Genes, Brain and Behavior
Official publication of the International Behavioural and Neural Genetics Society

Genes, Brain and Behavior (2013) ***12:*** *525–531* doi: 10.1111/gbb.12039

Impulsiveness mediates the association between *GABRA2* SNPs and lifetime alcohol problems

S. Villafuerte [†,‡,*], V. Strumba[†,‡,§], S. F. Stoltenberg[†,‡,¶], R. A. Zucker[‡,§] and M. Burmeister[†,‡,]**

[†]Molecular & Behavioral Neuroscience Institute , [‡]Department of Psychiatry , [§]Addiction Research Center, University of Michigan, Ann Arbor, MI, USA, [¶]Behavior Genetics Laboratory, Department of Psychology, University of Nebraska, Lincoln, NE, USA, and [**]Human Genetics, University of Michigan, Ann Arbor, MI, USA

**Corresponding author: S. Villafuerte, Molecular & Behavioral Neuroscience Institute, University of Michigan, 109 Zina Pitcher Place, BSRB 5063, Ann Arbor, MI 48109–2200, USA. E-mail: svillafu@umich.edu*

Received 13 November 2012, revised 12 February 2013 and 13 March 2013, accepted for publication 25 March 2013

Genetic variants in *GABRA2* have previously been shown to be associated with alcohol measures, electroencephalography (EEG) β waves and impulsiveness-related traits. Impulsiveness is a behavioral risk factor for alcohol and other substance abuse. Here, we tested association between 11 variants in *GABRA2* with NEO-impulsiveness and problem drinking. Our sample of 295 unrelated adult subjects was from a community of families with at least one male with DSM-IV alcohol use diagnosis, and from a socioeconomically comparable control group. Ten *GABRA2* SNPs (single-nucleotide polymorphisms) were associated with the NEO-impulsiveness ($P < 0.03$). The alleles associated with higher impulsiveness correspond to the minor alleles identified in previous alcohol dependence studies. All ten SNPs are in linkage disequilibrium (LD) with each other and represent one effect on impulsiveness. Four SNPs and the corresponding haplotype from intron 3 to intron 4 were also associated with Lifetime Alcohol Problems Score (LAPS, $P < 0.03$) (not corrected for multiple testing). Impulsiveness partially mediates (22.6% average) this relation between *GABRA2* and LAPS. Our results suggest that *GABRA2* variation in the region between introns 3 and 4 is associated with impulsiveness and this effect partially influences the development of alcohol problems, but a direct effect of *GABRA2* on problem drinking remains. A potential functional SNP rs279827, located next to a splice site, is located in the most significant region for both impulsiveness and LAPS. The high degree of LD among nine of these SNPs and the conditional analyses we have performed suggest that all variants represent one signal.

Keywords: Alcohol problems, *GABRA2*, impulsiveness, mediation, single-nucleotide polymorphisms

Impulsivity has repeatedly been identified as an important determinant of alcohol use and problems (Dick *et al*. 2010; Lejuez *et al*. 2010; Zucker *et al*. 2011) (unless otherwise differentiated, we hereafter refer to 'alcohol use and problems' as alcohol involvement). According to Whiteside and Lynam (2001) trait Impulsivity is a multidimensional behavioral construct which includes at least four different component traits: urgency (impulsiveness in NEO_PI), sensation seeking, lack of premeditation and lack of perseverance. These subscales show differential relations with alcohol involvement. Specific to alcohol problems, Magid and Colder (2007) found in a group of non-abstaining undergraduates that those with both high scores on the UPPS (Whiteside *et al*. 2005) urgency subscale (impulsiveness in the NEO) and low scores on perseverance had a higher level of alcohol problems. Both the urgency scale in the UPPS impulsivity scale and the NEO-impulsiveness (Costa & Mccrae, 1992) measure impulsive behaviors under conditions of negative affect which can be motivated by a coping strategy involving use of alcohol to deal with emotional distress, often with disregard for negative consequences (Dawe *et al*. 2004).

It is possible that impulsiveness and alcohol involvement may share a common underlying biological pathway such that particular genetic variants are associated with increased risk for alcohol involvement and that this effect is mediated by impulsiveness. The identification of the genetic risk for both impulsive behavior and alcohol involvement may help to identify the biological mechanisms underlying these complex traits. We previously reported (Villafuerte *et al*. 2012) an association between *GABRA2* and impulsiveness in a sample enriched for alcoholism. Gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter in the CNS, has a role in risk for developing alcohol use disorder (AUD) through the fast-acting receptor complex, $GABA_A$ (Grobin *et al*. 1998; Krystal *et al*. 2006). Repeated alcohol exposure affects the GABA system (Grobin *et al*. 1998) through binding sites at the GABA receptors, reducing neural inhibitory action. Genetic variation in *GABRA2* has been reproducibly associated with both alcoholism (Agrawal *et al*. 2006; Bauer *et al*. 2007; Covault *et al*. 2004; Edenberg *et al*. 2004; Enoch *et al*. 2006; Fehr *et al*. 2006; Lappalainen *et al*. 2005; Lind *et al*. 2008a,b; Pierucci-Lagha *et al*. 2005; Soyka *et al*. 2008) and an electroencephalography (EEG) measure, the β frequency band (Edenberg *et al*. 2004). Alcoholics (Rangaswamy *et al*. 2002) and their at-risk

