Novel Sequence Variations in the Brain-Derived Neurotrophic Factor Gene and Association With Major Depression and Antidepressant Treatment Response

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Context: Variations in the brain-derived neurotrophic factor gene (BDNF) have been associated with psychiatric disorders. Deep sequencing of the BDNF gene may identify new variations and bring further insight into psychiatric genetics.

Objective: To better characterize sequence variability in the BDNF gene by resequencing a genomic DNA region of 22 kilobases that contained all BDNF exons and their flanking regions.

Design: Case-control study.

Setting: University of California, Los Angeles, and University of Miami.

Participants: Two hundred sixty-four controls and 272 Mexican Americans with major depressive disorder (MDD) from Los Angeles who were assessed by the same bilingual clinical research team.

Main Outcome Measures: Identification of novel genetic polymorphisms in the BDNF gene and assessment of their frequencies and associations with MDD or antidepressant response.

Results: We identified 83 novel single-nucleotide polymorphisms (SNPs): 30 in untranslated regions, 4 in coding sequences, 37 in introns, and 12 in upstream regions; 3 of 4 rare novel coding SNPs were nonsynonymous. Association analyses of patients with MDD and controls showed that 6 SNPs were associated with MDD (rs12273539, rs11030103, rs6265, rs28722151, rs41282918, and rs11030101) and 2 haplotypes in different blocks (one including Val66, another near exon VIIIh) were significantly associated with MDD. One recently reported 5’ untranslated region SNP, rs61888800, was associated with antidepressant response after adjusting for age, sex, medication, and baseline score on the 21-item Hamilton Depression Rating Scale.

Conclusions: Our data support the concept that extensive resequencing of key candidate genes can lead to the discovery of substantial numbers of new variants. Further studies using larger independent samples are needed to confirm the association of the rs61888800 SNP with antidepressant response.

Trial Registration: clinicaltrials.gov Identifier: NCT00265291

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sively studied and implicated in the susceptibility to memory and hippocampal function impairments\(^3\) and several psychiatric disorders,\(^2\) such as obsessive-compulsive disorder,\(^2\) eating disorders,\(^2\) bipolar disorder,\(^2\) schizophrenia,\(^3\) major depression,\(^3\) and Alzheimer disease.\(^3\) Despite conflicting findings in replication studies for several of these associations, it is interesting that the less frequent variation, Met66, which is associated with poorer episodic memory and abnormal hippocampal activation on functional magnetic resonance imaging, generally confers a protective effect for neuropsychiatric conditions.

The genetic factors that contribute to human disease show enormous variation in the allelic spectra in number and population frequency of disease-predisposing alleles. Common and complex disorders are multifactorial and probably composed of both common genetic variants (common disease/common allele model) with small effect and rare sequence variants (rare variant/common disease model) with larger effect.\(^2\) Although the common allele is the prevalent view of these 2 competing models regarding the genetic basis of common and complex diseases, it has been predicted that resequencing studies may identify many rarer variants (>5%) of intermediate effect associated with common disorders; such efforts may also identify structural variations in genomic DNA, such as duplication and deletions of DNA sequences.\(^6\,\^7\)

Given the functional importance of BDNF in the CNS, the discovery of new BDNF allelic variants may be relevant to understanding the role of this gene in neurologic and psychiatric disorders. A number of studies have been conducted to examine the association of BDNF variants, but most of them have been focused on genotyping tag single-nucleotide polymorphisms (SNPs) or the functional coding SNP rs6265. To our knowledge, no study has comprehensively surveyed the entire BDNF exonic sequence variation through direct sequencing and correlated the identified genetic variants with disease susceptibility. To discover new BDNF genetic variants and detect rare variants, we sequenced a total 22-kilobase (kb) genomic DNA including all BDNF exons and their flanking regions in 536 DNA samples from 264 control subjects and 272 Mexican American individuals with major depressive disorder (MDD). We further investigated all of the identified genetic variants for association with risk for major depression and antidepressant treatment response.

