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REGULAR RESEARCH ARTICLE

Association of BDNF Val66Met Polymorphism and Brain BDNF Levels with Major Depression and Suicide

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Abstract

Background: Brain-derived neurotrophic factor is implicated in the pathophysiology of major depressive disorder and suicide. Both are partly caused by early life adversity, which reduces brain-derived neurotrophic factor protein levels. This study examines the association of brain-derived neurotrophic factor Val66Met polymorphism and brain brain-derived neurotrophic factor levels with depression and suicide. We hypothesized that both major depressive disorder and early life adversity would be associated with the Met allele and lower brain brain-derived neurotrophic factor levels. Such an association would be consistent with low brain-derived neurotrophic factor mediating the effect of early life adversity on adulthood suicide and major depressive disorder.

Methods: Brain-derived neurotrophic factor Val66Met polymorphism was genotyped in postmortem brains of 37 suicide decedents and 53 nonsuicides. Additionally, brain-derived neurotrophic factor protein levels were determined by Western blot in dorsolateral prefrontal cortex (Brodmann area 9), anterior cingulate cortex (Brodmann area 24), caudal brainstem, and rostral brainstem. The relationships between these measures and major depressive disorder, death by suicide, and reported early life adversity were examined.

Results: Subjects with the Met allele had an increased risk for depression. Depressed patients also have lower brain-derived neurotrophic factor levels in anterior cingulate cortex and caudal brainstem compared with nondepressed subjects. No effect of history of suicide death or early life adversity was observed with genotype, but lower brain-derived neurotrophic factor levels in the anterior cingulate cortex were found in subjects who had been exposed to early life adversity and/or died by suicide compared with nonsuicide decedents and no reported early life adversity.

Conclusions: This study provides further evidence implicating low brain brain-derived neurotrophic factor and the brain-derived neurotrophic factor Met allele in major depression risk. Future studies should seek to determine how altered brain-derived neurotrophic factor expression contributes to depression and suicide.

Keywords: brain-derived neurotrophic factor, single nucleotide polymorphism, depression, suicide, childhood adversity

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Significance Statement

This is the first study to examine the relationship between both brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and brain BDNF protein level and major depression, death by suicide, and reported childhood adversity. The Met allele (a minor variant of the BDNF gene) is associated with an increased risk for depression. Depressed patients also had lower BDNF levels in 2 brain regions, namely the anterior cingulate cortex (ACC) and caudal brainstem (pons), compared with nondepressed subjects. Additionally, lower BDNF levels in ACC were found in subjects who had been exposed to early life adversity and/or died by suicide compared with nonsuicide decedents and no reported childhood adversity. This study may aid in identifying the possible mechanism by which altered BDNF expression contributes to MDD and suicide.

Introduction

Suicide accounted for almost 1 million deaths worldwide in 2015 (WHO, 2016). Major depressive disorder (MDD) is the most prevalent psychiatric disorder in suicide decedents (Cavanagh et al., 2003). The stress diathesis model of suicidal behavior (Mann et al., 1999; Mann, 2003; van Heeringen and Mann, 2014) posits that the risk for suicide is determined, not only by the underlying psychiatric illness (stressor), but also by a trait-like diathesis. Identifying possible biological causes for this diathesis may aid in the development of prediction, prevention, and treatment strategies for suicide risk.

Some studies have reported thinner prefrontal and anterior cingulate cortex (ACC) in depressed suicide attempters (Wagner et al., 2011, 2012), lower density of neurons in dorsal and ventral prefrontal cortex (Underwood et al., 2012), and fewer mature granule cells in the dentate gyrus of depressed suicides (Boldrini et al., 2013), raising the possibility of a deficit in brain neurotrophic pathways. Brain-derived neurotrophic factor (BDNF) is one of the neurotrophins (Huang and Reichardt, 2001) that regulates neuron survival, plasticity (Morse et al., 1993; McAllister, 2001; Poo, 2001; Berton et al., 2006; Tsankova et al., 2006), and synaptic function (Lessmann et al., 2003). BDNF plays an integral role in differentiation during development (Alcántara et al., 2006; Engelhardt et al., 2007), is regulated by stress (Roceri et al., 2004), and is associated with the pathophysiology of mental disorders, particularly major depression (Russo-Neustadt, 2003). Thus, BDNF is a candidate molecule for contributing to the adverse brain effects of exposure to early life stress that may mediate the effects on adulthood risk of major depression and suicide.

