Sex-specific effect of the LDL-receptor rs6511720 polymorphism on plasma cholesterol levels. Results from the Czech post-MONICA study.

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***Abstract*—Aims:** Acute coronary syndrome is one of the most common causes of death in industrialized countries. From the conventional risk factors, high plasma cholesterol and dyslipidemia are probably the most commonly analyzed. Plasma cholesterol is influenced equally both by environmental (physical activity, dietary habits) and genetic factors.

Methods: Rs6511720 (G → T) variant was analyzed by PCR-RFLP in 2,559 adult individuals (aged 28-67 years, Czech post MONICA study) with known plasma lipid parameters. ANOVA was used for statistical analyses.

Results: Genotype distribution was similar to the neighboring countries and genotype frequencies (0.085 for minor T allele) were within the Hardy-Weinberg equilibrium (P = 0.44). Polymorphism has no effect on plasma lipid levels in males. In females, carrier's of the minor T allele has significantly lower concentration of total cholesterol (5.50 ± 1.07 mmol/L vs. 5.86 ± 1.16 mmol/L; P < 0.005) and LDL cholesterol (3.43 ± 1.00 mmol/L vs. 3.70 ± 1.05 mmol/L; P < 0.002). HDL cholesterol and triglycerides were not influenced by the analyzed polymorphism.

Conclusions: Rs6511720 (G → T) polymorphism within the LDL receptor gene has an effect on plasma cholesterol levels in Czech females, but not in males.

***Keywords*—** Czech population, LDL-receptor, plasma lipids, polymorphism.

I. Introduction

Coronary artery disease (CAD) and particularly acute coronary syndromes (ACS) are among the most frequent causes of death in developed industrial countries. Significant proportion (but definitely not all patients) of the ACS patients exhibit one on more from the five conventional risk factors (smoking, hypertension, type 2 diabetes, overweight/obesity and dyslipidemia) [1]. Dyslipidemia is characterized by high plasma total cholesterol and/or low density lipoprotein (LDL) - cholesterol, high levels of plasma triacylglyceroles (TG) and low levels of plasma high density lipoprotein (HDL) – cholesterol. Especially elevated plasma LDL cholesterol levels are associated with the ACS incidence [2].

High levels of plasma cholesterol result both from the unfavorable environmental factors (low physical activity and unhealthy dietary habits – low intake of fruits and vegetables and high intake of saturated fats) and from genetic predispositions (both in form of the common polymorphisms and rare mutations). It is estimated that both environmental and genetic factors influence final plasma cholesterol levels from 50%.

Seek for the genes (and variants) associated with plasma cholesterol levels was very successful in the case of genome wide association studies (GWAs). Couple of almost simultaneously published papers introduced dozens of variants associated with plasma lipid parameters [3]-[5]. Despite the fact, that the results were replicated on independent population, there is one major weakness of such studies. They have been performed mainly on west European populations with similar environmental influence and further replications are necessary [6].

Among the variants detected through the GWAs approach, are also variants within the LDL – receptor gene. LDL – receptor (OMIM acc. No. 606945) is the cell surface receptor which plays a very important role in cholesterol homeostasis. Binding to the apolipoprotein B, LDL – receptor internalize the cholesterol rich LDL – particles. One of the variant within the LDL – receptor gene, which was suggested by GWAs to influence significantly plasma cholesterol levels is the intronic rs6511720 polymorphism (G → T exchange).

To replicate the original finding, we have analyzed, if rs6511720 polymorphism at LDL – receptor locus is associated with plasma lipids in the adult Czech Slavonic population.

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II. Material and Methods

A. Analyzed individuals

In our study, 2,559 adult (29 - 68 years at the time of examination at 2000/2001, mean age 52.0 ± 10.2 years) individuals selected according to the WHO MONICA Project protocol were examined and analyzed [7]. All participants were of Caucasian ethnicity and all signed the informed consent. 1,191 males and 1,368 females were included in the study. The study was approved by the institutional Ethics committee and conducted according to the Good Clinical Practice guidelines.

