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Alcohol Drinking Habits and Genetic Polymorphism of Alcohol Metabolism Genes in West Siberia

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KEY WORDS Alcohol drinking habits; alcohol metabolism; genetic polymorphism; flushing; Siberia.

ABSTRACT In the present study we have determined alcohol drinking habits, alcohol-induced flushing response and polymorphism of alcohol metabolizing enzymes in a random female population sample from Novosibirsk (n=756, aged 25 to 65). Self-reported alcohol consumption and flushing response was measured with the help of a questionnaire. ADH2, ADH3, ALDH2 and CYP2E1 genotype frequencies were determined by DNA amplification followed by restriction enzyme digestion. Episodic consumption of alcohol (less than once per month and 1-2 times per month) was predominate (84.7% of the subjects) while only 7% reported regular consumption of alcohol (once per week and often). Current and past year abstainers constituted about 8.3%. The percentage of regular consumers of alcohol was greater among women with higher education. The average dose of alcohol consumed per drinking occasion was about 27.2 g pure alcohol. Alcohol-related flushing was experienced by about 30% of the subjects (13% often and 16% sometimes). The ALDH2 gene was found not to be polymorphic in this population sample. The frequencies of ADH2*2 and ADH3*2 alleles were 0.197 and 0.52, respectively. The frequency of the mutant allele (c2 allele) of CYP2E1 gene was found to be 0.03.

INTRODUCTION

The contribution that alcohol has made to the large fluctuations in mortality in Russia in recent years is now widely recognized. An association between heavy drinking and Russia is part of popular culture (Balkau 1999). Although a number of studies have been published concerning alcohol drinking patttern in Russian populations (Bobak et al. 1999; Nemtsov 2000), only few studies have looked into the relationship between alcohol consumption and alcohol-induced flushing. For example, alcohol drinking pattern and flushing response was investigated among indigenous natives of Chukotka, Siberia (Avksentyuk et al. 1995). However, no significant relationship between flushing, frequency and quantity of drinking and polymorphism of alcohol metabolizing enzymes was found. In another study (Kurilovich et al. 1998), the characteristics of alcohol-induced flushing response were investigated in some Siberian native populations (Chukchi, Eskimo, Jakuts, Udege, and Nanaian). Frequency of flushing response varied from 9.0% to 66.7%, and was more apparent among the females. Only the Nanaian demonstrated typical flushing, which did not allow them to consume high doses of alcohol. In the rest of the populations flushing was "atypical", i.e., appearing sometimes after high doses of alcohol but not interrupting the alcohol drinking, and not associated with the typical flushing response reported for Asians. However, frequency of alcohol problems, alcohol dependence symptoms, and somatic disorders (arterial hypertension, silent ischemia, diffuse liver lesions, and noncalculous cholecystitis) was higher among the flushers as compared to nonflushers.

Alcohol metabolism is one of the biological determinants that can significantly influence drinking behavior and the development of alcoholism and alcohol-induced organ damage. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the principal enzymes responsible for ethanol elimination in humans. Both the enzymes exhibit genetic polymorphism and racia/ethnic variation (Agarwal and Goedde 1990). The cytochrom P450 isoform, P4502E1 (CYP2E1), represents the second enzyme which can metabolize alcohol (Lieber 1999). Human liver contains several classes of ADH isozymes that exhibit high activity for ethanol oxidation. Three allelic variations occur at the gene locus of ADH2, i.e. ADH2*1, ADH2*2 and ADH2*3, which encode the subunits of ß1, ß2 and ß3,

