Human Molecular Genetics, 2008, Vol. 17, No. 6 **854–858**
doi:10.1093/hmg/ddm357
Advance Access published on December 4, 2007

Major genetic components underlying alcoholism in Korean population

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Received October 1, 2007; Revised and Accepted November 30, 2007

Alcohol metabolism is one of the biological determinants that could significantly be influenced by genetic polymorphisms in alcohol-metabolism genes. Alcohol dehydrogenase (ADH) converts alcohol to acetaldehyde, and aldehyde dehydrogenase (ALDH) converts acetaldehyde to acetate. The well-known genetic polymorphisms in *ADH1B*(His47Arg) and *ALDH2*(Glu487Lys) have dramatic effects on the rate of metabolizing alcohol and acetaldehyde, respectively. The protective allele of *ADH1B* (*ADH1B*47His*) encodes for a rapid ethanol-metabolizing enzyme, and the susceptible allele of the *ALDH2* (*ALDH2*487Lys*) is strongly associated with decreased rate of metabolizing acetaldehyde. However, the combined genetic effects of both functional polymorphisms have not been clarified. The combined analysis of two polymorphisms among a Korean population ($n = 1{,}032$) revealed dramatic genetic effects on the risk of alcoholism. Individuals bearing susceptible alleles at both loci have 91 times greater risk for alcoholism [odds ratio (OR) = 91.43, $P = 1.4 \times 10^{-32}$] and individuals bearing one susceptible and one protective allele at either loci have 11 times greater risk (OR = 11.40, $P = 3.5 \times 10^{-15}$) compared with subjects who have both protective alleles. The attributable fraction of those genetic factors, calculated based on population controls, indicates that alcoholism in 86.5% of alcoholic patients can be attributed to the detrimental effect of *ADH1B*47Arg* and/or *ALDH2*487Glu* in Korean population.

INTRODUCTION

Alcohol metabolism occurs in two major steps: oxidation of alcohol to acetaldehyde by the alcohol dehydrogenases (ADHs) enzymes, especially by ADH1B, and further oxidation of acetaldehyde into acetate by aldehyde dehydrogenase enzymes (ALDHs), mainly by ALDH2. Encoding genes for these two representative alcohol-metabolizing enzymes display polymorphisms (*ADH1B* His47Arg and *ALDH2* Glu487Lys) that show different alcohol/acetaldehyde oxidizing capability among individuals (1–4). The *ADH1B*47His* allele represents a much higher activity of ADH1B with ~40 times higher Vmax than the homozygotes for the *ADH1B*47Arg* form, which enables increased alcohol elimination from the blood after alcohol consumption (1,5). The *ALDH2*487Lys* allele encodes a catalytically inactive subunit (1,5), which causes alcohol-related adverse reactions including flushing, palpitation, nausea, headache, drowsiness, breathlessness and general discomfort (6). These adverse reactions in subjects with *ALDH2*487Lys*, as a result of excessive acetaldehyde accumulation, tend to reduce alcohol consumption, subsequently reducing the risk of alcoholism.

Many previous studies have reported genetic associations of *ADH1B* His47Arg and *ALDH2* Glu487Lys with alcoholism, especially in Asian populations (7–12). However, the combined genetic effects of these two loci have not yet been clarified. In the current study, the combined genetic effects of *ADH1B* and *ALDH2* genotypes were analyzed in a Korean population ($n = 1032$).

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Table 1. Analysis of genetic effect of *ADH1B* His47Arg and *ALDH2*Glu487Lys on the risk of alcoholism among Korean male subjects ($n = 1032$)

Loci	Genotype	Alcoholism, *n* (%)	HWE[a]	NC, *n* (%)	HWE[a]	OR (95% CI)[b]	*P*-value[b]	OR (95% CI)[c]	*P*-value[c]	Genetic effect
ADH1B His47Arg	His/His	217 (39.5)		298 (61.7)		1	–	1	–	Protective
	His/Arg	145 (26.4)	3.17×10^{-28}	155 (32.1)	0.110	1.29 (0.97–1.71)	0.09			Protective
	Arg/Arg	187 (34.1)		30 (6.2)		8.56 (5.61–13.07)	2.8×0^{-23}	7.80 (5.18–11.75)	8.2×0^{-23}	Susceptible
ALDH2 Glu487Lys	Glu/Glu	530 (96.5)		346 (73.6)		1	–	1	–	Susceptible
	Glu/Lys	19 (3.5)	0.680	122 (25.3)	0.297	0.10 (0.06–0.17)	4.5×10^{-19}	0.10 (0.06–0.15)	3.6×10^{-21}	Protective
	Lys/Lys	0 (0.0)		15 (3.1)		–	–			Protective

