# Genome-wide association study of comorbid depressive syndrome and alcohol dependence

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***Objective*** **Depression and alcohol dependence (AD) are common psychiatric disorders that often co-occur. Both disorders are genetically influenced, with heritability estimates in the range of 35–60%. In addition, evidence from twin studies suggests that AD and depression are genetically correlated. Herein, we report results from a genome-wide association study of a comorbid phenotype, in which cases meet the Diagnostic and Statistical Manual of Mental Disorders-IV symptom threshold for major depressive symptomatology and the Diagnostic and Statistical Manual of Mental Disorders-IV criteria for AD.**

***Methods*** **Samples ($N=467$ cases and $N=407$ controls) were of European-American descent and were genotyped using the Illumina Human 1M BeadChip array.**

***Results*** **Although no single-nucleotide polymorphism (SNP) meets genome-wide significance criteria, we identified 10 markers with $P$ values less than $1\times10^{-5}$, seven of which are located in known genes, which have not been previously implicated in either disorder. Genes harboring SNPs yielding $P$ values less than $1\times10^{-3}$ are functionally enriched for a number of gene ontology categories, notably several related to glutamatergic function. Investigation of expression localization using online resources suggests that these genes are expressed across a variety of tissues, including behaviorally relevant brain regions. Genes that have been previously associated with depression, AD, or other addiction-related phenotypes – such as *CDH13*, *CSMD2*, *GRID1*, and *HTR1B* – were implicated by nominally significant SNPs. Finally, the degree of overlap of significant SNPs between a comorbid phenotype and an AD-only phenotype is modest.**

***Conclusion*** **These results underscore the complex genomic influences on psychiatric phenotypes and suggest that a comorbid phenotype is partially influenced by genetic variants that do not affect AD alone.** ***Psychiatr Genet*** **22:31–41 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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## Introduction

Alcohol dependence (AD) and major depression (MD) are common psychiatric disorders that often co-occur. Alcohol researchers have frequently delineated different 'types' of AD, with a central distinction being the presence or absence of externalizing disorders, such as antisocial personality disorder, or internalizing disorders, including depression. For example, Cloninger *et al.* (1981) defined type I AD as that which is driven in part by drinking to self-medicate negative affects; Del Boca and Hesselbrock (1996) described four types of dependence, including an 'internalizing' type in which individuals exhibit high anxiety and/or depression and consume alcohol to alleviate anxiety or depression. Classes of alcohol-dependent individuals who could be broadly described as suffering from mood and/or anxiety disorders have also been defined by others (Lesch *et al.*, 1988; Windle and Scheidt, 2004).

The National Epidemiologic Survey of Alcoholism and Related Conditions, using a representative population-based sample, found that the lifetime prevalence of major depressive disorders classified under the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; American Psychiatric Association, 2000) is 13.2%, and the 12-month prevalence is 5.2% (Hasin *et al.*, 2005). The corresponding figures for DSM-IV AD are 12.5 and 3.8% (Hasin *et al.*, 2007). Among individuals with a lifetime diagnosis of major depressive disorder, 21% met criteria for AD (Hasin *et al.*, 2005), which is 1.7-fold that predicted if the disorders were independent. Similarly,

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individuals with a lifetime AD diagnosis are at an increased risk for MD (odds ratio = 2.2; Hasin *et al.*, 2007). Analyses of the treatment-based Sequenced Treatment Alternatives to Relieve Depression sample (Davis *et al.*, 2006) found that individuals comorbid for MD and a substance use disorder (not limited to alcohol) have an earlier age of onset of depression relative to noncomorbid depressed individuals. Such individuals exhibit more depressive symptoms, have higher levels of functional impairment, and suffer from concurrent anxiety disorders more frequently. Importantly, these individuals also present an increased suicide risk (Davis *et al.*, 2006). Given the significant economic, social, and health consequences associated with both disorders, the optimization of prevention and treatment efforts is crucial. An understanding of the biological underpinnings of the disorders is essential for such efforts.

Multiple genetic variants, the effects of which vary in direction and magnitude, likely influence manifestation of and variation in depression and AD. Furthermore, these genetic variants likely interact with one another (epistasis), may be involved in multiple phenotypes (pleiotropy), and are subject to environmental influences. Typically, genes associated with a particular complex trait are of small effect, individually accounting for only a very low proportion of total variance (Flint, 2003; Plomin and Davis, 2009).

Evidence from twin studies indicates that MD and AD are genetically correlated. Kendler *et al.* (1993) found that, in a population of US women, the genetic correlation between the disorders was approximately 0.4–0.6. A study of adult male twins (Lyons *et al.*, 2006) found that, although a reciprocal causation model (whereby AD increased the risk of MD and *vice versa*) provided the best fit for the data, genetic correlation between these traits could not be ruled out.

A previous investigation from the Collaborative Study on the Genetics of Alcoholism (COGA) identified a region on chromosome 1 that was linked to both AD and depressive syndrome (Nurnberger *et al.*, 2001). More recent studies have identified specific genes that are associated with both depression and AD, including *CHRM2* (Wang *et al.*, 2004; Edenberg and Foroud, 2006), *SLC6A4* (Dick *et al.*, 2007b; Gokturk *et al.*, 2008), *COMT* (Sery *et al.*, 2006; Baekken *et al.*, 2008), and *DRD2* (Koks *et al.*, 2006; Dick *et al.*, 2007c), although others failed to replicate these associations (e.g. Furlong *et al.*, 1998; Gillespie *et al.*, 2005; Serretti *et al.*, 2006; Cohen-Woods *et al.*, 2009). Furthermore, *DRD2*, *CHRM2*, *SLC6A4*, and *MAOA* have all been associated with comorbid conditions involving alcohol use and internalizing symptomatology in adolescents (Saraceno *et al.*, 2009). Thus, findings from molecular genetic studies, in conjunction with twin studies on genetic correlation between the phenotypes, represent converging evidence that comorbidity of these traits is genetically influenced. One would expect that genes associated with comorbidity could fall into one of several categories: genes influencing AD irrespective of depressive status, genes influencing depression irrespective of AD status, and genes that specifically influence a comorbid status but not either disorder on its own.

