

Confirmation of Association Between the Val66Met Polymorphism in the Brain-Derived Neurotrophic Factor (*BDNF*) Gene and Bipolar I Disorder

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Recent studies have indicated that the brain-derived neurotrophic factor (*BDNF*) gene is involved in the etiology of bipolar disorder (BPD). Two family-based association studies showed that the Val allele of the functional polymorphism Val66Met in the *BDNF* gene is associated with BPD; however, others could not confirm the results. Here we performed a replication study in an independent sample and tested the hypothesis that the Val66 allele in the *BDNF* gene confers susceptibility to bipolar I disorder (BPI). Six hundred twenty-one patients with BPI and 998 control subjects were genotyped for the Val66Met polymorphism. All cases and controls were of European descent. All BPI patients had a positive family history of affective disorder. The frequency of the Val allele was significantly increased in BPI patient when compared to controls ($\chi^2 = 4.8$; $df = 1$; $P = 0.028$; two-sided; OR = 1.22; 95% CI: 1.02–1.47). Results confirm previous findings and suggest that the Val allele increases risk for BPI in patients of European descent. Further studies are necessary to elucidate the involvement of the *BDNF* gene in the pathophysiology of BPD.

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KEY WORDS: polymorphism; genetics; association study; *BDNF*; mood disorder; susceptibility

INTRODUCTION

The brain-derived neurotrophic factor (*BDNF*) regulates neuronal survival, growth, and connectivity during development and participates in plasticity and maintenance of neurons throughout adulthood [Huang and Reichardt, 2001]. Growing evidence suggests that *BDNF* is involved in the etiology of mood disorders [Hashimoto et al., 2004] and

depressive personality traits [Lang et al., 2004]. Serum levels of *BDNF* were decreased in depressed patients when compared to controls [Shimizu et al., 2003], and postmortem brain studies in patients with bipolar disorder (BPD) showed decreased *BDNF* protein when compared to controls [Knable et al., 2004]. The use of antidepressants, electroconvulsive therapy, and mood stabilizers such as lithium increase *BDNF* gene transcription [Hashimoto et al., 2002]. Infusion of *BDNF* into rat brain has a direct antidepressant effect in animal models of depression [Shirayama et al., 2002]. Given its biological function, the *BDNF* gene is therefore an interesting candidate gene for BPD. The *BDNF* gene maps to the short arm of chromosome 11, a region where linkage studies have suggested a putative susceptibility locus for BPD [McInnes et al., 1996; Detera-Wadleigh et al., 1999]. Two family-based association studies showed that the Val allele of the functional polymorphism Val66Met in the *BDNF* gene [Egan et al., 2003] is associated with BPD [Neves-Pereira et al., 2002; Sklar et al., 2002]; however, others could not confirm the results [Hong et al., 2003; Nakata et al., 2003; Kunugi et al., 2004; Oswald et al., 2004; Neves-Pereira et al., 2005]. Here we performed a replication study in an independent sample and tested the hypothesis that the Val66 allele in the *BDNF* gene confers susceptibility to BPD.

METHODS AND MATERIALS

Subjects

Six hundred twenty-one unrelated bipolar I patients of European descent participated in this study. Patients were collected at centers participating in the National Institute of Mental Health (NIMH) Genetics Initiative on Bipolar Disorder and carried a diagnosis of bipolar I disorder (BPI) defined by DSM-IV criteria. The key criterion for admission of a family to the study was a diagnosis of BPI in two or more siblings. Background and detailed methodology for the NIMH Genetics Initiative are described elsewhere [NIMH Genetics Initiative Bipolar Group, 1997]. The patient group consisted of 37% males and 63% females. The average age at recruitment was 41.6 years. Psychotic symptoms were present in 66% of the patients at some point during their illness. Psychosis was defined as presence of auditory/visual hallucinations and/or paranoid/bizarre delusions.

Nine hundred ninety-eight control samples were obtained from healthy individuals with no history of psychiatric or chronic neurological disease. Controls were collected in response to advertisements and screened by a semi-structured interview for mood disorders and psychosis. All control subjects were of European descent and comprised 52% males and 48% females, with an average age at recruitment of 38.5 years. Informed consent was obtained from all individuals in accordance with Institutional Review Board (IRB) procedures.

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TABLE I. Genotype and Allele Frequencies of the Val66Met SNP in the *BDNF* Gene

| SNP | Sample | n | Genotype frequencies | | | P^* | Allele frequencies | |
|----------|----------|-----|----------------------|---------|---------|-------|--------------------|-------|
| | | | Val/Val | Val/Met | Met/Met | | f(Val) | P^* |
| rs6265 | | | | | | | | |
| Val66Met | Bipolar | 621 | 0.678 | 0.287 | 0.035 | 0.062 | 0.821 | 0.028 |
| | Controls | 998 | 0.620 | 0.339 | 0.041 | | 0.790 | |

*Type-I error rates for comparison of genotype and allele frequencies between bipolar I patients and controls.

