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Family-Based Association Analysis of Alcohol Dependence Criteria and Severity

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Background: Despite the high heritability of alcohol dependence (AD), the genes found to be associated with it account for only a small proportion of its total variability. The goal of this study was to identify and analyze phenotypes based on homogeneous classes of individuals to increase the power to detect genetic risk factors contributing to the risk of AD.

Methods: The 7 individual DSM-IV criteria for AD were analyzed using latent class analysis (LCA) to identify classes defined by the pattern of endorsement of the criteria. A genome-wide association study was performed in 118 extended European American families ($n = 2,322$ individuals) densely affected with AD to identify genes associated with AD, with each of the 7 DSM-IV criteria, and with the probability of belonging to 2 of 3 latent classes.

Results: Heritability for DSM-IV AD was 61% and ranged from 17 to 60% for the other phenotypes. A single nucleotide polymorphism (SNP) in the olfactory receptor *OR51L1* was significantly associated (7.3×10^{-8}) with the DSM-IV criterion of *persistent desire to, or inability to, cut down on drinking*. LCA revealed a 3-class model: the “low-risk” class (50%) rarely endorsed any criteria and none met criteria for AD; the “moderate-risk” class (33%) endorsed primarily 4 DSM-IV criteria and 48% met criteria for AD; and the “high-risk” class (17%) manifested high endorsement probabilities for most criteria and nearly all (99%) met criteria for AD. One SNP in a sodium leak channel *NALCN* demonstrated genome-wide significance with the high-risk class ($p = 4.1 \times 10^{-8}$). Analyses in an independent sample did not replicate these associations.

Conclusions: We explored the genetic contribution to several phenotypes derived from the DSM-IV AD criteria. The strongest evidence of association was with SNPs in *NALCN* and *OR51L1*.

Key Words: Alcohol Dependence Criteria, Latent Class Analysis, Family-Based Association, GWAS.

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ALCOHOL DEPENDENCE (AD) is one of the most common and costly public health problems in the United States and throughout the world, affecting 4 to 5% of the U.S. population in a 12-month period (Kessler, 2005; Li et al., 2007) and 12.5% across the lifetime (Hasin et al., 2007). Family, twin, and adoption studies have provided convergent evidence for the role of genetic factors in AD (Goodwin et al., 1974; Heath et al., 1997). Approximately 40 to 60% of the total variance in risk of AD is due to heritable influences (Prescott et al., 2006; Schuckit et al., 2001).

To date, a few genes have been shown to be associated with AD, including *ADH1B*, *ALDH2*, *ADH4*, *CHRNA5*, *CHRM2*, *GABRA2*, and *GABRG1* (Agrawal et al., 2012; Covault et al., 2008; Edenberg and Foroud, 2006; Edenberg et al., 2004, 2006; Enoch, 2008; Enoch et al., 2009; Luo et al., 2005; Wang et al., 2004, 2009); however, the variation in each of these genes accounts for only a small portion of the vulnerability to AD. This could be due to the study design, the gene coverage, or most likely the complex etiology and heterogeneity of AD.

The DSM-IV diagnosis of AD requires the manifestation of 3 or more of the following criteria at any time during the

same 12-month period: *tolerance, withdrawal, using alcohol more than intended, a persistent desire to cut down or inability to do so, spending a great deal of time obtaining alcohol or recovering from its effects, giving up activities to drink, and continuing alcohol use despite physical or psychological problems (DSM-IV)* (American Psychiatric Association, 1994). The requirement of *any 3* inherently creates a group of AD individuals that is clinically and likely genetically heterogeneous. Therefore, approaches that focus on potentially more homogeneous subgroups may increase the power to identify novel genetic variants by reducing the clinical (and therefore possibly the genetic) heterogeneity.

We used several complementary approaches to examine the genetic contributions to the risk of AD. We first analyzed the dichotomous phenotype of AD, because it is the most widely studied phenotype and most directly reflects the clinically relevant trait. We then examined each of the DSM-IV criteria for dependence individually, as one approach to reduce heterogeneity. We also employed latent class analysis (LCA) to identify subgroups of individuals defined on the basis of their pattern of endorsement of DSM-IV criteria and used the probability of class assignment as a quantitative phenotype for analysis. This approach may also reduce heterogeneity and increase the power to identify genetically similar subgroups.

We utilized a genome-wide approach to detect common genetic variants associated with the phenotypes. Unlike most studies that employ a case-control design, we performed a family-based genome-wide association study (GWAS) using extended families densely affected by AD. The sample was limited to European American families to reduce one source of potential heterogeneity.

MATERIALS AND METHODS

Sample

Alcoholic probands were recruited from inpatient and outpatient treatment facilities through 6 sites participating in the Collaborative Study on the Genetics of Alcoholism (COGA) (Begleiter et al., 1995; Foroud et al., 2000). The probands and their family members were administered a validated poly-diagnostic interview, the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) (Bucholz et al., 1994; Hesselbrock et al., 1999). Individuals below the age of 18 were administered an adolescent version of the SSAGA. Institutional review boards at all sites approved the study.

A subsample of COGA families was selected to maximize the contribution of genetic variants to the risk of AD. All families recruited by COGA were reviewed and prioritized based on, in preferential order: (i) the largest number of AD family members with DNA; (ii) the largest number of family members with electrophysiology data and DNA; and (iii) the largest number of family members with DNA regardless of other phenotypes. The final sample consisted of 118 large multiplex European American families, with DNA available from 2,322 individuals.

Phenotypes and Statistical Analysis

Phenotypic information of the members of these 118 families was collected using the SSAGA, with some individuals having multiple

assessments across time. If an individual was interviewed more than once, data from the SSAGA interview with the maximum total number of endorsed DSM-IV AD criteria were utilized.

Only individuals who reported drinking at least once per month for 6 months or more at any evaluation (regular drinking) were included in the analysis. Subjects who were 15 years or older and met DSM-IV criteria at any evaluation were classified as AD ($n = 687$), and individuals who were 23 years or older and did not meet criteria for DSM-IV AD were classified as unaffected ($n = 773$). All other individuals were classified as unknown ($n = 326$), including self-reported AD children at a very early age (<15 years) and unaffected individuals who were younger than 23 years and thus had not fully passed through the primary age of risk of AD and therefore might still develop it. These 326 individuals were included in analyses of all other phenotypes.

LCA was conducted to identify subgroups of individuals based on the pattern of endorsement of the 7 DSM-IV AD criteria (Muthén and Muthén, 1998–2011). LCA may be viewed as a version of nonparametric cluster analysis and makes the assumption of conditional independence (i.e., endorsement of one criterion within a class is uncorrelated with other criteria). There are 2 important estimates recovered from LCA: (i) the prevalence of individual classes of individuals with similar item endorsement profiles; and (ii) the likelihood of endorsing an item conditional on class membership. The Bayesian information criteria (BIC) and entropy (reflecting level of misclassification) were utilized to determine which model (i.e., number of latent classes) provided the best fit to the data.

