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Role of *GABRA2* in Moderating Subjective Responses to Alcohol

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Abstract

Background—Human twin studies have shown that certain responses to alcohol, including subjective perceptions, are genetically influenced. Previous studies have provided evidence that a low level of response to alcohol predicts future alcohol use disorders in humans. Recent genetic studies suggest an association between alcohol dependence and genetic variation in the γ-aminobutyric acid A ($GABA_A$) receptor $\alpha 2$ subunit gene (*GABRA2*). Based on a haplotypic association of alcohol dependence with *GABRA2*, we investigated whether *GABRA2* alleles are associated with the subjective responses to clamped alcohol concentration.

Methods—One hundred and ten healthy social drinkers (53 men) underwent the alcohol clamp. Fifteen minutes after the start of an intravenous infusion of alcohol, the breath alcohol concentration was clamped at a target of 50 ± 5 mg/dl for 165 minutes. Subjective physiologic responses to alcohol and stimulant and sedative effects of alcohol were measured repeatedly during the alcohol clamp. Because aldehyde dehydrogenase 2 (ALDH2) has been shown to have a great impact on the subjective responses to alcohol, we divided subjects by *ALDH2* genotype for further analyses. To examine the role of genetic variation in *GABRA2*, 7 single nucleotide polymorphisms (SNPs) that were informative in association studies were included as factors in the analysis.

Results—Among these 7 SNPs, 3 SNPs (rs279869, rs279858, and rs279837) located in the middle of the *GABRA2* gene showed significant associations with subjective effects of alcohol. Subjects with 1 or 2 copies of the more common allele showed greater subjective responses to alcohol than did individuals homozygous for the alcohol dependence–associated allele regardless of *ALDH2* genotype.

Conclusions—These findings confirm and extend the observation that the *GABRA2* alleles affect the subjective responses to alcohol, and suggest that the genetic variations in *GABRA2* might play a role in the risk of alcohol use disorders by moderating the subjective effects of alcohol.

Keywords

GABA; *GABRA2*; Alcohol; Subjective Response; Alcohol Clamping; *ALDH2*

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Alcohol use disorder is a common and complex disorder with a well-documented highly hereditary nature (Higuchi et al., 2006; Roh et al., 2008). Subjective response to alcohol is also known to be a genetically influenced characteristic (Schuckit et al., 2007; Viken et al., 2003). This suggests that genetic influences on individual variation in subjective response to alcohol may underlie the effects of genes on alcohol-related disorders. A low level of response to the acute effects of alcohol has been associated with an increased risk of both excessive alcohol intake (Hinckers et al., 2006; Schuckit et al., 2007) and alcohol dependence (Schuckit, 1994; Schuckit and Smith, 1996; Schuckit et al., 2004), which are well known to be highly heritable (Kendler, 2001).

There is consistent evidence that the $GABA_A$ receptor regulates the alcohol self-administration in animal models, probably by stimulating reward circuitry in the mesolimbic system (Chester and Cunningham, 2002; Eiler and June, 2007; Harvey et al., 2002; June et al., 2003). Several $GABA_A$ receptor subunits have been implicated in alcohol effects, so that the specific subunit composition of the receptor may be an important determinant of alcohol's CNS effects. The $\alpha 2$ subunit of the $GABA_A$ receptor mediates the anxiolytic effects of benzodiazepines (Low et al., 2000; Rudolph et al., 1999) and enhances the hypnotic, but not the sedative, effects of combined exposure to alcohol and benzodiazepines (Tauber et al., 2003). Two genome-wide scans in humans have provided evidence of linkage of alcohol dependence to a region of chromosome 4p that includes a cluster of 4 genes encoding γ-aminobutyric acid A ($GABA_A$) receptor subunits (Long et al., 1998; Reich et al., 1998). A previous study (Edenberg et al., 2004) found that 31 single nucleotide polymorphisms (SNPs) in $GABA_A$ receptor $\alpha 2$ subunit gene (*GABRA2*), but only 1 of the 20 SNPs in the flanking genes, showed significant association with alcohol dependence. Additional studies have provided the replication of this association in a region of *GABRA2* among various ethnic groups (Covault et al., 2004; Fehr et al., 2006; Lappalainen et al., 2005; Soyka et al., 2008); though, there is a negative association study between *GABRA2* and alcohol dependence (Matthews et al., 2007). Previous studies have also provided evidence that $GABA_A$ receptors mediate several behavioral effects of alcohol such as ethanol self-administration and motor impairment (Davies, 2003; Grobin et al., 1998; Hanchar et al., 2005). *GABRA2* gene was associated with the differences in the subjective effects of alcohol including blushing sensations, stimulant and sedative effects (Pierucci-Lagha et al., 2005), and the variance in drinking behavior (Bauer et al., 2007). In the former study (Pierucci-Lagha et al., 2005), the more common A allele of the rs279858 SNP within the *GABRA2* gene showed greater subjective effects of alcohol than did individuals with 1 or 2 copies of the alcohol dependence–associated G allele. These findings underscore the potential contributions of variation at *GABRA2* to the differences in the subjective responses to alcohol, the variance of drinking behavior, and the risk for alcohol dependence.