offspring (Rangaswamy *et al*. 2004) have increased power in the β frequency band (13–28 Hz). Furthermore, *GABRA2* has been associated with childhood conduct disorder symptoms (Dick *et al*. 2006) and trajectories of externalizing behavior (Dick *et al*. 2009). The single-nucleotide polymorphisms (SNPs) reported in these studies expand a region with two haplotype blocks in high linkage disequilibrium (LD) facilitating allele comparison across studies. The majority of studies on alcohol dependence or abuse (Agrawal *et al*. 2006; Bauer *et al*. 2007; Covault *et al*. 2004; Edenberg *et al*. 2004; Enoch *et al*. 2006; Fehr *et al*. 2006; Lappalainen *et al*. 2005; Lind *et al*. 2008a,b; Pierucci-Lagha *et al*. 2005; Soyka *et al*. 2008) reported the minor allele associated with the disorder.

Here we report genetic associations of eleven SNPs in *GABRA2* with both impulsiveness and the Lifetime Alcohol Problems Score (LAPS), a composite index of problem alcohol use over the life course, and carry out an analysis of the role of impulsiveness as a mediator of the relationship between *GABRA2* SNPs and LAPS. The study was carried out on the adult sub-sample of a population-based study of families which had at least one male with DSM-IV AUD diagnosis, or were from a comparable group of control families (Zucker *et al*. 2000).

Materials and methods

Subjects and assessment

The sample consisted of 295 (167 females) biologically unrelated adult subjects from the Michigan Longitudinal Study (MLS) who were genotyped for eleven *GABRA2* SNPs and for whom NEO-PI-R and the LAPS data were available. This is an ongoing multi-wave, community recruited prospective study of families of men with a drunk-driving conviction and AUD diagnosis who were living with a 3–5 year old son/daughter and the biological mother at time of recruitment (mean age 32; range 22–46 at baseline). The study began recruitment in 1985. In addition, control families without a history of substance abuse were recruited from the same or socioeconomically comparable neighborhoods. Families identified during the community canvass for controls who also had an AUD diagnosis were recruited as well (Zucker *et al*. 1996). For this study only the parents were selected as both personality traits are more stable in adulthood and alcohol problems are more evident compared to the youth population. One hundred twenty-five subjects (51 females) had a DSM-IV lifetime alcohol dependence/abuse diagnosis and 170 (116 females) did not have this diagnosis. All subjects were unrelated and represent parents and/or partners. The great majority were of Caucasian origin, with 3.0% (9) of other ethnicity (1 African American, 2 Native American and 6 Hispanic-Caucasian). All subjects were extensively assessed at 3-year intervals with behavioral and alcohol measures appropriate for age. Written informed consent was obtained from all participants after the nature of the study had been explained to them; the protocol was approved by the Institutional Review Board at the University of Michigan.

The LAPS (Zucker, 1991; Zucker *et al*. 1997) is a time-based, multi-dimensional measure of problem alcohol involvement which scales the extent to which alcoholism-related symptomatology has been salient over the life course. Lifetime Alcohol Problems Score is constructed of three component sub-scores which assess onset of first symptom (age of first drunkenness), variety of symptomatology (number of different alcohol related difficulties at any time) and life-invasiveness of the symptoms (index of life course duration of all drinking related problems from onset to present, corrected for period of risk exposure). Lifetime Alcohol Problems Score is the sum of these three (standardized) component scores. For the number of alcohol related difficulties, questions included missing school, lost friends, divorce or separation, getting fired or laid off, received ticket for drunk driving, had a car accident, kept drinking after promising to stay sober, number of times being admitted at the hospital among others. The index of life course duration of drinking related problems is the age at which these items occurred for the first and most recent times. Lifetime Alcohol Problems Score is an effective indicator to scale the extent of alcoholic risk load in preclinical stages of development for offspring, and for adults it is a metric of chronicity and severity. Measures of validity included discriminant analyses which revealed expected associations between LAPS and measures of cognitive functioning, family relationships, self-concept and temperament (Zucker, 1990; Zucker, 1991; Zucker *et al*. 1997). We used the maximum LAPS score across waves T1–T6 and used this variable for analysis.

Personality traits were assessed using the NEO-PI-R questionnaire (Costa & Mccrae, 1992) at all waves within a period of approximately 15 years. Impulsiveness is a facet from the neuroticism domain that measures the tendency to act on cravings and urges in response to distress along with later regret. As the scores on the impulsiveness facet from the neuroticism domain did not differ significantly across assessment waves, we composed a new dependent variable for the impulsiveness facet by averaging the individual scores across the different data waves (T4–T6). Subjects with Lifetime AUD diagnosis have higher impulsiveness score (16.5 ± 4.0; $P = 0.0003$) than those without AUD diagnosis (14.8 ± 3.9).

In summary, our sample consists of 295 subjects for which genotype, LAPS and impulsiveness data were available. The correlation between impulsiveness and LAPS is significant (0.193, $P = 0.001$). Also, the sample includes 110 couples (220 subjects). We found no significant correlation between husband and wife variables for both impulsiveness and LAPS. The presence of dyads does not affect standard errors in the model.

