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Polymorphisms of Alcohol Dehydrogenase Genes *ADH1B* and *ADH7* in Russian Populations of Siberia

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Abstract—Three Russian populations of Siberia were examined for allele and genotype frequency distributions of two alcohol dehydrogenase genes, *ADH1B* (exon 3 polymorphism A/G detectable with *Msl*I) and *ADH7* (intron 5 polymorphism G/C detectable with *Sty*I). No interpopulation or sex difference in allele frequencies was revealed. Allele *ADH1B*G* (+*Msl*I, A2) was rare (3.6–7.5%); the frequency of the "mutant" *ADH7* allele (–*Sty*I, B2) was 46.02% in the total sample (N = 339). The genotype frequencies obeyed the Hardy–Weinberg equilibrium and the alleles were in linkage equilibrium in each population. Frequency of *ADH7* allele *B2* increased beyond 40 years of age in the total sample (by 11%, P = 0.001) and in the Tomsk population (by 9%, P = 0.017). The *ADH1B* and *ADH7* polymorphisms had no effect on the antioxidant activity (AOA), which was inferred from the ability of serum to reduce the yield of thiobarbituric acid-reactive species in the Fe^{2+}–lecithin system. In the Tomsk population, carriers of *AHD1B* allele *A2* showed a significant increase in very low density lipoproteins (by 9.95%, P = 0.045) and a near significant increase in systolic pressure (by 6.8%, P = 0.068) and serum triglycerides (by 6.16%, P = 0.058).

Key words: human, population genetics, alcohol dehydrogenases, genetic polymorphism

INTRODUCTION

Alcohol dehydrogenases (ADH) [EC 1.1.1.1] of five classes catalyze oxidation of various alcohols to aldehydes. The enzymes are encoded by seven genes, which cluster in a region of about 380 kb on chromosome 4q21–23 [1]. Class I genes (*ADH1A*, *ADH1B*, *ADH1C*) form a 77-kb cluster, which has *ADH7* (class IV) at the 5' end and *ADH6*, *ADH4*, and *ADH5* (classes V, II, and III, respectively) at the 3' end [1]. Class I ADH display the highest similarity. It should be noted that the seven genes are all structurally similar, but are expressed in different tissues. Their protein products are similar in amino acid sequence, but differ in substrate specificity [2–4]. Class III ADH [EC 1.2.1.1] (*ADH5*) is to an extent an exception, acting mostly as glutathione-dependent formaldehyde dehydrogenase [5]. Class I ADH metabolize predominantly ethanol, and polymorphic alleles of their genes are associated with alcohol abuse incidence in populations. For instance, a substantial variation in frequency among ethnic groups has been reported for allele *ADH1B*2* (*ADH1B*47His*, earlier known as *ADH2-2*), which protects from alcoholism, coding for a protein with substitution Arg47 ⟶ His and, consequently, a higher enzymic activity [1, 6]. The allele is more common in East Asian populations. The frequency of this allele is high and alcoholism incidence low in Ashkenazi Jews [7]. *ADH7* is 17.3 kb and contains 9 exons; its protein product consists of 374 amino acid residues and has a high retinol dehydrogenase activity [8, 9]. Class I and class IV ADH originate from a common ancestor and share some functions, in particular, ethanol oxidation. One of the major functions of ADH7 is oxidation of retinol to retinal, which is involved in synthesis of retinoic acid, a hormone essential for cell differentiation. *ADH7* is expressed predominantly in the intestine and esophagus [8]; its product mostly oxidizes mixed alcohols with medium-sized chains (octanol) and aromatic alcohols. At high concentrations of ethanol, ADH7 participates in the first stage of its utilization along with class I ADH [5].

Thus, ADH are multifunctional enzymes that are involved in metabolism of retinoids, catabolism of neuromediators, transformation of steroid hormones and ω-oxy fatty acids, and synthesis of cholesterol and bile acids. In addition, the enzymes protect cells from various xenobiotics, exogenous ethanol, and endogenous toxins [5]. Genes for class I and IV ethanol-oxidizing ADH have been studied in most detail.