A functional polymorphism (rs6265) in the BDNF gene results in a valine-to-methionine substitution at codon residue 66 (Val66Met; Schumacher et al., 2005; Post, 2007; Rybakowski, 2008). The Met allele is associated with less BDNF activity (Egan et al., 2003) and lower serum levels (Ozan et al., 2010) and appears to be associated with major depression (Hwang et al., 2006), memory impairments (Egan et al., 2003; Hariri et al., 2003), reduced hippocampal activity (Chen et al., 2004), and anxiety-related behaviors in animal models (Chen et al., 2006), although not all studies agree (Sen et al., 2003; Cohen et al., 2004; Schumacher et al., 2005; Strauss et al., 2005; Surtees et al., 2007; Chen et al., 2008). Furthermore, Met allele increases the risk for suicidal behavior (Iga et al., 2007; Schenkel et al., 2010), particularly in depressed patients (Sarchiapone et al., 2008) and those exposed to early life stress (Pregelj et al., 2011).

Low plasma BDNF levels are reported in major depression (Karege et al., 2002; Sen et al., 2008; de Azevedo Cardoso et al., 2014) and suicidal behavior (Kim et al., 2007). Postmortem studies indicate low BDNF protein levels in amygdala (Guilloux et al., 2012) and decreased BDNF signaling in ACC (Tripp et al., 2012) of depressed patients. Low BDNF protein was also reported in hippocampus (Dwivedi et al., 2003; Karege et al., 2005; Banerjee et al., 2013) and prefrontal cortex (Dwivedi et al., 2003; Karege et al., 2005) of suicide decedents. Antidepressants increase BDNF blood levels in depressed patients (Chen et al., 2001; Gervasoni et al., 2005; Gonul et al., 2005) and animal models of depression (Nibuya et al., 1995; Russo-Neustadt et al., 1999). Moreover, in animal depression models, injection of BDNF into the hippocampus (Shirayama et al., 2002) and midbrain (Siuciak et al., 1997b) produces an antidepressant-like effect (e.g., decreased escape failure in the learned helplessness paradigm and decreased immobility in the forced swim test).

Exposure to stress decreases brain BDNF levels in rodents (Smith et al., 1995; Nibuya et al., 1999; Roceri et al., 2004). Stress, particularly in early life, can downregulate BDNF in depressed (Grassi-Oliveira et al., 2008) and suicidal patients (Dwivedi, 2010). Some, but not all (Perroud et al., 2008) studies show that exposure to early life stress in BDNF Met carriers predicts future depression (Aguilera et al., 2009; Gatt et al., 2009) and suicide (Pregelj et al., 2011).

In the current study, we sought to identify the inter-relationship of BDNF in suicide, major depression, and reported childhood adversity by examining the BDNF polymorphism and BDNF protein levels in prefrontal cortex (PFC; Brodmann Area 9), ACC (Brodmann area 24), and brainstem postmortem in cases of major depression, suicide decedents, and nonsuicide, nonpsychiatric sudden death comparison groups. We hypothesized that major depression would be associated with the Met allele and lower brain BDNF levels, and this association would be more pronounced in suicide and/or MDD. We also hypothesized that reported childhood adversity would also correlate with low BDNF protein level in depressed suicide decedents, consistent with a model that posits low BDNF expression at least partly mediates the effect of early life adversity on adult risk of major depression and suicide.

Methods

Subjects

Brain samples from 37 suicide decedents and 53 nonsuicide comparison subjects were studied, and clinical and demographic details are in Table 1. Procedures for collection and use of brain tissue were approved by the applicable Institutional Review Boards.

All suicide and nonsuicide subjects died suddenly, without a prolonged agonal period that might have an impact on the biological measures. Postmortem interval (PMI; time from death to freezing of brain samples) was limited to 24 hours. Brain samples were coded and assayed by laboratory personnel blind to the cause of death. The brainstem was dissected from the forebrain, which was bisected and cut into 2-cm slabs with the tissue blocks frozen for later sectioning. The brainstem was

Table 1. Demographic and Clinical Characteristics of non-MDD and MDD Subjects

Variable	Non-MDD (n=45)		MDD (n=45)		Analysis
	Mean	SD	Mean	SD	P[a]
Age (y)	40.4	17.7	47.1	17.8	.077
Alcohol level (%)	0.0143	0.0545	0.0079	0.0269	.520
pH of brain tissue	6.5	0.3	6.5	0.3	.638
Total aggression score	13.1	5.4	13.7	4.3	.576
PMI	15.2	4.8	16.3	6.7	.413
RIN	6.5	0.6	6	1	.280
	N	*%*	*N*	*%*	
Sex					
Female	6	13.3	10	22.2	.409
Male	39	86.7	35	77.8	
Race					
White	35	77.8	37	84.1	.792
Black	5	11.1	4	9.1	
Hispanic	5	11.1	3	6.8	
Tobacco smoking					
Yes	18	43.9	19	57.6	.350
No	23	56.1	14	42.4	
Death by suicide					
Yes	5	11.1	32	71.1	**<.001**
No	40	88.9	13	28.9	
Early life adversity					
Yes	15	33.3	17	39.5	.658
No	30	66.7	26	60.5	
History of personality disorder					
Yes	5	11.1	8	18.6	.378
No	40	88.9	35	81.4	