This study examined the moderating effects of *GABRA2* alleles on subjective and physiologic effects of alcohol in healthy social drinkers. We used the alcohol clamp method for alcohol administration, which uses an intravenous infusion of alcohol at rates adjusted online to close the gap between measurements of breath alcohol concentration (BrAC) and the target concentration. The clamp method reduces experimental variance in BrAC (O'Connor et al., 1998) which can be caused by the substantial pharmacokinetic variability following oral alcohol administration. Therefore, the alcohol clamp used in this study is a more exhaustive objective measure than the oral loading of alcohol that was used in the previous study of the effects of *GABRA2* on the subjective effects of alcohol assessed only during the ascending limb of the BrAC (Pierucci-Lagha et al., 2005). Our method allowed the evaluation of the initial response to alcohol following the ascending limb of the BrAC as well as the adaptive response to alcohol during the clamped BrAC interval. Based on the previous studies, we hypothesized that *GABRA2* alleles would moderate the subjective

responses to alcohol measured during not only the ascending limb of BrAC curve but also the clamped BrAC interval. As we expected, subjects with *ALDH2*1/*2* showed higher initial response than those with *ALDH2*1/*1* (Matsushita et al., manuscript in preparation). Therefore, we divided subjects by *ALDH2* genotype and performed further analyses separately in each *ALDH2* genotypic group.

SUBJECTS AND METHODS

Subjects

The study population consisted of 110 Japanese subjects (53 men, 48.2%) and was recruited from Yokosuka, Kanagawa, Japan. Subjects were aged 20 to 59 (mean age ± SD, 36.7 ± 10.5 years). All were healthy without apparent history of physical and psychiatric illness, determined by questionnaires about health status and past medical history. A questionnaire was used to quantify the subjects' alcohol use and to evaluate the family history of alcohol-related problems. Most of them were social drinkers but included small percentage of heavy drinkers who drink more than 4 drinks on any day or 14 per week in men (3 drinks on any day or 7 per week in women) as shown in Table 1. We compared initial response, which will be defined in the following, to alcohol between social and heavy drinkers. Because we did not find any significant differences in initial response to alcohol between them, we combined social and heavy drinkers and performed subsequent analyses. Exclusion criteria included apparent medical history of renal, hepatic, cardiovascular, pulmonary, or gastrointestinal disease; a personal history of any DSM-IV (American Psychiatric Association, 1994) axis I disorder, including alcohol-related disorders; homozygous for inactive aldehyde dehydrogenase 2 (*ALDH2*) allele (*ALDH2*2/*2*) because of the possibility of acute intoxication caused by ALDH deficiency. The Ethics Committee of the National Hospital Organization Kurihama Alcoholism Center approved the protocol, and all subjects gave written informed consent before participation.

Preparation for Testing

Subjects arrived in the Kurihama Alcoholism Center laboratory at 9:00 AM, having been instructed to abstain from alcohol for at least 36 hours and from food for at least 12 hours. Abstinence was verified by BrAC measurement. After measuring height, weight, body temperature, and blood pressure, subjects ate a 350-kilocalorie breakfast that was composed of cornflakes and milk. An indwelling catheter was inserted into a vein in the antecubital fossa of each arm, the dominant arm for the infusion and the nondominant arm for blood sampling.

Alcohol Administration by Alcohol Clamp

At 10:00 AM, the intravenous infusion of 6% (v/v) ethanol in Ringer's lactate was begun, using a precomputed rate profile. The profile was derived by forcing a physiologically based pharmacokinetic model of the individual's alcohol distribution and elimination to follow the desired time course of BrAC as a function of time: a linear ascending limb reaching 50 ± 5 mg/dl at 15 minutes, and then constant for 165 minutes by the method of Ramchandani et al. (1999a). We chose the target level, 50 mg%, as a low to moderate level that would be well tolerated by all subjects without major adverse effects and that would give us measurable effects with sufficient interindividual variance to examine the genetic determinants of interest. Based on serial BrAC measurement, small and intermittent adjustments of the infusion rate were made to maintain the clamped BrAC within 5 mg/dl of the target concentration. After 165 minutes of clamping, the infusion was stopped, the catheter was removed, and the subject was provided with lunch. The BrAC was tracked at 15-minute intervals until it fell below 20 mg/dl when the subject was dismissed from the laboratory.

The duration of a typical study session was 5 hours. The whole BrAC clamping paradigm is shown in Fig. 1.

Measures of Subjective Responses

A battery of dependent measures of subjective responses to alcohol was administered in the baseline condition before the infusion and every 30 minutes after the infusion. Subjective responses were measured using the following self-report questionnaires.

