Published in final edited form as:
Psychiatr Genet. 2012 February ; 22(1): 31–41. doi:10.1097/YPG.0b013e32834acd07.

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

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Abstract

Objective—Depression and alcohol dependence 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 alcohol dependence and depression are genetically correlated. Here we report results from a genome-wide association study (GWAS) of a comorbid phenotype in which cases meet the DSM-IV symptom threshold for major depressive symptomatology and DSM-IV criteria for alcohol dependence.

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 SNP meets genome-wide significance criteria, we identify ten markers with p-values $< 1 \times 10^{-5}$, seven of which are located in known genes, which have not been previously implicated in either disorder. Genes harboring SNPs yielding $p<1 \times 10^{-3}$ 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, alcohol dependence, 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 alcohol dependence-only phenotype is modest.

Conclusions—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 alcohol dependence alone.

Keywords

genetics of alcoholism; comorbidity; genetic risk; depressive syndrome

Introduction

Alcohol dependence (AD) and major depression (MD) are common psychiatric disorders that often co-occur. Alcohol researchers have frequently delineated different “types” of alcohol dependence, 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 alcohol dependence as that which is driven in part by drinking to self-medicate negative affect; Del Boca and Hesselbrock (1996) describe four types of dependence, including an “internalizing” type that exhibits high anxiety and/or depression, and consumes 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 DSM-IV (American Psychiatric Association, 2000) major depressive disorder is 13.2%, and the 12-month prevalence is 5.2% (Hasin *et al.*, 2005). The corresponding figures for DSM-IV alcohol dependence are 12.5% and 3.8% (Hasin *et al.*, 2007). Among individuals with a lifetime diagnosis of major depressive disorder, 21% met criteria for alcohol dependence (Hasin *et al.*, 2005), which is 1.7-fold that predicted if the disorders were independent. Similarly, individuals with a lifetime alcohol dependence diagnosis are at increased risk for major depression (odds ratio=2.2, (Hasin *et al.*, 2007). Analyses of the treatment-based Sequenced Treatment Alternatives to Relieve Depression (STAR*D) sample (Davis *et al.*, 2006) found that individuals comorbid for major depression and a substance use disorder (not limited to alcohol) have an earlier age of onset for depression relative to non-comorbid depressed individuals, exhibit more depressive symptoms, have higher levels of functional impairment, and more frequently suffer from concurrent anxiety disorders. Importantly, these individuals also present an increased suicide risk (Davis *et al.*, 2006). Given the dramatic 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, whose effects vary in direction and magnitude, likely influence manifestation of and variation in depression and alcohol dependence. 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 major depression and alcohol dependence are genetically correlated. Kendler and colleagues (1993) found that, in a population of US adult women, the genetic correlation between the disorders was ~0.4–0.6. A study of adult male twins men (Lyons *et al.*, 2006) found that, while a reciprocal causation model (whereby

alcohol dependence increased the risk of major depression and vice versa) provided the best fit to 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 alcohol dependence and depressive syndrome (Nurnberger *et al.*, 2001). More recent studies have identified specific genes that are associated with both depression and alcohol dependence, including *CHRM2* (Edenberg and Foroud, 2006; Wang *et al.*, 2004), *SLC6A4* (Dick *et al.*, 2007b; Gokturk *et al.*, 2008), *COMT* (Baekken *et al.*, 2008; Sery *et al.*, 2006), and *DRD2* (Dick *et al.*, 2007c; Koks *et al.*, 2006), although others report failures to replicate these associations (e.g., Cohen-Woods *et al.*, 2009; Furlong *et al.*, 1998; Gillespie *et al.*, 2005; Serretti *et al.*, 2006). 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 genetics 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 alcohol dependence irrespective of depressive status, genes influencing depression irrespective of alcohol dependence 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 prior hypotheses, and therefore 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 and colleagues (Sullivan *et al.*, 2009) reported results from a genome-wide association study (GWAS) on major depression. Although no 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* (*PCLO*). Muglia *et al.* (2008) also conducted a GWAS analysis for major depression 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 and colleagues (2006) and Treutlein and colleagues (2009a) have reported GWAS results for alcohol dependence. The former reported clusters of nominally significant SNPs, including some located in genes previously associated with addiction-related traits. Treutlein *et al.* (2009a) employed 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 alcohol dependence. In the current study, we describe the results from the first genome-wide association study of comorbid depressive syndrome and alcohol dependence.

