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ORIGINAL ARTICLE

Impulsiveness and insula activation during reward anticipation are associated with genetic variants in *GABRA2* in a family sample enriched for alcoholism

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Genetic factors, externalizing personality traits such as impulsivity, and brain processing of salient stimuli all can affect individual risk for alcoholism. One of very few confirmed genetic association findings differentiating alcoholics from non-alcoholics is with variants in the inhibitory γ-amino butyric acid α2 receptor subunit (*GABRA2*) gene. Here we report the association of two of these *GABRA2* variants with measures of alcohol symptoms, impulsivity and with insula cortex activation during anticipation of reward or loss using functional magnetic resonance imaging (fMRI). In a sample of 173 families (449 subjects), 129 of whom had at least one member diagnosed with alcohol dependence or abuse, carriers for the G allele in two single-nucleotide polymorphisms (SNPs) and haplotypes were more likely to have alcohol dependence symptoms (rs279858, $P=0.01$; rs279826, $P=0.05$; haplotype, $P=0.02$) and higher NEO Personality Inventory-Revised (NEO-PI-R) Impulsiveness scores (rs279858, $P=0.016$; rs279826, $P=0.012$; haplotype, $P=0.032$) with a stronger effect in women (rs279858, $P=0.011$; rs279826, $P=0.002$; haplotype, $P=0.006$), all *P*-values are corrected for family history and age. A subset of offspring from these families ($n=44$, 20 females), genotyped for *GABRA2*, participated in an fMRI study using a monetary incentive delay task. Increased insula activation during reward ($r^2=0.4$; $P=0.026$) and loss ($r^2=0.38$; $P=0.039$) anticipation was correlated with NEO-PI-R Impulsiveness and further associated with the GG genotype for both SNPs (*P*'s <0.04). Our results suggest that *GABRA2* genetic variation is associated with Impulsiveness through variation of insula activity responses, here evidenced during anticipatory responses.

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Keywords: alcohol dependence; fMRI; *GABRA2*; impulsiveness; insula; SNP

Introduction

γ-Aminobutyric acid (GABA), an inhibitory neurotransmitter in the central nervous system (CNS), has a role in risk for developing alcohol use disorder (AUD) through the fast-acting receptor complex, $GABA_A$.[1,2] Repeated alcohol exposure affects the GABA system[2] through binding sites at the GABA receptors reducing neural inhibitory action. Genetic variation in γ-amino butyric acid α2 receptor subunit (*GABRA2*), the gene encoding the $GABA_A\alpha2$ receptor subunit, has been reproducibly associated with both alcoholism[3–12] and an electroencephalography measure, the β-frequency band.[6] Alcoholics[13] and their at-risk offspring[14] have increased power in the β-frequency band (13–28 Hz). Increased electroencephalography-β activity is also a good predictor of relapse.[15] In cortical networks, GABAergic transmission is critical for the maintenance of the excitation–inhibition homeostatic balance.[16]

The insula cortex is an important area for integrating emotional and homeostatic information to and from limbic and cortical areas, and has been implicated in negative emotional states such as craving and anxiety. Evidence for a role of the insula in conscious urges to take drugs comes from studies involving damage to the insula in both human[17–19] and animals.[20] Furthermore, insula activation has been associated with cue-induced drug craving,[17,21,22] maintaining urge to the use of drugs[23] in addicts and anticipation of aversive exposure in individuals with anxiety disorder.[24] Studies in non-clinical samples have revealed an association between insula function and self-report measures of anxiety.[25] These emotional stages of urge, anxiety and anticipation of negative events may be related to urgency, an

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impulsiveness trait involving rash actions under intense negative affect.[26]

Impulsivity is a multidimensional behavioral construct that includes at least four different component traits: urgency, sensation seeking, lack of premeditation and lack of perseverance.[26] Urgency (called Impulsiveness in the NEO Personality Inventory (NEO-PI)[27]) is related to the development of alcohol problems that are motivated by a coping strategy involving use of alcohol to deal with emotional distress, often with disregard for negative consequences.[28]

