Family-Based Association Study of Lithium-Related and Other Candidate Genes in Bipolar Disorder

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Context: Association studies in bipolar disorder have been focused on a relatively narrow pool of candidate genes based on a limited understanding of the underlying pathophysiologic features. Recent developments suggest that a broader pool of genes may be associated with this disorder.

Objective: To examine the association between genes related to the lithium mechanism of action, as well as other positional and functional candidates, with bipolar I disorder.

Design: We examined a dense set of haplotype-tagging single-nucleotide polymorphisms using a gene-based test of association.

Participants: Three hundred seventy-nine parent-affected offspring trios.

Results: No genes specifically chosen to probe the action of lithium were associated with bipolar disorder. However, gene-based analysis of sialyltransferase 4A (SIAT4A), tachykinin receptor 1 (TACR1), and γ-aminobutyric acid, β2 receptor subunit (GABRB2) yielded evidence of association (empirical P value, <.005). Among 3 genes associated with schizophrenia or bipolar disorder in multiple previous studies, including dysbindin (DTNBP1), neuregulin (NRG1), and disrupted-in-schizophrenia 1 (DISC1), only DISC1 showed evidence of association in this cohort. In a secondary analysis of these 6 genes among parent-proband trios with a history of psychosis, evidence of the association with SIAT4A was strengthened.

Conclusions: These results suggest novel candidates and 1 gene (DISC1) previously associated with schizophrenia that merit further study in bipolar disorder. However, polymorphisms in major lithium-signaling genes do not appear to contribute substantially to bipolar liability.

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independent second-messenger signaling. In the second pathway, lithium acts as a selective inhibitor of glycogen synthase kinase 3B (GSK3B), influencing several downstream pathways including activation of the Wnt-signaling pathway. Perhaps most compellingly, mice that are haploinsufficient for GSK3B display behaviors similar to those of mice receiving long-term treatment with lithium. The GSK3B pathway has also been postulated to contribute to the observed neuroprotective effects of lithium. The 2 hypotheses are not mutually exclusive; for example, both pathways appear to converge on the serine/threonine kinase Akt-1 region. Genes in either of these 2 signaling pathways are therefore candidates for association with the risk for bipolar disorder.

Three other lines of evidence implicate additional candidate genes in bipolar disorder. First, messenger RNA expression studies or similar paradigms have identified additional genes not belonging to 1 of the 2 pathways noted. Some of these are differentially regulated by lithium or by other traditional mood stabilizers, differentially expressed in the brains of patients with bipolar disorder (hereinafter referred to as bipolar patients), or yield proteins that are otherwise implicated in the mechanism of action of mood stabilizers. For example, genes related to oligodendrocyte differentiation or function exhibited differential expression in a postmortem study of bipolar patients, whereas the traditional mood stabilizer valproate sodium appears to influence histone deacetylation. Second, a small number of genes known to be expressed in the central nervous system lie under bipolar linkage peaks on q6 and q8. Finally, a small number of genes have been shown in multiple studies to be associated with schizophrenia, including disrupted-in-schizophrenia 1 (DISC1), neuregulin (NRG1), and dysbindin (DTNB1), and an overlap in liability with bipolar disorder has been suggested. Several other genes have also been associated with the risk of, or the pathways implicated in, schizophrenia or affective illness.

Therefore, to identify genes associated with bipolar disorder liability, we conducted a family-based association study examining a select panel of candidate genes based on these hypotheses.

### METHODS

#### SAMPLE DESCRIPTION

Patient samples were selected from the National Institute of Mental Health [NIMH] Genetics Collaborative Study of Bipolar Disorder waves 1 through 4, details of which have been previously reported. In brief, that study ascertained subjects in the following 2 ways: (1) first-degree probands with bipolar I disorder (BPI) and at least 1 first-degree relative with BPI or schizoaffective disorder, bipolar type (SAB), and (2) 2 first- or second-degree relatives with BPI or SAB, with at least 2 additional members of the extended family with BPI, SAB, bipolar II disorder (BPII), or recurrent major depressive disorder. In either approach, subjects with 2 parents with BPI or SAB were excluded. Diagnosis was determined using the Diagnostic Interview for Genetic Studies with best-estimate diagnosis assigned by 2 independent psychiatrists based on the Diagnostic Interview for Genetic Studies result, family informants, and review of medical records.