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 (SSAGA, (Bucholz *et al.*, 1994), a validated poly-diagnostic 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 SSAGA.

Case-control sample collection and measures

For the GWAS sample (described in more detail in Edenberg *et al.*, 2010), unrelated alcohol dependent cases and non-alcohol dependent controls were selected from the pool of alcohol dependent and community-ascertained families. Cases all met DSM-IV criteria for alcohol dependence at some point during their lives. In situations where an alcohol dependent proband had been phenotypically assessed multiple times for their lifetime history, he/she had to have met diagnostic criteria for alcohol dependence 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, but 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 could 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 marijuana dependent; 40.7% (N=188) were cocaine dependent; 30.6% (N=140) were dependent on stimulants; 20.6% (N=87) were dependent on sedatives; and 18.8% (N=85) were opioid dependent. Because COGA probands were recruited in part from treatment centers, they likely represent relatively severe cases of alcohol dependence. In addition, the cases used in this study, who also meet Criterion A for a major depressive episode, endorsed more AD symptoms than 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 alcohol dependence, also met lifetime DSM-IV symptom threshold for a major depressive episode (≥5 out of 9 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, and thus 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±.63) than cases (41.4±.48). This was intentional, to ensure that "unaffected" individuals were further through the period of maximal risk for onset of alcohol dependence. Cases were more frequently male ($\chi^2=71.16$, $p<0.0001$); this is likely due to the selection of cases via alcohol dependence, which is more prevalent in men than in women. However, had selection been based on depressive syndrome primarily and alcohol dependence secondarily, the sample would likely have had a disproportionate number of women. Cases 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 (CEU, YRI, CHB, and JPT) to assign the study subjects 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. Futhermore, exclusion of depressed controls (N=144) and cases whose depression met bereavement exclusion criteria (N=26) resulted in a final GWAS sample size of N=467 comorbid cases (N=287 males and N=180 females) and N=407 unaffected controls (N=132 males and N=275 females). Of the 467 cases, N=181 met criteria for an independent depressive episode – one experienced outside the context of drug or alcohol use – while N=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 (CIDR). 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, CA, 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 due to poor sample quality. Blind duplicate reproducibility was >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, etc., resulting in the removal of 13 additional samples. SNPs with a call rate of ≥98.0% in the EA sample were included in analyses. SNPs were excluded if the minor allele frequency was <1% in the combined case and control dataset; further SNPs were excluded if significant ($p<10^{-4}$) deviation from Hardy Weinberg equilibrium were 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 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 $p<10^{-5}$ 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 $p<10^{-3}$ 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 $p<10^{-3}$ threshold, 538 are

located within 366 known genes; gene ontology information was available for 321 of these through ToppGene. We employed a FDR of $p<0.05$ as the significance criterion, and excluded categories that applied to fewer than 3 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 hundred genes that contained two or more markers with $p<10^{-3}$ 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-MD GWAS case-control sample

The genotyping rate in the EA sample was 99.7%. After applying several quality control steps (see Methods), 876,476 single nucleotide polymorphisms (SNPs) were analyzed. The sex and age covariates were statistically significant ($p<10^{-15}$ and $p<10^{-11}$, 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 (5×10^{-8}; Figure 1, Supplementary Table 1). Ten SNPs had p-values $<10^{-5}$ (Table 1). Seven of these all 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. *OPA3* (*optic atrophy 3*) is involved in sensory perception, specifically visual perception. *EFHA2* (*EF-Hand domain family, member 2*) 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. *WDR7* (*WD repeat domain 7*), 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 *SPATA13* (*spermatogenesis associated 13*).