Single-nucleotide polymorphism genotyping

We used the Illumina Addiction biology SNP array designed by Hodgkinson *et al*. (Hodgkinson *et al*. 2008). The panel includes SNPs from 130 candidate genes for alcoholism, addictions and disorders of mood and anxiety and is genotyped using the Illumina GoldenGate platform (Illumina, Inc., San Diego, CA, USA). Twelve SNPs from *GABRA2* were included in the panel. Three SNPs (rs10008315, rs9291283 and rs7678520) that were rare or had low call rates were excluded. In addition, we included two *GABRA2* SNPs, rs279826 (intron 4) and rs279858 (exon 5 and K132K), genotyped by Taqman (Villafuerte *et al*. 2012) previously associated with both alcohol measures and impulsiveness (Villafuerte *et al*. 2012). We included duplicates (78 for the array and 12 for the Taqman assay) and no discrepancies were observed. In summary, 11 SNPs are reported in the analyses.

Linkage disequilibrium (LD) between markers was calculated with Haploview (Barrett *et al*. 2005). All SNPs were in Hardy–Weinberg equilibrium.

Haplotypes were constructed for four SNPs (rs10805145, rs426463, rs279827 and rs279826) in high LD ($LD > 0.93$) located in the proximal block (Figure 1). Because of the high LD among these SNPs, haplotypes were estimated manually and without ambiguity for 288 subjects out of the 295 participants. Two major haplotypes, AAAA (54.3%) and the complementary CCGG (43.3%), were estimated without ambiguity for 288 subjects and were coded with 0, 1 and 2 if they have 0, 1 or 2 copies of the minor (risk) haplotype (CCGG), respectively.

Statistical analyses

Association analyses

General Linear Models (univariate) in SPSS was used to predict both impulsiveness and LAPS from *GABRA2* SNPs. Covariates in the model were gender, age, race and DSM-IV diagnosis of AUD. To test the independent effect of SNP rs1442060, not in LD with the other SNPs, rs279827 was included as a covariate. Independent *t*-tests were used to compare impulsiveness and LAPS differences between subjects with and without lifetime AUD diagnosis.

Multiple testing

We used the Single-Nucleotide Polymorphism Spectral Decomposition (SNPSpD) test, a simple correction for multiple testing of SNPs in LD with each other, on the basis of the spectral decomposition (SpD) of matrices of pairwise LD between SNPs. The user-friendly Web interface (http://gump.qimr.edu.au/general/daleN/SNPSpD/) (Nyholt, 2004) provides the effective number of independent marker loci and experiment-wide significance threshold required to keep type I error rate at 5%. For this study, including 11 SNPs, the effective number of independent marker loci is 5 and the experiment-wide significance threshold was 0.01.

Mediation analyses

To test the indirect effect, bootstrapping procedures in AMOS were used to examine whether impulsiveness mediated the relation between *GABRA2* variants and LAPS. Bootstrapping (MacKinnon *et al*. 2007) is a nonparametric method based on re-sampling with replacement done 2000 times. We used a 90% confidence interval. We tested the indirect effect of the four SNPs that were associated with both LAPS and impulsiveness.

Results

GABRA2 variants and the impulsiveness facet of the neuroticism domain (NEO-PI)

Eleven SNPs from the *GABRA2* gene spanning from intron 1 to intron 9 were analyzed with both the NEO-impulsiveness and the LAPSs in the 295 adult subjects. We found 10 SNPs associated with the NEO-impulsiveness ($P < 0.03$) (Table 1). Nine of these associated SNPs were in LD with each other (r^2 from 0.71 to 0.96) while one SNP, rs1442060, showed LD (r^2) values of less than 0.21 (D′ of 0.51) with the other nine SNPs. These polymorphisms explain between 2.6% and 4.8% of the total variance of impulsiveness. One of the strongest associated SNPs, rs279827 (impulsiveness ($P = 0.001$), is located next to a splice acceptor site (Figure 1). Similar results were obtained for the Caucasian group ($N = 286$) providing additional evidence that race does not confound our results. Four out of the 10 associated SNPs did not pass multiple correction. These SNPs are located between intron 4 and intron 9.

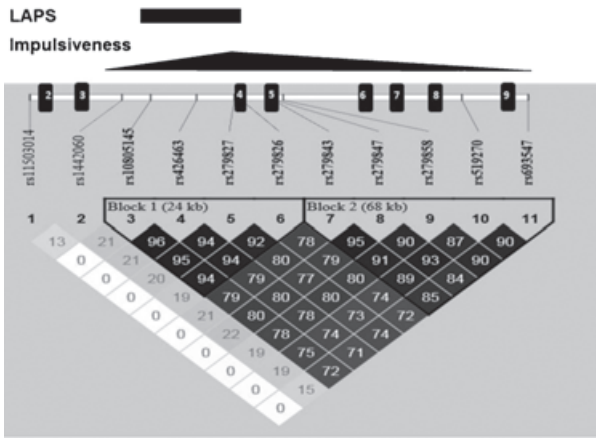

Figure 1: Location and LD of eleven genotyped SNPs within the *GABRA2* gene (not drawn to scale). The black boxes depict the exons. The thicker areas in the triangles indicate the strongest association signal for both impulsiveness and LAPS.

To determine the interdependence of the SNPs, we repeated the association test using one of the most significantly, rs279827, as a conditional factor (covariate). None of the SNPs were longer significant indicating that there are not two or more independent SNPs influencing the association of GABRA2 on impulsiveness. Figure 1 depicts the location of the 10 associated SNPs highlighting the strength of the association with impulsiveness around SNP rs279827.