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respectively. Two variants occur at the ADH3 locus, ADH3*1 and ADH3*2, coding for γ1 and γ2 subunits, respectively. The ADH2*2 and ADH3*1 alleles predominate in Asians and the resultant homodimeric ß2ß2 and γ1γ1 enzymes are 40- and 2-fold more active than the ß1ß1 and γ2γ2 enzymes, respectively (Peng et al. 1999). Approximately 90% of Orientals are "atypical" for the ADH2 locus, either heterozygous ADH21*2 or homozygous ADH22*2 status, and their liver ADH activity is significantly higher than that of the "usual" ADH21*1 subjects (Edenberg and Bosron, 1997). Two major ALDH isozymes are present in the liver; a cytosolic (ALDH1) and a mitochondrial (ALDH2). The mitochondrial ALDH i.e. ALDH2, exhibits an extremely low Km of 0.20 µM for acetaldehyde, as compared with the cytosolic ALDH1 with a Km of about 33 µM. Hence, the mitochondrial ALDH is the major enzyme form responsible for the metabolism of acetaldehyde in liver during alcohol consumption. In about 50% of Orientals the ALDH2 isozyme is inactive (Goedde et al. 1979). The catalytic deficiency of ALDH2 is caused by a point mutation at amino acid position 487, where a substitution of Lys for Glu that results from a transition of G (C) to A (T) at nucleotide 1510 (from initiation codon) has taken place (Yoshida et al. 1991). Individuals with a mutant form of mitochondrial ALDH2 exhibit the phenotype of alcohol-induced cutaneous flushing and other symptoms of vasodilatation, decreased voluntary alcohol consumption, and a reduced risk of developing alcoholism (Agarwal and Goedde 1990).

It is well documented that allele frequencies of ADH2*2, ADH3*1 and ALDH2*2 are significantly decreased in alcoholics as compared to Asian general population (Higuchi et al. 1995; Shen et al. 1997) suggesting that the high-activity ß2-ADH and γ1-ADH as well as the low-activity ALDH2 may play a protective roles against the development of alcoholism. The protective effect of ALDH2*2 appears to be substantially greater than that of ADH2*2 and ADH3*1 (Peng et al. 1999). Moreover, genetic factors may be of importance in determining inter-individual susceptibility to alcoholic liver disease (ALD) (Pirmohamed et al. 1995). In the liver, CYP2E1 represents a major P450 isoform. Past studies have shown that the rare mutant allele (termed as c2 allele) that lacks the Rsa I restriction site is associated with higher transcriptional activity, protein levels and enzyme activity than the more common wild-type allele (c1 allele) (Tsutsumi et al. 1994).

In the presence study we have examined the pattern of alcohol consumption and alcohol-related flushing response among a representative sample of women from Novosibirsk (West Siberia). The genotype distribution of the alcohol metabolizing enzymes, ADH2, ADH3, ALDH2 and CYP2E1 were determined to explore their possible relationship with alcohol drinking behavior.

MATERIALS AND METHODS

The study was carried out on samples collected under the WHO program MONICA ("Monitoring of Trend and Determinants in Cardiovascular Disease"). It was a larger project that included an investigation of cardiovascular disorders, diabetes mellitus, anemia, alcohol abuse and other medical anomalies. The study subjects comprised 875 women ranging in age from 25 to 65 with a mean age of 45,5 year. Women were included into the sample using 10-year age intervals to achieve approximately equal representation in each age group. All the subjects belonged to the urban white population (Caucasians). A questionnaire study about alcohol consumption and flushing response was conducted among 756 women. Respondents were asked about their current frequency of alcohol consumption (during the past year), past drinking habits, average dose per drinking occasion, and average past week dose. Information about loss of control and blackouts was achieved by standard questions (the study also included questions about problems in the family and job troubles caused by alcohol drinking habits). All participants were asked about the occurrence of alcohol-related facial flushing and the frequency of alcohol intake among their parents.

Venous blood was obtained for biochemical and genetic analysis. DNA samples of 299 women were obtained from clotted blood using conventional methods (treatment with proteinase K, phenol-chloroform extraction and

ethanol precipitation). All genotyping analyses were carried out in Hamburg (Germany).