For the present study, we initially identified all completely affected parent-proband trios for whom DNA was available from the Rutgers University repository (http://www.nimhgenetics.org) using a broad definition that included BPI, BPII, or SAB probands; from these, BPI parent-proband trios were selected for primary analyses, based on the most current phenotypic data available to us (NIMH release 3.05 [http://www.nimhgenetics.org]).

### GENE AND SINGLE-NUCLEOTIDE POLYMORPHISM SELECTION

Genes were selected from the following 3 broad categories based on a review of the literature: (1) implication in lithium signaling (n=91, including 17 related primarily to inositol 1,4,5-triphosphate, 39 related primarily to GSK3B/Wnt signaling, and 35 others implicated by messenger RNA expression data or related approaches); (2) location under a bipolar disorder linkage peak (n=10); and (3) previous evidence of association with or involvement in schizophrenia or mood disorders (n=23, including 3 with replicated association in schizophrenia [DISC1, DTNB1, and NRG1]). A fourth gene previously associated with schizophrenia, G72, was omitted because it had previously been studied in the NIMH cohort. In all, 124 genes spanning 11.8 megabases were selected with this approach (eTable 1; available at http://www.archgenpsychiatry.com).

The single-nucleotide polymorphisms (SNPs) within the candidate genes were selected using a haplotype-tagging (or locus variation-tagging) approach. This approach identifies a set of nonredundant "tag" SNPs that capture common genetic variation in the designated region, allowing a more efficient screen than typing all SNPs in a region. The tagging approach has been shown to be efficient and powerful for association studies. In selecting tags, priority was given to known or putative functional SNPs, including exonic SNPs or promoter-region SNPs. First, genotypic data for all SNPs in regions encompassing each gene (including 10-kilobase [kb] 5' and 10-kb 3' flanking regions) were obtained from the International HapMap Project phase lc public database (http://www.hapmap.org). The bioinformatics software TAMAL was also used to identify putative functional SNPs in the same gene regions. The SNPs selected from the HapMap and TAMAL databases were submitted to the program Tagger to identify the subset to be used for tagging; parameters included a minimum coefficient of determination (r2) threshold of 0.8 and minimum minor allele frequency of 0.05. The SNPs selected using TAMAL for their functional importance were forced into the final SNP set, regardless of their tagging performance. For 2 genes previously reported to be associated with schizophrenia, NRG1 and the ionotropic/kainate glutamate receptor 2 (GRIK2), the tagging approach was not applied because the large gene size and low linkage disequilibrium would have required a prohibitive number of SNPs to be genotyped; rather, SNPs were selected on the basis of those previously showing evidence of association with schizophrenia.

Genotyping was performed using a gene expression platform (Illumina BeadArray at the Center for Genotyping and Analysis of the Broad Institute). In total, 1536 SNPs were genotyped in 1302 samples; 1 control sample from the Centre d’Etude du Polymorphisme Humain set was also included on each 96-well plate. After data cleaning (eTable 2), the final sample included 1261 autosomal SNPs genotyped in 829 individuals from 225 families and yielded 379 affected-offspring parent-proband trios. Resulting genotype success rates for these SNPs were in excess of 99%, and mean genotyping rates were greater than 99% for all individuals (minimum, 94%). For the analyzable duplicate samples, interplate concordance was greater than 99.9% and concordance with published Centre d’Etude du Polymorphisme Humain genotypes was 100% (n=1025 genotypes in total).

To determine the informativeness of the resulting SNPs for the gene panel, Tagger was rerun with the same parameters but including only the passing SNPs. Although these SNPs were
identified using HapMap phase Ic data, the informativeness for the 1180 HapMap SNPs meeting quality control criteria was estimated using phase II data, released subsequent to our initial genotyping assay development (eTable 1). Of a total of 7762 HapMap phase II SNPs in the tagged genes, 77% were captured with $r^2 \geq 0.8$, with mean $r^2 = 0.83$. For the individual genes, 67 were captured with mean $r^2 \geq 0.8$ and 108 with mean $r^2 \geq 0.5$, suggesting that the tag SNPs adequately captured the common variation in these genes.

