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A BDNF Coding Variant is Associated with the NEO Personality Inventory Domain Neuroticism, a Risk Factor for Depression

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INTRODUCTION

Genetic factors influence vulnerability to depression (Sullivan *et al*, 2000), but no specific genes have been definitively implicated. One promising approach is to determine whether variations in specific (candidate) genes are associated not with disease *per se*, but with traits, such as personality factors, that are themselves associated with risk for the disorder (Lander and Schork, 1994; Stoltenberg and Burmeister, 2000). Often such traits have a higher heritability than the disease status (Almasy and Blangero, 2001). Neuroticism, as measured by the NEO personality inventory (NEO-PI) (Costa and McCrae, 1997), a psychometrically sound and widely used instrument, is one such trait. High scorers on the Neuroticism domain are characterized by frequent experience of 'negative emotionality' such as anxiety, low mood, and hostility. Converging lines of evidence point to brain-derived neurotrophic factor (BDNF) as a factor in the pathophysiology of depression. To explore the possibility that variation in the BDNF gene is, in part, responsible for the population variation in Neuroticism, we studied a community sample of 441 subjects, genotyping a G→A single-nucleotide polymorphism (SNP) responsible for a valine→methionine substitution in the prodomain of BDNF. The less common, nonconserved Met allele was associated with significantly lower mean Neuroticism scores ($p = 0.0057$). Our study provides further evidence and one possible mechanism linking BDNF to depression.

BDNF influences neuronal differentiation in development, as well as synaptic plasticity and neuronal survival in adulthood (Thoenen, 1995). Several results suggest that it may play a role in the pathophysiology of depression (Duman, 2002). Heterozygous BDNF knockout mice show behavioral abnormalities consistent with serotonergic dysfunction. These behavioral changes are corrected through antidepressant treatment (Lyons *et al*, 1999). Additional evidence connecting BDNF and depression comes from studies showing that infusion of recombinant BDNF into the mouse midbrain (Siuciak *et al*, 1997) or hippocampus (Duman, 2002) produces an antidepressant effect in both learned helplessness and forced swim models of depression. Furthermore, stress, a trigger for depression, lowers hippocampal transcription of BDNF in mice (Nibuya *et al*, 1995). In contrast, numerous antidepressants, including selective serotonin reuptake inhibitors, electroconvulsive therapy, lithium, and monoamine oxidase inhibitors (Nibuya *et al*, 1995; Russo-Neustadt *et al*, 1999), increase BDNF transcription. This transcriptional increase occurs after a delay similar to that seen in the onset of clinical effects of antidepressants (Nibuya *et al*, 1995).

The recent finding that there is significant neurogenesis in the hippocampi of adult primates suggests a mechanism through which BDNF might relate to depression (Gould *et al*, 1999). The hippocampi of depressed patients are significantly smaller than those of healthy individuals (Sheline, 2000). This reduced volume may be the result of decreased neurogenesis in depressed individuals, a possi-

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bility supported by the findings that hippocampal neurogenesis is reduced by stress (Gould *et al*, 1998) and increased by antidepressant treatment (Malberg *et al*, 2000). The parallel changes in BDNF levels and neurogenesis in response to stress and antidepressant treatment suggest a connection between BDNF and hippocampal neurogenesis. Together, these lines of evidence lead to the neurotrophic hypothesis of depression (Duman *et al*, 1997), suggesting that genetic variations in BDNF might influence hippocampal neurogenesis and ultimately vulnerability to depression.

Neuroticism is a strong marker for vulnerability to depression (Duggan *et al*, 1995; Kendler *et al*, 1993). Characteristics of Neuroticism make it particularly useful for genetic studies of depression. The reported heritability of Neuroticism is 40–50% (Jang *et al*, 1996; Lake *et al*, 2000), equal to or greater than heritibility estimates for depression (36%) (Kendler and Prescott, 1999). Furthermore, approximately 70% of the correlation between Neuroticism and depression risk is because of shared genetic risk factors (Kendler *et al*, 1993). Neuroticism is also a quantitative trait and stable through adulthood (Costa and McCrae, 1988). In this study, we explore whether a BDNF variant is associated with variation in Neuroticism.

