

Gender Differences in Alcohol Metabolizing Hepatic Enzyme Genotypes in Korean Patients with Alcohol Dependence

Abstract

There are a number of epidemiological studies concerning the gender differences in the genetic etiology of alcohol dependence (AD), with ADH2 and ALDH2 being strong candidates. The purpose of this study was to investigate gender differences in frequencies of ADH2 and ALDH2 genotypes in AD patients and in a normal control (NC) group of Koreans. Study subjects consisted of 228 AD patients (180 males, 48 females) and 138 NC (79 males, 59 females). For both male and female subjects, the frequency of the ADH2*1/1 genotype was significantly higher in AD patients compared to the NC group. However, the effect size of the ADH2*1/1 genotype on AD was much larger in females than in males. Furthermore, the ALDH2*1/1 genotype was positively associated with AD in male subjects but negatively associated with AD in female patients. Interestingly, AD in males was primarily determined by ALDH2 enzyme activity (92%), whereas female AD was primarily determined by ADH2 enzyme activity (60.4%). These results suggest that risk for the development of AD in males is mainly associated with the ALDH2*1/1 genotype, while in female patients, the ADH2*1/1 genotype was more highly associated with risk of AD. Overall, it is evident that gender differences associated with genetic risks for AD are present.

Keywords

Alcohol dependence • ADH2 • ALDH2 • Gender differences • Genetic risk

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Introduction

The strong familial transmission tendency in alcohol dependence (AD) has drawn researcher's attention to the existence of a genetic influence on alcoholism, which accounts for about 40-60% of the risk [1,2]. Among the the many candidate genes that have been proposed to be associated with the development of AD, peripheral alcohol metabolizing enzyme genes including alcohol dehydrogenase 2 (ADH2) and aldehyde dehydrogenase 2 (ALDH2) have been shown to be highly associated with risk for the development of AD [3].

Gender differences have been identified in the development and pathophysiology of alcoholism. For example, women are much less likely to develop problems with alcohol than men [4]. However, women are more sensitive to the pathologic effects of alcohol on body organs and there is a shorter duration between abusive drinking and treatment seeking in female alcoholics [5,6]. Despite these obvious gender differences, the majority of the studies examining the genetic etiology of alcohol dependence have been conducted using only male patients with alcohol dependence with relatively few studies examining the role of genetics in female alcohol-dependent patients. With so few studies, it is not surprising that the results of these studies are inconsistent. McGue *et al.* [7]

conducted a twin study including both genders and reported that genetic etiology is significant in male alcohol dependence but that heritability is lower in women than in men. However, Heath *et al.* [8] reported that genetic etiology accounts for up to 65% of the influence for both male and female patients with alcohol dependence, showing no difference between sexes. Unlike previous studies, Prescott *et al.* [9,10] recently carried out a study that included male-female dizygotic twins. The results of their study suggested that genetic etiology is significant for alcohol dependence in both genders but that the genetic etiology influencing male alcohol dependence and the genetic etiology influencing female alcohol dependence are only partially consistent with each other.

Furthermore, a limited number of studies examining gender differences in specific genes, such as the alcohol metabolizing enzyme genes, are available. Borrás *et al.* [11] reported that ADH2*1 was related to the risk of alcohol dependence in males but not in females. Cheng *et al.* [12] and Whitfield *et al.* [13] also reported that the genetic etiology of alcohol metabolizing enzymes may differ in male and female patients with alcohol dependence. However, these studies did not include ALDH2 [11-13]. In addition, no study has investigated the relationships between ADH2, ALDH2 and alcohol dependence with gender a Korean population [14-18].

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Therefore, studies concerning the gender differences and genetic influence of two major alcohol metabolizing enzymes on alcohol dependence at the same time are needed. The purpose of this study was to calculate the frequency of ADH2 and ALDH2 genotypes in Korean patients with alcohol dependence and in Korean subjects without alcohol dependence and to examine the differences in the frequencies of the genotypes between the two groups and between men and women.