A critical assumption of LCA is conditional independence, and violations can result in extraction of more classes than are required. To address this concern, we examined a factor mixture model where a single factor was nested within each latent class, relaxing the conditional independence assumption. The fit of this model was compared with the LCA. As the objective of this study was to classify individuals (i.e., person-centered) rather than symptoms (i.e., item-centered), we did not conduct factor analyses in the absence of LCA.

Based on the best-fitting model, class membership was utilized in a 2-step process. First, an individual was assigned to the latent class with the highest probability, and discrete class memberships were compared using post hoc validators such as gender, age at onset of regular drinking (from the first interview in which the individual began drinking regularly), and DSM-IV AD. Interpretation of the classes was based on the validators. Gender and DSM-IV AD differences between classes were assessed with a chi-squared test; number of DSM-IV criteria, age, and age at onset of regular drinking were tested using an analysis of variance, with gender and birth cohort as covariates. Tukey-adjusted *t*-tests were employed to test for pairwise differences when there was a significant ($p < 0.05$) main effect of latent class assignment.

To optimize the power for genetic analyses, we constructed secondary continuous phenotypes based on the probability of assignment to each of the 3 latent classes. As a result, information describing each latent class provided an interpretation of the 3 classes, while the probability of being in each class was employed as the phenotype in analysis. This approach avoids multiple pairwise comparisons of the 3 classes in analyses, avoids misclassification inherent in assigning individuals to a particular latent class, maximizes the number of subjects, and was shown previously to improve power to detect genetic association (Bureau et al., 2011).

Heritability was estimated using the polygenic option in SOLAR (Almasy and Blangero, 1998) for the following phenotypes: DSM-IV AD, each individual DSM-IV criterion, the 3 continuous phenotypes based on the LCA probabilities, and membership in each latent class. Sex and birth cohort, defined by year of birth (<1930, 1930 to 1949, 1950 to 1969, and ≥ 1970), were used as covariates for all heritability calculations to account for secular trends (Gruzca et al., 2008) in alcohol use and dependence.

Genotyping and Association Analysis

Genotyping for 2,105 subjects in these 118 families was performed at the Genome Technology Access Center at Washington University School of Medicine in St. Louis using the Illumina Human OmniExpress array 12.VI (Illumina, San Diego, CA). In addition, genotypes previously generated on the Illumina Human 1M-Duo BeadChip (Illumina) by the Center for Inherited Disease Research were also included for 224 subjects from these families (Edenberg et al., 2010). For quality control purposes, 51 of the 2,105 subjects were genotyped on both arrays and available for analysis, prior to sample exclusions ($n = 7$) due to Mendelian inconsistencies.

Quality control was first performed on genotype data generated from the OmniExpress. Genotypes for individuals with a genotype rate <98% were excluded from analysis, and single nucleotide polymorphisms (SNPs) with a genotyping rate <98% were excluded from the analysis. Further details can be found in Kang and colleagues (2012). Genotypes of the 51 overlapping individuals were then compared. There were 571 of 544,276 SNPs genotyped on both arrays with 2 or more nonmissing and different genotypes, which were excluded from all further analyses. There were 442 genotyped founders used to remove SNPs, which violated Hardy–Weinberg equilibrium ($p < 10^{-6}$). SNPs with minor allele frequency <5% were also removed from further analysis. After combining the data generated from the 2 arrays in the members of these 118 families, pedigree structure was reviewed and modified, as described in Kang and colleagues (2012).

The family-based generalized disequilibrium test, which employs information from all discordant relative pairs (Chen et al., 2009), was used to test for association with the qualitative traits: AD and each DSM-IV criteria. A linear mixed-effects model was employed using the kinship library (lmekin) implemented in R (<http://www.inside-r.org/packages/cran/kinship/docs/print.lmekin>) to test for association with the quantitative traits reflecting probability of membership in the latent classes. The linear mixed-effects model in the kinship function allows for the covariance matrix to be completely specified for the random effects. The result is that each family has a different covariance pattern based on the kinship coefficients, to model the familial genetic random effects. Sex, age at last interview, and birth cohort as well as the first principal component from the EIGENSTRAT (Price et al., 2006) analysis were evaluated as potential covariates. Any significantly associated covariate ($p < 0.05$) was included in all final association models. As described in Wang and colleagues (in press), imputed data were generated using BEAGLE 3.3.1 (Browning and Browning, 2009) in the chromosomal regions providing the greatest evidence of association with the genotyped SNPs.

Replication Study

The Study of Addiction: Genetics and Environment (SAGE) was used as an independent replication sample to evaluate the SNPs showing the greatest evidence of association in the COGA sample. This is a case-control sample derived from 3 large complementary studies: COGA, the Family Study of Cocaine Dependence, and the Collaborative Genetics Study of Nicotine Dependence (Bierut et al., 2010). The 129 individuals who overlapped between the 118 pedigrees and SAGE were removed from the replication data set. The remaining SAGE samples included 2,647 individuals of European American descent. AD and individual criteria were obtained from SSAGA data. Imputed dosage data were obtained using the same method as described above. PLINK (Purcell et al., 2007) was used in all analyses, employing age at interview and gender as covariates.

Results from LCA were derived separately in this sample using Mplus, as described above. LCA was also conducted in the SAGE+COGA combined sample and in the SAGE sample including the 129 individuals to examine whether latent class assignment was sample-dependent. This was tested with McNemar's change test.

RESULTS

Sample

Following quality control and the exclusion of some individuals after correction of pedigree inconsistencies, the final analytic sample included 2,322 genotyped individuals from 118 extended families, of which 1,786 individuals met phenotypic criteria for inclusion (Table 1). The average number of genotyped members per family was 19.6, with an average of 5.9 genotyped individuals per family meeting criteria for DSM-IV AD. A total of 707,557 autosomal SNPs passed quality review; 115,772 SNPs were excluded due to a low minor allele frequency (<5%). The final data set consisted of 591,785 SNPs, yielding a genome-wide threshold of $p = 8.45 \times 10^{-8}$. Because the phenotypes examined represent some facet of AD and additional Bonferroni correction of related phenotypes would be unnecessarily stringent, this p -value based on the number of SNPs was considered as the significance threshold.

Latent Class Analysis

The optimum latent class model (by BIC model fitting criteria) was a 3-class model. The sample-size-adjusted (ssadj) BIC decreased from 13,849 for a 3-class model to 13,827 for a 4-class model, with a corresponding drop in entropy of 0.77 to 0.68. The ssadj BIC from the factor mixed model was 13,830, with an entropy of 0.768. Due to the similar results between the 2 models, a more parsimonious 3-class model that assumed conditional independence was used in subsequent analyses.