METHODS

Subjects

The subjects, 268 female and 173 male, were from 257 families participating in the Family Blood Pressure Program at the Tecumseh, Michigan Site (Boerwinkle, 2002). In all, 99% of the subjects were non-Hispanic Caucasians. Family eligibility in this study was dependent on the availability of a proband between 25 and 40 years old with a systolic blood pressure in the upper 15% of blood pressure distribution in earlier rounds of examination. Parents and siblings of probands were studied when available.

Genotyping

In Genbank sequences and the public SNP database (http://www.ncbi.nlm.nih.gov/), we identified a common coding variant in the *BDNF* gene, a G→A polymorphism responsible for a Val66Met change (Cargill *et al*, 1999). Electronic restriction mapping showed that the 'A' allele creates a restriction site for the enzyme Hsp92II. We designed primers (SBDNF1-AAA GAA GCA AAC ATC CGA GGA CAA G; SBDNF2-ATT CCT CCA GCA GAA AGA GAA GAG G) resulting in a 274 bp PCR product. A PTC 100 thermal cycler (MJ Research, Watertown, MA, USA) was used for DNA amplification. Amplification reactions were performed in a total volume of 20 μl, containing approximately 50 ng of genomic template, 1 μM of each primer, 200 μM dNTP, 2 μl 10X Opti-Prime Buffer 6 (Stratagene, La Jolla, CA, USA) and 1 U of *Taq* polymerase. The PCR cycling conditions consisted of an initial denaturation for 2 min at 94°C followed by 35 cycles of 94°C for 1 min, 55°C for 2 min, and 72 for 2 min and a final extension at 72°C for 4 min. In the presence of the 'G' allele, Hsp92II digestion produced two products, 57 and 217 bp, whereas the 'A' allele produced 3 products, 57, 77, and 140 bp. The presence of a second Hsp92II site served as a restriction digest control, identifying incomplete digests for repeat analysis. PCR products were electrophoresed on a 2% agarose gel and visualized using the Gel-Star nucleic acid gel stain (BioWhittaker Molecular Applications, Rockland, ME, USA). Of all subjects, 91% were successfully genotyped.

Personality Inventory

The NEO-PI was administered to the subjects in the study. This inventory, consisting of 181 questions, assesses subjects on five global personality domains and breaks down three of these domains into six specific facets each. The NEO-PI is a well-established inventory designed through factor analytic strategies. The inventory provides high test–retest reliability and longitudinal stability (Costa and McCrae, 1997).

Statistical Analysis

The presence of association was determined using the QTDT Program version 2.1 (available at http://www.sph.umich.edu/statgen/abecasis/QTDT/). QTDT was used to fit a variance components model to account for familial resemblance because of kinship and linkage. However, rather than modeling allelic effects based on allelic transmission, we tested the overall additive genetic effect of each polymorphism (Abecasis *et al*, 2000a, b).

RESULTS

We analyzed DNA samples from 441 subjects in the Tecumseh, Michigan Blood Pressure Study (Boerwinkle, 2002) at a G→A polymorphism responsible for a Val66Met change (Cargill *et al*, 1999) in the prodomain of the BDNF gene. The frequency of the alleles (adjusted for familial correlations) were: Val = 0.75, Met = 0.25, similar to previously reported frequencies (Val = 0.68 and 0.83, Met = 0.32 and 0.17) (Cargill *et al*, 1999; Sklar, in press). In this sample there was no significant correlation between blood pressure and Neuroticism (diastolic blood pressure $r = -0.064$, $p = 0.116$; systolic blood pressure $r = -0.016$, $p = 0.696$). Since the mean Neuroticism score for the heterozygous Val/Met group was intermediate to the two homozygous groups, an additive model of genotype effect was used. In an analysis controlling for familial correlations, the Met allele showed a significant association with lower mean Neuroticism scores ($p = 0.0057$; Figure 1). No personality domain, other than Neuroticism, was associated with BDNF genotype (Table 1). Of the six Neuroticism facets, only depression (N3), self-consciousness (N4), anxiety (N1), and vulnerability (N6) were associated with BDNF genotype. Of the 12 facets comprising the other personality domains, only the openness facet, feelings (O3) was associated with BDNF genotype (Table 2). This association was modest. For all but one of the associated facets, the heterozygous Val/Met group score was intermediate to the two homozygous groups, supporting an additive model for genotype effect. For the O3 facet, the heterozygous Val/Met group score was similar to the Met/Met group score, supporting a model where the Met allele may have a dominant effect.