Covariates in the model (age, gender and race) were not significant and were not included in the final analysis. However, as expected, subjects with an AUD diagnosis had significantly higher impulsiveness scores (16.5 ± 0.40, mean $\pm$ SEM; $P = 0.0003$) compared to subjects with no AUD diagnosis (14.8 ± 3.9, mean $\pm$ SEM). In the full genetic model, lifetime alcohol use diagnosis as a binary covariate (diagnosis vs. no diagnosis) was significant ($P < 0.002$).

Homozygotes for the minor haplotype (CCGG/CCGG) showed significantly higher scores of impulsiveness ($P = 0.001$) (Table 2) but the haplotype was not a better variable than the most significant SNP alone.

GABRA2 variants and LAPSs

Next, as impulsiveness has been associated with alcohol problems, we also tested the association of these SNPs with the LAPS. Four SNPs, including the most strongly associated SNP mentioned above, spanning the region from intron 3 to intron 4 were associated with LAPS (Table 2). Although, none of these SNPs survives multiple testing, we consider to report this association for the mediation analysis that follows. As expected, the association was in the same direction, i.e. alleles associated with higher scores of impulsiveness were also associated with higher scores of LAPS (Table 2). The effect size of these SNPs varies between 2.4% and 2.5%, somewhat lower than the effect size for impulsiveness. Again SNP rs279827 showed one of the strongest effects on LAPS (2.5%). Gender, age and race as covariates were not significant and were not included in the final model. However, as expected LAPS scores were significantly higher in the AUD group (12.5 ± 2.1) compared to the non-AUD subjects (8.9 ± 1.9) ($P < 0.00001$). Figure 1 depicts the location of the four associated SNPs with LAPS, highlighting the strength of the association around SNP rs279827. Similar results were obtained In the Caucasian sample when we checked for possible stratification. Homozygotes for the minor haplotype (CCGG/CCGG) showed significantly higher scores of LAPS ($P = 0.024$) (Table 3), but the haplotype was not a better variable than the most significant SNP alone.

Of the three LAPS components (age of first drunkenness, number of different alcohol related difficulties at any time and index of life course duration of all drinking related problems from onset to present, corrected for risk exposure), the association with *GABRA2* SNPs was driven mainly by number of alcohol related difficulties ($P < 0.04$) and index of life course duration of all drinking related problems ($P < 0.05$), and not by age of first drunkenness.

Table 1: Means for impulsiveness by *GABRA2* SNPs and haplotypes

Markers	Position	Minor: major allele/MAF	Number of minor alleles* mean ± SEM (*N*) 0	1	2	Effect size %	Significance
rs11503014	Intron 1	C:G/0.30	15.73 ± 0.34 (141)	15.09 ± 0.35 (131)	16.43 ± 0.83 (23)		0.211
rs1442060	Intron 3	A:G/0.45	14.68 ± 0.51 (90)	15.13 ± 0.33 (144)	16.63 ± 0.42 (61)	3.7	0.004
rs10805145	Intron 3	C:T/0.46	14.7 ± 0.43 (83)	15.33 ± 0.31 (151)	17.0 ± 0.50 (61)	4.2	0.002
rs426463	Intron 3	C:A/0.46	14.62 ± 0.43 (84)	15.36 ± 0.32 (153)	17.14 ± 0.51 (58)	4.8	0.001
rs279827	Intron 3	G:A/0.46	14.58 ± 0.42 (86)	15.41 ± 0.32 (148)	17.01 ± 0.5 (61)	4.5	0.001
rs279826	Intron 4	G:A/0.46	14.62 ± 0.43 (84)	15.35 ± 0.32 (150)	17.06 ± 0.50 (61)	4.6	0.001
rs279843	Intron 4	T:G/0.44	14.64 ± 0.41 (91)	15.54 ± 0.32 (149)	16.81 ± 0.53 (55)	3.5	0.006
rs279847	Intron 4	A:C/0.44	14.75 ± 0.411 (92)	15.47 ± 0.33 (146)	16.78 ± 0.52 (57)	3.1	0.010
rs279858	Exon 5	G:A/0.43	14.75 ± 0.41 (94)	15.50 ± 0.33 (147)	16.78 ± 0.54 (54)	3.0	0.012
rs519270	Intron 8	T:C/0.43	14.85 ± 0.40 (96)	15.46 ± 0.33 (144)	16.72 ± 0.53 (55)	2.6	0.021
rs693547	Intron 9	T:A/0.43	14.83 ± 0.40 (97)	15.46 ± 0.33 (143)	16.78 ± 0.53 (55)	2.8	0.015
rs10805145-rs279826	Intron 3–Intron 4	CCGG/TAAA	14.70 ± 0.43 (83)	15.41 ± 0.32 (147)	17.14 ± 0.51 (58)	4.6	0.001

MAF, minor allele frequency.
*Impulsiveness: NEO-PI impulsiveness facet from the neuroticism domain, raw scores.