We examined the *ADH1B* and *ADH7* polymorphisms in ethnic Russians of three Siberian populations in order to study the Siberian gene pool and to evaluate the predisposition to alcohol abuse. The genetic structure was characterized for three (Tomsk, Seversk, and Kargala) populations of the Tomsk

Region; the variation of allele and genotype frequencies was analyzed as dependent on the age, sex, and population; and the polymorphisms were tested for linkage disequilibrium. Since the ADH functions are diverse and retinol (vitamin A) acts as an antioxidant, the *ADH1B* and *ADH7* polymorphisms were tested for contribution to the variation of traits reflecting the state of the antioxidant system (AOS) and lipid metabolism.

EXPERIMENTAL

DNA specimens of healthy people were obtained from the DNA bank of the Institute of Medical Genetics. The subjects were from the town of Tomsk (111 males and 2 females, ethnic Russians), the village of Kargala located 65 km away from Tomsk (46 males and 52 females, 98% ethnic Russians), and the town of Seversk located 10 km away from Tomsk (99 males and 79 females, 89% ethnic Russians). DNA was isolated from whole venous blood by the perchlorate method in the presence of phenol [10].

Genotyping of *ADH1B* and *ADH7* (NCBI assay ID ss2978360 for *ADH1B* and ss2978365 for *ADH7* [11]) involved PCR and RFLP analysis in 2% agarose gel. The PCR conditions and primers were as described earlier [1]. The A/G polymorphism of *ADH1B* exon 3 was detected with *Msl*I, the 685-bp product suggesting the wild-type allele *A1* and the fragments of 443 and 242 bp, the mutant allele *A2*. The G/C polymorphism of *ADH7* intron 5 was detected with *Sty*I, the fragments of 263 and 214 bp suggesting the wild-type allele *B1* and the intact 477-bp product, the mutant allele *B2*.

Serum antioxidant activity (AOA) was estimated spectrophotometrically by malonic dialdehyde content [12, 13], in samples of Seversk (101 people) and Kargala (51 people). Correction for seasonal and interpopulation variation employed an equation taking into account the standard deviations in the groups [14, 15].

In addition, Tomsk residents were tested for systolic and diastolic pressure; total cholesterol; triglycerides; and high, low, and very low density lipoproteins. The biochemical estimates were normalized with respect to the body mass index. Trait distributions were checked for normality and, when necessary, logarithmically brought to the normal one.

Statistical analysis. The *ADH1B* and *ADH7* alleles were tested for association with quantitative traits by one- or two-way ANOVA. Allele frequencies were computed, genotype distributions checked for deviation from the Hardy–Weinberg proportions, observed and expected heterozygosities compared, allele and genotype frequencies compared for individual groups, and linkage disequilibrium tests performed by the standard methods of population biometrics [16]. Analysis employed the STATISTICA 5.0 and Microsoft Excel 97 packages.

RESULTS AND DISCUSSION

Genetic Structures of the Populations

The genotype frequencies observed in the three individual populations and in the total sample obeyed the Hardy–Weinberg equilibrium (Table 1). No significant difference in *ADH1B* or *ADH7* allele frequencies was found for the populations or sex groups. A near significant increase in frequency of *ADH7* allele *B2* was detected in the Seversk population as compared with the populations of Kargala ($\chi^2 = 3.47$, $P = 0.063$) and Tomsk ($\chi^2 = 2.94$, $P = 0.087$) (Table 2). A possible cause is heterogeneity of the Seversk population, which mostly consists of recent migrants. This was supported by the near maximal estimates of heterozygosity and effective allele number (Table 2). The functional role of the *Sty*I polymorphism of *ADH7* is still unknown; the frequency of the mutant allele *B2* varies from 15% in the indigenous populations of North America to 70–75% in some populations of Africa and East Asia [1].

Homozygotes for the mutant A2 allele of *ADH1B* were not found in our samples (Table 1). A decrease in frequency of allele *A2* (3.5%) in the Kargala population was nonsignificant ($\chi^2 = 3.05$, $P = 0.081$ as compared with the Tomsk population; $\chi^2 = 1.64$, $P = 0.199$ as compared with the Seversk population) and was probably due to higher homogeneity of this rural population.

