

# Association of the Brain-Derived Neurotrophic Factor Val66Met Polymorphism With Reduced Hippocampal Volumes in Major Depression

Thomas Frodl, MD; Cornelius Schüle, MD; Gisela Schmitt, MD; Christine Born, MD; Thomas Baghai, MD; Peter Zill, PhD; Ronald Bottlender, MD; Rainer Rupprecht, MD; Brigitta Bondy, MD; Maximilian Reiser, MD; Hans-Jürgen Möller, MD; Eva M. Meisenzahl, MD

**Context:** Brain-derived neurotrophic factor (BDNF) modulates hippocampal plasticity, which is believed to be altered in patients with major depression.

**Objective:** To examine the effect of the *BDNF* Val66Met polymorphism on hippocampal and amygdala volumes in patients with major depression and in healthy control subjects.

**Design:** Cross-sectional comparison between patients and controls.

**Setting:** Inpatients with major depression from the Department of Psychiatry and Psychotherapy and healthy controls from the community were recruited.

**Participants:** The study population of 120 subjects included 60 patients with major depression and 60 healthy controls.

**Main Outcome Measures:** Using a combined strategy, hippocampal and amygdala volumes were esti-

mated on high-resolution magnetic resonance images, and genotyping was performed for the *BDNF* Val66Met polymorphism.

**Results:** Patients had significantly smaller hippocampal volumes compared with controls ( $P = .02$ ). Significantly smaller hippocampal volumes were observed for patients and for controls carrying the *Met-BDNF* allele compared with subjects homozygous for the *Val-BDNF* allele ( $P = .006$ ). With respect to amygdala volumes, no significant differences between patients and controls and no significant main effects for the *BDNF* Val66Met polymorphism were observed.

**Conclusions:** These genotype-related alterations suggest that *Met-BDNF* allele carriers might be at risk to develop smaller hippocampal volumes and may be susceptible to major depression. This study supports findings from animal studies that the hippocampus is involved in brain development and plasticity.

*Arch Gen Psychiatry.* 2007;64:410-416

## Author Affiliations:

Department of Psychiatry and Psychotherapy (Drs Frodl, Schüle, Schmitt, Baghai, Zill, Rupprecht, Bondy, Möller, and Meisenzahl) and Radiology (Drs Born and Reiser), Ludwig-Maximilians University, Munich, Germany, and East London and the City Mental Health NHS Trust, Department of Psychiatry, Newham Centre for Mental Health, London, United Kingdom (Dr Bottlender).

**M**AJOR DEPRESSION IS ONE of the most frequent human diseases, with a lifetime prevalence of 16% and a 12-month prevalence of 6.6%.<sup>1</sup> Dysfunction of neuronal plasticity or remodeling could contribute to the pathophysiology of mood disorders.<sup>2</sup> This hypothesis is supported by preclinical and clinical investigations demonstrating that stress and depression lead to reduction of the total volume of the hippocampus and to atrophy and loss of neurons in the adult hippocampus. Experimental studies<sup>3,4</sup> found that prolonged stress decreases the number of apical dendritic branch points and the length of apical dendrites, particularly in the laminar CA3 region of the hippocampus. This effect was glucocorticoid dependent and emerged after 3 weeks of experimental corticosterone treatment. Moreover, antidepress-

sants suppress the toxic effects of stress on the hippocampus and increase hippocampal neurogenesis.<sup>5</sup> In vivo investigations detected reduced hippocampal volumes in older patients<sup>6,7</sup> and in younger patients<sup>8-10</sup> with major depression. Two meta-analytic studies<sup>11,12</sup> confirmed that the hippocampus is consistently reduced in volume in patients with major depression, although there were some negative findings.

With respect to the neurotrophin hypothesis of depression, brain-derived neurotrophic factor (BDNF) is of major importance because it modulates hippocampal plasticity in physiological models and in animals.<sup>13</sup> Depressive states in animal models have been shown to be associated with reduced BDNF levels in the brain, and central administration of BDNF has been demonstrated to reverse such depressive states.<sup>14</sup> However, BDNF also seems to be impor-

tant for the manifestation of depressive states. In mice, BDNF function was required for the development of persistent social aversion in a social defeat paradigm. Chronic treatment then restored social functioning, and blockade of BDNF activity in the ventral tegmental area and projections to the nucleus accumbens exerted antidepressant-like effects.<sup>15</sup>

Haplotype analysis of the *BDNF* gene showed a robust association with major depression and with schizophrenia in the presence of depressive symptoms.<sup>16</sup> In another study,<sup>17</sup> no significant association was found between *BDNF* and unipolar depression. A common single nucleotide polymorphism consisting of a missense change (G196A) that produces a nonconservative amino acid change (valine to methionine) in the coding exon of *BDNF* at position 66 (Val66Met) was recently described as a functional polymorphism.<sup>18</sup> In a large community sample of 441 subjects, the Val-*BDNF* allele was associated with a high neuroticism score, which is a risk factor for depression. Other investigators failed to replicate this finding in 3 large populations.<sup>19</sup> Patients with geriatric depression in a Taiwanese veterans population showed a significant excess of the Met-*BDNF* allele compared with control subjects,<sup>20</sup> whereas no significant associations were detected in a Chinese population<sup>21,22</sup> or in a German population.<sup>16</sup> The *BDNF* Val66Met polymorphism was associated with cognitive performance during the Wisconsin Card Sorting Test in patients with bipolar disorder; patients homozygous for the Val-*BDNF* allele had a higher percentage of correct reaction in the task.<sup>23</sup> Recently, patients with schizophrenia carrying the Met-*BDNF* allele were observed to have more visuospatial impairment.<sup>24</sup> Overall, there is great diversity in the findings regarding the association between *BDNF* and depression.