Sensation scale (SS) (Maisto et al., 1980) consists of 26 items that are divided into 6 subscales measuring the subject's current perceptions about a variety of sensations that often are associated with alcohol but are not specifically attributed to alcohol during testing: Gastro-Intestinal subscale measures sensations felt in the stomach (4 items), Anesthetic subscale measures sensations associated with loss of feeling or decreased sensitivity to feelings (9 items), Central Stimulant subscale measures sensations involving effects on the brain, or what is commonly called "getting high" (4 items), Impaired Function subscale measures perceived changes in certain abilities or skills (3 items), Warmth/Glow subscale measures effects associated with blushing sensations (3 items), and Dynamic Peripheral subscale measures sensations associated with excitation, including changed breathing and heart rate (3 items). We used 5 subscales in our analysis and excluded the Gastro-Intestinal subscale that does not appear to discriminate effectively between subjects who consumed alcoholic and nonalcoholic beverages (Maisto et al., 1980). We added General subscale incorporating all items except the items "warm" and "relaxed" instead (Ramchandani et al., 1999b).

Biphasic alcohol effects scale (BAES) (Martin et al., 1993) is a 14-item unipolar adjective rating scale designed to measure both stimulant and sedative effects of alcohol. The BAES is composed of a 7-item Stimulant subscale and a 7-item Sedative subscale. Subjects rated the adjectives on a scale of 0 (not at all) to 10 (extremely). Item scores were summed into each subscale.

Assessment of Initial Response and Acute Adaptation

Initial and adaptive responses to alcohol were assessed using scalar indices of change (Morzorati et al., 2002; Ramchandani et al., 1999b). One index assessed "initial response" (improvements or impairments) in brain function after alcohol. The other index assessed "acute adaptation" (tolerance or sensitization) to alcohol while the brain's exposure to alcohol was held constant. We defined initial response as the difference in the SS or the BAES between the baseline and the first 30 minutes after the beginning of the infusion. The index of acute adaptation was computed as the changes in the measurement of subjective responses from 30 minutes to 4 hours after the infusion by measuring every 30 minutes 8 times total as shown in Fig. 1.

Genotyping

DNA was purified from venous blood samples using the DNA Extractor WB Kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). *ALDH2* genotyping was performed by a previously described polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method (Harada and Zhang, 1993). A total of 7 SNPs from *GABRA2* gene were selected according to the prior research (Lappalainen et al., 2005): rs567926, rs534459, rs529826, rs279869, rs279858, rs279837, and rs9291283. These SNPs were genotyped using the TaqMan method on an ABI GeneAmp PCR System 9700 apparatus (Applied Biosystems, Foster City, CA). The location of the 7 *GABRA2* SNPs is presented in the previous study (Covault et al., 2004).

Statistical Analyses

The differences in initial response by *ALDH2* and *GABRA2* genotypes were analyzed by analysis of variance (ANOVA), whereas the data for acute adaptation were examined using repeated measures ANOVA. We compared the differences in initial response and acute adaptation among subjects with different genotypes of 7 *GABRA2* SNPs divided by *ALDH2* genotype because *ALDH2* genotype has been proved to have strong effects on the level of response to alcohol (Cook et al., 2005; Luczak et al., 2002; Wall et al., 1999).

The Duncan test was used for the post hoc analysis. The dependent measures examined in the analyses were the initial response and acute adaptation by calculating 6 SS subscales scores and the 2 BAES subscale scores. The statistical analysis of the comparison data was performed using SAS program (version 9.1.3, SAS Institute Inc., Cary, NC). The statistical significance level was 0.05. We used the software Haploview to visualize linkage disequilibrium (LD) relationships between *GABRA2* SNPs (Barrett et al., 2005).

RESULTS

Drinking Features

Of participants, 2.6% had a positive family history of alcohol dependence. The demographic and drinking data of participants are given in Table 1. As shown in Table 1, the gender groups did not differ on demographic or drinking characteristics except drinking amount per occasion. The genotype frequencies of *ALDH2* and *GABRA2* SNPs are shown in Table 2.

Linkage Disequilibrium Within GABRA2

A high degree of LD was observed across the *GABRA2* gene in the subjects of this study. The estimated pairwise LD (D′) (Lewontin, 1988) values between SNPs 1 to 7 were 1 (SNPs 1 to 2), 1 (2 to 3), 1 (3 to 4), 1 (4 to 5), 0.92 (5 to 6), and 1 (6 to 7). Almost complete LD was observed among these SNPs. A detailed illustration of LD within *GABRA2* is presented in Fig. 2. Best estimates generated by Haploview for the 5-marker haplotypes defined by SNPs 1 to 5 identified 3 common haplotypes, TCACA, CTGAG, and TCAAG, which together represented 99.2% of chromosomes. The frequencies of each haplotype were 62.0, 25.9, and 11.3%, respectively.