DNA and Statistical Analyses

The Val66Met polymorphism was genotyped using the *Applied Biosystems*' "Assays-on-demand" single nucleotide polymorphism (SNP) genotyping assay as per manufacturers protocol (C_11592758/rs6265). Genotypes and allele frequencies were compared between groups using Chi square contingency analysis. A two-tailed type I error rate of 5% was chosen for the analysis. Our sample size had reasonable power to detect a disease association at a P -value less than or equal to 0.05, assuming an odds ratio of 1.5 (98% for a log additive mode of inheritance, 92% for a dominant, and 27% for a recessive mode of inheritance). Power analysis was performed using the *Quanto* program [Gauderman, 2002].

RESULTS

None of the genotype counts deviated significantly from those expected from Hardy–Weinberg equilibrium ($P = 0.544$). Allele frequencies of the tested SNP were consistent with those reported for Caucasians in the literature (Val = 77–83%), public (Val = 72%), and the Celera (Val = 78%) database. Genotype and allele frequencies are shown in Table I.

DISCUSSION

In this study, we confirm the association between the Val allele of the functional Val66Met polymorphism and BPI. Our study is the largest of *BDNF* and BPI to date and is consistent with previous findings by Neves-Pereira et al. [2002] and Sklar et al. [2002]; however, the effect of the Val66Met variation seems to be moderate (OR = 1.22; 95% CI: 1.02–1.47) and other groups failed to demonstrate association with disease [Hong et al., 2003; Nakata et al., 2003; Kunugi et al., 2004; Oswald et al., 2004; Neves-Pereira et al., 2005].

There are several possible reasons for the discrepancy in findings: Most of the negative replication studies were done in Asian populations and allele frequencies differ significantly between individuals of European descent (f(Val) = 77–83%) and individuals of Asian descent (f(Val) = 55–59%). This difference in polymorphism frequency, based on ethnic background, is difficult to control for and must be considered carefully in the interpretation of data gathered from such association studies. Thus, a negative replication study in a population with a different ethnic background than the initial one studied does not exclude an involvement of the gene variation per se. Population stratification is unlikely to play a major confounding role in our study population, since all cases and controls were of European ancestry and our observed allele frequencies are consistent with those observed for Caucasian in the literature [Neves-Pereira et al., 2002, 2005; Sklar et al., 2002; Oswald et al., 2004]; however, case-control association studies of subjects with self-reported ancestries are not immune to population stratification [Freedman et al., 2004].

Another possible factor contributing to the disparity in results between the present study and that of others might be limited statistical power. With the exception of Kunugi et al. [2004], who used 347 BPI patients, all other studies had few

BPI cases, thus making interpretation of negative findings difficult. In our study of 621 BPI patients and 998 controls, we had reasonable power to detect disease association assuming an odds ratio of 1.5; however, larger sample sizes, in thousands, are required for studies of genes with small effects.

In contrast to other studies, our sample of BPI patients had, as key criterion for admission, a positive family history of affective disorder, implicating higher genetic loading of the probands and thus a stronger genetic effect of the tested variation. Nevertheless, even with narrow defined entry criteria for the study, BPD is a spectrum disorder with likely multiple genes involved, each contributing only a small fraction to the overall risk. Inconsistent study design, including combination of bipolar I and bipolar II patients, different age of onset and possible co-morbidities might alter the ability to detect contributing risk factors. The importance of homogeneous patient groups is exemplified by recent reports that demonstrate association of the Val66Met SNP in early onset BPD [Geller et al., 2004; Strauss et al., 2004]. Further dissection of the patient group by using endo-phenotypes and additional clinical data would greatly increase the yield for specific candidate genes but would also require a much larger sample size.

Even though the Val66Met polymorphism has been shown to be functional with the Met66 allele affecting intracellular trafficking and activity-dependent secretion of *BDNF* protein [Egan et al., 2003], the biological relevance of the overrepresentation of the Val allele in BPD remains unknown. A recent study reports larger hippocampal volumes in homozygotes for the Val allele when compared to heterozygote subjects [Szeszko et al., 2005], thus increased Val allele protein expression among bipolar probands might alter hippocampal formation and predispose individuals to disease. It is furthermore possible that another variation in the vicinity of the Val66Met polymorphism is responsible for increased risk to BPD, as suggested by Neves-Pereira et al. [2002]. A "gene-based" approach, including additional marker and further haplotype analyses, as suggested by Neale and Sham [2004], might be necessary to effectively investigate the involvement of the *BDNF* gene in BPD.

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