**ANALYSIS**

As suggested by Neale and Sham, we considered the natural unit of analysis to be the single gene rather than the single SNP or haplotype. We therefore used a gene-based framework to aggregate the single SNP statistics and correct for multiple testing up to the level of the individual gene. Specifically, primary analysis screened for association among the 379 BPI trios using the set-based test implemented in the PLINK association analysis toolset (http://pngu.mgh.harvard.edu/~purcell/plink/) for all 124 genes. This test is similar to that described and shown to be highly efficient by Ott and Hoh: it computes the test statistic ($P$ values) for the best single SNP per region, for the best 2 SNPs per region, for the best 3 SNPs per region, and so forth. The significance of these set statistics is then estimated by permutation, which allows a determination of genewise significance, allowing for correlation between SNPs and tests while controlling for type I error at the single-gene level. For these analyses, the significance of the SNP combinations including 1 to 5 SNPs were estimated, using $50,000$ permutations. Although it would be possible to sum over all SNPs within 1 gene, rather than the best 5, this approach would tend to obscure associations if only 1 or a few SNPs show evidence of association, as we would expect. The transmission disequilibrium test is problematic as a test for association in multiplex families in the presence of linkage because transmissions to affected offspring are not independent. However, the determination of $P$ values by permutation allows transmissions to multiple affected siblings to be analyzed while taking into account this relatedness.

Choosing the correct test for testing multiple genes for association, within the constraints of available power, by setting a more stringent permuted genewise $P < .005$ as the threshold for significance in the gene-based test. This value was selected according to examination of thresholds for $70\%$ power to detect association in the single-SNP transmission disequilibrium test. Anticipating the need for replication of any suggestive finding, and recognizing the possibility of more than 1 true-positive association given the nature of the hypotheses under study, we elected not to choose a more stringent threshold. Tests of $DISC1$, $NRG1$, and $DTNBP1$, which we considered to have greater prior probability of association based on previous reports, used a less stringent threshold for statistical significance ($P < .05$) than the other genes.

For any gene with evidence of association in the gene-based test, we then examined 3-marker haplotypes within the gene using a sliding-window approach for illustrative purposes. For graphical illustration of SNP location within a subset of genes, the Haploview (http://www.broad.mit.edu/mpg/haploview/) and Locusview 2.0 (http://www.broad.mit.edu/mpg/locusview/) packages were used.

Finally, given emerging evidence of overlap between bipolar disorder and primary psychotic disorders, we performed a follow-up analysis of only those genes with evidence of association with BPI in our primary analysis, as well as the 3 replication genes ($DTNBP1$, $NRG1$, and $DISC1$). For this analysis, affection status was determined by psychosis (SAB or BPI with psychotic features in the proband) rather than BPI diagnosis, yielding 294 affected trios drawn from the larger cohort. To aid in the interpretation of results, the power for single-marker analyses was estimated using the Genetic Power Calculator for 379 trios assuming a discrete trait analyzed by means of the transmission disequilibrium test. For $\alpha = .005$ and an additive model with a genotypic risk ratio of 1.5, power was at least $75\%$ for minor allele frequency of $25\%$ or greater and at least $70\%$ for minor allele frequency of $20\%$ or greater. For $\alpha = .05$, as applied to the replication genes, and an additive model with a genotypic risk ratio of 1.4, power was at least $75\%$ for a minor allele frequency of $20\%$ or greater and at least $70\%$ for a minor allele frequency of $15\%$ or greater. These estimates do not consider the nonindependence of trios in multiplex families, although comparison of asymptotic and permuted $P$ values suggested little influence of nonindependence here. True power is likely to be substantially greater because of the use of the set-based test.

