Genes, Brain and Behavior (2008) **7:** 447–454

Human γ-aminobutyric acid A receptor α2 gene moderates the acute effects of alcohol and brain mRNA expression

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Received 2 May 2007, revised 24 October 2007, accepted for publication 28 October 2007

γ-aminobutyric acid A ($GABA_A$) receptors moderate several of the behavioral effects of alcohol. In fact, recent studies have shown an association between the gene for the α2-subunit of the $GABA_A$ receptor (*GABRA2*) and alcoholism. In the present study, we examined the functional relevance of the *GABRA2* gene in alcohol dependence by assessing brain *GABRA2* mRNA and $GABA_A$ α2-subunit protein levels in post-mortem prefrontal cortical tissue collected from control and alcohol-dependent individuals. In addition, using an endophenotype approach, we tested whether the *GABRA2* gene moderates sensitivity to the acute effects of alcohol in two independent samples from distinct human alcohol challenge studies. Results indicated that *GABRA2* mRNA levels significantly differed by *GABRA2* genotype. *GABRA2* single nucleotide polymorphisms (rs573400, rs279871 and rs279858) were significantly associated with sensitivity to the acute effects of alcohol. Specifically, there was a significant main effect of *GABRA2* × breath alcohol concentration on several measures of subjective responses to alcohol, including the hedonic value of alcohol. Importantly, reanalysis of a previous intravenous alcohol administration study confirmed the results of the oral alcohol challenge study. In summary, these results extend previous findings and provide new insights into the putative biobehavioral mechanisms that may moderate the association between the *GABRA2* gene, sensitivity to the acute effects of alcohol and ultimately alcohol dependence.

Keywords: Alcohol, alcoholism, endophenotype, GABA, GABRA2, haplotype, mRNA, polymorphism, SNP

[1]This author was an Outstanding Young Investigator Awardee, 8th Annual Meeting of the International Behavioural and Neural Genetics Society, Vancouver, BC, Canada, May 19–22, 2006.

Alcohol dependence is a complex, polygenic disease influenced by both genetic and environmental factors (Enoch & Goldman 2001; Han *et al.* 1999; Kendler *et al.* 1994; Prescott & Kendler 1999). Until recently, advances in our knowledge of genes associated with alcohol dependence have been slow to materialize. However, with the advent of high-throughput genotyping platforms, the identification of specific genes underlying alcohol dependence is now possible (for review, see Dick & Bierut 2006).

γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter within the central nervous system, mediates several of the behavioral effects of alcohol, including lack of motor coordination, sedation, anxiolysis, tolerance, ethanol preference and symptoms of withdrawal (Buck 1996; Grobin *et al.* 1998; Korpi *et al.* 1998). Initial evidence that $GABA_A$ receptors may contribute to alcohol dependence was provided when two genome-wide linkage studies found a linkage peak for alcohol dependence on chromosome 4p, positioned near a cluster of $GABA_A$ subunit genes (Long *et al.* 1998; Reich *et al.* 1998). Since then, several research groups have found similar linkage patterns for alcohol dependence/addiction (Foroud *et al.* 2000; Uhl *et al.* 2001; Uhl *et al.* 2002; Zinn-Justin & Abel 1999).

A fine-mapping study by Edenberg *et al.* (2004) examined 69 single nucleotide polymorphisms (SNPs) that spanned a cluster of $GABA_A$ receptor subunits (*GABRG1*, *GABRA2*, *GABRA4* and *GABRB1*) and found a three-SNP haplotype within *GABRA2* to be highly associated with electroencephalogram activity and alcohol dependence. These findings were replicated and extended by Covault *et al.* (2004) and others (Dick & Bierut 2006; Drgon *et al.* 2006; Enoch *et al.* 2006; Fehr *et al.* 2006; Lappalainen *et al.* 2005). Interestingly, Enoch *et al.* (2006) recently showed that the G allele (rs279858) may not be directly associated with alcoholism and that anxiety moderates this association (Table 1).