ADH2 and ADH3 Genotyping

For amplification of ADH2 the following primers (5'-ATTCTGTAGATGGTGGCTGT-3') and (5'-GAAGGGGGGTCACCAGGTTG-3') were used as forward and reverse, respectively. For amplification of ADH3 the following primers (5'-GCTTTAAGAGTAAATATT CTGTCCCC-3') and (5'-AATCTACCTCTTT CCAGAGC-3') were used as forward and reverse, respectively. 23.1 µl of the PCR reaction mixture contained 0.5 µl of 100 pM of each primer, 2.5 µl of PCR buffer (containing 15 mM $MgCl_2$), 5 µl of solution Q, 14 µl H2O, 0.5 µl of 10 mM solution of dNTP, 1-2 units of Taq polymerase and 1 µl of DNA. Amplification was performed in the following mode: 1 cycle: 94°C for 4 min; 35 cycles for 1 min at 95°C, 45 sec at 55°C, 45 sec at 72°C; 10 min at 72°C. Eight microliters of PCR product was digested with the restriction enzyme Mae III for overnight at 37°C for ADH2 and with the restriction enzyme Ssp I for overnight at 37°C for ADH3. The restriction fragements were separated on 8% polyacrylamide gels and visualized by silver staining.

ALDH2 Genotyping

For amplification the following primers (5'-CAAATTACAGGGTCAAGGGCT-3') and (5'-CCACACTCACATTTTCTCTT-3') were used as forward and reverse, respectively. Twenty five µl of the PCR reaction mixture contained 0.2 µl of 100 pM solution of each primer, 2.5 µl of PCR buffer, 19.6 µl of H2O, 0.8 µl of 50 mM $MgCl_2$, 0.5 µl of 10 mM solution of dNTP, 1-2 units of Taq plymerase and 1 µl of DNA. Amplification was performed in the following mode: 30 cycles of denaturation at 94°C for 90 sec; annealing at 58°C for 180 sec; extension at 72°C for 60 sec; 1 cycle at 72°C for 10 min. Eight µl of the PCR product was digested with the restriction enzyme Mbo II for overnight at 37°C. The restriction fragements were separated on 8% polyacrylamide gels and visualized by silver staining.

CYP2E1Genotyping

For amplification the following primers (5'-CCAGTCGAGTCTACATTGTCA-3') and (5'-TTCATTCTGTCTTCTAACTGG-3') were used as forward and reverse, respectively. Twenty five µl of the PCR reaction mixture contained 0.5 µl of 100 pM solution of each primer; 2.5 µl of PCR buffer; 19 µl of H_2O; 0.8 µl of 50 mM $MgCl_2$; 0.5 µl of 10 mM solution of dNTP; 1-2 units of Taq plymerase and 1 µl of DNA. Amplification was performed in the following mode: 35 cycles of denaturation at 94°C for 1 min; annealing at 52°C for 1 min; extension at 72°C for 60 sec. Eight µl of PCR product was digested with the restriction enzyme Rsa I for overnight at 37°C. The restriction fragements were separated on 8% polyacrylamide gels and visualized by silver staining. For statistical analyses SPSS program was used. Genotype and allele frequencies for each polymorphism were calculated by direct counting.

RESULTS

The study sample was divided into three groups according to the self-reported alcohol drinking habits (Table 1).

Table 1: Alcohol consumer groups according to their past and present alcohol drinking habits

Group 1	Current and past abstainers
Group 2	Episodic alcohol drinkers
Group 3	Regular alcohol drinkers

As shown in table 2, among women of Novosibirsk episodic consumption of alcohol (less than once per month and 1-2 times per month) predominated (84.7% of the subjects), while regular consumption of alcohol (once per week and often) was reported by only 7.0%, and the current and past year abstainers constituted about 8.3%. Regular consumers were more frequent in the lowest age group. Moreover, among the regular consumers of alcohol, women with higher education predominated.

