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No Evidence for Association between 19 Cholinergic Genes and Bipolar Disorder

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Abstract

Cholinergic dysfunction has been proposed for the pathogenesis of bipolar disorder (BD), and we have therefore performed a systematic association study of cholinergic system genes in BD (including schizoaffective disorder bipolar type). We genotyped 93 single nucleotide polymorphisms (SNPs) in 19 genes (*CHAT*, *CHRM1-5*, *CHRNA1-7*, *CHRNA9*, *CHRNA10*, and *CHRNB1-4*) in two series of samples: the National Institute of Mental Health (NIMH) Genetics Initiative pedigrees with 474 samples from 152 families, and the Clinical Neurogenetics (CNG) pedigrees with 83 samples from 22 multiplex families.

Sib-Transmission/Disequilibrium test (sib_TDT) analysis showed nominally significant transmission bias for four SNPs (*CHRNA2*: rs7017417, $P = 0.024$; *CHRNA5*: rs514743, $P = 0.031$; *CHRNB1*: rs2302762, $P = 0.049$; *CHRNB4*: rs1948, $P = 0.031$). Haploview analyses showed nominally significant transmission bias of several haplotypes in *CHRNA2*, *CHRNA7*, *CHRNB1* and *CHRNB4*, respectively. However, none of these associations reached gene-wide significance after correction by permutation. Alcohol dependence (including alcohol abuse) was not a significant covariate in the present genetic association analysis. Thus, it is unlikely that these 19 cholinergic genes play a major role in the predisposition to bipolar disorder in these pedigrees.

Keywords

single nucleotide polymorphism; linkage disequilibrium; haplotype; bipolar disorder; cholinergic

INTRODUCTION

Bipolar disorder (BD), also known as manic-depressive illness, is a devastating mental illness with a lifetime prevalence of approximate 1% in the general population [Weissman et al., 1996]. Although the pathophysiology of BD has not been elucidated as yet, family, twin and adoption studies clearly show that genetic factors play an important role in the etiology of this disease [Craddock and Jones, 2001; Craddock and Jones, 1999]. Recent linkage and association studies have identified several candidate chromosome regions and susceptibility loci for BD. However, the results are inconsistent and inconclusive, and no causative alleles or haplotypes

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have been found so far [Badner and Gershon, 2002; Maier et al., 2005; McQueen et al., 2005; Craddock et al., 2005; Kato et al., 2005; Segurado et al., 2003].

Human cholinergic receptor system includes muscarinic and nicotinic receptors, which belong to the superfamily of plasma membrane-bound G protein coupled receptors and ligand-gated ion channels, respectively. There is accumulating evidence suggesting that cholinergic receptors and choline acetyltransferase (CHAT) may be involved in the pathogenesis of mood disorder including BD [Bertrand, 2005; Bymaster and Felder, 2002; Janowsky and Overstreet, 1990; McEvoy and Allen, 2002; Shytle et al., 2002].

The first line of evidence is from pharmacologic studies. Preclinical and clinical pharmacologic studies on roles of the muscarinic acetylcholinergic receptors (mAchRs) in BD were reviewed in detail by Bymaster and Felder [Bymaster and Felder, 2002]. For example, cholinesterase inhibitors such as physostigmine have antimanic effects by stimulation of mAchRs, possibly through activation of M4 receptors. Muscarinic antagonists such as arecoline can cause depression-like symptoms. The mood stabilizer lithium increases acetylcholine synthesis and choline uptake, and also enhances the expression of muscarinic M3 receptors and decreases M2 receptor levels. On the other hand, some antidepressants may lessen depression and stabilize mood through inhibition of nicotinic acetylcholinergic receptors (nAchRs), as reviewed elsewhere [Shytle et al., 2002]. First, several classic tricyclic antidepressants such as imipramine and desipramine, and more atypical selective monoamine reuptake inhibitors such as fluoxetine and sertraline have nAchR inhibition activity. Second, depression associated hypercholinergic neurotransmission may be mediated by excessive activation of nAchRs. Third, nicotine dependence (including nicotine abuse) is very common in major depression and bipolar disorder [Lasser et al., 2000; Leonard et al., 2001; McEvoy and Allen, 2002], possibly due to antidepressant properties of nicotine. Fourth, nAchRs exist in many different subtypes constructed from lots of subunit combinations, and are distributed widely in axons, presynapses, and postsynapses in the central nervous system. They modulate neuronal excitability through regulating release of monoamines as well as glutamate, γ-aminobutyric acid, and various neuropeptides. Those neurotransmitters have been thought to contribute to the pathophysiology of BD. Fifth, antidepressants may lessen symptoms through blockade of nAchRs involved in stress-induced activation of the hypothalamic-pituitary axis, which is very common in unmedicated depressed patients. Sixth, nAchRs may regulate mood through modulation of midbrain dopaminergic neurons. Finally, nAchRs may also influence circadian rhythm, whose disruption is thought to be relevant to mood instability in patients with BD and underlie pathogenesis of the disease.