The *BDNF* Val66Met polymorphism in *BDNF* in the 5' signal domain has been shown to affect intracellular packaging and regulation of BDNF secretion<sup>25</sup> and human hippocampal function.<sup>26</sup> Healthy Met-*BDNF* allele carriers had substantial relative decreases in hippocampal volume that are gender and age independent, suggesting that these changes may occur before adulthood.<sup>27,28</sup> The Met-*BDNF* allele may be a vulnerability factor for the development of disease processes associated with dysfunction of this brain region. However, an exaggerated age-related volume reduction of the dorsolateral prefrontal cortex was found in healthy Met-*BDNF* allele carriers; therefore, subjects with the Met-*BDNF* allele might be more vulnerable to aging than individuals homozygous for the Val-*BDNF* allele.<sup>29</sup> Furthermore, stress decreases the expression of BDNF in the hippocampus.<sup>30</sup> So far, there is no study available (to our knowledge) that has investigated the effect of the *BDNF* polymorphism on hippocampal volumes in patients with depression and in healthy control subjects. A possible association between the *BDNF* polymorphism and hippocampal reductions that in turn have an effect on the development of depression may enhance our knowledge of the neurotrophic and neurogenic hypothesis of depression.<sup>31</sup>

Herein, we used a combined strategy of neuroimaging techniques and genetic analysis to identify the effect of the *BDNF* polymorphism on the hippocampus and amygdala in patients with major depression and in healthy controls. In a sample of 40 patients and 40 controls, pa-

**Table 1. Demographic and Clinical Characteristics of Study Subjects\***

Characteristic	Patients (n = 60)	Healthy Control Subjects (n = 60)	P Value
Age, y	44.2 ± 11.8	41.6 ± 12.3	.23
Female-male ratio	29:31	29:31	>.99†
Handedness (right/left), No. of patients	56/4	56/4	>.99†
Height, cm	171.2 ± 8.5	175.7 ± 9.3	.006
Weight, kg	70.8 ± 15.4	72.6 ± 13.8	.49
Alcohol intake, g/d	9.7 ± 15.9	10.1 ± 10.5	.87
Age at onset of depression, y	37.7 ± 11.7	...	
Illness duration, y	6.7 ± 8.7	...	
Hamilton Depression Rating Scale score	23.0 ± 6.3	...	
<i>BDNF</i> frequency, No. of patients			
Genotype			
Val/Val	37	40	.76†
Val/Met	21	19	
Met/Met	2	1	
Allele			
Val/Val	37	40	.57†
Met carriers	23	20	

Abbreviation: *BDNF*, brain-derived neurotrophic factor.

\*Data are given as mean ± SD unless otherwise indicated.

† $\chi^2$  Test. All other P values are by t test.

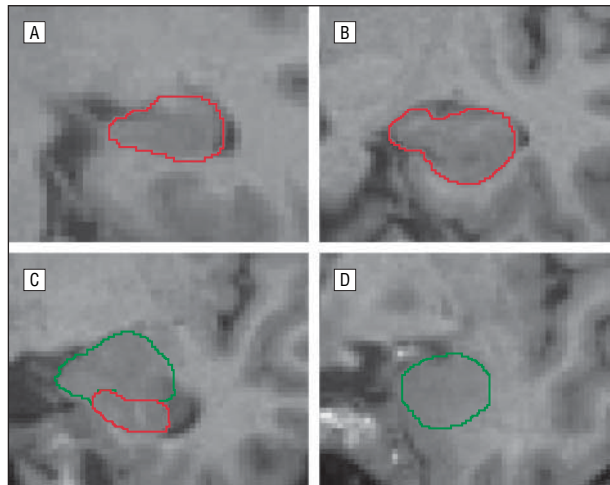
tients with major depression carrying the *L/L* genotype in the promoter region of the serotonin transporter gene (*5-HTTLPR*) were found to have reduced hippocampal volumes compared with healthy controls.<sup>32</sup> In the present study, we sought to test the following hypotheses: (1) In this large sample, hippocampal and amygdala volumes are reduced in patients with major depression compared with healthy controls. (2) Reduced hippocampal or amygdala volumes are related to the *BDNF* polymorphism among the patients.

## METHODS

### PARTICIPANTS

Sixty inpatients with major depression from the Department of Psychiatry and Psychotherapy, Ludwig-Maximilians University, Munich, Germany, were recruited (age range, 18-65 years; mean ± SD age, 44.2 ± 11.8 years) (**Table 1**). Psychiatric diagnoses based on *DSM-IV* criteria and on the Structured Clinical Interview for *DSM-IV* were determined by a consensus of at least 2 psychiatrists. Clinical variables were documented using the 21-item Hamilton Depression Rating Scale.<sup>33</sup>

All patients were inpatients. Thirty patients had a first depressive episode, and 30 patients had recurrent episodes. There were 8 patients with 1 earlier admission to a psychiatric hospital, 5 patients with 2 earlier admissions, 2 patients with 3 earlier admissions, and 1 patient each with 4, 7, 12, and 15 earlier admissions. Almost all patients, except those not taking antidepressant medication, were in an outpatient service and were treated with antidepressants before the current admission. They were hospitalized because their conditions did not improve. Magnetic resonance imaging was performed in the first 2 weeks after admission to the hospital.



