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Brain-derived neurotrophic factor (BDNF) Val66Met and adulthood chronic stress interact to affect depressive symptoms

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Abstract

Background—*BDNF* Val66Met by chronic stress interaction has been studied using childhood stress as a moderator, but has not been widely studied using chronic stress in adulthood.

Methods—Two independent samples were used: Duke-CG (238 Caucasians) and MESA (5524 Caucasians, African Americans and Hispanics). Chronic stress in Duke-CG was operationalized as having primary caregiving responsibility for a spouse or relative with diagnosed Alzheimer's disease or other major dementia; chronic stress in MESA was defined using chronic burden score constructed from self-reported problems of health (self and someone close), job, finance and relationships. CES-D scale was the measure of depression in both samples. The *BDNF* Val66Met by adulthood chronic stress interaction predicting CES-D was examined using linear regression, adjusted for covariates.

Results—The main effect of *BDNF* Val66Met genotype on CES-D scores was non-significant ($p > 0.607$) but the adulthood chronic stress indicator was significant ($p < 0.001$) in both samples. The *BDNF* Val66Met genotype by adulthood chronic stress interaction was also significant ($p < 0.039$) in both samples. The impact of chronic stress in adulthood on CES-D scores was significantly larger in Val/Val genotype individuals than Met carriers.

Conclusion—We found in two independent samples that depression levels increased significantly more as a function of adulthood chronic stress Val/Val genotype carriers than Met carriers. Individuals with the Val/Val genotype and chronic stress exposure could be targeted for interventions designed to reduce risk of depression if this finding is confirmed in future studies.

Keywords

BDNF Val66Met; Depression; Chronic stress; Rs6265; CES-D

1. Introduction

Brain-derived neurotrophic factor (BDNF) is one of the mammalian neurotrophin-family proteins that supports the survival of existing neurons, contributes to the growth and

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Contributors: RJ and BHB came up with the specific hypothesis of this study; ICS and RBW designed the study and collected data; RJ managed the literature searches and analyses, and undertook the statistical analyses; RJ, BHB, and MAB discussed and interpreted the data and results; RJ wrote the first draft of the manuscript; BHB, MAB, ICS and RBW edited the manuscript and gave suggestions. All authors contributed to and have approved the final manuscript.

Conflict of interest: Redford B. Williams is a founder and major stockholder in Williams LifeSkills, Inc.

differentiation of new neurons and synapses (Huang and Reichardt, 2001), and protects against stress-induced neuronal damage (Radecki et al., 2005). In the brain, it is active in the hippocampus, cortex, and basal forebrain – areas vital to learning, memory, and higher thinking (Yamada and Nabeshima, 2003). Studies have reported that BDNF may be involved in the pathophysiology of depression and/or the effective treatment of depression. The role of BDNF in depression is also supported by studies that indicate the variation in the human *BDNF* gene associated with depression, although the findings are mixed.

BDNF protein is encoded by the *BDNF* gene in humans on chromosome 11. *BDNF* Val66Met (rs6265) – is a SNP with a G/A allele polymorphism, resulting in variation between valine and methionine at codon66 (Bath and Lee, 2006; Egan et al., 2003). The Val/Val genotype has been reported to be associated with increased CNS gene expression relative to Val/Met and Met/Met (McHughen et al., 2010). Findings from studies that examine the association between this functional *BDNF* Val66Met and depression are mixed. Some studies have found such an association (Czira et al., 2011; Duncan et al., 2009; Hwang et al., 2006; Licinio et al., 2009; Ribeiro et al., 2007; Sen et al., 2003; Taylor et al., 2007; Verhagen et al., 2010), others have not (Chen et al., 2008; Gratacos et al., 2007; Hong et al., 2003; Schumacher et al., 2005; Surtees et al., 2007). Even among the studies finding an association between *BDNF* Val66Met and depression, the risk conferred by the genotype is inconsistent. Some studies showed that individuals with the Val/Val (G/G) genotype had an increased depressive trait, such as major depression disorder (Licinio et al., 2009; Ribeiro et al., 2007), scores on Beck Depression Inventory – II (BDI – II) (Duncan et al., 2009), and depression facet scores in neuroticism (Sen et al., 2003), whereas others report that Met/Met (A/A) subjects have more severe symptoms of depression (Czira et al., 2011; Hwang et al., 2006; Taylor et al., 2007; Verhagen et al., 2010). These reported associations of both alleles with increased levels of depression indicate that the reported association of the Val/Val genotype with increased CNS gene expression (McHughen et al., 2010) does not result in a straightforward association of that genotype with resistance to depression. Instead, they raise possibility that environmental factors moderate the influence of Val66Met on BDNF expression and hence the association of *BDNF* genotype with indices of depression.