Abbreviations: MDD, major depressive disorder; PMI, postmortem interval; RIN, RNA integrity number.
[a]Independent sample *t* test (df=81, df=88 for age) for continuous variables; chi-square analysis (df=1) for categorical variables.

cut in a cryostat in the horizontal plane into 20-µm-thick sections. Sections were selected that contained rostral and caudal levels of the dorsal raphe nucleus, the brain region containing serotonergic neurons that innervate the forebrain. The dorsal raphe nucleus was selected as a region of interest for the actions of BDNF because of the role of serotonin and serotonin-synthesizing neurons in mood disorders and suicide, and the reported relationship between BDNF and serotonergic neurons (Martinowich and Lu, 2008). The rostral and caudal levels are referred to as rostral and caudal brainstem, respectively. The caudal brainstem level corresponded to caudal pons and contained both dorsal and median raphe nuclei. Toxicological screening of body fluids (blood, bile, aqueous humor, and urine) as well as brain tissue was performed for cocaine, opiates, alcohol, cannabinoids, and other acidic and basic drugs, and major psychotropic drugs.

Psychological autopsies were completed and psychiatric diagnoses made using our validated method as previously described (Kelly and Mann, 1996). In brief, after giving written informed consent, at least one next of kin of subjects was interviewed. The Structured Clinical Interview for DSM-IV Axis I (SCID-I) (First et al., 1995), SCID-II (First et al., 1997), Brown-Goodwin Lifetime History of Aggression Scale (Brown et al., 1979), and Columbia Suicide History Form (Oquendo et al., 2003) were administered to the informant by a psychologist with at least a master's level degree. The Brown-Goodwin Lifetime History of Aggression Scale score was calculated, excluding the item regarding self-inflicted injury. Research diagnoses were based on DSM-IV criteria. The sample was divided clinically into subjects with a history of DSM-IV MDD (n=45) and those without such history (non-MDD) (n=45). History of early life adversity in terms of physical or sexual abuse or neglect before the age of 15 years was also obtained during the psychological autopsy interview using a checklist.

Exclusion criteria included: (1) cases of undetermined manner of death, (2) gross neuropathology, (3) positive toxicology screens for psychoactive and neurotoxic drugs (including antidepressants and alcohol), (4) alcohol use disorder, and (5) history of bipolar disorder or psychosis.

Genotyping

DNA was extracted from frozen brain tissue. The BDNF Val66Met polymorphism (GenBank dbSNP: rs6265) was typed by PCR protocol as published previously (Sublette et al., 2008). Briefly, the oligonucleotide primers, sense MannBF-1F (5'-ATCCCGGTGAAAGAAAGCCCTAAC-3′) and antisense MannBF-1R (5′-CCCCTGCAGCCTTCTTTTGTGTAA-3′), were used to amplify a PCR fragment of 673 bp length. PCR was carried out in a 20-µL volume, containing 100 ng DNA, 40 ng of each primer with HotStartTaq Plus Master Mix kit (Qiagen). Samples were processed in a BioRad T100 Thermal Cycler (BioRad). DNA samples were denatured first at 95°C for 6 min. Thirty temperature cycles, consisting of 30 s at 95°C, 40 s at 60°C, and 40 s at 72°C, were followed by a final extension step of 72°C for 4 min. The PCR fragments were digested with BsaA I restriction enzyme (NE Biolab), which produces 3 fragments of 275, 321, and 77 bp when guanine is present at nucleotide 1249, and 2 fragments of 321

and 352 bp if cytosine is present at this position. The digested PCR products were separated on a 1.2% agarose gel.

Western Blotting of BDNF

Frozen brain samples from Brodmann Area (BA) 9 of 85 subjects, BA24 of 51 subjects, caudal brainstem of 33 subjects, and rostral brainstem of 25 subjects were homogenized in cell lysis buffer (Cell Signaling) plus 2% phosphatase inhibitor cocktail 2, 2% phosphatase inhibitor cocktail 3, and 2% Protease Inhibitor cocktail (Sigma). The homogenate was centrifuged for 10 min at 15000 x g at 4°C, and the supernatant was transferred into Eppendorf tubes and stored at -80°C. Protein concentration was determined using Beckman DU530 spectrophotometer. Laemmli buffer with β-mercaptoethanol was added in Eppendorf tubes. After adding protein samples, water was added up to 20 µL to each tube. The samples were denatured for 5 min at 95°C and then centrifuged for 5 s. Protein samples (25 µg) were loaded onto 12% Mini-PROTEAN TGX gel. The gel was run with 10x Tris-glycine with SDS for 20 min at 40 V and increased to 100 V for 1 h and transferred in an enhanced chemiluminescence (ECL) nitrocellulose membrane (Amersham) for 1 h and 15 min at 90 volts with a transfer buffer (1x tris-glycine with 20% methanol) at 4°C. The membranes were washed with TBST buffer (10 mm Tris-base, 0.15 m NaCl, and 0.1% Tween-20) for 10 min. The blots were blocked by incubating with 5% nonfat milk in TBST for 1 h. Then the blots were incubated overnight at 4°C with primary polyclonal anti-BDNF antibody (Alomone Labs) at a dilution of 1:50000. The membranes were washed with TBST and incubated with horseradish-peroxidase secondary antibody (anti-rabbit immunoglobulin G; 1:5000] in 5% nonfat milk for 3 h at 4°C. The membranes were extensively washed with TBST and exposed to enhanced chemiluminescence autoradiography film. The same nitrocellulose membrane was stripped and reprobed with GAPDH antibody (Cell Signaling).