The criterion endorsement probabilities, conditional on class membership, is shown in Fig. 1 and reveals a range of severity. A low-risk class (50% of individuals) included individuals with negligible-to-low endorsement of the dependence criteria. A moderate-risk class (33% of individuals) was distinguished by individuals with low endorsement probability of *withdrawal*, *gave up activities to drink*, and *time spent obtaining or recovering from the effects of alcohol*, but higher (>0.51) probabilities of endorsing each of the remaining criteria. A high-risk class (17% of individuals) included individuals with high (>0.86) probability of endorsing the 7 dependence criteria.

Table 2A summarizes the characteristics of each of the latent classes and Table 2B provides summary statistics for the DSM-IV criteria for each latent class. As expected, there were significantly more DSM-IV AD individuals ($\chi^2 = 1,222.3$, $df = 2$, $p < 0.0001$) and significantly more males ($\chi^2 = 66.2$, $df = 2$, $p < 0.0001$) in the moderate and high-risk classes. The mean number of DSM-IV criteria was significantly different among the 3 classes, overall $F(6, 1,779) = 2,043.7$, $p < 0.0001$; $p < 0.0001$ for each pairwise comparison, with higher scores in the high-risk class, indicating that classes reflected a continuum of severity as indexed by number of DSM-IV criteria. There was a main effect of latent class for age at regular drinking onset, overall

Table 1. Demographic and Phenotypic Characteristics of the Sample. Percentages ≥ 50 are Italicized. Percentages ≥ 90 are Italicized Bold

DSM-IV criterion	N = # Individuals who endorsed this criterion	% Individuals who endorsed this criterion	% Who endorsed this criterion were male	% Who endorsed this criterion were DSM-IV alcohol dependent	% Who endorsed this criterion were in low-risk latent class	% Who endorsed this criterion were in moderate-risk latent class	% Who endorsed this criterion were in high-risk latent class
Withdrawal	327	18.3	66.1	94.8	0.0	22.3	77.7
Gave up activities to drink	357	20.0	64.7	95.0	0.3	26.3	73.4
Time spent obtaining, using, or recovering from alcohol	427	23.9	63.2	91.6	4.0	30.9	65.1
Drink despite physical/psychological problems	635	35.6	59.7	84.7	1.7	52.6	45.7
Persistent desire to cut down or inability to do so	829	46.4	57.4	71.5	13.0	52.2	34.7
Tolerance	947	53.0	58.9	63.2	28.3	42.6	28.6
Drink more than intended	1,085	60.8	54.9	60.8	23.0	50.3	26.6

$F(6, 1,779) = 83.0, p < 0.0001$; latent class $p < 0.0001$, and a marginal effect for age, overall $F(6, 1,779) = 2,394.0, p < 0.0001$; latent class $p = 0.045$. The low-risk class contained no individuals meeting criteria for AD, although some individuals endorsed *tolerance* (30%) or *drink more than intended* (28%). Individuals in this class began drinking regularly at a significantly older age (19.5 years) than those in the moderate ($p < 0.0001$) or high-risk ($p < 0.0001$) classes. In the moderate-risk class, 66% were AD, with 92% of this class endorsing *drink more than intended*, and more than 50% endorsing *use despite physical/psychological problems* (56%), *persistent desire/inability to cut down* (73%), or *tolerance* (68%). On average, they began regular drinking earlier (17.6 years) than their counterparts assigned to the low-risk class ($p < 0.0001$). The third class was composed primarily of AD (99%) individuals, with the majority endorsing *use despite physical/psychological problems* (98%), *drink more than intended* (98%), or *persistent desire/inability to cut down* (97%). These individuals began drinking at the youngest age (16.8 years) compared with their counterparts in the moderate ($p = 0.01$) and low-risk ($p < 0.0001$) classes. Individuals in this class were significantly older (55.0 years) than individuals in the low-risk (46.3 years, $p = 0.03$) but not the moderate-risk (49.3 years, $p = 0.19$) classes. There were no significant differences in age between the low- and moderate-risk classes ($p = 0.61$) or between the moderate- and high-risk classes ($p = 0.19$).

Genetic Analysis

Heritability was estimated for DSM-IV AD, each of the DSM-IV criteria, and the 3 traits defined by the probability of belonging to each of latent class (Table 3), after covarying for the effects of sex and birth cohort on the phenotype. Heritability for AD was estimated to be 61%. Estimates of heritability for the individual criteria ranged from 59% (*drinking more than intended*) to 29% (*tolerance*). Heritability of membership in the low- and high-risk classes was 52% and 53%.

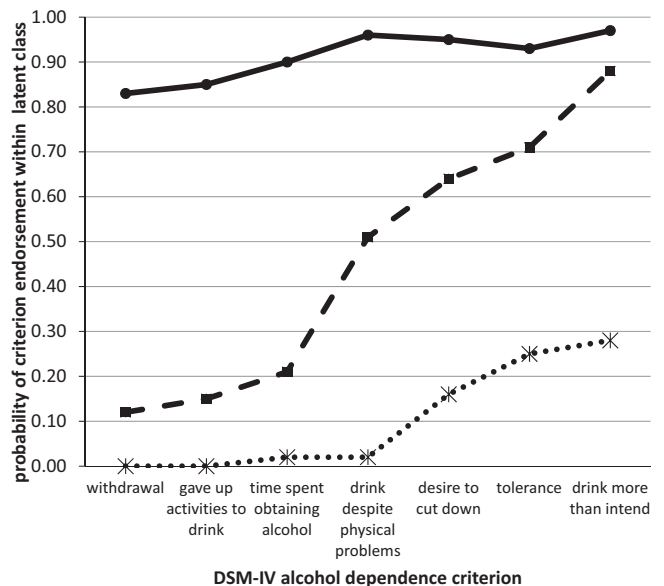


Fig. 1. Average probability of DSM-IV criterion endorsement within each latent class. The x-axis indicates the DSM-IV criteria. The y-axis denotes the probability of endorsing each criterion within each latent class. The low-risk class is depicted with a dotted line. The moderate-risk class is depicted with a dashed line. The high-risk class is depicted with a solid line.

Due to the redundancy of including all 3 probabilities and the low heritability of the moderate-risk class (17%), this phenotype was not included in the GWAS.

Genome-wide results of the association analyses of each phenotype are summarized in Fig. 2, and all associations with $p < 1.0 \times 10^{-6}$ are shown in Table 4; Table S1 contains results for all SNPs with $p < 1.0 \times 10^{-5}$. There was no evidence to suggest inflation of the association p -values ($0.984 < \lambda < 1.01$), validating the choice of the significance threshold. QQ plots are provided for each phenotype in Fig. S1. For DSM-IV AD, the best SNP reached 7.2×10^{-7} .