Chronic stress is one such environmental factor that is a trigger for several psychiatric disorders, including depression (Kendler et al., 1999). Chronic stress has neurotoxic effects, including damage to hippocampal cells (Sapolsky, 2000) that may underlie symptoms of depression. Existing evidence supports the involvement of *BDNF* Val66Met in stress-sensitivity, depressive states and the development of brain structures related to emotional processing and depression, like the hippocampus and amygdala (Gatt et al., 2007; Joffe et al., 2009; Shirayama et al., 2002; van Wingen et al., 2010). The association between *BDNF* Val66Met and intermediate traits or depressive symptoms may be moderated, therefore, by chronic stress. The effect of this interaction on depression remains unclear, but a few studies have found that childhood stress moderates *BDNF* Val66Met effects on depressive symptoms. Gatt et al. (Gatt et al., 2009) reported a significant interaction of *BDNF* Val66Met and early life stress predicting hippocampal and amygdala volumes and working memory among Europeans. Consistent with the reported increased gene expression associated with the Val/Val genotype, the combination of *BDNF* Met carriers and exposure to early life stress predicted reduced gray matter in hippocampus and poorer working memory. Another recent study found that childhood stressful life events were associated with an affective memory bias (a potential cognitive intermediate phenotype for depression) but only in European young men carrying the *BDNF* Met allele (van Oostrom et al., 2012). One study showed that childhood sexual abuse had a greater impact on depressive symptoms in Met carriers of *BDNF* gene than in Val/Val group among Spanish young adults (Aguilera et al., 2009). These findings suggest that effects of childhood stress on depression and related phenotypes are more pronounced in *BDNF* Met carriers.

It is possible, however, that the importance and the effects of chronic stress that occurs during childhood on adult depressive symptoms may be quite different from the effects of chronic stress in adulthood. Jaffee *et al.* (Jaffee et al., 2002) have shown that risk factors experienced during childhood (e.g., perinatal insults, caretaker instability) were associated with juvenile-onset depression, whereas, these risk factors were less related to adult-onset depression. BDNF levels fluctuate (lower in childhood and rise in early adulthood) and have different functions throughout development, suggesting that effects on gene expression vary during development and may have different effects on behavioral outcomes at different life stages (Perea et al., 2012). Therefore, the interaction of *BDNF* Val66Met and adulthood chronic stress may exhibit a different impact on depression compared with its interaction with childhood stress. Perea *et. al* (Perea et al., 2012) recently reported that among Spanish undergraduate students the chronic stress during college years (an adulthood stress) was associated with increased negative affectivity (an underlying construct for both depressive and anxiety disorders) regardless of the *BDNF* Val66Met genotype, while, in this same sample, greater stress during childhood was associated with greater negative affectivity only in Met carriers. In contrast to these studies showing a larger impact of childhood stress on depression among Met carriers, Perroud et al. (Perroud et al., 2008) reported a larger impact of childhood trauma on risk of violent suicide attempt in adulthood among persons with the Val/Val genotype.

Taken altogether, the findings reviewed above suggest that the *BDNF* Met allele might confer increased vulnerability to depression after chronic stress exposure during childhood, but the interaction between *BDNF* Val66Met and chronic stress in adulthood has received much less attention. Moreover, several studies (Duncan et al., 2009; Licinio et al., 2009; Ribeiro et al., 2007; Sen et al., 2003) that have shown that the Val/Val genotype per se was associated with increased depressive traits or symptoms as a main effect (not moderated by childhood stress exposure). In the present paper, therefore, we evaluate the impact of the interaction of *BDNF* Val66Met and chronic stress in adulthood on depressive symptoms, first, in a U.S. sample where chronic stress is defined as caregiving for a relative with Alzheimer's disease or other dementia; and, second, in a public-access database – the Multi-Ethnic Study of Atherosclerosis (MESA) – in which chronic stress is evaluated using a measure of chronic burden, *i.e.*, health problems (self and someone close), job, finance and relationship problems.