Statistical Analysis

The genotype frequency distributions of the SNPs were tested for Hardy–Weinberg equilibrium in the non-MDD, nonsuicide controls.

Data were statistically evaluated using IBM SPSS Statistics (SPSS Inc., Version 23.0). Comparison of demographic, clinical, and postmortem characteristics between the depressed and nondepressed subjects was performed using 2-tailed *t* tests for continuous variables and chi-square analyses for categorical variables. The differences in allele and genotype frequency distributions between the non-MDD vs MDD, nonsuicides vs suicides, as well as subjects with no history of early life adversity vs those with such history were calculated using Pearson's chi-square tests (df = 1 and df = 2, respectively). The difference in life time history of aggression among the 3 genotype groups was assessed using 1-way ANOVA.

For BDNF protein analyses, we first explored the effects of age, sex, RNA integrity number (RIN), PMI, and brain pH on the levels of BDNF protein using Pearson's correlation analyses. No effect of these variables was detected on BDNF protein levels in the 4 brain regions studied; therefore, they were not added as covariates in further analyses. A 2-tailed *t* test was used to compare BDNF protein levels between subjects with GG genotypes vs carriers of A allele (AA and AG), and non-MDD vs MDD in the 4 brain regions. We found an effect of depression diagnosis on BDNF protein levels, so we controlled for depression status in subsequent analyses (suicide vs nonsuicide, history of early life adversity vs no early life adversity) using an ANCOVA using MDD diagnosis as a covariate. We also examined the effect of ELA on BDNF protein levels among the depressed subjects using ANOVA to compare the BDNF protein levels among the 4 groups (nondepressed non-ELA, nondepressed ELA, depressed non-ELA, and depressed ELA) that was followed by posthoc Tukey's test whenever there was significant result in the initial model.

Additionally, we further classified the sample into 4 groups based on the manner of death (suicide or not) and history of exposure to early life adversity (nonsuicide, no adversity vs nonsuicide, adversity vs suicide, no adversity vs suicide, adversity) and examined the differences of BDNF protein levels among these 4 groups while controling for depression status using ANCOVA in each region separately. We also repeated the main analysis comparing the MDD group with nonpsychiatric controls. Correction for multiple comparisons was made using the Bonferroni method.

Results

Demographics and Clinical Characteristics

Demographic and clinical characteristics of the study sample (n = 90) are shown in Table 1. The MDD and non-MDD groups did not differ in terms of age, sex, or race. The MDD group were more likely to die by suicide than the non-MDD group ($\chi^2 = 33.46$, df = 1, $P < .001$). There were also no group differences in blood alcohol level, brain tissue pH, PMI, and RIN. There were also no group differences in lifetime history of aggression score, reported early life adversity, or comorbid personality disorders.

BDNF Val66Met Polymorphism Allele and Genotype Frequencies

Genotype frequencies in the non-MDD, nonsuicide control group were in Hardy-Weinberg equilibrium ($\chi^2 = 0.1117$). BDNF Val66Met genotype distribution differed between MDD and non-MDD groups ($\chi^2 = 7.91$, df = 2, $P = .019$), and posthoc testing showed that this was due to an association of Met allele (A) with the MDD group ($\chi^2 = 6.54$, df = 1, $P = .011$) (see Table 2). The BDNF polymorphism did not differ between suicide and nonsuicide decedents ($\chi^2 = 2.8$, df = 2, $P = .24$; Table 2). Reported early life adversity was not associated with genotype ($\chi^2 = 0.96$, df = 2, $P = .62$; Table 2). Subclassifying the carriers of Met allele into 2 groups according to reported exposure to early life adversity revealed no association with major depression ($\chi^2 = 0.09$, df = 1, $P = .77$; Table 3). The lifetime history of aggression score was not different between the three genotypes ($F = 1.42$, df = 2,79, $P = .25$).

BDNF Protein

No statistically significant effects of age, sex, RIN, PMI, or brain pH on the levels of BDNF protein were detected ($P > .05$ all variables) (see supplement).

