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Genetic polymorphism of alcohol-metabolizing enzyme and alcohol dependence in Polish men

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Abstract

Alcohol dependence poses a serious medical and sociological problem. It is influenced by multiple environmental and genetic factors, which may determine differences in alcohol metabolism. Genetic polymorphism of the enzymes involved in alcohol metabolism is highly ethnically and race dependent. The purpose of this study was to investigate the differences, if present, in the allele and genotype frequency of alcohol dehydrogenase 1B (ADH1B), ADH1C and the microsomal ethanol-oxidizing system (MEOS/CYP2E1) between alcohol-dependent individuals and controls and also to determine if these genotypes cause a difference in the age at which the patients become alcohol dependent. The allele and genotype frequencies of ADH1B, ADH1C, and CYP2E1 were determined in 204 alcohol dependent men and 172 healthy volunteers who do not drink alcohol (control group). Genotyping was performed by PCR-RFLP methods on white cell DNA. ADH1B*1 (99.3%) and ADH1C*1 (62.5%) alleles and ADH1B*1/*1 (N = 201) and ADH1C*1/*1 (N = 85) genotypes were statistically more frequent among alcohol-dependent subjects than among controls (99.3 and 62.5%, N = 201 and 85 *vs* 94.5 and 40.7%, N = 153 and 32, respectively). Differences in the CYP2E1 allele and genotype distribution between groups were not significant. The persons with ADH1C*1/*1 and CYP2E1*c1/*c2 genotypes became alcohol dependent at a considerably younger age than the subjects with ADH1C*1/*2, ADH1C*2/*2 and CYP2E1*c1/*c1 genotypes (28.08, 25.67 years *vs* 36.0, 45.05, 34.45 years, respectively). In the Polish men examined, ADH1C*1 and ADH1B*1 alleles and ADH1C*1/*1 and ADH1B*1/*1 genotypes favor alcohol dependence. The ADH1B*2 allele may protect from alcohol dependence. However, subjects with ADH1C*1/*1 and CYP2E1*c1/*c2 genotypes become alcohol dependent at a considerably younger age than the subjects with ADH1C*1/*2, ADH1C*2/*2 and CYP2E1*c1/*c1 genotypes.

Key words: CYP2E1 gene polymorphism; ADH gene polymorphism; Alcohol dependence; Age of dependence onset

Introduction

In Poland, about 10% of the population abuse alcohol and people treated for alcohol dependence account for about 2.5% of the population. Estimates show that 20-40% of Polish patients hospitalized have an alcohol problem and their illness often resulted from alcohol abuse. Alcohol dependence does not develop in all drinking individuals. In the Polish population, even among those consuming large amounts of alcohol systematically, dependence occurs only in 3% of cases. It is assumed that this fact may be associated, apart from multiple environmental factors, with differences in alcohol metabolism (1). Alcohol metabolism is one of the biological determinants that can significantly influence drinking behavior and the development of alcohol dependence (2). Alcohol dehydrogenase (ADH), aldehyde dehydrogenase and microsomal ethanol-oxidizing system (MEOS/CYP2E1) allele and genotypes occur at different frequencies in particular ethnic groups, and their role in the development of alcohol dependence differs between races and populations (3-11). These enzymes play a role in the variation in health effect outcomes seen in different populations owing to alcohol consumption. These include differences in expression of phenotype, in locus heterogeneity, in risk alleles, and in population structure (12). Genes coding for ethanol-metabolizing enzymes are referred to as alcoholic genes. Alcohol dependence is considered to be a multigene disease (13).

Knowledge of genetic predisposition to alcohol dependence can permit control this social phenomenon in the future. It may be the basis for the treatment of alcohol dependence by inhibition of alcohol gene expression in the genome, allowing the incorporation of therapeutic genes that can modify the expression of disease-predisposing

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genes, an effect that can last from months to years (14). For the implementation of this idea it is of a practical value to know the time frame for possible preventive treatment actions. It can be observed that age at which people become alcohol dependent varies widely. No reports have been published so far about the possible relationship between polymorphism of the alcohol-metabolizing gene and age of alcohol dependence onset.

The aim of the present study was to compare the differences, if present, in ADH1B, ADH1C, and CYP2E1 allele and genotype frequency between alcohol-dependent individuals and controls, and to examine if these genotypes are associated with a difference in the age at which the patient became alcohol dependent.

Material and Methods

A group of 204 men - alcohol-dependent subjects aged 20 to 78 years and 172 healthy male volunteers aged 18 to 72 years as a control group - participated in the study. The group of alcohol-dependent subjects included patients consuming more than 80 g pure ethanol a day for at least 2 years. Alcohol history and the data concerning the age at which the patients became alcohol dependent were obtained with a face-to-face interview. All subjects met the DSM-IV diagnostic criteria for alcohol dependence (15). The alcohol-dependent patients were recruited from the Department of Therapy of Addiction to Alcohol and Department of Gastroenterology, Medical University of Lublin. Their age at onset of alcohol dependence was determined on the basis of medical history concerning their alcohol intake, which sometimes was detailed by contacting family members or friends (after patient's consent), and was assumed to be the earliest date they met the DSM-IV diagnostic criteria for alcohol dependence.