Association Between GABRA2 and Initial Response

SNPs 1, 2, 3, and 7 showed no significant association with the initial response to alcohol (data not shown), whereas SNPs 4 to 6 were significantly associated with the initial response on several subscales in SS. As the distribution of genotypes between SNPs 4 and 5 is identical in all subjects, we combined the results from these 2 SNPs (SNP4 A-allele = SNP5 G-allele; SNP4 C-allele = SNP5 A-allele). Among individuals with *ALDH2*1/*2*, subjects with 1 or 2 copies of SNP4 C-allele (SNP5 A-allele) had greater increased scores in Anesthetic [$F(2, 20) = 5.23$, $p = 0.015$], Dynamic Peripheral [$F(2, 22) = 4.14$, $p = 0.030$], and General [$F(2, 18) = 4.82$, $p = 0.021$] subscales of SS than those homozygous for SNP4 A-allele (SNP5 G-allele) (Fig. 3*B*). In individuals with *ALDH2*1 /*1*, there was a trend for higher scores among SNP4 C-allele (SNP5 A-allele) carriers than SNP4 A-allele (SNP5 G-allele) homozygotes in Stimulant [$F(2, 74) = 2.74$, $p = 0.071$] subscale of the BAES (Fig. 4*A*). In SNP6, the above-mentioned 3 SS subscale scores were also statistically significant: In subjects with *ALDH2*1/*1*, there were significant differences in Anesthetic [$F(2, 74) = 3.58$, $p = 0.033$] and Dynamic Peripheral [$F(2, 77) = 4.39$, $p = 0.016$] subscale scores among TT, TC, and CC genotypes (Fig. 5*A*). Among subjects with *ALDH2*1 /*2*, the increase in Anesthetic [$F(2, 20) = 5.24$, $p = 0.015$], Dynamic Peripheral [$F(2, 22) = 4.09$, $p = 0.031$], and General [$F(2, 18) = 4.84$, $p = 0.021$] subscale scores following alcohol administration was greater in the T-allele carriers than in the C-allele homozygotes (Fig.

5*B*). Without regard to *ALDH2* genotype, all participants showed no significant differences in BAES by the genotype of *GABRA2* SNP6 (Fig. 6).

Association Between GABRA2 and Acute Adaptation

All but the SNP6 had no significant association with acute adaptation (data not shown). In the *GABRA2* SNP6, subjects with *ALDH2*1/*1* showed significant association with acute adaptation in Dynamic Peripheral [$F(2, 72) = 6.39$, $p = 0.003$] subscale of SS and Stimulant [$F(2, 70) = 4.01$, $p = 0.023$] and Sedative [$F(2, 69) = 5.49$, $p = 0.006$] subscales of BAES. By contrast, there were no significant effects of the SNP6 on the acute adaptation in those with *ALDH2*1/*2*. Significant levels of all these results are shown in Table 3.

DISCUSSION

This is the first association study of *GABRA2* polymorphisms with subjective responses to alcohol using the objective method of intravenous alcohol administration, i.e., the alcohol clamp. The most consistent finding in this study was an effect of *GABRA2* SNPs 4 to 6 (rs279869, rs279858, and rs279837) on subjective responses to alcohol in nondependent drinkers.

In the present study, subjects with 1 or more C allele at SNP4 (A allele at SNP5) and with *ALDH2*1/*2* reported greater initial responses to alcohol (as measured on the SS) compared with subjects who were homozygous for the A allele at SNP4 (G allele at SNP5). In subjects with *ALDH2*1 /*1*, however, there was no significant effect of the genotype of SNPs 4 and 5 on the initial response with the exception that SNP4 C-allele (SNP5 A-allele) carriers showed a trend for higher scores in Stimulant subscale of the BAES than SNP4 A-allele (SNP5 G-allele) homozygotes. On the other hand, subjects with the T allele at SNP6 presented greater subjective responses to alcohol than did individuals homozygous for the C allele, without regard to *ALDH2* genotype. The results for initial responses might be interpreted in the context that the role of *GABRA2* in the subjective responses to alcohol could be independent of *ALDH2*. As for the acute adaptation, only participants with *ALDH2*1/*1* reported significantly different subjective effects of alcohol according to the genotype of *GABRA2* SNP6. These results for acute adaptation can be interpreted in the following way: the effects of *ALDH2*1/*2* on the subjective responses to alcohol were so overwhelming that the effects of *GABRA2* might be masked in subjects with *ALDH2*1/*2*.