Subjects with GG genotype did not differ from carriers of A allele regarding BDNF levels in dorsolateral PFC (dlPFC) ($t_{(83)} = -0.32$, $P = .8$), ACC ($t_{(49)} = -0.73$, $P = .46$), rostral brain stem (BSr) ($t_{(23)} = 0.16$, $P = .87$), or caudal brain stem (BSc) ($t_{(31)} = -2.02$, $P = .052$). Likewise, there was no association between genotype (GG vs A carrier) and BDNF level in depression, suicide, or reported childhood adversity; multivariate analysis, mixed model analysis, and step-wise linear regression analysis all failed to detect an effect of genotype.

Table 2. BDNF Val66Met polymorphism allele and genotype frequencies

		Genotype n (%)					Allele n (%)			
rs6265	n	GG	AG	AA	χ^{2a}	P	G	A	χ^{2a}	P
Non-MDD	45	34 (75.6)	10 (22.2)	1 (2.2)	7.91	**.019**	78 (87)	12 (13)	6.54	**.011**
MDD	45	21 (46.7)	22 (48.9)	2 (4.4)			64 (71)	26 (29)		
Non-Suicide	53	36 (67.9)	16 (28.3)	1 (1.8)	2.8	.24	88 (83)	18 (17)	2.64	.1
Suicide	37	19 (51.4)	16 (43.2)	2 (5.4)			54 (73)	20 (27)		
No early life adversity	58	37 (63.8)	19 (32.8)	2 (3.4)	0.96	.62	93 (80)	23 (20)	0.32	.57
Early life adversity	32	18 (56.3)	13 (40.6)	1 (3.1)			49 (77)	15 (23)		

Abbreviation: MDD, major depressive disorder.
[a]Chi-square analysis (df = 1 for allele frequency comparisons, df = 2 for genotype frequency comparisons).

Table 3. Distribution of BDND Met Carriers with and without History of Early Life Adversity in Relation to MDD

Met Carriers	Non-MDD (n = 11)	MDD (n = 24)	Analysis P[a]
With ELA	4 (36.4%)	10 (41.7%)	.77
Without ELA	7 (63.6%)	14 (58.3%)	

Abbreviations: ELA, early life adversity; MDD, major depressive disorder.
[a]Chi-square analysis (df = 1).

However, BDNF protein level appeared to be lower in ACC ($t_{(49)} = 2.14$, $P = .04$ without Bonferroni correction) and caudal brainstem ($t_{(31)} = 2.37$, $P = .02$ without Bonferroni) in the MDD group compared with non-MDD group. No effect of major depression diagnosis was found on BDNF protein levels in dlPFC ($t_{(83)} = -0.72$, $P = .47$) or rostral brainstem ($t_{(23)} = 0.58$, $P = .57$) (see Figure 1).

Since there was an indication of a potential effect of MDD in ACC and caudal brainstem (vide supra), we controlled for MDD diagnosis in subsequent analyses of the effects of suicide and early life adversity by using ANCOVA. Classifying the study subjects based on manner of death (suicide or not) and exposure to early adversity or not (Figure 2), BDNF protein level in ACC in suicide decedents with adversity history was lower compared with controls without reported adversity ($F_{(3, 46)} = 5.84$, $P = .006$ uncorrected, $P = .048$ corrected), but not in the dlPFC ($F_{(3, 78)} = 0.89$, $P = .45$), BSc ($F_{(2, 27)} = 0.48$, $P = .63$), or BSr ($F_{(2, 20)} = 1.37$, $P = .28$).

There was no difference in BDNF levels between suicide decedents and nonsuicide decedents, with MDD diagnosis as a covariate, in dlPFC ($F_{(1, 82)} = 0.004$, $P = .95$), ACC ($F_{(1, 48)} = 0.52$, $P = .48$), BSc ($F_{(1, 30)} = 0.09$, $P = .76$), or BSr ($F_{(1, 22)} = 2.67$, $P = .12$). Likewise, there was no difference in BDNF levels between subjects with reported early life adversity compared with those without such a history in dlPFC ($F_{(1, 79)} = 1.99$, $P = .16$), ACC ($F_{(1, 48)} = 0.93$, $P = .34$), BSc ($F_{(1, 28)} = 0.91$, $P = .35$), or BSr ($F_{(1, 21)} = 1.89$, $P = .18$).

Comparing nondepressed non-ELA, nondepressed ELA, depressed non-ELA, and depressed ELA for BDNF protein levels showed an overall effect of group in the ACC ($f_{(3,43)} = 3.421$, $P = .025$). Posthoc Tukey's tests revealed that this effect was mainly derived from the difference between nondepressed non-ELA and depressed non-ELA groups ($P = .033$). The nondepressed non-ELA group showed no difference from the nondepressed ELA group ($P = .255$) or the depressed ELA group ($P = .974$). The depressed ELA group did not differ from the depressed non-ELA group ($P = .084$) or the nondepressed ELA group ($P = .481$). The depressed non-ELA showed no difference from the nondepressed ELA ($P = .681$). Taken together, this suggests no effect of ELA on BDNF protein level in BA24. Likewise, the same model revealed no significant differences in the caudal brain stem ($f_{(2,28)} = 2.188$, $P = .131$), rostral brain stem ($f_{(2,21)} = 1.04$, $P = .370$), or dlPFC ($f_{(3,78)} = 3.421$, $P = .025$).

