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HUMAN GENETICS

Association Analysis of Alcohol Metabolizing Enzymes *ADH1B, ADH7, CYP2E1* Gene Polymorphism with Risk for Coronary Atherosclerosis

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Abstract—The allele and genotype distribution of two alcohol dehydrogenase genes *ADH1B* (exon 3 polymorphism *A/G* (*47His*)), *ADH7* (intron 5 polymorphism *G/C*) and cytochrome P450 2E1 gene (*CYP2E1*; 5'-flanking region *G/C* and intron 6 T/A polymorphisms) were examined in Russian (Tomsk, $n = 125$) healthy population and in coronary atherosclerosis patients (CA, $n = 92$). The genotype frequencies followed the Hardy–Weinberg equilibrium and the alleles were in linkage equilibrium or gametic equilibrium in the control sample. Only two *CYP2E1* gene polymorphisms were in linkage disequilibrium. The frequencies of the derived alleles at *ADH1B*G* (+*Msl*I) allele, *CYP2E1*C2* (+*Pst*I) allele and *CYP2E1*C* (–*Dra*I) allele were 8.48 ± 1.86, 1.20 ± 0.69, and 10.00 ± 1.90%, respectively. The *ADH7* gene polymorphism showed a high level of heterozygosity; the frequency of the *ADH7*C* (–*Sty*I) allele was 44.58 ± 3.21%. A significantly higher frequency of *CYP2E1 Pst*I *C2* allele has been revealed in the CA group ($P = 0.043$; $OR = 4.23$; 95% CI 1.03–20.01). The tendency to significant effect of *A1A2* genotype in *ADH1B Msl*I polymorphism was observed for systolic blood pressure in the control group ($P = 0.068$). The statistically significant two-way interaction effects of *ADH7 Sty*I and *CYP2E1 Dra*I on diastolic blood pressure ($P = 0.029$) and on the serum high density lipoprotein level ($P = 0.042$) were also revealed. Association of *A1A2* genotype in *ADH1B Msl*I polymorphism with reduced amount in a serum of a very low density lipoprotein level ($P = 0.045$) have also been shown. This may result from multifunctional activity of alcohol metabolizing enzymes and their involvement in many metabolic and free radical reactions in the body.

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INTRODUCTION

To date, noninfectious diseases are the main cause of human lethality according to a WHO report (2003). For the majority of lethal cases, cardiovascular diseases (CVD) including hypertension, ischemic heart disease, cerebral-vascular diseases (stroke), peripheral blood diseases, heart insufficiency, rheumatic diseases, inherited heart defects, and cardiomyopathy are responsible [1].

Excessive alcohol consumption has been long associated with increased risk of CVD. Nevertheless recently it has been shown that moderate alcohol consumption have a cardio-protecting effect especially in respect to coronary heart disease and ischemic lesions [2]. Approximately 90% of ethanol degrades in human liver in oxidative and nonoxidative metabolic pathways [3]. The main enzymes metabolizing ethanol are considered to be alcohol dehydrogenase (ADH [1.1.1.1]), acetaldehyde dehydrogenase (ALDH [1.2.1.3]), and cytochrome P450 class 2E1 (CYP2E1 [1.14.14.1]). Catalase (CAT [1.11.1.6]) and fatty acid ethyl ethers synthase (FAEES [2.3.1.85]) are also involved in ethanol oxidation but are less important [3]. It is clear that genetic polymorphisms in these enzymes affect the rate of ethanol metabolism and are involved in the diseases induced by excessive alcohol consumption and the factors determining predisposition to CVD. It has been shown that Japanese men homozygous for wild-type class 1 ADH allele (*ADH1B*47Ala*; *ADH2*1*) have higher correlation between arterial blood pressure and daily alcohol consumption compared to hetero- and homozygous men with a mutant allele (*ADH1B*47His; ADH2*2*). The latter two isozymes are capable of oxidizing ethanol 100- and 200-fold faster compared to the wild-type homodimer, respectively [4, 5]. At the same time these correlations were similar in three genotypes of *ALDH2* gene in mitochondrial acetaldehyde dehydrogenase. Homo- and heterozygous carriers of mutant alleles (*ALDH2*2*) have respectively 19- and 6-fold higher concentration of acetaldehyde compared to the carriers of the wild-type homozygous alleles (*ALDH2*1*). This data may indicate that the increase of blood pressure depends on blood concentration of ethanol rather than acetaldehyde [5]. Hashimoto et al. [6] has also shown an association of genotypes *ADH1B* with arterial pressure fluctuations as well as serum triglyceride and urea concentrations in the Japanese population, consuming moderate and high amounts of ethanol (more than