B. Laboratory and clinical analyses

DNA was extracted from peripheral blood white cells. Rs6511720 was genotyped (for more details see [8]) using the nested polymerase chain reaction and restriction fragment length polymorphism (PCR – RFLP) analysis using the oligonucleotides 5´aac atc aca ttc tca gcc atc ccg g and 5´ ttg tag aga tga ggt ctc gct tgg. Product from the first PCR was diluted 1 : 50 and used as a template for the second amplification with oligonucleotides 5´ acc ggg gat gat gat gat tgc and 5´ ttg cct aag act tcc tta aca ttt g. Restriction enzyme MboI was used to distinguish the alleles. All chemicals were purchased from Fermentas (Burlington, Ontario, Canada). Common G allele was represented by uncut product (132 bp) while the presence of restriction fragments of 107 and 25 bp, represented the minor T allele.

C. Biochemical analyses

Lipoprotein parameters (assessed in plasma after an overnight fast) were measured using standard enzymatic methods in CDC Atlanta accredited laboratory.

D. Statistical analyses

The Hardy-Weinberg test (http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls) was applied to confirm the independent segregation of the alleles. ANOVA was used for analysis of the association between the genotypes and plasma lipids. Due to the low number of the minor allele homozygotes, they have been for the analysis pooled with heterozygotes. P-values less than 0.05 were considered to be significant.

III. Results and Discussion

The genotyping call rate was 97.7% for the entire population and genotypes distributions were within the Hardy Weinberg equilibrium (P = 0.66 for males; P = 0.54 for females and finally P = 0.44 for entire population). The minor allele (T) frequency did not significantly differ (P = 0.32) between males and females (for more details see Table I) and in entire population is almost identical with the frequencies described in the white Western populations (0.095 in Czechs and ~ 0.085 in Western Europeans; data from the HapMap project).

TABLE I.

Genotypes distribution of the LDL – receptor rs6511720 polymorphism in Czech general population.

Genotype	Males		Females	
	N	%	N	%
GG	943	83.2	1108	81.0
GT	180	15.9	244	17.8
TT	10	0.9	16	1.2

Among males (Table II), we have not detected any significant association between the rs6511720 polymorphism and plasma lipids. For LDL – cholesterol, there was a trend to the lower concentrations in T allele carriers.

TABLE II.

Effect of the LDL – receptor rs6511720 polymorphism on plasma lipid concentrations in Czech general population - males. Concentrations are given in mmol/L.

Males	Genotype		
	GG	+ T	P
N	943	190	
Total cholesterol	5.77 ± 1.04	5.70 ±1.11	0.28
LDL cholesterol	3.69 ± 0.94	3.55 ± 0.89	0.07
HDL cholesterol	1.23 ± 0.34	1.22 ± 0.37	0.85
Triglycerides	1.94 ± 1.20	2.18 ± 1.88	0.20

In contrast, variant was associated with plasma cholesterol levels in females. Both plasma concentration of total cholesterol (P < 0.005) and plasma LDL – cholesterol (P < 0.002) were significantly higher in common GG homozygotes than in carriers of the minor T allele (for more details, see Table III). Plasma levels of HDL – cholesterol and triacylglycerols were not influenced by the rs6511720 polymorphism.

TABLE III.

Effect of the LDL – receptor rs6511720 polymorphism on plasma lipid concentrations in Czech general population - females. Concentrations are given in mmol/L.

Females	Genotype		
	GG	+ T	P
N	1108	260	
Total cholesterol	5.86 ± 1.16	5.50 ± 1.07	0.001
LDL cholesterol	3.43 ± 1.00	3.43 ± 1.00	0.002
HDL cholesterol	1.50 ± 0.38	1.49 ± 0.36	0.89
Triglycerides	1.46 ± 0.80	1.46 ± 0.91	0.99

The significance of the obtained results has not changed (neither in males, nor in females) if adjustment for body mass index, smoking status and age was performed.

The finding, that the rs6511720 polymorphism within the LDL – receptor gene influence plasma lipids in females but not in males is not in agreements with originally published results

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[3]-[5], however, we have already confirmed some [9] but not all [10] [11], results from GWAs studies. Surprisingly it is a common feature of genetic association studies. Positive results published in high impact journals are more likely to have high bias scores (and smaller sample sizes) [6] [12] and thus are more prone to present incomplete and also false positive results.

Our results underline the importance of the replication studies, especially if they are performed on some clearly distinguished population subgroups (males vs. females, smokers vs. never smokers, etc.). They can point on some context dependent effect of the genetic polymorphisms on analyzed biochemical or anthropometrical parameter. Also the potentially different genetic background or different environment could play an important role in expression of the effects of the different alleles.

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