Mediation analyses

Given that four *GABRA2* SNPs were associated with both impulsiveness and LAPS, we tested whether impulsiveness may mediate the effect of *GABRA2* on LAPS. We selected the four SNPs that showed association with both impulsiveness and LAPS (rs10805145, rs426463, rs279827 and rs279826) to test for mediation. Table 3 reports the standardized results of the model-based bootstrap that directly tests the significance of mediation effects using 2000 samples from the original data. Evidence for partial mediation was observed for all four SNPs. Specifically, the indirect effect of *GABRA2* on LAPS was significant for all four SNPs and the direct effect of *GABRA2* on LAPS remained significant. On average, 22.6% of the relation of *GABRA2* SNPs was due to impulsiveness, with SNP rs279827 showing the highest mediated effect, of 23.2%.

Discussion

The evidence here indicates that a small part of the effect of *GABRA2* on alcoholism is mediated through a specific facet of behavior, namely, impulsiveness. The extent to which this behavioral trait has an underlying genetic component is just starting to be understood. Previously, we reported an association of two *GABRA2* SNPs with impulsiveness, a facet from the neuroticism domain in the NEO-PI-R. Here, we tested these two SNPs and nine additional SNPs with the NEO-impulsiveness, and also with a developmentally constructed, life course measure of alcohol problems, LAPS. Ten SNPs were associated with impulsiveness and four of these were also associated with LAPS, before correcting for multiple testing. The alleles associated with higher scores for both impulsiveness and LAPS correspond to the same

Table 2: Means for Lifetime Alcohol Problems Score (LAPS) by *GABRA2* SNPs and haplotypes

Markers	Position	Minor allele	Number of minor alleles mean ± SEM (*N*) 0	1	2	Effect size %	Significance
rs11503014	Intron 1	C	10.55 ± 0.55 (141)	10.53 ± 0.23 (131)	10.30 ± 0.23 (23)		0.746
rs1442060	Intron 3	A	10.39 ± 0.28 (90)	10.57 ± 0.22 (144)	10.10 ± 0.34 (61)		0.487
rs10805145	Intron 3	C	9.80 ± 0.29 (83)	10.55 ± 0.21 (151)	10.93 ± 0.34 (61)	2.4	0.027
rs426463	Intron 3	C	9.82 ± 0.29 (84)	10.53 ± 0.21 (153)	11.00 ± 0.35 (58)	2.5	0.025
rs279827	Intron 3	G	9.82 ± 0.28 (86)	10.56 ± 0.22 (148)	10.93 ± 0.34 (61)	2.4	0.029
rs279826	Intron 4	G	9.82 ± 0.29 (84)	10.53 ± 0.21 (150)	11.00 ± 0.34 (61)	2.5	0.027
rs279843	Intron 4	T	11.0 ± 0.36 (91)	10.50 ± 0.22 (149)	10.0 ± 0.28 (55)		0.072
rs279847	Intron 4	A	11.02 ± 0.35 (92)	10.40 ± 0.22 (146)	10.07 ± 0.28 (57)		0.105
rs279858	Exon 5	G	10.10 ± 0.27 (94)	10.42 ± 0.22 (147)	11.00 ± 0.36 (54)		0.182
rs519270	Intron 8	T	10.14 ± 0.27 (96)	10.43 ± 0.22 (144)	10.87 ± 0.36 (55)		0.268
rs693547	Intron 9	T	10.16 ± 0.27 (97)	10.46 ± 0.22 (143)	10.76 ± 0.38 (55)		0.387
rs10805145-rs279826	Intron 3–Intron 4	CCGG	9.80 ± 0.29 (83)	10.51 ± 0.22 (147)	11.0 ± 0.35 (58)	2.6	0.024

Table 3: Standardized values of bootstrap mediation analyses of impulsiveness on the effect of *GABRA2* SNPs on Lifetime Alcohol Problems Score (LAPS)

	Total effects LAPS			Direct effects			Indirect effects				
	Estimate	SE	*P*-value	Estimate	SE	*P*-value	Estimate	SE	Confidence interval 90%	*P*-value	Mediated effect %
rs10805145	0.152	0.058	0.010	0.119	0.061	0.047	0.033	0.016	0.011–0.064	0.006	22.4
rs426463	0.156	0.058	0.007	0.121	0.062	0.053	0.035	0.016	0.012–0.066	0.006	22.4
rs279827	0.151	0.058	0.011	0.116	0.061	0.051	0.035	0.016	0.012–0.067	0.006	23.2
rs279826	0.155	0.059	0.011	0.120	0.062	0.048	0.035	0.016	0.012–0.066	0.006	22.5
rs10805145-rs279826	0.160	0.058	0.004	0.126	0.061	0.033	0.034	0.017	0.011–0.065	0.006	21.3

haplotype (minor alleles) previously described in other studies reporting an association of *GABRA2* variation with both alcoholism (Edenberg & Foroud, 2006; Fehr *et al*. 2006) and brain oscillations (Edenberg *et al*. 2004). Motivated by our previous finding (Villafuerte *et al*. 2012) and by a recent report in a sample of college students where higher scores on the urgency (impulsiveness) scale predicted alcohol problems but not alcohol use (Magid & Colder, 2007), we conducted association analyses of *GABRA2* variation with both impulsiveness and alcohol problems. Previously, we reported moderate associations of two of these *GABRA2* SNPs with higher percentage of alcoholic symptoms (Villafuerte *et al*. 2012). Some of the items on that measure overlap to a small degree with items comprising one component of LAPS, thus forecasting the association we report here. Our results suggest that genetic variation in *GABRA2* in the region comprising intron 3 to intron 4 may influence both level of impulsiveness and also level of alcohol problems. Notably, the association of *GABRA2* is stronger with impulsiveness than with alcohol problems, showing that an intermediate trait such as a personality trait is closer to the genetic influence, hence to the biology, than the manifestation of alcohol problems which are the result of several factors.