The second line of evidence comes from linkage and association studies. Some known cholinergic system genes map to those putative BD linked regions (Table I). Recent studies have suggested variants in cholinergic receptor genes contribute susceptibility to normal behavior variation, neuropsychiatric diseases as well as addiction. A common single nucleotide polymorphism (SNP), A1890T, in the 3′untranslated region of the cholinergic muscarinic 2 receptor (*CHRM2*) gene, has been associated with intelligence quotient (IQ) [Comings et al., 2003], and with major depression in women [Comings et al., 2002]. Wang and colleagues [Wang et al., 2004] found that variants in *CHRM2* increased the risk for alcohol dependence as well as major depression. A recent study confirmed Wang et al's findings and also reported that the *CHRM2* gene predisposed to drug dependence and affective disorder including BD [Luo et al., 2005]. Several nicotinic acetylcholine receptor genes such as *CHRNA2*, *CHRNA4* and *CHRNB2* have been reported to be associated with nicotine dependence [Faraone et al., 2004;Feng et al., 2004;Li et al., 2005], and *CHRNA4* with alcoholism in Koreans [Kim et al., 2004]. A two base-pair deletion causing a frame-shift mutation in the nicotinic cholinergic receptor alpha polypeptide 7 (*CHRNA7*) gene was reported to influence the risk for major depression and BD in Chinese [Hong et al., 2004;Lai et al., 2001], and this gene may

also be linked to schizophrenia [De Luca et al., 2004a;De Luca et al., 2004b;Freedman et al., 2001;Leonard et al., 2002;Leonard and Freedman, 2003;Raux et al., 2002;Riley et al., 2000;Xu et al., 2001;Meyer et al., 2003].

There are also other findings implying that cholinergic genes are involved in the development of BD. Convergent Functional Genomics (CFG) is an approach for selecting candidate genes, which integrates brain gene expression data from a relevant pharmacogenomic animal model (e.g. treating mouse with amphetamine and/or valproate) with human data (e.g. linkage loci from human genome scans or gene expression alterations in postmortem brains from patients), as a Bayesian strategy of cross-validating findings. CFG produced a short list of BD candidate genes including *CHRM1* [Ogden et al., 2004].

In the present study, we performed an association study to test the hypothesis that cholinergic pathway gene variants may increase susceptibility to bipolar disorder. We also analyzed the possible influence of alcohol dependence on the association results, since alcohol dependence is a common comorbidity in bipolar disorder [Strakowski and DelBello, 2000; Sherwood et al., 2001; Brown, 2005], and several cholinergic gene have been reported to be associated with alcohol and/or mood disorder [Hong et al., 2004; Lai et al., 2001; Luo et al., 2005; Comings et al., 2002; Kim et al., 2004; Wang et al., 2004].