Previous association studies have shown the SNP4 A-allele, SNP5 G-allele, and SNP6 C-allele to be over-represented among subjects with alcohol dependence (Covault et al., 2004; Lappalainen et al., 2005). The risk of alcohol dependence has been associated with a low level of response to alcohol, as measured by subjective feeling of intoxication following an alcohol challenge (Bauer and Hesselbrock, 1993; Schuckit, 1984, 1994; Schuckit et al., 1996). A family history of alcohol dependence has also been associated with a diminished response to alcohol in nonalcoholics (Moss et al., 1989; Newlin and Thomson, 1990; O'Malley and Maisto, 1985; Pollock, 1992; Savoie et al., 1988; Schuckit, 1984; Schuckit et al., 2000). Monozygotic twins show greater similarity in sensitivity to an alcohol challenge than do dizygotic twins (Heath and Martin, 1992; Martin et al., 1985; Viken et al., 2003), providing evidence that alcohol sensitivity is an inherited trait. In view of this, our findings are consistent with the hypothesis that the risk of alcohol dependence associated with the A allele at SNP4, the G allele at SNP5, and the C allele at SNP6 in 2 population studies (Covault et al., 2004; Lappalainen et al., 2005) may, in part, be mediated by the decreased subjective responses to alcohol in individuals homozygous for these alleles.

Our results indicate that individuals with 1 or 2 copies of the more common SNP4 C-allele, SNP5 A-allele, or SNP6 T-allele experience a greater initial response after their BrAC rises

to the target level of 50 mg%. It is possible that, because of these greater initial subjective responses, such individuals may be less likely to continue drinking. On the other hand, individuals homozygous for the alcohol dependence–associated A allele at SNP4, the G allele at SNP5, or the C allele at SNP6 experience a lower initial response after their BrAC rises. Thus, these individuals may need to drink more alcohol to achieve a comparable level of alcohol-induced subjective response and may be more likely to drink if given access to alcohol. However, studies are needed to determine the effects of *GABRA2* alleles on drinking behavior as it occurs in natural settings or using self-administration paradigms.

Although genotypic differences at SNPs in the *GABRA2* gene are correlated with differences in subjective responses to alcohol, the functional effects of the allelic variation at *GABRA2* are not understood (Covault et al., 2004; Dick et al., 2006; Edenberg et al., 2004; Lappalainen et al., 2005; Soyka et al., 2008). To our knowledge, no common functional coding sequence variants have been described in the *GABRA2* gene. The SNPs 4 and 6 are located in introns 6 and 3 of *GABRA2*, respectively; thus, we know that these are not coding SNPs. The SNP5 is located in exon 5 of *GABRA2*, but this type of SNP is known as a silent mutation. There are no additional data on possible functions of these SNPs. It is unclear whether these particular SNPs are directly involved in the subjective responses to alcohol or whether the SNPs are in linkage disequilibrium with the actual variant in *GABRA2* that causes differences in the alcohol effects. Therefore, additional research is required to elucidate the potential mechanism by which the gene influences the subjective responses to alcohol.

One of the major strengths of this study is the use of the alcohol clamp as the method of alcohol administration. The alcohol clamp minimizes the experimental variance in the brain's exposure to alcohol and makes it possible to maintain long intervals at a target concentration that is the same for all subjects (O'Connor et al., 1998). By avoiding uncertainties associated with gastric emptying and absorption, the method allows the clamp to be established within 20 minutes after beginning ethanol infusion (Ramchandani et al., 1999a). Other strengths of the study include the choice of various SNPs within *GABRA2* to predict the responses to alcohol based on findings from recent association studies (Covault et al., 2004; Edenberg et al., 2004; Lappalainen et al., 2005) that yielded highly convergent results of an allelic association with alcohol dependence. In addition, the relatively large number of subjects that participated in this experiment compared with previous study (Pierucci-Lagha et al., 2005) made it possible to examine the separate effects of the alleles by using all 3 genotype groups to examine the responses to alcohol.

The results of our study must nonetheless be viewed in the context of the study's limitations. Although the instruments that were chosen are used widely to evaluate alcohol-induced subjective effects, the primary outcome measures used in this study were based on participants' self-report. Subsequent studies of the impact of *GABRA2* on behavioral measures of alcohol's effects in humans should also include physiologic measures that are sensitive to alcohol effects, such as static ataxia, and neuropsychological tests by which changes in cognitive function can also be assessed. Another limitation of the study could be that we included only Japanese subjects; therefore, we should be careful to generalize these findings to other ethnic groups. Despite these limitations, the results of this study suggest an important association between the subjective responses to alcohol and *GABRA2* polymorphisms. These findings provide further evidence for the role of *GABRA2* in the subjective effects of alcohol and in the development of alcohol use disorders.