Sensitivity to alcohol represents an established endophenotype for alcohol dependence and has been used to identify candidate genes that may contribute to the development of alcohol-use disorders (Hutchison *et al.* 2001; Hutchison *et al.* 2002; Lappalainen *et al.* 2005; Ray & Hutchison 2004). Alcohol sensitivity is a strong endophenotype as it is narrowly defined, is readily identifiable and gives rise to continuous quantifiable traits, which lend more power to detecting an effect than categorical variables (Gottesman & Gould 2003;

doi: 10.1111/j.1601-183X.2007.00369.x

Table 1: *GABRA2* risk allele discrepancies across all studies for SNP rs279858

		Alcoholic genotype			Control genotype		
Authors	Ethnicity	AA	AG	GG	AA	AG	GG
Edenberg *et al.* 2004*,†,‡	European American	0.37	0.49	0.15			
Covault *et al.* 2004§	European American	0.29	0.50	0.21	0.37	0.46	0.16
Lappalainen *et al.* 2005§	Russian	0.25	0.52	0.23	0.38	0.48	0.15
Fehr *et al.* 2006§	German	0.31	0.49	0.20	0.45	0.41	0.14
Enoch *et al.* 2006*	Finnish men	0.44	0.38	0.18	0.34	0.50	0.16
Enoch *et al.* 2006§,¶	Plains Indian men	0.14	0.43	0.43	0.06	0.62	0.32

*A allele associated with alcohol dependence.

†Family-based association study.

‡Edenberg and Foroud (2006), AA is actually GG when correcting for strand of gDNA.

§G allele associated with alcohol dependence.

¶Trend, $P = 0.097$, 2df.

Hines *et al.* 2005; Hutchison *et al.* 2002). Recently, Pierrucci-Lagha *et al.* (2005) used an endophenotype approach and showed that variation within the *GABRA2* gene moderated the acute subjective effects of alcohol.

In light of the existing literature, the present study applied a translational approach to elucidate the biological mechanism underlying the behavioral endophenotype of alcohol sensitivity by addressing these aims: (1) to determine the functional relevance of the *GABRA2* gene by post-mortem brain *GABRA2* mRNA and α2-subunit protein analyses and (2) to examine whether SNPs within the *GABRA2* gene, selected *a priori* on existing research, influence the acute subjective effects of alcohol. Our results seek to extend previous findings and examine the putative biobehavioral mechanisms by which the *GABRA2* gene may moderate sensitivity to the acute effects of alcohol.

Methods

Post-mortem brain tissue GABRA2 *mRNA analysis*

Prefrontal cortex (PFC; region BA 10) was obtained from the Australian Brain Donor Programs NSW Tissue Resource Centre, which is supported by The University of Sydney, National Health and Medical Research Council of Australia, Neuroscience Institute of Schizophrenia and Allied Disorders, National Institute of Alcohol Abuse and Alcoholism and NSW Department of Health. Quantitative real-time polymerase chain reaction (PCR) was performed using an ABI-7500 thermocycler and TaqMan reagents (ABI, Foster City, CA, USA), in 96-well plates according to the manufacturer's recommendations, except that the final reaction volume was 10 μl. Both *GABRA2* and *GAPDH* (control gene) expression assays were purchased from ABI (Assay ID: Hs00168069_m1 and PN 4310881E, respectively). All experiments were repeated two to three times for quality assurance (for details, please see Appendix S1.).

Post-mortem PFC GABA$_A$ α2-subunit protein levels

Western blotting

Protein was extracted from the PFC (BA9 and BA10) for the purposes of determining relative levels of the GABAα_2 subunit (for details please see Appendix S1).

Human laboratory studies and GABRA2 *SNPs*

Participants

Behavioral data from two distinct alcohol administration studies were analyzed for the present report. The University of Colorado Human Research Committee approved both studies, and all participants provided written informed consent after receiving a full explanation of the study. All participants were moderate-to-heavy drinkers [three or more drinks (two drinks for females) per occasion at least twice per week] and were recruited primarily from a college setting. Participants also scored ≥8 in the alcohol use disorders identification test (Allen *et al.* 1997), indicating a hazardous drinking pattern. Individuals who were trying to quit or had sought treatment for alcohol problems in the past were excluded from this study and offered appropriate treatment referrals. Participants completed either the oral alcohol ($n = 75$) or the intravenous (i.v.) alcohol ($n = 47$) administrations. For participant recruitment details, please see Appendix S1.

Overview of experimental procedures

In the oral alcohol study, all participants completed a two-session alcohol challenge, one in which they received alcohol and another in which they received a matched placebo. The sessions were randomized, participant-blind and separated by at least 1 week. The i.v. alcohol study used a one-session design. Data from the i.v. alcohol challenge represent a reanalysis of a previously published study of sensitivity to alcohol and the *OPRM1* gene (Ray & Hutchison 2004). This previous study is reported here as a replication sample. For a detailed description of the laboratory procedures performed during the *IV Alcohol Administration* and the *Oral Alcohol Administration Paradigms*, please see Appendix S1.

