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Association of the *CHRNA4* Neuronal Nicotinic Receptor Subunit Gene with Frequency of Binge Drinking in Young Adults

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Background: Binge drinking is responsible for over half of all alcohol-related deaths and results in significant health and economic costs to individuals and society. Knowledge of genetic aspects of this behavior, particularly as it emerges in young adulthood, could lead to improved treatment and prevention programs.

Methods: We have focused on the association of variation in neuronal nicotinic receptor subunit genes (*CHRN*s) in a cohort of 702 Hispanic and non-Hispanic White young adults who are part of the Social and Emotional Contexts of Adolescent Smoking Patterns (SECASP) study. Fifty-five single nucleotide polymorphisms (SNPs) covering the variation in 5 CHRNs (*CHRNA4*, *CHRNB2*, *CHRNA2*, *CHRNB3A6*, and *CHRNA5A3B4*) were studied.

Results: Frequency of binge drinking and other correlated alcohol consumption measures were significantly associated with SNPs in *CHRNA4* (*p*-values ranged from 0.0003 to 0.02), but not with SNPs in other *CHRN*s. This association was independent of smoking status in our cohort.

Conclusions: Variants in *CHRNA4* may contribute to risk of binge drinking in young adults in this cohort. Results will need to be confirmed in independent samples.

Key Words: Binge Drinking, Neuronal Nicotinic Receptor Genes, Genetic Association.

ALCOHOL-related disorders result in significant personal costs and burdens to society (Anthony and Echeagaray-Wagner, 2000), fueling the need for studies of initiation of alcohol use and of susceptibility factors leading to later dependence. Alcohol dependence and/or abuse are common, with a lifetime prevalence of 13% (Grant et al., 2004) and are the third leading preventable cause of death in the United States (McGinnis & Foege, 1993). Experimentation with alcohol often begins early, in adolescence and young adulthood. The 2012 data from the Monitoring the Future report (Johnston et al., 2013) show that 64% of 12th graders reported using alcohol, 45% reported having been drunk, and binge drinking occurred at a rate of 24% in 2012. Binge drinking, defined as the consumption within 2 hours of 4 drinks for females or 5 for males (National Institute on Alcohol Abuse and Alcoholism [NIAAA], www.niaaa.nih.gov), is an alcohol consumption behavior that is of major concern from a public health perspective. Binge drinking accounts for over half of all alcohol-related deaths and three-quarters of the estimated economic costs of excessive alcohol use (CDC-MMWR 2012, 2013). Discovering risk factors for this behavior in particular could have an important public health impact.

Binge drinking and other measures of heavy use have been of particular interest for studies of genetic risk. In a nonclinically ascertained adult study population, Kendler and colleagues (2010) showed that the highest association of any single alcohol behavior measure with alcohol dependence was frequency of being drunk. Furthermore, this phenotypic association had a strong genetic basis, consistent across both men and women. As a result, we have focused this genetic study on the frequency of binge drinking in our cohort.

Genetic studies of alcohol behaviors will benefit from a focus on young adulthood. While initiation and patterns of alcohol use in early adolescence have been shown to have substantial social and environmental components, alcohol use has increasingly strong genetic influence from the ages of 15 to 23 (Kendler et al., 2008), with a corresponding drop in familial environmental influences. This large twin study showed that heritable effects of alcohol use rose from about

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0% at age 13 years to the adult level of approximately 40 to 45% by about age 19 to 21 years. Importantly, this is the age of the subjects in our study (mean = 21.4 years). Similarly, the shared family environmental effects dropped from about 50% to about 10% over the same age range. Individual-specific environmental factors remained at a relatively steady 45%.