When we performed the analysis comparing the nonpsychiatric (non-MDD nonsuicide) controls (n = 40) with the MDD group, the results were similar. BDNF Val66Met genotype distribution differed between MDD and nonpsychiatric control groups ($\chi^2 = 7.1$, df = 2, $P = .029$), such that Met allele was associated with MDD. BDNF protein level was lower in ACC ($t_{(49)} = 2.14$, $P = .04$ without Bonferroni correction) and caudal brainstem ($t_{(31)} = 2.37$, $P = .02$ without Bonferroni) in the MDD group compared with nonpsychiatric controls. There was no effect of MDD diagnosis on BDNF protein levels in dlPFC ($t_{(83)} = -0.72$, $P = .47$) or rostral brainstem ($t_{(23)} = 0.58$, $P = .57$).

Discussion

The current study provides evidence for a role of BDNF in the pathophysiology of MDD. We found an association of both BDNF Met allele and lower brain BDNF protein level in the ACC and caudal brainstem with MDD, and there were lower BDNF levels in association with a reported history of childhood adversity and suicide as cause of death. Contrary to our hypothesis, we did not find BDNF brain levels associated with genotype.

BDNF Polymorphism

Our finding that the Met allele is associated with history of MDD is consistent with a reported association with geriatric depression (Hwang et al., 2006) and combined anxiety and depression diagnoses (Jiang et al., 2005; Taylor et al., 2007). A meta-analysis of Verhagen et al. (2010) also reported such association with depression in men only. Chen et al. (2006) found increased anxiety-related behaviors in $BDNF^{Met/Met}$ mice indicating a causal relationship. The BDNF Met allele is associated with less hippocampal activity (Egan et al., 2003; Hariri et al., 2003; Frey et al., 2007) and smaller volume (Duman, 2002; Pezawas et al., 2004; Szeszko et al., 2005; Frodl et al., 2007; Gonul et al., 2011). On the other hand, many studies did not find such association (Hong et al., 2003; Tsai et al., 2003; Gratacòs et al., 2007; Surtees et al., 2007). The differences in results from these studies may be due to differences in study design, sample characteristics (e.g., age groups, ethnicity or race) or because of the clinical and biological heterogeneity of major depression (Akiskal et al., 1981). Strauss et al. (2005) found an association of Val allele with childhood mood disorders, whereas Hwang et al. (2006) found that the Met allele is related to geriatric depression. Some studies conclude that a gene-gene or gene-environment interaction is

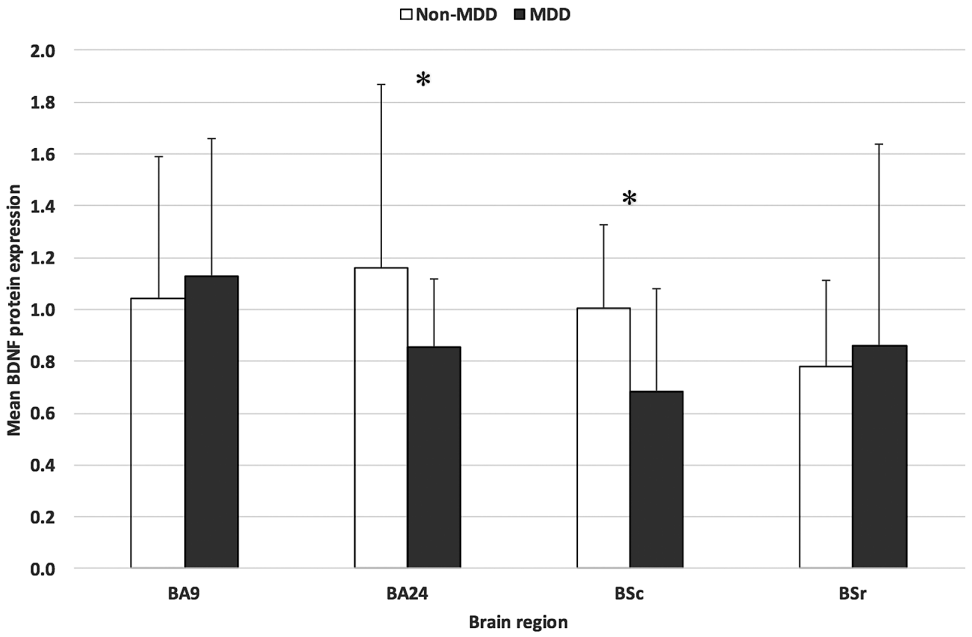

Figure 1. Bar chart of mean ± SD of brain-derived neurotrophic factor (BDNF) protein levels in Brodmann area (BA)9 (n = 85), BA24 (n = 51), BSc (n = 33), and BSr (n = 25) in non-major depressive disorder (MDD) and MDD groups. BDNF protein levels were lower in BA24 and caudal brain stem (BSc) of depressed patients compared to nondepressed subjects. No group differences were found in the BA9 or in rostral brain stem (BSr). *P < .05.

