Manuscript Number: PG-D-07-00053R1

Title: Evidence of association between brain-derived neurotrophic factor (BDNF) gene and bipolar disorder

Article Type: Original Study

Section/Category:

Keywords: brain-derived neurotrophic factor; bipolar disorder; association study; single nucleotide polymorphism

Corresponding Author: Dr Tatiana Foroud, PhD

Corresponding Author's Institution: Indiana University School of Medicine

First Author: Tatiana Foroud

Order of Authors: Tatiana Foroud; Lixiang Liu; Tatiana Foroud, PhD; Xiaoling Xuei; Wade Berrettini; William Byerley; William Coryell; Rif El-Mallakh; Elliot Gershon; John Kelsoe; William Lawson; Dean MacKinnon; Melvin McInnis; Francis McMahon; Dennis Murphy; John Rice; William Scheftner; Peter Zandi; Falk Lohoff; Alexander Niculescu; Eric Meyer; Howard Edenberg

Manuscript Region of Origin: UNITED STATES

Abstract: Objective   Brain-derived neurotrophic factor (BDNF) plays an important role in the survival, differentiation, and outgrowth of select peripheral and central neurons throughout adulthood. There is growing evidence suggesting that BDNF is involved in the pathophysiology of mood disorders.

Methods   Ten SNPs across the BDNF gene were genotyped in a sample of 1,749 Caucasian Americans from 250 multiplex bipolar families. Family-based association analysis was employed with three hierarchical bipolar disorder models to test for association between SNPs in BDNF and the risk for bipolar disorder. In addition, an exploratory analysis was performed to test for an association of the SNPs in BDNF and the phenotypes of rapid cycling and episode frequency.

Results Evidence of association (p<0.05) was found with several of the SNPs using multiple models of bipolar disorder; one of these SNPs also showed evidence of association (p<0.05) with rapid cycling.

Conclusion   These results provide further evidence that variation in BDNF affects the risk for bipolar disorder.

December 16, 2007

Professor Hugh Gurling
Windeyer Institute for Medical Sciences
46 Cleveland Street
London W1T 4JF
Office: 020 7679 9474
Molecular Psychiatry Laboratory: 020 7679 9436

Dear Dr. Gurling

Thank you for the review of manuscript #: PG-D-07-00053 entitled 'Evidence of association between brain-derived neurotrophic factor (*BDNF*) gene and bipolar disorder'. We appreciate the helpful comments of the reviewers and have revised the manuscript based upon their recommendations. Below, we have provided our response to each of the items raised by the two reviewers.

Reviewer #1:

1. **The authors should present genotype and allele counts in cases and internal controls for the examined SNPs in a model where the strongest evidence for association was obtained.**

Our manuscript is a family-based association study, and does not include a sample of cases and controls. Rather, the evidence of association is assessed based on the overtransmission of a particular allele to affected individuals in a family, as well as the greater frequency of that particular allele in affected individuals as compared with their unaffected siblings. Thus, the statistical evidence for the association cannot be displayed as a count of alleles and genotypes in cases and controls. To clarify to the readers that we are performing a family-based test of association, we have added sample size counts for the number of affected and unaffected individuals in the family using our most liberal (inclusive) definition of unaffected (see page 8 under the "phenotype definitions").

2. **The authors employed three different phenotype definitions and two definitions of unaffected individuals (i.e., 6 models in total), and then examined 10 SNPs. I think that the authors must describe how multiple testing was taken into account in the analysis.**

We have reviewed the genomic architecture in BDNF and found that the 10 SNPs genotyped within BDNF are all within a single block. Therefore, it would appear to be overly conservative to correct for the testing of 10 SNPs. However, we agree with the reviewer that we have tested 6 interdependent (i.e. nested) genetic models. Therefore, while over-conservative, we have applied a correction for the evaluation of 6 genetic models. We have added a paragraph in the Discussion to address this point.

3. **In the first paragraph of the discussion, the authors state that they found strong evidence for association with two SNPs; however, the obtained best p-value was p=0.002**

only when model 1 and the conservative definition of unaffected was used. I think that this evidence is not very strong.

We have altered the first paragraph and removed the word strong as an adjective describing the association.

4. **In the same paragraph, the authors state that their data are consistent with Sklar et al (2002); however, readers can not know how these data are consistent because allele counts on rs6265 (Val66Met) are not described at all in the manuscript. In addition, the same topic on rs6265 is later addressed in the 2nd paragraph on page 13 which include no data on the authors' own study. The authors state that "these conflicting reports could be due to the use of differing study designs, etc". However, the possible association between bipolar disorder and rs6265 has been completely refuted by two studies of Green et al (2006) for over 3000 individuals in UK and Kunugi et al (2004) for over 1000 individuals in Japan. I think that the obtained results (no significant association) in the authors' study are simply consistent with such previous negative results.**

Sklar and her colleagues reported allele frequencies and the number of transmitted and non-transmitted (T/U) for the common Val allele of rs6265. We provided the minor allele frequency (MAF) in our table 1, and we have added the number of T/U for the SNP in the first paragraph of the discussion section.

5. **The authors did not cite a recently published important paper of Okada et al (Mol Psychiatry 11:695-703, 2006) reporting a significant association between bipolar disorder and a novel functional microsatellite polymorphism of the BDNF gene.**

We apologize for overlooking this paper and have added a reference to it in the Introduction.

6. **The authors may describe any speculation on the possible mechanism underlying the relationship between genetic variation of the BDNF and rapid cycling.**

We believe it is premature to speculate on a mechanism given that our association is with a different SNP than the reports in the literature by Green et al. and Muller et al.

7. **Complete LD usually refers to LD=1.0 with any value of r2 while absolute LD refers to LD=1.0 and r2=1.0. Thus "absolute" rather than "complete" should be used (L13P11). rs1419480 should have been rs1519480 (L12P11).**

We have incorporated the reviewer's recommendations.

8. **In Figure 1, there are only two exons; however, there are much more exons in the BDNF gene in the recent literature. The gene structure in fig. 1 is too old and should be updated.**

There are six or more known alternative splicing forms of the BDNF gene. Each contains two or one exon. We do not believe that the exon variant of each transcript should be combined in one gene structure as drawn in Figure 1 of Okada et al., 2006. Instead, in Figure 1 of the revised manuscript, we added the structures of the other five transcripts above the main transcript (NM_170731) in scale to illustrate the location of each exon variant.

9. **Legends for Figures 2 and 3 are too short. Explanations for colors and empty boxes should be given. General readers do not know that empty boxes imply D'=1.0 in Figure 2. (R2) should be (r2) in the legend for Figure 3.**

We have provided additional text to more thoroughly explain Figures 2 and 3. We have also changed R2 to r2.