2. Methods

2.1. Participants

2.1.1. Duke CG—The Duke Caregiving (Duke-CG) participants were enrolled in 2001–2004 and detailed study procedures are described elsewhere (Kring et al., 2010). Briefly, caregivers, defined as having the primary responsibility for care of a spouse or relative with diagnosed Alzheimer's disease or other major dementia, were recruited using flyers, advertisements in the local media, and community outreach efforts. Controls were identified by asking caregivers to nominate two to five friends with similar demographic factors (*e.g.*, gender, age, and race) who lived in their neighborhood. Informed written consent forms approved by the Duke University Medical Center Institutional review board were obtained from all subjects. A questionnaire battery was given to participants during the home visit by a nurse and returned on their visit to the General Clinical Research Center at Duke University Medical Center. The clinic visit was scheduled during the same week as the in-home visit, and consisted of a general physical examination, and a blood sample was drawn for genotyping. The frequency of the Met allele in African Americans was insufficient (0 Met/Met and 6 Val/Met) for statistical analyses; therefore, African-Americans were excluded in the present study. The 238 Caucasians with non-missing values on all measures of interest were included in the present study, consisting of 122 caregivers and 116 controls.

2.1.2. MESA—MESA is a federally funded multi-center, longitudinal cohort study of the factors that influence the progression of mild subclinical cardiovascular disease (CVD) to severe subclinical and clinical CVD in a multi-ethnic group of subjects (Bild et al., 2002). Information on the sampling frame and study design has been previously reported (Bild et al., 2002). Briefly, subjects without a history of clinical CVD, were recruited from six U.S. communities. Institutional review board approval was obtained at all MESA sites. Adults weighing > 300 pounds were not eligible for participation. MESA data included four ethnic groups: Caucasian, Chinese, African-American and Hispanic. The frequency of the Met allele was much higher in an Asian sample (47%) than the frequencies of 17–21% that have been observed in western samples (Kim et al., 2007), therefore, we excluded Chinese samples in this study. The data used in the present study includes the first examination between July 2000 and August 2002 for self-reported ethnic groups Caucasian, African-American, and Hispanic. This resulted in 5524 MESA participants for the current study.

2.2. Depression measure

The Center for Epidemiologic Studies of Depression (CES-D) scale was used to measure depressive symptoms in both samples. CES-D is a widely used 20-item self-report scale designed to measure depressive symptoms in a general population (Radloff, 1977). Items were scored on a 4-point scale (0–3) with the total CES-D score ranging 0 to 60. CES-D scores were transformed using the square root value in both samples to better meet the distributional assumptions of the statistical model.

2.3. Genotyping

The genotyping of *BDNF* Val66Met (rs6265) for the Duke-CG sample was conducted using ABI 7900 Taqman system (Applied Biosystems, Carlsbad, California, USA) using standard genotyping protocols implemented at the Center of Human Genetics at Duke University Medical Center. Genotyping of *BDNF* Val66Met for the MESA sample was from genome-wide genotyping results performed at the Broad Institute of Harvard and MIT (Boston, Massachusetts, USA) and at Affymetrix (Santa Clara, California, USA) using the Affymetrix Genome-Wide Human SNP Array 6.0. Both assays met quality control standards established in both labs.

2.4. Chronic stress measurement

In the Duke-CG sample, caregiving stress was used as the chronic stress for adults. Caregivers and controls were based on the sampling design of the study. Caregivers were coded 1 and controls were coded 2.

In the MESA sample, chronic burden was assessed using a measure of adulthood chronic stress based on a 5-item self-report questionnaire related to health and life: “Please tell us whether any of the following has been a problem for you – serious ongoing health problem (yourself); serious ongoing health problem (someone close to you); ongoing difficulties with your job or ability to work; ongoing financial strain; and ongoing difficulties in a relationship with someone close to you. The response of “yes” to any of 5 questions was scored as 1 and 0 if response was “no”. The total score was calculated by summing the item responses; scores this ranged from 0 = lowest stress to 5 = highest stress. If a response was missing on any of the five items, no total score was coded.

2.5. Covariates

Covariates were selected a priori based on substantive knowledge. Use of medication commonly prescribed to treat depression was collected by self-report in both samples. This medication included tricyclic anti-depressants, tricyclic anti-depressant/anti-psychotic

combinations, MAO inhibitors, and non-tricyclic antidepressants other than MAO inhibitors (SSRIs) in both Duke-CG and MESA samples. Medication use was coded 1 if any of these medications were used, and 0 for none used. MESA data was collected from 6 sites which coded as 3 – Forsyth County, North Carolina; 4 – New York, New York; 5 – Baltimore City and Baltimore County, Maryland; 6 – St. Paul, Minnesota; 7 – Chicago, Illinois; and 8 – Los Angeles County, California.