Although genetic etiology for alcohol use and dependence has been well supported (Heath et al., 1997; Madden and Heath, 2002; Madden et al., 2000; Reich et al., 1998; Schuckit, 2009; Wolff, 1972), much work remains to elucidate particular genetic risk mutations and understand genetic mechanisms. Alcohol genetic studies have focused primarily on alcohol dehydrogenases and related genes and also on gamma-aminobutyric acid (*GABA*) genes. In this study, we have instead investigated neuronal nicotinic receptor (*CHRN*) subunit genes, which encode nicotinic acetylcholine receptors (Mineur and Picciotto, 2008). These receptors are implicated biologically in alcohol consumption. Alcohol causes an increase in the neurotransmitter acetylcholine (Ericson et al., 2003). Acetylcholine activates nicotinic receptors, which in turn modulate the dopaminergic activation that is associated with drug reward response (Hendrickson et al., 2010; Soderpalm et al., 2009). In addition to many associations with nicotine dependence and smoking behaviors, *CHRN* subunit genes, particularly *CHRNA4* and *CHRNB2*, have also been implicated in alcohol and other substance use, and *CHRN* gene variants may contribute to both conditions (Hoft et al., 2009; Schlaepfer et al., 2008a,b; Tuesta et al., 2011; Wang et al., 2009).

The parent study for this project is a longitudinal study of cigarette and other substance use in adolescents and young adults (Social and Emotional Contexts of Adolescent Smoking Patterns [SECASP] study; Dierker and Mermelstein, 2010; Selya et al., 2013). The relations between *CHRN* genes and smoking heaviness in this cohort have been reported previously (Cannon et al., 2013). Given the evidence for *CHRN* associations with smoking heaviness in youth (Cannon et al., 2013; Ducci et al., 2011; Rodriguez et al., 2011), the *CHRN* associations with alcohol (Hendrickson et al., 2010; Hoft et al., 2009; Tuesta et al., 2011), the comorbidity between cigarette and alcohol use in adolescents (Kendler et al., 2008), and tests of independent associations between *CHRN* genes and alcohol and smoking phenotypes are warranted.

MATERIALS AND METHODS

Subjects

The subjects in this study were participants in the SECASP study. The SECASP cohort (N = 1,263) was recruited from December 2004 through 2006 from 12,970 9th- and 10th-grade students in the greater Chicago area who were screened regarding tobacco use. The mean age at entry into the study was 15.6 years (range: 13.9 to 17.5 years). At the year 5 assessment, which is the focus of this study, the mean age was 21.4 years (range 19.2 to 23.7 years). Students were selected to create a high-risk sample for smoking, although the study also included a random sample of students who had never smoked or who had tried smoking more than 12 months prior to screening. SECASP participants have been followed longitudinally into young adulthood. After a baseline assessment, subsequent assessments occurred 6 and 15 months and 2, 3, 4, and 5 years later. Participants contacted during the 5th-year measurement wave (N = 1,027 = 81% of the total cohort) were asked to volunteer to participate in the genetic arm of the project and provide a saliva sample for DNA extraction, and 953 (93%) of those asked to volunteer did so.

The SECASP study subjects are representative of the Chicago greater metropolitan high school population in terms of race and ethnicity (56% non-Hispanic Whites, 13% Hispanic Whites, 17% non-Hispanic African Americans, and 14% all other categories). In preliminary analyses, we found highly significant effects of these 4 race categories on our alcohol measures and race/ethnicity differences in genotype frequencies across many of the chosen single nucleotide polymorphisms (SNPs). However, these effects were largely driven by differences between Blacks and other races when compared with the Hispanic and non-Hispanic Whites. To maximize sample size while minimizing basic phenotypic differences and possible genotypic population stratification effects (Pritchard and Rosenberg, 1999), we limited the current analyses to Hispanic (N = 115) and non-Hispanic Whites (N = 587) and included ethnicity as a covariate in all analyses. These 702 subjects were participants who responded that they had ever had a drink on the 5th-year measurement questionnaire. There were 292 males (41.60%) and 410 females (58.40%). Of interest, 294 (41.88%) of these subjects had not smoked at all in the past 30 days at the 5th-year measurement wave. The rest of the cohort for this analysis were light smokers, with an average of 6.45 cigarettes per day (CPD; standard deviation = 5.96, range 1 to 35).