Materials and Methods

Human Subjects

Korean patients who were diagnosed with alcohol dependence by psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) [19] and were currently admitted to a psychiatry ward in one of four hospitals (Pusan National University Hospital, Yangsan Hospital, Dasarang Hospital, and Maya Hospital) were eligible for participation in this study. Patients were excluded if they used substances other than nicotine or caffeine, or if they had major psychiatric disorders, such as schizophrenia, bipolar disorder and major depressive disorder. The final number of subjects with alcohol dependence was 228 (180 males and 48 females).

The control group included Korean individuals who visited Pusan National University Hospital for a comprehensive medical examination. Control subjects reported drinking no more than five standard drinks per month at any time during their lives. Control subjects were excluded if there was evidence of a lifetime history of a major psychiatric syndrome, including drug or alcohol abuse or dependence. Moreover, the control subjects had to be 50 years of age or older in order to lower the chance that they would develop alcohol dependence in the future. The final number of the subjects included in the normal control group was 138 (79 males and 59 females). All subjects consented to participation in accordance with the Institutional Review Board at the Pusan National University Hospital.

Clinical Assessments

Information collected for the alcohol-dependent group included the age at which drinking started, the age at onset of alcohol-related problems (ARP), the period from the age at which drinking started to the age at onset of ARP, the age of first admission to a psychiatric hospital for ARP, cases of more than one admission due to ARP, the average number of drinking days per month and drinks per drinking day during the 12-months just before the present admission, a history of severe alcohol withdrawal symptoms including seizures, hallucinations, or delirium, and the presence of a family history of alcohol dependence in a first-degree relative. ARP was defined according to the diagnostic criteria of DSM-IV for alcohol abuse.

DNA analysis

Approximately twenty milliliters of EDTA-treated venous blood was obtained for DNA extraction from each subject. Genomic DNA was extracted from blood samples using standard methods

[20]. ADH2 and ALDH2 were genotyped by polymerase chain reaction (PCR) amplification of DNA fragments with nonisotopic allele-specific oligonucleotides biotinylated at the 5' end containing targeted single base-pair differences based on the methods of Harada and Zhang [21] for ALDH2 and those of Groppi *et al.* [22] for ADH2.

Both genotype and allele frequency were considered. The specific alleles examined were ALDH2*1 and ALDH2*2, which correspond to the active and inactive subunits, respectively. Although it has been suggested that the heterozygous state may differ from the homozygous state, the mutant ALDH2 allele is dominant over the normal allele in most cases. Therefore, we consider the effects of homozygosity for the ALDH2*1 with those of heterozygosity or homozygosity for the ALDH2*2 allele. Similarly, the ADH2 alleles studied were ADH2*1 and ADH2*2. The phenotype of ADH2 superactivity is regarded as compatible with the possession of the ADH2*2/ADH2*2 genotype [23,24]. Patients homozygous for the ADH2*1 allele were compared to those heterozygous or homozygous for the ADH2*2 allele.

Statistical analysis

Descriptive analyses included means and standard deviations for continuous variables and frequencies for categorical variables. We performed chi-square tests and univariate logistic regressions to investigate the frequency differences and odds ratios, as well as the associated 95% confidence intervals for the ADH2 and ALDH2 genes, respectively, between the control group and alcohol-dependent group across genders. Multivariate logistic regression was used to assume the combined effects of the ADH2 and ALDH2 genes on the development of alcoholism in each gender. Statistical analysis was performed using SAS 8.1. All analyses used a two-sided test with the statistical significance set at below $P = 0.05$.

Results

Demographic characteristics and drinking history

The demographic and clinical characteristics of the alcohol-dependent subjects are presented in Table 1 according to gender. There were no differences in age, education, drinking-related clinical variables, except for the age at which drinking started, the age at onset of ARP, the period from the age at which drinking started to the age at the onset of ARP and the history of severe alcohol withdrawal. The age at which drinking started and the age at the onset of ARP were younger in the male subjects than in female subjects. The period from the age at which drinking started to the age at the onset of ARP was shorter and withdrawal symptoms were more common in women.

