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Confirmation of prior evidence of genetic susceptibility to alcoholism in a genome wide association study of comorbid alcoholism and bipolar disorder

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Abstract

Objectives—Alcoholism and affective disorders are both strongly comorbid and heritable. We have investigated the genetic comorbidity between bipolar affective disorder and alcoholism.

Methods—A genome-wide allelic association study of 506 patients from the University College London (UCL) bipolar disorder case control sample and 510 ancestrally-matched supernormal controls. 143 of the bipolar subjects fulfilled the research diagnostic criteria (RDC) diagnosis of alcoholism. 372,193 single nucleotide polymorphisms (SNPs) were genotyped. Genes previously shown to be associated with alcoholism and addiction phenotypes were then tested for association in the bipolar alcoholic sample using gene wise permutation tests of all SNPs genotyped within a 50kb region flanking each gene.

Results—Several CNS genes showed significant ($p<0.05$) gene wise evidence of association with bipolar alcoholism. The genes implicated which replicated previously identified associations with alcoholism were: cadherin 11 (*CDH11*), collagen type XI alpha 2 (*COL11A2*), neuromedin U receptor 2 (*NMUR2*), exportin7 (*XP07*) and semaphorin associated protein 5A (*SEMA5A*). The SNPs most strongly implicated in bipolar alcoholism, but which did not meet conventional genome-wide significance criteria were the insulin-like growth factor binding protein 7 (*IGFBP7*), carboxypeptidase O (*CPO*), cerebellin 2 (*CBLN2*), and the cadherin 12 (*CDH12*) genes.

Conclusions—We have confirmed the role of some genes previously shown to be associated with alcoholism in the comorbid bipolar alcoholism subgroup. In this subgroup bipolar disorder

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may lower the threshold for the phenotypic expression of these alcoholism susceptibility genes. We also show that some genes may independently increase susceptibility to affective disorder and alcoholism.

Keywords

Bipolar; alcoholism; genome wide association; comorbid; gene wise

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Introduction

Alcoholism and unipolar affective disorders are strongly comorbid (Davis et al., 2008, Pottenger et al., 1978, Helzer and Pryzbeck, 1988, Regier et al., 1990, McElroy et al., 2001, Kessler et al., 1997). Several studies have shown that up to 70% of cases of alcoholism also have major depressive disorder (Davis et al., 2008, Pottenger et al., 1978). This comorbid unipolar affective disorder has been repeatedly shown to be both primary and secondary to alcoholism (Gurling et al., 1984, Merikangas and Gelernter, 1990, Merikangas et al., 1985, Merikangas et al., 1994). Comorbidity between bipolar disorder (BPD) and alcoholism is well established from epidemiological, twin, family and linkage studies (O'Sullivan et al., 1988, Gurling et al., 1984, Merikangas and Gelernter, 1990, Preisig et al., 2001, Sonne and Brady, 2002, Berrettini et al., 1997, Winokur et al., 1996). These studies show that about 50% of individuals with bipolar disorder also manifest alcohol abuse or dependence and altogether about 75% have a substance use disorder.

The early twin and adoption studies can be interpreted as suggesting that there is a higher heritability for alcoholism combined with criminality than for other subtypes of alcoholism (Kaij, 1960, Gurling et al., 1984, Gurling and Cook, 1999). Men with alcohol dependence have much higher rates of dis-social personality disorder and criminality than women with alcoholism (Cloninger et al., 1978, Cloninger et al., 1986). Two important adoption studies from Sweden found replicated evidence for genetic effects associated with criminality and male alcoholism, known as type two alcoholism, which had an early age of onset and strong genetic transmission (Sigvardsson et al., 1982). Women with alcohol dependence have higher rates of depression and anxiety than men and the heritability may be higher (Klerman et al., 1996, Merikangas et al., 1985, Merikangas et al., 1994, Gurling and Cook, 1999, Kuo et al., 2006a, Bierut et al., 1999). The relationship between anxiety disorders and alcoholism seems to be very similar to the relationship between alcoholism and depression (Mullan et al., 1986, Merikangas et al., 1994).

Alcoholism is strongly recurrent in both the first and second degree relatives of alcoholic probands (Cotton, 1979). This is consistent with there being both genetic and cultural effects on risk. Genetic effects in alcoholism were first established using classical twin and adoption studies (Kaij, 1960, Bohman, 1978, Sigvardsson et al., 1996, Sigvardsson et al., 1982, Ball, 2005). Heritability estimates for human diseases have inherent limitations but one twin study of alcohol dependence estimated heritability to be between 50% and 61% (Hansell et al., 2008). The largest twin study, of 9,000 male pairs, found that alcoholism was 53% "heritable" (Kendler et al., 1997). However, twin studies do not always show substantial heritabilities or increased concordance for alcoholism in monozygotic (MZ) compared to dizygotic (DZ) female twins (Gurling et al., 1984, Pickens et al., 1991, Prescott et al., 2007, Prescott et al., 2005).

Genetic linkage studies on large samples of families have been carried out by several research groups in European and Native American populations (Cook et al., 1996, Guerrini et al., 2005, Kendler et al., 2006, Kuo et al., 2006b, Hesselbrock et al., 2001, Ehlers et al., 2004). The most important have been those from the USA Consortium on the Genetics of

Alcoholism (COGA) (Foroud et al., 2000). For example a lod above 3.00 for linkage with alcohol dependence syndrome (ADS) was found on chromosome 3. Another lod above 3.00 with ADS was found on chromosome 6 (Cantor and Lanning, 1999). Lods above 3.00 were found with alcoholism on chromosome 11 and 4 in Native American families (Long et al., 1998). Some of these positive findings were replicated in a linkage study in Ireland (Prescott et al., 2006). In the USA COGA study a combined ADS and conduct disorder (Dick et al., 2004) phenotype showed positive lod scores over a region of chromosome 2 that includes the Tachykinin A Receptor 1 (*TACR1*) locus. The *TACR1* locus was later reported to be associated with bipolar disorder and attention deficit hyperactivity disorder (ADHD) (Perlis et al., 2008, Ferreira et al., 2008, Yan et al., 2009). Lod scores greater than 3.0 were found on chromosomes 7, 2, 3, 5, 9, and 14 when antisocial families were selected to achieve greater homogeneity (Jacobson et al., 2008). Replication of the linkage to alcoholism on chromosome 1 originally found by the USA COGA study (Dick et al., 2002) has been achieved in Finland (Lappalainen et al., 2004) with further support from a linkage study in the UK (Guerrini et al., 2005). This locus appears to be showing linkage to a combined depression and alcoholism phenotype (Nurnberger et al., 2001).