To further examine the casual relation of these associations, we performed mediation analysis where impulsiveness mediated the relation between *GABRA2* and LAPS. Indeed, bootstrap analyses indicated that the effect of *GABRA2* on LAPS was partially mediated by impulsiveness. On average, 22.6% of the effect of *GABRA2* on LAPS is accounted for by the impulsiveness-mediating effect. The remaining effect (77.4%) may be mediated by other behavioral risk factors not yet identified, the direct effect of *GABRA2* on LAPS and measurement error. It is also possible that GABRA2 affects some factor that influences both impulsiveness and alcohol problem.

Furthermore, the associated gene region with both impulsiveness and LAPS comprises intron 3 to intron 4. No coding variation has been reported for exon 4. However, isoforms of human *GABRA2* mRNA where exon 4 (68 bp) has been spliced out were discovered in many brain regions. The product of this isoform would be a truncated protein (non-functional) of only 66 amino acids due to the creation of a stop codon (Tian *et al*. 2005). Notably, one of the associated SNPs rs279827 is located next to the acceptor splice site (Tian *et al*. 2005). It is not known if SNP rs279827 would have an effect on splicing. But it is intriguing to note that of 11 SNPs in the *GABRA2* gene tested, the SNP near this splice site is the most strongly associated. The haplotype analysis comprising the SNPs in the proximal block (Figure 1) shows similar results as the individual SNPs supporting the notion that in this study the haplotype does not unveil a better not genotyped SNP in the region or independent contribution of the genotyped SNPs.

GABRA2 has been extensively investigated for its role in alcoholism, alcohol sensitivity, anxiolytic effects of benzodiazepines and its effect on EEG β patterns. The evidence suggests that this gene may have a pleiotropic effect on brain function involving overlapping mechanisms. The presence of alcohol and benzodiazepine binding sites in this subunit reveals the role of GABRA2 on alcohol sensitivity and anxiety (Low *et al*. 2000; Uhart *et al*. 2013), while the effect of *GABRA2* genetic variation on the EEG β wave suggests a different mechanism, involving the excitation–inhibition homeostatic balance and impulsivity. Converging lines of evidence point to the role of GABRA2 on impulsive-related behavior. Both in ADHD children and alcoholics with impulsive behaviors show increase in the EEG β activity (Bauer & Hesselbrock, 1993; Clarke *et al*. 2001). The frontal region is associated with self-regulation and inhibition/control behavior. It appears that genetic variation in *GABRA2* that influences the β activity in EEG may also influence inhibitory control behaviors such as impulsiveness. The moody and temper tantrum behavior may be related to the kinds of impulsiveness we describe here, involving lack of control under distress.

These results contribute to a refined understanding of the genetic role of *GABRA2* on impulsiveness, a major precursive behavioral risk characteristic (Masten *et al*. 2008; Zucker *et al*. 2008) and on LAPS that measures a facet of subsequent alcohol problems that is specific to problems due to consumption.

References

Agrawal, A., Edenberg, H.J., Foroud, T., Bierut, L.J., Dunne, G., Hinrichs, A.L., Nurnberger, J.I., Crowe, R., Kuperman, S., Schuckit, M.A., Begleiter, H., Porjesz, B. & Dick, D.M. (2006) Association of GABRA2 with drug dependence in the collaborative study of the genetics of alcoholism sample. *Behav Genet* **36**, 640–650.

Barrett, J.C., Fry, B., Maller, J. & Daly, M.J. (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**, 263–265.

Bauer, L.O. & Hesselbrock, V.M. (1993) EEG, autonomic and subjective correlates of the risk for alcoholism. *J Stud Alcohol* **54**, 577–589.

Bauer, L.O., Covault, J., Harel, O., Das, S., Gelernter, J., Anton, R. & Kranzler, H.R. (2007) Variation in GABRA2 predicts drinking behavior in project MATCH subjects. *Alcohol Clin Exp Res* **31**, 1780–1787.

Clarke, A.R., Barry, R.J., Mccarthy, R. & Selikowitz, M. (2001) Excess beta activity in children with attention-deficit/hyperactivity disorder: an atypical electrophysiological group. *Psychiatr Res* **103**, 205–218.

Costa, P.T.J. & Mccrae, R.R. (1992) *Revised NEO Personality Inventory (NEO-PI-R) and NEO Five-Factor Inventory (NEO-FFI) professional manual*. Psychological Assessment Resources, Odessa, FL.

Covault, J., Gelernter, J., Hesselbrock, V., Nellissery, M. & Kranzler, H.R. (2004) Allelic and haplotypic association of GABRA2 with alcohol dependence. *Am J Med Genet B* **129B**, 104–109.

Dawe, S., Gullo, M.J. & Loxton, N.J. (2004) Reward drive and rash impulsiveness as dimensions of impulsivity: implications for substance misuse. *Addict Behav* **29**, 1389–1405.