Average daily dose of alcohol consumed during the past week was 18.94 ± 1.4 grams of pure alcohol. Average dose per drinking occasion was 27.2 ± 0.86 grams of pure alcohol. For the type of alcohol beverages preferentially consumed, vodka and dry wine predominated (both in weekly average dose and the dose per drinking

Table 2: Alcohol consumption habits in different age groups (n=756)

Age group	*Alcohol consumer group*					
	Group 1		*Group 2*		*Group 3*	
	n	*%*	*n*	*%*	*n*	*%*
25-34	7	3.7	153	80.5	30	15.8
35-44	7	4.7	134	89.3	9	6.0
45-54	11	5.3	185	89.8	10	4.9
55-64	38	18.1	168	80	4	1.9
Total	63	8.3	640	84.7	53	7.0

P<0.001. The differences between the groups are statistically significant (Chi-Square test).

occasion). When asked about the past alcohol consumption (during life time) the percentage of regular consumers of alcohol was slightly lower than the current abstainers (7% vs. 8.3%). The causes of reduction in alcohol consumption were given as social causes (10.6% of the subjects), health grounds (6.8% of the subjects) such as cardiovascular diseases (2.8%), gastroenterological disorders (2.2%), and neurological pathology (1.8%).

The percentage of manifestations related to alcohol dependence such as the loss of control and blackouts were reported by only 1.6% and 1.7% of the subjects, respectively. However, 1.1% of the women reported that they had troubles in the family due to their drinking habits; 7.0% felt annoyance or guilt due to their drinking; 5.8% had sometimes the desire to reduce their alcohol consumption. On the following day after alcohol drinking, 6.7% of the females reported feeling sick in the morning after drinking; 13.9% reported dizziness, headache, and bad mood; 6.4% experienced tachycardia or heartache; 2.6% of the females took morning drinks to relieve such feelings.

Out of a total of 726 females, 28.5% reported that they experienced facial flushing; 15.6% experienced flushing "sometimes" and 12.9% experienced flushing "often". The incidence of flushing response in different groups is presented in table 3.

Table 3: The incidence of flushing response in alcohol drinkers and nondrinkers (n=726)

Facial	*Alcohol consumer groups*							
Flushing	*Group 1*		*Group 2*		*Group 3*		*Total*	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Never	34	6.6	449	86.5	36	6.9	519	71.5
Sometimes	1	2.6	97	85.8	15	13.3	113	15.6
Often	3	3.2	90	95.7	1	1.1	94	12.9

P<0.01. The differences between the groups are statistically significant (Chi-Square test).

The majority of those females who experienced flushing (83.8%) were able to continue drinking after flushing began. However, 8.1% were not able to continue drinking and 8.1% could continue only sometimes. Many females reported to have adverse manifestations after alcohol ingestion (symptoms which usually accompany flushing). Nausea was reported by 0.3% of the often-flushed and by 2.3% of females who flushed sometimes only; in females reporting often and sometimes flushing, feeling drowsy was reported by 1.1% and 5.9%; feeling dizziness by 0.3% and 3.5%; increased heart beat by 0.6% and 2.2%; sweating by 0.3% and 1.8%, headache by 1.0% and 3.0%, respectively. Many of the flushers noted that not all types of drinks caused flushing but it was more often induced by vodka (42.6% cases) and sparkling wine (champagne) (32.6% cases).

The distribution of the ADH2, ADH3 and CYP2E1 genotypes among the Novosibirsk sample is presented in table 4.

Homozygous genotype, ADH2*2/*2, was present in 8.7% of the cases; heterozygous genotype, ADH2*1/*2, was present in 22.07% of the cases and homozygous genotype ADH2*1/*1 was present in 69.23% of the cases. The

Table 4: Frequency distribution of ADH2 and ADH3 genotypes (n=299)

Genotype	*Genotype frequency %, (n)*	*Allele frequency*	
ADH2		*ADH2[1]*	*ADH2[2]*
1-1	69.23 (207)	0.803	0.197
1-2	22.07 (66)		
2-2	8.7 (26)		
Genotype	*Genotype frequency %, (n)*	*Allele frequency*	
ADH3		*ADH3[1]*	*ADH3[2]*
1-1	20.06 (60)	0.48	0.52
1-2	56.19 (168)		
2-2	23.75 (71)		
Genotype	*Genotype frequency %, (n)*	*Allele frequency*	
CYP2E1		*c 1*	*c 2*
1-1	93.98 (281)	0.969	0.030
1-2	6.02 (18)		
2-2	-		

frequency of the ADH2*2 allele was found to be 0.197. The ADH3*2 allele was predominate (0.52). The ALDH2 gene was found to be not polymorphic in this population sample. Only ALDH2 1*1 genotype was detected in the total sample. The more common wild-type allele (c1 allele) and the mutant allele (c2 allele) of CYP2E1 were found to be 0.969 and 0.030, respectively.