Outcome measures: subjective effects of alcohol

During both the alcohol administration studies, the following outcome measures of alcohol sensitivity and urge to drink were administered at baseline (i.e. prealcohol) and at each of the following three target breath alcohol concentrations (BrACs): 0.02, 0.04 and 0.06. Target BrACs were identical in both studies.

The short version of the *Profile of Mood States* (POMS) was used to collect information on mood changes. The POMS is a reliable and valid measure of various mood states, including positive mood, negative mood, tension and vigor (Johanson & Uhlenhuth 1980; McNair *et al.* 1971).

Alcohol urge questionnaire. The *alcohol urge questionnaire* (AUQ) (Bohn *et al.* 1995) was used to assess urge to drink. The AUQ consists of eight items related to urge to drink that are rated on a 7-point Likert scale with the extremes anchored by *Strongly Disagree*

and *Strongly Agree*. The AUQ has shown good internal consistency and reliability.

Biphasic alcohol effects scale. The biphasic alcohol effects scale (BAES) (Martin *et al.* 1993) was used to collect information on changes in self-reported stimulation and sedation after alcohol administration. The BAES has previously shown reliability and validity in investigations of the effects of alcohol (Erblich & Earleywine 1995).

DNA extraction and genotyping

Cheek swabs were collected and genomic DNA extracted following published procedures (Freeman *et al.* 1997; Hutchison *et al.* 2002; Walker *et al.* 1999). Genomic DNA was also extracted from post-mortem PFC tissue using the QIAzol (Qiagen, Valencia, CA, USA). The *GABRA2* TaqMan® SNP Assays (rs573400, rs279871, rs279858, rs2119767 and rs1372472) were purchased from ABI and assessed using the ABI-7500 thermocycler (Livak 1999 for description of the TaqMan assay).

Data analysis

For both alcohol studies, analyses of the effects of *GABRA2* were conducted using a 3 (rs279858 genotype: AA vs. AG vs. GG) by 3 (drink: drink 1 vs. drink 2 vs. drink 3; oral study) or (BrAC: 0.02 vs. 0.04 vs. 0.06; i.v. study) mixed-design ANOVA with repeated measures on the drink/BrAC variable. In the oral study, the dependent variable was a difference score calculated by subtracting the placebo beverage score from the alcohol beverage score for each of the three drinks. The dependent measures examined were alcohol-induced changes in mood, stimulation, sedation and the hedonic effects of alcohol (e.g. 'liking' of the alcohol exposure). A univariate general linear model was used to test for *GABRA2* mRNA and protein-level differences. pH was used as a covariate in the mRNA analysis. SAS version 9.1 was used to conduct all analyses.

Results

GABRA2 *gene, SNPs evaluated and linkage disequilibrium map*

As shown in Fig. 1a, the *GABRA2* gene is located on chromosome 4p12, in a cluster of three other $GABA_A$ receptor subunit genes. Figure 1b graphically illustrates the linkage disequilibrium (LD) of each SNP investigated within this study. As shown in Fig. 1b, the five SNPs evaluated fall into two distinct haplotype blocks. In addition, within this study, SNPs within each haplotype block, block 1: SNPs 1, 2 and 3 and block 2: SNPs 4 and 5, were found to be in complete LD with each other. No significant effects were observed for the *GABRA2* block 2 SNPs on any measure of sensitivity to alcohol. In contrast, block 1 SNPs were found to moderate several of the alcohol-induced effects on mood and on the hedonic value of alcohol. Because SNPs 1–3 were found to be in complete LD (genotype at SNP 1 predicted genotype at SNP 2 and 3 100% of the time), we have chosen to graphically illustrate the results of *GABRA2* SNP 3 (rs279858) for all levels of analyses. In addition, the participants in the i.v. alcohol study, the replication sample, were only genotyped for SNP 3. All SNPs analyzed were found to be in Hardy–Weinberg equilibrium. Hardy–Weinberg equilibrium for each SNP and LD between the SNPs were analyzed using the HAPLOVIEW 3.32 software (http://www.broad.mit.edu/mpg/haploview/) (Barrett *et al.* 2005). HAPLOVIEW was also used to generate the *GABRA2* LD map shown in Fig. 1b.