[a]*P*-value of genotype distribution deviation from Hardy–Weinberg equilibrium.
[b]Logistic regression models were used for calculating ORs (95% CI) and corresponding *P*-values. The common alleles were used as the referent genotype to the heterozygote and homozygote of the minor allele.
[c]OR and *P*-value of referent analysis between susceptible versus protective genotype groups.

RESULTS

In the Korean population, the *ADH1B* His47Arg polymorphism showed, as expected, a dramatic genetic association with the risk of alcoholism. Referent analysis of *ADH1B* His47Arg revealed that its genetic mode for the risk of alcoholism is apparently susceptible and recessive, when comparing the strength and magnitude of associations of heterozygotes and homozygotes for *ADH1B*47Arg*. Only 6.2% of normal controls had the *ADH1B*47Arg/Arg* genotype, compared with 34.1% in alcoholics [recessive mode; odds ratio (OR) = 7.80 95% confidential interval (CI), 5.18–11.75, $P = 8.2 \times 10^{-23}$]. In addition, the genotype distribution of *ADH1B* His47Arg showed severe deviation from Hardy–Weinberg equilibrium (HWE) ($P = 3.17 \times 10^{-28}$) in alcoholics, whereas no deviation was detected among normal controls. The deviation from HWE occurring only in alcoholics strongly suggests that a selection bias (or pressure) is involved, which would be additional direct evidence of association of this polymorphism with the risk of alcoholism (Table 1).

The *ALDH2Glu487Lys* also showed apparent genetic effects on the risk of alcoholism [dominant mode; OR = 0.10 (95% CI, 0.06–0.15) and $P = 3.6 \times 10^{-21}$]. In contrast to *ADH1B*47Arg*, the genetic mode of *ALDH2*487Lys* clearly appeared to be protective and dominant in the referent analysis. Interestingly, subjects bearing *ALDH2*487Lys/Lys* appeared to have complete genetic protection against alcoholism (no homozygotes in alcoholics) (Table 1).

Next, in order to examine whether these two functional polymorphisms have any interaction effects on the risk of alcoholism, subgroup analysis according to genetic effects of each loci was performed. The results were that similar magnitudes of genetic effects with those of all subjects were shown for the two polymorphisms (Supplementary Materils, Table S1), suggesting clearly independent genetic effects on the risk of alcoholism.

To examine the combined genetic effect of *ADH1B* His47Arg and *ALDH2* Glu487Lys, subjects were subdivided by genetic effects of both loci. Combined analysis revealed huge genetic effects on the risk of alcoholism, e.g. individuals bearing susceptible alleles at both loci were found to have 91 times greater risk for alcoholism (OR = 91.43, $P = 1.4 \times 10^{-32}$) and individuals bearing one susceptible and one protective allele at either loci had 11 times greater risk (OR = 11.40, $P = 3.5 \times 10^{-15}$) compared with subjects who have both protective alleles (Table 2). The results of this combined analysis are concordant with those of the separated analysis (independent effects; Supplementary Materils, Table S1) and clearly showed additive effects on the risk of alcoholism development.

The attributable fraction (AF) was calculated by OR (10.53) and frequency (subjects with one or two susceptible alleles for either loci; 75.2%) based on population controls (Table 2). AF indicates that alcoholism in 86.5% of alcoholic patients in the Korean population can be attributed to the detrimental effects of *ADH1B*47Arg* and/or *ALDH2*487Glu*.

DISCUSSION

Alcoholism [MIM# 103780] is a leading cause of morbidity and premature death. Several lines of evidence suggest a substantial genetic component to the risk for alcoholism. Alcoholism is believed to be a multifactorial and polygenic disorder involving complex gene-to-gene and gene-to-environment interactions. According to the National Council of Alcoholism and Drug Dependence (NCADD) and the American Society of Addiction Medicine (ASAM), 'alcoholism is a primary, chronic disease with genetic, psychosocial and environmental factors influencing its development and manifestations. The disease is often progressive and fatal. It is characterized by continuous or periodic impaired control over drinking, preoccupation with the drug alcohol, use of alcohol despite adverse consequences and distortion in thinking, most notably denial'.