Gene expression in “top hits”

FHIT expression is consistent across most tissues, but was 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 is available. *OXTR* is highly expressed in lymphoblasts, although datamining 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, but especially in the amygdala, prefrontal cortex, and hypothalamus. *SPATA13* exhibits increased expression in various blood cells, though expression is also detected across brain tissues. *OPA3* is expressed consistently across brain tissues, though its highest detected expression levels are in cancer cells. No expression information is available for *EFHA2*.

Gene ontology analysis

We selected SNPs with $p<10^{-3}$ (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 – e.g., 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 $p<10^{-3}$ have been previously associated with alcohol dependence, 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.

One hundred five genes contained two or more SNPs with $p<10^{-3}$. We evaluated linkage disequilibrium (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 alcohol dependence phenotype, rather than by the comorbid phenotype, we ran a parallel analysis comparing N=354 individuals with alcohol dependence but without depressive syndrome to N=407 controls who had neither disorder (the same controls that were used in the primary analysis). We then compared p-values from that analysis to those in our original list of SNPs with $p<10^{-3}$. Only 52 of the 938 markers reported here met the same criteria in the alcohol dependence-only analysis; 44.3% (416/938) had $p<0.05$. The direction of the allelic effect was reversed in 12 (of 938) cases, but none of those 12 markers had $p<0.05$ in the secondary analysis.

Discussion

We present the first report of a genome-wide association analysis of comorbid depressive syndrome and alcohol dependence. No marker met genome-wide significance criteria, but 10 had p-values $<10^{-5}$, and 938 had p-values $<10^{-3}$. Indeed, given the genomic complexity and phenotypic heterogeneity of alcohol dependence and depressive syndrome, we might not expect that the effect size of any individual marker is large enough to reach genome-wide significance criteria in a study of this size; rather, many common variants of small effect likely influence these traits, with affected individuals each harboring an overlapping but unique set of risk-conferring alleles (Purcell *et al.*, 2009; Wellcome Trust Case Control Consortium, 2007).

For markers with a p-value $<10^{-3}$, 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 the 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, alcohol dependence (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 alcohol dependence, but *GRID1* was modestly associated with major depression in a genome-wide meta-analysis (Muglia *et al.*, 2008). 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 alcohol dependence (Johnson *et al.*, 2006; Treutlein *et al.*, 2009a) and major depression (Muglia *et al.*, 2008), respectively. Seven genes implicated in the current report – *CTNNA2*, *ESRRG*, *FBXO21*, *GALNT2*, *GRID1*, *IGSF21*, and *SMARCA2* – were reported previously to be proximal (within 250kb) and in reasonably high LD ($r^2 \geq 0.5$) with markers associated with major depression (Sullivan *et al.*, 2009). *AGTR1*, *CSMD2*, and *NMUR2* were nominally associated with alcohol dependence (via "clustered positive SNPs") in a report by Johnson and colleagues (2006).

Muglia and colleagues (2008) reported that the protein tyrosine phosphatase receptor *PTPRN* was significantly associated with major depression: in the current report, two other protein tyrosine phosphatase receptors, *PTPRD* and *PTPRS*, are 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 $p<10^{-3}$ to determine whether it has 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 $p<10^{-3}$. Lee and colleagues (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 versus controls) in that study, although it was 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 that we are detecting independent signals within *HTR1B*.

Analysis of gene expression localization revealed that genes spanning markers with $p<10^{-5}$ 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 $p<10^{-3}$ (data not shown). We would hypothesize that genes relevant to depressive syndrome and alcohol dependence would be expressed preferentially in the brain or in tissues relevant to the stress response, and indeed that is the case for many, though not all, of these genes.

Our analysis of linkage disequilibrium in the 105 genes containing multiple significant markers suggests that in nearly half (N=48) of these we are detecting 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.