Many small and medium scale case control genetic association studies have already shown that genetic effects on susceptibility to alcoholism are partly derived from neurotransmitter receptor variants (Kohnke, 2008), from alcoholism metabolism genes (Dick et al., 2007, Kuo et al., 2008, Luo et al., 2005) and from other central nervous system (CNS) related genes. Most striking in alcoholism is the protective effect of the alcohol-metabolising aldehyde dehydrogenase 2 (*ALDH2*) variants in Japanese and Chinese Asian populations (Yoshida, 1992, Agarwal and Goedde, 1987). The possible involvement of the alcohol dehydrogenase (*ADH*) gene cluster has been reinforced by linkage studies repeatedly implicating chromosome 4 (Luczak et al., 2006, Edenberg et al., 2006) and by several allelic assocation studies (Kuo et al., 2008, Edenberg et al., 2006, Ball, 2005). The GABA receptor alpha2 subunit (*GABRA2*) gene is the most replicated gene increasing risk of alcoholism (reviewed in Enoch (2010) and recently examined in Bierut et al., 2010). The Bierut study was a GWAS of alcohol dependence syndrome using 1,897 alcohol dependent individuals drawn from the COGA study, the Family Study of Cocaine Dependence (FSCD) study and the Collaborative Genetic Study of Nicotine Dependence (COGEND) study datasets in which *GABRA2* was also evaluated independently. In this study five SNPs at *GABRA2* were nominally associated (uncorrected $P < 0.05$), with odds ratios between 1.11 and 1.16 (Bierut et al., 2010). Another GWAS of probands drawn from the COGA study reported association with alcohol dependence and a cluster of genes on chromosome 11. This study also implicated rs35164 (just downstream of *CDH11*) in early onset alcoholics and found evidence to support associations of alcohol dependence with carboxypeptidase E (*CPE*), deoxyribonuclease II beta (*DNASE2B*), solute carrier family 10 (sodium/bile acid cotransporter family), member 2 (*SLC10A2*), ADP-ribosylation-like factor 6 interacting protein 5 (*ARL6IP5*), inhibitor of DNA binding 4, dominant negative helix-loop-helix protein (*ID4*), GATA binding protein 4 (*GATA4*), spectrin repeat containing, nuclear envelope 1 (*SYNE1*), and adenylate cyclase 3 (*ADCY3*) (Edenberg et al., 2010). Other genes possibly mediating risk of alcoholism are discussed in Edenberg et al 2010. In a further GWAS analysis of probands from the COGA study using event-related brain oscillations as an endophentype, association between the serotonin receptor 7 (*HTR7*) and the endophenotype as well as with alochol dependence alone was reported (Zlojutro et al., 2010).

A GWAS from Germany with 487 alcohol dependent males and 1358 controls followed up analysis of 140 SNPs in a further 1024 male patients and 996 age-matched controls. The SNPs in the follow up study were made up of 121 SNPs that showed a nominal significance of $P<10^{-4}$ in the original GWAS and 19 SNPs in the human homologues of rat genes whose

brain expression was altered after chronic alcohol exposure (Treutlein et al., 2009). In the data from the combined study two intergenic SNPs located on chromosome 2q35 met genome-wide significance (rs7590720, $P = 9.72 \times 10^{-9}$; rs1344694, $P = 1.69 \times 10^{-8}$) in a region which has been implicated in linkage studies for alcohol phenotypes. Nine further SNPs were located in genes, including *CDH13* and *ADH1C*, which have been reported to be associated with alcohol dependence.

An Australian and Dutch pooling-based GWAS of nicotine dependence (ND; 1273 cases and 1113 controls) and alcohol dependence (AD; 1224 cases and 1162 controls) revealed three SNPs for comorbid AD/ND (rs7530302 near MARK1 on chromosome 1 ($P = 1.90 \times 10^{-9}$), rs1784300 near DDX6 on chromosome 11 ($P = 2.60 \times 10^{-9}$) and rs12882384 in KIAA1409 on chromosome 14 ($P = 4.86 \times 10^{-8}$). None of the SNPs achieved genome wide significance in a Australian/Dutch meta-analysis in the same paper, but a gene network diagram based on the top results revealed in this study overrepresentation of genes coding for ion-channels and cell adhesion molecules {Lind, 2010 #91}.

Additionally a genome-wide association of pooled DNA from alcoholics was compared to quantitative trait loci implicated in mouse addiction phenotypes. This convergent strategy identified brain-expressed susceptibility genes involved in cell adhesion, enzyme activity, protein and transcriptional processes, neuroreceptors, ion channel and transport processes and cell structure. The set of proteins implicated was described as proving a “connectivity constellation” for susceptibility to addiction (Liu et al., 2006, Uhl et al., 2008a, Liu et al., 2005).

Family, adoption, twin, linkage and allelic association studies have contributed to the evidence for genetic susceptibility to bipolar affective disorder with markers at several genes showing replicated association with bipolar disorder (Baum et al., 2008, Craddock et al., 2008, Moskvina et al., 2009, Ferreira et al., 2008, Askland, 2006, Askland et al., 2009). Recently, Johnson and colleagues found several overlapping clusters of single nucleotide polymorphisms (SNPs) when comparing the genome-wide findings of four studies on bipolar disorder and one on substance dependence, including alcoholism and illicit drug use (Johnson et al., 2009). There have been no previous genome-wide association studies of bipolar alcoholism. This study aims to identify genetic regions associated with bipolar disorder that are comorbid with alcoholism and also to distinguish between genes involved in alcoholism and those involved in bipolar affective disorder.

Materials and methods

Subjects

The BPD cases and control subjects were from the University College London (UCL) bipolar disorder sample comprising 506 BPD cases and 510 controls. Cases were of Caucasian English, Irish, Scots or Welsh ancestry, with a maximum of one grandparent from Western Europe permitted.

Cases were interviewed using the Schizophrenia and Affective Disorders Schedule – Life Time version (SADS-L) and diagnosed according to ICD-10 diagnostic criteria, (ICD-DCR), Research Diagnostic Criteria (RDC) and DSM-III-R (Spitzer and Endicott, 1977, Spitzer et al., 1978). All cases met the ICD-10–DCR criteria for bipolar affective disorder and DSM-III-R criteria for bipolar I disorder. We did not take illicit drug use into account. The control subjects were recruited from London branches of the National Health Service (NHS) blood transfusion service, from local NHS family doctor clinics and from university student volunteers. All control subjects were interviewed with the SADS-L to exclude all psychiatric disorders including alcohol dependence and drug misuse according to RDC/

DSMIIIR criteria as well as drinking above the upper limit for safe drinking of 21 units per week for males and 14 units for females as defined by the UK Royal College of Physicians (where a unit is 8g of ethanol). The control subjects were further selected on the basis of not having a family history of bipolar disorder, schizophrenia or alcoholism. All subjects gave informed consent prior to inclusion, and study approval was obtained from local and central NHS research ethics committees.

Genomic DNA was isolated from blood samples using a standard detergent cell lysis, proteinase K, phenol chloroform, ethanol precipitation method (Sambrook, 2001). Genotyping was performed using the Affymetrix Gene Chip Human Mapping 500K Array Set at the Broad Institute of Harvard and MIT using standard protocols and quality control, and addressing potential population stratification as previously described (Sklar et al., 2008, Ferreira et al., 2008). SNPs were excluded for the following reasons: (1) call rate of less than 95% (n = 23 673), (2) minor allele frequency of less than 1% (n = 67 661), (3) Hardy–Weinberg equilibrium $P<1 \times 10^{-6}$ in controls (n = 11 671) and (4) differential rates of missing genotypes between cases and controls, using Fisher's exact test, $P<1 \times 10^{-3}$ (n = 388). Following the above exclusions, genotype data was obtained for 372,193 single nucleotide polymorphisms (SNPs).