Reviewer #2:

i) **Sample details need to be more thoroughly explained, for example diagnostic assessment, distribution according to DSM-IV criteria, age, sex.**

We have added more sample details under the Material and Method section.

ii) **If the rapid cycling variable met DSM-IV criteria please state.**

The rapid cycling variable met DSM-IV criteria, we have added this under the Phenotype Definition section in this revised version of the manuscript.

iii) **Phenotype models - were BPI individuals tested on their own?**

BPI subjects were tested together with SABP in model 1, however, BPI subjects made up about 95% of the subjects in this disease model.

iv) **Please give more details on the association seen with rapid cycling and state if multiple testing was taken into account.**

We have added genotypic counts for the cases and controls for the rapid cycling phenotype. Only one phenotype was analyzed and a different type of analysis was employed, so there was no need to correct for multiple testing.

v) **Models for definition of unaffected individuals need to be explained within the sample details.**

As recommended by this reviewer, we have moved the definition of the unaffected family members to the section of the manuscript describing the sample.

vi) **Be more cautious when explaining the "strong" evidence of association with two SNPs.**

As suggested by Reviewer 1 as well, we no longer describe the evidence of association as "strong".

vii) Clarify how the 10 SNPs genotyped were chosen. If some of the genotyped SNPs were known to be in high LD in terms of both D' and r2 within the CEU individuals in Hapmap, was using tag SNPs selected using haploview considered?

SNPs were selected prior to the availability of the more complete HapMap data, wich is currently available. The SNPs were selected either from dbSNP, such as Val66Met (rs6265), or from HapMap for tag SNPs ($r^2 = 0.8$, MAF ≥ 0.05) within the CEU population. We have added this information to the Materials and Methods.

In addition to answering the reviewers' questions, we also added text to the Discussion regarding two recently published genome-wide association studies. Four SNPs in the BDNF intron region were associated ($p<0.05$) with bipolar disorder in three independent datasets. These data were added to Figure 1.

We thank the reviewers for their helpful suggestions which have improved the revised manuscript. If you have any questions, please feel free to contact me at tforoud@iupui.edu. We hope that these changes will allow the manuscript to be accepted by *Psychiatric Genetics*.

Sincerely,

Tatiana Foroud, Ph.D.

* Manuscript (All Manuscript Text Pages in MS Word format, including Title Page, Refere...

1

Evidence of association between brain-derived neurotrophic factor (*BDNF*) gene and bipolar disorder

Running title: *BDNF* and bipolar disorder

Lixiang Liu[a], Tatiana Foroud[a], Xiaoling Xuei[a], Wade Berrettini[b], William Byerley[c], William Coryell[d], Rif El-Mallakh[e], Elliot S. Gershon[f], John R. Kelsoe[g], William B. Lawson[h], Dean F. MacKinnon[i], Melvin McInnis[j], Francis J. McMahon[k], Dennis L. Murphy[k], John Rice[l], William Scheftner[m], Peter P. Zandi[i], Falk Lohoff[b], Alexander B. Niculescu[a], Eric T. Meyer[a], Howard J. Edenberg[a], John I. Nurnberger Jr.[a]

[a]Indiana University, Indianapolis, IN 46202; [b]University of Pennsylvania, Philadelphia, PA 19104; [c]University of California Irvine, Irvine, CA 92697; [d]University of Iowa, Iowa City, IA 52242; [e]University of Louisville, Louisville, KY 40292; [f]University of Chicago, IL 60637; [g]University of California San Diego, La Jolla, CA 92093; [h]Howard University, Washington, DC 20059; [i]The Johns Hopkins Hospital, Baltimore, MD 21287; [j]University of Michigan, Ann Arbor, MI 48109; [k]National Institute of Mental Health, National Institute of Health, Bethesda, MD 20892; [l]Washington University St. Louis, St. Louis, MO 63110; [m]Rush University Medical Center, Chicago, Illinois 60612

Correspondence to: Tatiana Foroud, Ph.D.

Department of Medical and Molecular Genetics

Indiana University School of Medicine

Health Information and Translational Sciences HS 4000

410 West 10th Street

Indianapolis, IN 46202-3002

Tel: 317-278-1291

Fax: (317)-278-1100

E-mail: tforoud@iupui.edu

Abstract

Objective   Brain-derived neurotrophic factor (*BDNF*) plays an important role in the survival, differentiation, and outgrowth of select peripheral and central neurons throughout adulthood. There is growing evidence suggesting that *BDNF* is involved in the pathophysiology of mood disorders.

Methods   Ten SNPs across the *BDNF* gene were genotyped in a sample of 1,749 Caucasian Americans from 250 multiplex bipolar families. Family-based association analysis was employed with three hierarchical bipolar disorder models to test for association between SNPs in *BDNF* and the risk for bipolar disorder. In addition, an exploratory analysis was performed to test for an association of the SNPs in *BDNF* and the phenotypes of rapid cycling and episode frequency.

Results   Evidence of association ($p<0.05$) was found with several of the SNPs using multiple models of bipolar disorder; one of these SNPs also showed evidence of association ($p<0.05$) with rapid cycling.

Conclusion   These results provide further evidence that variation in *BDNF* affects the risk for bipolar disorder.

Keywords: brain-derived neurotrophic factor; bipolar disorder; association study; single nucleotide polymorphism

Introduction

While family, twin, and adoption studies have provided strong evidence for a significant genetic component in bipolar disorder (Craddock and Jones, 1999), it is clear that multiple genes must contribute to disease susceptibility (McGuffin and Katz, 1989; Gershon, 1990). Brain-derived neurotrophic factor (*BDNF*), which belongs to the neurotrophin family, plays an important role in the survival, differentiation, and outgrowth of select peripheral and central neurons throughout adulthood (Schinder, 2000; Huang and Reichardt, 2001). There is growing convergent evidence suggesting that *BDNF* is involved in the pathophysiology of mood disorders and in the mechanism of action of therapeutic agents (Sheline *et al*., 1996, 2003; Chen *et al*., 2001; Fukumoto *et al*., 2001; Duman, 2002; Karege *et al*., 2002; Nestler, 2002; Shirayama *et al*., 2002; Coyle *et al*., 2003; Shimizu *et al*., 2003; Chen *et al*., 2004; Ogden *et al*, 2004).

Several human studies have shown that serum BNDF levels were decreased in patients with major depressive disorder when compared with the control group (Karege *et al*., 2002; Shimizu *et al*., 2002). Increased levels of BDNF immunoreactivity were found in postmortem hippocampal tissue among antidepressant treated bipolar patients, compared with antidepressant untreated bipolar subjects (Chen, *et al*., 2001). Neuroimaging studies suggested that decreased BDNF levels might account for structural brain changes in bipolar patients (Sheline *et al*., 1996, 2003; Manji, *et al*., 2001).

Studies in animal models have suggested that *BDNF* expression might be a downstream target of antidepressant treatments and that *BDNF* might provide protection against stress-induced neuronal damage (Duman, 2002; Nestler *et al*., 2002; Coyle *et al.*, 2003). In animal models of depression, an antidepressant-like effect was produced by the infusion of BDNF into the rat midbrain (Siuciak *et al*., 1997; Shirayama *et al*., 2002). The level of BDNF mRNA in the

rat hippocampus was increased by electroconvulsive seizures, and the down-regulation of BDNF mRNA was blocked by the chronic administration of electroconvulsive seizures (Lindeforts, *et al*., 1995; Nibuya *et al*., 1995). Chronic administration of lithium or valproate increased *BDNF* expression in rat brain (Fukumoto *et al*., 2001). *BDNF* ablation affects the reactivity of the serotoninergic system to the stress hormone corticosterone (Hensler *et al*., 2007).