The majority of previous research has relied on candidate genes, particularly those genes involved in neurotransmitter systems known to be involved in the etiology of addiction or depression. In contrast, genome-wide scans do not rely on earlier hypotheses, and therefore they represent a useful method by which to identify novel variants influencing the phenotype of interest. In addition, they can provide further support for previously implicated genetic loci. Recently, Sullivan *et al.* (2009) reported results from a genome-wide association study (GWAS) on MD. Although no single-nucleotide polymorphism (SNP) met criteria for genome-wide significance ($P < 5 \times 10^{-8}$), four of their most significant markers were in the gene coding for the presynaptic protein piccolo. Muglia *et al.* (2010) also conducted a GWAS analysis for MD using two separate samples and reported no markers meeting genome-wide significance criteria. However, secondary analyses by this research group suggested that genes previously implicated in mood disorders were significantly ($P < 0.0001$) associated with depression when the two samples were combined.

Johnson *et al.* (2006) and Treutlein *et al.* (2009a) have reported GWAS results for AD. The former reported clusters of nominally significant SNPs, including some located in genes previously associated with addiction-related traits. Treutlein *et al.* (2009a) used a two-stage approach and identified two intergenic markers reaching genome-wide significance, as well as nominally significant SNPs located within genes previously implicated in AD. In the current study, we describe the results from the first GWAS of comorbid depressive syndrome and AD.

## Methods

### Sample

Alcohol-dependent probands were ascertained by COGA through alcohol treatment programs and evaluated at multiple centers in the US: Indiana University, State University of New York Health Science Center Brooklyn, University of Connecticut, University of Iowa, University of California-San Diego, Washington University in St. Louis, and Howard University. The Institutional Review Boards of all participating institutions approved the study. After participants provided informed consent, probands and their relatives were administered the Semi-Structured Assessment for the Genetics of Alcoholism (Bucholz *et al.*, 1994), a validated polydiagnostic instrument. Details of ascertainment and assessment have been previously described (Nurnberger *et al.*, 2004). In addition, community probands were recruited at each site using a variety of

methods, including through driver's license records, random mailings to employees and students at a university, and attendees at medical and dental clinics. Again, after providing informed consent, community probands and their family members were administered the Semi-Structured Assessment for the Genetics of Alcoholism.

### Case–control sample collection and measures

For the GWAS sample (described in more detail in Edenberg *et al.*, 2010), unrelated alcohol-dependent cases and nonalcohol-dependent controls were selected from the pool of alcohol-dependent and community-ascertained families. All cases met DSM-IV criteria for AD at some point during their lives. In situations in which an alcohol-dependent proband had been phenotypically assessed multiple times for his or her lifetime history, he or she had to have met the diagnostic criteria for AD at each assessment to be a GWAS 'case'. Controls were selected from both the community-recruited families and those recruited through an alcohol-dependent individual; however, they could not share a common ancestor with a case. In addition, controls were required to have consumed alcohol but to have never met criteria for any alcohol-related diagnosis (abuse or dependence). In addition, controls should not have met diagnostic criteria for abuse or dependence of cocaine, stimulants, sedatives, opioids, or marijuana. Because AD is so frequently comorbid with other types of substance dependence, cases meeting diagnostic criteria for other types of dependence were not excluded: 49.9% ($N = 226$) of cases were dependent on marijuana; 40.7% ($N = 188$) were dependent on cocaine; 30.6% ($N = 140$) were dependent on stimulants; 20.6% ($N = 87$) were dependent on sedatives; and 18.8% ($N = 85$) were dependent on opioids. Because COGA probands were recruited in part from treatment centers, they likely represent relatively severe cases of AD. In addition, the cases used in this study, who also meet criterion A for a major depressive episode, endorsed more AD symptoms than did cases without a history of depression (see Discussion). Thus, the cases included in this analysis likely represent an extreme phenotype.

For the GWAS analysis in this report, we defined as 'cases' those individuals who, in addition to meeting lifetime DSM-IV criteria for AD, also met lifetime DSM-IV symptom threshold for a major depressive episode (at least five of nine symptoms within a 2-week period, one of which had to be sadness or anhedonia). Individuals were excluded if symptoms were due to bereavement, but not if symptoms were experienced under the influence of drugs and/or alcohol. For the sake of simplicity, we will refer to the depression phenotype as 'depressive syndrome' (as in Nurnberger *et al.*, 2001); the reader should note that cases were required to meet criterion A for a major depressive episode (in addition to being alcohol dependent) but were not required to have experienced these symptoms independent of alcohol or other substances. Thus, they do not necessarily meet full criteria for a major depressive episode (see below for additional details). Controls were excluded if they met our criteria for depressive syndrome. Cases and controls differed significantly by age ($t = 7.87$, $P < 0.001$), with controls being older ($47.5 \pm 0.63$) than cases ($41.4 \pm 0.48$). This was intentional, to ensure that 'unaffected' individuals had passed the period of maximal risk for onset of AD. Cases were more frequently male ($\chi^2 = 71.16$, $P < 0.0001$); this was likely because of the selection of cases on the basis of AD, which is more prevalent in men than in women. However, had selection been based on depressive syndrome primarily and AD secondarily, the sample would likely have had a disproportionate number of women. Cases had completed significantly fewer years of school and had lower current household income ($P < 0.0001$ in both cases). Cases had been admitted to an inpatient psychiatric ward/chemical dependency treatment facility more frequently than controls (cases: mean = 4.4, range 0–60; controls: mean = 0.02, range 0–3; $P < 0.0001$).