We defined two phenotypes: bipolar disorder cases that had comorbid RDC alcoholism (BPALC) and bipolar disorder cases without comorbid RDC alcoholism (non-alcoholic bipolar disorder, NABPD). Tests of allelic association were carried out in the UCL BPALC sample by comparing allele frequencies with the UCL supernormal control sample. The NABPD subgroup was also compared to the control sample. Both chi square analyses were performed using PLINK (Purcell et al., 2007). We set a threshold of p = 0.01 to declare a SNP as showing nominal evidence for association with the bipolar alcoholism group compared to controls (Uhl et al., 2008a). Assessment of previous SNP and gene associations was explored using information from previous association studies of alcoholism listed in the Genetic Association Database (www.geneticassociationdb.nih.gov/), the Ethanol-Related Gene Resource (www.bioinfo.vipbg.vcu.edu/), the HuGE navigator (www.hugenavigator.net/), PubMed, and hand-searched selected articles. The search for implicated genes was completed on 16 December 2009. Replication genes were those implicated in association studies for alcoholism ("alcohol dependence" or "alcohol dependency" or "alcoholism") and related addictions phenotypes ("drug" or "opiate" or "cocaine" or "cannabis" and "dependency" or "addiction"). If a prior report implicated a gene found in the present study we conducted an empirical gene-wise analysis in order to take into account the multiple markers genotyped at each gene locus in our sample. The relationship between SNP markers to genes was defined using Ensembl gene set 34_35g. Gene-wise significance was calculated using COMBASSOC 1.2 for all SNPs located within 50 Kb of both ends of each implicated gene (Curtis et al., 2008). COMBASSOC computes an empirical p value at the level of the whole gene by combining individual p values from all markers across the gene and then carrying out a permutation test, for which we used 10,000 replicates. COMBASSOC requires a minimum of 2 SNPs. Lastly, a Bonferroni correction for the number of genes tested was applied to genes with gene wise $p<0.05$. Power estimation for the experiment was calculated using the QpowR program (https://www.msu.edu/~steibelj/JP_files/QpowR.pdf). Given a total heritability of 0.05, a sample size of 640, an experiment-wise error rate of 1×10^{-7} (with an alpha of 0.05 and 500,000 tests) power was 0.68 under an additive model and 0.58 assuming a dominance model. The genomic inflation factors (lambdas) for the two analyses were 1.012 for BPALC vs controls, and 1.011 for NABPD vs controls. These were calculated in R using the GenABEL v. 1.6-4 package. We used R to generate the Manhattan and Q-Q plots using the GGPLOT2 package (http://had.co.nz/ggplot2/) and with additional R scripts from (http://gettinggeneticsdone.blogspot.com/2010/01/gwas-manhattan-plots-and-qq-plots-using.html).

Supplementary material Figures 2 and 3 are quantile-quantile (Q-Q) plots for BPALC vs controls and NAPBD vs controls, respectively, both showing a normal distribution. Supplementary Figures 4 and 5 report the Manhattan plots for BPALC vs controls and NAPBD vs controls, respectively.

Results

The main aim of our study was to detect those SNPs and genes previously associated with alcoholism that could be replicated in our subgroup of alcoholic bipolar research subjects. Demographic breakdown of the groups was as follows: i) BPALC male 80 (56%), female 63 (44%). ii) NABPD, male 113 (31%), female 250 (69%). iii) Controls male 217 (43%), female 293 (57%).

There were 3799 SNPs nominally associated with alcoholic bipolar subjects with significance p <0.01. 2136 of these SNPs were within 50kb of a gene and 1491 were within the gene. The nominally significant associations are reported in Table 1 and Supplementary Table 1. Twelve SNPs attained p-values of $<1.0 \times 10^{-4}$ but no markers attained conventional genome-wide significance levels. Table 1 lists the tests of association with SNPs associated with bipolar alcoholism and in the non alcoholic bipolar group (NABPD) and this enables us to differentiate genetic effects on bipolar alcoholism from genetic effects on pure bipolar disorder (ie NABPD). Supplementary Table 1 reports the full genome wide findings, arranged by BPALC p-value, where SNPs had p<0.01.

As described in the methods section, in order to confirm the involvement of genes previously implicated in alcoholism and addictions phenotypes, we employed gene wise permutation tests on all SNPs within and next to a previously implicated gene and corrected for multiple tests . The results from these tests are shown in Table 2. Replications with gene wise significance at p<0.05 for association with the bipolar alcoholic group included the cadherin 11 (*CDH11*), collagen type XI alpha 2 (*COL11A2*), neuromedin U receptor 2 (*NMUR2*), exportin7 (*XP07*) and semaphorin associated protein 5A (*SEMA5A*) genes. Only the gene wise association with CDH11 remained significant after Bonferroni correction for the genes tested (n=38). Also shown in Table 2 are the tests of association with SNPs in the alcoholism associated genes in the bipolar research subjects after the alcoholic bipolar subjects have been removed, denoted once again as the NABPD group.

Lastly, genes which were implicated by SNP associations and replication, but which did not achieve gene-wise significance in our sample are also detailed in Table 2. The most replicated alcoholism genes *ALDH, ADH* and *GABRA2* were not implicated at SNP or gene wise level in the BPALC phenotype.

We also note that the remaining SNPs which we found were nominally associated with BPALC and which confirmed previous reported gene associations with addiction phenotypes in the “connectivity constellation” failed to show gene-wise significance in our study (data not shown). These connectivity genes were: the neural cell adhesion molecule (*NRCAM*) gene, brain-specific angiogenesis inhibitor 3 (*BAI3*), contactin associated protein-like 2 (*CNTNAP2*), CUB and Sushi multiple domains 1 (*CSMD1*), catenin (cadherin-associated protein), alpha 2 and 3 (*CTNNA2 & 3*), Down syndrome cell adhesion molecule (*DSCAM*), protein tyrosine phosphatase, receptor type, D (*PTPRD*), sarcoglycan zeta (*SGCZ*), brain-specific angiogenesis inhibitor 3 (*BAI3*), astrotactin 2 (*ASTN2*), ankyrin repeat and sterile alpha motif domain containing 1B (*ANKS1B*), contactin 4 and 6 (*CNTN4 & 6*) (Uhl et al., 2008b). The SNPs associated with these genes are listed in Supplementary Table 1.

The top-ranked (by genome wide significance values) alcoholic bipolar SNP associations and the genes they represent are listed in Table 1 and continued in Supplementary Table 1. Selected findings are noted below. The most significant SNP ($p=4.54\times10^{-6}$) was rs8062326, a single SNP placed midway between the synaptotagmin 17 (*SYT17*) and Inositol 1,4,5-triphosphate receptor-interacting protein-like 2 (*ITPRIPL2*) genes. One of two SNPs intronic to the collagen, type I, alpha 2 (*COL1A2*, highest ranked SNP rs369982, $p = 2.06 \times 10^{-5}$) gene was ranked second most associated and when all SNPs in this gene were analysed the gene-wise test was significant at $p = 5 \times 10^{-4}$. Ranked 29th, the first of forty five SNPs nominally associated with BPALC were located in and around cadherin 11 (*CDH11*, highest ranked SNP rs429065, $p = 1.0 \times 10^{-4}$, gene-wise $p = 6 \times 10^{-4}$). rs429065 is located 22 Kb downstream of *CDH11* (Figure 1). Eleven intronic SNPs at *CDH11* were found to be associated with BPALC of which two were also nominally associated with NABPD. Two of the associated SNPs from a genome-wide study of pooled DNA from the COGA sample (Johnson et al., 2006) were also nominally associated in our sample. These were intronic SNP rs35200 ($p = 1.9 \times 10^{-2}$) and rs35164 ($p = 1.4 \times 10^{-4}$) which is 5,484 base pairs downstream from *CDH11*. rs25164 was also implicated with early age of onset alcoholism in the GWAS by Edenberg and colleagues (Edenberg et al., 2010). Excluding the CDH11 SNPs replicated above, no strongly associated SNPs from previous GWAS replicated in this study. Eighteen SNPs within the introns of cadherin 12 (*CDH12*, Table 3) and 10 of 14 SNPs genotyped within the introns of cadherin 13 (*CDH13,* Table 2) were nominally associated with BPALC. In total eighty four SNPs in or near cadherin-family genes were nominally associated with BPALC.

Table 1 and Supplementary Table 1 also report the top-ranked BPALC-associated SNPs which were also nominally significant in NABPD genetic associations. Most significant and ranked 2nd genome wide was a SNP intronic to musashi homolog 2 (*MSI2*, highest ranked SNP rs1024820, $p = 1.24 \times 10^{-5}$). However these SNPs were not in LD with the *MSI2* gene, nor was the gene associated with bipolar alcoholism using gene-wise analysis. There were six BPALC associated SNPs in or within 25 Kb of tetraspanin 8 (*TSPAN8*, highest ranked SNP rs1705236, p = 1.24 × 10-4; gene wise p non significant) three of which were also associated with NABPD.