As for *ADH1B*, allele *A2* (*ADH1B*47His*, *ADH2*2*) codes for the enzyme with a higher activity and substantially varies in frequency among populations. Thus the frequency of allele *A2* is more than 33% in populations of East Asia and less than 25% in other regions [1]. Our estimates agree with the frequency reported for ethnic Russians earlier (6.6%, $N = 46$) [1]. According to published data, the frequency of the mutant allele is rather high (41%) in healthy Muscovites ($N = 50$) and 18.9% in alcoholics without cirrhosis ($N = 37$) [17].

To study the allele frequency distributions of *ADH1B* and *ADH7* as dependent on the age, groups of subjects below or beyond the median age were isolated in each sample and compared with each other. No association was observed between the *ADH1B* allele frequencies and the age. Yet the frequency of *ADH7* allele *B2* was elevated in older subjects of the Tomsk population (15.11%) and of the total sample (8.9%), which was mostly due to a higher frequency of homozygotes B2/B2 (Table 3). It should be noted that the frequency of allele *B2* increased with age in the Seversk and Kargala populations, but the increase was nonsignificant possibly because the age range was shifted to an older age in our samples from these populations. The null hypothesis that the age distribution

Table 1. Genotype frequency distributions of *ADH1B* and *ADH7* polymorphisms in the three populations of the Tomsk Region

Sample	Gene, genotype	Total cases	Frequency, %	χ^2	*P*	Mean age (range)	*N*
Seversk	*ADH1B**					52.65 ± 0.92	128
	A1/*A1*	112	87.50	0.57	0.451	(12–75)	
	A1/*A2*	16	12.50				
	ADH7						
	B1/*B1*	32	25.00	0.27	0.600		
	B1/*B2*	61	47.66				
	B2/*B2*	35	27.34				
Kargala	*ADH1B**			0.13	0.714	37.39 ± 0.74	98
	A1/*A1*	91	92.86			(10–50)	
	A1/*A2*	7	7.14				
	ADH7			2.19	0.139		
	B1/*B1*	29	29.59				
	B1/*B2*	55	56.12				
	B2/*B2*	14	14.29				
Tomsk	*ADH1B**			0.75	0.387	40.64 ± 0.76	113
	A1/*A1*	96	84.96			(28–50)	
	A1/*A2*	17	15.04				
	ADH7			0.23	0.633		
	B1/*B1*	35	30.97				
	B1/*B2*	58	51.33				
	B2/*B2*	20	17.70				
Total	*ADH1B**			1.33	0.248	44.23 ± 0.60	339
	A1/*A1*	299	88.20			(10–75)	
	A1/*A2*	40	11.80				
	ADH7			0.37	0.542		
	B1/*B1*	96	28.32				
	B1/*B2*	174	51.33				
	B2/*B2*	69	20.35				

Note: *P* is the significance of correspondence of the observed genotype frequencies to the Hardy–Weinberg equilibrium.
* Homozygotes for *ADH1B* allele *A2* were not found.

is normal was confirmed for the sample from Tomsk. The coefficient of age variation was about 19% in each of the three samples and 25% in the total sample.

An increase in frequency of allele *B2* in older subjects may be related to a diversity of functions played by class IV ADH (detoxification of xenobiotics, synthesis of retinoids, etc. [5]). Possibly, allele *B2* is not selectively neutral for individual survival beyond 40–42, if the corresponding polymorphism is linked to another one or the allele function depends on the genetic environment, epistatic interactions, or conformational changes in the mRNA. It is clear that our findings need verification with other populations.