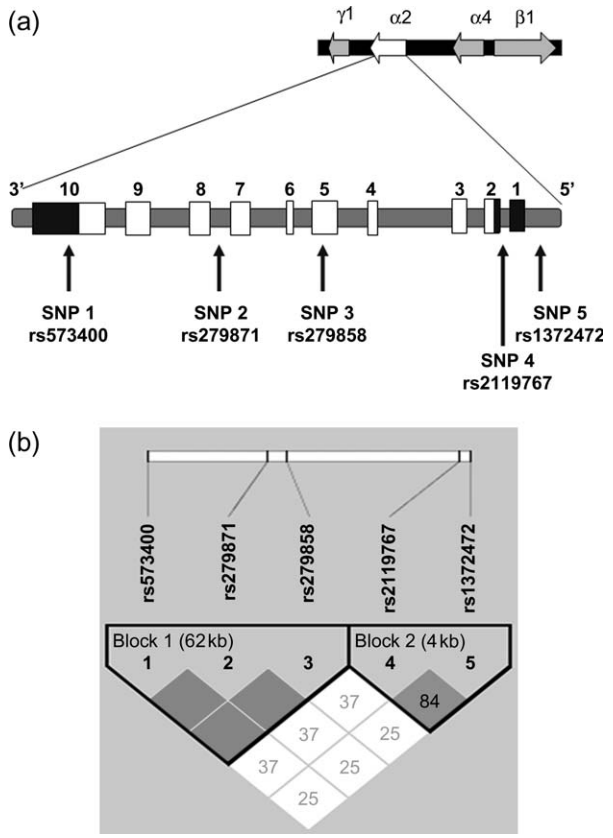

Figure 1: The *GABRA2* gene. (a) Schematic of the human *GABRA2* gene structure highlighting the positions of the five SNPs investigated within this study. Boxes are exons, and gray shaded areas are untranslated regions. (b) Linkage disequilibrium structure of the *GABRA2* gene using HAPLOVIEW 3.32 software; dark boxes denote high LD, whereas white denotes low or no LD.

Quantification of post-mortem GABRA2 *mRNA levels*

To examine the possibility that differences in *GABRA2* mRNA levels were moderating the association between the *GABRA2* gene and alcohol dependence, we obtained post-mortem PFC tissue samples from 20 age-matched alcoholic (AD) and 20 control (C) male individuals. Real-time reverse transcriptase-PCR analysis showed no significant differences in *GABRA2* mRNA levels between alcoholic and control samples within the PFC (data not shown). There was, however, a significant main effect of *GABRA2* genotype on PFC *GABRA2* mRNA levels [$F_{2,35} = 4.68$, $P < 0.02$] while using pH as a covariate in the analysis of variance. Of the 40 samples analyzed, three samples failed to amplify and as such were excluded from the analysis. The genotyping (SNP 3) results of the 37 samples that did amplify indicated that 10 (5 AD and 5 C) individuals were AA, 20 (11 AD and 9 C) were AG and 7 (5 AD and 2 C) were GG. Analyses indicated that the

AA genotype was associated with significantly greater mRNA levels in the PFC as compared to the AG genotype (Fig. 2a).

Quantification of post-mortem GABA$_A$ α2-subunit protein levels

To determine whether the observed *GABRA2* mRNA-level differences were indicative of differences in GABA$_A$ α2-subunit protein levels, a western blot analysis was performed using 10 alcoholic and 10 controls from the above post-mortem PFC tissue samples. Because of the limited quantity of tissue obtained from the Australian Brain Bank, we were only able to extract protein from a subset of the samples used in the mRNA study, from 10 controls and 10 alcohol-dependent patients; of these, only 18 samples were quantifiable. The genotyping results indicated that of the 18 samples analyzed, 5 (4 AD and 1 C) individuals were AA, 8 (4 AD and 4 C) were AG and 5 (4 AD and 1 C) were GG. Analyses indicated that there were no significant differences in PFC GABA$_A$ α2-subunit protein levels by *GABRA2* genotype (Fig. 2b). In addition, no group by *GABRA2* α2-subunit protein-level differences were observed (data not shown).