We found six nominally BPALC-associated SNPs near or within the GABA-A beta-3 receptor (*GABRB3*, highest ranked SNP $p = 2.6 \times 10^{-4}$, uncorrected gene-wise significance $p = 2 \times 10^{-3}$). The tachykinin receptor 1 (*TACR1*, also known as neurokinin-1 receptor, NK1R) was nominally associated with BPALC with three SNPs, one of which, rs3771829 ($p = 3 \times 10^{-3}$) was also associated with NABPD ($p = 1.7 \times 10^{-2}$). There were eight SNPs nominally associated with BPALC near the gene for insulin-like growth factor 1 (*IGF1*, highest ranked SNP: rs12426318, 154 kb downstream, $p = 1.8 \times 10^{-3}$). Neither *TACR1* nor *IGF1* survived gene wise testing for either phenotype.

Lastly we performed gene wise testing on the remaining genes implicated by clustered (7 or more) nominal SNP associations with the BPALC phenotype, but which had no prior literature association with alcoholism or related phenotypes. These represent novel BPALC candidate genes (Table 3). Three genes survived Bonferroni correction: *KIAA1772, DC2* protein, and zinc finger and BTB domain containing 16 (*ZBTB16*).

Discussion

This genome-wide study comparing individuals with bipolar disorder with and without alcoholism diagnoses has replicated five previous associations with alcoholism and addiction phenotypes by gene wise analysis that appear to be independent of bipolar disorder. Our most significant SNP associations with BPALC were in or near genes

involved in cell adhesion, neurotransmitter pathways, enzymatic activity, cellular messengers, connective tissue, and cell regulation but none of these reached genome wide significance ($p<10^{-8}$) and can therefore the associations can only be described as suggestive. The presence of association between these SNPs and the BPALC cases but not in the NABPD cases suggests genetic effects on alcoholism independent of bipolar affective disorder.

First we discuss SNP associations then gene wise associations. Although we found a SNP strongly associated with BPALC at Synaptotagmin 17 (*SYT17*, ranked 1st genome-wide) we could not be certain that it was actually this gene that was implicated because it was equidistant between *SYT17* and Inositol 1,4,5-triphosphate receptor-interacting protein-like 2 (*ITPRIPL2*). *SYT17* is expressed abundantly in the frontal and temporal lobes, hippocampus, hypothalamus, amygdala, substantia nigra, and pituitary. Synaptotagmins are considered to be important in the docking and fusion of synaptic vesicles and plasma membrane in neurotransmitter release and are thus worthy candidate genes (Fukuda et al., 1994). *ITPRIPL2* is also expressed in the brain but little is known about its function.

The simultaneous presence of nominal SNP association between the markers in both the BPALC and NABPD groups suggests putative genetic effects on alcoholism through an affective disorder mechanism. One such gene, *MSI2* (the gene nearest the highest-ranked SNP in the NABPD phenotype) encodes a brain-expressed protein which may play a role in post transcriptional gene regulation. Another such gene, tetraspanin 8 (*TSPAN8)* is independently associated with bipolar disorder, and encodes a protein which mediates signal transduction events that play a role in the regulation of cell development, activation, growth and motility. Our most significant *TSPAN8* SNP was rs1705236 and this was found to be the most well associated SNP in a recent large whole genome association study of bipolar disorder, which notably included the UCL sample used in this study (Sklar et al., 2008). *TSPAN8* was nominally associated with NABPD at uncorrected gene wise level ($p=3.9 \times 10^{-3}$) but not for the BPALC phenotype. This further reinforces the role of *TSPAN8* as a bipolar disorder candidate gene.

Genes implicated by gene-wise significance in the present study include those that are functionally involved in second-messenger systems, ion and neurotransmitter function, neuronal adhesion, differentiation and architecture. These are considered part of the "connectivity constellation" of genes (Uhl et al., 2008b). We applied a Bonferroni correction to the gene wise association test presented in table 2 in an attempt to correct for the multiple tests performed. And indeed it is encouraging to see that the result for *CDH11* survives this correction. However, this correction is almost certainly overly conservative given the prior evidence for association at each of the loci tested in table 2. The cadherin (*CDH*) gene family are a useful example of connectivity genes. These genes encode a large group of transmembrane proteins that mediate calcium-dependent cell-cell adhesion and the generation of synaptic complexity in the developing brain {Benson, 1998 #93}. Cadherins have been implicated in mnemonic processes, addictions and bipolar disorder (Johnson et al., 2009, Uhl et al., 2008b). We discuss the cadherins of relevance to the present paper here.

CDH11 (Tables 1, 2) was implicated in a genome-wide study of pooled COGA DNA (Johnson et al., 2006), a recent GWA of alcoholism from COGA families (Edenberg et al., 2010),and was implicated in our study with positive corrected gene-wise significance tests and high genome-wide ranking. Twenty two *CDH11* SNPs in the UCL sample were nominally implicated in BPALC and seven were associated with NABPD in the UCL sample. Despite evidence that our strongest *CDH11* SNP, rs429065 may be nominally associated with bipolar disorder in the Wellcome Trust Case Control Consortium ($p=1.2 \times$

10-2) and STEP-BD samples ($p = 2.7 \times 10^{-2}$ (WTCCC, 2007; Sklar et al., 2008). *CDH11* was however not associated with NABPD at the level of the gene

CDH12 (gene wise $p = 3.5 \times 10^{-3}$) is found near a linkage peak for alcohol dependence symptom count (Hansell et al., 2010), was found over-expressed in both post-mortem brain studies of individuals with a history of alcohol misuse or dependence (Sokolov et al., 2003) and violent suicide victims (Thalmeier et al., 2008), and is now included on a custom SNP array chip for genotyping of candidate addiction genes (Hodgkinson et al., 2008).

CDH13 was not implicated in the present study but was recently associated with bipolar disorder and addictions in a study of GWA data for both disorders (Johnson et al., 2009) and in a GWA of alcohol dependence (Treutlein et al., 2009). *CDH11* is implicated, and *CDH12* tentatively implicated, in alcoholism in bipolar subjects in the present study. Thus the cadherin family remain robust candidate genes through replication and offering plausible pathways for alterations in synaptic neuroplasticity in individuals with alcohol dependence (Sokolov et al., 2003).

In the present study the remaining connectivity genes implicated by gene wise testing include: i) a member of the semaphorin protein family, Semaphorin associated protein 5A (*SEMA5A*). This gene is involved in axonal guidance during neural development (Adams et al., 1996). *SEMA5A* was previously associated with both bipolar disorder and substance dependence (Johnson et al., 2009) and successful smoking cessation (Uhl et al., 2008c). ii) neuromedin U receptor 2 (*NMUR2)* is a brain expressed protein found in the hypothalamus which was implicated in the pooled COGA genome-wide association data (Johnson et al., 2006). iii) Collagen type XI alpha 2 (*COL11A2*) has been implicated in sensorineural deafness but has not been reported as being associated with addictions or affective disorder phenotypes. iv) Exportin 7 (*XPO7*) mediates the nuclear export of proteins with broad substrate specificity and was implicated in the pooled COGA genome-wide association data (Johnson et al., 2006).

Three genes *KIAA1772,* oligosaccharyltransferase complex subunit *(OSTC),* and zinc finger and BTB domain containing 16 (*ZBTB16*) with novel gene wide association results remain unconfirmed candidate genes implicated by multiple SNP clustering and corrected significance values and as such they are worthy of further investigation.