Two family studies of bipolar disorder have reported linkage to chromosome 11p13, where *BDNF* is located (Detera-Wadleigh *et al*., 1999; McInnes *et al*., 1996). Significant association of bipolar disorder with polymorphic markers genotyped in *BDNF* has been reported in two family based studies (Neves-Pereira *et al*., 2002; Sklar *et al*., 2002). Both studies genotyped the Val66Met polymorphism (rs6265) and reported over-transmission of the common Val66 allele to individuals with bipolar disorder. This finding was confirmed in a case-control study of unrelated subjects from the NIMH Genetics Initiative (Lohoff *et al*., 2005). Further evidence of association of the Val66Met polymorphism was reported in three small childhood-onset samples: two using a family-based design (Geller *et al*., 2004; Strauss, 2005) and one employing a case-control approach (Strauss *et al*., 2004). In addition to the Val66Met polymorphism, Strauss *et al* found significant evidence of association between bipolar disorder and four other *BDNF* SNPs (Strauss, 2005). A novel microsatellite polymorphism, which is located about 1.0 kb upstream of the translation initiation site of the *BDNF* gene, was reported to confer susceptibility to bipolar disorder (Okada *et al*., 2006). In contrast to these positive findings, a number of groups have failed to replicate the association of *BDNF* with the risk of bipolar disorder (Hong *et al*., 2003; Nakata *et al*., 2003; Kunugi *et al*., 2004; Oswald *et al*., 2004; Skibinska *et al*., 2004; Neves-Pereira *et al*., 2005; Green *et al*., 2006; Liu *et al*., 2007; Kanazawa *et al*., 2007). Several investigators also studied the association between *BDNF* and rapid-cycling:

Muller *et al* found several markers across *BNDF*, including the Val66Met polymorphism , were significantly associated with both bipolar disorder and rapid-cycling (Muller *et al*., 2006); Green and colleagues did not find evidence of association between bipolar disorder and the Val66Met polymorphism, but their study of a subset of bipolar subjects with rapid cycling showed significant association with this missense polymorphism (Green *et al*., 2006).

Most association studies of bipolar disorder have limited their analyses of *BDNF* polymorphisms to a single marker: the Val66Met polymorphism (Geller *et al*., 2004; Green *et al*., 2006; Kunugi *et al*., 2004; Skibinska *et al*., 2004; Lohoff *et al*., 2005). The sample sizes for most studies were relatively small. To ensure a rigorous evaluation of the role of *BDNF* in bipolar disorder susceptibility, the present study has analyzed a large sample of American Caucasian multiplex bipolar disorder families. Ten single nucleotide polymorphisms (SNPs) that spanned *BDNF*, including Val66Met, were genotyped, and family-based association analysis was employed. Using this rigorous approach, we found that several *BDNF* SNPs were associated with the risk for bipolar disorder.

Materials and Methods

Sample

Multiplex families with bipolar disorder were recruited at 10 centers across the United States: Indiana University (with a satellite site at University of Louisville), Johns Hopkins University, the NIMH Intramural Research Program, Rush-Presbyterian Medical Center in Chicago, University of California at Irvine, University of California at San Diego, University of Chicago, University of Iowa, University of Pennsylvania, and Washington University in St. Louis. All participating sites' institutional review boards approved this study. All subjects

received a personal assessment with the Diagnostic Interview for Genetic Studies (DIGS) by a trained interviewer. Medical records were sought for any subject with a history of mental health treatment. First-degree relatives of each proband were interviewed as well with the DIGS and the FIGS (family interview for genetic studies). Pedigree extension through affecteds was carried out as much as possible given resources available to the sites. Each participant was diagnosed by a best estimate procedure involving two senior clinicians reviewing all available information and agreeing on all Axis I diagnoses. A third clinician tiebreaker was consulted when consensus was not reached (about 1-2% of cases). All affected subjects were at least 16 years of age. The ascertainment and assessment of the study participants were previously described (NIMH Genetics Initiative Bipolar Group, 1997).

A whole genome screen was completed in 644 families consisting of 2,909 individuals (Edenberg *et al*., 1997; Detera-Wadleigh *et al*., 1997; Rice *et al*., 1997; Stine *et al*., 1997; Foroud *et al*., 2000; Dick *et al*., 2003; Willour *et al*., 2003; Zandi *et al*. 2003). Each family was carefully examined for its informativeness for family-based association study. A total of 250 multiplex families, consisting of 1,749 individuals (771 males, 978 females), were selected for genotyping based on two criteria: all family members were European American; and DNA was available from both parents for at least one affected individual in each family. This selection not only provided a more ethnically homogeneous sample, but also maximized its power for a family-based test of association.

Phenotype Definitions

A set of three hierarchical disease definitions were used in the statistical analysis for bipolar disorder. Model 1 was the narrowest disease definition and included as affected only

those individuals meeting criteria for either bipolar I or schizoaffective bipolar disorders (657 affected). Model 2 included as affected individuals meeting criteria for model 1, plus individuals diagnosed with bipolar II disorder (785 affected). Model 3 was the broadest disease definition and included as affected all individuals meeting criteria for models 1 and 2, plus individuals with recurrent unipolar depression (950 affected). We considered two models for our definition of unaffected individuals. In the first, we used very liberal criteria: for each hierarchical disease model, an unaffected individual was defined as anyone who was not affected under that model (Model 1: 1,053 unaffected; Model 2: 925 unaffected; Model 3: 760 unaffected). In the second, more conservative definition, we did not consider anyone to be unaffected; rather, the individuals who did not meet criteria for being affected were classified as unknown.

Rapid cycling was defined as the occurrence of four or more distinct episodes of depression and mania or hypomania within twelve months. The rapid cycling variable met DSM-IV criteria. Episode frequency was defined as the total number of distinct episodes suffered by the bipolar patient divided by the total number of years since the first episode occurred.

SNP genotyping

BDNF is located on chr11p13 (Maisonpierre, *et al*., 1991) and is approximately 67 kb in size (NM_170731). SNPs across *BDNF* (Figure 1) were selected from public databases, such as dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) and HapMap (http://www.hapmap.org/). Tag SNPs were chosen within the CEU population of the HapMap database ($r^2 = 0.8$, $MAF \geq 0.05$). The Val66Met polymorphism (rs6265) was among the genotyped SNPs. SNP genotyping was performed using a modified single-nucleotide extension reaction, with allele detection by mass spectrometry (Sequenom MassArray system, Sequenom, San Diego, CA). To determine allele

frequencies and to test the assays, SNPs were genotyped in a set of 40 unrelated Caucasian individuals obtained from Coriell and examined for deviations from Hardy-Weinberg equilibrium. SNPs with minor allele frequencies greater than 0.10 were preferentially genotyped in the study sample; all SNPs were in Hardy-Weinberg equilibrium. All genotypic data on the subjects were checked for Mendelian inheritance errors using the program PEDCHECK (O'Connell and Weeks, 1998). Marker allele frequencies and heterozygosities were estimated using the USERM13 (Boehnke, 1991) option of the MENDEL linkage computer program. Deviation from Hardy-Weinberg equilibrium was assessed for each SNP using Haploview (Barret, *et al*., 2005). The genotyped SNPs in *BDNF*, along with their chromosomal positions and minor allele frequencies, are shown in Table 1.