A principal component-based analysis was performed in PLINK (Purcell *et al.*, 2007) to cluster these samples along with HapMap reference samples to assign the study patients to groups of predominantly European and African ancestry. We conducted analyses on the European-American (EA) subsample ($N = 1399$) in the interest of reducing genetic and etiological heterogeneity. The somewhat restricted nature of inclusion criteria necessarily limits both case and control sample sizes. Furthermore, exclusion of depressed controls ($N = 144$) and cases whose depression met bereavement exclusion criteria ($N = 26$) resulted in a final GWAS sample size of 467 comorbid cases (287 men and 180 women) and 407 unaffected controls (132 men and 275 women). Of the 467 cases, 181 met criteria for an independent depressive episode – one experienced outside the context of drug or alcohol use – whereas 286 reported moderate-to-heavy alcohol or drug use during the time they experienced depressive symptoms.

### Genome-wide association analysis

Genotyping was performed by the Center for Inherited Disease Research. DNA was obtained from blood or lymphoblast cell lines. Genotyping was performed using the Illumina Infinium II assay protocol with hybridization to Illumina HumanHap 1M BeadChips (Illumina, San Diego, California, USA). A subset of the data is available through dbGaP (*http://www.ncbi.nlm.nih.gov/sites/entrez?db=gap*; accession number: phs000125.v1.p1). Twenty-seven samples were removed because of poor sample quality. Blind duplicate reproducibility was more than 99.9%. Samples with genotypes for at least 98.0% of the markers were considered for inclusion in analyses and were

screened for cryptic relatedness, population stratification, and so on, resulting in the removal of 13 additional samples. SNPs with a call rate of 98.0% or more in the EA sample were included in the analyses. SNPs were excluded if the minor allele frequency was less than 1% in the combined case and control data set; further SNPs were excluded if significant ($P < 10^{-4}$) deviation from Hardy–Weinberg equilibrium was observed. Additional details are provided in Edenberg *et al.* (2010). The GWAS analysis was conducted in PLINK version 1.05 for all autosomes and the X chromosome, with age and sex included as covariates. An additive model was assumed, and, because of the binary outcome variable, logistic regression was used. Annotations are based on assembly GRCh37/hg19. Gene names were assigned to markers based on RefSeq gene sequences.

### Additional analyses

We used several approaches to determine whether genes implicated by our results (i.e., those harboring markers with $P < 10^{-3}$) had been previously associated with psychiatric phenotypes: this included a manual literature search in PubMed (*http://www.ncbi.nlm.nih.gov/pubmed/*), querying the National Center of Biotechnology Information (NCBI) Gene database (*http://www.ncbi.nlm.nih.gov/gene*), and querying the NCBI Association Results browser (*http://www.ncbi.nlm.nih.gov/projects/gapplusprev/sgap_plus.htm*). Information obtained through the Association Results browser is limited to the results of genome-wide screens. Note that the Association Results browser returns records for genes whose associations have been 'pre-computed' at NCBI but for which no publication is available. Those results are cited here as being reported by NCBI.

We investigated gene expression in markers with a $P$ value less than $10^{-5}$ using the online database BioGPS (Wu *et al.*, 2009), which includes expression information across 79 tissue types (Su *et al.*, 2004). We also conducted secondary analyses of markers with a $P$ value of less than $10^{-3}$ to assess whether these markers were enriched for gene ontology categories, using the online database ToppGene (Chen *et al.*, 2009). Of the 938 markers meeting the $P$ value of less than $10^{-3}$ threshold, 538 are located within 366 known genes; gene ontology information was available for 321 of these through ToppGene. We used a false discovery rate of $P$ value less than 0.05 as the significance criterion and excluded categories that applied to fewer than three genes. We note that this method does not take into account the fact that larger genes are likely to span more markers than small genes and are thus more likely to harbor a marker meeting our $P$ value threshold by chance alone.

Finally, we used PLINK to assess linkage disequilibrium (LD) in the 100 genes that contained two or more markers with a $P$ value less than $10^{-3}$ to investigate whether different markers were likely to represent independent signals. A threshold of $r^2 < 0.5$ was used as an indication of independent signals within a gene.

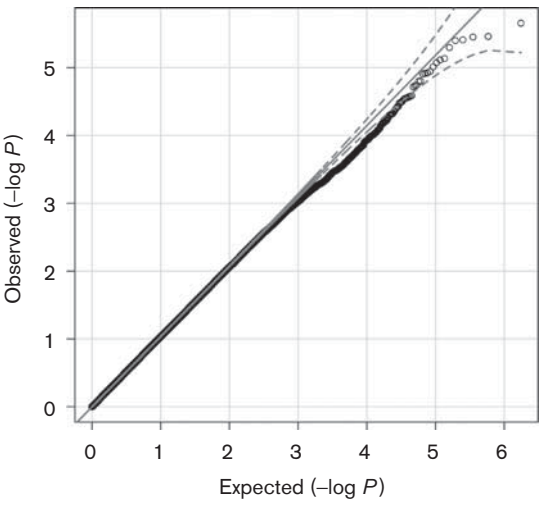
## Results

### Association analysis in the AD-depressive syndrome genome-wide association study case–control sample

The genotyping rate in the EA sample was 99.7%. After applying several quality control steps (see Methods), 876,476 SNPs were analyzed. The sex and age covariates were statistically significant ($P < 10^{-15}$ and $10^{-11}$, respectively) for all screened SNPs (sex was not a covariate for SNPs on the X chromosome). None of the SNPs analyzed met criteria for genome-wide significance ($5 \times 10^{-8}$; Fig. 1, Supplementary Table 1). Ten SNPs had $P$ values of less than $10^{-5}$ (Table 1). Seven of these fall within known genes: *OXTR*, *FAF1*, *OPA3*, *WDR7*, *SPATA13*, *EFHA2*, and *FHIT*. The remaining three are located on the X chromosome and are not near any known gene.