Dick, D.M., Bierut, L., Hinrichs, A., Fox, L., Bucholz, K.K., Kramer, J., Kuperman, S., Hesselbrock, V., Schuckit, M., Almasy, L., Tischfield, J., Porjesz, B., Begleiter, H., Nurnberger, J. Jr., Xuei, X., Edenberg, H.J. & Foroud, T. (2006) The role of GABRA2 in risk for conduct disorder and alcohol and drug dependence across developmental stages. *Behav Genet* **36**, 577–590.

Dick, D.M., Latendresse, S.J., Lansford, J.E., Budde, J.P., Goate, A., Dodge, K.A., Pettit, G.S. & Bates, J.E. (2009) Role of GABRA2 in trajectories of externalizing behavior across development and evidence of moderation by parental monitoring. *Arch Gen Psychiatry* **66**, 649–657.

Dick, D.M., Smith, G., Olausson, P., Mitchell, S.H., Leeman, R.F., O'malley, S.S. & Sher, K. (2010) Understanding the construct of impulsivity and its relationship to alcohol use disorders. *Addict Biol* **15**, 217–226.

Edenberg, H.J. & Foroud, T. (2006) The genetics of alcoholism: identifying specific genes through family studies. *Addict Biol* **11**, 386–396.

Edenberg, H.J., Dick, D.M., Xuei, X.L., Tian, H.J., Almasy, L., Bauer, L.O., Crowe, R.R., Goate, A., Hesselbrock, V., Jones, K., Kwon, J., Li, T.K., Nurnberger, J.I., O'connor, S.J., Reich, T., Rice, J., Schuckit, M.A., Porjesz, B., Foroud, T. & Begleiter, H. (2004) Variations in GABRA2, encoding the alpha 2 subunit of the GABA(A) receptor, are associated with alcohol dependence and with brain oscillations. *Am J Hum Genet* **74**, 705–714.

Enoch, M.A., Schwartz, L., Albaugh, B., Virkkunen, M. & Goldman, D. (2006) Dimensional anxiety mediates linkage of GABRA2 haplotypes with alcoholism. *Am J Med Genet B* **141B**, 599–607.

Fehr, C., Sander, T., Tadic, A., Lenzen, K.P., Anghelescu, I., Klawe, C., Dahmen, N., Schmidt, L.G. & Szegedi, A. (2006) Confirmation of association of the GABRA2 gene with alcohol dependence by subtype-specific analysis. *Psychiatr Genet* **16**, 9–17.

Grobin, A.C., Matthews, D.B., Devaud, L.L. & Morrow, A.L. (1998) The role of GABA(A) receptors in the acute and chronic effects of ethanol. *Psychopharmacology (Berl)* **139**, 2–19.

Hodgkinson, C.A., Yuan, Q., Xu, K., Shen, P.H., Heinz, E., Lobos, E.A., Binder, E.B., Cubells, J., Ehlers, C.L., Gelernter, J., Mann, J., Riley, B., Roy, A., Tabakoff, B., Todd, R.D., Zhou, Z. & Goldman, D. (2008) Addictions biology: haplotype-based analysis for 130 candidate genes on a single array. *Alcohol* **43**, 505–515.

Krystal, J.H., Staley, J., Mason, G., Petrakis, I.L., Kaufman, J., Harris, R.A., Gelernter, J. & Lappalainen, J. (2006) Gamma-aminobutyric acid type A receptors and alcoholism: intoxication, dependence, vulnerability, and treatment. *Arch Gen Psychiatry* **63**, 957–968.

Lappalainen, J., Krupitsky, E., Remizov, M., Pchelina, S., Taraskina, A., Zvartau, E., Somberg, L.K., Covault, J., Kranzler, H.R., Krystal, J.H. & Gelernter, J. (2005) Association between alcoholism and gamma-amino butyric acid alpha 2 receptor subtype in a Russian population. *Alcohol Clin Exp Res* **29**, 493–498.

Lejuez, C.W., Magidson, J.F., Mitchell, S.H., Sinha, R., Stevens, M.C. & De Wit, H. (2010) Behavioral and biological indicators of impulsivity in the development of alcohol use, problems, and disorders. *Alcohol Clin Exp Res* **34**, 1334–1345.

Lind, P.A., Macgregor, S., Agrawal, A., Montgomery, G.W., Heath, A.C., Martin, N.G. & Whitfield, J.B. (2008a) The role of GABRA2 in alcohol dependence, smoking, and illicit drug use in an Australian population sample. *Alcohol Clin Exp Res* **32**, 1721–1731.

Lind, P.A., Macgregor, S., Montgomery, G.W., Heath, A.C., Martin, N.G. & Whitfield, J.B. (2008b) Effects of GABRA2 variation on physiological, psychomotor and subjective responses in the alcohol challenge twin study. *Twin Res Hum Genet* **11**, 174–182.

Low, K., Crestani, F., Keist, R., Benke, D., Brunig, I., Benson, J.A., Fritschy, J.M., Rulicke, T., Bluethmann, H., Mohler, H. & Rudolph, U. (2000) Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* **290**, 131–134.

Mackinnon, D.P., Fairchild, A.J. & Fritz, M.S. (2007) Mediation analysis. *Annu Rev Psychol* **58**, 593–614.

Magid, V. & Colder, C.R. (2007) The UPPS impulsive Behavior scale: factor structure and associations with college drinking. *Pers Indiv Differ* **43**, 1927–1937.

Masten, A.S., Faden, V.B., Zucker, R.A. & Spear, L.P. (2008) Underage drinking: a developmental framework. *Pediatrics* **121** (Suppl. 4), S235–S251.