Statistical analyses

Linkage disequilibrium (LD) was evaluated between the SNPs using the program Haploview (Barret, *et al*., 2005). The extent of LD was evaluated using Lewontin's standardized disequilibrium coefficient (D') and delta-squared (r^2). The program Tagger (de Bakker *et al*., 2005) was used to examine how well the genotyped SNPs captured information from the ungenotyped SNPs. The Haploview program was also used to examine the haplotype block structures, where the blocks were defined using the criteria proposed by Gabriel (Gabriel, *et al*., 2002; Reich, *et al*., 2001).

Family-based association was performed for each of the genotyped SNPs using the three hierarchical bipolar disorder models. The pedigree disequilibrium test (PDT; Martin *et al*., 2000) implemented in the UNPHASED package (Dudbridge, 2003) was used to test SNPs for their association with bipolar disorder. The PDT utilizes data from all available trios in a family, as

well as discordant sibships. Evidence for association is assessed based on the overtransmission of a particular allele to affected individuals, and the greater frequency of that particular allele in affected individuals than in their unaffected siblings. In the liberal definition of unaffected, the PDT statistic would include both concordant and discordant transmissions. When using the conservative definition of unaffected, the PDT statistic would only include transmissions to affected individuals. In all analyses, the "average-PDT" statistic, which averages the association statistic across all families, was used to test for association between the SNPs and bipolar disorder (Martin *et al*., 2000).

There were only 24 families in our sample with at least one member having the rapid cycling phenotype. As a result, there was no power to perform family-based association analyses. Therefore, a case-control approach was taken by selecting one rapid cycling case from each of the 24 families. A control was defined as a subject with bipolar disorder who did not have rapid cycling and one such subject was randomly selected from each of the remaining 226 families. The Pearson Chi-square test was performed to test for an association between the *BDNF* SNPs and the rapid cycling diagnosis.

Exploratory analyses were also performed on the phenotype of episode frequency. From each family, the bipolar subject with the greatest episode frequency was selected for analysis. Due to the skewness of the distribution, the distribution was dichotomized. Those patients in the top 1/3 of the distribution (83 subjects) were classified as having high episode frequency. Those patients in the bottom 1/3 of the distribution (83 patients) were classified as having low episode frequency. The Pearson Chi-square test was used to test for an association between episode frequency classification and *BDNF* SNP genotype.

Results

   Ten SNPs were genotyped across *BDNF*. The pairwise LD among adjacent SNPs was

high (D' > 0.98) and all the SNPs fell into a single haplotype block (Figure 2). To determine

whether these SNPs adequately assessed the variation within *BDNF*, the program Tagger (de

Bakker *et al*., 2005) was used to examine how well the genotyped SNPs capture information

from ungenotyped SNPs. Eight of the ten genotyped SNPs were in the HapMap database and

could be assessed; these 8 SNPs captured 77.3% of all HapMap alleles (of MAF $\geq$ 0.05) at $r^2 >$

0.8, and 88.6% alleles at $r^2 > 0.5$; the mean r^2 was 0.86. Two SNPs were not included in HapMap

and therefore could not be assessed; therefore, these numbers underestimate the coverage of

BDNF.

   Initial PDT analyses employing a liberal definition of unaffected detected some evidence

of association (p<0.03) with two SNPs (rs1519480 and rs7127507) in *BDNF* across all three

hierarchical bipolar disorder models. However, when PDT analyses were performed using the

conservative definition of unaffected, much stronger evidence of association (p<0.005) with

these two SNPs was observed using the narrowest disease model (Table1). Two additional SNPs

(rs2030324 and rs2883187) provided evidence of association (p<0.05) when the two narrower

models of disease were employed, and one more SNP (rs12273363) provided evidence only for

the narrowest model.

   Several of the SNPs between rs1519480 and rs7127507 did not provide evidence of

association using any of the three disease models. To better understand how a gene that appeared

to be in a single block based on D' estimates of LD could have such a pattern of association, we

reviewed the pattern of LD using the r^2 statistic. As shown in Figure 3, an unusual pattern of LD

was identified. The two SNPs with the greatest evidence of association with bipolar disorder,

rs1519480 and rs7127507, were in very high LD with each other (r^2=0.88), but had little

evidence of LD with the SNPs between them (r^2<0.14). The intervening SNPs were in high LD

with each other (r^2>0.87) and with the SNP flanking rs7127507 (r^2>0.87). The genotypes at

rs1519480 and rs7127507 were 97% concordant. Data from HapMap were reviewed and these

two SNPs are in nearly absolute LD in that dataset as well.

SNP rs7127507 was also found to be associated with the phenotype of rapid cycling (p <

0.05; genotype counts in controls: CC:CT:TT=25:87:98; genotype counts in rapid cycling

patients: CC:CT:TT=6:5:8). No other SNP provided evidence of association with rapid cycling.

The previously reported Val66Met polymorphism (rs6265) was not significantly associated with

either bipolar disorder or rapid cycling; there was a trend (p=0.06) toward an overtransmission of

the common Val allele using the broadest bipolar disorder model. None of the SNPs were

associated with episode frequency.

Discussion

This study presents a thorough evaluation of the evidence for association between bipolar

disorder and the *BDNF* gene. We found several SNPs showing evidence of association with

bipolar disorder. However, when we use the same phenotypic definition as Sklar and colleagues

(Sklar *et al*., 2002), our results with SNP rs6265 using Model 1 are consistent with the previous

report (p=0.40, T/U=654/644, where T=transmissions from heterozygous parent to affected

offspring, U=non-transmissions). The study of Sklar et al, employed about 25% of the same

subjects used in this study. We also analyzed the data by excluding the first 97 families

ascertained trios from which were included in a previous report (Sklar *et al*., 2002). The same

LD structure and a similar pattern of significant association results were obtained in this sample

independent of the Sklar study. SNPs rs1519480 and rs7127507 were still significant across all three hierarchical bipolar disorder models (p<0.05); rs2030324 and rs2883187 still showed evidence of association (p<0.05) in the two narrower disease models.

It is not clear how best to define unaffected persons in multiplex pedigrees for bipolar disorder. Onset of bipolar disorder can occur throughout the life span; however, the median age of onset for the first major mood episode is about 20. Some authors report multiple peaks in age of onset data, with a subgroup of patients having their first episode in the 30's or later (Leboyer *et al.*, 2005; Lin *et al.*, 2006). It is also clear that bipolar illness represents the extreme endpoint of a spectrum of mood instability, and that minor variants may not come to clinical attention (Angst, 1998; Akiskal and Pinto, 1999). In this context it is not unreasonable to define only affected individuals and regard undiagnosed persons as having an unknown affected status. The fact that *BDNF* produced more significant evidence for association in this model may suggest that our family database includes a number of persons with subclinical bipolar phenotypes carrying *BDNF* "risk" variants, who detracted from the association evidence when they were classed as unaffected.