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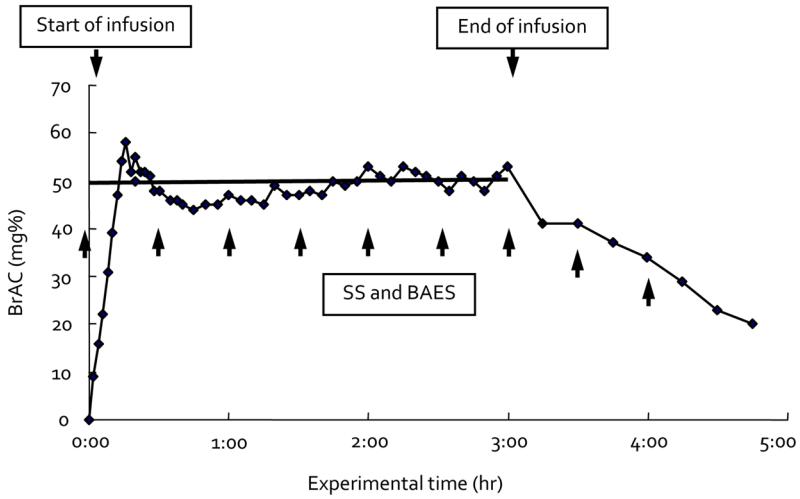

Fig. 1.

Representative sample of breath alcohol concentration clamping paradigm. SS, Sensation Scale; BAES, Biphasic Alcohol Effects Scale.

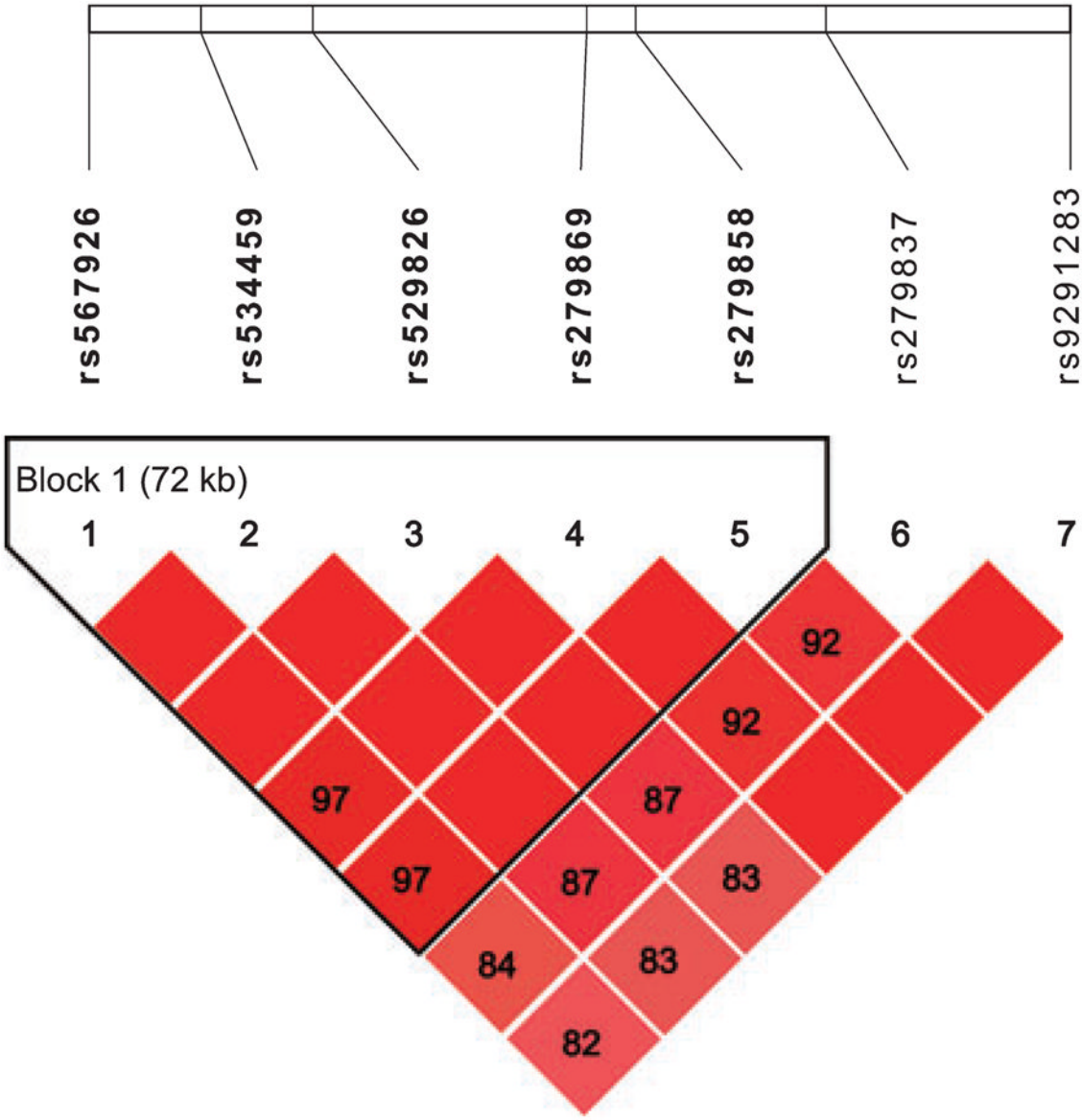

Fig. 2.