**Figure 1.** Magnetic resonance imaging sections. A and B, Coronal sections through occipitorostral parts of the hippocampus. The shape of the hippocampus may be compared with that of a rabbit with the head directed vertically (B). C, Section through the posteromedial part of the amygdala. D, Section through the anteromedial part of the amygdala.

On the day of magnetic resonance imaging, patients were taking the following medications: 17 patients were taking serotonin reuptake inhibitors (sertraline [n=7], citalopram hydrobromide [n=7], paroxetine hydrochloride [n=2], and fluvoxamine maleate [n=1]), 11 patients were taking tricyclic antidepressants (amitriptyline hydrochloride [n=4], doxepin hydrochloride [n=5], maprotiline hydrochloride [n=1], and trimipramine maleate [n=1]), and 23 patients were taking other antidepressants (venlafaxine hydrochloride [n=7], reboxetine [n=9], and mirtazapine [n=7]). Nine patients were not taking antidepressant medication at the time of magnetic resonance imaging. Patients taking antidepressants had a mean  $\pm$  SD duration of treatment of  $15.2 \pm 12.9$  days.

For comparison, 60 healthy controls were matched with respect to gender, handedness, and age (age range, 22-64 years; mean  $\pm$  SD age,  $41.6 \pm 12.3$  years) (Table 1). A structured interview was used to assess medical history, trauma, and other exclusion criteria. Neither the control subjects nor their first-degree relatives had a history of neurological or mental illness. Exclusion criteria for patients and controls were previous head injury with loss of consciousness, cortisol medication in the medical history, and previous alcohol or other substance abuse. Other mental illnesses including personality disorders as well as neurological diseases were also exclusion criteria. No subject had received electroconvulsive therapy. Handedness was determined using the Edinburgh Inventory.<sup>34</sup>

After an extensive description of the study to the patients with major depression and the healthy controls, written informed consent was obtained. The study design was approved by the local ethics committee and was prepared in accord with the ethical standards laid down in the Declaration of Helsinki.

## MAGNETIC RESONANCE IMAGING PROCEDURES

Magnetic resonance images were obtained (1.5-T Magnetom Vision; Siemens, Erlangen, Germany) using a coronal T2-weighted and proton density-weighted dual-echo sequence (repetition time, 3710 milliseconds; echo times, 22 milliseconds [first echo] and 90 milliseconds [second echo]; total acquisition time, 9 minutes; number of acquisitions, 1; field of view, 230 mm; matrix,  $240 \times 256$  pixels; and section thickness, 3 mm) and a 3-dimensional magnetization prepared rapid acquisition gradient-echo sequence (repetition time, 11.6 milliseconds; echo time, 4.9

milliseconds; total acquisition time, 9 minutes; number of acquisitions, 1; field of view, 230 mm; matrix,  $512 \times 512$  pixels; and section thickness, 1.5 mm). A commercial software package was used for further image processing (Analyze; Biomedical Imaging Resource, Mayo Foundation, Rochester, Minn), with size reduction from 16 to 8 bits and transformation to a uniform matrix of  $256 \times 256$  pixels on 192 sections of 1.0-mm thickness. All data sets were realigned and resampled 3-dimensionally for the anterior commissure to posterior commissure line according to Talairach coordinates using a software program (BRAINS; Brain Research: Analysis of Images, Networks and Systems; developed by Andreasen et al<sup>35</sup>). This program allowed control of the regions of interest for the sagittal and transverse sections simultaneously, as well as control of the segmentation for calculating intracranial content and gray and white matter volumes (in cubic centimeters) within the defined region of interest.

## DEFINITION OF THE HIPPOCAMPAL AND AMYGDALA FORMATION

We used the definition of the hippocampus according to Niemann et al<sup>36</sup> and the detection of the hippocampal-amygdala border from the description of Convit et al.<sup>37</sup> The evaluation staff (T.F.) was blinded to each subject's study group status. The amygdala was outlined manually using a mouse-driven cursor. The definition of the amygdala according to the criteria established by Convit et al<sup>37</sup> was applied. To obtain the most anterior boundary, the definition in accord with Altshuler et al<sup>38</sup> was used (for a detailed description, see Frodl et al<sup>9,39</sup>). The hippocampus and amygdala are illustrated in **Figure 1**.