Phenotypes

The frequency of binge drinking was assessed at the year 5 assessment with this question: "During the last 12 months, how often did you have 5 or more drinks (males) or 4 or more drinks (females) containing any kind of alcohol within a 2-hour period?" These amounts and the time period are consistent with the definition recommended by NIAAA as reflecting a drinking episode likely to result in a blood alcohol concentration of 0.08 g/dl or higher. The binge frequency variable was coded as 0 (never in the last year), 1 (1 or 2 days), 2 (3 to 12 days), 3 (2 to 4 days a month), 4 (twice a week), and 5 (more than twice a week). Other correlated measures of drinking quantity and drinking frequency were also studied for genotypic associations (a description of these measures and association results can be found in Tables S2–S4).

Secondary analyses were carried out using smoking status as a covariate. Because we included 294 nonsmokers who used alcohol in this study, we adjusted for a dichotomous yes/no smoking variable. We also determined the quantitative correlation between CPD and binge drinking for the subset of the sample that smoked. In this analysis, the distribution of CPD for the 408 subjects with CPD > 0 was log transformed ($\log_{10}$ [CPD + 1], referred to as log CPD) to improve the distribution in this group of light smokers.

Genotyping

We chose tag SNP markers from the *CHRNA5A3B4* and *CHRNB3A6* gene clusters and the *CHRNA4*, *CHRNB2,* and *CHRNA2* genes (Cannon et al., 2013). A SNP pairwise correlation (r^2) of > 0.64 and a minor allele frequency of > 0.05 were used to group SNPs into linkage disequilibrium (LD) bins (Carlson et al., 2004) using 261 of the 371 European 1000 Genomes Phase 1 individuals (this subset was chosen because they are unrelated). Tag SNP selection was further refined by including potentially functional variants with prior evidence for association with adult smoking

phenotypes (e.g., the *CHRNA5* SNP rs16969968 and the *CHRNB2* 3′ UTR SNP rs2072661; Conti et al., 2008). Additional markers in the *CHRNA5A3B4* and *CHRNB3A6* gene clusters were added to capture finer detail in the LD structures found in these regions. A total of 55 SNPs were included in this study (see Table S1).

Genomic DNA was purified from saliva samples collected with Oragene OG-500 self-collection kits (DNA Genotek, Inc., Kanata, Ontario, Canada) under the supervision of the field study team. DNA was isolated according using Gentra PureGene Blood kit reagents (Qiagen, Valencia, CA) following the manufacturer's instructions for purification from body fluids. Genotyping was carried out using a combination of fluorescent dideoxy-DNA sequencing, TaqMan SNP genotyping, and Fluidigm SNPtype assays (Cannon et al., 2013). The mean call rate was 0.96 (range = 0.90 to 1.00), and all SNPs reported in this study passed a Hardy–Weinberg equilibrium filter at $p > 0.05$ and 2 SNPs at $p > 0.01$.

In this study, we chose to analyze Hispanic and non-Hispanic Whites in our cohort together, partly due to a lack of significant effects of ethnicity on our phenotype of interest (see Results). We also demonstrate that the SNPs that we chose for our most significant gene finding (*CHRNA4*) have similar allele frequencies in Hispanic and non-Hispanic Whites (Fig. S1) and tag-related high-frequency haplotypes in non-Hispanic Whites (Fig. S2). Finally, we show similar substantive results stratified by ethnicity (Table S5).

Analyses

We explored descriptive characteristics of the sample and phenotypes using the SAS software package (www.sas.com). For genetic analyses, we used PLINK (http://pngu.mgh.harvard.edu/purcell/plink; Purcell et al., 2007). This software allows tests of sets of SNPs and individual SNPs accounting for covariates and provides calculation of empirical *p*-values. Phenotypes were analyzed using a quantitative model, including tests of both additive and nonadditive allele effects. Empirical significance testing was carried out using simulations assuming the most significant model; in all cases, this was the additive model. All analyses were adjusted for chronological age, gender, and ethnicity. Secondary analyses were adjusted additionally for smoking status to determine independence of genetic associations from this basic smoking phenotype.