Pattern of linkage disequilibrium (LD) within *GABRA2* identified by SNPs 1 to 7. The numbers on the top correspond to each SNP as named in Table 2. Each number in the diamonds represents LD (D′) values for the respective SNP pairs. ◆, Absolute LD ($D' = 1$); 97, $D' = 0.97$ between SNPs 1 and 4.

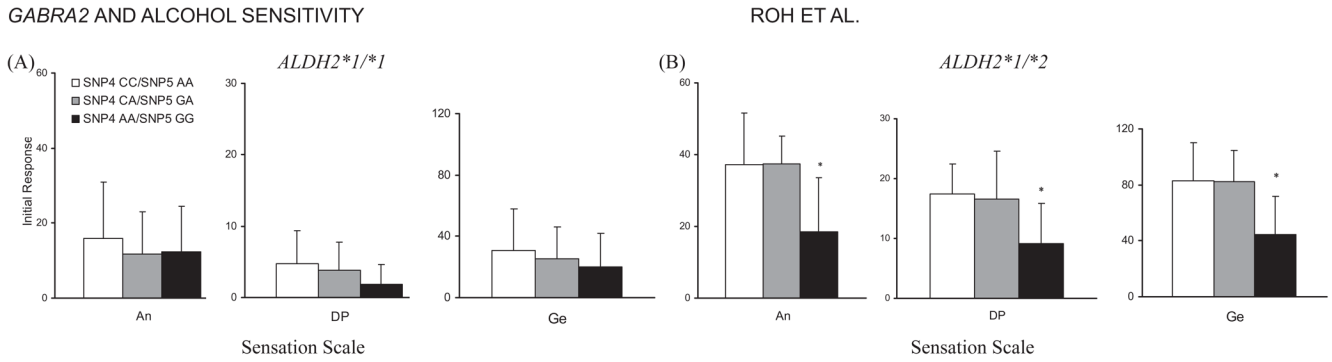

Fig. 3.

Estimated mean (SD) for the initial response measured by Sensation Scale by genotypes of *ALDH2* and *GABRA2* SNPs 4 and 5. (**A**) Among subjects with *ALDH2*1 /*1*, initial responses did not differ by the genotype of *GABRA2* SNPs 4 and 5. (**B**) Among subjects with *ALDH2*1 /*2*, those with 1 or 2 copies of the SNP4 C-allele (SNP5 A-allele) of *GABRA2* showed significantly more increased initial responses in Anesthetic ($p = 0.015$), Dynamic Peripheral ($p = 0.030$), and General ($p = 0.021$) subscales than those homozygous for the SNP4 A-allele (SNP5 G-allele). Asterisks indicate significant differences ($p < 0.05$). An, Anesthetic subscale; DP, Dynamic Peripheral subscale; Ge, General subscale.

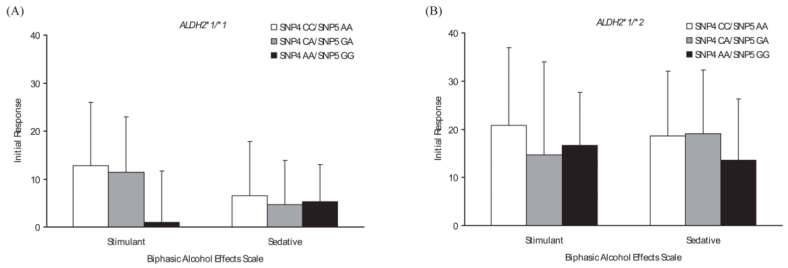

Fig. 4.

Estimated mean (SD) for the initial response measured by Biphasic Alcohol Effects Scale by genotypes of *ALDH2* and *GABRA2* SNPs 4 and 5. (**A**) Among subjects with *ALDH2*1 / *1*, there was a trend for higher initial responses in Stimulant ($p = 0.071$) subscale among the SNP4 C-allele (SNP5 A-allele) carriers than the SNP4 A-allele (SNP5 G-allele) homozygotes. (**B**) Among subjects with *ALDH2*1 /*2*, there were no significant differences in the initial responses by the genotypes of *GABRA2* SNPs 4 and 5.

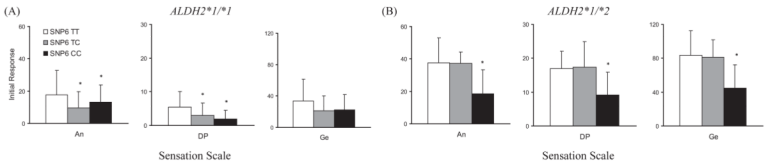

Fig. 5.