As variation in *GABRA2* has been associated with childhood conduct disorder symptoms, electroencephalography-β and alcoholism, we hypothesized that it may also influence behavioral traits such as impulsivity, an externalizing risk behavior for AUD. Here we report testing for a genetic association between two *GABRA2* single-nucleotide polymorphisms (SNPs) and the NEO-PI-Revised (NEO-PI-R) Impulsiveness facet. The test was carried out in a community-recruited sample of families that had at least one member with Diagnostic and Statistical Manual, Fourth Edition AUD diagnosis or were from an age and socioeconomically comparable group of control families.[29] A subset of this sample comprising adolescent and young adult offspring underwent an functional magnetic resonance imaging (fMRI) study using a modification of the monetary incentive delay task.[30] Given the role of the anterior insula cortex in the representation of interoceptive responses to emotionally salient and rewarding stimuli, the blood-oxygenation-level-dependent activation of this region was obtained during anticipation of a monetary reward or loss. We anticipated that genetic variation in *GABRA2* would be associated with Impulsiveness, as well as with insula cortex activation, which in turn would be positively correlated with Impulsiveness.

Materials and methods

Subjects and assessment

The population sample consisted of 449 subjects from the Michigan Longitudinal Study (MLS) who were genotyped for two *GABRA2* SNPs and for whom NEO-PI-R data were available. This is an ongoing multiwave, community-recruited prospective study of families of men with a drunk-driving conviction and AUD diagnosis who were living with a 3- to 5-year-old son/daughter and the biological mother at the 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.[31] Both the parental (246) and the offspring (203) generation are being followed (see Table 1 for detailed demographic information). Three hundred and twenty-three subjects were from 129 families with risk for alcoholism and 126 from 44 control families. The great majority were of Caucasian origin, with only 1.8% (8) of other ethnicity. All subjects were extensively assessed at 3-year intervals with behavioral and alcohol measures appropriate for age. One hundred and thirty (51 female; 14 adult offspring) subjects had a lifetime alcohol dependence/abuse diagnosis.

For this study, we used the maximum value (best estimate of maximum level of alcoholic symptomatology capable of being achieved by the individual) from all waves of the percentage of alcohol dependence symptoms (tolerance, withdrawal, craving) and problems (legal and social complications) reported from a 31-item checklist, the MLS Drinking and Drug History,[32] which contains a list of all symptoms listed in Diagnostic and Statistical Manual, Fourth Edition and others from earlier diagnostic systems[33] (see Supplementary materials). It incorporates non-overlapping items from the 1978 National Institute on Drug Abuse Survey,[34] from the American Drinking Practices Survey[35] and from the VA Medical Center (University of California, San Diego, CA, USA) Research Questionnaire for Alcoholics.[36] They provide data on quantity, frequency and variability of alcohol consumption, frequency of drug use, and multiple questions on symptoms, consequences and troubles related to the use of these substances, and have been extensively used in a variety of national surveys and clinical settings.[37] The modal value is

Table 1 Summary of the sample demographics and traits by AUD family history and diagnosis

	*No AUD family history (*N = *126)*	*AUD family history (*N = *323)*	*DSM-IV lifetime AUD diagnosis (*N = *130)*
Adult age at the time of last interview (mean, s.d.)	*N* = 67.48 ± 4.1	*N* = 179.47 ± 5.7	*N* = 116.47.3 (6.17)
Adult offspring age at the time of last interview (mean, s.d.)	*N* = 59.20 ± 1.0	*N* = 144.20 ± 0.98	*N* = 14.16.9 ± 1.02
Females	47	131	51
Males	79	192	79
% AD symptoms (mean, s.d.)	0.05 ± 0.12	0.21 ± 0.26	0.38 ± 0.29
Impulsiveness (mean, s.d.)	15.66 ± 4.08	16.04 ± 3.71	16.17 ± 3.92

Abbreviations: AD, alcohol dependence; AUD, alcohol use disorder; DSM-IV, Diagnostic and Statistical Manual, Fourth Edition; s.d., standard deviation.

zero. Alcohol dependence and abuse lifetime diagnosis was made by a trained clinician based on DSM-IV criteria at each wave using three instruments: Diagnostic Interview Schedule (DIS-III), the Short Michigan Alcohol Screening Test (SMAST) and the Drinking and Drug History. On the basis of information collected by all three instruments, a lifetime diagnosis was made ($\kappa = 0.81$).

Personality traits were assessed using the NEO-PI-R questionnaire[27] at all waves for parents and starting at wave 6 (older than age 18) for offspring. Analyses compared the percentage of alcohol dependence symptoms with the personality trait Impulsiveness, a Neuroticism facet of the NEO-PI-R[27] and two *GABRA2* SNPs.