MATERIALS AND METHODS

Subjects and Phenotypes

Two series of pedigrees with BD were included in this study: the Clinical Neurogenetics (CNG) pedigrees with 83 samples from 22 multiplex families, including 6 trios and 8 quads; and the National Institute of Mental Health (NIMH) Genetics Initiative pedigrees waves I and II, with 474 samples from 152 families, including 23 trios and 83 quads. The CNG pedigrees were described in detail elsewhere [Berrettini et al., 1991]. Background and detailed clinical assessment for the NIMH Genetics Initiative were described previously [Nurnberger, Jr. et al., 1997]. Briefly, all subjects were assessed with the Diagnostic Instrument for Genetic Studies (DIGS) [Nurnberger, Jr. et al., 1994] and the Family Interview for Genetic Studies (FIGS) by a clinically trained professional. Subsequently, two clinicians made separate reviews of all available information including DIGS and FIGS data and medical records, and made a final diagnosis using a best-estimate procedure. The nuclear trio- and quad-families in these two series included 201 affected children. Of them, 156 met DSM-III-R criteria for bipolar I disorder, 30 for bipolar II disorder, and 15 for schizoaffective disorder bipolar type. All were Caucasian except for four African, three Hispanic, two Asian and one unknown. One hundred and nine were females and 92 were males. The NIMH waves I and II samples have alcohol dependence (alcohol abuse included) diagnostic information available but not that of nicotine dependence, while the CNG samples lack information on both alcohol and nicotine dependence.

Gene and SNP Selection

Nineteen genes in the cholinergic pathway were selected in the present study, including 5 muscarinic receptor (*CHRM1-5*) genes, 9 nicotinic receptor alpha subunit (*CHRNA1-7*, *CHRNA9* and *CHRNA10*) genes, 4 nicotinic receptor beta subunit (*CHRNB1-4*) genes, and CHAT gene (Table I). SNPs were chosen from public databases using a series of bioinformatics tools, including SNP Information Mining Pipeline (SIMP) and Gene Information Mining Pipeline (GIMP) developed in our laboratory (http://bioinfo.bsd.uchicago.edu/index.html). First, we used GIMP to get correct mapping information in the human genome for the 19 genes. Then, we downloaded the genotype data from HapMap release 16c.1 (http://www.hapmap.org/index.html.en) for these genes including SNPs within the genomic

sequence from the 5' transcription start site through the 3' transcription end site plus 10 kilobases of flanking sequence at both ends of each gene. SNPs were included if the minor allele frequency (MAF) in Caucasians was greater than 0.1. Finally, the LD-Select algorithm was used to select tag SNPs (tSNPs) to represent SNPs in linkage disequilibrium (LD) with each other [Carlson et al., 2004]. This method clusters the SNPs into different bins according to the pairwise correlation coefficient r^2 values. We used a criterion of $r^2 \geq 0.85$ to define the bins. We selected tSNPs, singleton SNPs (only one SNP in each bin), and several SNPs which have been analyzed in other reports [Luo et al., 2005;Wang et al., 2004]. A total of 93 SNPs were chosen for study (Table II).

Genotyping

All SNPs were genotyped with the TaqMan 5' exonuclease assay in a 384-well microplate format [Applied Biosystems Inc. (ABI), Foster City, CA]. Briefly, 20 ng of DNA was amplified in a total volume of 5 μl containing major and minor allele probes labeled with 5' VIC or 5' FAM fluorophore and 2.5 μl of TaqMan universal PCR master mix. Amplification reaction conditions were 10 min at 95°C, followed by 40 cycles of 92°C for 15 sec and 60°C for 1 min. Allelic discrimination analysis was performed on the Prism 7900HT Fast Real-Time PCR system using the software SDSv2.2.1 (ABI). TaqMan probes and primers were obtained from Applied Biosystems Assay-by-Demand, Assay-by-Design, or Pre-Designed Services for SNP genotyping. We used PedCheck1.1 to detect any Mendelian inconsistencies or genotyping errors [O'Connell and Weeks, 1998] and used Merlin to avoid unlikely genotypes [Abecasis et al., 2002]. All genotyping errors were manually resolved by checking the raw genotype data and individuals who were either blanked (zeroed) or corrected prior to statistical analysis. The average rate of success for each genotyped SNP was > 98.2%.