Two genotyped SNPs reached genome-wide significance ($p < 8.4 \times 10^{-8}$). The most significant result was for a SNP

Table 2. (A) Phenotypic Characteristics of the 3 Latent Classes. Percentages ≥ 50 are italicized. Percentages ≥ 90 are italicized Bold. (B) Summary Statistics Describing DSM-IV Criteria Endorsement for the 3 Latent Classes

(A)												
Latent class	N	% Male	% DSM-IV AD	Age of onset regular drinking (years)	Current age (years)	% Class who endorsed withdrawal	% Class who endorsed gave up activities to drink	% Class who endorsed time spent obtaining/recovering from alcohol	% Class who endorsed use despite physical/psychological problems	% Class who endorsed persistent desire/inability to cut down	% Class who endorsed tolerance	% Class who endorsed drink more than intended
Low-risk	896	40.5	0	19.5	46.3	0.0	0.1	1.9	1.2	12.1	30.5	27.9
Moderate-risk	594	53.0	66.3	17.6	49.3	12.3	15.8	22.2	56.2	72.9	67.9	91.9
High-risk	296	66.6	99.0	16.8	55.0	85.8	88.5	93.9	98.0	97.3	91.6	97.6

(B)				
Latent class	N	Mean # criteria	Standard error	Median # criteria
Low-risk	896	0.74	0.025	1
Moderate-risk	594	3.39	0.014	3
High-risk	296	6.53	0.037	7

N, sample size; AD, alcohol dependence.

on chromosome 13 in *NALCN* (sodium leak channel, nonselective), rs17484734 ($p = 4.1 \times 10^{-8}$; Fig. 3A) for the probability of high-risk membership. This SNP also showed evidence of association with *withdrawal* ($p = 1.3 \times 10^{-7}$). This SNP is in a recombination hot spot and thus is not in linkage disequilibrium (LD) with the surrounding genotyped SNPs. There were no other genotyped SNPs in this region demonstrating association with these phenotypes. One imputed SNP, rs1151384, in moderate LD with rs17484734 ($r^2 = 0.80$) provided supporting evidence of association with both phenotypes ($p \leq 1.4 \times 10^{-6}$ for probability of high-risk membership and *withdrawal*). No other imputed SNPs were in LD with these 2 SNPs ($r^2 < 0.20$).

The DSM-IV criterion *persistent desire/inability to cut down on drinking* also exceeded genome-wide significance thresholds for rs11035102, in the olfactory receptor *OR51L1* (olfactory receptor, family 51, subfamily L, member 1; $p = 7.3 \times 10^{-8}$). Evaluation of imputed SNPs in this region revealed supporting evidence of genome-wide association for rs11035097 ($p = 8.3 \times 10^{-8}$) in addition to moderate association with 6 other SNPs all in high LD with the genotyped SNP ($r^2 \geq 0.80$, all $p < 9.7 \times 10^{-7}$). *OR51L1* is located in a region of chromosome 11 with many other olfactory receptors (Fig. 3B). The genotyped SNP also demonstrated suggestive association with *drinking despite physical problems* ($p = 2.1 \times 10^{-6}$) and with the high-risk latent class probability trait ($p = 4.9 \times 10^{-5}$).

Replication of Association Results

LCA was performed in the SAGE sample with comparable results to the COGA sample, including probabilities of criteria endorsement. When examining class assignments of (i) SAGE subjects in the SAGE+COGA versus the primary SAGE sample and (ii) the 106 overlapping COGA subjects in SAGE (23 individuals were excluded in the COGA analysis due to the restriction on regular drinking), a McNemar's change test demonstrated no significant change in class assignment in the SAGE sample compared with SAGE+COGA nor in the 106 overlapping individuals (all $p > 0.72$). Nine of the 10 SNPs with top association results in Table 4 were genotyped in the SAGE data, and imputed dosage data were used for rs12006002, which had not been genotyped in SAGE. The SNPs did not demonstrate association with any of the phenotypes (all $p > 0.16$).

DISCUSSION

This is the first GWAS to our knowledge that examines each individual criterion of the DSM-IV AD diagnosis as well as phenotypes defined by the probability of latent class assignment. As such, it is the first association study demonstrating genome-wide significance with 1 of the DSM-IV AD criteria as well as with the phenotype derived from the probability of membership in the high-risk class. This suggests that characterizing more homogeneous subgroups of individ-

Table 3. Heritability Estimates and Significance of Covariates

Phenotype	h^2	SE (h^2)	h^2 p -value	Sex p -value	1930 to 1949 cohort p -value	1950 to 1969 cohort p -value	≥ 1970 cohort p -value	r^2
DSM-IV alcohol dependence	0.61	0.08	2.8×10^{-17}	1.6×10^{-24}	2.7×10^{-3}	1.4×10^{-9}	2.4×10^{-10}	0.07
Withdrawal	0.49	0.08	1.5×10^{-11}	5.2×10^{-11}	0.36	0.03	0.28	0.05
Gave up activities to drink	0.50	0.08	6.1×10^{-14}	1.3×10^{-10}	0.11	4.2×10^{-4}	0.77	0.06
Time spent obtaining/using or recovering from alcohol	0.41	0.07	2.4×10^{-12}	3.7×10^{-12}	0.50	1.1×10^{-4}	0.39	0.05
Use despite physical/psychological problems	0.51	0.07	7.8×10^{-17}	2.3×10^{-14}	0.03	4.1×10^{-7}	0.05	0.08
Persistent desire to cut down or inability to do so	0.47	0.06	3.8×10^{-17}	5.8×10^{-16}	0.04	0.04	0.07	0.04
Tolerance	0.29	0.06	6.7×10^{-9}	1.0×10^{-24}	9.1×10^{-3}	8.2×10^{-9}	2.7×10^{-8}	0.05
Drink more than intended	0.59	0.09	1.8×10^{-18}	1.2×10^{-14}	8.7×10^{-6}	5.6×10^{-16}	5.3×10^{-5}	0.06
Probability of assignment to low-risk latent class	0.34	0.04	2.1×10^{-31}	0.09	6.7×10^{-5}	1.4×10^{-16}	3.0×10^{-3}	0.08
Membership in low-risk latent class	0.52	0.06	4.4×10^{-25}	1.9×10^{-17}	6.7×10^{-4}	7.9×10^{-13}	4.2×10^{-3}	0.06
Probability of assignment to moderate-risk latent class	0.12	0.03	2.0×10^{-7}	1.1×10^{-5}	2.6×10^{-3}	1.7×10^{-6}	6.2×10^{-3}	0.02
Membership in moderate-risk latent class	0.17	0.03	1.8×10^{-5}	3.3×10^{-5}	0.03	1.9×10^{-4}	0.025	0.01
Probability of assignment to high-risk latent class	0.19	0.03	2.9×10^{-18}	1.2×10^{-14}	0.24	8.8×10^{-6}	0.58	0.07
Membership in high-risk latent class	0.53	0.08	3.5×10^{-15}	3.5×10^{-12}	0.11	9.9×10^{-5}	0.72	0.07