Table 1. Polymorphism of the ethanol-metabolizing enzymes, examined in this study

Gene	Chromosomal localization	Polymorphism	Mutant allele	Function	Expression	Reference
CYP2E1	10*q*24.3-qter	–/+ *Pst*I in 5'-UTR (–1293); rs 3813867; *G/C*	*CYP2E1*5B*; *C2* (+*Pst*I)	Ethanol-induced microsomal monooxygenase, detoxication of xenobiotics, ethanol oxidation	Liver, partially kidney, nose mucosa, lungs, intestine, lymphocytes	[10–13]
		+/–*Dra*I in intron 6 (7632); rs 6413432; *T/A*	*CYP2E1*6*; *C* (–*Dra*I)			
ADH1B	4*q*21–23	–/+*Msl*I in exon 3; rs 1229984; *A/G*	*ADH1B*47 His*; *A2* (+*Msl*I)	Ethanol and other aliphatic and aromatic alcohols oxidation	Liver, negligible in other tissues	[14–18]
ADH7	4*q*21–23	+/–*Sty*I in intron 5; rs 1154458; *G/C*	*ADH7*B2* (–*Sty*I)	Oxidation of retinol, high concentrations of ethanol, the mixture of middle--chain alcohols and aromatic alcohols	Intestine, esophagus	[14, 18–21]

300 g/week), and having the values of more that 2/3 percentile (in higher 1/3 of values in variation rows of characters). Thus, this study has shown for the first time an association of homozygous *ADH1B*47His* allele with the risk of development of coronary heart disease [6]. The association of moderate ethanol consumption and low risk of coronary heart disease may result from ethanol positive effects, which involve changes in cholesterol content in lipoproteins of high and low density, insulin susceptibility, thrombocyte aggregation, blood coagulation and fibrinolysis. Ethanol increases concentration of lipoproteins of high density (HDL) fraction, which protect from coronary heart disease as well as produce anti-thrombotic effect [7]. However alcohol can exert an adverse effect as it increases arterial blood pressure and triglyceride and urea concentration [6].

Whitfield et al. [8] did not find any effects of genotypes *ADH1B* and *ADH1C* on alcohol consumption and HDL in plasma although concentration of all measured components of HDL increased with the level of alcohol consumption. There is no evidence at present for the linkage of genetic polymorphism of ethanol–metabolizing enzymes with the risk of CVD development as well as direct evidence of ethanol metabolizing enzyme genetic polymorphism involvement in the variability of quantitative traits determining sensitivity to these diseases.

Cytochrome P-450 enzymes, namely CYP2E1, are localized in the liver and blood vessel walls and are involved in the oxidation of low-density lipoproteins (LDL) and, hence, in atherogenesis [9]. The genes that we studied in this work are presented in Table 1. ADH represents a group of polyfunctional enzymes, which participate in cholesterol and bile acid synthesis as well as in the metabolism of retinoid, neuromediators and steroid hormones [14]. Seven ADH genes form a 380-kb cluster localized on chromosome 4 at q21–q23 [15]. Polymorphism *ADH1B*47His* is the most widespread and is associated with alcoholism and alcohol-induced diseases in many populations [16, 17].

The objective of this study is to evaluate the risk of coronary atherosclerosis development associated with polymorphism of ethanol metabolizing enzymes genes, namely *ADH1B, ADH7,* and *CYP2E1*, as well as to search for association of this enzyme genotypic variability with arterial blood pressure and lipid metabolism markers in the Russian population of West-Siberian region.

MATERIALS AND METHODS

The objects of the study. The group of patients from Tomsk with the ischemic heart disease and coronary atherosclerosis (KA), documented by angiographic studies of coronary blood vessel, consisted of 80 men and 12 women (age from 30 to 70 years, mean age 49.44 ± 0.98 years). The control group was represented by healthy 121 men and 4 women (age 20–58 years, mean 41.09 ± 0.70 years). The groups were homogenous ethnically, containing more than 90% of Russians.