The distribution of ADH2 and ADH3 genotypes in groups with different alcohol consumption habits are presented in table 5. The differences in genotype distribution among different alcohol drinking groups were not statistically significant.

Table 5: The distribution of ADH2 and ADH3 genotypes in different alcohol consumer groups

Genotype	*Alcohol consumer group*		
ADH2	*Group 1 (%)*	*Group 2 (%)*	*Group 3 (%)*
1-1	4.9	84.2	10.8
1-2	4.5	83.3	12.1
2-2	8.0	88.0	4.0
ADH3	*Group 1 (%)*	*Group 2 (%)*	*Group 3 (%)*
1-1	5.0	78.3	16.7
1-2	5.4	86.8	7.8
2-2	4.5	83.6	11.9

The differences between the groups are not statistically significant (Chi-Square test)

The average daily dose during the past week was slightly lower than the average dose per drinking occasion both in ADH2 and ADH3 genotypes (Table 6).

Moreover, incidence of flushing reaction was quite similar in different ADH2 genotypes. However, subjects with ADH3*2 allele reported a lower incidence of flush response to alcohol. The data are presented in table 7.

Table 6: The mean daily dose consumed during the past week and average dose per drinking occasion in different ADH2 and ADH3 genotypes

Genotype	*Average daily dose in the past week (g)*	*Average dose per drinking occasion (g)*
ADH2		
1-1	17.75 ± 35.7	29.89 ± 23.5
1-2	24.49 ± 40.8	27.63 ± 19.5
2-2	13.16 ± 20.0	26.61 ± 23.5
ADH3		
1-1	26.53 ± 49.9	28.53 ± 18.6
1-2	15.74 ± 15.7	29.71 ± 25.3
2-2	19.80 ± 19.8	28.09 ± 18.8

Table 7: Flushing reaction vs. genotype

Genotype	*Flush (-) (%)*	*Flush (+) (%)*
ADH2		
1-1	70.6	29.4
1-2	69.2	30.8
2-2	70.8	29.2
ADH3		
1-1	55.4	44.6
1-2	75.0	25.0
2-2	71.2	28.8

$P < 0.05$. The differences between the groups are statistically significant (Chi-Square test).

A larger percentage of subjects with ADH*2 and ADH3*1 allele reported their inability to continue drinking after experiencing the alcohol-induced flush reaction.

DISCUSSION

Alcohol abuse, alcohol intolerance, alcohol dependence and other alcohol-related disabilities are some of the most challenging public health problems facing our modern-day society. Many recent investigations have shown that genetic variations in alcohol and acetaldehyde metabolism may indeed be responsible for individual and racial differences in acute reactions to ethanol, alcohol drinking habits and vulnerability to alcohol-related organ damage (Agarwal and Goedde 1990; Agarwal et al. 1997).

The results of the present study about the genetic polymorphism of alcohol metabolism genes in a female population sample from Siberia is comparable with the frequency distribution of ADH2, ADH3, ALDH2 and CYP2E1 genotypes in Caucasian populations reported earlier. The distribution of the ADH2*2 and ADH3*2 alleles was higher (0.197 and 0.52, respectively) than in other Caucasoids populations (0.01-0.13 and 0.4, respectevly). The prevalence of the mutant allele (c2 allele) of CYP2E1 gene (0.030) was similar to that found in the Caucasians population (0.04).