Linkage Disequilibrium of the *ADH1B* and *ADH7* Polymorphisms

We did not reveal a linkage disequilibrium for *ADH1B* and *ADH7* in the individual populations or the total sample (Table 4). The two genes are linked together and are about 101 kb apart. Our data agree with a lack of linkage disequilibrium between the two genes in the European populations other than Catalonian [1]. A significant linkage disequilibrium has been observed for the Chinese population of San Francisco and some other populations with the bottle-neck effect (a dramatic decrease in population size), a small size, or a high coefficient of inbreeding (e.g., Ethiopian Jews, Samarians, Micronesians, Druses) [1, 18]. Pos-

Table 2. Genetic variation and mutant allele frequencies of *ADH1B* and *ADH7* in the tree populations of Siberia

Gene, allele	Sample	Frequency, %	H_{obs}	H_{exp}	D	N_e
ADH1B (*+MslI*)/*A2*	Seversk	0.0625 ± 0.0151	0.1250 ± 0.0292	0.1172 ± 0.0264	0.0667 ± 0.1138	1.132
	Kargala	0.0357 ± 0.0133	0.0714 ± 0.0260	0.0689 ± 0.0245	0.0370 ± 0.1357	1.0740
	Tomsk	0.0752 ± 0.0175	0.1504 ± 0.0336	0.1391 ± 0.0297	0.0813 ± 0.1185	1.1616
	Total	0.0590 ± 0.0090	0.1180 ± 0.0175	0.1110 ± 0.0159	0.0627 ± 0.0703	1.1249
ADH7 (*–StyI*)/*B2*	Seversk	0.5117 ± 0.0312	0.4766 ± 0.0441	0.4997 ± 0.0031	–0.0464 ± 0.0843	1.9989
	Kargala	0.4235 ± 0.0353	0.5612 ± 0.0501	0.4883 ± 0.0113	0.1494 ± 0.0866	1.9542
	Tomsk	0.4336 ± 0.0330	0.5133 ± 0.0470	0.4912 ± 0.0092	0.045 ± 0.0900	1.9654
	Total	0.4602 ± 0.0191	0.5133 ± 0.0271	0.4968 ± 0.0032	0.0331 ± 0.0525	1.9874

Note: Estimates (mean + standard error) are given for observed (H_{obs}) and expected (H_{exp}) heterozygosities, relative deviation (D) of H_{obs} from H_{exp}, and effective allele number (n_e).

Table 3. Frequency of *ADH7* allele *B2* in different age groups

Sample	Me	Frequency B2, %		χ^2	P
		≤Me	>Me		
Seversk	55	48.39 ± 4.49 (62)	53.79 ± 4.34 (66)	0.75	0.388
Kargala	38	40.22 ± 5.11 (45)	44.23 ± 4.87 (53)	0.00	0.974
Tomsk	40	32.76 ± 4.36 (58)	54.55 ± 4.75 (55)	10.91	0.001
Total	42	41.47 ± 2.67 (170)	50.59 ± 2.72 (169)	5.68	0.017

Note: Me is the median age. Frequencies are given as mean ± standard error; sample size is indicated in parentheses.

sibly, linkage disequilibrium was undetectable in our sample because of the low frequency of *ADH1B* allele *A2* or panmixy of Russian populations. We think it noteworthy that the probability of linkage disequilibrium was estimated at 80–85% for all but the Kargala population as a result of the low frequency of haplotype A2/B1. Presumably, the interpopulation variation of linkage disequilibrium parameters of *ADH1B* and *ADH7* and estimates of relative risk of alcoholism is explained by social factors [1, 6, 18].

Association of the *ADH1B* and *ADH7* Alleles with Serum AOA and Lipid Metabolism Parameters

We have earlier observed a seasonal variation of AOA and a difference in AOA between the urban (Seversk) and rural (Kargala) populations [13, 15]. In view of this, the AOA estimates were corrected with a formula taking account of the AOA standard deviation in the samples [14, 15]. No significant association was detected between AOA and the *ADH1B* or *ADH7* alleles in the Seversk (N = 101, P = 0.927 and 0.936, respectively) or Kargala (N = 51, P = 0.939 and 0.583) populations and in the pooled sample from the two populations (N = 152, P = 0.915 and 0.935). The effect of allele interaction on AOA in the pooled sample was nonsignificant (P = 0.381).