It is interesting to see that SNP rs7127507 is significantly associated with both bipolar disorder and rapid cycling in our study. Muller and colleagues also reported that several markers on the gene *BNDF* had significant association with both bipolar disorder and rapid cycling, and suggested that their significant findings on bipolar disorder were mainly driven by the rapid cycling patients (Muller *et al*., 2006). Since we have a relatively small number of rapid cycling patients in our sample, and there is only one SNP associated with rapid cycling, we plan to collect more rapid cycling cases to confirm this observation.

Although we have performed the association tests for 10 SNPs, all these SNPs are in high LD, and fall in one haplotype block, as indicated in Figure 2; therefore, it would not be appropriate to correct for 10 tests, since these SNPs are highly correlated. However, since we employed three different nested phenotype definitions and two nested definitions of unaffected individuals (i.e., 6 models in total), an overly conservative Bonferroni correction was applied to our results, Both SNP rs1519480 and rs7127507 are still significantly associated with bipolar disorder after this correction (P<0.05). Since there is only one phenotype model for rapid cycling and these analyses were exploratory, we did not perform any statistical correction to that association result.

Many previous studies have focused on the missense polymorphism Val66Met (rs6265) (Geller *et al*., 2004; Kunugi *et al*., 2004; Neves-Pereira *et al*., 2002; Skibinska *et al*., 2004; Lohoff *et al*., 2005; Green *et al*., 2006; Liu *et al*., 2007; Kanazawa *et al*., 2007). Neves-Pereira *et al*. (2002) reported a significant association (p-value=0.00064) using 283 trios. Lohoff *et al*. (2005) performed a case-control (621 BPI cases vs. 998 controls) study and reported the Val allele was significantly increased in BPI patients when compared to the controls. Among their 621 BPI cases, 213 were used in this study, which accounts for 12% of this sample. Sklar *et al* (2002) found a marginally significant overtransmission of the Val allele to the bipolar proband (p-value=0.04) in their Hopkins sample (136 trios); however, this finding was not confirmed when they employed 189 trios from the NIMH bipolar initiative (p-value=0.09), which accounted for about 25% of our sample. They also did not find significant association in their UK sample (p-value=0.24) with a sample 145 trios. There are also a number of case-control studies which have failed to replicate the association of bipolar disorder with *BDNF* (Hong *et al*., 2003; Nakata *et al*., 2003; Kunugi *et al*., 2004; Oswald *et al*., 2004; Skibinska *et al*., 2004;

Neves-Pereira *et al*., 2005; Green *et al*., 2006; Liu *et al*., 2007). These conflicting reports could

be due to the use of differing study designs, variable ancestry of the samples, and the wide range

of sample sizes which directly affect the power of the samples to detect association.

Two of the SNPs providing evidence of association with bipolar disorder, rs1519480 and

rs12273363, are located in highly conserved regions when compared with other vertebrate

genomes in the 17-way Vertebrate MultiAlignment (UCSC Genome Browser, v. 141). This

suggests that these two markers may play some important functional role. None of the SNPs

genotyped in this study was on or near the alternative splicing sites that give rise to 5 known

transcription isoforms of *BDNF* (www.ncbi.nlm.nih.gov). Therefore, the three significantly

associated markers, rs7127507, rs2030324, and rs2883187, in the intronic region of the gene

may play some other functional role(s) than alternative splicing. Such mechanisms are yet to be

discovered. Interestingly, in two recently published genome-wide association studies (Baum *et

al*., 2007; The Wellcome Trust Case Control Consortium, 2007), four additional SNPs in this

intronic region (rs16917234, rs12291186, rs11030108, rs12291063) were found to be

significantly associated with bipolar disorder in three independent datasets: NIMH, German and

Wellcome Trust Case Control Consortium (Personal communication with Dr. Francis McMahon

for the NIMH and German datasets). These four SNPs are shown in Figure 1. In contrast with the

inconsistent reports on the Val66Met polymorphism, the cumulative evidence of association in

the intron region suggests that mRNA processing, stability and *BDNF* expression levels play a

role in the susceptibility for bipolar disorder.

The current study has several strengths. First, we employed a sample of 250 multiplex,

well-characterized bipolar disorder families, with 402 trios in model 1, 471 trios in model 2 and

522 trios in model 3. Second, analyses were performed in a single ethnic group (European

Americans). Third, we employed family-based tests of association on bipolar disorder, which minimize the effects of potential population stratification. Fourth, the most significant association analyses used only affected individuals. While this limits the informativeness of the PDT analysis by eliminating the contribution of discordant sib pairs, we have reduced the possible effects of erroneous subject classification. Fifth, 10 SNPs were genotyped across *BDNF*, with high LD between the SNPs. This ensured good coverage of the gene and maximized our power to detect association anywhere in *BDNF*.

In summary, we have found evidence of association between bipolar disorder and SNPs in *BDNF*, including SNPs near both ends of this gene. The evidence provided by us in this paper supports a role for *BDNF* as a pathophysiologic factor in mood disorders.

grant from the Indiana Genomics Initiative (INGEN). INGEN is supported in part by the Lilly

Endowment, Inc.

References

Akiskal HS, Pinto O (1999). The evolving bipolar spectrum. Prototypes I, II, III, and IV.

Psychiatr Clin North Am **22**:517-34.

Angst J (1998). The emerging epidemiology of hypomania and bipolar II disorder. *J Affect*

Disord **50**:143-51.

Barrett JC, Fry B, Maller J, Daly MJ (2005). Haploview: analysis and visualization of LD and

haplotype maps. *Bioinformatics* **21**:263–265.

Baum AE, Akula N, Cabanero M, Cardona I, Corona W, Klemens B, Schulze TG, Cichon S,

Rietschel M, Nöthen MM, Georgi A, Schumacher J, Schwarz M, Abou Jamra R, Höfels S,

Propping P, Satagopan J, Detera-Wadleigh SD, Hardy J, McMahon FJ. A genome-wide

association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the

etiology of bipolar disorder. *Mol Psychiatry*. 2007 May 8

Boehnke M. Allele frequency estimation from data on relatives (1991). *Am J Hum Genet* **48**:22–

25.

Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT (2001). Increased hippocampal

BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol. Psychiatry*

50:260–265.

Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS (2004). Variant brain-

derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-

dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J Neurosci* **24(18)**:4401-11.

Coyle JT, Duman RS (2003). Finding the intracellular signaling pathways affected by mood disorder treatments. *Neuron* **38**:157-60.

Craddock N, Jones I (1999). Genetics of bipolar disorder. *J Med Genet* **36**:585–594.

de Bakker PIW, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D (2005). Efficiency and power in genetic association studies. *Nature Genet* **37**:1217–1223.

Detera-Wadleigh SD, Badner JA, Yoshikawa T, Sanders AR, Goldin LR, Turner G, Rollins DY, Moses T, Guroff JJ, Kazuba D, Maxwell ME, Edenberg HJ, Foroud T, Lahiri D, Nurnberger JI Jr, Stine OC, McMahon F, Meyers DA, MacKinnon D, Simpson S, McInnis M, DePaulo JR, Rice J, Goate A, Gershon ES (1997). Initial genome scan of the NIMH genetics initiative bipolar pedigrees: chromosomes 4, 7, 9, 18, 19, 20, and 21q. *Am J Med Genet* **74**:254-62.