*OXTR*, which encodes the oxytocin receptor, is involved in a variety of biological processes including muscle contraction, regulation of blood pressure, and processes relevant to reproduction, such as lactation. It binds vasopressin as well as oxytocin. *FAF1*, or Fas (*TNFRSF6*)-associated factor 1, has been implicated in the regulation of apoptosis. Optic atrophy 3 (*OPA3*) is involved in sensory perception, specifically visual perception. EF-hand domain family, member 2 (*EFHA2*), is involved in calcium binding. *FHIT*, the fragile histidine triad gene, plays a role in the cell cycle and metabolic processes, as well as in cation and metal binding. WD repeat domain

**Fig. 1**




Q–Q plot for association analysis of alcohol dependence with comorbid depressive syndrome ($\lambda = 1.0363$). The solid red line represents the expected –log $P$ values; black dots represent the observed –log $P$ values; the dashed red lines represent 95% confidence intervals.

**Table 1 Information on single-nucleotide polymorphisms with a *P* value of less than $10^{-5}$ from genome-wide association analysis of comorbid depressive syndrome and alcohol dependence in the European-American Collaborative Study on the Genetics of Alcoholism sample**

| Chr | Single-nucleotide polymorphism | BP | A1/A2 | Minor allelic frequency | Gene | Odds ratio | L95 | U95 | *P* value |
|---|---|---|---|---|---|---|---|---|---|
| 3 | rs237899 | 8783515 | A/G | 0.3687 | *OXTR* | 1.692 | 1.361 | 2.104 | 2.207e-6 |
| X | rs5968205 | 82841146 | C/T | 0.1549 | n/a | 0.4544 | 0.3256 | 0.634 | 3.461e-6 |
| X | rs5922858 | 82857664 | G/T | 0.1505 | n/a | 0.4509 | 0.322 | 0.6314 | 3.525e-6 |
| 1 | rs3827730 | 50710426 | C/T | 0.332 | *FAF1* | 1.716 | 1.364 | 2.158 | 3.888e-6 |
| 19 | rs8111589 | 50726398 | C/T | 0.4365 | *OPA3* | 1.641 | 1.329 | 2.025 | 3.988e-6 |
| X | rs5922838 | 82757851 | G/A | 0.1555 | n/a | 0.4615 | 0.331 | 0.6433 | 5.038e-6 |
| 13 | rs9805786 | 23556356 | G/T | 0.4103 | *SPATA13* | 1.649 | 1.325 | 2.053 | 7.331e-6 |
| 18 | rs17750015 | 52548620 | C/T | 0.3498 | *WDR7* | 0.5955 | 0.4745 | 0.7473 | 7.691e-6 |
| 8 | rs10090288 | 16974006 | C/A | 0.07225 | *EFHA2* | 0.3928 | 0.2603 | 0.5926 | 8.439e-6 |
| 3 | rs1735460 | 60666645 | T/C | 0.02538 | *FHIT* | 0.1918 | 0.09224 | 0.3986 | 9.728e-6 |

7 (*WDR7*) has a role in proteolysis and has been associated with multiple sclerosis in another GWAS (Baranzini *et al.*, 2009). No gene ontology information is available for spermatogenesis-associated 13 (*SPATA13*).

### Gene expression in 'top hits'

*FHIT* expression is consistent across most tissues but is highest in CD4 T-cells; modestly increased expression is observed in a variety of other tissues/cells, including CD34 cells, the hypothalamus, and whole brain; expression is also observed in every brain region for which data are available. *OXTR* is highly expressed in lymphoblasts, although data-mining resources (*http://www.ncbi.nlm.nih.gov/unigene*) also indicate increased expression in the brain, skin, and breast. As with *FHIT*, *OXTR* is modestly expressed in all brain regions reported in BioGPS. *FAF1* expression is increased in testis tissues, as well as in various blood cells and cancerous cells. *WDR7* expression is highest across multiple brain regions, especially in the amygdala, prefrontal cortex, and hypothalamus. *SPATA13* exhibits increased expression in various blood cells, although expression is also detected across brain tissues. *OPA3* is expressed consistently across brain tissues, although its highest detected expression levels are in cancer cells. No expression information is available for *EFHA2*.

### Gene ontology analysis

We selected SNPs with a *P* value of less than $10^{-3}$ (Supplementary Table 2) to assess potential gene ontology enrichment using the online database ToppGene (see Methods). One biological process gene ontology category – response to drug – was significantly overrepresented. Eight molecular function categories were enriched. Several of these were very closely related – for example, ionotropic glutamate receptor activity, extracellular-glutamate-gated ion channel activity, glutamate receptor activity, and excitatory extracellular ligand-gated ion channel activity – and were each populated by the same five glutamate-related genes: *GRIN2A*, *GRIN2C*, *GRID1*, *GRIA1*, and *GRIA4*. Each of these genes encodes a glutamate receptor. In addition, a number of cellular component categories related to neural function were statistically overrepresented. All enriched categories are detailed in Table 2.

### Additional secondary analyses

We conducted literature and database searches to determine whether any of the 366 genes spanning markers with a *P* value less than $10^{-3}$ have been previously associated with AD, depression, or other potentially relevant phenotypes (particularly those related to addiction or internalizing). We found that over 60 genes had a history of association with phenotypes of interest (Table 3). Some of these have been implicated in phenotypes related to both addiction and internalizing characteristics.