### METHODS

#### PARTICIPANTS

Participants were 264 controls and 272 patients with MDD, aged 19 to 68 years. This study was approved by the institutional review boards of the University of California, Los Angeles, and the University of Miami. Subjects gave written informed consent. All participants were Mexican Americans and had at least 3 grandparents born in Mexico. The definition of MDD was a DSM-IV diagnosis of current, unipolar major depressive episode and a score of 18 or greater on the 21-item Hamilton Depression Rating Scale (HAM-D21) with item 1 (depressed mood) rated 2 or greater. All patients with MDD were enrolled in a pharmacogenetic study of antidepressant treatment response as previously described and registered at http://clinicaltrials.gov (No. NCT00265291).\(^6\,\^7\) The demographic characteristics and the numbers of subjects in each subgroup are presented in eTable 1 (http://www.archgenpsychiatry.com) and a flowchart (eFigure 1). Briefly, all patients with MDD had a comprehensive psychiatric and medical assessment in their primary language based on diagnostic and ratings instruments that had been fully validated in English and in Spanish. Exclusion criteria included active medical illnesses that could be etiologically related to the ongoing depressive episode, current or active suicidal ideation with a plan and strong intent, pregnancy/lactation, current use of medications with significant CNS activity that interfere with activity on an electroencephalogram (eg, benzodiazepines) or any other antidepressant treatment within the 2 weeks before enrollment, illicit drug use and/or alcohol abuse in the preceding 3 months, or current enrollment in psychotherapy. Control individuals for our genomic studies were in general good health but were not screened for medical or psychiatric illness; they were age- and sex-matched and recruited from the same Mexican American community in Los Angeles by the same bilingual clinical research team.

### ANTIDEPRESSANT TREATMENT

All patients had an initial comprehensive psychiatric and medical assessment and, if enrolled, had weekly structured follow-up assessments for 9 weeks. The study consisted of 2 phases: a 1-week single-blind placebo lead-in phase to minimize the impact of placebo responders, followed (if subjects continued to meet the inclusion criteria after phase 1) by random assignment to 1 of the 2 treatment groups: fluoxetine hydrochloride, 10 to 40 mg/d, or desipramine hydrochloride, 50 to 200 mg/d, administered in a double-blind manner for 8 weeks. Our primary clinical outcome measure was HAM-D21 score, and clinical remission with antidepressants was defined as having a final (week 8) HAM-D21 score less than 8.\(^6\) In addition, the relative response change was also computed as the difference in HAM-D21 score between pretreatment and posttreatment divided by the pretreatment HAM-D21 score.

### GENOMIC DNA COLLECTION AND SEQUENCING

At the initial visit, blood samples were collected under informed consent from the participating individuals into EDTA (K2EDTA BD Vacutainer EDTA tubes; Becton Dickinson, Franklin Lakes, New Jersey), and genomic DNA was isolated by using DNA purification kits (Puregene; Gentra Systems, Indianapolis, Indiana). BDNF (OMIM 113505) DNA sequencing was completed to identify genetic polymorphisms in exonic or flanking exons by the Wellcome Trust Sanger Institute following their ExoSeq protocol (http://www.sanger.ac.uk/humgen/exoseq/). A 22-kb genomic DNA region, containing the entire BDNF exons and their flanking regions, was sequenced. Briefly, DNA sequences were extracted from the Vega database (http://vega.sanger.ac.uk/index.html). Primers were designed automatically by means of Primer3 (http://frodo.wi.mit.edu/) to amplify DNA, and primer pairs were checked for uniqueness before ordering and prescreened to determine the optimum conditions for amplification. After amplification, a sample of the products was visualized on an agarose gel to confirm the size of the polymerase chain reaction product. The remaining polymerase chain reaction product was then cleaned up by means of 2 enzymes, exonuclease 1 and shrimp alkaline phosphatase. Bi-directional sequencing of amplicons was carried out with a cycle sequencing kit (Big Dye Terminator, version 3.1; Applied Biosystems, Foster City, California). The SNPs were called by means of ExoTrace, a Web site algorithm (http://www.sanger.ac.uk/...
NUCLEOTIDE DIVERSITY AND POPULATION DIFFERENTIATION ESTIMATION

Nucleotide diversity (θ) and its standard deviation (S(θ)) were calculated by SNP class under the assumption of an infinite neutral allele model as follows:46,47:

\[ \theta = \frac{K}{aL}, \]

\[ S(\theta) = \frac{\sqrt{aL + b(\theta L)}}{aL}, \]

\[ a = \sum_{i=2}^{n} \frac{1}{(i-1)}, \]

\[ b = \sum_{i=2}^{n} \frac{1}{(i-1)^2}, \]

where \( K \) represents the number of observed SNPs among \( L \) base pairs of genomic sequence in a sample of \( n \) alleles. All calculations were based on \( n = 990 \) for all the sites given in which the average sample size was 495 individuals across all the polymorphisms. The pairwise population differentiation (\( F_{ST} \)) values were estimated for the database SNPs (dbSNPs) that were both detected in our Mexican American sample and reported in the HapMap sample and were calculated as described by Weir and coworkers.48-50

HARDY-WEINBERG EQUILIBRIUM TEST AND POPULATION STRATIFICATION ANALYSIS

Case-control study design is an efficient method for examining associations between candidate alleles and disease. However, to compare allele frequencies and to be able to treat chromosomes as independent observations, the genotype frequencies must be in Hardy-Weinberg equilibrium.51 Deviation from Hardy-Weinberg equilibrium was tested separately for healthy individuals by using the PLINK program. In the allelic association analysis, each polymorphism was tested in controls to ensure the fitting with Hardy-Weinberg equilibrium; the odds ratio (OR) on the \( 2 \times 2 \) contingency table of allele counts and its 95% confidence interval (CI) were also estimated for the polymorphism associated with the diagnosis of depression. In the genotypic association analysis, the SNP effects were tested under a co-dominant model on the \( 2 \times 3 \) contingency table of genotype counts. In addition, logistic regression analyses were performed to test whether the observed SNP-depression association remained valid after controlling for age and sex by means of the SAS package (SAS Institute Inc, Cary, North Carolina).

