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. Borras 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].
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 then 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
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 but a negative influence on alcoholism in the females. The ALDH2*1/1 (faster), it also has a positive influence on alcoholism (Wald \(\chi^2 = 18.6898, p < 0.001\)) and when the ALDH2 genotype is 1/1 (slower) state, it has a positive 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.

In the males, the assumed logit model is \(\ln \left( \frac{P_x}{1-P_x} \right) = 0.6181 + 2.5678 \text{ADH2} + 3.3526 \text{ALDH2}. \) 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 \(\ln \left( \frac{P_x}{1-P_x} \right) = 2.7243 + 2.7589 \text{ADH2} – 1.3807 \text{ALDH2}. \) 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 \(\chi^2 = 6.0578, p<0.05\)). 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. Borras 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>
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<th>Alleles</th>
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<th>χ²</th>
<th>Odds Ratio (95% CI)</th>
<th>Wald statistic</th>
<th>p</th>
<th>Alcohol dependent Women N=48(%)</th>
<th>Normal control subject N=59(%)</th>
<th>χ²</th>
<th>Odds Ratio (95% CI)</th>
<th>Wald statistic</th>
<th>p</th>
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<td>29(60.4)</td>
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<td>16.534</td>
<td>&lt;.001</td>
<td>86.703**</td>
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* 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.
The frequency of the ALDH2*1/1 genotype was significantly higher (89.4% to 99.2%) in the alcohol-dependent groups, showing that the role of ALDH2 enzymes in female alcohol dependence was more significant than in males. The previous Korean studies concerning the role of ALDH in alcohol dependence have also consistently reported that faster ALDH2*1/1 genotype activity increases the risk of alcohol dependence. However, most of the previous studies have been primarily focused on male subjects. 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.

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 ADH2 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. Therefore, female alcoholics can be exposed to higher blood alcohol levels than male alcoholics.


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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.

References