In summary, our sample consists of 449 subjects with both genotype and Impulsiveness data. Impulsiveness and percentage of alcohol dependence symptoms were available for 415 subjects. Genotype data and percentage of alcohol dependence symptoms were available for 448 subjects.

Participants in the fMRI study

Twenty-nine young adult children of alcoholics (17 males and 12 females; mean age: 20.1 ± 1.2; range: 18–22) and 15 controls (7 males and 8 females; mean age 20.0 ± 1.3; range: 18–22) who were genotyped for the *GABRA2* SNPs also participated in the fMRI study. All were Caucasian, right-handed, and were a subset of unrelated MLS offspring. All had passed the exclusionary screen criterion of no fetal alcohol effects when recruited as children. Control subjects also were unrelated and had no parental history of AUD.

Exclusion criteria for this study were as follows: any neurological, acute, uncorrected or chronic medical illness; any Axis I psychiatric or developmental disorders; any current or recent (within 6 months) treatment with centrally active medications; a history of psychosis or schizophrenia in first-degree relatives; and a positive urine drug screen on the day of the study. Three of the participants had a diagnosis of alcohol abuse; this was not exclusionary given our interest in predicting range of variation in risk for AUD. Written informed consent, approved by the University of Michigan Medical School Institutional Review Board, was obtained before the study.

SNP genotyping and analysis

We selected two SNPs, rs279826 (intron 4) and rs279858 (exon 5, K132K) that capture the information of a long haplotype block of 109 kb where previous associations have been reported with alcohol dependence and related traits. The two SNPs (rs279826 and rs279858) were genotyped by TaqMan using inventoried assays of primers and probes (Applied Biosystems, ABI, Foster City, CA, USA). The PCR reactions were run in 5 μl volume using ABI standard protocol 2.5 μl of 2 × Master Mix, 0.25 μl of 20 × primers and probes and 10–20 ng of genomic DNA. The cycling conditions for PCR consisted of 95 °C for 10 min, followed by 40 cycles of 92 °C for 15 s and 60 °C for 1 min. The reactions and fluorescence scan were run on an ABI PRIZM 7900HT Sequence Analyzer. Allelic determination was carried out using SDS 2.1 (ABI). Probe sequences and allele information are publicly available online www.appliedbiosystems.com. We included 12 duplicates and no discrepancies were observed.

Linkage disequilibrium between markers was analyzed with Haploview.[38] Both SNPs were in Hardy–Weinberg equilibrium. To test for an association between the SNPs and Impulsiveness, we applied a regression test implemented in MERLIN-1.1-alpha program that takes family relationships into account in the association test.[39] First, 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's scores across the different data waves. Second, we analyzed the correlation between the Impulsiveness scores and percentage of alcohol dependence symptoms using Spearman's correlation as the alcohol variable was not normally distributed. To test the association of *GABRA2* SNPs and percentage of alcohol dependence symptoms, a zero-inflated Poisson regression model was applied to account for the excessive number of zeros (240/448) using SAS. The zero-inflated Poisson test includes all subjects. The zero-inflated Poisson regression generates two separate models and then combines them. First, a model is generated for the 'certain zero' cases. Then, a Poisson model is generated to predict the counts for those subjects who are not certain zeros. Finally, the two models are combined. This test does not consider family relationship.

Neuroimaging measures

Brain response during anticipation of incentive stimuli was probed during fMRI using a modified version of the Monetary Incentive Delay task.[30] Modifications were designed to eliminate the memory component from the original task, by replacing symbols for 'reward' and 'loss' trials with text stating trial type. A schematic of the modified paradigm is presented in Figure 1. Each 6 s trial consisted of four events. First, subjects were presented with an incentive cue (2000 ms) of five possible values (gain of US$0.2, $5.0; loss of $0.2, $5.0; or no change $0). This was followed by a 2000 ms anticipation delay. Next, a target appeared for a variable length of time (200–300 ms) during which subjects made a button press response in an attempt to gain or avoid losing said incentive. Subjects were instructed to respond to neutral targets despite the lack of incentive value. A feedback message then informed subjects of the trial outcome. The incentive trials were presented contiguously in a pseudorandom order. Subjects performed two runs of the task, each lasting 5 min. A total of 20 trials of each condition were recorded. The duration of the response target was calculated