Initial global explorations of gene associations with frequency of binge drinking were carried out based on the set of SNPs defined by each gene or gene cluster, followed by more detailed univariate tests of individual SNPs within a set if the global set test was significant. Five SNP sets were created: (i) 5 SNPs in the *CHRNA4* gene, (ii) 7 SNPs in *CHRNB2* gene, (iii) 12 SNPs in *CHRNA2* gene, (iv) 12 SNPs in the *CHRNB3A6* gene cluster, and (v) 19 SNPs in the *CHRNA5A3B4* gene cluster (see Table S1). The SNP set tests began with calculation of LD among SNPs within a set, excluding a SNP from a set if LD was above $r^2 = 0.5$. Within each set, univariate analyses were then performed with each SNP to determine association with binge frequency using a linear model with specified covariates. Five or fewer SNPs within each set were selected by PLINK with univariate *p*-values <0.05, with the most significant SNP selected first, then other SNPs included in order of decreasing significance, after removing SNPs in LD with any previously selected SNPs. The set statistic was then calculated as the mean of these best single SNP statistics; 10,000 permutations of the data were performed by flipping alleles of each subject with a 50:50 probability. The LD between SNPs was kept constant by applying the same 50:50 flip choices to all SNPs within the same permutation replicate. The association analysis was repeated for each simulated replicate. An empirical *p*-value for the set statistic was then defined as the number of times the permuted set statistic exceeded the original statistic for the set. The set tests provided a single empirical *p*-value based on a multivariate estimate of gene (or gene cluster) independent of number of SNPs tested and controlling for correlation among the SNPs within the set.

After performing these global tests of each of the 5 sets of SNPs, we pursued detailed further univariate SNP analyses with sets where the global test resulted in significance of $p < 0.05$. Univariate tests of SNPs within these sets were explored using linear modeling in PLINK. Empirical significance statistics based on 10,000 simulations are also reported for these univariate SNP analyses. Finally, we used SAS general linear models to calculate least squares means of binge frequency by genotype, adjusted for effects of age, gender, and ethnicity to determine directions of the genetic effects. Additional analyses of correlated drinking quantity and drinking frequency measures using set tests and univariate tests are presented in Tables S2–S4. The supplementary data also present results within the Hispanic and non-Hispanic ethnic groups in Table S5 and Figs S1 and S2.

Effect sizes of SNP effects were calculated using the f^2 statistic (Cohen, 1988), which reflects the magnitude of the additional variance explained (R^2) by the SNP in a model including the other covariate predictors, as follows:

$$f^2 = (R^2_{SC} - R^2_C)/(1 - R^2_{SC}),$$

where R^2_{SC} is the variance explained by the SNP and the covariates, and R^2_C is the variance explained by only the covariates. We also explored the association of significance and effect size for each SNP and its LD with the most significant SNP in the set.

RESULTS

Descriptive Results

Table 1 shows the descriptive phenotypic characteristics of the 702 individuals in this study (for details regarding quality control statistics of the SNPs used in this study, see Cannon et al., 2013). Of the sample, 45.3% reported binging at least twice a month, and 18.4% binged twice a week or more. In a model predicting binge frequency using age, gender, ethnicity, and dichotomous smoking/nonsmoking status, both gender and smoking status were significant, although age and ethnicity were not. Binge drinking was more frequent among males. Binge drinking was also more frequent among smokers than nonsmokers (see Fig. 1). However, the phenotypic correlation between binge frequency and our quantitative smoking measure (log CPD) was −0.02 (NS) for the subset of 408 alcohol-using subjects who smoked at least 1 cigarette in the 30-day period.