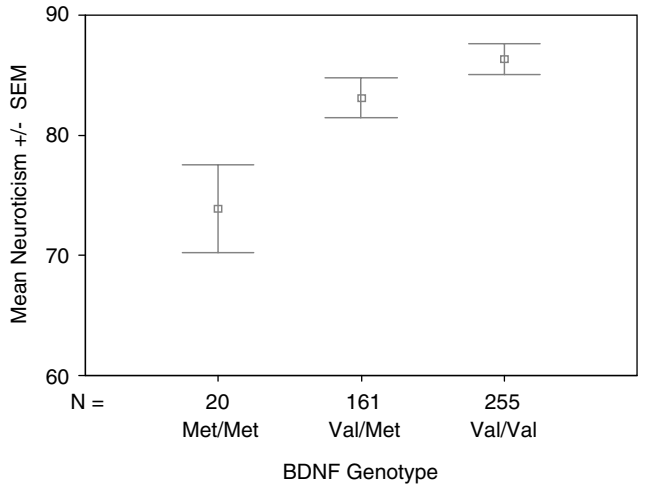

Figure 1 NEO-PI neuroticism scores as a function of BDNF genotype.

Table 1 NEO-PI domain scores as a function of BDNF genotype

Domain	BDNF genotype	*N*	Mean	SEM	*p* value
Neuroticism	Val/Val	255	86.35	1.27	0.0057
	Val/Met	161	83.11	1.67	
	Met/Met	20	73.90	3.66	
Extraversion	Val/Val	255	107.22	1.07	0.329
	Val/Met	162	108.17	1.40	
	Met/Met	20	111.18	2.76	
Openness	Val/Val	254	104.19	1.07	0.197
	Val/Met	160	101.90	1.23	
	Met/Met	20	102.18	3.69	
Conscientiousness	Val/Val	258	48.10	0.50	0.736
	Val/Met	161	47.53	0.66	
	Met/Met	20	48.53	1.95	
Agreeableness	Val/Val	259	47.19	0.40	0.721
	Val/Met	162	47.21	0.55	
	Met/Met	20	48.05	1.94	

Table 2 NEO-PI facet scores[a] as a function of BDNF genotype in Tecumseh population

Facet	BDNF genotype	*N*	Mean	SEM	*p* value
N1—anxiety	Val/Val	258	15.83	0.31	0.0020
	Val/Met	161	14.94	0.38	
	Met/Met	20	13.52	1.02	
N2—hostility	Val/Val	258	12.18	0.28	0.597
	Val/Met	161	11.98	0.37	
	Met/Met	20	11.77	0.82	
N3—depression	Val/Val	255	14.39	0.34	0.0058
	Val/Met	161	13.34	0.46	
	Met/Met	20	11.39	1.04	
N4—self-consciousness	Val/Val	258	15.74	15.74	0.0086
	Val/Met	161	14.86	14.86	
	Met/Met	20	13.05	13.05	
N5—impulsiveness	Val/Val	258	16.72	0.23	0.497
	Val/Met	161	16.91	0.31	
	Met/Met	20	15.15	1.13	
N6—vulnerability	Val/Val	259	11.41	0.23	0.0390
	Val/Met	162	11.08	0.34	
	Met/Met	20	9.00	0.70	
O3—feelings	Val/Val	254	20.90	0.24	0.0215
	Val/Met	161	19.78	0.28	
	Met/Met	20	19.92	0.83	

[a]All six Neuroticism facets are shown along with O3, the only facet from the other domains that reached nominal significance.