Differences in the frequencies of the ADH and ALDH2 genotypes

As shown in Table 2, there were significant differences in the frequencies of the three ADH2 genotypes between the alcohol dependence and control subjects in both genders. Similar and more robust results were demonstrated by comparing subjects

Table 1. Comparison of demographic data and alcohol history between male and female subjects with alcohol dependence.

	Male (n=180)	Female (n=48)	t or χ^2	P value
Age (year)	47.3±9.4	44.9±10.5	1.56	0.120
Education (year)	9.5±4.3	9.0± 4.4	0.73	0.468
Age at which-drinking started (year)	20.0±3.9	27.3± 9.6	-5.21	<0.001*
Age at the onset of ARP (year)	34.1±8.6	37.8±10.1	-2.51	0.013*
Age at the onset of ARP - Age at the onset of ARP (year) ^a	14.1±8.4	10.5± 8.9	2.73	0.009*
Age at the 1 st adm. to hospital for ARP (year)	41.9±9.8	42.9±10.0	-0.65	0.519
More than one admission due to ARP	138(76.7%)	35(72.9%)	0.29	0.589
Drinking day/month	15.5±9.6	15.5±8.8	0.02	0.983
Drinks/drinking day (SD)	12.6±7.6	10.7±5.7	1.92	0.058
History of severe alcohol withdrawal	65(36.1%)	34(70.8%)	18.60	<0.001*
Family history of ARP	98(54.4%)	27(56.3%)	0.05	0.823

a. Age at the onset of ARP - Age at the onset of ARP: Period from between age at the onset of ARP to age at the onset of ARP.
ARP, alcohol related problem; adm, admission; SD, standard drink

with type 1/1 and those with the other types (1/2+2/2, hereinafter 2+) or those with the type 1 and 2 alleles. The ADH2*1/1 (slower) genotype positively influenced the development of alcoholism in both genders. However, the effect size was considerably larger in females than in males (OR, female vs. male, 16.484 vs 8.986). The gender differences in the frequency of the ADH2 genotype became more distinct when making comparisons between male and female alcohol-dependent subjects directly as described above.

In the case of ALDH2, there were also significant differences in the frequencies of the 1/1, 1/2 and 2/2 genotypes, the frequency of homozygosity for the 1/1 state, and the frequency of the two alleles of 1 and 2 between the alcohol-dependent and control subjects in both genders. However, the ALDH2 gene had opposite effects on the development of alcoholism in males and females. The ALDH2*1/1 (faster) genotype had a positive influence on alcoholism in the male subjects and a negative influence on the female subjects. Thus, the gender difference in genotype frequency between the alcohol dependence and control groups was more prominent in the ALDH2 enzyme than the ADH2 enzyme.

The probability of developing alcohol dependence with the ADH2 + ALDH2 genotypes in each gender and the comparison of the probability between genders

Gender differences in the influence of the ADH2 and ALDH2 enzyme genes on the development of alcoholism were revealed more distinctly when comparing the two gene combination set. The results are shown in Table 3. Alcohol dependency in males was mainly determined by the activity of the ALDH2 enzyme. According to logistic regression analysis, the two gene combination sets most closely associated with the greatest probability of developing alcohol dependence in males (ADH2*1/1-ALDH2*1/1 and ADH2*2+-ALDH2*1/1) both had the ALDH2*1/1 genotype, which accounted for 92.2% of all males

with alcohol dependence. Female alcohol dependence was mostly associated with the activity of the ADH2 enzyme. The two gene combination sets most closely associated with female alcohol dependence (ADH2*1/1-ALDH2*1/1 and ADH2*1/1-ALDH2*2+) both had the ADH2*1/1 genotype and accounted for 60.5% of the females with alcohol dependence.