SNP Analyses

The SNP set test adjusting for age, gender, and ethnicity as covariates was significant only for *CHRNA4* (Set 1), $p = 0.01$. Including smoking status as an additional covariate did not change the *CHRNA4* set effect ($p = 0.01$), suggesting that the *CHRNA4* association with the frequency of binge drinking is independent of the significant phenotypic relationship between smoking status and binge drinking. The *p*-values for other sets adjusted for age, gender, and ethnicity were all nonsignificant, *p*-values more than 0.15. When

Table 1. Descriptive Characteristics of 702 Hispanic and Non-Hispanic White Participants Who Ever Had an Alcoholic Drink at Assessment Year 5 of the Study

Variable	Mean (SD, range)	Frequency
Hispanic only, $N = 115$ (16.38% of sample)		
Age	21.43 (0.86, 19.8 to 23.7)	
Gender		40.0% male
Smoking status		55.7% smokers
Binge frequency (qualitative)[a]		0:10.4%, 1:21.7%, 2:24.4%, 3:26.2%, 4:13.9%, 5:4.4%
Binge frequency (quantitative)	2.24 (1.34, 0 to 5)	
Non-Hispanic only, $N = 587$ (83.62% of sample)		
Age	21.44 (0.82, 19.2 to 23.7)	
Gender		41.9% male
Smoking status		58.6% smokers
Binge frequency (qualitative)[a]		0:12.1%, 1:17.6%, 2:24.7%, 3:27.3%, 4:12.6%, 5:5.8%
Binge frequency (quantitative)	2.28 (1.37, 0 to 5)	
All subjects, $N = 702$		
Age	21.44 (0.83, 19.2 to 23.7)	
Gender		41.6% male
Smoking status		58.1% smokers
Binge frequency (qualitative)[a]		0:11.8%, 1:18.2%, 2:24.6%, 3:26.9%, 4:12.8%, 5:5.6%
Binge frequency (quantitative)	2.27 (1.37, 0 to 5)	

Results from linear phenotypic models predicting binge frequency

Subsample	F for age (*p*-value)	F for gender (*p*-value)	F for smoking status (*p*-value)	F for ethnicity (*p*-value)
Hispanic only	1.53 (NS)	4.12 (0.05)	11.21 (0.001)	–
Non-Hispanic only	0.35 (NS)	20.77 (<0.0001)	16.07 (<0.0001)	–
All subjects	1.01 (NS)	25.73 (<0.0001)	24.84 (<0.0001)	0.02 (NS)

[a]Binge frequency = frequency in the past year of having 5 or more drinks (males) or 4 or more drinks (females) containing any kind of alcohol within a 2-hour period. Coding was 0 (never in the last year), 1 (1 or 2 days), 2 (3 to 12 days), 3 (2 to 4 times a month), 4 (twice a week), and 5 (more than twice a week).

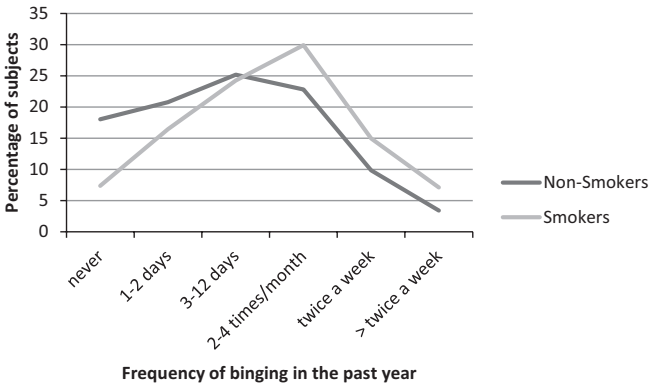

Fig. 1. Distribution of the binge frequency variable by smoking status.

additionally adjusted for smoking status, results remained nonsignificant, although we note that in these analyses the *CHRNB2* set test resulted in a *p*-value of 0.07, suggesting a possible weak effect of this gene. Set tests with other correlated alcohol consumption phenotypes described in Table S2 gave similar SNP results for drinking frequency measures (see Table S3), although effects were weaker than those seen for the frequency of binge drinking. Set tests within the Hispanic and non-Hispanic White groups were also similar (Table S5).