For determination of interrater reliability, 10 brains were randomly chosen, and regions of interest were determined by 2 raters independently. The intraclass correlations for the interrater reliability and the intrarater reliability in randomly chosen brains were high.<sup>9,39</sup>

## LABORATORY ANALYSIS

DNA was extracted from a 5-mL blood sample using a kit (QIAamp Blood Isolation Kit; QIAGEN GmbH, Hilden, Germany) following the instructions of the supplier. All genotyping were performed by the fluorescence resonance energy transfer method using a commercially available system (Light Cycler System; Roche Diagnostics, Mannheim, Germany). For the G196A polymorphism in *BDNF*, the following conditions were applied: forward primer, 5'-TCA TAC TTT GGT TGC ATG AAG G-3'; reverse primer, 5'-AGA AGA GGA GGC TCC AAA GG-3'; and acceptor hybridization probe, 5'-LCRed604-TGT TGG ATG AGG ACC AGA AAG TTC GGC-p-3'. Polymerase chain reaction was performed using 50 ng of DNA in a total volume of 20  $\mu$ L containing 2  $\mu$ L of reaction mix (0.4  $\mu$ M each primer, 0.2  $\mu$ M each hybridization probe, and 2  $\mu$ M magnesium chloride) according to the manufacturer's instructions for 40 cycles of denaturation (95°C for 0 seconds), annealing (64°C for 10 seconds), and extension (72°C for 10 seconds), with ramp rates of 20°C/s. After amplification, a melting curve was generated by holding the reaction at 40°C for 20 seconds and then by heating slowly to 95°C with a ramp rate of 0.2°C/s. The fluorescence signal was plotted against temperature to give melting curves of each sample. Peaks were obtained at 52°C for the A allele and at 57°C for the G allele.

## STATISTICAL ANALYSIS

All statistical tests were considered significant at  $P < .05$ . Morphometric measurements in both study groups were tested for normal distribution and for homogeneity of variance. Depar-

**Table 2. Repeated-Measures Analysis of Covariance Results for Hippocampal Volumes**

Variable	Gray Matter		White Matter	
	F <sub>1,115</sub> Score	P Value	F <sub>1,115</sub> Score	P Value
Hemisphere	0.41	.53	0.04	.84
Diagnosis	5.7	.02	18.3	<.001
<i>BDNF</i> allele	7.8	.006	2.00	.16
Intracranial content	44.4	<.001	15.0	<.001
Diagnosis × <i>BDNF</i> allele	0.03	.86	0.34	.56
Diagnosis × hemisphere	0.39	.54	4.3	.04
Diagnosis × <i>BDNF</i> allele × hemisphere	0.08	.79	0.05	.82

Abbreviation: BDNF, brain-derived neurotrophic factor.

ture from Hardy-Weinberg equilibrium was tested using  $\chi^2$  test. *t* Test and analysis of variance were used to test for differences in demographic variables between patients and controls and among the genotypes.  $\chi^2$  Test was used to compare the genotype frequencies between patients and controls. Hippocampal and amygdala volumes were subjected to analysis of covariance assessing the main and interaction effects of the within-subject factor of hemisphere (left or right) and the between-subject factors of diagnosis (patients or controls) and *BDNF* allele (Met carriers or Val/Val) using intracranial content as the cofactor. Post hoc analyses were carried out using analysis of covariance and *t* test to test hippocampal and amygdala volumes for differences between genotypes.

## RESULTS

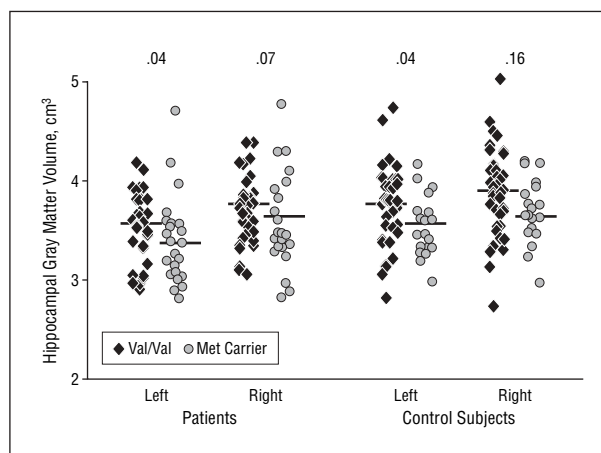
Patients and controls did not differ with regard to demographic variables (Table 1). The *BDNF Val66Met* genotype distributions for the patients and controls were in Hardy-Weinberg equilibrium. Age and weight were not different between patients and controls for each *BDNF* allele group (Met carriers or Val/Val). Illness duration ( $F_{1,58}=3.3, P=.07$ ), age at onset ( $F_{1,58}=1.7, P=.20$ ), and depression severity (Hamilton Depression Rating Scale,  $F_{1,58}=0.01; P=.91$ ) were not significantly different between patients who were *Met-BDNF* allele carriers and patients who were homozygous for the *Val-BDNF* allele.

Furthermore, the antidepressant medications taken (none, serotonin reuptake inhibitors, tricyclic antidepressants, or other antidepressants) were not significantly different among patients with the various *BDNF* genotypes ( $\chi^2_{58}=2.1, P=.50$ ). The *BDNF* genotype frequencies are given in Table 1. They did not differ between patients and controls.

### HIPPOCAMPUS

The analysis of covariance results for the gray matter and the white matter of the hippocampus are given in **Table 2**. A significant main diagnosis effect was found indicating smaller hippocampal gray and white matter volumes in patients with major depression compared with healthy controls. Moreover, there was a significant effect of *BDNF* allele on the hippocampal gray matter volume (**Figure 2**).