2.6. Statistical analysis

The primary analysis was carried out using multiple linear regression models. In a preliminary phase of analysis, we evaluated non-additivity by testing effects on depressive symptom levels of three-way interactions of *BDNF* Val66Met by caregiving status/chronic burden by each of the three demographic variables, race, gender, and age. If the three-way terms were not significant, it indicated that gene effects on depressive symptoms were not moderated by race, gender or age and the three-way interactions can be removed from the model. Analyses then tested a model predicting CES-D scores, with main effects terms for *BDNF* Val66-Met genotype and chronic stress, the *BDNF* Val66Met $\times$ chronic stress interaction term. In the MESA sample, the model also included the adjustment covariates gender, age, BMI, antidepressant use, race, and site. Race and site were not included in the model for the Duke-CG sample, as the Duke-CG sample is comprised of only white participants and was obtained at a single site. Due to the low frequency of Met/Met carriers, Met/Met and Val/Met were collapsed into a single group, which we labeled as Met carriers. Therefore the analysis compares Val/Val with the combined group of Met/Met and Val/Met genotypes. Regression analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, North Carolina). In a supplementary analysis to rule out gene/environment associations, we also examined the association between *BDNF* Val66Met genotype and caregiving status or chronic burden. A significance level of 0.05 was used for all analyses.

3. Results

3.1. Participant characteristics

Table 1 shows the demographic characteristics, genotype frequencies and chronic stress distributions for the two samples. There were more women than men in the Duke-CG sample, whereas in MESA gender was relatively balanced. The mean age was early sixties and non-significantly different ($p = 0.490$) between the Duke-CG and MESA samples. The mean BMI was larger ($p < 0.001$) in the MESA sample than Duke-CG sample. A greater ($p < 0.001$) percentage of subjects in the Duke-CG sample used anti-depressants, as compared to the MESA sample. In the Duke-CG sample the number of caregivers was similar to the number of controls. In MESA, about one third of subjects reported no chronic burden. CES-D scores were significantly higher ($p < 0.001$) in the Duke-CG sample, as compared to in the MESA sample. The frequencies of *BDNF* Val66Met did not differ between Duke-CG and MESA samples. The overall genotype distribution met Hardy–Weinberg equilibrium (HWE) in the Duke-CG sample ($p = 0.52$) but not in the MESA sample ($p < 0.0001$). The deviation in the MESA sample may be due to genotyping error or sample stratification. The *BDNF* genotype was obtained from public dbGap data which had been examined for genotyping error by published criteria. We do not have access to the raw data to further evaluate genotyping error as the source of HWE disequilibrium. The genotype distribution in Table 1 included Caucasians, African Americans and Hispanics. We tested HWE stratified by ethnic group and found that the deviation from HWE was only seen in the Caucasians ($p = 0.001$), which seems to be driving the deviation from HWE in the overall dataset. We further examined HWE in Caucasians by collection site. Site 3 and Site 4 showed a deviation from HWE suggesting that HWE could also be due to differences in the sites. If we excluded data at these two sites, and there was no longer a deviation from HWE

($p = 0.204$). Our further analyses were adjusted for site to control for these potential differences.

3.2. Main and interaction effects of BDNF Val66Met and adulthood chronic stress

In the Duke-CG sample, none of the three way interactions were significant ($ps > 0.1$). The *BDNF* Val66Met genotype was not associated with caregiving status ($p = 0.241$) indicating at best a very modest correlation between *BDNF* Val66Met genotype and caregiving status. The main effect of caregiving stress group was statistically significantly associated with CES-D scores ($p < 0.001$), but the main effect of *BDNF* Val66Met genotype was not significant ($p > 0.607$). The interaction of *BDNF* Val66Met $\times$ caregiving stress status was significantly associated with CES-D scores ($p = 0.039$, Table 2). This interaction is illustrated in Fig. 1, where it can be seen that the increase in CES-D scores as a function of caregiving stress was larger in participants with the Val/Val genotype ($p < 0.001$, Cohen's $d = 0.72$) than those carrying the Met allele ($p = 0.045$, Cohen's $d = 0.49$).

In MESA, the three-way interactions again were not significant ($ps > 0.194$). The correlation between *BDNF* Val66Met genotype and chronic burden in MESA also was non-significant ($p = 0.523$).