The control group included healthy volunteers who do not drink alcohol at all or occasionally drink such a small quantity of alcohol (no more than 10 g pure ethanol a year) that they can be considered to be non-drinkers. This information was collected with a face-to-face interview. Control subjects had no evidence of chronic disease or of the presence of disease at physical examination and had normal laboratory test results. They were recruited from the students and personnel of the Medical University of Lublin.

All the patients in this study were Caucasians. The study was approved by the Bioethics Committee of the Medical University of Lublin, Poland, and all subjects gave written informed consent to participate.

Genomic DNA was isolated from peripheral blood with 0.5 M EDTA (16). For the detection of polymorphism in the ADH1B gene the polymerase chain reaction-restriction fragment length polymorphism technique was used on white cell DNA (17). The primers for amplification were ADH1B 247, ADH1B 303, ADH1B 290, ADH1B 424, and ADH1B 352. The amplified product was digested with the *Mae*III enzyme (Roche Applied Science, USA) and with the *Alu*I enzyme (MBI Fermantas, Germany), subjected to 4% agarose or 12% polyacrylamide gel electrophoresis, and stained with ethidium bromide or silver nitrate (17).

For the detection of polymorphisms in the ADH1C gene the primers used for amplification were ADH1C 321 and ADH1C 351 (17). The amplified product was digested with *Ssp*I enzyme (MBI Fermantas), subjected to 3% agarose gel electrophoresis and stained with ethidium bromide (17).

For the detection of CYP2E1 gene polymorphisms in the 5'-flanking region of the P4502E1 gene, CYP2E J8 and CYP2E J9 were used as the primers for amplification (18). The amplified product was digested with *Pst*I (MBI Fermantas) or *Rsa*I enzyme (MBI Fermantas), subjected to 2% agarose gel electrophoresis, and stained with ethidium bromide (18).

Statistical analysis

The χ^2 test or the Fischer exact test was used to compare genotypes and alleles between the groups. The Mann-Whitney test was used to determine if genotype was associated with the age at which the patients became alcohol dependent. The level of significance was set at $P < 0.05$ in all analyses. χ^2 goodness of fit tests were used to study agreement with Hardy-Weinberg equilibrium. All calculations were done using the Statistica PL software.

Results

In the alcohol-dependent group, ADH1C*1 allele was detected in 62.5% of the subjects, and ADH1C*1/*1 genotype in 85 patients, a significantly higher frequency compared to the control group (Table 1). Similarly, ADH1B*1 allele and ADH1B*1/*1 genotype frequencies were significantly higher in the group of alcohol-dependent subjects than among non-drinkers. However, ADH1B*2 allele and ADH1B*1/*2 heterozygotic genotype were significantly more frequent in the control group than among the alcohol-dependent subjects. Differences in CYP2E1 allele and genotype distribution between alcohol-dependent subjects and controls were not statistically significant. The genotype distribution of all groups studied fitted the expected Hardy-Weinberg equilibrium.

The patients with homozygotic ADH1C*1/*1 genotype became alcohol dependent significantly earlier than the patients with ADH1C*1/*2 and ADH1C*2/*2 genotypes (Table 2). The average age of the patients with ADH1B*1/*1 genotype who became alcohol dependent was 28.93 years and was lower than the age of those with the ADH1B*1/*2 genotype. However, it was impossible to analyze the results statistically since the groups differed in size, nor was it possible to state conclusively if a certain type of ADH1B*1/*2 genotype affected the age at which the patients became alcohol dependent. The patients with the CYP2E1*c1/*c2

genotype became alcohol dependent significantly earlier than the persons with homozygotic CYP2E1*c1/*c1 genotype.

Table 1. ADH1C, ADH1B and CYP2E1 allele and genotype frequencies in alcohol-dependent patients and controls.

	Alcohol-dependent subjects (N = 204)	Controls (N = 172)	P
ADH1C alleles (%)			<0.001
*1	62.5%	40.7%	
*2	37.5%	59.3%	
ADH1C genotypes (N)			<0.001
*1/*1	85	32	
*1/*2	88	76	
*2/*2	31	64	
ADH1B alleles (%)			<0.001
*1	99.3%	94.5%	
*2	0.7%	5.5%	
ADH1B genotypes (N)			<0.001
*1/*1	201	153	
*1/*2	3	19	
*2/*2	0	0	
CYP2E1 alleles (%)			>0.05
*c1	97.8%	98.8%	
*c2	2.2%	1.2%	
CYP2E1 genotypes (N)			>0.05
*c1/*c1	195	168	
*c1/*c2	9	4	
*c2/*c2	0	0	

Statistical comparisons between groups were made with the χ^2 test or the Fischer exact test.