In the Tomsk sample, no significant association was found between the *ADH1B* or *ADH7* alleles and blood pressure or most parameters of lipid metabolism (Table 5). Yet a near significant decrease in systolic pressure was observed in heterozygous carriers of *ADH1B* allele *A2* (P = 0.07). Polymorphism of this gene accounted for 3.05% of the total variance of systolic pressure. The ADH activity at pH 7.5 is about fivefold higher in heterozygotes as compared with homozygotes for the wild-type allele [19]. It is well documented that elevated blood pressure is associated with higher alcohol consumption and that allele *A2* is

Table 4. Analysis of linkage disequilibrium between *ADH1B* and *ADH7* alleles in the three populations of Siberia

Sample	d	χ^2	P	N	ρ
Seversk	–0.0134 ± 0.0107	1.56	0.212	128	–0.1105
Kargala	0.0048 ± 0.0096	0.27	0.603	98	0.0528
Tomsk	–0.0176 ± 0.0114	2.01	0.152	113	–0.1349
Total	–0.0088 ± 0.0062	1.93	0.1648	339	–0.0756

Note: We estimated linkage disequilibrium parameter d (mean ± standard error), significance P of the difference of d from zero, and coefficient of correlation (ρ) of allele frequencies for the two genes.

Table 5. Analysis of variance of the association of the *ADH1B* and *ADH7* alleles with blood pressure and lipid metabolism parameters in the Tomsk population

Parameter	*ADH1B* (+/– *MslI*)			*ADH7* (–/+ *StyI*)		
	F	*P*	*N*	*F*	*P*	*N*
Systolic pressure	3.39	0.068	110	1.27	0.284	118
Diastolic pressure	2.05	0.154	110	1.54	0.220	118
Total cholesterol	0.04	0.836	112	1.18	0.309	120
Triglycerides	3.68	0.058	112	0.36	0.701	120
High density lipoproteins	0.25	0.616	111	0.13	0.879	119
Low density lipoproteins	0.44	0.509	111	0.63	0.534	119
Very low density lipoproteins	4.10	0.045	112	0.33	0.719	120

Note: Analysis of variance employed Fishers' test (*F*).

protective [6]. Hence, a decrease in blood pressure in carriers of allele *A2* may be explained by a high activity of the enzyme and rapid degradation of ethanol. The change in blood pressure may also be related to other ADH functions, e.g., to its role in catabolism of neuromediators [5]. A significant decrease (by 9.95%) in very low density lipoproteins and a near significant decrease (by 6.16%) in triglycerides were observed in carriers of allele *A2* from the Tomsk population (Table 5). The *ADH1B* polymorphism accounted for 3.59 and 3.24% of the total variance of these parameters, respectively. A possible explanation is that ethanol oxidation by ADH yields NADH. The NADH/NAD ratio increases, which expedites fatty acid synthesis [20]. Possibly, this triggers compensatory mechanisms reducing the pressure, triglycerides, and very low density lipoproteins in carrier of the mutant allele *A2*. We did not observe a significant association between the polymorphic *ADH7* alleles and any of the traits under study, nor a combined effect of *ADH1B* and *ADH7* alleles was detected. Genetic polymorphism of *ADH1B* has first been associated with coronary heart disease in Japanese who consumed alcohol in moderate or large amounts (>300 g weakly): these subjects had risk factors higher than 2/3 percentile (i.e., falling to the upper one-third of the variation range) [21]. A significant ($P < 0.05$) increase in systolic pressure, serum triglycerides, and uric acid has been observed in homozygotes A2/A2 compared with homozygotes A1/A1. It has been assumed that the increase is independent of aldehyde accumulation in blood and results from metabolic changes accompanying ethanol oxidation (e.g., an increase in NADH/NAD and reactive oxygen species) or other factors. This assumption still needs verification.

To summarize, the three Russian populations of Siberia did not differ in allele frequencies of *ADH7* and *ADH1B*. The mutant *ADH1B* allele *A2* occurred at a low (3.6–7.5%) frequency, the Hardy–Weinberg equilibrium was obeyed, and no linkage disequilibrium was observed. The frequency of the mutant *ADH7* allele *B2* (a lack of the *Sty*I site) was significantly increased in older subjects, as found for the Tomsk population and for the total sample. The *ADH1B* and *ADH7* polymorphisms were not associated with AOA. Carriers of allele *A2* from Tomsk displayed a significant decrease in very low density lipoproteins and a near significant increase in systolic pressure and triglycerides.

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