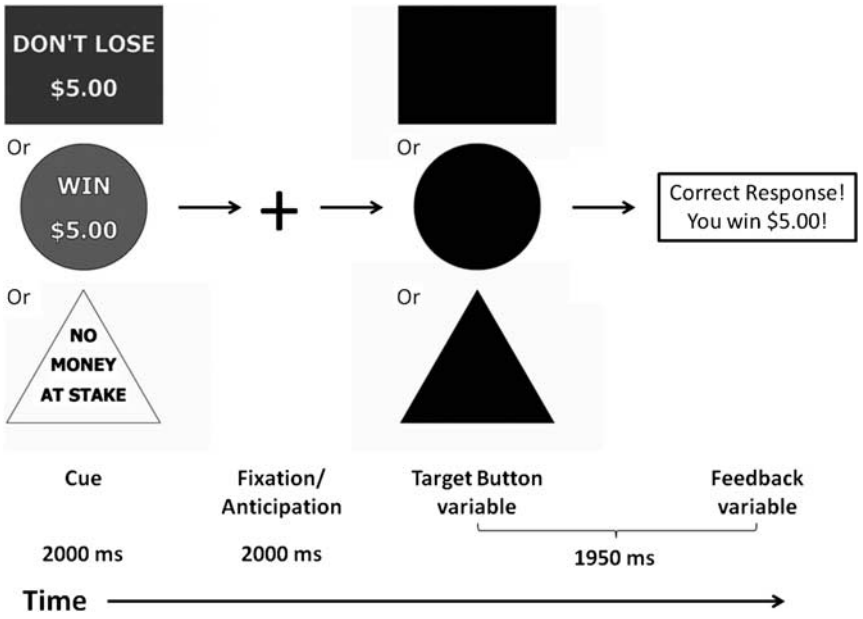

Figure 1 A schematic illustration of the monetary incentive delay task performed by subjects in the fMRI scanner.

based on individual subject's reaction time during a practice session before scanning. The allotted duration was calibrated such that the overall success rate was approximately 60%. Participants were paid a fixed rate to participate in the study and additionally received any money they won during the task. Reaction time and success rate for each incentive condition were calculated. Independent sample *t*-tests were used to assess possible performance differences between *GABRA2* A-carriers and GG genotype groups for both SNPs and haplotypes.

Whole-brain blood oxygen level-dependent functional images were acquired on a 3.0 T GE Signa scanner (Milwaukee, WI, USA) using a T2*-weighted single-shot combined spiral in/out sequence[40] with the following imaging parameters: repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, flip angle (FA) = 90°; field of view (FOV) = 200 mm; matrix size = 64 × 64; in-plane resolution = 3.12 × 3.12 mm; and slice thickness = 4 mm. A high-resolution anatomical T1 scan was obtained for spatial normalization (three-dimensional spoiled gradient recalled echo, TR = 25 ms, min TE, FOV = 25 cm, 256 × 256 matrix, slice thickness = 1.4 mm). Participant motion was minimized with the use of foam pads placed around the head along with a forehead strap. In addition, the importance of keeping as still as possible was emphasized during the informed consent process and at scanner entry.

Functional images were reconstructed using an iterative algorithm,[41] which is more robust against image distortions caused by off-resonance effects than conventional methods. Subject head motion and slice-acquisition timing were corrected using the FSL 4.0 analysis tools library (Analysis Group, FMRIB, Oxford, UK).[42] Analysis of estimated motion parameters confirmed that overall head motion within each run did not exceed 2 mm translation or 2° rotation in any direction. All remaining image processing was completed using statistical parametric mapping SPM2 package (Wellcome Institute of Cognitive Neurology, London, UK). Functional images were spatially normalized to a standard stereotactic space as defined by the Montreal Neurological Institute. A 6 mm full-width half-maximum Gaussian spatial smoothing kernel was applied to improve signal-to-noise ratio and to account for individual differences in anatomy.

Statistical analysis

Individual analysis was completed using a general linear model in SPM2. Five regressors of interest (anticipation of win US$0.2, win $5.0, lose $0.2, lose $5.0 and neutral $0) were convolved with the canonical hemodynamic response function. Motion parameters were modeled as nuisance regressors to remove residual motion artifacts. Scanner drift and other low-frequency noise were removed from the image time series using a 128 s high-pass filter. Two contrasts of interest, anticipation of reward (US$0.2 and $5.0 combined) minus neutral incentive and anticipation of loss (US $0.2 and $5.0 combined) minus neutral incentive, were calculated for each individual.