The *CHRNA4* SNP set showed a significant global test, so we performed detailed univariate analyses by SNP, adjusted for covariate effects as described above (see Table 2). All *p*-values presented in this table were again empirically derived using 10,000 permutations of the data. Primary age-gender- and ethnicity-adjusted results are presented, together with results adjusted additionally for smoking status. Smoking status did not substantively affect the results. Univariate SNP associations with other correlated measures of drinking frequency and quantity are shown in Table S4. For *CHRNA4*, the minor allele was associated with an increase in the frequency of binging, with differences between means of the major allele and minor allele homozygotes of about one-third to one-half of a standard deviation (Fig. 2). SNPs rs4809538, rs4522666, and rs1044396 all remain significant if a Bonferroni adjustment is made for multiple testing. While significant, effect sizes range from 0.02 to 0.03, classified as "small" effects (Cohen, 1988), explaining an additional 2 to 3% of the variance in the phenotype.

The most significant SNP in this set was rs4522666 ($p = 0.0003$, effect size = 0.03). The significance and effect sizes for other SNPs were directly related to the strength of LD with rs4522666. The SNP rs1044396 was the next most significant and has the highest LD with rs4522666 ($r^2 = 0.40$ in non-Hispanic Whites, and $r^2 = 0.25$ in Hispanic Whites). The other 3 SNPs all have lower significance and also proportionally lower LD with rs4522666. In descending order of significance, the LD with rs4522666 for rs4809538, rs755203, and rs2236196 was $r^2 = 0.25$, $r^2 = 0.22$, and $r^2 = 0.14$ in non-Hispanic Whites, and $r^2 = 0.14$, $r^2 = 0.13$, and $r^2 = 0.06$ in Hispanic Whites.

Table 2. Univariate Associations of Frequency of Binge Drinking with Single Nucleotide Polymorphisms (SNPs) in *CHRNA4*

SNP	Location[a]	MAF [b]	*p*-Value (adj. age, gender, ethnicity)	*p*-Value (adj. age, gender, ethnicity, smoking status)	Size of SNP effect (f^2)
rs4809538	61,440,620	0.20	0.01***	0.008***	0.02
rs4522666	61,444,924	0.37	0.0003***	0.0003***	0.03
rs2236196	61,448,000	0.31	0.02	0.04	0.02
rs1044396	61,451,578	0.47	0.002***	0.005***	0.03
rs755203	61,464,708	0.43	0.02	0.03	0.02

[a]Locations are based on dbSNP build 137 (hg19).
[b]Minor allele frequency observed.
***Significant at $p < 0.05$ if a Bonferroni adjustment is made based on 5 SNP tests.

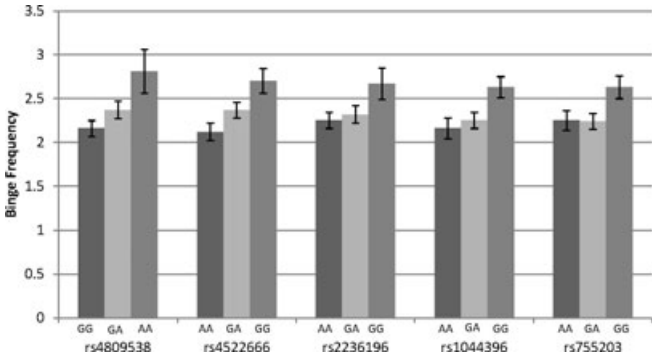

Fig. 2. Least squares means by genotype of the binge frequency variable, adjusted for age, gender, and ethnicity. Major/minor alleles for each single nucleotide polymorphism were rs4809538 (G/A), rs4522666 (A/G), rs2236196 (A/G), rs1044396 (A/G), and rs755203 (A/G). Frequency of binge drinking was coded as follows: 0 (never in the last year), 1 (1 or 2 days), 2 (3 to 12 days), 3 (2 to 4 times a month), 4 (twice a week), and 5 (more than twice a week).

