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The brain-derived neurotrophic factor rs6265 (Val66Met) polymorphism and depression in Mexican-Americans

Luciana Ribeiro^a, João V. Busnello^a, Rita M. Cantor^b, Fiona Whelan^c, Pamela Whittaker^d, Panos Deloukas^d, Ma-Li Wong^a, and Julio Licinio^{a,e}

^aDepartment of Psychiatry & Behavioral Sciences, Center on Pharmacogenomics, University of Miami Miller School of Medicine, Miami, Florida

^bDepartment of Human Genetics, David Geffen School of Medicine, California, Los Angeles, USA

^cSemel Institute of Neuroscience and Human Behavior at University of California, California, Los Angeles, USA

^dThe WellcomeTrust, Sanger Institute, Hinxton, Cambridge, UK

^eFederal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

Abstract

The hypothesis that brain-derived neurotrophic factor (BDNF) is involved in the pathogenesis of major depression is supported by several research findings; however, genetic studies assessing the relationship between BDNF and psychiatric disorders have produced conflicting results. We examined the effect of a BDNF polymorphism on depression susceptibility in Mexican-Americans. The single nucleotide polymorphism (Val66Met), which has been shown to have functional and behavioral effects, was genotyped in 284 depressed participants and 331 controls, showing association with depression ($P=0.005$). Individuals homozygous for the major allele (GG) had an increased chance of being depressed (OR=1.7 95% CI 1.17-2.47). Our findings support the association of BDNF single nucleotide polymorphism rs6265 and depression, suggesting that this polymorphism may increase susceptibility to major depression in Mexican-Americans.

Keywords

BDNF (brain derived neurotrophic factor); major depressive disorder; Mexican-Americans; pharmacogenetics; polymorphism; psychiatry; single nucleotide polymorphism; rs6265; Val66Met

Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin superfamily responsible for promoting and modifying growth, development, and survival of neuronal populations [1]. The BDNF gene consists of five alternatively spliced 5' exons and one major 3' exon, which result in at least six BDNF transcripts leading to three preproprotein isoforms that differ in the lengths of the signal peptides. For this reason, sequence variations in BDNF may lead to variations in gene expression or protein metabolism causing selective

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Correspondence to Dr Luciana Ribeiro, MD, PhD, Department of Psychiatry & Behavioral Sciences, University of Miami, Miller School of Medicine, Batchelor Children's Research Institute, 1580 NW, 10th Avenue, 6th floor, Miami, FL 33136-1013, USA, Tel: + 305 243 4723; fax + 305 243 9708; e-mail: lribeiro@med.miami.edu.

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neuronal vulnerability. The single nucleotide polymorphism (SNP) rs6265, which causes a valine-to-methionine substitution at codon 66 (Val66Met) in the *BDNF* gene, has been shown to affect intracellular trafficking and activity-dependent secretion of BDNF [2]. Met-BDNF carriers demonstrate substantial relative decreases in hippocampal volume, and Val/Met-BDNF affects the volume of grey matter in the cerebral neocortex of normal humans. Furthermore, Met-BDNF is associated with brain volume reduction, particularly in the lateral convexity of the prefrontal cortex [3]. Kleim and colleagues [4] showed that the amplitude of evoked potentials and motor map reorganization during motor learning tasks was reduced in healthy humans who are carriers of the Val66Met polymorphism. It is therefore justifiable that questions about a possible role of BDNF in psychiatric disorders were raised, with several reports supporting its involvement in the pathogenesis of major depressive disorder [5-11]. A number of conflicting results, however, exist in the literature, and are summarized here.

Schumacher *et al.* [12] performed one large genetic case-control study for *BDNF*, including 1097 controls and 465 patients with major depressive disorder from a German population. In the analysis of two SNPs and a dinucleotide repeat [rs988748, (GT)_n, rs6265 (Val66Met)] in the *BDNF* gene they obtained positive association results at a haplotype level, with haplotypes G-174bp-A, G-170bp-A and C-174bp-G emerging as important discriminators between patients and controls ($P=0.00065$). They subsequently studied an independent sample of 312 patients with major depressive disorder and 444 controls. The single-marker analysis did not provide strong evidence for association, but haplotype analysis of the combination of markers rs988748-(GT)_n-rs6265 produced expressive associations with major depression (global P value=0.00006). Two other genetic association studies, both in Chinese populations studied by Tsai *et al.* (2003) [13] and Hong *et al.* (2003) [14], focused on the investigation of the single variant (rs6265) and failed to establish *BDNF* as a risk factor for major depressive disorder. In this study we examined *BDNF* polymorphism rs6265, also known as Val66Met, in depressed Mexican-Americans.