For haplotype-based association analysis, haplotype blocks were identified by searching for a “spine” of strong linkage disequilibrium (LD) running from one marker to another along the legs of the triangle in the LD chart, and haplotype population frequencies were estimated by using an expectation maximization algorithm performed in the computer program Haploview (Version 4; Broad Institute, http://www.broad.mit.edu/mpg/haplovew/). haplotype frequencies were compared between depressed and control individuals to test whether a certain haplotype was associated with a diagnosis of depression.

To correct for multiple testing, 20,000 permutations were performed to estimate the adjusted \( P \) values for both single SNP-based analyses and haplotype-based analyses by using Haploview.

GENETIC ASSOCIATION ANALYSES OF RESPONSE TO ANTIDEPRESSANTS

Data analyses were performed using both intention-to-treat (ITT) and completed-treatment samples. The ITT sample consisted of patients who were randomized to 1 arm and received at least 1 dose of antidepressant medication, and the completed-treatment sample consisted of patients who completed 8 weeks of antidepressant treatment. The last observation carried forward approach was used to input missing outcome in the ITT analysis. For discrete outcome (remission vs nonremission), we investigated the allelic and genotypic association with the response to antidepressant treatment by using approaches similar to those in the analyses of cases and controls. For the quantitative outcome (relative reduction percentage in HAM-D21 scores between pretreatment and posttreatment), we conducted the analyses on the basis of 3 genetic models (additive, dominant, and recessive) and first performed the analyses by using the combined samples of patients treated with desipramine or fluoxetine.
We then performed the analyses separately by antidepressant medication (desipramine only, fluoxetine only). We used a multiple linear regression model to examine the association between genotype and relative HAM-D21 score reduction by controlling for age, sex, and baseline (pretreatment) HAM-D21 score using the PLINK program.

POWER CALCULATION

Power to test the allelic association with depression was estimated with a range of effect size (OR) between 1.35 and 2.25 and minor allele frequency between 0.1 and 0.25 using the PAWE program.35 Power analyses showed that, at a 2-sided significance level of .05, sample sizes of 265 cases and 265 controls can achieve 80% power to detect an allelic OR of 1.68, 1.57, 1.50, and 1.46 with a minor allele frequency of 0.09, 0.15, 0.20, and 0.25, respectively. Power calculations for the association of BDNF variants with antidepressant treatment continuous outcome were given for a range of allele frequencies and Cohen effect sizes (mean difference in unit of standard deviation) based on the dominant genetic model and using the Quanto (Version 1.2.3) program.36,37 Sample size is assumed to be 200 for the combined sample and 100 for each antidepressant treatment group based on an ITT design. Power analyses showed that, at a 2-sided significance level of .05 and when the allele frequency is 0.15 or more, the power is greater than or equal to 89% to uncover a moderate effect size of 0.5 for a sample of 200 patients and greater than or equal to 78% to detect a medium effect size of 0.6 for a sample of 100 patients.

DETECTION OF SEQUENCE VARIATION

Approximately 22 kb of BDNF exonic sequence and its flanking regions was systematically screened for novel nucleotide sequence variations in the sample of 536 Mexican American individuals. A total of 130 nucleotide sequence variations were identified (Table 1). They included 83 novel SNPs and 47 dbSNPs: 40 in untranslated regions (UTRs), 6 in coding sequences, 62 in intronic sequences, and 22 in the flanking regions. Among 6 coding SNPs, 3 novel nonsynonymous SNPs (NT_009237.17_26467094 [Ala/Thr], NT_009237.17_26467235 [His/Gly], and NT_009237.17_26467246 [Gly/Asp]), and 1 synonymous SNP (NT_009237.17_26466614) were found, and their minor allele frequencies were 0.0019, 0.0019, 0.001, and 0.001, respectively, in the combined sample of cases and controls. Seventy-nine other novel polymorphisms included 30 UTR SNPs, 37 intronic SNPs, and 12 upstream SNPs (eTable 2). The minor allele frequencies for the novel polymorphisms ranged from 0.0009 to 0.2445 with an allele distribution as follows: less than or equal to 0.01, 37.6%; greater than 0.01 and less than or equal to 0.05, 50.5%; and greater than 0.01, 11.9% in the combined sample of cases and controls.