That so few (<6.0%) of the markers associated with the comorbid phenotype at $p<10^{-3}$ 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 alcohol dependence alone are highly correlated, but also that the former is also influenced by many genetic variants that are not independently associated with alcohol dependence on its own. Thus, many of the variants reported here might be specific to susceptibility for only the comorbid phenotype, while others predispose to alcohol dependence *or* depressive syndrome in the absence of the other. This finding is consistent with previous reports. Many genes have been associated with alcohol dependence *or* depression but not the other (for reviews, see (Gelernter *et al.*, 2009; Levinson, 2006), 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 to 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), as compared to 67% of the comorbid cases. However, the comorbid cases did endorse significantly more alcohol dependence symptoms than cases with alcohol dependence-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 alcohol dependence. 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 European Americans and might not be generalizable to other populations. Replication in other samples is essential. Second, our gene ontology analysis might not be entirely unbiased, since 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 (N=85 females and N=201 males of the total N of 467 cases). It is unclear how such a distinction might influence our results. Previous work suggests that substance-induced and 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 alcohol dependence 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/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 and mood-related problems could have developed through high levels of disinhibition, which is manifesting in various ways. Again, due to sample size limitations, we are unable to address this directly.

In summary, we report results from the first GWAS of a comorbid depressive syndrome/alcohol dependence phenotype. Although we did not identify markers meeting genome-wide significance criteria, nominally significant markers implicate genes that have been previously implicated in alcohol dependence, 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 somewhat distinct from those influencing alcohol dependence on its own. We feel that these results provide an important step toward understanding the genetic influences on comorbidity between depressive syndrome and alcohol dependence, and more generally toward our understanding of the biological etiology of these disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The Collaborative Study on the Genetics of Alcoholism (COGA), Principal Investigators B. Porjesz, V. Hesselbrock, H. Edenberg, L. Bierut, includes ten different centers: University of Connecticut (V. Hesselbrock); Indiana University (H.J. Edenberg, J. Nurnberger Jr., T. Foroud); University of Iowa (S. Kuperman, J. Kramer); SUNY Downstate (B. Porjesz); Washington University in St. Louis (L. Bierut, A. Goate, J. Rice, K. Bucholz); University of California at San Diego (M. Schuckit); Rutgers University (J. Tischfield); Southwest Foundation (L. Almasy), Howard University (R. Taylor) and Virginia Commonwealth University (D. Dick). Other COGA collaborators contributing to the GWAS data set include: L. Bauer (University of Connecticut); D. Koller, S. O'Connor, L. Wetherill, X. Xuei (Indiana University); Grace Chan (University of Iowa); N. Manz, M. Rangaswamy (SUNY Downstate); A. Hinrichs, J. Rohrbaugh, J-C Wang (Washington University in St. Louis); A. Brooks (Rutgers University); and F. Aliev (Virginia Commonwealth University). A. Parsian and M. Reilly are the NIAAA Staff Collaborators. We continue to be inspired by our memories of Henri Begleiter and Theodore Reich, founding PI and Co-PI of COGA, and also owe a debt of gratitude to other past organizers of COGA, including Ting-Kai Li, currently a consultant with COGA, P. Michael Conneally, Raymond Crowe, and Wendy Reich, for their critical contributions. This national collaborative study is supported by NIH Grant U10AA008401 from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) and the National Institute on Drug Abuse (NIDA). ACE received funding from the National Institute of Mental Health (MH020030).