DISCUSSION

This work presents a significant association between SNPs in *CHRNA4* and the frequency of alcohol binging in 702 Hispanic and non-Hispanic White young adults who are participants in the SECASP longitudinal study of nicotine (Dierker and Mermelstein, 2010; Selya et al., 2013). We chose to focus on this measure for its impact on public health (CDC-MMWR 2012, 2013), its association with alcohol dependence, and its potential as a genetic trait (Kendler et al., 2010). Several correlated measures of alcohol quantity and frequency also showed associations with *CHRNA4* (see detailed results in Tables S3 and S4), although the strongest findings occurred with binge frequency. We analyzed Hispanic and non-Hispanic Whites together because this ethnic distinction did not significantly impact the binge frequency phenotype. In addition, the SNPs that we chose for *CHRNA4* tag the same underlying haplotype structure for the Hispanic and non-Hispanic Whites (see Fig. S2). Substantive results were similar when analyses were carried out stratified by ethnicity (Table S5).

Somewhat surprisingly, our study identified a genetic effect of *CHRNA4* on alcohol binge frequency independent of smoking. While we demonstrated a significant phenotypic association between binge frequency and smoking status in our study cohort, adjustment for smoking did not substantively affect the genetic association with *CHRNA4*. This result suggests that the genetic association is independent of the well-documented association between smoking and alcohol use. This independence is also consistent with previous results in our cohort showing genetic associations between cigarette use and SNPs in *CHRNB3A6, CHRNA5A3B4,* and *CHRNA2*, but not *CHRNA4* or *CHRNB2* (Cannon et al., 2013). Conversely, the present study of this cohort focusing instead on alcohol found no association with SNPs in *CHRNB3A6, CHRNA5A3B4*, or *CHRNA2*, but a robust association with *CHRNA4*. We acknowledge a modest increase in the chance of observing a positive result with our *CHRN* SNPs by testing 2 phenotypes in our cohort (cigarette use and binge frequency).

Our SNP effect sizes were small, explaining 2 to 3% of the variance in frequency of binging. There was a direct relationship between strength of association and strength of LD with the strongest SNP, rs4522666, suggesting that these other results are secondary to the rs4522666 result. In Tables S2–S4, significant associations were again found between other measures of drinking frequency and *CHRNA4,* but evidence was not as strong as that found for frequency of binging. For all of the SNP associations with *CHRNA4* in our cohort, the minor alleles were associated with greater frequency of binging, resulting in an increase of about one-third to one-half of a standard deviation from the major allele homozygotes to the minor allele homozygotes.

Previous studies of human subjects reveal an interesting mix of evidence for effects of *CHRNA4* on alcohol and nicotine phenotypes. A study of Korean adult alcoholics revealed an association with *CHRNA4*, in addition to significant effects of several other candidate genes (Kim et al., 2004). Focusing on the subjective responses to nicotine and alcohol, Ehringer and colleagues (2007) showed a modest *CHRNA4* association with alcohol (but not nicotine) in Caucasian adolescents recruited from substance use treatment centers, the criminal justice system, and community-based studies. Subsequent studies from this research group using this cohort have gone on to reveal modest associations between *CHRNA4* and adolescent nicotine dependence (Kamens et al., 2013). Similar associations between *CHRNA4* and nicotine dependence have been reported in studies of adults

(Breitling et al., 2009; Feng et al., 2004; Li et al., 2005). Several studies have also demonstrated associations between *CHRNA4* and subjective effects of nicotine (Picciotto et al., 1998; Tapper et al., 2004). Many of these associations involve high-frequency SNPs within the same LD bins measured in this study, including rs1044396 and rs2236196. The SNPs rs4522666 and rs2236196 are both located in the *CHRNA4* 3′ UTR, and functional studies have implicated rs2236196 in gene expression levels (Hutchison et al., 2007). The SNP rs4522666 has not been associated with smoking phenotypes, but in an exploratory analysis of risk-taking constructs it was associated with harm avoidance in young adults (Roe et al., 2009).