Sex and birth cohort were included as covariates in all analyses, with <1930 as the reference cohort. r^2 = proportion of variance accounted for by the covariates. Significant heritabilities ($p < 0.05$) are bolded.

uals, defined either by a specific AD criterion or by the probability of belonging to a latent class, increased the power to detect genetic association relative to the diagnosis of AD, a particularly heterogeneous phenotype. For example, estimated power was equivalent for the 2 phenotypes DSM-IV and *persistent desire/inability to cut down*, yet one of the strongest association results was with the *persistent desire/inability to cut down*, $p = 7.3 \times 10^{-8}$, while there was modest evidence of association with DSM-IV ($p = 1.5 \times 10^{-5}$; Table S1).

We estimated the heritability of AD, the individual DSM-IV criteria, as well as 2 continuous phenotypes based on the LCA probabilities. The estimated heritability for AD (61%) was similar to previous reports (Prescott et al., 2006). However, the heritability estimates for the individual DSM-IV criteria were slightly larger than previously reported (Slutske et al., 1999). This may reflect the effects of shared environment, which is known to inflate the estimate of the genetic contribution to the trait (Tenesa and Haley, 2013). Although the phenotypes based on the probabilities of class assignment were less heritable ($\leq 34\%$), actual membership in each class was more heritable ($\geq 52\%$). This may be related to the entropy such that misclassification may have favored the assignment of related individuals into the same class. The less heritable moderate-risk class (17%) could be a result of environmental influences and therefore was not examined further.

As seen in Table 4, the individual DSM-IV criteria provided stronger evidence of association than DSM-IV AD. This suggests that individuals who endorse a specific criterion may have a more common genetic etiology compared with the heterogeneous group of AD individuals, who are diagnosed by endorsing different combinations of DSM-IV criteria. Furthermore, association was observed with a quantitative trait derived from the probability of belonging to the high-risk latent class, despite its lower heritability estimate

compared with the heritability of AD. This is consistent with Kuo and colleagues (2008), who found that the first factor derived in a factor analysis was a better fit to a general population of social drinkers when combined with a latent class framework of 3 classes of drinkers (severe, moderate, and non-problem drinkers).

Our goal was to classify individuals, not symptoms, and hence, we employed a latent class approach rather than a factor analysis model defining 1 continuous latent trait. It is noteworthy, however, that the pattern of LCA results obtained here (high, moderate, low, vs subtypes) are typical of data that are well-suited to factor analysis. The use of LCA has successfully been employed to examine patterns of alcohol abuse and dependence symptoms (Beseler et al., 2012; Bucholz et al., 1996; Ko et al., 2010; Moss et al., 2008) as well as other addictions such as nicotine dependence (Agrawal et al., 2011) and opioid dependence (Shand et al., 2011). The majority of these studies also found a 3-class model was the best fit, with the classes representing a severity range from low risk to high risk. There are several advantages to examining individuals according to their risk profile, including identifying individuals in a trajectory toward future dependence, distinguishing the best clinical or pharmacological treatment for moderate- or at-risk individuals, and—importantly for this study—reducing genetic and phenotypic heterogeneity.

One SNP, rs11035102, which is located in a region of several olfactory genes, showed genome-wide significant association with *having a persistent desire to cut down/inability to do so* criteria for AD, with strong support from several imputed SNPs in high LD (Fig. 3B). This SNP was also moderately associated with the criterion of *drinking alcohol despite physical/psychological consequences* in the present study and with the total number of DSM-IV criteria (symptom count) in the same COGA family sample ($p = 3.8 \times 10^{-5}$; Wang et al., in press; Table S1). This gene