In the males, the assumed logit model is $\ln \frac{P_x}{1-P_x} = 0.6181 + 2.5678 \text{ ADH2} + 3.3526 \text{ ALDH2}$. When the ADH2 genotype is 1/1 (slower) state, it has a positive influence on alcoholism (Wald $-\chi^2 = 18.6898$, $p < 0.001$) and when the ALDH2 genotype is 1/1 (faster), it also has a positive influence on alcoholism (Wald $-\chi^2 = 61.0371$, $p < 0.001$). In the females, the assumed logit model is $\ln \frac{P_x}{1-P_x} = 2.7243 + 2.7589 \text{ ADH2} - 1.3807 \text{ ALDH2}$. When the ADH2 genotype is 1/1 (slower), it has a positive influence on alcoholism (Wald $-\chi^2 = 23.4045$, $p < 0.001$), and when the ALDH2 genotype is 1/1 (faster), it has a negative influence on alcoholism (Wald $-\chi^2 = 6.5078$, $p < 0.05$). This suggests that there are gender differences in the genetic risk for alcohol dependence.

Discussion

This study found that the homozygosity for ADH2*1/1 had a positive influence on the development of alcoholism in both genders, but the degree of influence was larger in the females than in the males. The influence of homozygosity for ALDH2*1/1 on the development of alcohol dependence differed in the males and females; it had a positive influence on alcoholism in the males but a negative influence on alcoholism in the females.

The correlation between slower ADH enzyme (ADH2*1/1) activity and the development of alcohol dependence in males has been consistently reported. However, in females, the role of ADH in alcohol dependence has been unclear or underestimated until now. Borrás *et al.* [11] investigated ADH2 gene polymorphisms in 876 white subjects and reported that the frequency of the ADH2*1 allele was significantly higher in the male alcohol-dependent

Table 2. The frequency of the ADH2 and ALDH2 in the male and female alcohol-dependent subjects and control subjects.

	Alleles	Alcohol dependent Men N=180(%)	Normal control subjects N=79(%)	χ^2	Odds Ratio (95% CI)	Wald statistic	P	Alcohol dependent Women N=48(%)	Normal control subject N=59(%)	χ^2	Odds Ratio (95% CI)	Wald statistic	P	χ^2_a (M/F)	
ADH2	1/1	68(37.7)	5(6.3)	28.316**	2.550 (1.749,3.721)	23.549	<.001	29(60.4)	5(8.5)	37.170**	6.111 (3.106,12.025)	27.477	<.001	10.882*	
	1/2	55(30.6)	30(38.0)					14(29.2)	23(39.0)						
	2/2	57(31.7)	44(55.7)					5(10.4)	31(52.5)						
	ADH2	1/1	68(37.7)	5(6.3)	26.827**	8.986 (3.460,23.338)	20.328	<.001	29(60.4)	5(8.5)	32.939**	16.484 (5.578,48.713)	25.696	<.001	7.945*
		1/2 or 2/2	112(62.3)	74(93.7)					19(39.6)	54(91.5)					
		1	191(53.1)	40(25.3)					72(75.0)	33(28.0)					
ADH2		2	169(46.9)	118(74.7)	34.196**	3.334 (2.204,5.044)	32.492	<.001	24((25.0)	85(82.0)	46.857**	7.727 (4.189,14.255)	42.828	<.001	14.951**
		1/1	166(92.2)	27(34.2)					27(56.3)	50(84.7)					
		1/2	14(7.5)	47(59.5)					16(33.3)	7(11.9)					
	ALDH2	2/2	0(0.0)	5(6.3)	98.083**	21.708 (10.611,44.410)	71.024	<.001	5(10.4)	2(3.4)	10.659*	0.340 (0.163,0.709)	8.267	.004	43.352**
		1/1	166(92.2)	27(34.2)					27(56.3)	50(84.7)					
		1/2 or 2/2	14(7.5)	52(65.8)					21(43.7)	9(15.3)					
ALDH2		1	332(92.2)	54(34.2)	194.831**	12.124 (6.464,22.741)	60.456	<.001	54(62.8)	100(87.7)	21.319**	0.340 (0.202,0.571)	16.534	<.001	86.703**
		2	28(17.8)	104(65.8)					32(37.2)	14(12.3)					

* p<0.05, ** p<0.01

a. comparing between male and female alcohol dependent subject
Chi-square tests and univariate logistic regressions with ADH2 and ALDH2 genotypes respectively.