This report focuses only on differences in the insula, owing to *a priori* interest in this brain region. Volume of interest analyses of blood-oxygenation-level-dependent signal data were conducted on bilateral insula. Left and right insula masks were generated based on the Anatomical Automatic Labeling atlas using WFU PickAtlas software toolbox.[43–45] Effect sizes for reward and loss anticipation activation

in the volume of interests were extracted from individual contrasts of interest using MarsBaR Region of interest toolbox.[46] *T*-tests were conducted in SPSS to compare the insula effect sizes between A-carriers and GG *GABRA2* genotypes and haplotypes. Pearson's correlation was conducted in SPSS to test the relationship between Impulsiveness and insula effect sizes.

Results

GABRA2 variants, Impulsiveness and alcoholism

Personality and genotype data were available for 436 subjects (rs279826) and 431 subjects (rs279858), respectively (449 total). Linkage disequilibrium for both SNPs in our sample is $r^2 = 0.78$, resulting in two major haplotypes, A–A and G–G, corresponding to 93% of the total haplotypes. We found both *GABRA2* SNPs and the haplotype associated with the Impulsiveness facet of the Neuroticism domain in our overall sample (Table 2). These polymorphisms explain about 1.6% of the total variance of Impulsiveness. Overall, subjects with the GG genotype for both SNPs and haplotypes showed higher scores on the NEO-PI-R Impulsiveness facet (rs279858, F = 6.3, $P = 0.02$; rs279826, F = 6.9, $P = 0.008$; haplotype, F = 5.2, $P = 0.01$). These results remain significant when controlled for family history and age (rs279858, $P = 0.016$; rs279826, $P = 0.012$; haplotype, $P = 0.032$). Regressing out the effect of each SNP confirms that they are not independent. Genotype × sex interaction was observed as a secondary analysis for rs279826 (F = 2.9, $P = 0.05$), indicating that the genetic effect was observed in women (rs279858, $P = 0.012$; rs279826, $P = 0.006$; haplotype, $P = 0.009$) and the association was stronger when controlled for family history and age (rs279858, $P = 0.011$; rs279826, $P = 0.002$; haplotype, $P = 0.006$) (Table 2).

As *GABRA2* variants were previously found to be associated with alcoholism, we also tested these Impulsiveness-associated SNPs for association with percentage of alcohol dependence symptom. In the presence of any alcoholic symptoms, the GG genotype for both SNPs rs279858 ($N = 443$, $\underline{\beta} = -0.676$, $P = 0.02$) and rs279826 ($N = 448$, $\beta = -0.562$, $P = 0.05$) and GG/GG haplotype ($N = 408$, $\beta = -0.703$, $P = 0.02$) were likely to have alcohol dependence symptoms compared with A carriers. In the case of the haplotype, this association became significant when gender was included as covariate where women with the GG/GG haplotype were likely to have a higher percentage of alcoholic symptoms compared with A carriers ($\beta = 0.7653$; $P = 0.0053$).

The initial stimulus for this study was the well-established association of Impulsiveness with AUD.[47] Percentage of alcohol symptoms ($N = 415$, $r^2 = 0.128$, $P = 0.009$) was significantly positively correlated with Impulsiveness (Spearman's ρ correlation). No gender differences were observed for Impulsiveness.

Insula activity during anticipation of monetary reward and loss and GABRA2 variation

During reward anticipation, individuals with the GG genotype of either SNP and carriers of the G–G