Detera-Wadleigh SD, Badner JA, Berrettini WH, Yoshikawa T, Goldin LR & Turner G (1999). A high-density genome scan detects evidence for a bipolar-disorder susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. *Proc Natl Acad Sci* **96**: 5604–5609.

Dick DM, Foroud T, Flury L, Bowman ES, Miller MJ, Rau NL, Moe PR, Samavedy N, El-Mallakh R, Manji H, Glitz DA, Meyer ET, Smiley C, Hahn R, Widmark C, McKinney R, Sutton L, Ballas C, Grice D, Berrettini W, Byerley W, Coryell W, DePaulo R, MacKinnon DF, Gershon ES, Kelsoe JR, McMahon FJ, McInnis M, Murphy DL, Reich T, Scheftner W, Nurnberger JI Jr. (2003). Genomewide linkage analyses of bipolar disorder: a new sample of 250 pedigrees from the National Institute of Mental Health Genetics Initiative. *Am J Hum Genet* **73**:107-14. Erratum in: *Am J Hum Genet* **73**:979.

Dudbridge F (2003). Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol*
25:115–121.

Duman RS (2002). Genetics of childhood disorders: XXXIX. Stem cell research, Part 3:
regulation of neurogenesis by stress and antidepressant treatment. *J Am Acad Child Adolesc
Psychiatry* **41**:745–748.

Edenberg HJ, Foroud T, Conneally PM, Sorbel JJ, Carr K, Crose C, Willig C, Zhao J, Miller M,
Bowman E, Mayeda A, Rau NL, Smiley C, Rice JP, Goate A, Reich T, Stine OC, McMahon
F, DePaulo JR, Meyers D, Detera-Wadleigh SD, Goldin LR, Gershon ES, Blehar MC,
Nurnberger JI Jr. (1997). Initial genomic scan of the NIMH genetics initiative bipolar
pedigrees: chromosomes 3, 5, 15, 16, 17, and 22. *Am J Med Genet* **74**:238-46.

Foroud T, Castelluccio PF, Koller DL, Edenberg HJ, Miller M, Bowman E, Rau NL, Smiley C,
Rice JP, Goate A, Armstrong C, Bierut LJ, Reich T, Detera-Wadleigh SD, Goldin LR,
Badner JA, Guroff JJ, Gershon ES, McMahon FJ, Simpson S, MacKinnon D, McInnis M,
Stine OC, DePaulo JR, Blehar MC, Nurnberger JI Jr. (2000). Suggestive evidence of a locus
on chromosome 10p using the NIMH genetics initiative bipolar affective disorder pedigrees.
Am J Med Genet **96**:18-23.

Fukumoto T, Morinobu S, Okamoto Y, Kagaya A, Yamawaki S (2001). Chronic lithium
treatment increases the expression of brain-derived neurotrophic factor in the rat brain.
Psychopharmacology **158**:100–106.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B (2002). The structure of
haplotype blocks in the human genome. *Science* **296**:2225–2229.

Geller B, Badner JA, Tillman R, Christian SL, Bolhofner K & Cook EH, Jr. (2004). Linkage
disequilibrium of the brain-derived neurotrophic factor Val66Met polymorphism in children

with a prepubertal and early adolescent bipolar disorder phenotype. *Am J Psychiatry* **161**: 1698–1700.

Gershon ES (1990). Genetics. In: Gooswin FK, Jamison KR (eds) *Manic-depressive illness.* New York, Oxford University Press, pp 373–401.

Green EK, Raybould R, Macgregor S, Hyde S, Young AH, O'Donovan MC, Owen MJ, Kirov G, Jones L, Jones I, Craddock N (2006). Genetic variation of brain-derived neurotrophic factor (BDNF) in bipolar disorder: case-control study of over 3000 individuals from the UK. *Br J Psychiatry* **188**:21-5.

Hensler JG, Advani T, Monteggia LM (2007). Regulation of Serotonin-1A Receptor Function in Inducible Brain-Derived Neurotrophic Factor Knockout Mice After Administration of Corticosterone. *Biol Psychiatry* in Press.

Hong CJ, Huo SJ, Yen FC, Tung CL, Pan GM & Tsai SJ (2003). Association study of a brain-derived neurotrophic-factor genetic polymorphism and mood disorders, age of onset and suicidal behavior. *Neuropsychobiology* **48**: 186–189.

Huang EJ, Reichardt LF (2001). Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci* **24**:677-736.

Kanazawa T, Glatt SJ, Kia-Keating B, Yoneda H, Tsuang MT (2007). Meta-analysis reveals no association of the Val66Met polymorphism of brain-derived neurotrophic factor with either schizophrenia or bipolar disorder. *Psychiatr Genet* **17**:165-170.

Karege F, Bondolfi G, Gervasoni N, Schwald M, Aubry JM, Bertschy G (2002). Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psychiatry Res* **109**:143–148.

Kunugi H, Iijima Y, Tatsumi M, Yoshida M, Hashimoto R & Kato T (2004). No association between the Val66Met polymorphism of the brain-derived neurotrophic factor gene and bipolar disorder in a Japanese population: a multicenter study. *Biol Psychiatry* **56**: 376–378.

Leboyer M,Henry C,Paillere-MartinotML, Bellivier F (2005). Age at onset in bipolar affective disorders: a review. *Bipolar Disord* **7:**111-8.

Lin PI, McInnis MG, Potash JB, Willour V, MacKinnon DF, DePaulo JR, Zandi PP (2006). Clinical correlates and familial aggregation of age at onset in bipolar disorder. *Am J Psychiatry*. **163**:240-6.

Lindefors N, Brodin E, Metsis M (1995). Spatiotemporal selective effects on brain-derived neurotrophic factor and trkB messenger RNA in rat hippocampus by electroconvulsive shock. *Neuroscience* **65**:661–670.

Liu M, Ling SH, Li WB, Wang CY, Chen DF, Wang G (2007). An association study between GRIN1, BDNF genes and bipolar disorder. *Hereditas* **29**:41-6.

Lohoff FW, Sander T, Ferraro TN, Dahl JP, Gallinat J, Berrettini WH (2005). Confirmation of association between the Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene and bipolar I disorder. *Am J Med Genet B Neuropsychiatr Genet* **139**:51-3.

Manji HK, Duman RS (2001). Impairments of neuroplasticity and cellular resilience in severe mood disorders: implications for the development of novel therapeutics. *Psychopharmacol Bull* **35(2)**:5-49.

Maisonpierre PC, Le Beau MM, Espinosa R, Ip NY, Belluscio L, de la Monte SM, Squinto S, Furth ME, Yancopoulos GD (1991). Human and rat brain-derived neurotrophic factor and neurotrophin-3: gene structures, distributions, and chromosomal localizations. *Genomics* **10**:558–568.

Martin ER, Monks SA, Warren LL, Kaplan NL(2000). A test for linkage and association in general pedigrees: The Pedigree Disequilibrium Test. *Am J Hum Genet* **67**:146–154.

McGuffin P, Katz R (1989) The genetics of depression and manic-depressive disorder. *Br J Psychiatry* **155**:294–304

McInnes LA, Escamilla MA, Service SK, Reus VI, Leon P & Silva S (1996). A complete genome screen for genes predisposing to severe bipolar disorder in two Costa Rican pedigrees. *Proc Natl Acad Sci* **93**:13060–13065.