A total of 105 genes contained two or more SNPs with a *P* value less than $10^{-3}$. We evaluated LD among markers within each of these genes to assess whether these were redundant or suggestive of multiple, independent signals. Forty-eight genes met these criteria, including four genes (*FAF1*, *OPA3*, *OXTR*, and *SPATA13*) implicated by our most significant markers, as well as *GRIN2A* and *HTR1B*. A full list is provided in Table 4.

To assess whether our results were driven primarily by the AD phenotype, rather than by the comorbid phenotype, we ran a parallel analysis comparing 354 individuals having AD but without depressive syndrome with 407 controls who had neither disorder (the same controls that were used in the primary analysis). We then compared *P* values from that analysis with those in our original list of SNPs with a *P* value less than $10^{-3}$. Only 52 of the 938 markers reported here met the same criteria in the AD-only analysis; 44.3% (416/938) had a *P* value of less than 0.05. The direction of the allelic effect was reversed in 12 (of 938) cases, but none of those 12 markers had a *P* value less than 0.05 in the secondary analysis.

## Discussion

We present the first report of a genome-wide association analysis of comorbid depressive syndrome and AD. No marker met genome-wide significance criteria, but 10 had *P* values less than $10^{-5}$ and 938 had *P* values less than $10^{-3}$. Indeed, given the genomic complexity and phenotypic heterogeneity of AD and depressive syndrome, we might not expect the effect size of any individual marker to be large enough to reach genome-wide significance in a

**Table 2 Categories functionally enriched among genes containing markers with a *P* value of less than $10^{-3}$, based on 321 genes for which annotations were available in ToppGene (Chen *et al.*, 2009)**

| Category name | False discovery rate *P* value | Genes in category |
|---|---|---|
| Molecular functions | | |
| Ionotropic glutamate receptor activity | 0.0000036 | *GRIN2C, GRIN2A, GRID1, GRIA1, GRIA4* |
| Extracellular-glutamate-gated ion channel activity | 0.0000048 | *GRIN2C, GRIN2A, GRID1, GRIA1, GRIA4* |
| Glutamate receptor activity | 0.000052 | *GRIN2C, GRIN2A, GRID1, GRIA1, GRIA4* |
| Drug transporter activity | 0.000302 | *SLC22A5, ABCB1, ABCB4, SLC46A2* |
| Alkali metal ion binding | 0.000366 | *SLC9A8, SLC22A5, KCNA3, SLC5A11, IMPA2, SCN5A, SLC24A5, KCNT2, KCNS3, KCNS2, SLC17A6* |
| Transmembrane transporter activity | 0.000562 | *SLC9A8, GRIN2C, GRIN2A, NMUR2, SLC22A5, KCNA3, ABCB1, ABCB4, SLC5A11, SLC1A3, SCN5A, SLC14A2, GRID1, TRPC4, SLC46A2, SLC24A5, TOMM20, GRIA4, GRIA1, SLCO4A1, KCNT2, KCNS3, KCNS2, UQCRFS1, TAP2, SLC17A6* |
| Excitatory extracellular ligand-gated ion channel activity | 0.000558 | *GRIN2C, GRIN2A, GRID1, GRIA1, GRIA4* |
| Calcium ion binding | 0.000329 | *EFEMP1, CCBE1, CLSTN2, THBS2, FREM1, GALNTL4, GPR98, SNTB1, ITSN1, GALNS, GALNT2, EFHA1, DMD, F9, EYS, CAB39, PCDH19, SPTA1, ASPH, SLC24A5, RAB11FIP4, CDH13, TRPC4, CDH4, CADM3, GRIN2A, GRIN2C* |
| Biological process | | |
| Response to drug | 0.000016 | *SLC22A5, CDKN1A, SMPD1, OXTR, SLC1A3, ABCB4, ABCB1, UQCRFS1, SNX27, DPYD, EMX2, SRP54, SLC46A2, GRIN2A* |
| Cellular components | | |
| Cell junction | 0.000000031 | *ITSN1, GRIN2C, MAGI2, GRIN2A, CTNNA2, ZNF236, EGFLAM, PKP4, LZTS1, GPHN, ABCB1, ABCB4, PDZD2, RHOU, SNTB1, SCN5A, CADM3, VAPA, GRID1, DLGAP1, GRIA4, GRIA1, PARD3B, EVL, SLC17A6, OXTR, DMD* |
| Ionotropic glutamate receptor complex | 0.0000574 | *GRIN2C, GRIN2A, GRIA4, GRIA1* |
| Synapse | 0.0000025 | *ITSN1, GRIN2C, MAGI2, GRIN2A, EGFLAM, LZTS1, GPHN, CLSTN2, SNTB1, GRID1, DLGAP1, GRIA4, GRIA1, CAV3, SLC17A6, DMD, SLC1A3, SDC2, EPHA7, EFNA2* |
| Postsynaptic membrane | 0.0000725 | *GRIN2C, GRIN2A, LZTS1, GPHN, CLSTN2, GRID1, DLGAP1, GRIA4, GRIA1, EPHA7* |
| Cell-cell junction | 0.0007714 | *CTNNA2, PKP4, ABCB1, ABCB4, PDZD2, SCN5A, CADM3, VAPA, PARD3B, OXTR* |
| Postsynaptic density | 0.001317 | *GRIN2C, GRIN2A, LZTS1, DLGAP1, GRIA1, GRIA4* |
| Outer membrane-bounded periplasmic Space/periplasmic space | 0.000293 | *GRID1, GRIN2A, GRIN2C* |
| Cell envelope | 0.000678 | *GRID1, GRIN2A, GRIN2C* |
| External encapsulating structure part | 0.001056 | *GRID1, GRIN2A, GRIN2C* |
| External encapsulating structure | 0.001286 | *GRID1, GRIN2A, GRIN2C* |
| Dystrophin-associated glycoprotein complex | 0.002158 | *SNTB1, DMD, CAV3* |

Genes spanning more than one marker meeting our significance criterion were only submitted once. As in the NCBI Gene database (*http://www.ncbi.nlm.nih.gov/gene*), gene ontologies are divided into three primary categories: molecular functions, biological processes, and cellular components. Genes implicated by markers meeting our significance criterion fall into the categories in Table 2 significantly more frequently than would a random selection of the same number of genes. See Methods for further details.

study of this size; rather, many common variants of small effect likely influence these traits, with each affected individual harboring an overlapping but unique set of risk-conferring alleles (Wellcome Trust Case Control Consortium, 2007; Purcell *et al.*, 2009).