**RESULTS**

Three genes ($SIAT4A$, $TACR1$, and $GABRB2$) yielded permuted $P < .005$ in gene-based association tests, corrected for all tests within the gene; the 5 SNPs in each gene that contribute to this association are listed in the Table. The association between BPI and $SIAT4A$, $TACR1$, $GABRB2$, and $DISC1$ was further characterized using 3-marker sliding-window haplotypes because the set-based test does not localize association within a gene (Figure). In $GABRB2$, multiple haplotypes were differentially transmitted to bipolar offspring; the strongest association was observed with an overtransmitted 3-marker haplotype of rs556547, rs967771, and rs10515828 (transmitted to nontransmitted [T:NT] ratio, 203:139; $\chi^2 = 11.98, P < .001$). In $SIAT4A$, the strongest association was observed with a 3-marker haplotype of rs2075823, rs6986303, and rs9643297, which was undertransmitted (T:NT ratio, 190.5:249.7; $\chi^2 = 7.96, P = .005$). Finally, for $TACR1$, a 3-marker combination of rs3771809, rs6546952, and rs3771811 was undertransmitted (T:NT ratio, 190.5:249.7; $\chi^2 = 7.96, P = .005$).

In $DISC1$, an overtransmitted haplotype of rs10495308, rs2793091, and rs2793085 (T:NT ratio, 98:65; $\chi^2 = 6.67, P = .01$) showed the greatest evidence of association. (To aid in the comparison of these results with previous studies and to facilitate future ones, the odds ratios and $P$ values [in terms of $\log (P)$] from single-SNP tests are also presented in the eFigure and supplemental eTable 4). Notably, the gene-based test explicitly considers the single-SNP results (ie, single-SNP transmission disequilibrium test; see SNP 1 in the Table) and thus does not require further correction for these single-SNP tests.

Because of the reported overlap in schizophrenia and bipolar liability, we performed a secondary analysis examining gene-based associations using the lifetime presence or absence of psychotic symptoms, regardless of diagnosis, as the phenotype. This analysis included 294 affected-offspring trios drawn from the larger cohort, including BPI and SAB offspring. For $SIAT4A$, the gene-based test yielded $P < 4 \times 10^{-5}$; for the other genes, re-
Results were essentially unchanged from the primary analysis of the bipolar phenotype (eTable 5). Three-marker sliding-window results for this gene are shown in the Figure (top half of SIAT4A panel [A]).

COMMENT

In this large-scale family-based association study of bipolar disorder, we identified evidence of association using a gene-based test for 3 genes from a panel of 124. One gene, SIAT4A, is in a region of chromosome 8 implicated in a meta-analysis of linkage data in bipolar disorder; a second, TACR1, was identified in an expression study of bipolar disorder; and a third, GABRB2, was previously implicated in 2 association studies of schizophrenia. None of the genes related to lithium signaling demonstrated evidence of association.

### Table. Gene-Based Test for Association With Bipolar Disorder

<table>
<thead>
<tr>
<th>Gene</th>
<th>Position</th>
<th>No. of SNPs Tested</th>
<th>Mean r²</th>
<th>Proportion of HapMap SNPs Captured With r² &gt; 0.8</th>
<th>SNP Rank Order</th>
<th>SNP</th>
<th>Mean χ² Test</th>
<th>P Value Gene</th>
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Replication genes:

**DISC1** | chr1:228073965-228483722 | 54 | 0.84 | 0.75 | SNP 1: rs11577215 | 6.62 | .08 |  |
|         |            |                   |         | SNP 2: rs10864702 | 6.05 | .04 |  |
|         |            |                   |         | SNP 3: rs1025101 | 5.38 | .03 |  |
|         |            |                   |         | SNP 4: rs1934909 | 4.86 | .02 |  |
|         |            |                   |         | SNP 5: rs2759346 | 4.43 | .01 |  |

**DTNB1** | chr6:19626511-15780272 | 19 | 0.91 | 0.87 | SNP 1: rs1000117 | 3.46 | .38 |  |
|         |            |                   |         | SNP 2: rs1011313 | 3.08 | .27 |  |
|         |            |                   |         | SNP 3: rs1997679 | 2.72 | .21 |  |
|         |            |                   |         | SNP 4: rs760666 | 2.50 | .14 |  |
|         |            |                   |         | SNP 5: rs9296983 | 2.36 | .09 |  |