Important negative findings include the genes of the Gamma-aminobutyric acid (GABA) system which encode receptors for the major inhibitory transmitter of the nervous system. GABA receptors are of strong interest in addictions, alcoholism, and affective disorders (Agrawal et al., 2008, Bierut et al., 2010). GABA receptor alpha 2 (*GABRA2*) is the most replicated gene in alcoholism (Bierut et al., 2010). *GABRB3* polymorphisms while less robustly implicated in alcoholism than *GABRA2* have been associated with mood-related alcohol expectancy (Young et al., 2004) and are more common in the children of alcoholics than children of non alcoholics (Namkoong et al., 2008). In the present study *GABRB3* showed gene wise association with BPALC but did not survive Bonferroni correction. None of the GABA receptor genes including *GABRA2* were implicated at corrected gene wise level in the present study. This may reflect the small study size, genetic heterogeneity, or possibly that alcoholism in bipolar disorder is mediated by different pathways.

The relationship between bipolar and unipolar affective disorder and alcohol dependence is bidirectional (Sonne and Brady, 2002) and there is little room for doubt that the two disorders have reciprocal effects on each other. Alcohol dependence may also change expression and function of the same neurotransmitters as those involved in bipolar disorder, thereby “prompting” the symptoms of unipolar and bipolar affective disorder (Tohen et al., 1998).

The present study has benefitted from a well-characterized, ancestrally homogenous and ancestrally-matched control sample. The main drawback was the small sample size of bipolar alcoholics for a genome-wide study.

Although low significance values are needed for convincing GWAS results the fact that genes previously implicated in the genetics of alcoholism were found by us also to be associated with bipolar-alcoholism after gene wise association tests suggests that our results are robust. The “connectivity cluster” genes are strongly represented in this study. We also report tentative new alcoholism gene associations in a bipolar-alcoholic sample which are in need of further investigation in additional alcoholic bipolar and alcoholism samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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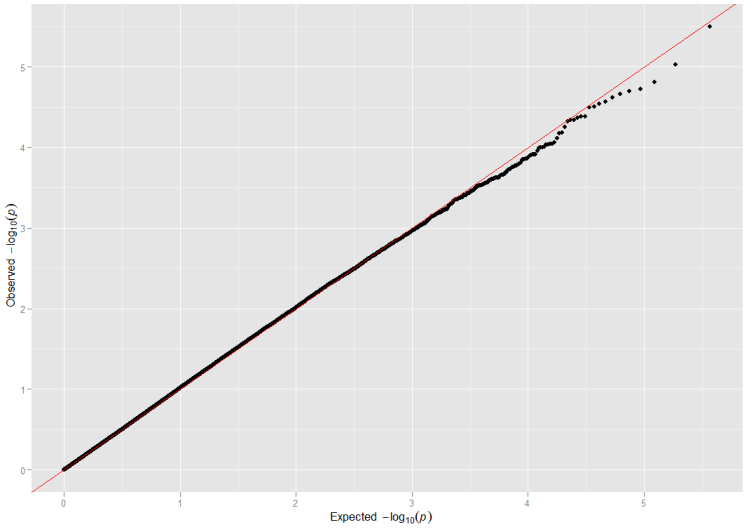

Figure 1.
The CDH11 SNP (rs429065) with the most significant BPALC association is shown (large red diamond) with its P-value. Estimated recombination rates (taken from HapMap) are plotted to reflect LD structure. LD between rs429065 and the other SNPs genotyped in the study are shown; red: r2 ≥ 0.8; orange:0.5 ≤ r2 <0.8; yellow:0.2 ≤ r2 < 0.5; white: r2 < 0.2.

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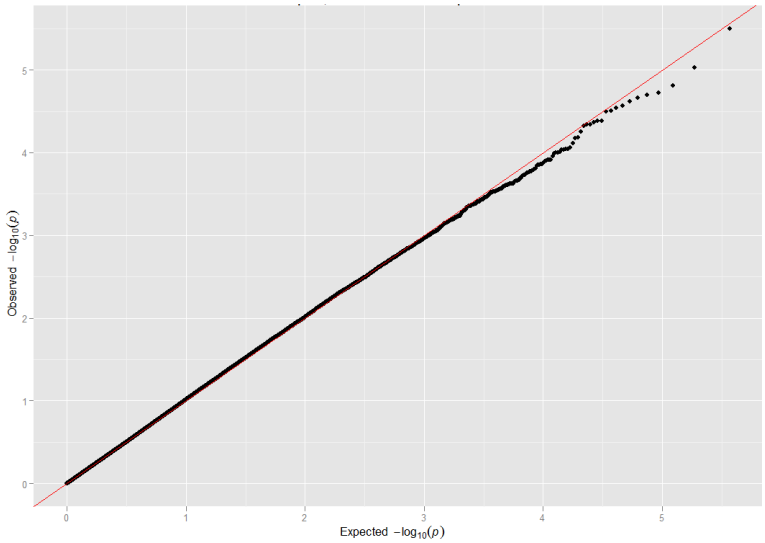

Figure 2.
Quantile-quantile plots of P values of autosomal single-nucleotide polymorphisms (SNPs) for BPALC vs controls

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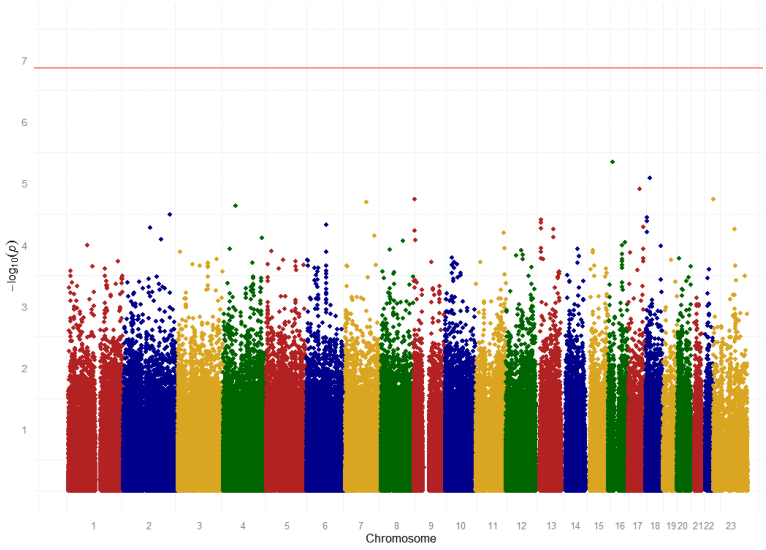

Figure 3.
Quantile-quantile plots of P values of autosomal single-nucleotide polymorphisms (SNPs) for NABPD vs controls

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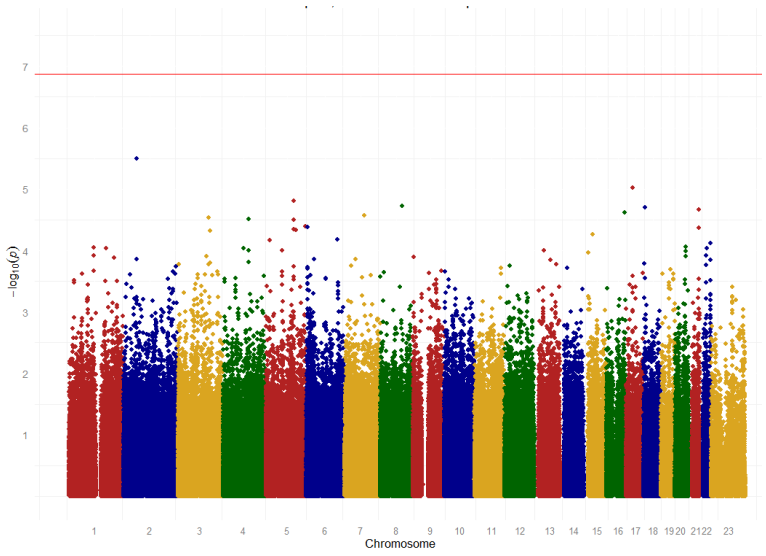

Figure 4.
Manhattan plot of BPALC vs controls. The figure shows a genome-wide synopsis of the genome-wide association study findings.