For markers with a *P* value of less than $10^{-3}$, additional analyses were carried out. These genes are functionally enriched for a number of molecular function categories related to glutamate activity, as well as for categories involving transport activity. In addition, a disproportionate number of these genes fall into cellular component categories such as cell junction, postsynaptic membrane, ionotropic glutamate receptor complex, and synapse. Overall, these results suggest that genes associated with the comorbid phenotype are involved in neural processes. Specifically, the glutamatergic system is strongly implicated, which is not surprising given its previous association with depression, AD (for reviews, see Kohnke, 2008; McNally *et al.*, 2008), and alcohol response (Joslyn *et al.*, 2010). Most of the glutamate-related genes implicated in the current study (*GRIN2C*, *GRIN2A*, *GRIA1*, and *GRIA4*) have not previously been associated with depression or AD, but *GRID1* was modestly associated with MD in a genome-wide meta-analysis (Muglia *et al.*, 2010). In addition, all but *GRIN2C* have been associated with schizophrenia (Carter, 2007; O'Connor and Hemby, 2007; Treutlein *et al.*, 2009b), and *GRIN2A* has been implicated in heroin addiction among African Americans (Levran *et al.*, 2009).

The results reported here represent some level of replication for other genes as well: *CDH13* and *VGLL4* have been implicated previously in GWAS analyses for AD (Johnson *et al.*, 2006; Treutlein *et al.*, 2009a) and MD (Muglia *et al.*, 2010), respectively. Seven genes implicated in the current report – *CTNNA2*, *ESRRG*, *FBXO21*, *GALNT2*, *GRID1*, *IGSF21*, and *SMARCA2* – were reported previously to be proximal (within 250 kb) and in reasonably high LD ($r^2 \geq 0.5$) with markers associated with MD (Sullivan *et al.*, 2009). *AGTR1*, *CSMD2*, and *NMUR2* were nominally associated with AD (by 'clustered positive SNPs') in a report by Johnson *et al.* (2006).

**Table 3 Genes harboring markers with a *P* value of less than 0.001 in the current study, that have been previously associated with alcohol dependence, depression, or other relevant psychiatric phenotypes (see Methods for details)**

| Gene | Alcohol dependence | Depression | Other relevant psychiatric phenotypes |
|---|---|---|---|
| *AGTR1* | Johnson *et al.* (2006) | | |
| *ALPK2* | | Shyn *et al.* (2011) | |
| *C6orf204* | | | Smoking cessation[a] (Rose *et al.*, 2010) |
| *CCBE1* | | | Smoking cessation (Uhl *et al.*, 2008) |
| *CDH4* | | Rietschel *et al.* (2010) | |
| *CDH13* | Johnson *et al.* (2006); Treutlein *et al.* (2009a) | Muglia *et al.* (2010) | Smoking cessation (Uhl *et al.*, 2008); ADHD (Lesch *et al.*, 2008); schizophrenia (NCBI, 2011) |
| *CDKAL1* | | Shyn *et al.* (2011) | |
| *CLSTN2* | | | Smoking cessation (Uhl *et al.*, 2008) |
| *CREB5* | | | Smoking cessation (Uhl *et al.*, 2008); ADHD (Lesch *et al.*, 2008) |
| *CSMD2* | Johnson *et al.* (2006) | Shyn *et al.* (2011) | |
| *CTNNA2* | | Sullivan *et al.* (2009) | Smoking cessation (Uhl *et al.*, 2008); ADHD (Lesch *et al.*, 2008) |
| *DMD* | | Shyn *et al.* (2011) | Bipolar disorder (NCBI, 2011) |
| *EGFLAM* | | Shyn *et al.* (2011) | |
| *EMX2* | | | Conduct disorder (Dick *et al.*, 2011) |
| *EPHA7* | | Shyn *et al.* (2011) | Smoking cessation (Rose *et al.*, 2010) |
| *ESRRG* | | Sullivan *et al.* (2009) | |
| *EVL* | | | Smoking cessation (Rose *et al.*, 2010) |
| *FAF1* | | | Smoking cessation (Rose *et al.*, 2010) |
| *FGF9* | | NCBI (2011) | |
| *FBXO21* | | Sullivan *et al.* (2009) | |
| *GALNT2* | | Sullivan *et al.* (2009) | |
| *GPC6* | | | Neuroticism (Calboli *et al.*, 2010); ADHD (Lesch *et al.*, 2008) |
| *GRIA1* | | | Schizophrenia (Carter, 2007) |
| *GRIA4* | | | Schizophrenia (Carter, 2007; O'Connor and Hemby, 2007) |
| *GRID1* | | Muglia *et al.* (2010); Sullivan *et al.* (2009) | Bipolar disorder & schizophrenia (Carter, 2007; Treutlein *et al.*, 2009b) |
| *GRIN2A* | | | Smoking cessation (Uhl *et al.*, 2008); bipolar disorder & schizophrenia (Carter, 2007) |
| *HTR1B* | Sun *et al.* (2002) | (Lopez-Figueroa *et al.*, 2004); substance abuse disorder with depression (Huang *et al.*, 2003) | Depressed/anxious vs. antisocial subtypes of alcohol dependence (Lee *et al.*, 2009); heroin addiction (Proudnikov *et al.*, 2006); anorexia nervosa (Kiezebrink *et al.*, 2010; Pinheiro *et al.*, 2010); suicidal ideation in MD (Wang *et al.*, 2009) |
| *IGSF21* | | Sullivan *et al.* (2009) | |
| *IMMP2L* | | | Cognitive performance (Need *et al.*, 2009) |
| *KCNA3* | | Shyn *et al.* (2011) | Panic disorder (Otowa *et al.*, 2009) |
| *KCNT2* | | Shyn *et al.* (2011) | |
| *LRFN5* | | Rietschel *et al.* (2010) | |
| *MACROD2* | | | Autism (Anney *et al.*, 2010); schizophrenia (NCBI, 2011) |
| *MBOAT1* | | | ADHD (Lasky-Su *et al.*, 2008) |
| *MPHOSPH6* | | | Cognitive performance (Need *et al.*, 2009) |
| *NDNL2* | | | ADHD (Lasky-Su *et al.*, 2008) |
| *NKAIN2* | | | Neuroticism (Calboli *et al.*, 2010) |
| *NMUR2* | Johnson *et al.* (2006) | | |
| *OXTR* | | | Symptoms of depression (Thompson *et al.*, 2011); depressive temperament (Kawamura *et al.*, 2010) |
| *PELI1* | | Schol-Gelok *et al.* (2010) | |
| *PITRM* | | | ADHD (Anney *et al.*, 2008) |
| *PTPRD* | | | Smoking cessation (Uhl *et al.*, 2008) |
| *RAB11FIP4* | | | ADHD (NCBI, 2011) |
| *RANBP3L* | | | ADHD (NCBI, 2011) |
| *RGNEF* | | | Smoking cessation (Rose *et al.*, 2010) |
| *RLBP1L1* | | | Smoking cessation (Rose *et al.*, 2010) |
| *SEMA5A* | | | smoking cessation (Uhl *et al.*, 2008) |
| *SEMA6A* | | | Smoking cessation (Rose *et al.*, 2010) |
| *SMARCA2* | | Sullivan *et al.* (2009) | |
| *SORCS2* | | Rietschel *et al.* (2010) | |
| *SOX5* | | | Smoking cessation (Uhl *et al.*, 2008) |
| *TRAF3* | | | Schizophrenia (Potkin *et al.*, 2009) |
| *TSHZ2* | | | Smoking cessation (Rose *et al.*, 2010) |
| *UQCRFS1* | | | Schizophrenia (NCBI, 2011) |
| *VGLL4* | Treutlein *et al.* (2009a) | | Bipolar disorder (NCBI, 2011) |
| *WDR7* | | | Smoking cessation (Rose *et al.*, 2010) |
| *ZNF285A* | Bierut *et al.* (2010) | | |
| *ZNF385B* | | | ADHD (NCBI, 2011) |
| *ZNF532* | | | Smoking cessation (Rose *et al.*, 2010) |