NUCLEOTIDE DIVERSITY

The nucleotide diversity was estimated in each class of sites (coding, 3’ UTR, 5’ UTR, and intronic) by correcting for both sample size and the length of the screened site (Table 1). The mean (SD) nucleotide diversities were comparable for coding (0.0010 [0.0005]), 3’ UTR (0.0011 [0.0003]), and 5’ UTR (0.0010 [0.0003]) regions, but the estimate showed some lower nucleotide diversity in the intronic region (0.0008 [0.0002]) and upstream region (0.0006 [0.0002]). For the type of substitution, all of the identified coding polymorphisms were transition, whereas the transition rates were 71.0%, 69.6%, 72.2%, and 68.2% for intronic, 3’ UTR, 5’ UTR, and upstream regions, respectively.

POPULATION DIFFERENTIATION

Among the 47 dbSNPs detected, 18 were reported in 3 HapMap ethnic groups: white (CEU), black (YRI), and Asian (CHB + JPT) in the National Center for Biotechnology Information database as of June 25, 2008. Pairwise FST values between Mexican Americans and each HapMap ethnic sample were computed for the shared 18 SNPs and are shown in Table 2. Overall, the greatest similarity in allele frequencies was found between Mexican Americans and whites, with a lower mean FST in Mexican Americans vs whites of 0.03, compared with 0.10 in Mexican Americans vs blacks and 0.09 in Mexican Americans vs Asians. For the single-locus estimates of FST values, large FST values (>0.1) were observed at 4 SNPs (rs7124442, rs1819808, rs4923468, and rs7931755) in Mexican Americans vs blacks (22.2%) and at 5 SNPs (rs62635, rs11030102, rs11030104, rs988748, and rs10767664) in Mexican Americans vs Asians (27.8%), but less often (5.5%) in Mexican Americans vs whites (1 SNP: rs12273539).
Analyses of SNP-based allelic associations showed that 6 polymorphisms were associated with MDD (rs12273539, \(P = 0.001\); rs11030103, \(P = 0.008\); rs6265, \(P = 0.009\); rs28722151, \(P = 0.01\); rs41282918, \(P = 0.01\); and rs11030101, \(P = 0.02\)) (Table 3). All of these 6 SNPs had a minor allele frequency of 0.14 or greater, and their genotypes were in Hardy-Weinberg equilibrium in controls. Genotyped-based analyses also showed that the 6 polymorphisms were associated with depression status with \(P = 0.04\) (Table 3). Among the 6 associated SNPs, 4 were intronic variants with ORs ranging from 1.37 to 1.80; 1 SNP was a 3' UTR variant (rs41282918) with an effect of OR = 2.13 (95% CI, 1.18-3.86); and 1 SNP was a nonsynonymous variant (rs6265) with an effect of OR = 1.66 (95% CI, 1.14-2.41). Logistic regression analyses did not show a significant difference in age or sex between cases and controls, and the associations of the 6 SNPs with depression remained similar after adjusting for age and sex. Permutation analysis showed that only SNP rs12273539 remained significant after adjusting for multiple tests with a corrected \(P\) value of 0.002.

**Haplotype-based association analyses of cases and controls**

The figure shows that 7 haplotype blocks were identified by searching for the solid spine of strong LD. Among the 130 detected polymorphisms, 33 SNPs with a minor allele frequency of 1.5% or greater were included in the haplotype analyses. Several haplotypes were found to be associated with the diagnosis of depression in block 3 (5 SNPs: rs66920186, rs6265, rs11030101, rs28722151, and rs11030102) and block 4 (4 SNPs: rs57085135, NT_009237.17_26469156, rs110303103, and rs12273539). Block 3 included 3 SNPs associated with depression (Table 3). The most significant association in block 3 was found for a common haplotype TGACC, and