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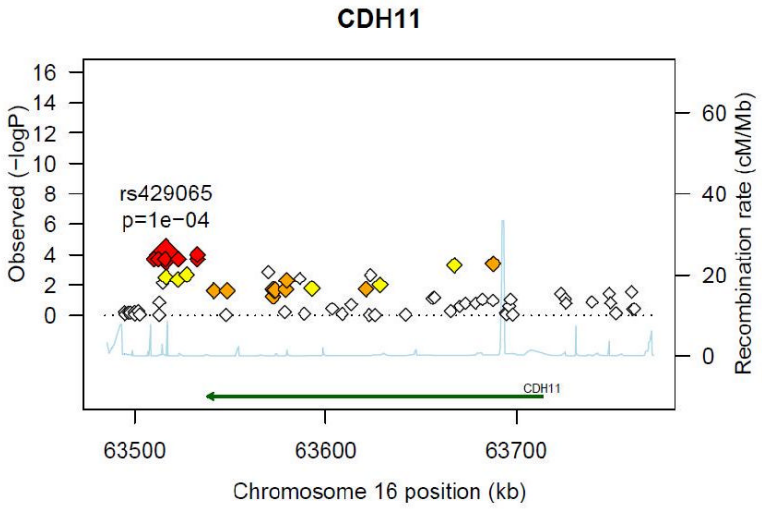

Figure 5.
Manhattan plot of NABPD vs controls. The figure shows a genome-wide synopsis of the genome-wide association study findings.

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Table 1

Genome wide association results for positive SNPs in bipolar alcoholism (BPALC) and in non-alcoholic bipolar disorder (NABPD) compared with controls

SNP	Chr	Position (base pairs)	Assoc Allele	AF: Cases	AF: Controls	NABPD P	BPALC P	Assoc SNPs: BPALC (NABPD)	Distance to nearest gene (bp)	Nearest gene or transcript	Nearest Gene name
rs8062326	16	19063517	T	0.077	0.022	N/S	4.54×10^{-06}	1(0)	23622	SYT17	Synaptotagmin XVII
rs1024820	17	52962590	T	0.433	0.295	3.75×10^{-03}	1.24×10^{-05}	2 (1)	intron	MSI2	Musashi homolog 2
rs17051551	23	3152375	C	0.374	0.233	6.77×10^{-03}	1.85×10^{-05}	5 (2)	−111628	ARSF	Arylsulfatase F
rs10967953	9	2746638	C	0.262	0.153	4.08×10^{-02}	1.86×10^{-05}	3 (2)	−47514	KIA0020	KIAA0020
rs369982	7	93886418	T	0.535	0.394	N/S	2.06×10^{-05}	2(0)	intron	COL1A2	Collagen, type I, alpha 2
rs17498319	4	57842471	C	0.245	0.14	N/S	2.34×10^{-05}	1(0)	−263459	IGFBP7	Insulin-like growth factor binding protein 7
rs4673364	2	207441356	T	0.206	0.109	N/S	3.27×10^{-05}	3(0)	71213	CPO	Carboxypeptidase O
rs280983	18	4441489	T	0.731	0.597	N/S	3.62×10^{-05}	10(0)	−785874	C18orf18	Uncharacterized protein C18orf18
rs4770393	13	22590789	A	0.063	0.018	N/S	3.91×10^{-05}	-	−33938	CR744339	CR744339
rs280984	18	4441130	G	0.734	0.602	N/S	4.17×10^{-05}	10(0)	−786233	C18orf18	Uncharacterized protein C18orf18.
rs7318572	13	22576935	T	0.047	0.01	1.38×10^{-02}	4.41×10^{-05}	3 (1)	−47792	CR744339	CR744339
rs6921115	6	85602229	C	0.354	0.233	N/S	4.71×10^{-05}	8(0)	71273	TBX18	T-box transcription factor TBX18
rs17836920	17	69590521	G	0.72	0.588	N/S	5.17×10^{-05}	5 (1)	258961	C17orf54	Chromosome 17 open reading frame 54
rs10205049	2	119616403	G	0.531	0.398	N/S	5.30×10^{-05}	1(0)	−147697	MARCO	Macrophage receptor/collagenous structure
rs7322449	13	22591091	A	0.06	0.017	3.85×10^{-02}	5.51×10^{-05}	-	239664	--	No known gene
rs1330910	13	77480045	A	0.112	0.047	N/S	5.55×10^{-05}	-	−347320	--	No known gene
rs5920654	23	97511317	C	0.832	0.701	N/S	5.62×10^{-05}	3(0)	−520646	Fc1Env	HERV-F provirus anc. Env polyprotein
rs7857841	9	2746756	A	0.147	0.071	N/S	5.92×10^{-05}	3 (2)	−47396	KIAA0020	KIAA0020 protein
rs9951521	18	4443498	G	0.731	0.601	N/S	6.25×10^{-05}	10(0)	558693	C18orf18	U6 spliceosomal RNA, near gene C18orf18
rs7104304	11	124788472	C	0.651	0.518	N/S	6.45×10^{-05}	3(0)	intron	PKNOX2	PBX/knotted 1 homeobox 2
rs6960667	7	130573985	C	0.101	0.041	4.89×10^{-02}	7.20×10^{-05}	1 (1)	89190	MKLN1	Muskelin 1
rs17068683	13	77466872	T	0.112	0.048	N/S	7.61×10^{-05}	-	46964	--	No known gene
rs17661170	4	171411895	A	0.804	0.684	5.88×10^{-04}	7.79×10^{-05}	14 (6)	163948	AADAT	Aminoadipate aminotransferase
rs12479292	2	169069788	C	0.678	0.548	N/S	8.37×10^{-05}	5(0)	intron	LASS6	LAG1 homolog, ceramide synthase 6

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SNP	Chr	Position (base pairs)	Assoc Allele	AF: Cases	AF: Controls	NABPD P	BPALC P	Assoc SNPs: BPALC (NABPD)	Distance to nearest gene (bp)	Nearest gene or transcript	Nearest Gene name
rs16925783	9	7278704	T	0.119	0.053	2.35×10^{-02}	8.45×10^{-05}	9 (4)	−113056	JMJD2C	Jumonji domain containing 2C
rs514589	8	97136355	T	0.412	0.29	7.56×10^{-03}	8.82×10^{-05}	-	−455732	--	U6 spliceosomal RNA
rs889420	16	76789480	T	0.171	0.09	N/S	9.33×10^{-05}	9(0)	intron	WWOX	WW domain containing oxidoreductase
rs313708	1	86091476	A	0.255	0.156	N/S	1.00×10^{-04}	3(0)	intron	COL24A1	Collagen, type XXIV, alpha 1
rs429065	16	63516143	G	0.371	0.254	N/S	1.00×10^{-04}	45 (2)	−22043	CDH11	Cadherin 11, type 2
rs17288584	18	67285642	A	0.101	0.042	N/S	1.00×10^{-04}	20 (2)	−1069253	CBLN2	Cerebellin 2 precursor
rs17078254	13	22576405	T	0.045	0.011	3.84×10^{-02}	1.14×10^{-04}	-	224978	--	No known gene
rs11606448	11	129767811	C	0.878	0.774	4.44×10^{-03}	1.16×10^{-04}	2 (1)	790	--	No known gene

Key: bp: base pairs. Chr: chromosome. NABPD: Non-alcoholic Bipolar Disorder. BPALC: Bipolar alcoholism. AF: Allele Frequency. Assoc: associated. N/S: Not significant. env: envelope.