Statistical Analysis

Pairwise linkage disequilibrium between any two markers in one or several closely contiguous genes on one chromosome was analyzed. The standard LD coefficient D' was calculated using the program Haploview (version 3.31) [Barrett et al., 2005]. The transmission disequilibrium test (TDT) was used to test for individual SNP transmission distortion. The sib_tdt program from the ASPEX 2.3 analysis package (ftp://lahmed.stanford.edu/pub/aspex) was used to analyze all affected sibs with significance for the individual SNP estimated by permutation, which holds the observed sharing of the marker between sib pairs constant, to correct for the effects of linkage. TDT analyses of individual SNPs and haplotype blocks were performed using Haploview software [Barrett et al., 2005]. Haploview can correctly analyze only trios for association. Therefore, all the genotyped sibs were used to construct haplotypes using the "solid spine of LD" but only one affected sib was listed as affected in the analysis ["first" (by post MAKEPED ID number) affected sib in sibship was chosen]. Nominal *P*-values were estimated from the chi-square (1 df) and the *P*-values for SNPs would not necessarily agree with those from ASPEX/sib_tdt. *P*-values were corrected for multiple testing of all the SNPs and haplotypes within a gene through 1000 permutations to obtain a "gene-wide" *P*-value.

To evaluate interactions between genes without significant main effects, we used the "focused interaction testing framework" method (FITF) [Millstein et al., 2006]. This approach tests for association of the combination of 1, 2, and 3 loci. For the tests of interactions of 2 or 3 loci, combinations are prescreened using a chi-square goodness of fit test in the entire sample (transmitted vs. non-transmitted genotypes), regardless of the transmission status of the genotype. It tests for deviations from the expected genotype distribution and selects those that exceed a particular threshold. Since the phenotype is not used in the prescreening, the prescreening does not produce biased results. In this analysis, there were 93 SNPs, 4378 possible pairs, and 129,766 possible 3-way combinations. Prescreening reduced this to 93 SNPs, 688 possible pairs, and 24,144 possible 3-way combinations. The resulting tests were

corrected by the false discovery rate (FDR), which is designed to reduce the proportion of all positive results that are false and is less conservative (has a less negative effect on power) than the Bonferroni correction [Benjamini et al., 2001].

Next, we performed an association analysis of the NIMH waves I and II data, using alcohol dependence as a covariate. It is unclear whether the best way to analyze this type of covariate should be based on the status of the individual alone or should be applied to anyone in the family. Thus, we performed the analysis in both ways. For the individual covariate analysis, individuals with both BD and alcohol dependence were coded as affected in the alcohol dependence group, and individuals with BD were coded as affected in the non-alcohol dependence group. For the nuclear family covariate analysis, individuals with BD were coded as affected in the alcohol dependence group if any member of their nuclear family had alcohol dependence, and individuals with BD were coded as affected in the non-alcohol dependence group only if no one in their family had alcohol dependence. For both analyses, only one affected offspring was analyzed by Haploview [Barrett et al., 2005]. All SNPs and haplotypes were compared between the alcohol and non-alcohol dependence group using chi-square tests to see if there was a significant difference in the proportion of alleles/haplotypes transmitted between the two groups.

RESULTS

The distribution of genotype frequency of all SNPs was in Hardy-Weinberg Equilibrium, and the LD structure for the SNPs in each gene or gene cluster was plotted by Haploview (Supplementary figure I). Table II shows the results of TDT tests on all SNPs. SNPs rs7017417 in *CHRNA2*, rs514743 in *CHRNA5*, rs2302762 in *CHRNB1*, and rs1948 in *CHRNB4* showed nominally significant association with BD. But these associations failed to maintain significance after correction for multiple testing by permutation (data not shown).

We also found a few haplotypes that showed nominally significant association with BD by Haploview analyses where the most significant results are summarized in Table III. However, the significance did not remain after permutation testing (Table III).

Moreover, we did not find any evidence of interaction among these 19 cholinergic genes by FITF analysis and FDR correction (Supplementary table I).

There were some SNPs that showed nominally significant transmission bias when using alcohol dependence as a covariate (data not shown). We compared the proportion of transmissions between the alcohol and the non-alcohol dependence groups for both the individual covariate analysis and the nuclear family covariate analysis (Table IV). None of the SNPs showed a significant difference in either analysis between two the groups after FDR correction for multiple testing (corrected $P > 0.05$).