Estimated mean (SD) for the initial response measured by Sensation Scale by genotypes of *ALDH2* and *GABRA2* SNP6. (**A**) Among subjects with *ALDH2*1 /*1*, there were significant differences in Anesthetic ($p = 0.033$) and Dynamic Peripheral ($p = 0.016$) subscale scores among the SNP6 TT, TC, and CC genotypes. (**B**) Among subjects with *ALDH2*1 /*2*, those with 1 or 2 copies of the SNP6 T-allele of *GABRA2* showed significantly more increased initial responses in Anesthetic ($p = 0.015$), Dynamic Peripheral ($p = 0.031$), and General ($p = 0.021$) subscales than those homozygous for the SNP6 C-allele. Asterisks indicate significant differences ($p < 0.05$). An, Anesthetic subscale; DP, Dynamic Peripheral subscale; Ge, General subscale.

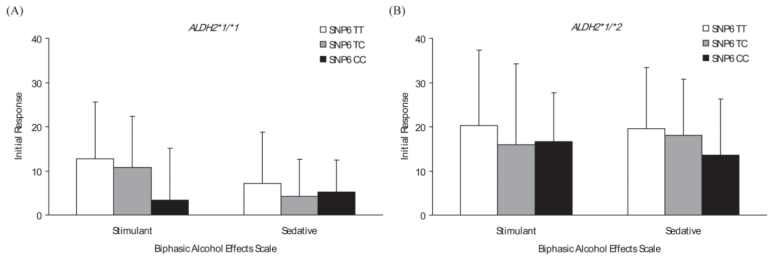

Fig. 6.

Estimated mean (SD) for the initial response measured by Biphasic Alcohol Effects Scale by genotypes of *ALDH2* and *GABRA2* SNP6. (**A**) Among subjects with *ALDH2*1 /*1*, there were no significant differences in the initial responses by *GABRA2* SNP6. (**B**) Among subjects with *ALDH2*1 /*2*, there were also no significant differences in the initial responses by the genotype of *GABRA2* SNP6.

Table 1

Demographic and Drinking Characteristics of Subjects

	Prevalence (%)	
Variables	**Male (*n* = 53)**	**Female (*n* = 57)**
Gender	48.2	51.8
Age [Mean (SD), years][a]	36.8 (10.5)	36.7 (10.6)
Family history of alcohol problems[a]		
Alcohol abuse	5.6	8.3
Alcoholic liver disease	1.9	3.3
Alcohol dependence	3.7	1.7
Drinking frequency[a]		
1–3/month	30.6	48.0
1–2/week	16.7	28.0
3–4/week	25.0	16.0
5–6/week	11.1	4.0
Everyday	16.7	4.0
Amounts of alcohol consumption per occasion[b]		
<10 g	0.0	13.0
<30 g	55.6	58.7
<40 g	2.8	6.5
<50 g	22.2	17.4
<60 g	0.0	4.4
≤100 g	8.3	0.0
>100 g	11.1	0.0

[a]No significant differences between gender groups.

[b]There were significant differences between gender groups ($p = 0.013$).

Table 2

Genotype Frequencies of *ALDH2* and *GABRA2* SNPs

Gene	SNP name (this study)	AB[a] assay ID	NCBI[b] SNP[c] reference ID	Genotype	Frequency (%)
ALDH2[d]				*ALDH2*1/*1:ALDH2*1/*2*	76.4:23.6
GABRA2[e]	1	7537087	rs567926	TT:TC:CC	57.8:31.4:10.8
	2	8262855	rs534459	TT:TC:CC	9.2:32.1:58.7
	3	1836784	rs529826	GG:GA:AA	9.4:30.2:60.4
	4	8262927	rs279869	CC:CA:AA	41.4:43.3:15.4
	5	2073557	rs279858	GG:GA:AA	15.4:43.3:41.4
	6	8263070	rs279837	TT:TC:CC	39.5:44.0:16.5
	7	8262290	rs9291283	TT:TC:CC	0.9:7.3:91.7

[a] Applied Biosystems.

[b] National Center for Biotechnology Information.

[c] Single nucleotide polymorphism.

[d] Aldehyde dehydrogenase 2 gene.

[e] γ-Aminobutyric acid A receptor α2 subunit gene.

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Table 3

Significance Levels[a] of Association Between the SNP6 in *GABRA2* and Acute Adaptation by *ALDH2* Genotype

	ALDH2*1 /*1	ALDH2*1 /*2
Sensation scale		
Anesthetic	0.067	0.579
Central stimulant	0.093	0.483
Impaired function	0.063	0.355
Warmth/glow	0.566	0.055
Dynamic peripheral	0.003*	0.284
General	0.203	0.730
Biphasic alcohol effects scale		
Stimulant	0.023*	0.092
Sedative	0.006*	0.657

[a]Significance levels were expressed in *p*-value by repeated measures ANOVA.

Significant *p*-values are indicated with an asterisk.