### Table 2. Allele Frequencies and FST Values for BDNF dbSNPs Shared by Mexican Americans and HapMap Samples

<table>
<thead>
<tr>
<th>SNP</th>
<th>Major/Minor Allele</th>
<th>Cases</th>
<th>Controls</th>
<th>All</th>
<th>MA vs CEU</th>
<th>MA vs YRI</th>
<th>MA vs HCB + JPT</th>
<th>FST</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7124442</td>
<td>T/C</td>
<td>0.23</td>
<td>0.26</td>
<td>0.25</td>
<td>0.37</td>
<td>0.54</td>
<td>0.07</td>
<td>0.03</td>
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<tr>
<td>rs11030101</td>
<td>A/T</td>
<td>0.26</td>
<td>0.33</td>
<td>0.29</td>
<td>0.40</td>
<td>0.12</td>
<td>0.31</td>
<td>0.02</td>
</tr>
<tr>
<td>rs11819808</td>
<td>C/T</td>
<td>0.01</td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
<td>0.28</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>rs11030102</td>
<td>C/G</td>
<td>0.17</td>
<td>0.18</td>
<td>0.18</td>
<td>0.29</td>
<td>0.07</td>
<td>0.01</td>
<td>0.04</td>
</tr>
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<td>rs2273539</td>
<td>C/T</td>
<td>0.25</td>
<td>0.23</td>
<td>0.29</td>
<td>0.00</td>
<td>0.34</td>
<td>0.13</td>
<td>0.20</td>
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<td>0.00</td>
<td>0.49</td>
<td>0.01</td>
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<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>rs987874</td>
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<td>0.16</td>
<td>0.15</td>
<td>0.22</td>
<td>0.05</td>
<td>0.49</td>
<td>0.01</td>
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<tr>
<td>rs492346</td>
<td>C/A</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.19</td>
<td>0.02</td>
<td>0.00</td>
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<tr>
<td>rs10767664</td>
<td>A/T</td>
<td>0.14</td>
<td>0.16</td>
<td>0.15</td>
<td>0.20</td>
<td>0.04</td>
<td>0.49</td>
<td>0.00</td>
</tr>
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<td>rs7931755</td>
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<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.25</td>
<td>0.00</td>
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<td>rs2030324</td>
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<td>0.41</td>
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<td>0.55</td>
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<td>0.16</td>
<td>0.16</td>
<td>0.19</td>
<td>0.07</td>
<td>0.01</td>
<td>0.00</td>
</tr>
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<td>rs908667</td>
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<td>0.04</td>
<td>0.05</td>
<td>0.04</td>
<td>0.12</td>
<td>0.10</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>rs7931247</td>
<td>C/T</td>
<td>0.37</td>
<td>0.42</td>
<td>0.39</td>
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<td>0.14</td>
<td>0.15</td>
<td>0.15</td>
<td>0.19</td>
<td>0.07</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>rs11030123</td>
<td>G/A</td>
<td>0.04</td>
<td>0.05</td>
<td>0.04</td>
<td>0.12</td>
<td>0.10</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**Abbreviations:** BDNF, brain-derived neurotrophic factor gene; CEU, Utah residents with ancestry from northern and western Europe in the United States; CHB, Han Chinese in Beijing; dbSNP, database single-nucleotide polymorphism (SNP); FST, population differentiation; JPT, Japanese in Tokyo; MA, Mexican American; YRI, Yoruba in Ibadan, Nigeria.

### Table 3. BDNF Polymorphisms Associated With Depression

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr Position</th>
<th>SNP Type</th>
<th>Risk/Nonrisk Allele</th>
<th>Control Risk Allele Frequency</th>
<th>OR (95% CI)</th>
<th>(P) Value*[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs41282918</td>
<td>27635356</td>
<td>3' UTR</td>
<td>A/C</td>
<td>0.84</td>
<td>2.13 (1.18-3.86)</td>
<td>.01 (.02)</td>
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<td>rs6265</td>
<td>27636492</td>
<td>Nonsynonymous</td>
<td>G/A</td>
<td>0.85</td>
<td>1.66 (1.14-2.41)</td>
<td>.009 (.008)</td>
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<tr>
<td>rs11030101</td>
<td>27637320</td>
<td>Intronic</td>
<td>A/T</td>
<td>0.67</td>
<td>1.37 (1.05-1.78)</td>
<td>.02 (.04)</td>
</tr>
<tr>
<td>rs28722151</td>
<td>27637752</td>
<td>Intronic</td>
<td>C/T</td>
<td>0.68</td>
<td>1.48 (1.10-1.99)</td>
<td>.01 (.009)</td>
</tr>
<tr>
<td>rs11030103</td>
<td>27638909</td>
<td>Intronic</td>
<td>G/A</td>
<td>0.19</td>
<td>1.80 (1.18-2.74)</td>
<td>.008 (.03)</td>
</tr>
<tr>
<td>rs12273539</td>
<td>27639887</td>
<td>Intronic</td>
<td>T/C</td>
<td>0.23</td>
<td>1.75 (1.32-2.31)</td>
<td>&lt;.001 (&lt;.001)</td>
</tr>
</tbody>
</table>

**Abbreviations:** BDNF, brain-derived neurotrophic factor gene; Chr, chromosome; CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism; UTR, untranslated region.