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Table 2

Gene-wise permutation tests of association for genes both i) implicated by SNP markers for bipolar alcoholism (BPALC) and ii) replicating associations with alcoholism and related phenotypes; contrasted with SNP and gene wise data in non-alcoholic bipolar disorder (NABPD)

Gene	Chr	BPALC (n=143)					NABPD (n=377)				Replication study reference and/or phenotype/study type
		Gene-wise permutation p	Bonferroni corrected p	Best SNP p	Best SNP ID*	# SNPs (p≤0.01)	Gene-wise permutation p	Best SNP p	Best SNP ID*	# SNPs (p≤0.01)	
CDH11	16	**6.00×10^{-04}**	**2.28×10^{-2}**	1.03×10^{-4}	rs429065	22	7.14×10^{-1}	3.38×10^{-02}	rs12446448	7	[c] (Johnson et al., 2006); [i] (Edenberg, 2010)
COL11A2	6	**1.04×10^{-02}**	3.95×10^{-1}	2.83×10^{-3}	rs3130159	2	6.85×10^{-2}	3.45×10^{-02}	rs3130159	2	[f] (Johnson et al., 2009)
NMUR2	5	**2.04×10^{-02}**	7.75×10^{-1}	1.43×10^{-2}	rs17113138	1	5.56×10^{-1}	-	Not associated	0	[c] (Johnson et al., 2006)
XPO7	8	**2.45×10^{-02}**	9.31×10^{-1}	7.99×10^{-3}	rs7013323	7	7.14×10^{-1}	-	Not associated	0	[c] (Johnson et al., 2006)
SEMA5A	5	**3.27×10^{-02}**	1.24	2.11×10^{-4}	rs2256569	1	1	-	Not associated	0	[f] (Johnson et al., 2009)
OPRD1	1	5.43×10^{-02}	-	2.35×10^{-2}	rs419335	5	7.14×10^{-1}	-	Not associated	0	[b] (Zhang et al., 2008).
CPE	4	5.95×10^{-02}	-	2.67×10^{-3}	rs6855889	3	4.17×10^{-1}	2.30×10^{-02}	rs11724658	2	[c] (Johnson et al., 2006)
GABRB1	4	6.76×10^{-02}	-	1.44×10^{-2}	rs7665508	5	7.14×10^{-1}	-	Not associated	0	[g] (Parsian and Zhang, 1999)
ZNF533	2	7.94×10^{-02}	-	7.67×10^{-3}	rs12693204	2	8.33×10^{-1}	3.13×10^{-03}	rs12693204	9	[c] (Johnson et al., 2006)
CDH13	16	9.80×10^{-02}	-	2.30×10^{-3}	rs17675094	14	1	2.00×10^{-02}	rs7499397	1	[f] (Johnson et al., 2009); [i] (Treutlein, 2009)
OPRM1	6	1.04×10^{-01}	-	3.54×10^{-2}	rs634479	4	6.25×10^{-1}	3.21×10^{-02}	rs599945	4	[g] (Bart et al., 2004; Nishizawa et al., 2006); [b] (Kranzler et al., 1998); [e+b] (Hoehe et al., 2000; Luo et al., 2003; Zhang et al., 2006)
SORCS2	4	1.22×10^{-01}	-	6.08×10^{-3}	rs12571469	2	2.27×10^{-1}	-	Not associated	0	[f] (Johnson et al., 2009)
IL1RN	2	1.43×10^{-01}	-	2.90×10^{-2}	rs315920	2	**1.95×10^{-2}**	8.41×10^{-03}	rs444413	7	[h] (Pastor, 2000)
LRRTM4	2	1.52×10^{-01}	-	3.21×10^{-3}	rs1922805	8	4.55×10^{-1}	3.92×10^{-04}	rs17752074	10	[c] (Johnson et al., 2006)
CHRM2	7	1.67×10^{-01}	-	2.12×10^{-4}	rs10488600	5	6.25×10^{-1}	2.05×10^{-02}	rs1111418	1	[a] (Wang et al., 2004); [b] (Dick et al., 2007)
HTR2A	13	2.00×10^{-01}	-	4.20×10^{-3}	rs1002513	7	2.27×10^{-1}	1.12×10^{-02}	rs9526245	3	[d] (Hwu and Chen, 2000) (Nakamura et al., 1999)
HNMT	2	2.00×10^{-01}	-	2.44×10^{-2}	rs1020678	2	3.57×10^{-1}	4.92×10^{-03}	rs1996872	3	[h] (Oroszi et al., 2005); [g] (Reuter et al., 2007)
ALDH1A1	9	2.63×10^{-01}	-	2.07×10^{-2}	rs8187895	2	8.33×10^{-1}	-	Not associated	0	[g] (Ehlers et al., 2004; Moore et al., 2007; Spence et al., 2003)
CHES1	14	2.94×10^{-01}	-	1.99×10^{-2}	rs17125470		1	-	Not associated	0	[f] (Johnson et al., 2009)

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Gene	Chr	BPALC (n=143)					NABPD (n=377)				Replication study reference and/or phenotype/study type
		Gene-wise permutation p	Bonferroni corrected p	Best SNP p	Best SNP ID*	# SNPs (p≤0.01)	Gene-wise permutation p	Best SNP p	Best SNP ID*	# SNPs (p≤0.01)	
COMT	22	3.33×10^{-01}	-	2.11×10^{-2}	rs4646312	1	7.14×10^{-1}	-	Not associated	0	[c] (Liu et al., 2005b); [h] (Sery et al., 2006; Tiihonen et al., 1999),
USH2A	1	3.57×10^{-01}	-	1.46×10^{-2}	rs10864235	10	1	4.49×10^{-03}	rs17581848	3	[c] (Johnson et al., 2006)
MBP	18	3.57×10^{-01}	-	3.64×10^{-3}	rs2156646	2	3.13×10^{-1}	-	Not associated	0	[f] (Johnson et al., 2009)
EFNA5	5	3.84×10^{-01}	-	9.49×10^{-3}	rs10043302	1	2.63×10^{-1}	1.57×10^{-02}	rs153118	3	[c] (Johnson et al., 2006)
CNR1	6	4.17×10^{-01}	-	1.13×10^{-2}	rs2057276	3	4.17×10^{-1}	3.54×10^{-02}	rs470744	2	[b] (Zuo et al., 2007)
LGI2	4	4.54×10^{-01}	-	9.79×10^{-3}	rs4697534	1	-	-	Not associated	0	[f] (Johnson et al., 2009)
DGKB	2	5.00×10^{-01}	-	1.78×10^{-2}	rs2357842	5	3.13×10^{-1}	1.06×10^{-02}	rs4584047	3	[c] (Johnson et al., 2006)
PTGDR	14	5.00×10^{-01}	-	1.81×10^{-2}	rs4898758	1	5.56×10^{-1}	-	Not associated	0	[c] (Johnson et al., 2006)
BDNF	11	5.00×10^{-01}	-	2.95×10^{-2}	rs6265	1	8.33×10^{-1}	-	Not associated	0	[d] (Matsushita et al., 2004)
RBM19	12	5.56×10^{-01}	-	7.20×10^{-3}	rs999444	1	3.57×10^{-1}	1.74×10^{-03}	rs999444	1	[c] (Johnson et al., 2006)
LRP1B	2	7.14×10^{-01}	-	5.71×10^{-3}	rs13432762	12	1.92×10^{-1}	1.56×10^{-03}	rs13032395	15	[c] (Johnson et al., 2006)
GFRA1	10	7.14×10^{-01}	-	7.02×10^{-3}	rs7907314	1	5.56×10^{-1}	-	Not associated	0	[f] (Johnson et al., 2009)
GRIN2B	12	8.33×10^{-01}	-	6.24×10^{-3}	rs4522263	2	4.17×10^{-1}	1.80×10^{-02}	rs1805476	3	[g] (Kim et al., 2006; Wernicke et al., 2003)
PDYN	20	8.33×10^{-01}	-	2.05×10^{-2}	rs6045664	1	**3.01×10^{-2}**	1.46×10^{-03}	rs6045664	1	[c] (Johnson et al., 2006)
ADH4	4	8.33×10^{-01}	-	3.05×10^{-2}	rs2851253	3	-	-	Not associated	0	[g] (Agrawal et al., 2006; Guindalini et al., 2005; Kuo et al., 2008; Luo et al., 2005)
CSMD1	2	1	-	3.30×10^{-3}	rs17079637	9	**3.01×10^{-2}**	5.87×10^{-03}	rs17079506	4	[c] (Johnson et al., 2006)
SESTD1	2	1	-	3.88×10^{-2}	rs16866688	1	1.11×10^{-1}	-	Not associated	0	[c] (Johnson et al., 2006)
ATXN1	6	1	-	7.84×10^{-4}	rs6459492	5	5.56×10^{-1}	-	Not associated	0	[f] (Johnson et al., 2009)
NRXN3	14	1	-	1.53×10^{-4}	rs11159379	4	8.33×10^{-1}	-	Not associated	0	[b] (Hishimoto et al., 2007; Lachman et al., 2007; Liu et al., 2005a; Uhl et al., 2008b)