In an exploratory analysis, the factor of first depressive episode vs recurrent episodes did not reveal significant interactions for hippocampal gray matter volume.



**Figure 2.** Scattergram of left and right hippocampal gray matter volumes showing *BDNF* (brain-derived neurotrophic factor) polymorphisms of patients with depression and of healthy control subjects. Horizontal lines indicate the mean for each group. *P* values are shown with intracranial content as a cofactor in the analysis of covariance. For the numbers of subjects in each group, see Table 1.

However, there was a significant 3-way interaction between diagnosis, *BDNF* allele, and the factor episode for hippocampal white matter volume ( $F_{1,111}=5.1, P=.03$ ). Post hoc testing revealed a significant effect among the patients with a first depressive episode that demonstrated smaller hippocampal white matter volume in patients carrying the *Met-BDNF* allele ( $F_{1,27}=11.2, P=.003$ ), without a significant effect in matched controls ( $F_{1,27}=0.08, P=.78$ ). In patients with recurrent episodes, no significant effect was found for *BDNF* allele ( $F_{1,27}=0.81, P=.38$ ). An effect between diagnosis and the factor of first depressive episode vs recurrent episodes was not detected.

### AMYGDALA

Left and right amygdala volumes did not show a significant effect for diagnosis ( $F_{1,115}=1.9, P=.20$ ) or for *BDNF* allele ( $F_{1,115}=1.1, P=.31$ ). Nor did they show a significant interaction between *BDNF* allele and diagnosis ( $F_{1,115}=1.1, P=.31$ ).

In an exploratory analysis, gender had a significant effect on these results. There was a significant 3-way interaction between diagnosis, *BDNF* allele, and gender for amygdala volumes ( $F_{1,111}=3.8, P=.05$ ). Post hoc analy-

sis detected that women had a significant interaction between diagnosis and *BDNF* allele ( $F_{1,53}=6.4, P=.01$ ), whereas men did not show such an effect ( $F_{1,57}=0.19, P=.66$ ). Female patients with the *Val/Val* genotype had significantly larger amygdala volumes compared with female controls with the same genotype ( $F_{1,27}=8.9, P=.006$ ), whereas this was not the case for female patients with the *Met* allele compared with female controls with the same allele ( $F_{1,25}=0.3, P=.62$ ). The exploratory analysis that included the factor of first depressive episode vs recurrent episodes did not show any significant interactions for amygdala volumes.

## CORRELATIONS TO CLINICAL VARIABLES

There were no significant correlations between hippocampal or amygdala volume and illness duration. Furthermore, there was no significant correlation between hippocampal or amygdala volume and severity of depression as measured by the Hamilton Depression Rating Scale.

### COMMENT

#### HIPPOCAMPUS

Our study identified for the first time (to our knowledge) an effect of the functional variation in the *Val66Met* locus in the 5' prodomain of *BDNF* on hippocampal morphologic structure in patients with major depression. The observed decreases in hippocampal volumes were independent of whether subjects were depressed or not and may occur during development of the hippocampus. This view is supported by the finding that the reduction of hippocampal volumes in healthy *Met-BDNF* allele carriers is age and gender independent.<sup>27</sup> The *BDNF* *Val66Met* polymorphism seems to be associated with altered hippocampal morphologic structure, which might have an effect on the susceptibility to or the expression of illnesses such as major depression that involve hippocampal neuronal integrity. Indeed, reduced hippocampal volumes affect the course of depressive illnesses,<sup>40</sup> they are related to executive dysfunctioning,<sup>41</sup> and changes in hippocampal neuronal integrity are associated with poor episodic memory.<sup>25</sup>

Patients with a first depressive episode did not differ from patients with recurrent episodes for hippocampal gray matter volumes and for the association between the *BDNF* *Val66Met* polymorphism and hippocampal gray matter volumes. However, hippocampal white matter volumes were smaller in patients with a first depressive episode who were *Met* carriers compared with those with the *Val/Val* genotype, whereas no such difference was detected in patients with recurrent episodes. The number of subjects may have been too small for a subgroup analysis, so this result should be interpreted with caution. In patients with recurrent episodes, other depression-related effects on hippocampal volumes may be present,<sup>6</sup> whereas this may not be the case at the beginning of the disease. Therefore, patients with a first depressive episode may show a greater effect of the *BDNF* polymorphism on hippocampal volumes.

It can be theorized that synaptic and cellular plasticity changes in the hippocampus might result in cognitive deficits. Brain-derived neurotrophic factor seems to play an important role in the early and late phases of long-term potentiation.<sup>13</sup> In the late phase, cyclic adenosine monophosphate- and the cyclic adenosine monophosphate-responsive element binding protein signaling pathway are recruited to direct protein synthesis-dependent changes in structure and function of the hippocampal synapsis.<sup>42</sup> In vitro investigations demonstrated that depolarization-induced secretion was reduced in *Met-BDNF*-transfected neurons compared with *Val-BDNF* analogues, leading to decreased *BDNF* activity in subjects with the *Met-BDNF* allele compared with those with the *Val-BDNF* allele.<sup>25</sup> Therefore, *Met-BDNF* allele carriers might manifest decreased synaptic and cellular plasticity during development and may have reduced hippocampal volumes.