[a] Results are based on the Fisher exact test for comparisons of allele and genotype (in parentheses) frequencies between depressed patients and controls.
the haplotype frequency was 0.453 in cases and 0.316 in controls ($\chi^2 = 20.80, P < .001$; permutation adjusted $P < .001$). In block 4, the most significant association was found for haplotype CTGT, and the haplotype frequency was 0.337 in cases and 0.229 in controls ($\chi^2 = 15.06, P < .001$; permutation adjusted $P = .002$). No other haplotypes were associated with depression after adjusting for multiple testing in the permutation tests.

GENETIC ASSOCIATION ANALYSES OF RESPONSE TO ANTIDEPRESSANTS

In the present study, there were 200 patients with MDD who received at least 1 dose of antidepressant treatment (ITT sample of 103 received desipramine and 97 received fluoxetine) and 142 patients with MDD who completed 8-week antidepressant treatment (completed-treatment sample of 68 with desipramine and 74 with fluoxetine). For the discrete outcome (remission vs non-remission), no detected polymorphisms were found to be significantly associated with the remission status in allelic and genotype-based analyses with the use of ITT or completed-treatment samples. For the quantitative outcome (relative reduction in HAM-D21 score), 1 newly reported 5' UTR SNP, rs61888800, was found to be associated with the better response to antidepressant treatment ($P = .02$) after adjusting for age, sex, medication, and baseline HAM-D21 score in the combined sample of pa-
tients treated with desipramine or fluoxetine in completed-treatment sample analysis. Patients who had GG genotype showed a larger average reduction of HAM-D21 score of 66.3% (95% CI, 62.0%-70.7%) compared with those who had non-GG genotype and had an average relative reduction of HAM-D21 score of 56.5% (95% CI, 48.6%-64.57%). For the medication-specific analyses, 8 BDNF polymorphisms were found to be associated with the HAM-D21 score reduction among the patients treated with desipramine in both ITT and completed-treatment analyses with \( P \leq 0.05 \) after controlling for age, sex, and baseline HAM-D21 score (Table 4). Among the 8 SNPs associated with response to desipramine treatment, all showed a 14% larger reduction in HAM-D21 score in the patients homozygous for a major allele except rs12273539, which showed 14% smaller reduction in patients homozygous for a major allele in completed-treatment analysis and showed a similar pattern but with a smaller reduction in ITT analysis. No polymorphism associated with desipramine treatment response remained significant after adjusting for multiple testing through permutation, and no detected SNPs were found significantly associated with the reduction of HAM-D scores in the fluoxetine-treated group.

**COMMENT**

Our results provide a detailed description of BDNF sequence variations in Mexican Americans. Among the 130 SNPs that we detected in this study, 83 are novel and only 47 have been reported in the National Center for Biotechnology Information dbSNP database, which has collected 254 BDNF SNPs to date (http://www.ncbi.nlm.nih.gov/projects/SNP). Most of these new polymorphisms (89%) are rare variants with a minor allele below 1% (eTable 2). This is not surprising because our study was conducted in a large sample of 537 subjects of a specific ethnic group that has not been investigated extensively. The overall nucleotide diversity in that genomic region is 0.0008. Pairwise \( F_{ST} \) values showed a substantial population differentiation in 18 dbSNPs using frequency data available from the National Center for Biotechnology Information database of 3 ethnic groups (CEU, YRI, and JPT). For example, a high divergence of allele frequency was noted for nonsynonymous SNP rs6265 across ethnic populations; minor allele (A allele) frequencies of 0.12, 0.18, 0.00, and 0.48 were found in Mexican Americans, whites, blacks, and Asians, respectively. Our findings suggest that the genetic variation in the BDNF gene across different populations may be large, and this heterogeneity may contribute to explain controversial findings in associations of BDNF with depressed patients from different populations.

It is noteworthy that rare variants in relevant genes in neurodevelopmental pathways have been associated with schizophrenia, further supporting the rare variant/common disease model. The discovery of 83 mostly rare variants in BDNF, a gene that is found to be relevant to several psychiatric disorders, may therefore be of widespread interest.

We report herein that 5 SNPs in the BDNF gene were significantly associated with depression, in addition to the nonsynonymous SNP rs6265 that we reported previously. Among the 6 SNPs, rs12273539, an intronic variant located 3.4 kb away from rs6265 and near alternative 5′ exon VIIIh (Figure), showed the most significant association with depression and remained significant after adjustment for multiple testing through permutation, and no detected SNPs were found significantly associated with the reduction of HAM-D scores in the fluoxetine-treated group.