Possible clues to the potential mechanisms underlying these various associations may lie in the animal and drug response studies. Focusing on alcohol, animal models have demonstrated that nicotinic receptor genes moderate the ethanol-induced release of dopamine (Larsson et al., 2005; Soderpalm et al., 2000), and ethanol may also interact directly with the function of nicotinic acetylcholine receptors (Liu et al., 1994; Wood et al., 1995). In particular, animal studies of nicotinic receptors containing the alpha4 subunit have shown a significant relationship with the reward response to alcohol (Liu et al., 2013) and to alcohol withdrawal (Butt et al., 2004). Further evidence is provided in recent animal studies of varenicline, an alpha4–beta2 nicotinic receptor partial agonist used in smoking cessation. This drug reduces not only nicotine dependence, but also alcohol consumption in rats (Steensland et al., 2007). Hendrickson and colleagues (2010) showed that this effect depends specifically on activation of alpha4 nicotinic receptors. In a mouse knockout line that did not express alpha4, varenicline did not reduce alcohol consumption. Conversely, in another mouse line that overexpressed alpha4, a very low dose of varenicline that was not efficacious in wild-type animals dramatically reduced alcohol consumption.

Human studies of the alpha4–beta2 partial agonist varenicline have again implicated alpha4 in alcohol consumption. Epidemiological data have shown that varenicline is associated with significant reduction in drinking as compared with nicotine replacement or no smoking cessation medication (McKee et al., 2013). Several controlled trials in humans have also demonstrated significant decreases in alcohol consumption in smokers (Fucito et al., 2011; McKee et al., 2009; Mitchell et al., 2012).

Reward response may provide an important common denominator in the *CHRNA4* genetic findings for alcohol and nicotine. While associations in our cohort with variables reflecting frequency of use showed independence between alcohol and nicotine, it is possible that a future focus on phenotypes that reflect reward response may reveal associations across substances. Additional phenotypes may provide further insights into these potential mechanisms. Field and colleagues (2013) have reported a significant correlation between alcohol binging, smoking, and obesity in adolescents. Consistent with this observation, Landgren and colleagues (2009) report an interesting association with *CHRNA4* and obesity, particularly within heavy alcohol users. This result may not be surprising given that the cholinergic dopaminergic reward effect seen with alcohol can also be seen with food (Larsson et al., 2005). Associations between *CHRNA4* and attention deficit hyperactivity disorder (Todd et al., 2003) and cognitive attention (Greenwood et al., 2012) may suggest yet other mechanisms underlying the relationship between *CHRNA4* and frequency of binging. The data in the SECASP longitudinal study will allow future genetic explorations of additional phenotypes related to reward response for both nicotine and alcohol. In addition, it will be possible to explore dependence of associations on correlated measures of temperament, such as impulsivity.

Limitations

Because of the longitudinal nature of SECASP, it was not feasible to ascertain the very large sample sizes of many contemporary genetic association studies. It is possible therefore that we may have missed effects due to lack of statistical power. To minimize this limitation, we focused on a candidate gene system with prior evidence of involvement in substance use, restricting the number of tests performed. We also note that our sample detected the small effects of these SNPs. While we may have missed other SNP effects, such effects would have to have explained less than 2% of the variance in the phenotype and may therefore be of lower interest as contributing risk factors. In addition, our findings require replication in other samples and, in particular, in samples with subjects of other ethnicities/races.

Summary

These findings suggest that variants in *CHRNA4* may contribute to risk of binge drinking, although confirmation of this association awaits replication in independent samples. Further study of this association could be important in the understanding and control of this significant alcohol behavior. Our longitudinal sample cohort will allow us to test for associations with other related phenotypes and to determine whether the strength of these associations weakens or strengthens over time.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Average genotypic frequency differences by race/ethnicity.

Fig. S2. 1KGP haplotypes, zoomed view at *CHRNA4*.

Table S1. List of all SNPs tested in this study, organized by gene or gene cluster and by chromosomal location.

Table S2. Descriptive characteristics of 702 participants with alcohol phenotypes.

Table S3. *p*-Values from PLINK multivariate tests of sets of SNPs defined by each nicotinic receptor gene or gene cluster with 2 levels of covariate adjustment.

Table S4. Individual univariate SNP tests with correlated alcohol quantity and frequency variables for *CHRNA4* for maximum frequency and drink frequency.

Table S5. Set tests for binge frequency by Hispanic ethnicity.