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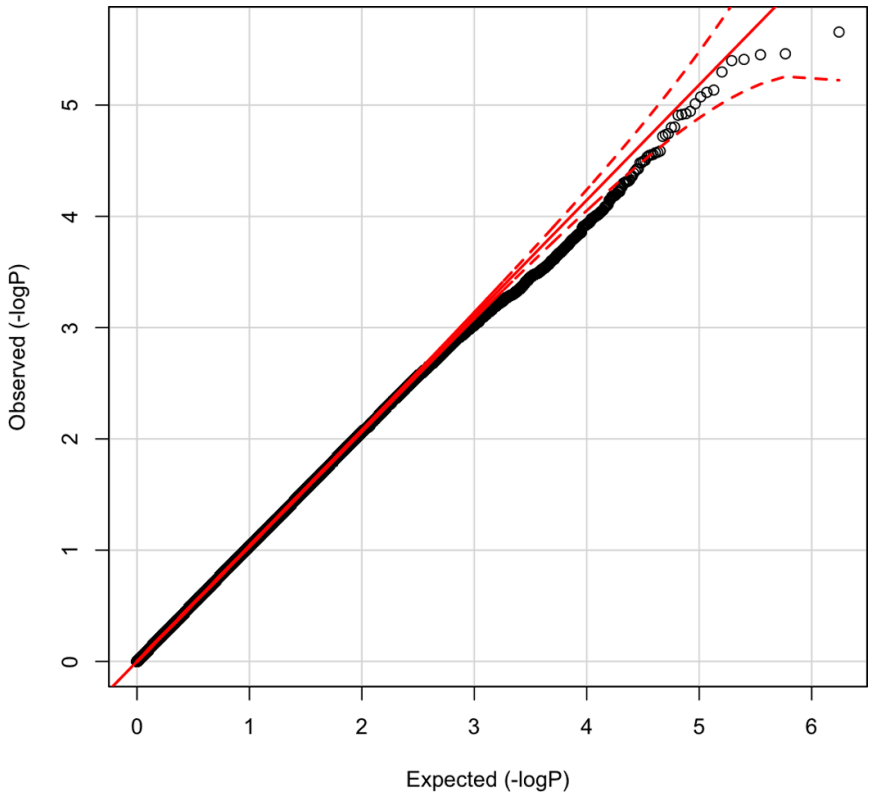

Figure 1.
Q-Q plot for association analysis of alcohol dependence with comorbid depressive syndrome (λ=1.0363). The solid red line represents the expected –log p-values; the black dots represent the observed –log p-values; the dashed red lines represent 95% confidence intervals.

Psychiatr Genet. Author manuscript; available in PMC 2013 February 1.

Table 1

Chr	SNP	BP	A1/A2	MAF	Gene	OR	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

Information on SNPs with $p<10^{-5}$ from genome-wide association analysis of comorbid depressive syndrome and alcohol dependence in European American COGA sample.

Table 2

Molecular Functions

Category Name	FDR p-value	Genes in Category
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

Category Name	FDR p-value	Genes in Category
response to drug	0.000016	*SLC22A5, CDKN1A, SMPD1, OXTR, SLC1A3, ABCB4, ABCB1, UQCRFS1, SNX27, DPYD, EMX2, SRP54, SLC46A2, GRIN2A*

Cellular Components

Category Name	FDR p-value	Genes in Category
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*

Molecular Functions		
Category Name	**FDR p-value**	**Genes in Category**
dystrophin-associated glycoprotein complex	0.002158	*SNTB1*, *DMD*, *CAV3*

Categories functionally enriched among genes containing markers with $p<10^{-3}$, based on 321 genes for which annotations were available in ToppGene (Chen *et al.*, 2009). 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.

Table 3

Gene	Alcohol Dependence	Depression	Other Relevant Psychiatric Phenotypes
AGTR1	Johnson *et al.*, 2006		
ALPK2		(Shyn *et al.*, 2011)	
C6orf204			smoking cessation* (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., 2008	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.*, 2010)
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 *et al.*, 2007)
GRIA4			schizophrenia (Carter *et al.*, 2007; O'Connor and Hemby, 2007)
GRID1		Muglia *et al.*, 2008; Sullivan *et al.*, 2009	bipolar disorder & schizophrenia (Carter *et al.*, 2007; Treutlein *et al.*, 2009b)
GRIN2A			smoking cessation (Uhl *et al.*, 2008); bipolar disorder & schizophrenia (Carter *et al.*, 2007)
HTR1B	(Sun *et al.*, 2002)	(Lopez-Figueroa *et al.*, 2004); substance abuse disorder with depression (Huang *et al.*, 2003)	depressed/anxious *versus* 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)

Gene	Alcohol Dependence	Depression	Other Relevant Psychiatric Phenotypes
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)

* Rose and colleagues 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 means only that the gene was included in the risk score.

Genes harboring markers with $p<0.001$ in the current study, that have been previously associated with alcohol dependence, depression, or other relevant psychiatric phenotypes. See Methods for details.

Table 4

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 spanning multiple, potentially independent ($r^2<0.5$) SNPs at $p<10^{-3}$. Genes implicated by our most significant ($p<10^{-5}$) markers are denoted with an asterisk.