**Table 4. BDNF Polymorphisms Associated With Response to Antidepressant Treatment With Desipramine**

<table>
<thead>
<tr>
<th>SNP (Typea) Chr Position</th>
<th>Genotype</th>
<th>No.</th>
<th>Mean (SD)</th>
<th>( \beta ) (95% CI)</th>
<th>Value</th>
<th>No.</th>
<th>Mean (SD)</th>
<th>( \beta ) (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7124442 (3′ UTR)</td>
<td>CC/CT</td>
<td>43</td>
<td>37.97 (30.35)</td>
<td>14.88 (2.99 to 26.77)</td>
<td>0.02</td>
<td>28</td>
<td>50.17 (25.58)</td>
<td>14.60 (3.09 to 26.11)</td>
<td>0.02</td>
</tr>
<tr>
<td>rs1100301 (intron)</td>
<td>GG/GC</td>
<td>35</td>
<td>36.89 (30.30)</td>
<td>15.12 (3.14 to 28.31)</td>
<td>0.02</td>
<td>21</td>
<td>46.64 (27.59)</td>
<td>15.09 (3.08 to 27.11)</td>
<td>0.02</td>
</tr>
<tr>
<td>rs12272539 (intron)</td>
<td>TT/TC</td>
<td>53</td>
<td>37.97 (30.30)</td>
<td>-13.70 (-25.95 to -1.45)</td>
<td>0.03</td>
<td>28</td>
<td>46.64 (27.49)</td>
<td>-14.16 (-26.10 to -2.23)</td>
<td>0.02</td>
</tr>
<tr>
<td>rs61888600 (5′ UTR to intron)</td>
<td>AA/AG</td>
<td>28</td>
<td>36.05 (30.88)</td>
<td>15.02 (3.64 to 28.40)</td>
<td>0.02</td>
<td>18</td>
<td>48.57 (28.44)</td>
<td>14.12 (1.20 to 27.04)</td>
<td>0.04</td>
</tr>
<tr>
<td>rs86133711 (intron)</td>
<td>GG</td>
<td>30</td>
<td>36.05 (30.88)</td>
<td>-13.70 (-25.95 to -1.45)</td>
<td>0.03</td>
<td>28</td>
<td>46.64 (27.49)</td>
<td>-14.16 (-26.10 to -2.23)</td>
<td>0.02</td>
</tr>
<tr>
<td>rs2030324 (intron)</td>
<td>AA/AG</td>
<td>28</td>
<td>36.05 (30.88)</td>
<td>15.02 (3.64 to 28.40)</td>
<td>0.02</td>
<td>18</td>
<td>48.57 (28.44)</td>
<td>14.12 (1.20 to 27.04)</td>
<td>0.04</td>
</tr>
<tr>
<td>rs1227363 (upstream)</td>
<td>GG</td>
<td>39</td>
<td>49.79 (32.27)</td>
<td>12.81 (0.34 to 25.29)</td>
<td>0.05</td>
<td>37</td>
<td>52.44 (24.80)</td>
<td>14.20 (2.78 to 25.62)</td>
<td>0.02</td>
</tr>
<tr>
<td>rs7931247 (upstream)</td>
<td>TT/TC</td>
<td>33</td>
<td>39.90 (30.23)</td>
<td>13.20 (0.92 to 25.49)</td>
<td>0.04</td>
<td>38</td>
<td>51.72 (24.86)</td>
<td>14.28 (1.74 to 27.09)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Abbreviations: BDNF, brain-derived neurotrophic factor gene; Chr, chromosome; CI, confidence interval; dbSNP, database single-nucleotide polymorphism (SNP); UTR, untranslated region.

a Intron-exon boundaries were based on multiple alternative 5′ exons in the National Center for Biotechnology Information AceView Database.

b Means are average relative reductions in 21-Item Hamilton Depression Rating Scale (HAM-D21) score. \( \beta \) values are regression coefficients for allele effect based on the dominant model after adjusting for sex to age and baseline HAM-D21 score.

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Val66 allele (G) in exon IX in block 3 and CTGT in block 4 near exon VIIIh, were found to be significantly associated with increased risk of depression after correcting for multiple testing.

We also found that 8 SNPs were associated with response to desipramine treatment in both ITT and completed-treatment samples, although the association did not remain significant after adjustment for multiple testing. Among the 8 SNPs, there were one 3’ UTR SNP (rs71244442) in block 1, 2 newly reported SNPs (5’ UTR SNP rs61888800 in exon Vh and intronic SNP56133711) in block 6, 3 SNPs (rs2030324 in the intron; and rs12273363 and rs7931247 in the upstream region) in block 7, and 1 in each of block 3 (rs11030102) and block 4 (rs12273539) (Figure). Interestingly, SNP rs12273539, which showed the most significant association with depression status, was also associated with the drug response to desipramine treatment ($\beta = -14.16\%$; $P = .02$) in 8-week completers.