Muller DJ, de Luca V, Sicard T, King N, Strauss J, Kennedy JL (2006). Brain-derived neurotrophic factor (BDNF) gene and rapid-cycling bipolar disorder: family-based association study. *Br J Psychiatry* **189**:317-23

Nakata K, Ujike H, Sakai A, Uchida N, Nomura A & Imamura T (2003). Association study of the brain-derived neurotrophic factor (BDNF) gene with bipolar disorder. *Neurosci Lett* **337**: 17–20.

Nestler EJ, Gould E, Manji H, Buncan M, Duman RS, Greshenfeld HK, Hen R, Koester S, Lederhendler I, Meaney M, Robbins T, Winsky L, Zalcman S (2002). Preclinical models: status of basic research in depression. *Biol. Psychiatry* 52:503–528.

Neves-Pereira M, Cheung JK, Pasdar A, Zhang F, Breen G & Yates P (2005). BDNF gene is a risk factor for schizophrenia in a Scottish population. *Mol Psychiatry* **10**: 208–212.

Neves-Pereira M, Mundo E, Muglia P, King N, Macciardi F & Kennedy JL (2002). The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. *Am J Hum Genet* **71**: 651–655.

Nibuya M, Morinobu S, Duman RS (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J. Neurosci.* **15**:7539–7547.

Niculescu AB (2005). Genomic studies of mood disorders -- the brain as a muscle? *Genome Biol* **6**:215.

NIMH Genetics Initiative Bipolar Group (1997). Genomic survey of bipolar illness in the NIMH genetics initiative pedigrees: a preliminary report. *Am J Med Genet* **74**:227-237.

Nurnberger JI Jr, Blehar MC, Kaufman CA, York-Cooler C, Simpson SG, Friedman JH, Severe JB, Malaspina D, Reich T, Miller M, Bowman E, DePaulo R, Cloninger R, Robinson G, Mildin S, Gershon E, Maxwell E, Guroff J, Kirch D, Wynne D, Berg K, Tsuang M, Faraone S, Pepple J, Ritz AL (1994). Diagnostic interview for genetic studies. *Arch Gen Psychiat* **51**:849-849.

O'Connell JR, Weeks DE (1998). PedCheck: A program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* **63**:259–266.

Ogden CA, Rich ME, Schork NJ, Paulus MP, Geyer MA, Lohr JB, Kuczenski R, Niculescu AB (2004). Candidate genes, pathways and mechanisms for bipolar (manic-depressive) and related disorders: an expanded convergent functional genomics approach. *Mol Psychiatry* 9:1007-29.

Okada T, Hashimoto R, Numakawa T, Iijima Y, Kosuga A, Tatsumi M, Kamijima K, Kato T, Kunugi H (2006). A complex polymorphic region in the brain-derived neurotrophic factor (BDNF) gene confers susceptibility to bipolar disorder and affects transcriptional activity. *Mol Psychiatry*. 11:695-703.

Oswald P, Del-Favero J, Massat I, Souery D, Claes S & van Broeckhoven E (2004). Non-replication of the brain-derived neurotrophic factor (BDNF) association in bipolar affective disorder: a Belgian patient-control study. *Am J Med Genet* **129B**: 34–35.

Rabinowitz D & Laird N (2000). A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. *Hum Hered* **50**:211–223.

Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ, Lavery T, Kouyoumjian R, Farhadian SF, Ward R, Lander ES (2001). Linkage disequilibrium in the human genome. *Nature.* **411(6834)**:199-204

Rice JP, Goate A, Williams JT, Bierut L, Dorr D, Wu W, Shears S, Gopalakrishnan G, Edenberg HJ, Foroud T, Nurnberger J Jr, Gershon ES, Detera-Wadleigh SD, Goldin LR, Guroff JJ, McMahon FJ, Simpson S, MacKinnon D, McInnis M, Stine OC, DePaulo JR, Blehar MC, Reich T (1997). Initial genome scan of the NIMH genetics initiative bipolar pedigrees: chromosomes 1, 6, 8, 10, and 12. *Am J Med Genet* **74**:247-53.

Schinder AF, Poo M (2000). The neurotrophin hypothesis for synaptic plasticity. *Trends Neurosci* **23(12)**:639-45.

Sheline YI, Gado MH & Kraemer HC (2003). Untreated depression and hippocampal volume loss. *Am J Psychiatry* **160**:1516–1518.

Sheline YI, Wang PW, Gado MH, Csernansky JG & Vannier MW (1996). Hippocampal atrophy in recurrent major depression. *Proc Natl Acad Sci* **93**: 3908–3913.

Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C, Nakazato M, Watanabe H, Shinoda N, Okada S, Iyo M (2003). Alterations of serum levels of brain-

derived neurotrophic factor (BDNF) in depressed patients without or with antidepressants. *Biol. Psychiatry* **54**:70–75.

Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *J. Neurosci.* **22**:3251–3261.

Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM (1997). Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). *Pharmacol. Biochem. Behav.* **56**:131–137.

Skibinska M, Hauser J, Czerski PM, Leszczynska-Rodziewicz A, Kosmowska M & Kapelski P (2004). Association analysis of brain-derived neurotrophic factor (BDNF) gene Val66Met polymorphism in schizophrenia and bipolar affective disorder. *World J Biol Psychiatry* **5**: 215–220.

Sklar P, Gabriel SB, McInnis MG, Bennett P, Lim YM & Tsan G (2002). Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. *Mol Psychiatry* **7**:579–593.

Stine OC, McMahon FJ, Chen L, Xu J, Meyers DA, MacKinnon DF, Simpson S, McInnis MG, Rice JP, Goate A, Reich T, Edenberg HJ, Foroud T, Nurnberger JI Jr, Detera-Wadleigh SD, Goldin LR, Guroff J, Gershon ES, Blehar MC, DePaulo JR (1997). Initial genome screen for bipolar disorder in the NIMH genetics initiative pedigrees: chromosomes 2, 11, 13, 14, and X. *Am J Med Genet* **74**:263-9.

Strauss J, Barr CL, George CJ, Devlin B, Vetro A, Kiss E, Baji I, King N, Shaikh S, Lanktree M, Kovacs M, Kennedy JL (2005). Brain-derived neurotrophic factor variants are associated with childhood-onset mood disorder: confirmation in a Hungarian sample. *Mol Psychiatry.* **10**:861-7.

Strauss J, Barr CL, George CJ, King N, Shaikh S & Devlin B (2004). Association study of brain-derived neurotrophic factor in adults with a history of childhood-onset mood disorder. *Am J Med Genet (Neuropsychiatr Genet)* **131B**: 16–19.

Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*. 2007 Jun 7;447(7145):661-78.

Willour VL, Zandi PP, Huo Y, Diggs TL, Chellis JL, MacKinnon DF, Simpson SG, McMahon FJ, Potash JB, Gershon ES, Reich T, Foroud T, Nurnberger JI Jr, DePaulo JR Jr, McInnis MG (2003). Genome scan of the fifty-six bipolar pedigrees from the NIMH genetics initiative replication sample: chromosomes 4, 7, 9, 18, 19, 20, and 21. *Am J Med Genet B Neuropsychiatr Genet* 121:21-7.