Methods

Genotyping of the rs6265 SNP of the *BDNF* gene was carried out in 284 Mexican-American depressed participants and 331 Mexican-American controls matched for age and sex to evaluate the SNPs association with a diagnosis of major depression.

The Institutional Review Boards of the University of California, Los Angeles and University of Miami approved the study. After complete description of the study to participants, written informed consent was obtained. Inclusion criteria allowed the participation of men and women between the ages of 18 and 70 years, with at least three grandparents born in Mexico, who had a diagnosis of major depression according to DSM-IV and a minimum score of 18 points in the HAM-D 21. The Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) for axis I disorders was used to diagnose major depressive disorder. All assessments were performed in the individual's primary language, by experienced clinical investigators, formally trained in the application of the rating instruments. Evaluators were not board-certified psychiatrists.

Blood samples were collected into BD Vacutainer EDTA tubes (Becton Dickinson, Franklin Lakes, New Jersey, USA), and genomic DNA was isolated using Gentra Puregene DNA purification kits (Gentra Systems, Indianapolis, Indiana, USA). The rs6265 (Val66Met) SNP of the *BDNF* gene was genotyped as described previously [15]. The threshold for retaining individual genotype calls was set to a Genecall score of 0.25. SNP quality control steps included (i) data quality, assessed by duplicate DNAs ($n=26$) across all the plates. (ii) The Hardy-Weinberg equilibrium equation, used to determine the probable genotype frequencies

in our study population. Deviation from HWE was tested separately for the control and depressed groups by using the ALLELE procedure in SAS/Genetics 9.1.3 (SAS Institute, Cary, North Carolina, USA).

Statistical analyses

Allele, genotype and allelic trend association tests were performed by using PROC CASE CONTROL in SAS/Genetics 9.1.3 (SAS Institute Inc.). Single marker association test was performed with Haploview [16], version 3.2 (Broad Institute, Cambridge, Massachusetts, USA). We compared the odds of having depression given the homozygous major, homozygous minor or heterozygous genotype for SNPs associated with diagnoses of depression. Odds ratios were calculated by using Prism program version 4.0 (GraphPad Software Inc., San Diego, California, USA).

Results

The rs6265 SNP was in HWE in depressed and control groups: 278 patients and 320 controls had data available for this analysis. SNP rs6265 in *BDNF* showed significant association with the diagnosis of depression at the significance level of $P=0.005$ for the single marker association test between control and depressed groups. The associated allele is G (major allele) with case: control ratio of 0.892: 0.836 ($P=0.005$). The odds ratio for being depressed was 1.7 (95% CI 1.17-2.47) for individuals homozygous for the major allele (G/G) at rs6265 in the *BDNF* ($P=0.0053$). An odds ratio of 1.7 indicates that a G/G homozygous is 70% more likely to be in the depressed group than not (Table 1).

Discussion

We report here the association of the methionine (Met) substitution for valine (Val) at codon 66 (Val66Met) of the *BDNF* gene (rs6265) with major depression in Mexican-Americans. This same SNP is associated with alterations in brain anatomy and memory, and recently Chen *et al.* [17] showed that a variant BDNF mouse (BDNF^{Met/Met}) reproduced the phenotypic hallmarks of humans with this variant allele. They showed that BDNF^{Met} was expressed in brain at normal levels, but its secretion from neurons was defective. Interestingly, when placed in stressful settings, BDNF^{Met/Met} mice exhibited increased anxiety-related behaviors that were not normalized by antidepressant treatment, which suggests that this may be a required pathway for antidepressant response.