Table 2. ADH1C, ADH1B and CYP2E1 genotypes and mean age when 204 patients became alcohol dependent.

	N	Age (years)	P
ADH1C genotypes			<0.001
*1/*1	85	28.08 ± 5.73	
*1/*2	88	36.0 ± 5.47	
*2/*2	31	45.05 ± 4.74	
ADH1B genotypes			ND
*1/*1	201	28.93 ± 8.0	
*1/*2	3	35.0 ± 0	
CYP2E1 genotypes			<0.001
*c1/*c1	195	34.45 ± 8.0	
*c1/*c2	9	25.67 ± 3.56	

Age reported as means ± SD. Statistical comparisons were made with the Mann-Whitney test. ND = statistical analysis not possible.

Discussion

The key issue in alcohol dependence is to identify predisposing factors in addition to defining those that lead to alcoholic damage to the digestive organs. Race and gender differences in alcohol tolerance and differences in individual predisposition to develop alcohol addiction have resulted in progress in research. Predisposition to alcohol dependence is affected by multiple environmental and genetic factors in a complicated way (19). Family and twin studies estimated about a 60% hereditary rate for alcohol dependence. Many lines of evidence obtained by different methods, e.g., gene-manipulated animals, linkage of human genome and genetic association studies, are accumulating information regarding the genetic components involved in alcohol dependence (20). Genetic polymorphisms of the genes encoding alcohol metabolism enzymes and neurotransmitter signaling molecules in dopamine, GABA, opioid, and serotonin systems have been shown to be involved substantially in individual variation of susceptibility to alcohol dependence (19,21).

Studies regarding ADH1C*1 among whites are more contradictory and show ambiguous findings: no correlation (13,22,23), protection against alcohol dependence (3) or inconclusive results (24,25). Borras at al. (3) and Espinos et al. (22), who studied Europeans, found that the ADH1C*1 allele was more frequent in alcohol-dependent subjects than among non-drinkers. Similarly, the results of the present study show that the ADH1C*1 allele and ADH1C*1/*1 genotype were detected significantly more frequently in the alcohol-dependent group than in the control group. These data suggest that the ADH1C*1 allele and ADH1C*1/*1 genotype are likely to favor alcohol dependence. Different results were obtained in studies of Asian populations (26-28), which showed that the ADH1C*1 allele has protective effects against excessive alcohol consumption.

The results of studies of Asian and European populations show that ADH1B*1 predominated among alcohol addicts and suggest a high risk of alcohol addiction (9,26-29). Hence, these investigators emphasize the importance of ADH1B*2 protection against developing alcohol dependence (3,24,30-37). Our results agree with those of other studies presented above and disagree with the study by Vidal et al. (38) on the Spanish population. We found that the ADH1B*2 alleles were significantly rarer among alcohol-dependent subjects than among the controls. Thus, we believe the ADH1B*2 alleles may decrease the risk of developing alcohol dependence in Polish individuals.

In all populations, the CYP2E1*c2 allele is generally considerably less frequent than the CYP2E1*c1 allele (9). The CYP2E1*c2 alleles are more common among Asians than Caucasians (7). Studies evaluating the association between CYP2E1 polymorphism and alcohol dependence in different races have provided contradictory results. Some studies found the CYP2E1*c2 allele as a risk factor

for alcoholism (18,33,39), while others did not detect this relationship (38,40). Our study did not find differences in CYP2E1 allele or genotype distribution between alcohol-dependent subjects and controls.

There has been no research published on the influence of certain genetic factors on the age at which people become alcohol dependent. Our results here show that the ADH1C*1/*1 and CYP2E1*c1/*c2 genotypes incline the patients to become alcohol dependent at a significantly younger age compared to those who have the ADH1C*1/*2, ADH1C*2/*2 or CYP2E1*c1/*c1 genotype. This observation can be of fundamental importance for the use of screening procedures in possible prevention against alcohol dependence. Since no similar studies regarding other races and populations are available, it is not possible to state if similar conclusions would apply to patients from other geographical regions such as Asia, Africa or other countries in Europe. Many factors, such as environmental, familial and somatic ones, may combine to affect the age at onset of alcohol dependence. Our findings may be limited to the sample studied and it is an open question whether they can be generalized. Further investigations on larger samples and different populations should shed light on this issue.

In conclusion, ADH1C*1 and ADH1B*1 alleles and ADH1C*1/*1 and ADH1B*1/*1 genotypes favor alcohol dependence among Polish men. On the other hand, the ADH1B*2 allele may protect from alcohol dependence. The persons with ADH1C*1/*1 and CYP2E1*c1/*c2 genotypes become alcohol dependent at a considerably younger age than subjects with ADH1C*1/*2, ADH1C*2/*2 and CYP2E1*c1/*c1 genotypes.

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