Table 2 *GABRA2* SNPs and Impulsiveness

	N	*Overall mean (s.e.m.)*	*Impulsiveness*			
			N	*Females, mean (s.e.m.)*	N	*Males, mean (s.e.m.)*
rs279858						
GG	81	16.8 (0.41)	35	17.3 (0.66)	46	16.4 (0.53)
GA	212	16.0 (0.26)	84	16.6 (0.43)	128	15.7 (0.32)
AA	138	15.5 (0.32)	55	15.3 (0.53)	83	15.7 (0.39)
P-value		**0.02 (*0.016)**		**0.012 (*0.011)**		0.422 (*0.379)
rs279826						
GG	99	16.6 (0.38)	42	17.2 (0.60)	57	16.1 (0.49)
GA	214	15.9 (0.26)	84	16.7 (0.42)	130	15.5 (0.32)
AA	123	15.2 (0.34)	48	14.7 (0.56)	75	16.1 (0.49)
P-value		**0.008 (*0.012)**		**0.006 (*0.002)**		0.557 (*0.588)
Haplotype						
GG/GG	83	16.7 (0.43)	35	17.2 (0.69)	48	16.3 (0.54)
AA/GG	198	16.0 (0.28)	76	16.6 (0.47)	122	15.6 (0.34)
AA/AA	124	15.2 (0.35)	50	14.7 (0.57)	74	15.6 (0.44)
P-value		**0.01 (*0.032)**		**0.009 (*0.006)**		0.490 (*0.531)

Abbreviations: *GABRA2*, γ-amino butyric acid α2 receptor subunit gene; s.e.m., standard error of mean; SNP, single-nucleotide polymorphisms
**P*-values adjusted for family history and age.
Bold values are significant *P*-values.

Table 3 Insula activation and *GABRA2* genotypes

Left insula	N	*Overall, mean (s.e.m.)*	N	*Females, mean (s.e.m.)*	N	*Males, mean (s.e.m.)*
Mean effect size for reward anticipation						
rs279858						
GG	10	0.73 (0.36)	5	1.03 (0.35)	5	0.44 (0.65)
AA /GA	32	−0.12 (0.18)	15	0.02 (0.21)	17	−0.25 (0.28)
P-value		**0.028 (*0.027)**		**0.027 (*0.018)**		0.279 (*0.318)
rs279826						
GG	11	0.77 (0.32)	6	1.05 (0.28)	5	0.44 (0.65)
AA/GA	33	−0.19 (0.17)	14	−0.05 (0.21)	19	−0.29 (0.25)
P-value		**0.009 (*0.016)**		**0.008 (*0.006)**		0.229 (*0.226)
Haplotype						
GG/GG	10	0.73 (0.36)	5	1.03 (0.35)	5	0.44 (0.65)
AA/AA _AA/GG	31	−0.17 (0.18)	13	−0.03 (0.23)	18	−0.28 (0.27)
P-value		**0.020 (*0.028)**		**0.023 (*0.011)**		0.250 (*0.252)
Right insula						
rs279826						
GG	11	0.46 (0.37)	6	0.77 (0.43)	5	0.08 (0.65)
AA/GA	33	−0.29 (0.18)	14	−0.16 (0.22)	19	−0.38 (0.27)
P-value		**0.05 (***0.07)		**0.046 (*0.043)**		0.462 (*0.490)
Mean effect size for loss anticipation						
rs279826						
GG	11	0.0.39 (0.36)	6	0.57 (0.24)	5	0.18 (0.78)
AA/GA	33	−0-0.35 (0.14)	14	−0.29 (0.14)	19	−0.40 (0.22)
P-value		**00.024 (*0.026)**		**0.004 (*0.004)**		0.335 (*0.337)
Haplotype						
GG/GG	10	0.30 (0.39)	5	0.42 (0.23)	5	0.17 (0.78)
AA/AA_AA/GG	31	−0-0.31 (0.12)	13	−0.36 (0.13)	18	−0.26 (0.19)
P-value		**0.0.05 (*0.058)**		**0.007 (*0.007)**		0.418 (*0.429)

Abbreviations: *GABRA2*, γ-amino butyric acid α2 receptor subunit gene; s.e.m., standard error of mean; SNP, single-nucleotide polymorphisms.
**P*-values adjusted for family history and age.
Bold values are significant *P*-values.

haplotype showed significantly higher activation in the left insula compared with those with other genotypes/haplotypes (rs279858, $P=0.028$; rs279826, $P=0.009$; haplotype, $P=0.020$) and remained significant after corrected for family history and age (rs279858, $P=0.049$; rs279826, $P=0.016$; haplotype, $P=0.028$) (Table 3). In the right insula, rs279826 G homozygotes showed higher activation ($P=0.05$) during reward anticipation, whereas neither rs279858 nor the haplotype were associated (data not shown). During loss anticipation, rs279826 G homozygotes and carriers of the G–G haplotype were associated with higher left insula activation (rs279826, $P=0.024$; haplotype, $P=0.05$). We report no association for either SNP or the haplotype with right insula activation during loss anticipation. The association between rs279826 and right insula response to reward anticipation loses some significance when controlling for family history and age ($P=0.07$), whereas the remainder of the findings remain significant ($P<0.05$). However, children of alcoholics showed greater activation of right insula to reward ($P=0.022$) and loss ($P=0.038$). By regressing out each SNP, we confirm that they are not independent. For both SNPs, we observed that A homozygotes and heterozygotes have similar means and, because of the small sample size in the fMRI study, grouped them together and compared them with GG mean values.