Table 3 Probability of developing alcohol dependence with the ADH2 + ALDH2 genotypes combination set in each gender.

ADH2	ALDH2	Male			female		
		Alcohol N=180(%)	Control N=79(%)	Probability	Alcohol N=48(%)	Control N=59(%)	Probability
1/1(slower)	1/1(faster)	60(33.3)	2(2.5)	0.98149	15(31.2)	5(8.5)	0.79309
	2+(slower)	8(4.4)	3(3.8)	0.64979	14(29.2)	0(0.0)	0.93845
2+(faster)	1/1(faster)	106(58.9)	25(31.6)	0.80265	12(25.0)	45(76.3)	0.19541
	2+(slower)	6(3.3)	49(62.0)	0.12459	7(14.6)	9(15.3)	0.49136

Multivariate logistic regression including ADH2 and ALDH2 genotypes.

group than in the male control group. They also reported that there was no significant difference between the female alcohol-dependent group and the female control group. Whitfield *et al.* [13], who investigated ADH2 gene polymorphisms in 412 European-Australian twins, reported that 26.3% of men with the ADH2*1/1 genotype were diagnosed with alcohol dependence, while only 5.3% of men with the ADH2*1/2 genotype were diagnosed with alcohol dependence, which indicates that there was a significantly higher percentage of alcohol dependence in the subjects with the ADH2*1/1 genotype. However, they reported comparative figures in women suggesting that there is no significant difference between males and females. Cheng *et al.* [12] conducted a survey on ADH2 genes in 353 Taiwan subjects. According to this study, the possibility of being diagnosed with alcohol dependence was 23.5% for men with the ADH2*2/2 genotype and 42.4% for men with ADH2*1+ genotype (that is, ADH2*1/1 + 1/2), showing a significantly smaller percentage in the subjects with the ADH2 2/2 genotype. However, again, comparative figures in women were reported, indicating that there was no significant difference between ADH2*2/2 genotype and ADH2*1+ genotype. The findings of these studies suggest the possibility that there may be a gender difference in the significance of ADH2 to the risk of alcohol dependence, which is consistent with the results presented in the present study. However, there are some noticeable differences between this study and the previous studies. In our study, the role of ADH2 in alcohol dependence was relatively more significant in females than in males.

Previous studies concerning the role of ALDH in alcohol dependence have also consistently reported that faster ALDH enzyme (ALDH2*1/1) activity increases the risk of alcohol dependence, which is consistent with the findings in our study. However, most of the previous studies have been primarily focused on male subjects. Thus, the results in female alcoholics are lacking, and role of ALDH in female alcohol dependence has been unclear until now. Here, the role of ALDH2 enzyme activity on the development of alcohol dependence in females seemed to be opposite to the role of ALDH2 in males.

The previous Korean studies concerning the role of ALDH in alcohol dependence reported that the frequencies of ALDH2*1/1 ranged from 48.4% to 73.6% in the control groups and from 89.4% to 99.2% in the alcohol-dependent groups, showing that the frequency of the ALDH2*1/1 genotype was significantly higher in alcohol-dependent subjects [14,15,17,25]. Previous studies

conducted on Chinese men [26] and Japanese men [27,28] also demonstrated an association between the ALDH2*1/1 genotype and alcohol dependence in males. It is noteworthy that the results of this study found that the frequency of the ALDH2*1/1 genotype in the male control group was lower (34.2%) than that in the control groups of previous studies conducted on Korean male subjects [14,15,17,25]. This may be attributed to the fact that, unlike in previous studies, the control group of this study represented a super control group that consisted of only those who consumed less than 5 standard drinks per month throughout their lives.