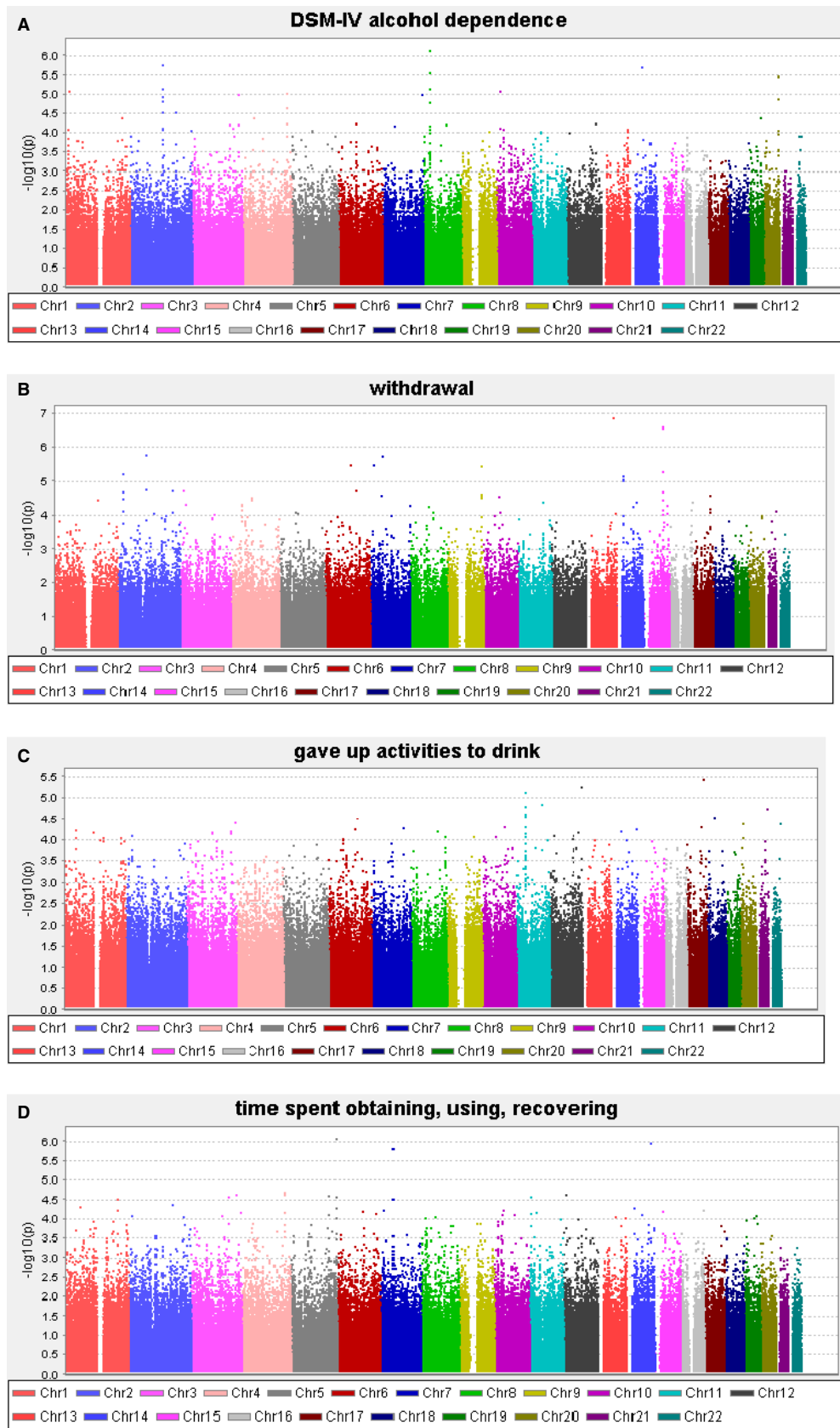


Fig. 2. Genome-wide association study results for the phenotypes. (A) alcohol dependence, (B) *withdrawal*, (C) *gave up activities to drink*, (D) *time spent obtaining/using/recovering from alcohol*, (E) *drink despite physical/psychological problems*, (F) *persistent desire/inability to cut down*, (G) *tolerance*, (H) *drink more than intended*, (I) low-risk latent class probability, (J) moderate-risk latent class probability, and (K) high-risk latent class probability.