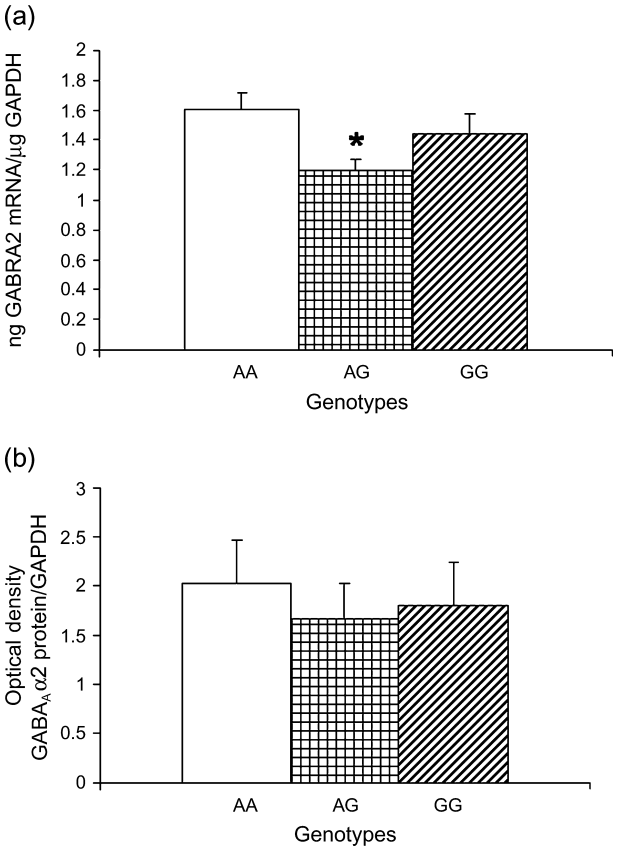

Figure 2: Post-mortem PFC *GABRA2* mRNA levels (a) and GABA$_A$ α2-subunit protein levels (b) by *GABRA2* rs279858 genotype. Data from two independent experiments with determinations in 10 AA, 19 AG and 7 GG individuals are expressed as ng *GABRA2*/μg GAPDH in (a) and determinations in 5 AA, 8 AG and 5 GG individuals expressed as optical density of GABA$_A$ α2-subunit protein/GAPDH in (b). Columns represent the means and vertical lines 1 SEM. *Value for AG individuals that is significantly different from AA individuals ($P < 0.02$).

Oral alcohol study

Pretest comparisons

The first set of analyses examined differences among genotype (AA, AG and GG) groups on baseline demographics and alcohol/drug-use variables that might confound the main analyses. As depicted in Table 2, there were no differences in self-reported ancestry, age, gender or quantity of alcohol use in the last 30 days. There were no differences in BrAC by genotype ($P > 0.05$).

GABRA2 *and sensitivity to alcohol*

A significant main effect of genotype on the POMS positive mood subscale ($F_{2,69} = 3.17$, $P < 0.04$) and a genotype × drink interaction ($F_{4,69} = 2.74$, $P < 0.04$) were also found. As shown in Fig. 3a, GG individuals reported significantly greater alcohol-induced increases in positive mood across alcoholic drinks as compared to the AG individuals. A significant main effect of genotype on the POMS subscale of vigor was found, such that GG and AA individuals reported significantly greater vigor after each drink of alcohol as compared to the AG individuals ($F_{2,69} = 3.37$, $P < 0.048$; Fig. 3b). There was neither a main effect of *GABRA2* nor a genotype × drink interaction for the tension or depression subscales of the POMS as well as the BAES.

Intravenous alcohol study

Participant characteristics

Demographic information and pretest comparisons are presented in Table 3. The first set of analyses tested for differences among AA, AG and GG groups on baseline demographics and alcohol-use variables that might confound the main analyses. There were no differences among the three genotype groups on any of the demographic or drinking variables assessed.

GABRA2 *and sensitivity to alcohol*

As shown in Fig. 4a, there was a trend toward a significant genotype × BrAC interaction ($F_{2,47} = 2.30$, $P < 0.057$) on the POMS subscale of vigor, such that GG and AA individuals reported significantly greater vigor across rising levels of BrAC as compared to the AG individuals. There was neither a main effect nor a genotype × BrAC interaction for *GABRA2* on the POMS subscales of tension, depression or positive mood. A significant genotype × BrAC interaction on alcohol-induced stimulation, measured by the BAES, was found, such that GG individuals reported higher increases in alcohol-induced stimulation across BrACs as compared to AG ($F_{2,47} = 3.71$, $P < 0.01$; Fig. 4b). No significant effects of

Table 2: Pretest differences of demographic and drinking characteristics by *GABRA2* genotype groups in the oral alcohol study

Variable	AA ($n = 21$)	AG ($n = 35$)	GG ($n = 19$)	Test for the difference
Gender (% male)	81	66	68	$\chi^2(2)$ 1.5; $P = 0.46$
Race (% Caucasian)	86	97	100	$\chi^2(2)$ 4.8; $P = 0.09$
Age (years)	22.4	21.8	21.1	$F_{2,74} < 1.0$; $P = 0.38$
Alcohol problems (RAPI) (0–69)	26.6	22.7	25.1	$F_{2,74} < 1.0$; $P = 0.54$
Average number of drinks per occasion in past 30 days	9.95	8.32	7.67	$F_{2,74} = 1.4$; $P = 0.25$