When stratifying by gender, the genetic difference in insula activation was apparent in women, but not in men. Women with the GG genotype for both SNPs and haplotype showed higher left insula activation during reward anticipation compared with the A carriers (rs279858, $P=0.027$; rs279826, $P=0.008$; haplotype, $P=0.023$) and remain significant when corrected for family history and age (rs279858, $P=0.036$; rs279826, $P=0.006$; haplotype, $P=0.011$), whereas men did not show genetic differences in activation (all P's >0.23) (Table 3). Female rs279826 G homozygotes also showed greater right insula activation to reward anticipation ($P=0.046$; corrected for family history and age, $P=0.043$). Also in women, greater left insula activation to loss anticipation was observed for rs279826 G

homozygotes (corrected, $P = 0.004$) and haplotype (corrected, $P = 0.007$), whereas men showed no differences (all P's > 0.34). Given the association of these alleles with Impulsiveness, we examined whether insula activation was also related to Impulsiveness. Indeed, Impulsiveness was correlated with left insula activation during reward anticipation ($r^2 = 0.399$, $P = 0.026$) and loss anticipation ($r^2 = 0.361$, $P = 0.046$). Impulsiveness and right insula activation approached significant correlation during reward anticipation ($r^2 = 0.338$, $P = 0.063$) and was significantly correlated during loss anticipation ($r^2 = 0.373$, $P = 0.039$). There were no significant differences between children of alcoholics and controls or for gender for these correlations.

Discussion

This study was stimulated by three previously established findings: (a) an association of *GABRA2* variants with alcoholism; (b) an association of impulsive behavior with alcoholism; and (c) an emerging role of the insula in conscious urges to take drugs and maintain the addictive behavior.[18] We thus sought to determine whether alcoholism-associated *GABRA2* variants were also associated with impulsive behaviors and with insula activation during reward and loss anticipation. In this study, the G allele and corresponding haplotype (GG/GG) for both SNPs, previously described as the high-risk haplotype by four studies,[5,6,8,9] are associated with higher probability to have alcoholic symptoms, higher scores of the NEO-PI-R Impulsiveness facet and with greater insula activation during anticipation of reward and loss, and this activation is correlated with NEO-PI-R Impulsiveness scores. The convergence of these findings suggests that variation in *GABRA2* contributes to the risk of alcoholism through the influence on impulsive behaviors and supports previous studies that relate *GABRA2* with impulsive-related traits such as conduct disorder,[48] and this effect may take place at least in part in the insula.

As expected from the extensive literature, we observed that subjects with higher Impulsiveness scores also had higher percentage of alcohol dependence symptoms, replicating the established evidence of the relationship between Impulsiveness and alcoholism.[28,47,49]