Hong and colleagues [14] were the first to suggest that associations of polymorphisms in the *BDNF* gene and mood disorders may be dependent on ethnicity. They relied on the fact that the positive association between *BDNF* gene rs6265 polymorphism and bipolar disorder had only been demonstrated for a Caucasian population but not for a Japanese or Chinese sample. Discrepant results on the association of the rs6265 polymorphism in different psychiatric disorders may reflect this fact. In major depression alone, results have been published supporting as well as discarding the association of rs6265 with the disorder [13,18]. This work offers a new type of sample on which to test whether this particular SNP has an impact on major depressive disorder.

Our recruitment was specific for ethnic background, an important fact that differs our approach from others that typically recruit individuals from the same nationality and geographical area, but do not have ethnicity as the characteristic on which their recruitment strategy is built upon. To the best of our knowledge, this is the first report of association of a *BDNF* gene polymorphism and major depressive disorder in Mexican-Americans.

An important feature of this work is the uniformity of assessments. To fulfill the large sample sizes required by genetic research rapid diagnoses are often conducted at multiple sites by various teams, resulting in inconsistencies in phenotype characterization and reduced reproducibility of findings. A key feature of this study is that this sample was all studied in Spanish by a small group of highly trained and experienced research personnel.

Our findings suggest that in these ethnic groups, patients with major depression have an increased probability of carrying the G/G genotype. This finding is in contrast to that of Hwang *et al.* [18] in a population of Taiwanese elders that had carriers of the A/A form as having an increased risk for depression. When comparing the two studies it is important to remember that the A/A genotype is extremely rare in non-Asiatic populations. Our sample had only two individuals with this genotype, precluding us from evaluating its effect on major depressive disorder. In another study involving a large sample of participants from the European Prospective Investigation into Cancer and Nutrition in Norfolk (EPIC) study, no association was found between the rs6265 polymorphism and diagnoses of depression by self-assessed instruments [19]; however, those types of assessment do not have diagnostic reliability, and they do not characterize the phenotype of major depression. In a study similar in design to ours, Tsai and colleagues [18] reported a lack of association between depression and the rs6265 polymorphism [13]. In a two-site European study assessing three polymorphisms in the *BDNF* gene, including rs6265, Schumacher and colleagues [12] did not find them individually associated with depression. Replication of these negative findings was obtained when the sample of one of the sites was analyzed separately. However, when performing a haplotype analysis considering these polymorphisms a positive association was identified with major depressive disorder but not with the other psychiatric disorders studied.

Conclusion

We found that the *BDNF* SNP rs6265 (Val66Met), which has functional and behavioral effects, is associated with major depressive disorder in Mexican-Americans. Individuals with the major allele (G/G) had an odds ratio of 1.7. Our work reports on a carefully evaluated sample of a well-defined ethnic group and it addresses an existing area of controversy; however, our sample is limited in power by its size. The contributions of other markers at the *BDNF* locus should be further examined. Our results provide justification for future large-scale comprehensive studies, with well-characterized samples, focused on the role of different polymorphisms of the *BDNF* gene, including rs6265, on the susceptibility to major depressive disorder.

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Table 1

Upper part: Demographic data and clinical characteristics of depressed patients in the three rs6265-genotype groups. Bottom part: Brain-derived neurotrophic factor genotype distribution at rs626

Group	Genotypes			All groups
	G/G	A/G	A/A	
Sex (F, %)	36.5	75.0	100.0	66.1
Age, (years), mean (SD)	39.1 (9.6)	38.3 (11.2)	49.0 (11.3)	39.0 (9.9)
HAM-D score, mean (SD)	25.5 (5.0)	25.0 (5.1)	20.0 (1.4)	25.3 (5.0)
HAM-A score, mean (SD)	22.3 (7.5)	19.3 (8.8)	11.0 (5.6)	21.6 (7.9)
Beck score, mean (SD)	28.8 (9.3)	28.9 (8.2)	25.5 (3.5)	28.8 (9.0)
CES-D score, mean (SD)	35.1 (26.9)	32.2 (8.0)	26.5 (2.1)	34.4 (24.2)
	<i>P</i> (G/Gvs. other)			
Patients, <i>n</i> (%)	220 (79.1)	56 (20.1)	2 (0.7)	0.005
Controls, <i>n</i> (%)	221 (69.1)	93 (29.1)	6 (1.9)	

HAM-A, Hamilton Anxiety Scale; HAM-D, Hamilton Depression Rating Scale; CES-D, Center for Epidemiologic Studies Depression Scale.