GABRA2 on self-reported feelings of alcohol-induced sedation were observed. As shown in Fig. 4c, a significant main effect of genotype on the hedonic value of the alcohol was observed for the item 'Overall, how pleasant was the exposure to alcohol?' Specifically, GG individuals reported greater 'liking' of the exposure to alcohol across rising BrAC levels, as compared to the AG and AA individuals ($F_{2,47} = 3.25$, $P < 0.05$). Lastly, we controlled for drinking variables such as Rutgers Alcohol Problem Index (RAPI) and drinks per episode and doing so did not change any of the results reported herein.

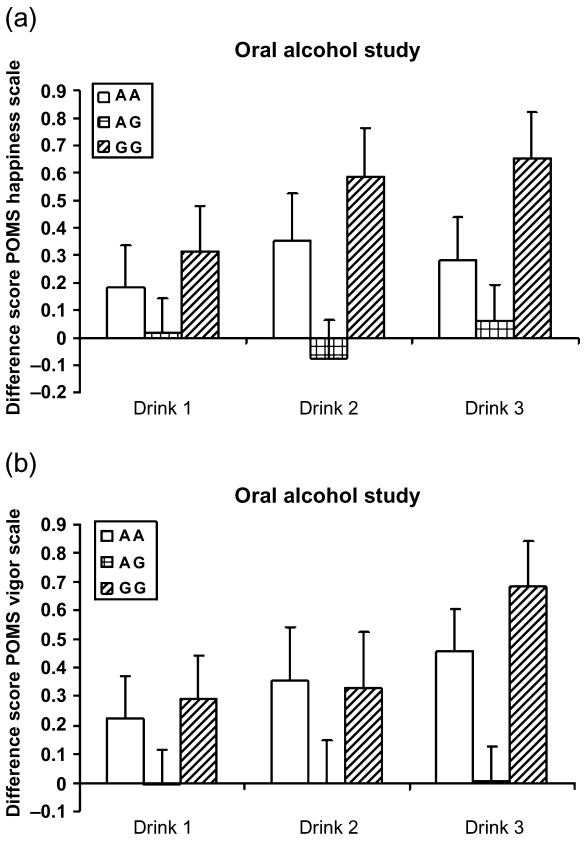

Figure 3: Sensitivity to oral alcohol by *GABRA2* genotype. Individuals with the GG genotype show significantly greater happiness (a) and greater vigor (b) after alcohol consumption.

Discussion

This study combined molecular and behavioral human laboratory approaches to examine the functional relevance underlying the association of the *GABRA2* gene with alcohol dependence and to determine the gene's influence on sensitivity to the acute effects of alcohol. Importantly, this study examined the functional significance of the genotypes tested for their association with the laboratory endophenotypes. The major findings of this study were the following: (1) levels of PFC α2-subunit mRNA and protein did not differ between alcohol-dependent and control individuals, (2) PFC α2-subunit mRNA levels differed between *GABRA2* genotypes, (3) sensitivity to the acute effects of alcohol was moderated by the *GABRA2* gene and (4) these findings were replicated by reanalyzing data from an i.v. alcohol-administration study (Ray & Hutchison 2004).

Although the recent literature has consistently shown an association between the *GABRA2* gene and alcohol dependence, the functional SNP(s) within the *GABRA2* loci have yet to be identified and the biological mechanisms underlying this association have yet to be elucidated. Our first aim was to determine whether the association of alcohol dependence with the *GABRA2* gene could be because of alterations in receptor function caused by changes in gene transcription and/or translation. The preliminary findings indicate that within the PFC, *GABRA2* mRNA levels significantly differed by *GABRA2* genotype (AA > AG). However, the difference in expression level was less than twofold and should, therefore, be interpreted with caution. In contrast, there were no differences in PFC α2-subunit protein levels by *GABRA2* genotype. However, the pattern of results mimicked those of the mRNA data, suggesting that perhaps with a larger sample size, these results may have reached significance. One speculative interpretation of these results could be that $GABA_A$ receptor subunit assembly/function is predetermined by *GABRA2* genotype. Because of the small sample size of these analyses, and given the difficulty in obtaining post-mortem brain tissue, the present findings clearly await replication. Moreover, these results illustrate the need to systematically examine all brain regions implicated within the drug reward/addiction pathway to more adequately examine the complex nature of the consequences of long-term alcohol abuse.