Control patients were checked for systolic and diastolic pressure (SP and DP), content of total cholesterol (TC), triglycerides (TG), lipoproteins of a high, low and very low density (HDL, LDL and VLDL, respectively) in serum. All the parameters excluding arterial blood pressure were normalized for body mass index (BMI). For the statistical calculations, the distributions of all the quantitative parameters were tested for normality and when necessary were adjusted to normal distribution using log scale. KA patients were tested only for the serum TC and TG content.

Genotyping of ADH1B, ADH7, and CYP2E1 polymorphisms. Genotyping of four mononucleotide polymorphisms *ADH1B, ADH7, CYP2E1, Pst*I, and *Dra*I (NCBI Assay ID rs1 229 984, rs1 154 458, rs3 813 867,

and rs6 413 432, respectively) were conducted by polymerase chain reaction (PCR) and analysis of restriction fragments length polymorphism (RFLP) of amplicons in 2–3% agarose gels. PCR protocols and primers were described earlier [15, 21–23]. Primers were provided by MEDIGEN Lab (Novosibirsk). Restriction enzymes were purchased from SibEnzim (Novosibirsk). G ⟶ A polymorphism in the third exon of *ADH1B* gene, which resulted in the appearance of restriction site *Msl*I and in *Arg47His* substitution was presented by two alleles: *A1*—the wild type (685 bp) and A2—mutant allele (443 + 242 bp; +*Msl*I; *47His*; *ADH1B*2*). Polymorphism C ⟶ G in intron 5 of the *ADH7* gene, which was revealed by restriction site *Sty*I, resulted in the appearance of *B1* (wild type, 263 + 214 bp) and *B2* (mutant, 477 bp, –*Sty*I) alleles. Polymorphism G ⟶ C in the 5'–flanking region of the *CYP2E1* (–1293 G > C) gene was registered by the *Pst*I restriction site and was represented by ancestral *C1* (410 bp) and derivative *C2* (290 + 120 bp, *CYP2E1*5B*, +*Pst*I) alleles. In intron 6 of the *CYP2E1* gene polymorphism T ⟶ A (7632T > A) was revealed by the loss of the *Dra*I restriction site, namely wild-type allele *D* (235 + 351 bp) and mutant allele *C* (686 bp, *CYP2E1*6*, –*Dra*I).

Statistical analysis. The accordance of genotype distribution to Hardy–Weinberg equilibrium, of observed and expected heterozygosities, comparison of allele and genotype frequencies, check for linkage disequilibrium (gametic disequilibrium) was done using traditional methods of population biometry [24]. The search of associations of genetic variability with the risk of coronary atherosclerosis development was conducted by comparison of allele frequencies in the groups of affected patients and healthy controls using Fisher's exact test with two-tailed evaluation of the level of significance (*P*) or according to χ^2 Pearson's test. The difference in genotype frequencies was estimated by statistics of maximum likelihood test χ^2 (ML χ^2). We also determined the odds ratio (OR) of the disease development and its 95% confidence interval (95% CI). Correlation of genotypic variability in ethanol metabolizing enzymes with quantitative patterns was evaluated using one- or two-way analysis of variance (ANOVA). *P* values less than 0.05 were considered statistically significant. Microsoft Office Excell 2003 and Statistica 5.5A software were used.

RESULTS AND DISCUSSION

Both examined groups have shown Hardy–Weinberg equilibrium at all the studied locuses (Table 2). Both samples were characterized by low mutant allele frequencies and therefore low genetic variability, excluding the *ADH7 Sty*I polymorphism, which exhibited maximum heterozygosities in both groups. The frequencies of the "derivative" alleles corresponded to those observed in many Caucasoid populations [15, 25].

In the control group, gametic equilibrium (linkage equilibrium) was observed at all the combinations of locus pairs excluding gene *CYP2E1 Pst*I and *Dra*I polymorphisms, which are linked and located at a distance of ≈8.9 kb (the disequilibrium index *D* was +0.006307 ± 0.006870; $\chi^2 = 4.76$; $P = 0.029$; correlation coefficient ρ between the loci was +0.19451).

Comparison of genotype frequencies in control and CA patients revealed ($P = 0.08$) a difference in genotype frequencies in polymorphic variant *CYP2E1 Pst*I. For the other loci, no genotype variability among groups was observed (Table 3).

The increased risk of CA development has been found in the carriers of *C2* allele of polymorphic variant *CYP2E1 Pst*I (Table 4). The increased frequency of *CYP2E1*C2* allele (fourfold or by 3.69%) in the CA patients was observed. Nevertheless this data should be reexamined in other populations and other ethnical groups, as the occurrence of mutant alleles in other regions can be high. Therefore the functional significance of the examined polymorphic variants for the development of atherosclerotic lesions cannot be excluded.