There were also several haplotypes showing nominally significant difference in transmission between the alcohol dependent and non-alcohol dependent groups for both the individual covariate analysis and the nuclear family covariate analysis (Table V). Again, the results did not withstand FDR correction for the many genes tested (corrected $P > 0.05$).

DISCUSSION

Although dysfunction of the cholinergic system has been suggested in the pathogenesis of BD, very few association studies on cholinergic genes in BD have been performed, much less a consistent or convincing genetic relationship between variants in these genes and the disease [Hong et al., 2004; Luo et al., 2005; Lohoff et al., 2005]. Here we did a systematic screen of 93 SNPs in 19 cholinergic genes in two series of BD samples. We found neither allelic nor

haplotypic association between these genes and BD in the combined sample, and we did not observe any interaction between alcohol dependence and cholinergic genes in increasing the risk for BD in the NIMH samples. Thus, it seems unlikely that these cholinergic genes contribute a major role in the predisposition to BD in these pedigrees.

Caution should be taken when viewing our negative results because of several limitations in the present study. First, based on Caucasian SNPs with MAF ≥ 0.1 and LD information for nineteen cholinergic genes in the HapMap project (release 16c.1) and SNPs tested in the present study, most genes have achieved sufficient physical and/or LD coverage of SNPs, except for several genes including *CHAT*, *CHRM2*, *CHRM3*, *CHRM4*, *CHRNA6* and *CHRNB2* (Table I and Supplementary figure I). These genes either have very few SNPs in the HapMap data to work with, or are too large to have complete coverage due to excessive costs. Thus susceptibility variants for BD may be missed in genes with poor SNP coverage. Better SNP coverage will be achieved with rapidly accumulating genotype data from the HapMap project and other human resequencing or genotyping projects. Secondly, genotype data from the HapMap project is good at capturing common variants, but not for variants with MAF < 0.05, and the choice of SNP tagging strategy may also affect the efficiency and power of capturing un-genotyped rare variants by LD [Zeggini et al., 2005;de Bakker et al., 2005]. We selected tSNPs with criteria of MAF > 0.1 and $r^2 \geq 0.85$, thus it is possible that we missed detection of association between rare variants in cholinergic genes and BD. Thirdly, our sample has limited capacity to detect alleles with small effects. For example, with 91 quads and 29 trios, $P < 0.0005$ (to correct for 93 SNPs), $r^2 = 0.85$ and a multiplicative model, we used PBAT software (http://www.biostat.harvard.edu/~clange/default.htm) to estimate the minimum odds ratios (ORs) for allele frequencies of 0.1 and 0.5 that we would have 80% power to detect. We could detect ORs of 2.2 and 2.0 for allele frequencies of 0.1 and 0.5, respectively. Finally, considering nicotine or alcohol dependence as a substantial comorbidity in mood disorders [Lasser et al., 2000;Leonard et al., 2001;McEvoy and Allen, 2002], and recent studies that have implicated cholinergic genes as a risk factor for these disorders [Feng et al., 2004;Hong et al., 2004;Li et al., 2005;Luo et al., 2005;Wang et al., 2004], the conclusion will be more convincing if the influence of nicotine dependence as well as alcohol dependence is assessed in larger samples.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE I

SNPs in 19 Cholinergic Genes Selected for BD Association Study

Gene Symbol	Region	BD linked study	Length (kb) [a]	Selected SNPs [b]	SNPs typed [c]	kb/SNP
CHAT	10q11.2	[Faraone et al., 1998]	76	13	7	10.9
CHRM1	11q13	NA	33	5	3	11
CHRM2	7q31-q35	[Detera-Wadleigh et al., 1999]	168	24	13	12.9
CHRM3	1q41-q44	NA	300	39	9	33.3
CHRM4	11p12-p11.2	NA	21	1	1	21
CHRM5	15q26	[Maziade et al., 2005]	67	5	5	13.4
CHRNA1	2q24-q32	[Cichon et al., 2001]	37	4	4	9.3
CHRNA2	8p21	[Cichon et al., 2001; Park et al., 2004]	38	3	5	7.6
CHRNA3	15q24	NA	48	2	2	24
CHRNA4	20q13.2-q13.3	[Nurnberger, Jr. et al., 1997]	37	4	2	18.5
CHRNA5	15q24	NA	49	5	7	7
CHRNA6	8p11.21	[Maziade et al., 2005]	36	4	1	36
CHRNA7	15q14	[Nurnberger, Jr. et al., 1997]	159	14	10	15.9
CHRNA9	4p14	[Nurnberger, Jr. et al., 1997]	40	7	5	8
CHRNA10	11p15.5	[Zandi et al., 2003]	26	4	5	5.2
CHRNB1	17p13.1	NA	33	7	5	6.6
CHRNB2	1q21.3	NA	29	1	2	14.5
CHRNB3	8p11.2	[Maziade et al., 2005]	60	5	3	20
CHRNB4	15q24	NA	17	2	4	4.3
Total or Average	...	...	1274	149	93	13.7