The second aim of this study was to determine whether a series of candidate SNPs within the *GABRA2* gene, selected *a priori* based on existing research, influence the acute subjective effects of alcohol. The present results confirm the nature of the *GABRA2* haplotype block structure

Table 3: Pretest differences of demographic and drinking characteristics by *GABRA2* genotype groups in the i.v. alcohol study

Variable	AA ($n = 11$)	AG ($n = 25$)	GG ($n = 11$)	Test for the difference
Gender (% male)	54.6	52.0	54.6	$\chi^2(2) < 1.0$; $P = 0.98$
Race (% Caucasian)	90.9	95.8	90.9	$\chi^2(2) < 1.0$; $P = 0.99$
Age (years)	21.45	22.16	21.45	$F_{2,46} = 1.25$; $P = 0.30$
Alcohol problems (RAPI) (0–69)	23.09	21.20	20.73	$F_{2,46} < 1.0$; $P = 0.92$
Average number of drinks per occasion in past year	4.64	3.98	4.82	$F_{2,46} = 1.17$; $P = 0.32$

as described by Covault *et al.* (2004) wherein intron 3 past the 3′ region of the gene is associated with alcohol dependence. In addition, this study supports the findings of Pierrucci-Lagha *et al.* (2005) in which the *GABRA2* gene moderated sensitivity to the acute effects of alcohol. The results of our current study indicate that GG and AA individuals reported greater alcohol-induced positive mood and feelings of vigor after an oral alcohol challenge as compared to AG individuals. In support of these findings, a similar pattern of results was found for the i.v. alcohol infusion study, such that, over rising BrAC levels, GG individuals reported feeling greater stimulation, more vigor and greater 'liking' of the alcohol exposure as compared to AG individuals. This difference was most pronounced at the 0.06 level of BrAC. Taken together, these data suggest that GG and AA individuals may be more sensitive than AG individuals to the rewarding effects of alcohol, thereby incurring a higher or lower risk for developing alcoholism, respectively.

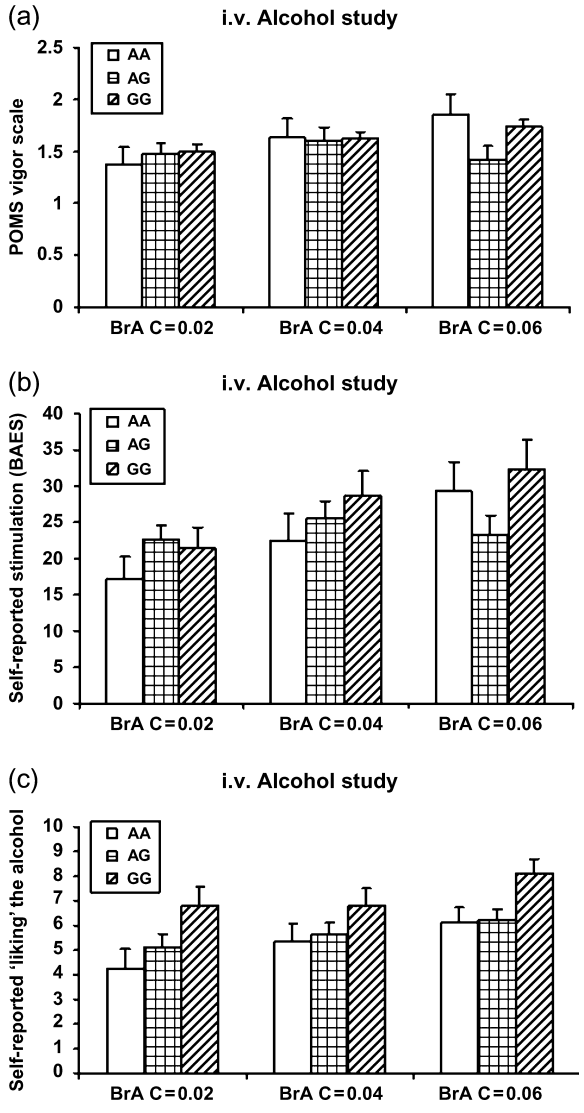

Figure 4: Sensitivity to i.v. alcohol by *GABRA2* genotype. In this replication sample, GG individuals also showed increased vigor (a), greater stimulation (b) as well as greater 'liking' of the exposure to alcohol (c).