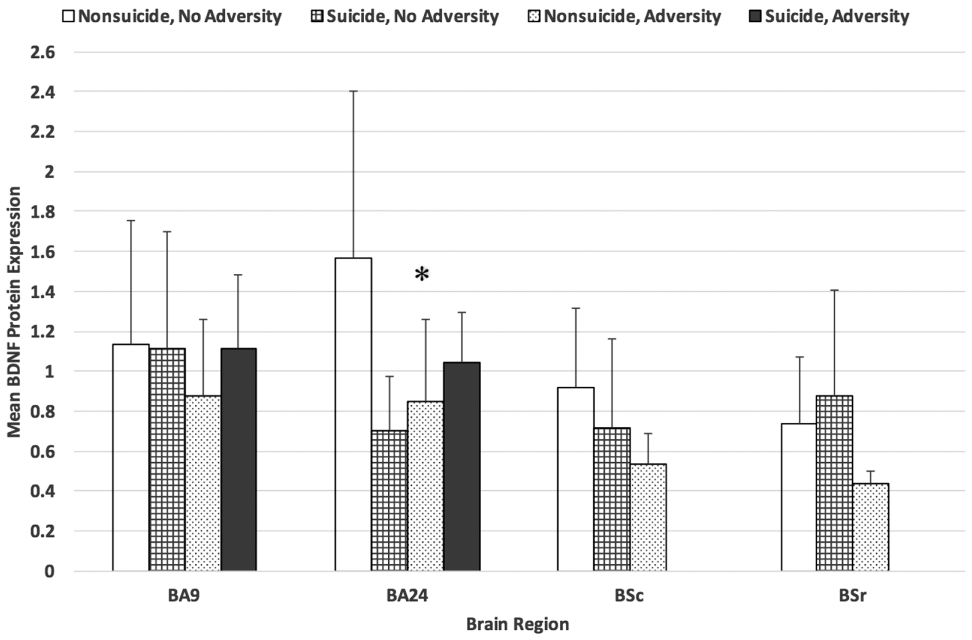

Figure 2. Bar chart of mean ± SD of brain-derived neurotrophic factor (BDNF) protein levels in Brodmann area (BA)9 (n = 85), BA24 (n = 51), caudal brain stem (BSc) (n = 33), and rostral brain stem (BSr) (n = 25) in various groups. BDNF protein levels in anterior cingulate corte (ACC) (BA24) in the group without history of suicide and adversity was higher than other groups. No group difference was found in the BA9, BSc, or BSr. *P < .05.

required for this BDNF polymorphism to contribute to major depression (Kaufman et al., 2006; Wichers et al., 2008; Belsky et al., 2009). We found association of both Met allele and BDNF protein levels in some brain regions with MDD; however, genotype appears to be unrelated to BDNF protein. We did not find a relationship between reported childhood adversity and genotype. The genotype antedates the childhood adversity and so genotype may theoretically predispose to ELA or affect the consequences of ELA. The absence of an association is the evidence we have that the polymorphism does not produce a phenotype that increases the risk for ELA. An interaction effect would mean the genotype affects the impact of ELA on the biological or behavioral phenotype.

We did not find an association between the val66met BDNF polymorphism and death by suicide, consistent with previous studies on suicide decedents (Zarrilli et al., 2009; Pregelj et al., 2011; Ratta-Apha et al., 2013). The Met allele is reportedly associated with nonfatal suicide attempts (Sarchiapone et al., 2008).

This may add further evidence to the hypothesis that fatal and nonfatal suicide attempts are only partially overlapping phenomena with different underlying genomic components (Mann et al., 2009).

BDNF Level in Brain

The MDD group had lower BDNF protein level in ACC and caudal brainstem, but not in dlPFC or rostral brainstem, compared with the non-MDD control group and this was not mediated by exposure to reported ELA. Our findings in ACC are consistent with Tripp et al. (2012), who found that BDNF receptor signaling and BDNF-dependent gene expression are lower in ACC of depressed compared with nondepressed subjects. That same study reported no differences in BDNF mRNA levels, which suggests that differences in BDNF protein levels may be due to defective translation of BDNF, but not to gene transcription. Therefore, the functional effect of BDNF genotype appears linked to BDNF protein (Egan et al., 2003; Chen et al., 2004) and not mRNA levels.

The ACC is implicated in the pathophysiology of mood disorders (Drevets et al., 2008). Functional neuroimaging studies show that the ACC, particularly its rostral/subgenual part, has a role in emotion regulation (Vogt et al., 1992; Davidson et al., 2002). Volume reduction in this area is a consistent finding in mood disorders (Drevets et al., 1997). Low BDNF levels in ACC may, therefore, contribute to its functional and structural deficits in MDD.