Table 5 shows the results of the one-way ANOVA for genotypic variability linkage of *ADH1B, ADH7* and *CYP2E1* with variation of some quantitative patterns, responsible for susceptibility to some types of CVD in the control group of Tomsk inhabitants. In the CA patients, no associations were found between all studied genotypes and triglyceride and total cholesterol in serum. However, the results showed close to statistically significant association of the *ADH1B*47His* (**A2*) polymorphism with systolic pressure (SP; $P < 0.07$). In the carriers of the *A1A1* genotype ($n = 91$) SP was on average 128.7 mm Hg, while individuals with *A2A2* genotype ($n = 19$) had SP of 119.95 mm Hg (Table 5). In addition, this allele affects the amount of serum triglycerides, which was decreased in the carriers of genotype *A1A2* ($P < 0.06$; *A1A1* ($n = 93$): *A1A2* ($n = 19$) = 123.34 : 87.06 μM/ml. A significant decrease in concentration of the very low-density lipoproteids was recorder in the carriers of the *A1A2* genotype ($P < 0.06$; *A1A1* : *A2A2* = 24.67 : 17.41 μM/ml).

Saito et al. [5] using 335 randomly selected Japanese subjects (aged 40–69 years) have found stronger regressive dependence of diastolic pressure on alcohol consumption in men with genotype *A1A1* (8.4%) compared to the group of men with genotypes *A1A2* (34.9%) or *A2A2* (56.7%). These authors have also shown that individuals with genotype *A1A1*, which were capable of oxidizing ethanol 100–200-fold slower compared to individuals with genotypes *A1A2* and *A2A2*, respectively [4], can maintain high blood ethanol concentration for a long time after its consumption, which can induce an increase of arterial pressure [5].

Yamada et al. [26] have shown that 855 middle-aged Japanese men did not show any significant association of the *ADH1B* and *ALDH2* genotypes with arterial pressure but have found an increased arterial pressure in individuals having *C2C2* genotype with *CYP2E1 Pst*I

Table 2. Frequencies of mutant alleles (%) and genetic variability of ethanol-metabolizing enzymes ADH1B, ADH7, and CYP2E1 in CA patients and healthy controls

Gene/allele	Sample	Frequency, %	H_{obs}	H_{exp}	χ^2	P	n
ADH1B	Controls	8.48 ± 1.86	16.96	15.53	0.96	0.327	112
(+*Msl*I/*A2*)	CA patients	4.89 ± 1.59	9.78	9.30	0.24	0.624	92
ADH7	Controls	44.58 ± 3.21	55.83	49.41	2.03	0.155	120
(–*Sty*I/*B2*)	CA patients	45.65 ± 3.67	50.00	49.62	0.01	0.920	92
CYP2E1	Controls	1.20 ± 0.69	2.40	2.37	0.02	0.892	125
(–*Pst*I/*C2*)	CA patients	4.89 ± 1.59	7.61	9.30	3.05	0.081	92
CYP2E1	Controls	10.00 ± 1.90	20.00	18.00	1.54	0.214	125
(–*Dra*I/*C*)	CA patients	8.70 ± 2.08	17.39	15.88	0.83	0.362	92

Note: H_{obs}, observed heterozygosity H_{exp}, expected heterozygosity, χ^2, P values of χ^2 and achieved level of the correspondence of the observed genotype distribution to the expected at Hardy–Weinberg equilibrium; n, sample size; CA, coronary atherosclerosis.

Table 3. Distribution and frequencies of genotypes *ADH1B*, *ADH7*, and *CYP2E1* in CA patients and healthy controls

Gene	Genotypes	Control		CA		χ^2	P
		number	frequency, %	number	frequency, %		
*ADH1B** (*Msl*I)	*A1A1*	93	83.04	83	90.22	2.25	0.133
	A1A2	19	16.96	9	9.78		
ADH7 (*Sty*I)	*B1B1*	33	27.50	27	29.35		
	B1B2	67	55.83	46	50.00	0.84	0.656
	B2B2	20	16.67	19	20.65		
CYP2E1 (*Pst*I)	*C1C1*	122	97.60	84	91.30		
	C1C2	3	2.40	7	7.61	5.04	0.080
	C2C2	0	0.00	1	1.09		
*CYP2E1** (*Dra*I)	*DD*	100	80.00	76	82.61	0.24	0.627
	DC	25	20.00	16	17.39		