These results are, to some extent, in agreement with Pierrucci-Lagha *et al.* (2005), who showed that AA individuals at SNP rs279858 reported greater stimulant and gastrointestinal effects of alcohol compared with AG/(GG, $n = 2$) individuals after an oral alcohol challenge. It is noteworthy that these two studies used different assessment tools and outcome measures. Therefore, a direct comparison between these studies does not seem warranted. What is most consistent between these two oral alcohol studies is the fact that the AG individuals were less responsive to an acute alcohol challenge than AA individuals were.

To date, four association studies (Covault *et al.* 2004; Edenberg *et al.* 2004; Fehr *et al.* 2006; Lappalainen *et al.* 2005) have found the G allele at SNP rs279858 confers risk to alcohol dependence. Intriguingly, however, Enoch *et al.* (2006) found that the *GABRA2* risk haplotype was opposite in two different populations (Finnish Caucasian men and Plains Indian men), was based on the common allele and was moderated by anxiety. Enoch *et al.* (2006) also found that single-SNP locus analyses showed that the association in Finns was driven by an increase in both homozygotes among alcoholics. The findings of the present study support those of Enoch *et al.* (2006) and suggest that perhaps possessing either the AA or the GG genotype at rs279858, in combination with environmental provocation (e.g. stress, anxiety and family history), may lead to an increased risk for alcoholism. Interestingly, differences in levels of anxiety within our study might help explain our findings from the oral alcohol study in which AG individuals were less responsive to alcohol on measures of mood than either AA or GG individuals. However, because neither of our studies included an anxiety measure, we are unable to assess the role of anxiety within the context of the present findings.

Importantly, the findings of the present study do not conform to a simple genetic model whereby the heterozygote group is expected to fall between both homozygote groups. To the contrary, the current findings highlight the complexity of processes such as gene expression and genetic association findings for complex phenotypes such as sensitivity to the effects of alcohol. Given that the functional SNP(s) have yet to be identified and because there is very high LD starting from intron 3 of the *GABRA2* gene that extends through the *GABRG1* gene, we cannot assume that this difference in expression is driven by a single SNP. Conversely, these results may be driven by a haplotype of SNPs, which may be additive, non-additive or the result of epistatic interactions. Furthermore, there may be underlying environmental differences between the genotypes that could also be contributing to these intriguing findings. In summary, the results of mRNA levels and behavioral outcomes do not fit a simple model of genetic inheritance and call into attention the complexity of the mechanisms underlying the GABA-mediated behavioral effects of alcohol.

In conclusion, the findings of this study extend previous studies such that the mRNA results suggest that the *GABRA2* genotype may influence innate $GABA_A$ receptor subunit assembly and function, which in turn may lead to a differential liability to alcoholism as a function of *GABRA2* genotypes. Position emission tomography (PET) studies using specific $GABA_A$ α2-subunit ligands would be very useful for determining the biological mechanism by which AG individuals show less reward than either homozygote. Based on our preliminary mRNA results, we would hypothesis that AG individuals display less reward from alcohol because they have less $GABA_A$ α2-containing receptors than either homozygote. Thus, future studies using PET to test this hypothesis are warranted. Moreover, the results of the two human laboratory endophenotype studies show that AG individuals may be less sensitive to the acute effects of alcohol. These results may be suggesting that both AA and GG individuals may be at a higher risk for the future development of alcoholism, whereas the AG genotype may in turn be protective against the disorder. An alternative explanation is that other polymorphisms within the *GABRA2* gene are driving these results by virtue of being in LD with the polymorphisms currently studied. Future longitudinal studies utilizing this endophenotype approach are needed to elucidate which individuals (more sensitive vs. less sensitive on measures of mood and hedonic value of alcohol) are at greater risk for developing alcohol dependence. Finally, studies focused on identifying the functional variant(s) within the *GABRA2* gene are needed and should ultimately inform the development of new therapeutics useful for the treatment of alcoholism.

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Acknowledgments

This research was supported by a grant from the National Institute on Alcoholism and Alcohol Abuse; AA015331 (H.H.) and AA012238 (K.H.). Tissues were received from the New South Wales Tissue Resource Centre and/or the Prince of Wales Medical Research Institute Tissue Resource Centre, which is supported by the National Health and Medical Research Council of Australia, The University of Sydney, Prince of Wales Medical Research Institute, Neuroscience Institute of Schizophrenia and Allied Disorders, National Institute of Alcohol Abuse and Alcoholism and NSW Department of Health.

Supplementary material

The following supplementary material is available for this article:

Appendix S1: *GABRA2* moderates subjective effects of alcohol.

This material is available as part of the online article from http://www.blackwell-synergy.com/doi/abs/10.1111/j.1601-183X.2007.00369.x

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