Considering the result of this study in the view of alcohol hepatic metabolism, males who have enzymes that are genetically advantageous in the elimination of acetaldehyde to acetate have a higher risk of developing alcoholism. In other words, male alcoholism is positively reinforced predominantly by the low level of negative feedback from acetaldehyde. These inferences are supported by the result that approximately 92.2% of the male alcohol-dependent subjects showed a higher enzyme activity in ALDH2 and approximately 62.3% of the male alcohol-dependent subjects showed a higher enzyme activity in ADH2. Therefore, alcohol could be metabolized into acetaldehyde rapidly due to ADH2*2+. However, blood acetaldehyde could not be increased due to ALDH2*1/1. This indicates that the drinking amount eventually increases when the activity of both ADH2 and ALDH2 have increased.

Female subjects who metabolize alcohol slowly can easily become intoxicated, and they are more likely to become dependent on alcohol. Female alcoholism is positively reinforced, predominantly by the high level of positive feedback from alcohol. Approximately 60.4% of the female alcohol-dependent subjects showed a slower ADH enzyme. The faster ALDH2*1/1 genotype, which was significant in the male alcohol dependence groups, was more frequent in the female control group than in the female alcohol-dependent group (84.7% vs 56.3%). Therefore, female alcoholics can be exposed to higher blood alcohol levels than male alcoholics.

Heath *et al.* [8,29,30] asserted that the gender differences in the role of this gene result from the interplay of genetic and environmental risk factors. Socio-culturally, the ability to drink large amounts of alcohol is considered to be representative of masculinity. In addition, considering that alcohol is frequently used in the mediation of social relationships, male, who have enzymes that are advantageous, are reinforced by alcohol

drinking behavior and may eventually develop alcoholism. Meanwhile, there are some reports that neurotic symptoms are more frequent in female alcoholics than in male alcoholics [31-34]. According to the tendency of female alcoholics to self-medicate preponderant neurotic symptoms, female subjects that are reinforced by alcohol drinking behavior and are more likely to develop alcohol dependence. Several studies have reported that women usually start drinking heavily at older ages than men but that they are likely to become dependent on alcohol more quickly than men, that is, in a matter of several years. In addition, alcohol-dependent women suffer from more physical disorders earlier than men [5,6]. This rapidly progressing phenomenon is referred to as the 'telescoping effect'. The difference in the progression of alcohol dependence between the sexes is related to the fact that women have a higher blood alcohol concentration (BAC) than men, even when they consume the same amount of alcohol. According to several related studies, this can be attributed to the fact that women have smaller bodies than men, they have less water and more fat in their bodies than men, and their primary alcohol metabolizing capabilities are lower than those in men [35]. The mechanism of this telescoping effect in female alcohol-dependent patients can alternatively be explained by our finding that females have a different motivation for drinking than males and that female subjects with a lower degree of ADH2 activity are more likely to become alcohol-dependent and to be exposed to alcohol's toxicity. Although not a random sample, the average age at which women started drinking was 27 years compared to 20 years for men, and the average age at the onset of ARP was 38 years for women (10.5 years after first drinking) and 34 years for men (about 14 years after first drinking), suggesting that the

progression of alcohol dependence is faster in women than in men.

Based on the above results, we conclude that ADH2 has a greater influence on the development of alcohol dependence in female subjects, while ALDH2 contributes more to the development of alcoholism in male subjects. To explain this result, we cautiously infer that males and females use alcohol for different purposes. Males who drink heavily and do not get easily intoxicated due to the rapid metabolism of alcohol are more likely to become alcohol-dependent while females who get easily intoxicated are more likely to become alcohol-dependent over time. The difference in drinking motivation by gender and the role of genetic markers on drinking motivation need further investigation.

Limitations

Limitations of this study include the small number of female alcohol-dependent subjects compared to that of male alcohol-dependent subjects and the question of whether subjects selected from several mental hospitals can represent all of the alcohol-dependent patients in Korea. Therefore, further studies including more female alcohol-dependent subjects and more data collection are necessary. However, this study is significant in that its subject population included Korean female alcohol-dependent patients for the first time.

Declaration of conflicts of interest

The authors declared no conflicts of interest.

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