Nyholt, D.R. (2004) A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* **74**, 765–769.

Pierucci-Lagha, A., Covault, J., Feinn, R., Nellissery, M., Hernandez-Avila, C., Oncken, C., Morrow, A.L. & Kranzler, H.R. (2005) GABRA2 alleles moderate the subjective effects of alcohol, which are attenuated by finasteride. *Neuropsychopharmacology* **30**, 1193–1203.

Rangaswamy, M., Porjesz, B., Chorlian, D.B., Wang, K., Jones, K.A., Bauer, L.O., Rohrbaugh, J., O'connor, S.J., Kuperman, S., Reich, T. & Begleiter, H. (2002) Beta power in the EEG of alcoholics. *Biol Psychiatry* **52**, 831–842.

Rangaswamy, M., Porjesz, B., Chorlian, D.B., Wang, K., Jones, K.A., Kuperman, S., Rohrbaugh, J., O'connor, S.J., Bauer, L.O., Reich, T. & Begleiter, H. (2004) Resting EEG in offspring of male alcoholics: beta frequencies. *Int J Psychophysiol* **51**, 239–251.

Soyka, M., Preuss, U.W., Hesselbrock, V., Zill, P., Koller, G. & Bondy, B. (2008) GABA-A2 receptor subunit gene (GABRA2) polymorphisms and risk for alcohol dependence. *J Psychiatr Res* **42**, 184–191.

Tian, H., Chen, H.J., Cross, T.H. & Edenberg, H.J. (2005) Alternative splicing and promoter use in the human GABRA2 gene. *Brain Res Mol Brain Res* **137**, 174–183.

Uhart, M., Weerts, E.M., Mccaul, M.E., Guo, X., Yan, X., Kranzler, H.R., Li, N. & Wand, G.S. (2013) GABRA2 markers moderate the subjective effects of alcohol. *Addict Biol* **18**, 357–369.

Villafuerte, S., Heitzeg, M.M., Foley, S., Wendy Yau, W.Y., Majczenko, K., Zubieta, J.K., Zucker, R.A. & Burmeister, M. (2012) Impulsiveness and insula activation during reward anticipation are associated with genetic variants in GABRA2 in a family sample enriched for alcoholism. *Mol Psychiatry* **17**, 511–519.

Whiteside, S.P. & Lynam, D.R. (2001) The Five Factor Model and impulsivity: using a structural model of personality to understand impulsivity. *Pers Indiv Differ* **30**, 669–689.

Whiteside, S.P., Lynam, D.R., Miller, J.D. & Reynolds, S.K. (2005) Validation of the UPPS impulsive behaviour scale: a four-factor model of impulsivity. *Eur J Personality* **19**, 559–574.

Zucker, R. A. (1990). The concept of risk and the etiology of alcoholism: a probabilistic developmental perspective. D. J. Pittman & H.R. White *Society, Culture and Drinking Patterns Re Examined*, Piscataway, NJ: Rutgers Center of Alcohol Studies.

Zucker, R.A. (1991) Scaling the developmental momentum of alcoholic process via the lifetime alcohol problems score. *Alcohol* **1**, 505–510.

Zucker, R.A., Ellis, D.A., Bingham, C.R., Fitzgerald, H.E. & Sanford, K.P. (1996) Other evidence for at least two alcoholisms, II: life

course variation in antisociality and heterogeneity of alcoholic outcome. *Dev Psychopathol* **8**, 831–848.

Zucker, R.A., Davies, W.H., Kincaid, S.B., Fitzgerald, H.E. & Reider, E.E. (1997) Conceptualizing and scaling the developmental structure of behavior disorder: the lifetime alcohol problems score as an example. *Dev Psychopathol* **9**, 453–471.

Zucker R. A., Fitzgerald H.E., Refior S.K., Puttler L.I., Pallas D.M. & Ellis D.A. (2000) The clinical and social ecology of childhood for children of alcoholics: description and implications for a differentiated social policy. In Fitzgerald, H.E., Lester, B.M. & Zuckerman, B.S. (eds), *Children of Addiction: Research, Health and Policy Issues*, Routledge/Falmer, New York, pp. 109–141.

Zucker, R.A., Donovan, J.E., Masten, A.S., Mattson, M.E. & Moss, H.B. (2008) Early developmental processes and the continuity of risk for underage drinking and problem drinking. *Pediatrics* **121** (Suppl. 4), S252–S272.

Zucker, R.A., Heitzeg, M.M. & Nigg, J.T. (2011) Parsing the undercontrol/disinhibition pathway to substance use disorders: a multilevel developmental problem. *Child Dev Perspect* **5**, 248–255.

Acknowledgments

We thank all the participants in the Michigan Longitudinal Study for their commitment to the study over the years. We thank Dr. Jennie Jester from the MLS and Brady West from the Center for Statistical Consultation and Research (CSCAR) at the University of Michigan for their excellent statistical support. This work was supported in part by grants R37 AA07065 and R01 DA02726 (to R.A.Z), R03 AA01957601 to S.V. and a pilot grant from the University of Michigan MICHR CTSA program supported in part by the National Center for Research Resources, grant UL1RR024986, and is now at the National Center for Advancing Translational Sciences, grant 2UL1TR000433. The authors declare no conflict of interest.