**NRF1** | chr8:31618809-32720283 | 11 | 0 | 0 | SNP 1: rs3923490 | 3.21 | .41 |  |
|         |            |                   |         | SNP 2: rs967205 | 1.97 | .51 |  |
|         |            |                   |         | SNP 3: rs246089 | 1.43 | .55 |  |
|         |            |                   |         | SNP 4: rs1481743 | 1.15 | .54 |  |
|         |            |                   |         | SNP 5: rs1687838 | 0.98 | .51 |  |

**Abbreviations:** chr, chromosome; SNP, single-nucleotide polymorphism.

*Mean r² between genotyped SNPs and all HapMap (International HapMap Project) SNPs in the gene region.

bProportion of HapMap SNPs in the gene region captured with r² > 0.8 by the genotyped (tag) SNPs.

Indicates the number of SNPs (eg, SNP 3 indicates the third most significant SNP in the set).

Indicates P value is adjusted for all single- and multiple-SNP tests.

Gene-based test for association with bipolar disorder phenotypes among nonreplication candidate genes with P < .005.

Gene-based test for association with bipolar disorder phenotypes among 3 replication candidate genes.

Tagging approach was not applied because of gene size.

SIALYLTRANSFERASE 4A

Sialyltransferase 4A (Online Mendelian Inheritance in Man *607187*), also referred to as ST3 β-galactoside α-2,3-sialyltransferase 1 (ST3GAL1), codes for one of a family of proteins that transfer sialic acid to glycoprotein or glycolipid carbohydrate groups. It was included herein because of its location under a bipolar disorder linkage peak on 8q identified in a pooled analysis of linkage data and prioritized among positional candidates because nerve cell adhesion molecules, key to cell-cell interaction in the developing brain, are modified by the addition of polysialic acid by sialyltransferases. Changes in the expression of nerve cell adhesion molecule 1 have been noted in the hippocampus in postmortem studies of bipolar patients. The gene coding for another glycosyltransferase, a mannosyltransferase at 11q23, was shown to be dis-
ruptured by a translocation break point cosegregating with bipolar disorder in one family. Finally, a recent report described an association between another sialyltransferase, SIA
t8B, and schizophrenia risk. Sialyltransferase 4A is known to be expressed in brain and many other tissues but is otherwise not well characterized. Our finding that the association is strongest among psychotic patients, particularly in the context of the SIA8B association, suggests that SIA4A should be studied among schizophrenia cohorts as well.

**TACHYKININ RECEPTOR 1**

Substance P, alternately referred to as neurokinin and tachykinin, and its primary receptor tachykinin receptor 1 (TACR1) (Online Mendelian Inheritance in Man 600232) have previously been associated with pain and more broadly with stress response, as well as motivation and reward/aversion circuits. Studies in mood disorders are limited, but a neuropathology study found differences in the expression pattern of TACR1 among unipolar but not bipolar subjects. Substance P antagonists are also known to have antidepressant and anxiolytic effects in animal models, although human studies remain inconclusive.

Tachykinin receptor 1 itself was initially reported to be one of a subset of genes regulated by lithium in cultured lymphoblastoid cells. After an examination of messenger RNA expression data suggested the gene coding for substance P as a high-priority target in bipolar disorder, a small population-based association study failed to find an association in 20 SNPs in 4 genes related to substance P with affective illness, although coverage of TACR1 itself was limited and the cohort was quite small. Otherwise, to our knowledge, this gene has not been studied in mood disorder cohorts, and no studies have examined the effects of substance P in bipolar disorder itself.