There are several implications to our findings. First, they support the concept that BDNF genetic variants may differ in frequency and/or effect among different ethnic groups. For example, our data support that, in the variant rs6265 (Val66Met), the Val (G allele) carriers are at increased risk for depression, which is consistent with the data of several studies in whites.39-61 However, several studies in Asians have reported no association between depression and Val66Met62-66 or the association of the Met (A allele) variant with susceptibility to depression.65,66 Our population differentiation analysis also showed that Mexican Americans and whites have a comparable Val66Met allele frequency ($F_{ST} = 0.01$), but they have substantial allele difference when compared with Asians ($F_{ST} = 0.31$). The observed results across ethnic groups may suggest heterogeneity in BDNF allele frequencies and genetic polymorphisms among populations. Second, they suggest that other BDNF genetic variants besides Val66Met may contribute to susceptibility to depression. In this survey, we found 6 BDNF polymorphisms that were associated with depression risk. The strongest association was found to an intronic variant: rs12273539. We also identified 2 haplotypes in different haplotype blocks, one containing rs6265 and the other containing rs12273539, that are significantly associated with depression after multiple testing adjustment ($P \leq .002$). Third, they suggest that the association of BDNF genetic variants with drug response to antidepressant treatment may be medication-specific and do not support a major role of Val66Met variant in antidepressant action in this population. Among the 6 polymorphisms associated with depression in this study, only SNP rs12273539 was found to be associated with HAM-D21 score reduction in desipramine treatment in our sample. However, 7 other SNPs were found to be associated with desipramine treatment by showing greater than or equal to 14% more average reduction in patients who are homozygous for a major allele.

Three studies62,64,67 have recently assessed the association between Val66Met polymorphism and antidepressant response in patients with MDD, but only 1 reported that Met carriers had a better response to 8-week citalopram hydrobromide treatment.62 Gratacóos et al62 reported an SNP rs908867 and a haplotype (TAT at rs12273363, rs908867, and rs1491850) in 5’ upstream region associated with antidepressant response. Interestingly, in this region, we found 3 SNPs (rs2030324, rs12273363, and rs7931247 in block 7) associated with desipramine treatment, although the association of rs908867 with response to antidepressant treatment was not significant in our study. The differential findings could be due to a number of factors such as medication type, outcome assessment, sample size, population structure, and, very importantly, the complexity and rich diversity in the regulation of BDNF multiple transcripts, in the coding and noncoding sequences, and in the proBDNF and mature BDNF translation product sequences.7,68

Limitations of this study are related to the sample size, which is relatively small, particularly for analyses of antidepressant treatment response. Power analyses showed that, at a single 2-sided significance of .05 and allele frequency of 0.15 or more, a sample size of 200 patients can achieve 89% power to detect a moderate effect size of 0.5, which is close to what we observed in the desipramine group; however, the power should be much lower if the genetic effect is medication-specific, as our results suggest. Another limitation is that the population stratification analysis was not based on ancestral informative markers, and the potential risk of hidden population substructures in this admixed sample could not completely be eliminated. Given the relatively small sample size, the lack of a replication sample, and the potential risk of a population substructure, the observed association should be interpreted with much caution and considered exploratory.

In conclusion, we have identified 83 novel BDNF genetic variants. Our data support the concept that extensive resequencing of key candidate genes can lead to the discovery of substantial numbers of new variants. Our results further implicate BDNF in the susceptibility to MDD and in the therapeutic response to antidepressants. To our knowledge, this work is the most comprehensive genetic association study to date to have examined the association between BDNF sequence variation with both depression and antidepressant response. Given that a number of alternative BDNF transcripts have been found to display complex splicing and expression patterns and that the findings in different studies remain inconsistent, further comprehensive studies in larger independent samples are clearly warranted for conclusive results. Moreover, we suggest that deep sequencing of relevant genes in large numbers of patients can disclose substantial numbers of novel variants that may be useful targets for future association studies.

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Author Contributions: Dr Licinio had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Rattiner LM, Davis M, Ressler KJ. Brain-derived neurotrophic factor in amygdala.

Pang PT, Lu B. Regulation of late-phase LTP and long-term memory in normal control human brain, and in Alzheimer's disease and Parkinson's disease.

Schinder AF, Poo M. The neurotrophin hypothesis for synaptic plasticity.

Poo MM. Neurotrophins as synaptic modulators.


Prussin P, Kazantzis A, Aid T, Palm K, Timmusk T. Dissecting the human brain-derived neurotrophic factor (BDNF) gene located at chromosome 17q11-q12 in Alzheimer's disease and depression in Mexican-Americans.


Weese-Mayer DE, Bolk S, Silveri JM, Chakravarti A. Idiopathic congenital cen-tral hypoventilation syndrome: evaluation of brain-derived neurotrophic factor (BDNF) gene in a child with central hypoventilation syndrome.