The *L/L* genotype of the serotonin transporter polymorphism in the promoter region of the serotonin transporter gene (*5-HTTLPR*) was associated with reduced hippocampal volumes in patients with major depression but not in control subjects.<sup>32</sup> Moreover, patients with late-onset geriatric depression who were homozygous for the *L* allele of *5-HTTLPR* exhibited smaller hippocampal volumes than other subject groups, whereas a significant association between the *S* allele of *5-HTTLPR* and smaller hippocampal volumes was observed in patients with early onset.<sup>43</sup> Disease-specific effects other than a genetic susceptibility might contribute to this effect of *5-HTTLPR*. Stress may be a prominent factor that can result in neuroplastic changes in the hippocampus.<sup>2</sup>

The presence of the *Met-BDNF* allele may independently reduce hippocampal volumes and increase the susceptibility to develop depression via altered hippocampal functioning. However, this is weakened by a study<sup>18</sup> among a community sample demonstrating that the *Val-BDNF* allele was associated with a high neuroticism score (which may be a risk factor for depression), by another study<sup>19</sup> that showed no association between the *BDNF* *Val66Met* polymorphism and neuroticism. In line with our hypothesis, another study<sup>20</sup> found an association between the *Met-BDNF* allele and geriatric depression. Moreover, among patients with bipolar disorder, the *Met-BDNF* allele was associated with executive dysfunctioning.<sup>23</sup> However, in another population, no significant relationship between the *BDNF* polymorphism and depression was found.<sup>21,22</sup> Our investigation supports the view that the *Met-BDNF* allele results in reduced hippocampal volumes (eg, via neuroplastic effects), which, in turn, increases the risk for depression. This indirect relationship may explain the inconsistent findings among previous *BDNF* studies.

#### AMYGDALA

We found no significant differences between patients and controls with respect to amygdala volumes in this largest sample of patients and controls to date (to our knowledge). Two other studies failed to find altered total amygdala volumes. The first study<sup>44</sup> did not show significantly smaller total amygdala volumes in 20 patients with depression compared with 20 healthy controls, whereas

amygdala core volumes were found to be significantly smaller in the patients. In the second study<sup>10</sup> among 34 drug-resistant patients with major depression, no significant differences in amygdala volumes were observed compared with 17 age-matched healthy controls. In an earlier investigation, no significant differences in amygdala volumes in patients with recurrent depression compared with healthy controls were observed.<sup>45</sup>

However, larger amygdala volumes have been detected in patients with a first episode of major depression,<sup>39</sup> in patients with bipolar disorder,<sup>46</sup> and in patients with borderline personality disorder.<sup>47</sup> An explanation for differences between patients with a first depressive episode and those with recurrent episodes may be an increased amygdala volume at the beginning of the disease and a reduction to normal size during the course of the disease.<sup>45</sup> In the present study, we focused on the genetic effects among patients and not on the differences between patients with a first depressive episode and those with recurrent episodes.

There was no significant main effect of the *BDNF* polymorphism in patients or in controls. In an exploratory analysis, there was a significant 3-way interaction between diagnosis, *BDNF* allele, and gender. However, this result should be interpreted with caution because a statistical correction for a post hoc analysis would result in nonsignificant post hoc effects. This finding must be replicated before it can be interpreted.

## CONCLUSIONS

The primary limitation of this study is its case-control design, which is sensitive to population stratification. This is unlikely to be problematic herein because the patients and controls with each *BDNF* allele did not differ for age, gender, origin, illness duration, age at onset, or medication use. There was a small number of subjects with the *Met-BDNF* allele. It could be argued that equal numbers of individuals with the genetic subtype should have been sampled. This would have required a very large number of subjects.

Our findings suggest that *Met-BDNF* allele carriers may be at risk of developing smaller hippocampal volumes and might be susceptible to developing major depression. The association between hippocampal volumes and the *BDNF* Val66Met polymorphism underline the hippocampal involvement in brain development and plasticity. This in vivo study confirms in vitro findings that *BDNF* is required for neuroplastic changes and brain development and further supports the neurotrophic and neurogenic hypothesis of depression.<sup>31</sup>

**Submitted for Publication:** May 16, 2006; final revision received July 28, 2006; accepted July 31, 2006.

**Correspondence:** Thomas Frodl, MD, Department of Psychiatry and Psychotherapy, Ludwig-Maximilians University, Nussbaumstrasse 7, 80336 Munich, Germany (Thomas.Frodl@med.uni-muenchen.de).

**Financial Disclosure:** None reported.

**Funding/Support:** This study was supported by the German Federal Research Ministry within the German Re-

search Networks in Medicine promotion as part of the German Research Network on Depression project.