ADHD, attention-deficit hyperactivity disorder.
[a]Rose *et al.* (2010) used a genetic risk score, based on previously implicated genes, to predict smoking cessation success. Reference to that study for a particular gene in this table only means that the gene was included in the risk score.

**Table 4 Genes spanning multiple, potentially independent ($r^2 < 0.5$) single-nucleotide polymorphisms at a *P* value of less than $10^{-3}$**

| | | | | |
|---|---|---|---|---|
| *ASPH* | *ENOX1* | *LOC389386* | *NSMCE2* | *SCN5A* |
| *BANF2* | *EPC1* | *LOC389970* | *OPA3** | *SMPD1* |
| *BTG1* | *EPHA7* | *LOC391048* | *OSBPL5* | *SNX30* |
| *C6orf204* | *FAF1** | *LOC392180* | *OXTR** | *SPATA13** |
| *CARD11* | *GALNT2* | *LOC401646* | *PITRM1* | *TSHZ2* |
| *CDKAL1* | *GRIN2A* | *LOC644192* | *POU3F4* | *TUSC3* |
| *CREB5* | *GPR101* | *LOC646388* | *PRDM5* | *VAPA* |
| *DIAPH2* | *HTR1B* | *LOC730134* | *PRKAR1B* | *ZNF236* |
| *EFEMP1* | *ITSN1* | *LOC730239* | *RANBP3L* | |
| *EGFLAM* | *LAPTM4B* | *MAGI1* | *RGNEF* | |

*Genes implicated by our most significant ($P < 10^{-5}$) markers.

Muglia *et al.* (2010) reported that the protein tyrosine phosphatase receptor *PTPRN* was significantly associated with MD: in the current report, two other protein tyrosine phosphatase receptors, *PTPRD* and *PTPRS*, were found to be significantly associated with the comorbid phenotype. In addition, nine genes identified in the current study have been associated with smoking cessation success (Uhl *et al.*, 2008): *CDH13*, *CTNNA2*, *CLSTN2*, *SEMA5A*, *PTPRD*, *CREB5*, *SOX5*, *GRIN2A*, and *CCBE1*. Perhaps, these genes are generally associated with addiction-related traits. These results are summarized in Table 3, which also includes the results of a systematic search of the NCBI Gene database and the NCBI Association Results browser of genes harboring a marker with a *P* value less than $10^{-3}$ to determine whether they have been previously associated with phenotypes related to substance use problems or internalizing symptoms.

Two markers in the *HTR1B* gene, which encodes a serotonin receptor, had a *P* value of less than $10^{-3}$. Lee *et al.* (2009) reported that allele frequency at a different marker in this gene, rs130058, differed significantly between individuals categorized as having an anxious/depressed alcoholism subtype versus an antisocial subtype of alcoholism. The SNP was not associated with the alcoholism phenotype *per se* (in cases vs. controls) in that study, although it was observed in another study (Sun *et al.*, 2002). This marker was not genotyped in the current sample, and the SNPs most proximal to rs130058 are not in high LD with the markers implicated in the current study ($r^2 = 0$–$0.01$). LD is also low ($r^2 = 0.13$) between the markers reported here, suggesting the detection of independent signals within *HTR1B*.