[a] genomic sequence plus 10 kb flanking sequence at each ends.

[b] original number of tag plus singleton SNPs selected by using GIMP and SIMP.

[c] tested SNPs include most SNPs from original selection and several SNPs studied by other research groups, see details in the text. NA indicates that no BD linkage signal in that region.

Am J Med Genet B Neuropsychiatr Genet. Author manuscript; available in PMC 2008 October 30.

TABLE II

Sib_TDT Tests of Allelic Association with Bipolar Disorder in the Combined Sample

Chromosome	Gene Symbol	dbSNP ID	Position [a]	Allele 1	Allele 2	Allele 1 frequency	TR [b]	*P*- value [c]
1	*CHRNB2*	rs2072660	151765513	T	C	0.20	0.44	1
		rs1127317	151772832	T	G	0.74	0.52	1
1	*CHRM3*	rs6700643	236840070	C	T	0.43	0.48	0.64
		rs6688537	236891737	A	C	0.53	0.52	1
		rs1019882	236940005	C	T	0.43	0.55	0.73
		rs543307	236968759	C	T	0.79	0.52	0.28
		rs658842	236989784	A	T	0.46	0.54	0.75
		rs621060	237033435	A	G	0.86	0.55	0.42
		rs528011	237059456	G	T	0.52	0.46	0.32
		rs10495447	237092489	A	G	0.29	0.50	0.66
		rs7520974	237108409	A	G	0.59	0.48	0.77
2	*CHRNA1*	rs6708544	175806738	G	A	0.66	0.52	0.47
		rs6732758	175812503	A	G	0.81	0.43	0.32
		rs3755486	175834430	C	T	0.52	0.54	0.43
		rs935863	175839345	A	G	0.68	0.51	0.83
4	*CHRNA9*	rs10805117	40248380	A	T	0.26	0.51	0.28
		rs6823439	40252142	s	A	0.43	0.49	0.22
		rs4861065	40259937	C	T	0.31	0.52	0.4
		rs6819483	40264884	C	G	0.24	0.47	1
		rs4264869	40270668	C	T	0.53	0.53	0.13
7	*CHRM2*	rs6957496	135967879	A	G	0.89	0.64	0.27
		rs4475425	135991241	A	G	0.27	0.51	0.94
		rs7810473	136012499	A	G	0.62	0.55	0.35
		rs1364409	136028075	T	A	0.33	0.46	0.41
		rs978437	136030220	C	T	0.33	0.45	0.42
		rs7782965	136040175	C	T	0.68	0.55	0.56
		rs1455858	136047745	C	T	0.67	0.56	0.33
		rs1824024	136059736	A	C	0.67	0.55	0.19
		rs2061174	136077442	A	G	0.67	0.57	0.26
		rs324614	136080016	G	C	0.26	0.44	0.25
		rs2350786	136092612	A	G	0.29	0.46	0.95
		rs324650	136109703	A	T	0.51	0.53	1
		rs8191992	136117350	A	T	0.53	0.52	0.82
8	*CHRNA2*	rs9314347	27341715	A	G	0.91	0.31	0.52
		rs891397	27346277	A	C	0.14	0.45	0.66
		rs2741339	27356879	A	G	0.73	0.51	1
		rs2741335	27359701	A	C	0.75	0.58	0.47
		rs7017417	27367215	A	G	0.88	0.55	**0.024**
8	*CHRNB3*	rs7004381	42568529	A	G	0.21	0.55	0.43
		rs11783289	42591964	G	A	0.13	0.56	0.6
		rs7017612	42616613	A	C	0.84	0.43	0.5
	CHRNA6	rs10107450	42647263	G	A	0.78	0.41	0.35
10	*CHAT*	rs885834	50160115	A	G	0.61	0.50	0.89
		rs10082479	50173822	A	T	0.87	0.52	0.76
		rs3793791	50186307	C	T	0.05	0.42	1
		rs7094248	50199971	C	G	0.47	0.47	0.63
		rs1917813	50202438	C	T	0.46	0.49	0.61
		rs6537546	50208646	A	T	0.92	0.47	0.68
		rs1917810	50214674	A	G	0.55	0.54	0.82
11	*CHRNA10*	rs7123164	3642755	A	G	0.13	0.48	0.38
		rs2271583	3649964	A	G	0.16	0.43	0.086
		rs2741862	3652294	A	G	0.73	0.51	1
		rs2672213	3655726	C	T	0.85	0.45	0.56