**Acknowledgment:** We would like to thank Anton Strauß, MD, and Bernhard Burgermeister for technical support.

## REFERENCES

1. Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, Rush AJ, Walters EE, Wang PS. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *JAMA*. 2003;289:3095-3105.
2. Duman RS. Pathophysiology of depression: the concept of synaptic plasticity. *Eur Psychiatry*. 2002;17(suppl 3):306-310.
3. Woolley CS, Gould E, McEwen BS. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Res*. 1990;531:225-231.
4. Reagan LP, McEwen BS. Controversies surrounding glucocorticoid-mediated cell death in the hippocampus. *J Chem Neuroanat*. 1997;13:149-167.
5. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science*. 2003;301:805-809.
6. Sheline YI, Sanghavi M, Mintun MA, Gado MH. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *J Neurosci*. 1999;19:5034-5043.
7. Steffens DC, Byrum CE, McQuoid DR, Greenberg DL, Payne ME, Blitchington TF, MacFall JR, Krishnan KR. Hippocampal volume in geriatric depression. *Biol Psychiatry*. 2000;48:301-309.
8. Bremner JD, Narayan M, Anderson ER, Staib LH, Miller HL, Charney DS. Hippocampal volume reduction in major depression. *Am J Psychiatry*. 2000;157:115-118.
9. Frodl T, Meisenzahl EM, Zetsche T, Hohne T, Banac S, Schorr C, Jager M, Leisinger G, Bottlender R, Reiser M, Möller HJ. Hippocampal changes in patients with a first episode of major depression. *Am J Psychiatry*. 2002;159:1112-1118.
10. Mervaala E, Fohr J, Kononen M, Valkonen-Korhonen M, Vainio P, Partanen K, Partanen J, Tiihonen J, Viinamaki H, Karjalainen AK, Lehtonen J. Quantitative MRI of the hippocampus and amygdala in severe depression. *Psychol Med*. 2000;30:117-125.
11. Campbell S, Marriott M, Nahmias C, MacQueen GM. Lower hippocampal volume in patients suffering from depression: a meta-analysis. *Am J Psychiatry*. 2004;161:598-607.
12. Videbech P, Ravnikle B. Hippocampal volume and depression: a meta-analysis of MRI studies. *Am J Psychiatry*. 2004;161:1957-1966.
13. Lu B, Gotschalk W. Modulation of hippocampal synaptic transmission and plasticity by neurotrophins. *Prog Brain Res*. 2000;128:231-241.
14. Nibuya M, Morinobu S, Duman RS. Regulation of *BDNF* and *trkB* mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci*. 1995;15:7539-7547.
15. Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ. Essential role of *BDNF* in the mesolimbic dopamine pathway in social defeat stress. *Science*. 2006;311:864-868.
16. Schumacher J, Jamra RA, Becker T, Ohlraun S, Klopp N, Binder EB, Schulze TG, Deschner M, Schmal C, Hofels S, Zobel A, Illig T, Propping P, Holsboer F, Rietschel M, Nothen MM, Cichon S. Evidence for a relationship between genetic variants at the brain-derived neurotrophic factor (*BDNF*) locus and major depression. *Biol Psychiatry*. 2005;58:307-314.
17. Oswald P, Del-Favero J, Souery D, Claes S, Van Broeckhoven C, Mendlewicz J. No implication of brain-derived neurotrophic factor (*BDNF*) gene in unipolar affective disorder: evidence from Belgian first and replication patient-control studies. *Eur Neuropsychopharmacol*. 2005;15:491-495.
18. Sen S, Nesse RM, Stoltenberg SF, Li S, Gleiberman L, Chakravarti A, Weder AB, Burgermeister M. A *BDNF* coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. *Neuropsychopharmacology*. 2003;28:397-401.
19. Willis-Owen SAG, Fullerton J, Surtees PG, Wainwright NWJ, Miller S, Flint J. The Val66Met coding variant of the brain-derived neurotrophic factor (*BDNF*) gene does not contribute toward variation in the personality trait neuroticism. *Biol Psychiatry*. 2005;58:738-742.
20. Hwang JP, Tsai SJ, Hong CJ, Yang CH, Lirng JF, Yang YM. The Val66Met polymorphism of the brain-derived neurotrophic-factor gene is associated with geriatric depression. *Neurobiol Aging*. 2006;27:1834-1837.
21. Tsai SJ, Cheng CY, Yu YW, Chen TJ, Hong CJ. Association study of brain-derived neurotrophic-factor genetic polymorphism and major depressive disorder.