The recent reports of an association of the ADH2*2 allele with a reduced ethanol consumption in Jewish men (Neumark et al. 1998) and Australian men of European origin (Whitfield et al. 1998) support the concept that the effect of ADH2 polymorphism on alcohol drinking behavior is widespread. However, it is obvious

that only a relatively small number of Europeans will be protected against alcoholism by possessing ADH2*2 because of the lower number of individuals with this allele (Borras et al. 2000). The distribution of ADH3 alleles is also homogeneous among European populations, with global frequencies of 55.6% for ADH3*1 and 44.4% for ADH3*2, in agreement with a previous report (Chen et al. 1999). The fact that the allele frequencies are practically identical between the alcoholic and nonalcoholic individuals suggests that the influence of ADH3 polymorphism on predisposition to alcohol abuse is small, in agreement with most previous studies in Europeans. The European populations show that linkage disequilibrium between ADH2 and ADH3 are associated, therefore the probability that both alleles are simultaneously found in an individual is higher than in the case of random allele segregation (Borras et al. 2000). Evidence for this allele linkage has been found also in Asian populations (Chen et al. 1999; Osier et al. 1999).

Most of the previous reports did not compare the effects of ADH polymorphism in men and women. ADH2 genotype had no detectable effects on alcohol consumption or the number of dependence symptoms in women (Whitfield et al. 1998). The rate of decrease in blood alcohol concentration after a test dose was the same in men and women in this group of subjects (Martin et al. 1985). There have been a number of claims that sex hormones affect alcohol metabolism (Mezey et al. 1988) or ADH activity (Mardh et al. 1986), or that the hormones are associated with differences in post-alcohol acetaldehyde concentrations (Eriksson et al. 1996).

CONCLUSIONS

The major objective of the present study was to document drinking patterns and flushing response in relation to polymorphisms of alcohol metabolism genes among the female urban white population living in Novosibirsk. The results of the study clearly show that daily dose of alcohol consumed was much less among the white urban population than that reported earlier for the native populations in Siberia (Avksentyuk et al. 1995). A lack of mutation in ALDH2 gene further supports the notion that this mutation is limited to the mongoloid populations. Though a relatively high prevalence of flushing response was observed, more than 80% of the flushers were able to continue drinking after flushing began, irrespective of their ADH2 or ADH3 genotype. Taken together, ADH2, ADH3 and CYP2E1 genes were found to be polymorphic among the female residents of West Siberia but no significant relationship between flushing response and frequency and quantity of drinking and the prevalence of alcohol-related symptoms was observed.

REFERENCES

Agarwal DP, Goedde HW 1990. *Alcohol Metabolism, Alcohol Intolerance and Alcoholism.* Berlin Heidelberg: Springer-Verlag.

Agarwal DP, Buda B, Czeizel AE, Goedde HW 1997. *Alcohol Consumption and Alcoholism in Hungary: Ethnocultural, Epidemiological and Biomedical Aspects.* Budapest: Akadémiai Kiadó.

Avksentyuk AV, Kurilovich SA, Duffy LK, Segal B, Voevoda MI, Nikitin YP 1995. Alcohol consumption and flushing response in natives of Chukotka, Siberia. *J Stud Alcohol,* **56**: 194-201.

Balkau B 1999. All the evidence points to alcohol being implicated in the recent changes in mortality in Russia. *Ann Epidemiol,* **9**: 339-340.

Bobak M, McKee M, Rose R, Marmot M 1999. Alcohol consumption in a national sample of the Russian population. *Addiction,* **94**: 857-866.

Borras E, Coutelle C, Rosell A et al. 2000. Genetic polymorphism of alcohol dehydrogenase in Europeans: the ADH2*2 allele decreases the risk for alcoholism and is associated with ADH3*1. *Hepatology,* **31**: 984-989.

Chen C-C, Lu R-B, Chen Y-C, Wang M-F, Chang Y-C, Li T-K, Yin S-J 1999. Interaction between the functional polymorphisms of the alcohol metabolism genes in protection against alcoholism. *Am J Hum Genet,* **65**: 795-807.

Edenberg HJ, Bosron WF 1997. Alcohol dehydrogenase. In: FP Guengerich (Ed.): *Comprehensive Toxicology,* Vol 3. New York: Elsevier Science Inc, pp 119-131.

Eriksson CJ, Fukunaga T, Sarkola T, Lindholm H, Ahola L 1996. Estrogen-related acetaldehyde elevation in women during alcohol intoxication. *Alcohol Clin Exp Res,* **20**: 1192-1195.