Am J Med Genet B Neuropsychiatr Genet. Author manuscript; available in PMC 2008 October 30.

Chromosome	Gene Symbol	dbSNP ID	Position [a]	Allele 1	Allele 2	Allele 1 frequency	TR [b]	*P*- value [c]
		rs6578411	3660281	A	G	0.88	0.61	0.33
11	*CHRM4*	rs2279465	46363847	A	G	0.83	0.59	0.15
11	*CHRM1*	rs2507821	62441966	G	T	0.41	0.50	0.95
		rs4963323	62449963	C	G	0.83	0.48	0.62
		rs1938677	62472311	A	G	0.45	0.55	0.71
15	*CHRNA7*	rs965435	30033515	C	T	0.70	0.54	0.47
		rs1913456	30048509	A	T	0.18	0.47	0.48
		rs4779971	30066020	A	G	0.25	0.43	0.49
		rs1355920	30073784	G	A	0.11	0.54	0.34
		rs12441060	30089749	G	A	0.45	0.55	0.5
		rs8035668	30107402	C	T	0.19	0.63	0.27
		rs1500946	30121476	A	G	0.80	0.54	0.3
		rs13329490	30127449	G	T	0.15	0.59	1
		rs904951	30134094	C	T	0.48	0.50	0.39
		rs2337980	30160252	A	G	0.49	0.57	0.66
15	*CHRM5*	rs2684953	32021787	C	T	0.84	0.54	0.86
		rs2702285	32030359	C	T	0.19	0.44	0.74
		rs4779656	32040659	A	G	0.81	0.43	0.52
		rs2684941	32047838	A	G	0.60	0.50	1
		rs499167	32064394	A	C	0.26	0.51	1
15	*CHRNA5*	rs4275821	76565360	G	A	0.33	0.45	0.19
		rs2036527	76567434	A	G	0.37	0.47	0.93
		rs684513	76574219	C	G	0.79	0.46	0.25
		rs680244	76627822	C	T	0.61	0.53	0.073
		rs951266	76594360	A	G	0.37	0.53	0.36
		rs514743	76600046	A	T	0.69	0.59	**0.031**
		rs615470	76601807	C	T	0.69	0.56	0.092
	CHRNA3	rs11637630	76615538	A	G	0.77	0.48	0.23
		rs6495309	76631064	G	A	0.80	0.48	0.2
	CHRNB4	rs1948	76633218	A	G	0.29	0.42	**0.031**
		rs7178270	76636896	G	C	0.59	0.46	0.46
		rs1316971	76646329	A	G	0.19	0.49	0.43
		rs11633223	76651295	A	G	0.63	0.47	0.67
17	*CHRNB1*	rs4151120	7542713	A	T	0.35	0.49	0.19
		rs3853818	7546867	C	T	0.53	0.47	0.3
		rs2302765	7551540	C	T	0.15	0.38	0.29
		rs2302762	7559426	C	T	0.67	0.40	**0.049**
		rs9217	7563653	C	T	0.33	0.41	0.3
20	*CHRNA4*	rs2273502	62714170	C	T	0.94	0.46	0.28
		rs755203	62720612	A	G	0.54	0.53	0.69

[a] Based on human genome sequence (NCBI Build 34, July 2003);

[b] TR: the proportion of times allele 1 is transmitted.