- ders, symptomatology, and antidepressant response. *Am J Med Genet B Neuropsychiatr Genet.* 2003;123:19-22.
22. Hong CJ, Huo SJ, Yen FC, Tung CL, Pan GM, Tsai SJ. Association study of brain-derived neurotrophic-factor genetic polymorphism and mood disorders, age of onset and suicidal behavior. *Neuropsychobiology.* 2003;48:186-189.
  23. Rybakowski JK, Borkowska A, Skibinska M, Szczepankiewicz A, Kapelski P, Leszczynska-Rodziewicz A, Czernski PM, Hauser J. Prefrontal cognition in schizophrenia and bipolar illness in relation to Val66Met polymorphism of the brain-derived neurotrophic factor gene. *Psychiatry Clin Neurosci.* 2006;60:70-76.
  24. Ho BC, Milev P, O'Leary DS, Librant A, Andreasen NC, Wassink TH. Cognitive and magnetic resonance imaging brain morphometric correlates of brain-derived neurotrophic factor Val66Met gene polymorphism in patients with schizophrenia and healthy volunteers. *Arch Gen Psychiatry.* 2006;63:731-740.
  25. Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell.* 2003;112:257-269.
  26. Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF, Weinberger DR. Brain-derived neurotrophic factor val<sup>66</sup>met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J Neurosci.* 2003;23:6690-6694.
  27. Pezawas L, Verchinski BA, Mattay VS, Callicott JH, Kolachana BS, Straub RE, Egan MF, Meyer-Lindenberg A, Weinberger DR. The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology. *J Neurosci.* 2004;24:10099-10102.
  28. Bueller JA, Aftab M, Sen S, Gomez-Hassan D, Burmeister M, Zubieta JK. BDNF Val66Met allele is associated with reduced hippocampal volume in healthy subjects. *Biol Psychiatry.* 2006;59:812-815.
  29. Nemoto K, Ohnishi T, Mori T, Moriguchi Y, Hashimoto R, Asada T, Kunugi H. The Val66Met polymorphism of the brain-derived neurotrophic factor gene affects age-related brain morphology. *Neurosci Lett.* 2006;397:25-29.
  30. Vollmayr B, Keck S, Henn FA, Schloss P. Acute stress decreases serotonin transporter mRNA in the raphe pontis but not in other raphe nuclei of the rat. *Neurosci Lett.* 2000;290:109-112.
  31. Warner-Schmidt JL, Duman RS. Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. *Hippocampus.* 2006;16:239-249.
  32. Frodl T, Meisenzahl EM, Zill P, Baghai T, Rujescu D, Leinsinger G, Bottlender R, Schüle C, Zwanzger P, Engel RR, Rupprecht R, Bondy B, Reiser M, Möller HJ. Reduced hippocampal volumes associated with the long variant of the serotonin transporter polymorphism in major depression. *Arch Gen Psychiatry.* 2004;61:177-183.
  33. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry.* 1960;23:56-62.
  34. Oldfield RC. The assessment and analysis of handedness: the Edinburgh Inventory. *Neuropsychologia.* 1971;9:97-113.
  35. Andreasen NC, Cohen G, Harris G, Cizadlo T, Parkkinen J, Rezaei K, Swayze VW II. Image processing for the study of brain structure and function: problems and programs. *J Neuropsychiatry Clin Neurosci.* 1992;4:125-133.
  36. Niemann K, Hammers A, Coenen VA, Thron A, Klosterkötter J. Evidence of a smaller left hippocampus and left temporal horn in both patients with first episode schizophrenia and normal control subjects. *Psychiatry Res.* 2000;99:93-110.
  37. Convit A, McHugh P, Wolf OT, de Leon MJ, Bobinski M, De Santi S, Roche A, Tsui W. MRI volume of the amygdala: a reliable method allowing separation from the hippocampal formation. *Psychiatry Res.* 1999;90:113-123.
  38. Altshuler LL, Bartzokis G, Grieder T, Currant J, Mintz J. Amygdala enlargement in bipolar disorder and hippocampal reduction in schizophrenia: an MRI study demonstrating neuroanatomic specificity. *Arch Gen Psychiatry.* 1998;55:663-664.
  39. Frodl T, Meisenzahl E, Zetzsche T, Bottlender R, Born C, Groll C, Jäger M, Leinsinger G, Hahn K, Möller HJ. Enlargement of amygdala volumes in patients with a first episode of major depression. *Biol Psychiatry.* 2002;51:708-714.
  40. Frodl T, Meisenzahl EM, Zetzsche T, Hohne T, Banac S, Schorr C, Jäger M, Leinsinger G, Bottlender R, Reiser M, Möller HJ. Hippocampal and amygdala changes in patients with major depressive disorder and healthy controls during a 1-year follow-up. *J Clin Psychiatry.* 2004;65:492-499.
  41. Frodl T, Schaub A, Banac S, Charypar M, Jäger M, Kümmler P, Bottlender R, Zetzsche T, Leinsinger G, Reiser M, Möller HJ, Meisenzahl EM. Reduced hippocampal volume correlates with executive dysfunctioning in major depression. *J Psychiatry Neurosci.* 2006;31:316-323.
  42. Kandel ER. The molecular biology of memory storage: a dialogue between genes and synapses. *Science.* 2001;294:1030-1038.
  43. Taylor WD, Steffens DC, Payne ME, MacFall JR, Marchuk DA, Svenson IK, Krishnan KR. Influence of serotonin transporter promoter region polymorphisms on hippocampal volumes in late-life depression. *Arch Gen Psychiatry.* 2005;62:537-544.
  44. Sheline YI, Gado MH, Price JL. Amygdala core nuclei volumes are decreased in recurrent major depression. *Neuroreport.* 1998;9:2023-2028.
  45. Frodl T, Meisenzahl EM, Zetzsche T, Born C, Jäger M, Groll C, Bottlender R, Leinsinger G, Möller HJ. Larger amygdala volumes in first depressive episode as compared to recurrent major depression and healthy control subjects. *Biol Psychiatry.* 2003;53:338-344.
  46. Strakowski SM, DelBello MP, Sax KW, Zimmerman ME, Shear PK, Hawkins JM, Larson ER. Brain magnetic resonance imaging of structural abnormalities in bipolar disorder. *Arch Gen Psychiatry.* 1999;56:254-260.
  47. Zetzsche T, Frodl T, Preuss UW, Schmitt G, Seifert D, Leinsinger G, Born C, Reiser M, Möller HJ, Meisenzahl EM. Amygdala volume and depressive symptoms in patients with borderline personality disorder. *Biol Psychiatry.* 2006;60:302-310.