Our finding of low BDNF brainstem levels in depressed subjects is consistent with Kozicz et al. (2008) who reported low BDNF expression in midbrain of male depressed patients. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra (Hyman and Hofer, 1991) and has a powerful positive effect on the survival of human and rat mesencephalic dopaminergic neurons in culture (Zhou et al., 1994). Chronic infusion of BDNF into the rat midbrain increases the turnover of serotonin and levels of noradrenaline in many forebrain areas, including the neocortex, basal ganglia, and hippocampus (Altar et al., 1994; Siuciak et al., 1996), and infusion into periaqueductal gray and dorsal raphe or into the substantia nigra had antidepressant-like effects in several behavioral tasks (Siuciak et al., 1997a).

We did not find an effect of suicide, by itself as a cause of death, on BDNF protein levels across the 4 brain regions examined. However, we did find that suicides, when considered with respect to exposure to early life adversity, did have lower BDNF level in ACC, but not in the other brain regions. Some previous studies found lower BDNF protein levels in the PFC of suicide decedents (Dwivedi et al., 2003; Karege et al., 2005; Pandey et al., 2010). However, the findings of these studies may be confounded by the underlying psychiatric diagnosis or exposure to early life adversity. Our current study included only subjects with history of MDD, and we controlled for MDD diagnosis status. The sample in the Pandey et al. (2010) study was exclusively teenage suicide decedents with potentially different characteristics, risk factors, and pathophysiology from adult suicide (Brent et al., 1993). Our findings indicate that low brain BDNF levels may contribute to the pathophysiology of MDD, which in turn is a major risk factor for suicide.

The connection between low BDNF, suicide, and early life adversity may come about by a dysregulated stress response system in which BDNF translation is altered by the chronic effects of stress resulting from maltreatment in early life, and also with the acute effects of stress accompanying suicide behavior. Infant maltreatment results in methylation of BDNF DNA, resulting in reduced BDNF gene expression in the adult prefrontal cortex (Roth et al., 2009). Interestingly, BDNF promoter/exon IV, which plays a critical role in BDNF gene regulation (Dennis and Levitt, 2005), is frequently hypermethylated in the Wernicke area of the postmortem brain of suicide subjects (Keller et al., 2010). Additionally, overactive hypothalamic-pituitary-adrenal axis has been linked to death by suicide (Mann et al., 2006), and abnormal stress response may result in part from a loss of neuronal plasticity that could be relevant in suicidal behavior (Duman and Monteggia, 2006). Therefore, in addition to its involvement in depression, BDNF may play an important role in the neurobiology of suicidal behavior in the context of a psychosocial stress (Deveci et al., 2007). However, further investigation in a larger sample is required to draw definitive conclusions about associations with suicide independently of a diagnosis of MDD.

Genotype and BDNF Level

We did not find association between the BDNF Val66Met polymorphism and brain BDNF protein levels, consistent with the Lee et al. (2005) study of temporal cortex of Alzheimer's patients. Our finding is also consistent with other (Duncan et al., 2009; Zou et al., 2010; Yoshimura et al., 2011), but not all (Ozan et al., 2010) studies that examined the effect of BDNF Val66Met polymorphism on blood BDNF levels. However, we found association of Met allele as well as low ACC and brainstem BDNF levels in MDD. It has been reported that Met allele contributes to the defect in activity-dependent BDNF secretion such that Met carriers have abnormal intracellular trafficking and packaging of pro-BDNF, reducing the depolarization-dependent secretion of the mature peptide (Egan et al., 2003; Chen et al., 2004). This suggests that the relationship between BDNF Val66Met polymorphism and brain BDNF levels is complex. This polymorphism may be in linkage disequilibrium with another unidentified functional polymorphism. This explanation is supported by Bhang et al. (2011) who reported that healthy volunteers who were homozygous for S at 5-HTTLPR and the Met allele of the BDNF Val66Met polymorphism displayed significantly lower serum BDNF levels. Future studies should examine the effect of combination of functional polymorphisms on brain BDNF levels.

Limitations

Despite the evidence found in our study for the association of BDNF with MDD, there is uncertainty about the mechanism through which BDNF contributes to depressive pathophysiology. Alleles at the Val66Met locus may also be in linkage disequilibrium with other risk alleles in different genes. Future studies should consider the associations and interactions of multiple genes simultaneously to identify their relative contribution to MDD, suicide, and early childhood adversity experiences.

Supplementary Material

Supplementary data are available at *International Journal of Neuropsychopharmacology* online.

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Statement of Interest

Dr. Mann receives royalties for the commercial use of the C-SSRS from the Research Foundation for Mental Hygiene. Other authors declare no conflicts of interest.

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