parameters over 8 years was also not dependent on this polymorphism.

On the other hand, the HL polymorphism significantly influenced the total cholesterol levels in males and HDL cholesterol levels in females, an effect seen only in 1996.

Between 1988 and 1996, a marked change in some of the lipid parameters was observed (Table 1), probably as a consequence of the life style change (predominantly diet change – lower animal fat and egg intake) since 1989 (Bobak *et al*. 1997).

It was reported, that postmenopausal women are at greater atherogenic risk than premenopausal women. Compared to the premenopausal women, they have increased levels of total and LDL cholesterol, increased triglycerides and decreased HDL cholesterol (Hallbert and Svanborg 1967, Campos *et al.* 1988). In our sample, the hormonal status of the women is quite heterogeneous, including both pre- and postmenopausal individuals and substantial number of the women had changed menopausal status over the 8-year follow-up.

Postmenopausal status was found to be an important and significant predictor of plasma lipids in women in this sample. If the postmenopausal status was entered in the ANOVA analysis as covariate, the effect of HL polymorphism on plasma HDL cholesterol was expressed both in pre- and postmenopausal women. Pre-menopausal carriers of the allele T have the highest and postmenopausal –480CC homozygotes the lowest levels of the HDL cholesterol. Postmenopausal carriers of the allele T have the same intermediate, level of plasma HDL cholesterol as premenopausal –480CC homozy-gotes (for more detail see Fig. 1).

To date, a number of studies have been reported which explored possible associations between the LPL polymorphism and lipid parameters or myocardial infarction (for review see Fisher *et al.* 1997). The results are not consistent and suggest that the effect of this variant was context-dependent (ethnicity and sex).

The previously published results suggested that the HL allele –480T is associated with a non-favorable plasma lipid profile (Jansen *et al.* 1997, 1999). In our study, in contrast, female carriers of this allele have higher plasma HDL cholesterol levels. An explanation for this inconsistency could be based on the different genetic background of this cohort. Different allele frequencies of other common polymorphisms in Czech population (Hubáček *et al.* 1999, Hubáček and Poledne, unpublished results) in other lipid-modulating genes could mask the influence of the lipase polymorphisms. It is also likely that changes in diet (higher animal fat intake, at least in 1988) of the Czech population could mask the effect of the LPL polymorphism and modulates the influence of the HL polymorphism on lipid metabolism.

We conclude, that in the Czech Slavic population, common polymorphism in the hepatic lipase (-480C/T), but not in the lipoprotein lipase gene (S447X), is a determinant of plasma HDL cholesterol in females and plasma total cholesterol in males, indicating the influence of gender- and diet-associated effects in the genetic determinants of plasma lipid parameters.

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Reprint requests

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