[c] Nominally significant *P*-values are highlighted in bold.

TABLE III

Haploview Tests of Most Significant Haplotyic Association with Bipolar Disorder

Gene	SNPs	Haplotype	Uncorrected *P*-value [a]	Gene-wide Permutation *P*-value [b]
CHRNA2	rs9314347 rs891397	GC	**0.029**	0.349
CHRNA7	rs8035668 rs1500946	CA	**0.03**	0.554
CHRNB4	rs1948 rs7178270 rs1316971 rs11633223	GCGG	**0.017**	0.382
CHRNB1	rs4151120 rs3853818 rs2302765 rs2302762 rs9217	TTTTT	**0.011**	0.249

[a] Nominally significant *P*-values are highlighted in bold.

[b] 1000 permutations performed.

TABLE IV

Allelic Transmission Bias between the Alcohol and Non-alcohol Groups*

Gene Symbol	SNP	Analysis by individuals [a]		Analysis by families [a]	
		Chi-square	*P*-value [b]	Chi-square	*P*-value [b]
CHRNB2	rs1127317	5.19	**0.023**	0.30	0.582
CHRM3	rs10495447	4.55	**0.033**	3.46	0.063
CHRNA1	rs6708544	3.34	0.068	4.60	**0.032**
CHRNA1	rs6732758	5.00	**0.025**	2.90	0.089
CHRM2	rs6957496	3.85	**0.050**	0.38	0.537
CHRM2	rs2350786	0.50	0.480	7.34	**0.007**
CHAT	rs7094248	0.02	0.876	4.58	**0.032**
CHRNA10	rs2741862	4.40	**0.036**	0.23	0.629
CHRNB4	rs7178270	1.15	0.284	4.24	**0.040**
CHRNB4	rs1316971	0.32	0.574	3.89	**0.048**

*The transmissions between the alcohol and non-alcohol dependence groups were compared.

[a]One analysis has alcohol dependence as an individual covariate and the other analysis has alcohol dependence as a nuclear family covariate; see details for data analyses in the text.

[b]Nominally significant *P*-values are highlighted in bold.

TABLE V

Haplotypic Transmission Bias between the Alcohol and Non-alcohol Dependence Groups*

Gene Symbol	SNPs	Haplotype	Analysis by individuals [a]		Analysis by families [a]	
			Chi-square	*P*-value [b]	Chi-square	*P*-value [b]
CHRNB2	rs2072660 rs1127317	CT	4.86	**0.028**	0.34	0.557
CHRM3	rs528011 rs10495447	TA	3.97	**0.046**	5.96	**0.015**
CHRNA1	rs6708544 rs6732758 rs3755486 rs935863	GGTA	1.39	0.238	4.69	**0.030**
CHRNA9	rs4861065 rs6819483 rs4264869	CCC	2.64	0.104	5.15	**0.023**
CHRM2	rs2061174 rs324614	AC	0.11	0.737	7.24	**0.007**
CHRNA10	rs2271583 rs2741862 rs2672213 rs6578411	GACA	10.61	**0.001**	1.05	0.305
CHRNA7	rs965435 rs1913456 rs4779971 rs1355920 12441060	TTGGG	7.50	**0.006**	0.00	1.000
CHRNA5 [c]	rs4275821					
CHRNA3 [c]	rs2036527 rs684513 rs680244 rs951266 rs514743 rs615470 rs11637630 rs6495309	AGGCGACGG	0.00	0.982	4.48	**0.034**
CHRNB4	rs1948 rs7178270 rs1316971 rs11633223	GCAA	0.25	0.615	4.43	**0.035**

*The transmissions between the alcohol and non-alcohol dependence groups were compared.

[a] One analysis has alcohol dependence as an individual covariate and the other analysis has alcohol dependence as a nuclear family covariate; see details for data analyses in the text.

[b] Nominally significant *P*-values are highlighted in bold.

[c] contiguous genes on 15q24.