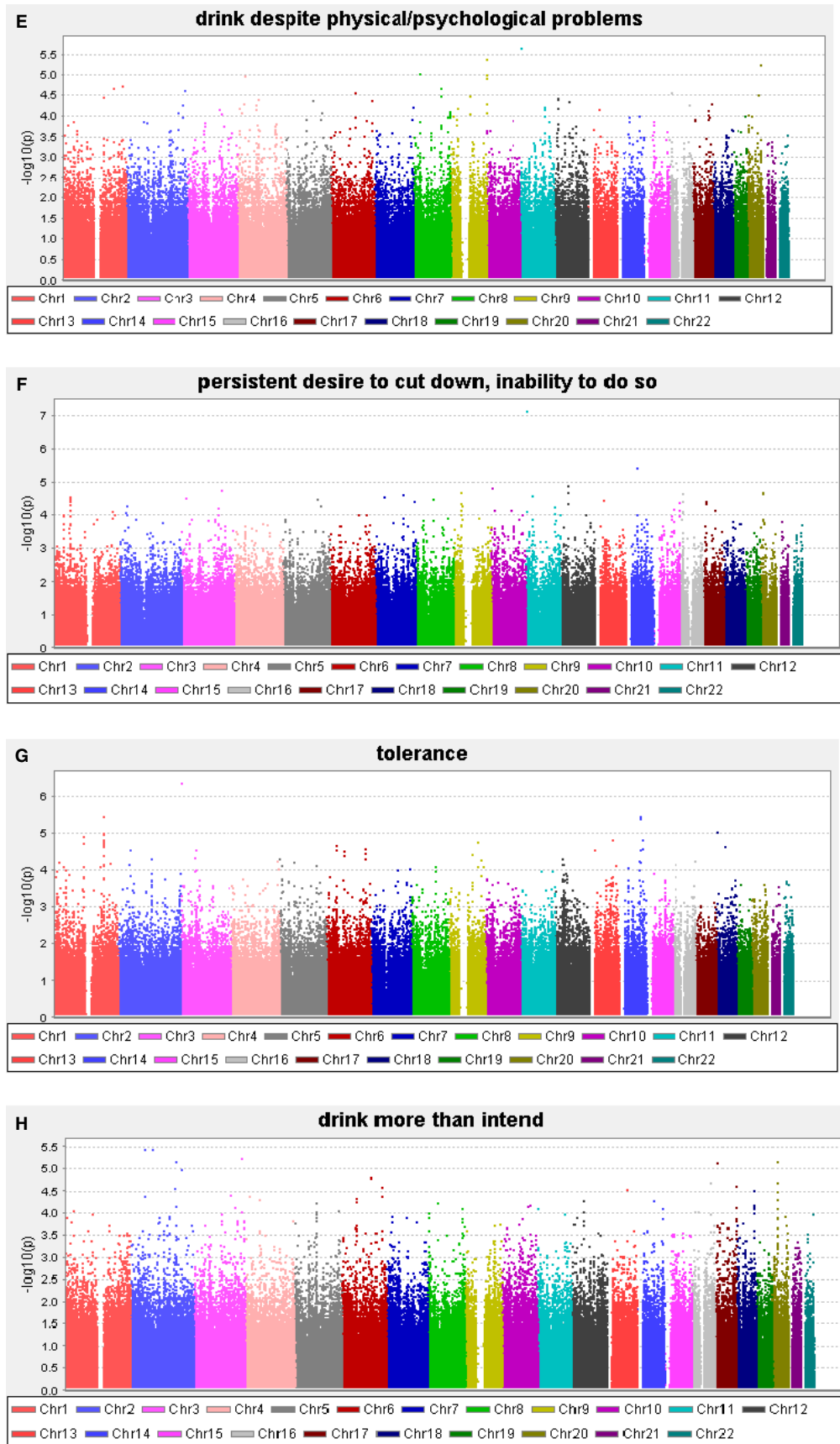


Fig. 2. Continued

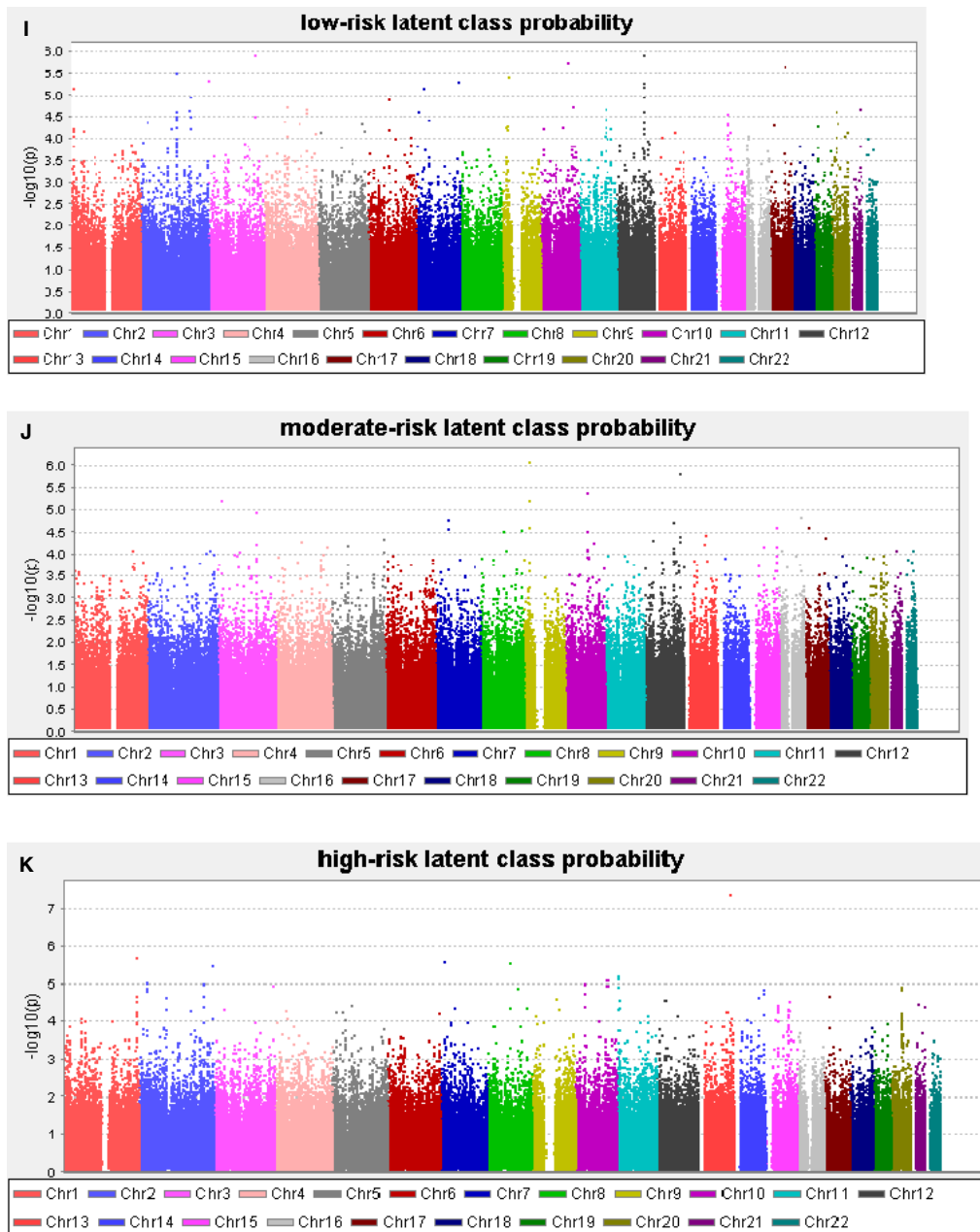


Fig. 2. Continued.

is near 2 olfactory receptors (*OR52E2* and *OR52J3*, seen in Fig. 3B) shown previously to be significantly associated with number of cigarettes smoked per day in a pathway analysis across 3 independent cohorts ascertained on nicotine or AD, one of which was the COGA sample (Harari et al., 2012). In a study of opioid-dependent individuals, the *desire/inability to cut down* was found to be 1 of the most prevalent substance use disorder criteria across 5 substances of abuse, including alcohol (Wu et al., 2012). Although this was not the most prevalent criterion in our sample, it was the criterion with the most diverse group of people, with 7% of the individuals endorsing this criterion being in the low-risk class, 53% from the moderate-risk class, and 40% from the

high-risk class. There is strong evidence supporting a relationship between learned olfactory sensory cues and alcohol consumption and dependence. It was shown that after alcohol odor stimuli, alcoholics found it more difficult to resist a drink than before the stimuli (Stormark et al., 1995). Priming subjects with an alcohol odor also increased activation of the limbic system (Bragulat et al., 2008) and led to enhanced craving for alcohol (Kareken et al., 2004, 2010).

The strongest association was between a SNP in a non-selective sodium leak channel *NALCN* and the probability of high-risk membership. This SNP, rs17484734, is in a recombination hotspot (Fig. 3A). As a result, only 1 imputed SNP provided supporting evidence for associa-

Table 4. Summary of Association Results with $p < 10^{-6}$ for any Trait in the COGA Sample and Corresponding Results from the SAGE Replication Sample. Supporting Phenotypes are Provided in Additional Rows. Genome-Wide Significant Results are in Bold

Chr ^a	Position ^b	SNP ^c	MAF ^d	Gene ^e	Phenotype	COGA p -value	SAGE p -value
3	3,692,598	rs1873023	0.12	<i>LRRN1</i> (148 kb)	<i>Tolerance</i>	4.2×10^{-7}	0.77
5	175,991,998	rs6892681	0.44	<i>CDHR2</i>	<i>Time spent obtaining/recovering from alcohol</i>	8.3×10^{-7}	0.16
8	26,894,971	rs13251780	0.14	<i>LOC100132229</i> (25 kb)	DSM-IV	7.2×10^{-7}	0.89
9	18,166,899	rs12006002	0.29	<i>LOC100421790</i> (12 kb)	Probability of moderate-risk class	8.3×10^{-7}	0.35
11	5,029,457	rs11035102	0.13	<i>OR51L1</i> (6 kb)	<i>Desire/inability to cut down</i>	7.3×10^{-8}	0.66
13	101,766,174	rs17484734	0.06	<i>NALCN</i>	Probability of high-risk class	4.1×10^{-8}	0.32
13	101,766,174	rs17484734	0.06	<i>NALCN</i>	<i>Withdrawal</i>	1.3×10^{-7}	0.88
15	75,415,962	rs2029519	0.25	<i>LOC100128721</i> (8 kb)	<i>Withdrawal</i>	2.6×10^{-7}	0.22
15	75,422,131	rs4479194	0.24	<i>LOC100128721</i> (14 kb)	<i>Withdrawal</i>	2.4×10^{-7}	0.19
15	75,424,593	rs7172677	0.24	<i>LOC100128721</i> (17 kb)	<i>Withdrawal</i>	2.8×10^{-7}	0.24

DSM-IV, alcohol dependence; COGA, Collaborative Study on the Genetics of Alcoholism; SAGE, Study of Addiction: Genetics and Environment.

^aChromosome.

^bChromosomal position (base pairs) based on human genome build 19, dbSNP 137.

^cSingle nucleotide polymorphism.

^dMinor allele frequency estimated on founders.

^eGene name and distance to nearest gene.

tion. This channel can be activated by several peptides including acetylcholine and substance P, both of which have been shown to interact with alcohol. Acetylcholine is released when alcohol binds to the nicotinic acetylcholine receptors as an agonist (Bito-Onon et al., 2011; Feduccia et al., 2012). Substance P, through interaction with the neurokinin 1 receptor, has been implicated in alcohol consumption, preference, and reinstatement and is a potential therapeutic target for AD (Schank et al., 2012). One of the components of the *NALCN* complex is *UNC79*, the homolog of which was shown to increase alcohol consumption in mice and led to increased sensitivity to alcohol in *Caenorhabditis elegans* (Specia et al., 2010).