In this study, by analyzing the well-known genetic polymorphisms in *ADH1B*(His47Arg) and *ALDH2*(Glu487Lys) in a large Korean sample ($n = 1032$), we were able to show that the combined effect of the two alleles has a huge impact on disease phenotype. The employment of the large sample gives the study a great statistical power, and the evidences from this study might be, therefore, indisputable. In terms of combined analysis of two functional polymorphisms, <5% of people in the Korean population (disease and population controls) have susceptible/susceptible genotypes. In addition, although considerable allele frequency variation occurs even among East Asian populations, up to 25% of Asians have protective genotypes at both loci, whereas very

Table 2. Combined analysis of association of *ADH1B* His47Arg and *ALDH2* Glu487Lys with the risk of alcoholism in Korean male subjects ($n = 1032$)

Genotype *ADH1B* His47Arg	*ALDH2* Glu487Lys	No. of subjects Alcoholism, *n* (%)	NC, *n* (%)	PC[e], *n* (%)	OR (95% CI)[a]	*P*-value[a]	OR (95% CI)[b]	*P*-value[b]	OR (95% CI)[c]	*P*-value[c]	*P*-value[d]
Protective (His/His or His/Arg)	Protective (Glu/Lys or Lys/Lys)	12 (2.2)	128 (26.5)	95 (24.8)	1	–	1		1		
Protective (His/His or His/Arg)	Susceptible (Glu/Glu)	350 (63.7)	325 (67.3)	263 (68.5)	11.49 (6.24–21.16)	4.7×10^{-15}	11.40 (6.19–20.99)	3.5×10^{-15}			2.2×10^{-31}
Susceptible (Arg/Arg)	Protective (Glu/Lys or Lys/Lys)	7 (1.3)	9 (1.9)	9 (2.3)	8.30 (2.62–26.24)	0.0003			16.14 (8.80–29.60)	2.6×10^{-19}	
Susceptible (Arg/Arg)	Susceptible (Glu/Glu)	180 (33.8)	21 (4.3)	17 (4.4)	91.43 (43.42–192.50)	1.4×10^{-32}	91.43 (43.42–192.50)	1.4×10^{-32}			

[a]OR and *P*-value for referent analysis of protective/protective genotype group.
[b]OR and *P*-value of genotype groups with one and two copy of susceptible alleles versus protective/protective genotype group.
[c]OR and *P*-value of genotype groups with one or two copies of susceptible allele versus protective/protective genotype group.
[d]Global *P*-value.
[e]Population controls ($n = 384$). Population controls were used for attributable fraction (AF, 87.7%) calculation [OR (10.53) and frequency (75.2% of PC) of subjects with one or two susceptible alleles for either loci)

low numbers of European or African populations have been reported to have these protective genotypes (13,14). The much higher frequencies of the protective *ADH1B* and *ALDH2* alleles (*ADH1B*47His* and *ALDH2*487Lys*) in some Asian populations, therefore, clearly provide some East Asians, especially Koreans and Japanese (6), with protection against alcoholism, compared with most European or African populations who have susceptible alleles (*ADH1B*47Arg* and *ALDH2*487Glu*).

Interestingly, the genotype distribution of both *ADH1B* and *ALDH2* differs greatly between Asian, especially East Asian, and other major populations. Recently, the global frequencies of *ADH1B*47His* have been well-defined. The frequency of the *ADH1B*47His* allele is particularly high in East/West Asian populations and moderate in southeast Asian and North African populations, but the allele is almost absent in sub-Saharan African, European and Native American populations (13,15). Similarly, *ALDH2*487Lys* has only been detected among East Asian populations (14). Although other social/cultural factors might be involved, these different genetic backgrounds regarding alcohol metabolism could very likely explain the lower incidence of alcohol abuse in some East Asian populations, including the Korean population (16).