Analysis of gene expression localization revealed that genes spanning markers with a *P* value of less than $10^{-5}$ are expressed across many different tissues, and in some cases expression does not appear to be increased in any particular tissue. The same was true of the broader list of genes with a *P* value of less than $10^{-3}$ (data not shown). We hypothesized that genes relevant to depressive syndrome and AD would be expressed preferentially in the brain or in tissues relevant to the stress response, and indeed that was the case for many, although not all, of these genes.

Our analysis of LD in the 105 genes containing multiple significant markers suggests that in nearly half ($N = 48$) of these we can detect multiple independent signals. Included among these are genes implicated by four of our seven most significant markers, as well as possible candidate genes such as *GRIN2A* and *HTR1B*. These genes should be prioritized for replication attempts in future studies.

The fact that so few (< 6.0%) of the markers associated with the comorbid phenotype at a *P* value of less than $10^{-3}$ met the same criteria in the analysis of alcohol-dependent-only individuals is intriguing. However, 45.0% (391/868) of our significant SNPs met much less stringent criteria ($P < 0.05$) in the alcohol-dependent-only analysis. *P* values were significantly correlated across the analyses ($P = 0.0016$, $F = 10.0$, adjusted $r^2 = 0.010$), suggesting not only that the comorbid phenotype and AD alone are highly correlated but also that the former is also influenced by many genetic variants that are not independently associated with AD on its own. Thus, many of the variants reported here might be specific to susceptibility for only the comorbid phenotype, whereas others predispose to AD or depressive syndrome in the absence of the other. This finding is consistent with previous reports. Many genes have been found to be associated with AD or depression but not with both [for reviews, see Levinson (2006); Gelernter *et al.* (2009)], and some genes – notably those related to monoaminergic neurotransmitters – have been associated with both [for a review, see Saraceno *et al.* (2009)]. Our results are indicative of an additional layer of complexity – the existence of genetic variants predisposing specifically to comorbidity but which are not associated with either disorder on its own. We also explored the possibility that the comorbid cases simply represent a more severe subset of the alcohol-dependent cases. Among the full COGA GWAS sample, 66% of the total alcohol-dependent cases met criteria for an illicit drug dependence [a phenotype known to capture a more severe subset of cases in COGA (Dick *et al.*, 2007a)], compared with 67% of the comorbid cases. However, the comorbid cases did endorse significantly more AD symptoms compared with cases with AD-only in the full EA sample (mean symptom count = 5.8 and 5.3, respectively; $P < 0.01$); additional analyses indicate that the male portion of the sample drove this difference. Thus, it is possible that some of our results are attributable to a slightly more severe level of AD. We also note that, because the controls used for the AD + / MD – analysis are the same as those used in the primary analysis, these results should not be considered unbiased, as a portion of the overlap between results could be attributable to idiosyncrasies of the control sample.

We recognize a number of limitations to the current study. First, our analyses were limited to EAs and might not be generalizable to other populations. Replication in other samples is essential. Second, our gene ontology analysis might not be entirely unbiased, as they do not adjust for gene size (Wang *et al.*, 2010): relatively large genes (including many of those expressed in the brain) span more markers than small genes and are thus more likely to harbor markers meeting our significance criterion by chance alone. Third, our 'cases' include individuals who do not meet full DSM-IV criteria for an independent major depressive episode, in that many experienced depressive symptoms under the influence of alcohol or drugs (85 women and 201 men of the total 467 cases). It is unclear how such a distinction might influence our results. Previous work suggests that substance-induced and substance-independent depressions might be etiologically distinct (Schuckit *et al.*, 2007); in addition, although the comorbid phenotype might have a heritable component (Nurnberger *et al.*, 2002), the genetics underlying this phenotype could be distinct from those underlying a comorbid phenotype of AD and independent depression. In this case, the lack of distinction between independent and induced depression in the current study could be problematic. Unfortunately, our sample sizes are not large enough to conduct meaningful analyses on depressive symptoms that occur only within or only outside the context of alcohol or drug use. Furthermore, the mixed nature of the depressive episodes, and the fact that a number of cases met diagnostic criteria for abuse or dependence on other substances, reflects the nature of these disorders; they often appear in conjunction with other psychiatric problems, particularly in a clinical setting. To this end, we also recognize the possibility that the genes implicated in the current report are actually indexing risk to behavioral disinhibition rather than comorbid AD and depressive syndrome *per se*; the high prevalence of illicit substance use disorders among cases suggests that these individuals' various substance-related and mood-related problems could have developed through high levels of disinhibition, which is manifesting in various ways. Again, because of sample size limitations, we are unable to address this directly.

In summary, we report results from the first GWAS of a comorbid depressive syndrome/AD phenotype. Although we did not identify markers meeting genome-wide significance criteria, nominally significant markers implicate genes that have been previously implicated in AD, depression, and other psychiatric disorders. Multiple genes involved in glutamate function are associated with case/control status in our sample, as are other genes involved in neural processes. These results suggest that the comorbid phenotype is influenced by genetic variants that are somewhat distinct from those influencing AD on its own. We feel that these results provide an important step toward understanding the genetic influences on comorbidity between depressive syndrome and AD, and more generally toward our understanding of the biological etiology of these disorders.

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### Conflicts of interest

Dr. Laura Bierut is listed as an inventor on a patent, 'Markers of Addiction' (US 20070258898), covering the use of certain SNPs in determining the diagnosis, prognosis, and treatment of addiction. Dr. Bierut acted as a consultant for Pfizer, Inc. in 2008.

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