A concern for this type of study is the potential presence of population stratification resulting in the appearance of false associations (Lander and Schork, 1994). To assess if the reported results were because of stratification, STRUCTURE, a program designed to infer population structure utilizing genotypes from numerous unlinked markers as genomic controls, was implemented (Pritchard and Rosenberg, 1999). Weak evidence for clustering into two groups was found ($p = 0.02$) (Theil and Schork, personal communication; data available upon request). There were no significant differences in either BDNF allele frequency or any of the personality traits between the two clusters. Furthermore, the population cluster that was nonsignificantly higher for mean Neuroticism scores had a nonsignificantly greater Met allele frequency. Thus, these reported results are unlikely to be the product of population stratification (data available on request). An additional concern for this study is that the population sample studied was chosen through the presence of a moderately hypertensive proband. Although there is no association in this sample between blood pressure and either BDNF or Neuroticism, the hypertensive nature of the sample should be noted. Lastly, we have demonstrated a role for this BDNF variant in personality variation in a rural Michigan, non-Hispanic Caucasian sample, but its role in other populations is not known.

DISCUSSION

Our results indicate that this BDNF polymorphism may account for a small, but significant proportion of the population variation in Neuroticism. This variant explains about 4% of the genetic variance in our sample. There are several reasons to think that these results for Neuroticism are relevant to depression. First, Neuroticism is strongly associated with depression (Duggan *et al*, 1995; Kendler *et al*, 1993). This established association between Neuroticism and depression is mediated entirely by four of the six Neuroticism facets: anxiety (N1), depression (N3), self-consciousness (N4), and vulnerability (N6) (Bagby *et al*, 1996). These four facets are precisely those that associate with BDNF genotype in the Tecumseh sample. The other two Neuroticism facets, hostility (N2) and impulsiveness (N5), are not associated with depression and show no association with BDNF in this study (Table 2). Outside of the Neuroticism domain, the facets that have been

associated with depression are aesthetics (O2) and feelings (O3) (Bagby *et al*, 1996; Wolfenstein and Trull, 1997). In our sample, the feelings (O3) facet is significantly associated with BDNF while aesthetics (O2) shows a trend towards an association ($p<0.10$). The reported p values were not corrected for multiple testing. However, when the BDNF association with Neuroticism is corrected for the 5 NEO-PI domains, the results remain significant. This is conservative since we went into this study with an *a priori* hypothesis that BDNF may be a candidate gene for Neuroticism, not the other domains. Since the facets are strongly associated with each other, it is difficult to assess how to correct for further multiple testing.

Further evidence that the Val allele might increase the risk of depression comes from a study showing that this Val allele was preferentially transmitted to bipolar probands in families (Sklar, in press). The finding that the Val allele is associated with the risk of clinical bipolar disorder supports our findings that the same allele is associated with increased scores on personality facets associated with depression in a community sample. While the effect size of this BDNF variant for Neuroticism is not large, the effect is in the same direction as in bipolar disorder and of the magnitude expected for an intermediate phenotype in a polygenic trait.

The functional significance of this Val→Met substitution, if any, is unknown. Protein sequence comparisons (data not shown) reveal that the common Val allele is completely conserved among >70 species including mammals, birds and fish (Murphy *et al*, 2001). That is, the 'protective' Met allele appears to be novel in evolutionary history. Our results, together with the neurotrophic hypothesis of depression (Duman *et al*, 1997), predict that the Met allele of BDNF produces higher activity or more efficient processing of BDNF. Given the proximity of this variant to a BDNF cleavage site, it is possible that this variant affects the efficiency of cleavage at this site (Mowla *et al*, 2001). The variant may also be involved in determining the efficiency of mature BDNF folding. Alternatively, the variant may have no functional consequence, but be in linkage disequilibrium with a nearby functional polymorphism. These models can be tested *in vitro* or in animal models. Given the increase of BDNF transcription in response to antidepressant treatment, further work is also indicated to determine if this genetic variation predicts clinical response to specific antidepressants.

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