p-values in **bold** are where gene-wise permutation analysis was <0.05. No NABPD gene wise tests withstood Bonferroni correction for 38 tests.

Key: Bp = base pair; Chr = chromosome; SNP = single nucleotide polymorphism;

= number..

* Association defined as nominally significant SNP (p<=0.01) in or flanking gene(+−50Kb) according to Single Nucleotide Polymorphism database (dbSNP), May 2006 annotation. p-values in **bold** are where gene-wise permutation analysis was <0.05. No NABPD gene wise tests withstood Bonferroni correction.

[a] Alcoholism with or without major depression

[b] Alcohol and/or dug dependence

[c] Pooled association study of 100K SNPs including COGA sample

[d] Male alcohol abuse and behaviour problems

[e] Haplotypic association

[f] Convergent addictions (including alcohol dependence) and bipolar genes

[g] Alcohol dependence

[h] DSM-IIIR alcoholism

[i] GWA.

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Table 3

Novel genes, implicated by clustering of 7 or more SNPs (p<0.01) and associated on gene wise testing in the bipolar alcoholic sample

Gene	BPALC								NABPD	
	SNPs						Gene-wise permutation significance	Bonferroni correction	SNPs	
	# Tested	# <50 Kb from gene	# Intronic	GWAS highest ranked SNP p	GWAS highest ranked SNP id	GWAS highest SNP rank			<50 Kb from gene	GWAS highest ranked SNP p
KIAA1772	27	12	12	1.25×10^{-3}	rs2117317	495	2.00×10^{-4}	**6.00×10^{-3}**	1	1.57×10^{-2}
DC2 protein	17	7	0	2.50×10^{-3}	rs2851365	920	9.49×10^{-4}	**2.85×10^{-2}**	1	7.90×10^{-3}
ZBTB16	75	9	9	5.43×10^{-4}	rs620095	225	1.61×10^{-3}	**4.83×10^{-2}**	0	N/A
GARNL1	18	9	9	3.97×10^{-4}	rs4982303	169	1.68×10^{-3}	5.04×10^{-2}	0	N/A
ZNF37A	24	8	2	1.35×10^{-3}	rs2505179	531	1.79×10^{-3}	5.37×10^{-2}	6	1.32×10^{-3}
GABRB3	23	6	5	2.60×10^{-4}	rs2114485	103	2.00×10^{-3}	6.00×10^{-2}	4	2.56×10^{-3}
HAAO	24	8	4	6.42×10^{-4}	rs7583085	259	2.29×10^{-3}	6.87×10^{-2}	0	N/A
ARHGEF12	34	13	13	1.24×10^{-3}	rs4938805	492	2.83×10^{-3}	8.49×10^{-2}	0	N/A
ADCY3	18	8	8	3.51×10^{-3}	rs10198275	1282	3.10×10^{-3}	9.30×10^{-2}	0	N/A
CDH12	128	18	18	1.30×10^{-4}	rs6879343	39	3.50×10^{-3}	1.05×10^{-1}	0	N/A
CLCN6	28	7	7	2.68×10^{-4}	rs2076001	106	3.71×10^{-3}	1.11×10^{-1}	0	N/A
AVEN	42	9	9	1.24×10^{-4}	rs517078	37	3.76×10^{-3}	1.13×10^{-1}	0	N/A
LRRC52	35	7	0	3.22×10^{-4}	rs166949	129	4.38×10^{-3}	1.31×10^{-1}	0	N/A
MS4A4A	32	7	0	1.27×10^{-2}	rs2868121	4233	5.00×10^{-3}	1.50×10^{-1}	0	N/A
STK4	25	9	8	2.74×10^{-3}	rs4549170	1013	6.12×10^{-3}	1.84×10^{-1}	0	N/A
GRM5	76	9	8	8.18×10^{-3}	rs973716	3119	7.00×10^{-3}	2.10×10^{-1}	0	N/A
KLHL1	76	9	9	2.95×10^{-3}	rs2225529	1084	7.63×10^{-3}	2.29×10^{-1}	1	3.28×10^{-2}
NEK11	48	8	8	1.98×10^{-4}	rs2718874	70	8.29×10^{-3}	2.49×10^{-1}	1	4.52×10^{-2}
SLCO3A1	93	11	9	2.46×10^{-4}	rs1400784	99	8.67×10^{-3}	2.60×10^{-1}	4	7.73×10^{-3}
EGF	25	9	9	3.66×10^{-3}	rs2250616	1352	1.00×10^{-2}	3.00×10^{-1}	0	N/A
ADARB2	132	8	8	1.70×10^{-3}	rs2813427	655	1.22×10^{-2}	3.66×10^{-1}	1	1.06×10^{-2}
PDE5A	33	12	8	1.56×10^{-3}	rs10009566	605	1.24×10^{-2}	3.72×10^{-1}	0	N/A

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Gene	BPALC								NABPD	
	SNPs						Gene-wise permutation significance	Bonferroni correction	SNPs	
	# Tested	# <50 Kb from gene	# Intronic	GWAS highest ranked SNP p	GWAS highest ranked SNP id	GWAS highest SNP rank			<50 Kb from gene	GWAS highest ranked SNP p
SDK1	217	21	21	1.16×10^{-3}	rs6965800	452	1.29×10^{-2}	3.87×10^{-1}	4	1.76×10^{-2}
PET112L	30	10	10	6.40×10^{-3}	rs1896883	2392	1.32×10^{-2}	3.96×10^{-1}	0	N/A
LOH12CR1	44	9	9	3.67×10^{-3}	rs4763829	1359	1.61×10^{-2}	4.83×10^{-1}	0	N/A
RORA	177	10	10	6.99×10^{-4}	rs11854222	281	1.98×10^{-2}	5.94×10^{-1}	0	N/A
FBN1	46	15	14	2.52×10^{-3}	rs12902728	929	2.18×10^{-2}	6.54×10^{-1}	10	3.25×10^{-3}
FSIP1	41	8	8	5.70×10^{-3}	rs2664124	2117	2.33×10^{-2}	6.99×10^{-1}	0	N/A
DAAM1	50	7	6	9.37×10^{-4}	rs10873114	385	2.56×10^{-2}	7.68×10^{-1}	3	1.55×10^{-2}
SYN3	144	14	14	2.55×10^{-4}	rs9606993	102	3.03×10^{-2}	9.09×10^{-1}	0	N/A
SLC8A3	70	12	12	3.14×10^{-3}	rs999618	1163	3.18×10^{-2}	9.54×10^{-1}	3	3.33×10^{-2}

Key: BPALC, bipolar alcoholic phenotype; NABPD, non-alcoholic bipolar phenotype

#, number; GWAS, genome wide association study; SNP id, dbSNP RS ID