We also replicate the association of two *GABRA2* SNPs and the haplotype with the presence of alcoholic symptoms. A large number of subjects in this study do not have any alcoholic symptom, resulting in an excessive number of zeroes (240/448) for which a zero-inflated Poisson test was applied. Subjects with the GG allele for both SNPs and haplotype are more likely to have any alcoholic symptom compared with A carriers. Several studies have reported the association of *GABRA2* variants and alcohol-related traits, but the direction of the association is not consistent. Two major haplotypes have been identified for this high linkage disequilibrium region. The haplotype containing the G allele for both SNPs analyzed in this study has been associated with alcohol dependence in four studies[5,6,8,9] and with the complementary haplotype (the A allele from both SNPs) in three studies.[3,11,12] The studies from Edenberg *et al*.[6] and Agrawal *et al*.[3] used the same data set from the Collaborative Study on the Genetics of Alcoholism, where Agrawal reported the A allele for both SNPs rs279858 and rs279826 associated with co-morbid illicit drug dependence, whereas Edenberg and Foroud[50] later clarified that the G-allele-containing haplotype was associated with alcohol dependence. When the SNP rs279858 was compared with the efficacy of three psychosocial treatments, different drinking outcomes among treatments were observed for subjects with the A allele, whereas no difference in outcome among treatments was observed for G allele carriers.[4] The authors suggested that an elevated anxiety level driven by the presence of the G allele may compromise the outcome as a result of enhanced stress and impulsivity. Individuals with the G allele may need to drink more alcohol to achieve the desired effects compared with AA subjects who experienced a higher subjective response to alcohol.[4] One[11] of the three studies that reported the A allele for the two SNPs in this study associated with alcohol-related measures found the haplotype containing the A allele for both SNPs associated with alcoholism compared with controls, but when the SNPs were analyzed independently, no association was observed. Our results may contribute to a better understanding of *GABRA2* variation on Impulsiveness measures. The G allele of both SNPs and corresponding G–G haplotype was associated with a specific kind of Impulsiveness that assesses rash action in response to distress—with rashness indicating impulsive action along with later regret.

Furthermore, we established an association between the NEO-PI-R Impulsiveness facet and activation of the insula cortex during anticipation of reward and loss in a subset of samples. *GABRA2* SNPs were associated with both the Impulsiveness facet in the large sample and with the insula cortex activation during reward and loss anticipation in the subset of samples. Greater insula activation during anticipation of reward and loss was observed in individuals with the G allele for both SNPs and this activation correlated with Impulsiveness scores. One SNP in particular, the rs279826 G homozygotes, was associated with left and right insula activation during reward anticipation and with the left insula during loss anticipation. This suggests that the effect of genetic variation in *GABRA2* on rash actions under distress may occur via modulation of insula activation. The insula translates interoceptive signals into conscious feelings, which can lead to subjective pleasure and cue-induced urges, anxiety and biased decision-making in the face of uncertain risk and reward.[18] The present findings add to the weight of evidence for the role of the insula on emotional stages

of urge to take drugs,[18] and altered related anticipatory processing in anxious individuals.[24] These traits are related to Impulsiveness, which in turn is positively correlated with insula activation in our sample.

Interestingly, the genetic effect of *GABRA2* on percentage of alcohol dependence symptom, Impulsiveness and insula activation was observed in women and not in men. No difference for this genetic effect was observed between subjects with higher risk for AUD compared with low-risk subjects. Enoch *et al.*[7] has reported association of anxiety disorders (lifetime Diagnostic and Statistical Manual, Third Edition, Revised anxiety disorders) in an haplotype containing the rs279858 G allele in women in a case–control sample of plain Indian alcoholics. This sex difference may be explained by the effect of neurosteroids, such as progesterone, on the GABA system modulating an array of behaviors including anxiety.[51] Prolonged exposure to progesterone increased anxiety-like behavior in female rats.[52] In addition, several cross-sectional, longitudinal and ecological momentary assessment studies have found that the association between distress and alcohol consumption was greater in women than in men.[53–55] This may suggest that *GABRA2* genetic variation may differentially influence the negative effect component in impulsive behavior in women; this in turn is an additional risk factor for problem alcohol consumption. Our results suggest that the alcohol dependence *GABRA2* G–G haplotype captures the genetic susceptibility for Impulsiveness driven by anxiety in women.

We propose that the *GABRA2* G–G haplotype influences the homeostatic imbalance toward excitation, as evidenced by the electrophysiological anomalies associated with this haplotype.[6] This imbalance may intensify the activation of the interoceptive representations within the insula (higher activation for GG during cue-induced urges), thus contributing to impulsive behaviors[48,56,57] related to distress. However, the applicability of these results across the entire sample must be treated with caution given that differences have been observed in the role of *GABRA2* in alcoholism risk across development. Specifically, a strong association with alcohol dependence may not emerge until the mid-1920s, whereas association with conduct disorder symptoms is present at earlier ages.[48] We interpret these results to indicate an influence of *GABRA2* on the underlying neural system that influences both early risk factors as well as later alcohol dependence. However, prospective, longitudinal studies will be required to support this. Larger samples along with replication of our findings are necessary to elucidate the specific mechanism by which *GABRA2* exerts its effect on Impulsiveness-related traits and its role in alcohol addiction.

Conflict of interest

The authors declare no conflict of interest.

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