* No homozygotes for the mutant allele were observed; χ^2, P, values of the maximum likelihood χ^2 test and the achieved level of significance of the differences in genotype frequencies between the coronary atherosclerosis (CA) patients and healthy controls.

polymorphism. However, after leveling of genotype groups by age, body mass index and the amount of consumed alcohol by multiple regression analysis, the effect of *CYP2E1 Pst*I genotypes on arterial pressure appeared to be insignificant. The authors conclude on the lack of the linkage of ethanol metabolizing enzyme *ADH1B, ALDH2* and *CYP2E1* polymorphism and the arterial pressure in Japanese men [26].

The opposite effect of *ADH1B* on systolic pressure has been shown in the study by Hashimoto et al. [6], who received the results comparable to our data presented here. Thus, in that study [6] has been shown that the occurrence of *A2A2* genotype at gene locus *ADH1B* was statistically significant in men who had systolic pressure higher of 1/3 variation row of the character (≥138 mm Hg) compared to individuals having SP in lower 2/3 of variation row (<138 mm Hg). However, Hashimoto et al. [6] have studied 133 men working in the city hospitals, which did not suffer hypertension, hyperlipidemia or hyperurikemia but consuming more than 300 g of ethanol per week. In contrast to our results, these authors [6] have shown that among the individuals which have serum triglycerides and uric acid higher than 2/3 percentiles as they were in the top 1/3 of variation rows, the men with genotype *A2A2*, rather than *A1A1*, predominated.

The effect of genotype variability of ethanol metabolizing enzymes on CVD and the risk factors of its development had not been studied in Caucasian populations probably due to the low frequencies of mutant alleles in Caucasoids.

The mechanisms of ethanol effects on arterial pressure are not clear. In the present work, the 5% level of statistical significance of *ADH7 Sty*I, *CYP2E1 Pst*I, and *Dra*I polymorphisms with neither of the examined quantitative patterns was not passed. However, exclud-

Table 4. Odds ratio for coronary atherosclerosis development according to the polymorphic variants of ethanol-metabolizing enzyme genes *ADH1B*, *ADH7,* and *CYP2E1*

Gene	Alleles	Number of alleles (%)		χ^2	*P*	*OR* (95% CI)
		controls	CA			
ADH1B	*A1*	205 (91.52)	175 (95.11)	2.04	0.153	0.55
(*Msl*I)	*A2*	19 (8.48)	9 (4.89)			(0.23–1.33)
ADH7	*B1*	133 (55.42)	100 (54.35)	0.05	0.827	1.02
(*Sty*I)	*B2*	107 (44.58)	84 (45.65)			(0.86–1.21)
CYP2E1	*C1*	247 (98.80)	175 (95.11)	4.09*	0.043	4.23
(*Pst*I)	*C2*	3 (1.20)	9 (4.89)			(1.03–20.01)
CYP2E1	*D*	225 (90.00)	168 (91.30)	0.21	0.646	0.86
(*Dra*I)	*C*	25 (10.00)	16 (8.70)			(0.42–1.73)

* χ^2 was calculated with Yeates correction for continuity; *OR* (95%CI) is the odds ratio and 95% confidence interval.

Table 5. One-way ANOVA of the association between gene polymorphisms *ADH1B*, *ADH7,* and *CYP2E1* with age, arterial pressure, lipid metabolism parameters, and bone mass index in the healthy controls from Tomsk