Goedde HW, Harada S, Agarwal DP 1979. Racial differences in alcohol sensitivity: a new hypothesis. *Hum Genet,* **51**: 331-334.

Hayashi S, Watanabe J, Kawajiri K 1991. Genetic polymorphisms in the 5'-flanking region change transcriptional regulation of the human cytochrome P4502E1 gene. *J Biochem,* **110**: 559-565.

Higuchi S, Matsushita S, Murayama M, Takagi S, Hayashida M 1995. Alcohol and aldehyde dehydrogenase polymorphism and the risk for alcoholism. *Am*

J Psychiatry, **152**: 1219-1221.

Kurilovich SA, Jakuschenko IA, Egorova NG, Avksentyuk AV, Trusov VB 1998. Flushing response and its role in alcohol disease in Siberian populations. *Int J Circumpolar Health*, **57** Suppl 1: 454-458.

Lieber CS 1999. Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968-1998)—a review. *Alcohol Clin Exp Res*, **23:** 991-1007.

Mardh G, Falchuk KH, Auld DS, Vallee B 1986. Testosterone allostericcally regulates ethanol oxidation by homo- and heterodimeric γ-subunit containing isozymes of human alcohol dehydrogenase. *Proc Natl Acad Sci USA*, **83**: 2836-2840.

Martin NG, Perl J, Oakeshott JC, Gibson JB, Starmer GA, Wilks AV 1985. A twin study of ethanol metabolism. *Behav Genet*, **15**: 93-109.

Mezey E, Oesterling JE, Potter JJ. 1988. Influence of male hormones on rates of ethanol Elimination in man. *Hepatology*, **8**: 742-744.

Neumark YD, Friedlander Y, Thomasson HR, Li T-K 1998. Association of the ADH2*2 allele with reduced ethanol consumption in Jewish men in Israel. *J Stud Alcohol*, **59**: 133-139.

Nemtsov AV 2000. Estimates of total alcohol consumption in Russia, 1980-1994. *Drug Alcohol Depend*, **58**: 133-142.

Osier M, Patstis AJ, Kidd JR, Lee J-F, Yin S-J, Ko H-C, Edenberg HJ 1999. Linkage disequilibrium at the ADH2 and ADH3 loci and risk of alcoholism. *Am J Hum Genet*, **64**: 1147-1157.

Peng G-S, Wang M-F, Chen C-Y, Luu S-U, Chou H-C, Li T-K, Yin S-J. 1999. Involvement of acetaldehyde for full protection against alcoholism by homozygosity of the variant allele of mitochondrial aldehyde dehydrogenase gene in Asians. *Pharmacogenetics*, **9**: 463-476.

Pirmohamed M, Kitteringham NR, Quest L, Allot R, Green V, Gilmore I, Park K 1995. Genetic polymorphism of cytochrome P4502E1 and risk of alcoholic liver disease in Caucasians. *Pharmacogenetics*, **5**: 351-357.

Shen Y-C, Fan J-H, Edenberg HJ, Li T-K, Cui Y-H, Wang Y-F, Tian C-H, Zhou C-F, Zhou R-L, Wang J, Zhao Z-L, Xia G-Y 1997. Polymprphism of ADH and ALDH genes among four ethnic groups in China and effects upon the risk for alcoholism. *Alcohol Clin Exp Res*, **21**: 1272-1277.

Tsutsumi M, Wang JS, Takase S, Takada A 1994. Hepatic messenger RNA contents of cytochrome P4502E1 in patients with different P4502E1 genotypes. *Alcohol Alcohol*, **29** Suppl 1:29-32.

Whitfield JB, Nightingale BN, Bucholz KK, Madden PAF, Heath AC, Martin NG 1998. ADH genotypes and alcohol use and dependence in Europeans. *Alcohol Clin Exp Res*, **22**: 1463-1469.

Yoshida A, Hsu LC, Yasunami M. 1991. Genetics of human alcohol-metabolizing enzymes. *Prog Nucleic Acid Res Mol Biol*, **40**: 255-287.