**GABA<sub>2</sub> RECEPTOR**

The γ-aminobutyric acid<sub>2</sub> (GABA<sub>2</sub>) receptor subunit gene (Online Mendelian Inheritance in Man 600232) is located...
in a region of chromosome 5q identified in a previous meta-analysis of linkage data in schizophrenia; linkage has also been reported in studies of families of Portuguese descent and those of Colombian and Costa Rican descent. One study reported the association of GABRB2 with schizophrenia in a cohort of Chinese patients with schizophrenia, and a subsequent family-based study identified a single overtransmitted SNP (rs168697) in GABRB2 in 2 independent cohorts, 1 of Portuguese and 1 of German descent. Our result does not directly replicate either of the schizophrenia findings. Of the SNPs associated in the Chinese cohort, none was associated in the present study. For rs194072 and rs252944, the T:NT ratio was 101:97 (P > .5); for rs187269, 154:158 (P > .5). Although rs1816071 was not directly genotyped, a 2-marker haplotype (rs1644454 and rs187269) served as a proxy with r² > 0.6; again, no significant evidence of association (for all comparisons, P > .5) was identified. The single SNP associated in the Portuguese and German cohorts was not successfully genotyped in our study, nor could a tagging SNP be identified in HapMap.

Although abnormalities in GABAergic neurotransmission have been best described in schizophrenia, differences have also been reported in affective illness. Changes in the expression of multiple GABA receptor subunits were noted in a cohort of individuals with major depressive disorder, particularly in suicides; similar changes were noted in another postmortem study. Increased immunolabeling of GABAβ2/3 subunits was increased in bipolar patients compared with control subjects. Finally, another BPI postmortem study found a decreased in bipolar patients compared with control subjects.88

Pharmacotherapies with known efficacy in bipolar disorder appear to influence GABAergic neuron development or GABAergic neurotransmission. Antipsychotics that antagonize the dopamine D2 receptor lead to upregulation of glutamic acid decarboxylase 67, the rate-limiting step in GABA synthesis. Valproic acid stimulates GABAergic neurogenesis in rat forebrain. In rat hippocampus, lithium treatment appears to enhance the firing of GABAergic interneurons. In bipolar patients, lithium (although not valproate) treatment was associated with decreases in a measure of glutamate and GABA levels on magnetic resonance spectroscopy; elevated levels of this measure had been observed in bipolar patients not receiving medication.

Finally, 2 sets of clinical observations in bipolar disorder implicate GABAergic neurotransmission. First, drugs such as benzodiazepines that act primarily on the GABA receptor are widely used adjunctively in mania, and a recent meta-analysis suggests efficacy for at least 1 of them. Second, studies of bipolar patients indicate extremely high rates of anxiety comorbidity.

Confining our analysis to individuals with psychotic disorders yielded minimal change in the evidence of association for GABRB2. This suggests that reports of association in schizophrenia and bipolar disorder may not simply indicate that this gene is a psychosis risk gene per se.
of space constraints. We identified no evidence of association for upstream or downstream genes in that pathway. Finally, although the SNP tagging approach was generally informative for most genes, we cannot exclude the possibility that rarer variation in these genes, or SNPs that were not adequately tagged, are those that confer bipolar risk.

We were unable to detect any significant evidence of association for 2 other genes implicated first in schizophrenia and later as bipolar candidates, NRG1\(^{19,39,113}\) and DTNB1,\(^{114}\) in the gene-based test. In DTNB1, we directly genotyped or captured by tagging \((r^2 = 1.0)\) the 8 SNPs with evidence of association in schizophrenia, including all of the haplotype-tagging SNPs identified by Mix et al.\(^{103}\) In this our results are consistent with negative results from other groups.\(^{105,113}\) We also did not detect an association with the set-based test for other genes previously implicated in bipolar disorder, including brain-derived neurotrophic factor (BDNF), the dopamine transporter (SLC6A3), and the serotonin transporter (SLC6A4).\(^{116-121}\)

We note 2 primary limitations in this study. First, although one of the larger reported cohorts of bipolar patients, the power to detect moderate effects is still only fair; thus, the possibility of type II error must be considered. Second, all of the reported associations will require replication because, even where the genes implicated overlap with previous reports, the specific SNPs or haplotypes conferring risk apparently do not. Nonetheless, if replicated, these genes may represent novel targets for the development of treatments and diagnostic tools in bipolar disorder.

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Additional Information: The eTables and eFigure are available at http://www.archgenpsychiatry.com.

REFERENCES