Several plausible evidences for natural selection of this allele in Koreans, Japanese and Han Chinese have been suggested (13,17). The researchers proposed interesting hypotheses that might explain the high frequencies of *ADH1B*47His* and *ALDH2*487Lys* in East Asian populations. Among them, the hypothesis of selection pressure for higher ADH1B (mediated by *ADH1B*47His)* and lower ALDH2 (mediated by *ALDH2*487Lys)*, against some endemic disease(s), including parasitism, in area with high frequencies of those alleles, might be the most plausible explanation, although the nature of the selective pressure and the time period during which it operates are still unknown. Interestingly, Li *et al*. (13) presented very convincing evidence that the distribution of *ADH1B*47His* increased in frequency independently in West and East Asia after humans had spread across Eurasia. Similarly, natural selection of *ALDH2*487Lys* in East Asian populations was also suggested (14).

In summary, we re-confirmed that the well-known *ADH1B* His47Arg and *ALDH2* Glu487Lys have the dramatic genetic associations with the risk of alcoholism, and that their genetic effects are clearly independent among Korean population. When combined together, those two loci revealed huge genetic effects on the risk of alcoholism. In addition, AF indicates that alcoholism in 87.7% of alcoholic patients in the Korean population can be attributed to the detrimental effect of *ADH1B*47Arg* and/or *ALDH2*487Glu*, which suggesting that the two polymorphisms are major genetic components for alcoholism, at least, in Korean population.

MATERIALS AND METHODS

Study subjects and genotyping of polymorphisms

The patients used in this study ($n = 549$, all males; mean age = 46.1, range = 20–73), all of whom were

alcohol-dependent according to the *Diagnostic and Statistical Manual of Mental Disorders IV* (DSM-IV) criteria (American Psychiatric Association, 1994), were recruited from Hangang Sacred Heart Hospital, Yong-In Mental Hospital, Hanmaum Hospital, Gumin Hospital, Hando Hospital (Hanllym University groups) and another network of multicenter mental hospitals in Korea (Holy Family Hospital, Chamsarang Mental Hospital, Chunchon Mental Hospital; Catholic University groups). The patients had neither major medical nor co-morbid psychiatric illnesses other than alcohol-related disorders and/or nicotine dependence (43%). The controls were unrelated healthy male employees of Hangang Sacred Heart Hospital ($n = 483$, all male; mean age = 33.2 years, range = 20–77). Most of the participating employees were non-drinkers; only some were occasional light drinkers, as revealed by a drinking habit questionnaire. Subjects who had first-degree relatives with major psychiatric disorders, such as schizophrenia, mood disorders or substance-use disorders other than nicotine dependence, were excluded. Additional population controls ($n = 384$, all males) were used for AF calculation. The Institutional Review Board of each hospital approved the study, and informed consents were obtained.

The *ADH1B* His47Arg and *ALDH2* Glu487Lys polymorphisms were genotyped using the TaqMan method (18), which has been described in our previous work (9,19).

Statistics

χ^2 tests were used to determine if the individual variants were in HWE. Logistic regression analyses, controlling for age as covariate, were used to calculate ORs and the *P*-values for case–control analysis. The AF was calculated by the formula $AF = f(R-1)/(1+f(R-1))$, where f is the frequency of the risk factor in the population and R is the measure of the OR (20). In combined analysis (Table 2), several facts were considered when choosing a method of analysis, including (i) the two SNPs were not linked to each other, (ii) no significant interactions were detected through interaction analysis ($P > 0.36$, data not shown) and (iii) both SNPs have obvious effects and their genetic modes are apparent [susceptible/recessive (*ADH1B*47Arg*) and protective/dominant (*ALDH2*487Lys*) in this study of a Korean population]. Based on these considerations, we have adopted one of the simplest methods of analysis, e.g. susceptible versus protective at two loci (four subgroups). The protective/protective subgroup was used as the referent to the other subgroups. Logistic regression analyses, controlling for age as covariate, were used to calculate ORs and the *P*-values for case–control analysis. All statistical analyses were performed using SAS 9.1 (SAS Institute, Inc., Cary, NC, USA).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

Conflict of Interest statement. None declared.

FUNDING

This work was supported by a grant from the National Research Lab Program as part of the National Research and Development Program of the Ministry of Commerce, Industry and Energy of Korea (M1-0302-00-0073). This study was also partly supported by a grant from Biomedical Brain Research Center (A040042) and a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare (03-PJ10-PG13-GD01-0002) funded by the Ministry of Health & Welfare, Republic of Korea.

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