Six of the SNPs presented in Table 4 also demonstrated moderate association with symptom count (rs17484734, rs12006002, rs2029519, rs4479194, rs7172677, rs11035102; all $p < 7.9 \times 10^{-5}$) (Wang et al., in press). The most significant association reported with the symptom count phenotype was with rs12903120 in *C15orf53*. This SNP was not associated with any of the phenotypes examined in this study ($0.36 \leq p \leq 2.7 \times 10^{-5}$). Because the symptom count phenotype is the total number of endorsed DSM-IV criteria, we might have expected more overlap between results with the individual criterion. However, there are several possible explanations for the observed differences. First, if our hypothesis is correct that different genetic factors may contribute to each criterion, then analyzing them separately would be a more powerful approach to identify the individual genetic effects. Second, there are some differences in the sample used in these 2 analyses as well as the analytic methods. For example, in the analyses reported herein, we employed a stricter inclusion criterion that only allowed regular drinkers. Previous analyses of alcohol symptom count utilized a more inclusive criterion that included all individuals reporting to have ever consumed alcohol.

We did not find additional support in the SAGE sample. This may be due to several factors. First, the power

to replicate findings of relatively small effect, such as those identified in this sample, is modest in replication samples of the size of SAGE. Second, the COGA family GWAS sample is quite unique in that the families were selected because of their large size and density of AD. Much of the SAGE sample consists of individuals recruited for nicotine and cocaine and then selected for comorbid AD. The risk factors contributing to AD and our AD-related phenotypes may be different from those more commonly found in sporadic AD or AD associated with other substance dependence. Finally, the lack of replication may be that our initial COGA findings are false-positive associations. Clearly, further studies, ideally in family samples ascertained for a high level of AD, are necessary.

The design of this study has several strengths. First, because most families have a strong history of AD, the sample is likely enriched for a greater genetic contribution to risk. Second, family-based analysis is robust to population substructures such as slight differences in ethnicity. This family-based design has been previously used to test for association with event-related brain oscillations correlated with deficits in alcoholics and offspring who are at risk of AD, with genome-wide significant association ($p = 4.7 \times 10^{-10}$) of SNPs in *KCNJ6* (Kang et al., 2012). We did not correct the significance threshold for the number of phenotypes analyzed; however, there was no indication of inflated association results, and the correlated phenotypes are all related to AD. A weakness of the study is the cross-sectional nature of the phenotypes employed. Thus, a longitudinal investigation into follow-up of individuals in the low-risk and especially those in the moderate-risk classes who are not yet alcohol dependent could provide insight as to clusters of individuals who change latent classes over time, and further evidence of the validity of employing homogenous phenotypes and suggest a genetic mechanism of liability.

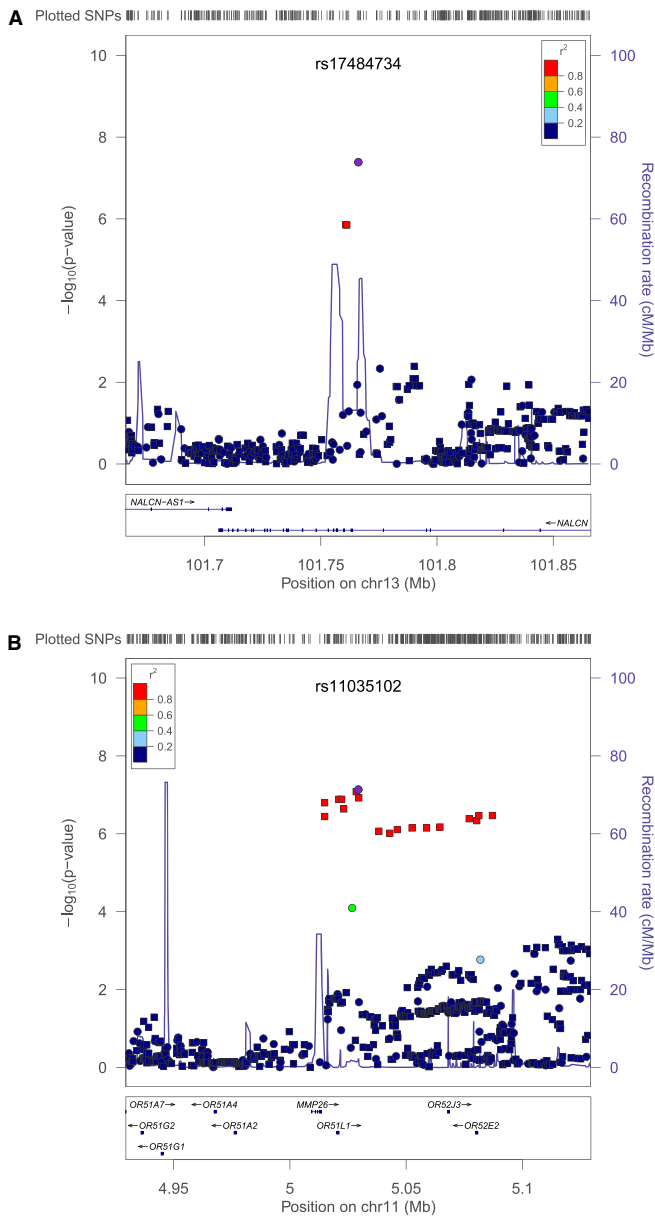


Fig. 3. Association results with key phenotypes. **(A)** High-risk latent class probability with single nucleotide polymorphisms (SNPs) in the region around rs17484734 in *NALCN*. **(B)** *Persistent desire/inability to cut down* with SNPs in the region around rs11035102 in *OR51L1*. Y-axis denotes the $-\log_{10}(p\text{-value})$ for association. X-axis is the physical position on the chromosome (Mb). The most significantly associated SNP is shown in purple. The extent of linkage disequilibrium (LD; as measured by r^2) between each SNP and the most significantly associated SNP is indicated by the color scale at top right. Larger values of r^2 indicate greater LD. Genotyped SNPs are indicated as circles and imputed SNPs by squares.

In summary, this family-based GWAS demonstrated 2 genome-wide significant associations, one of a polymorphism in an olfactory receptor with the *persistent desire or inability to cut down* on alcohol consumption, and the other with a sodium leak channel and the high-risk latent class trait. These results validate the advantage of defining a more homogenous sample in which to analyze genetic associations.

These results combined with those of other recent studies (Kang et al., 2012; Wang et al., in press) also demonstrate the valuable resource of the densely affected European American families of COGA.

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SUPPORTING INFORMATION

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

Fig. S1. Q-Q plots for the genome-wide association study results.

Table S1. Summary of association results with $p < 1.0 \times 10^{-5}$ for any trait in the COGA sample.