Zandi PP, Willour VL, Huo Y, Chellis J, Potash JB, MacKinnon DF, Simpson SG, McMahon FJ, Gershon E, Reich T, Foroud T, Nurnberger J Jr, DePaulo JR Jr, McInnis MG; National Institute of Mental Health Genetics Initiative Bipolar Group (2003). Genome scan of a second wave of NIMH genetics initiative bipolar pedigrees: chromosomes 2, 11, 13, 14, and X. *Am J Med Genet B Neuropsychiatr Genet* **119**:69-76.

Figure Legend

Figure 1. *BDNF* gene structure and SNP location. The *BDNF* gene structure is drawn
based on the transcript NM_170731 (encoding isoform b), with five alternative forms shown
above: NM_170732 (isoform a), NM_170733 (isoform a), NM_001709 (isoform a),
NM_170734 (isoform c), NM_170735 (isoform a), in order from bottom to top; other
splicing forms have also been reported. SNP positions are shown below. Exons are
numbered and indicated in black, the untranslated regions in dark gray and introns as light
gray bars connecting the 2 exons. The direction of transcription of all isoforms is right to left,
as indicated by the horizontal arrow. The SNPs which demonstrated significant association
with bipolar disorder are underlined. The SNPs showing evolutionary conservation are
indicated by asterisks. Four SNPs showing nominal significance ($p < 0.05$) in recent GWAS
studies are in italics and boxed (Personal communication with Dr. Francis McMahon; Baum
et al., 2007; The Wellcome Trust Case Control Consortium, 2007). The size of the gene is
scaled by a 2 kb bar at lower right.

Figure 2. Pairwise linkage disequilibrium (*D*') between the SNPs genotyped in *BDNF*.
Empty boxes indicate SNPs are in complete LD (*D*'=1), dark grey boxes are in high linkage
disequilibrium.

Figure 3. Pairwise linkage disequilibrium (r^2) between the SNPs genotyped in *BDNF*. Color
scheme: white ($r^2=0$), shades of grey ($0<r^2<1$), black ($r^2=1$).

Table

Table 1   *BDNF* SNPs: chromosome position, minor allele frequency and association with
bipolar disorder

					Average PDT[c]		
SNP #	**SNP**	**Position[a]**	**Location†**	**MAF[b]**	**Model 1**	**Model 2**	**Model 3**
1	rs1519480	27,632,288	downstream	0.30	**0.005**	**0.02**	**0.03**
2	rs6265	27,636,492	exon 2 Val66met	0.21	0.40	0.26	0.06
3	rs11030104	27,641,093	intron 1	0.22	0.78	0.87	0.35
4	rs7103411	27,656,701	intron 1	0.23	0.64	0.69	0.27
5	rs7127507	27,671,460	intron 1	0.32	**0.002**	**0.009**	**0.009**
6	rs988748	27,681,321	intron 1	0.23	0.55	0.58	0.21
7	rs2030324	27,683,491	intron 1	0.47	**0.02**	**0.04**	0.16
8	rs7934165	27,688,559	intron 1	0.49	0.12	0.24	0.58
9	rs2883187	27,697,668	intron 1	0.47	**0.02**	**0.04**	0.14
10	rs12273363	27,701,435	upstream	0.20	**0.05**	0.12	0.10

[a]Positions along Chromosome 11 are based on dbSNP build 125/ human genome build 34.1.

†Position within or near gene

[b]Minor allele frequency in European Americans in this study

[c]P-value of Average PDT statistic for association between the SNPs and bipolar disorder.
Significantly associated SNPs are shown in bold. Analyses are reported for the narrow
definition of unaffected in which everyone who is not affected is classified as unknown
rather than unaffected.

Figure 1
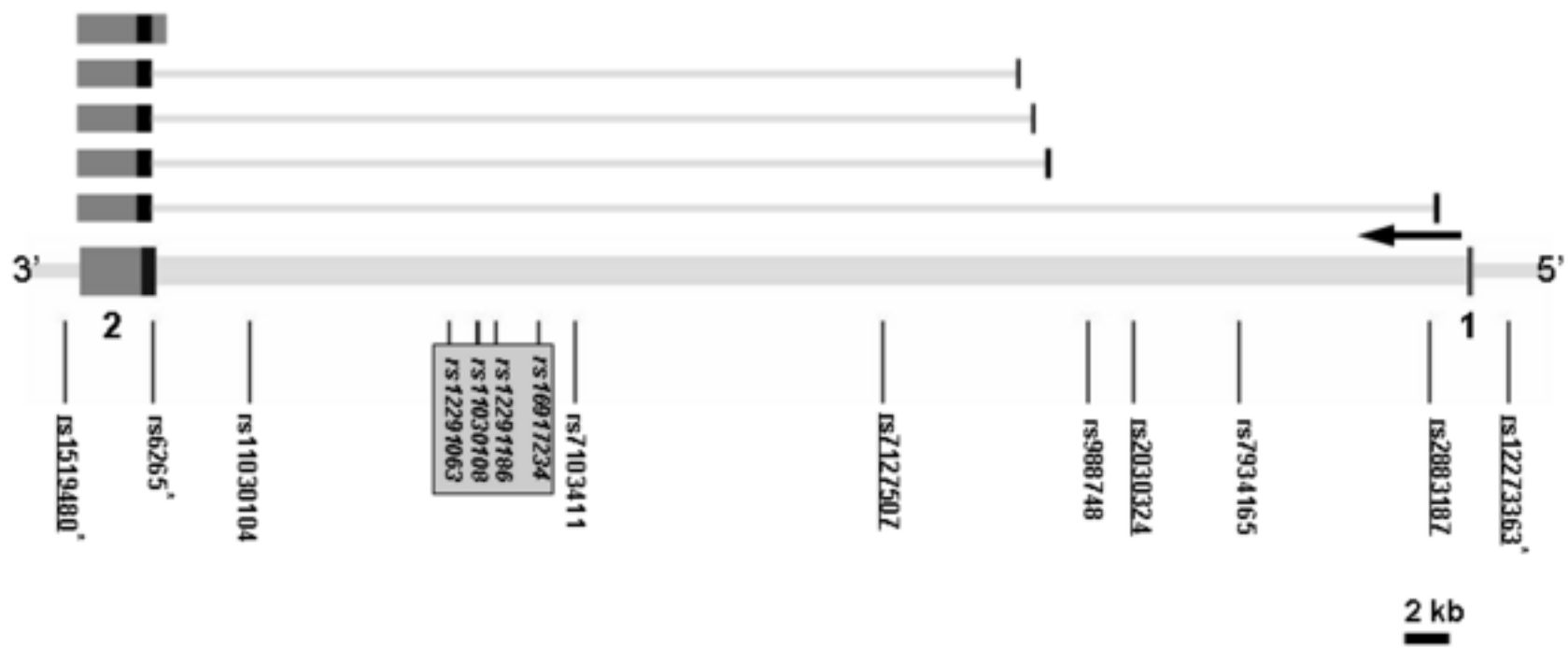**Click here to download high resolution image**

Figure 1