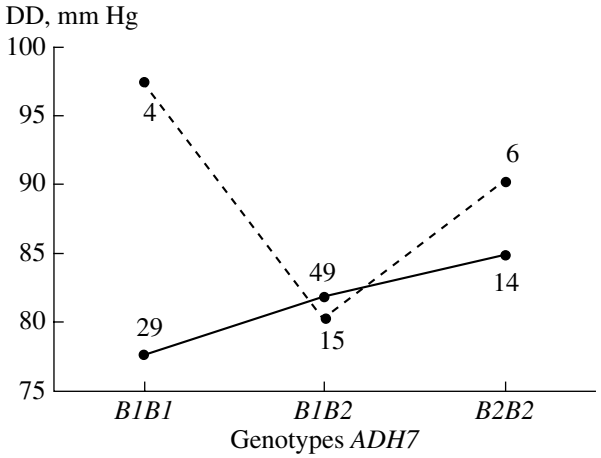
Character	*ADH1B* (+/–*Msl*I)			*ADH7* (+/–*Sty*I)			*CYP2E1* (+/–*Pst*I)			*CYP2E1* (+/–*Dra*I)		
	F	*P*	*n*	*F*	*P*	*n*	*F*	*P*	*n*	*F*	*P*	*n*
Age	1.99	0.161	118	1.59	0.207	126	0.02	0.960	130	0.30	0.586	130
BMI	0.17	0.683	112	0.78	0.463	120	1.47	0.227	125	0.17	0.677	125
SP	3.39	*0.068*	110	1.27	0.285	118	0.37	0.545	122	1.43	0.235	123
DP	2.05	0.155	110	1.53	0.220	118	0.86	0.356	122	1.66	0.200	123
HDL	0.25	0.616	111	0.13	0.879	119	1.05	0.308	124	0.78	0.380	124
LDL	0.64	0.424	111	1.06	0.351	118	0.95	0.331	123	0.00	0.975	124
VLDL	4.10	**0.045**	112	0.33	0.719	120	0.05	0.821	125	0.13	0.723	125
TC	0.04	0.836	112	1.28	0.281	119	1.29	0.258	124	0.04	0.851	125
TG	3.68	*0.058*	112	0.36	0.701	120	0.02	0.892	125	0.19	0.661	125

Note: *F*, *P*, *n* are Fisher's parameter for ANOVA, statistical significance and sample size, respectively. BMI, bone mass index; SP, systolic pressure; DP, diastolic pressure; HDL, high-density lipoproteins; LDL, low density lipoproteins; VLDL, very low-density lipoproteins; TC, total cholesterol; TG, triglycerides.

ing the genetic variation of *CYP2E1 Pst*I it is possible to suggest 70–80% statistically significant association of the polymorphic markers examined with both systolic and diastolic pressure (Table 1).

Two-way ANOVA of the interaction effects of pairwise combinations of the examined loci revealed a statistically significant effect of *ADH7 Sty*I and *CYP2E1 Dra*I interactions and diastolic pressure (figure; $F = 3.64$; $P = 0.029$; $n = 117$) and on the concentration of high-density lipoproteins ($F = 3.27$; $P = 0.042$; $n = 118$). The latter fact as well as the linkage of *ADH1B Msl*I polymorphism to variability of triglyceride content and very low-density lipoproteins is likely a result of NADH production during ethanol oxidation by ADH. The NADH/NAD ratio increases, promoting fatty acid synthesis [27]. This may be related to the multiple functions of these enzymes in the body, which may involve catabolism of neuromediators [14].

In conclusion, we would like to note the following. Firstly, the mutant alleles of the *ADH1B Msl*I, *CYP2E1 Pst*I, and *Dra*I genes occur at a low frequency, which limits their usage for molecular genetic diagnostics in



Combined effect of polymorphic variants *ADH7 Sty*I and *CYP2E1 Dra*I on diastolic pressure (DP) in control group ($F = 3.64$; $P = 0.029$; $n = 117$). Solid line corresponds to genotype *DD*, dotted line, to *DC* of polymorphism *CYP2E1 Dra*I; dots on the lines are average values of DP for pairwise combinations of corresponding phenotypes *ADH7*; numerals show the number of individuals in the subgroups.

respect to CVD susceptibility. Second, in the group of coronary atherosclerosis patients, a statistically significant ($P < 0.05$) increase in frequency (approximately fourfold) of the *C2* allele of the *CYP2E1 Pst*I polymorphism, which is related to higher transcriptional and enzymatic activity, was observed. However, this does not permit the usage of this variant for identifying risk groups of susceptibility to CA due to the low incidence of this allele in the Tomsk population (<2%) and high variability of the 95% confidence interval of the odds ratio in the disease development. Third, the close to statistically significant effect of *A1A2* genotype of *ADH1B Msl*I polymorphism to the decrease of systolic pressure ($P < 0.07$). Fourth, the revealed statistically significant effects of interacting loci *ADH7 Sty*I and *CYP2E1 Dra*I on diastolic pressure ($P = 0.029$) and on the high density lipoproteins ($P = 0.042$) as well as *A1A2* genotype *ADH1B Msl*I polymorphism on the low amounts of very low-density lipoproteins resulted from multiple functions of these enzymes.

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