Similar to the findings in the Duke-CG sample, the main effect of chronic burden on CES-D scores was statistically significant ($p < 0.001$) in MESA, and the main effect of *BDNF* Val66Met polymorphism was not significant ($p = 0.680$). Again, as in the Duke-CG sample, the interaction of *BDNF* Val66Met $\times$ chronic burden was significantly associated with CES-D scores ($p = 0.005$, Table 2). Although there is a deviation from HWE in the MESA sample probably driven by the differences at site 3 and site 4 in Caucasians, we adjusted for site in the analyses to control these differences. We also performed the analyses excluding data at these two sites and the interaction remained significant, suggesting that the lack of HWE is not driving the significant result. As shown in Fig. 2, in subjects with lower chronic burden scores, CES-D scores did not differ between *BDNF* Val66Met genotype, but as chronic burden increased, CES-D scores increased, and the increase in CES-D scores as a function of chronic burden was larger in participants with the Val/Val genotype than those carrying the Met allele. Among individuals with high chronic burden = 5, the predicted mean CES-D scores for Val/Val carriers was higher than Met carriers ($p = 0.005$, Cohen's $d = 0.60$), while among subjects with low chronic burden = 0, mean CES-D scores did not differ significantly between Val/Val carriers and Met carriers ($p = 0.090$, Cohen's $d = 0.16$). The mean CES-D score of 17.6 among those with chronic burden = 5 and the Val/Val genotype is in the clinically significant range (>16) for this scale (Radloff, 1977), thereby highlighting the potential clinical significance of the larger impact of Val/Val genotype in persons exposed to high chronic stress levels.

4. Discussion

Our results show a consistent pattern of moderation of the association between chronic stress in adulthood and depressive symptom levels by *BDNF* Val66Met genotype in two independent samples. In contrast to the pattern observed for childhood stress in other studies, where persons carrying the Met allele have exhibited impaired memory (Gatt et al., 2009), decreased recall of positive words (van Oostrom et al., 2012) and increased depressive symptoms (Aguilera et al., 2009), we found in two samples that depressive symptom levels increased significantly more as a function of chronic stress among those with the Val/Val genotype than those carrying the Met allele. Observing a similar pattern in two independent samples makes a strong case that the association between *BDNF* Val66Met genotype and depressive symptoms is moderated by adulthood chronic stress in a way that differs from the moderation of *BDNF* Val66Met by childhood stress.

In addition to differential moderation of gene effects by chronic stress occurring in childhood vs. adulthood, the present findings also may explain the inconsistent main effects of *BDNF* Val66Met genotype on depressive symptom levels in prior research with some studies showing higher depression levels in Met carriers (Czira et al., 2011; Hwang et al., 2006; Taylor et al., 2007; Verhagen et al., 2010). Other studies, however, found the main effect of Val/Val genotype associated with higher levels of depressive symptoms or disorders. One used a sample of 189 cigarette smokers who smoked an average of 16 cigarettes per day (Duncan et al., 2009), one used a family sample of subjects with a systolic blood pressure in the upper 15% of blood pressure distribution at earlier examination (Sen et al., 2003), and the samples in two others (Licinio et al., 2009; Ribeiro et al., 2007) consisted of Mexican-Americans living in the Los Angeles area. Each of these samples probably could be considered a group exposed to chronic stress, whether that accompanying the habit of cigarette smoking or having high blood pressure or of being a member of a minority group in a large metropolitan area (Farley et al., 2005; Finch and Vega, 2003). The present study's findings of increased impact of chronic stress on depressive symptoms in persons with the Val/Val genotype in two independent samples could account for the finding of increased depressive symptoms/disorders in these four studies (Duncan et al., 2009; Licinio et al., 2009; Ribeiro et al., 2007; Sen et al., 2003) using samples that likely to be characterized as exposed to increased levels of chronic stress in adulthood. In contrast, studies finding no effect of Val/Val genotype, or even an effect of the Met allele, to increase depressive symptoms were conducted in samples of older adults not likely to be selected for exposure to chronic stress (Czira et al., 2011).

There are additional prior studies that found significant *BDNF* Val66Met genotype $\times$ childhood adversity effects on risk of violent suicide attempt or depressive symptoms in adulthood (Aguilera et al., 2009; Gatt et al., 2009; Perroud et al., 2008; van Oostrom et al., 2012; Wichers et al., 2008). Similar to our findings, one study found increased risk of violent suicide attempts in persons with the Val/Val genotype and exposure to sexual abuse in childhood, but no effect of childhood trauma in Met carriers (Perroud et al., 2008). However, our finding that Val/Val conferred higher risk (bigger effect size) for depression among groups chronically stressed in adulthood was inconsistent with the findings in other studies that found a larger impact of childhood sexual abuse on adult depressive symptoms in Met carriers than in Val/Val group (Aguilera et al., 2009; Gatt et al., 2009; van Oostrom et al., 2012; Wichers et al., 2008). This inconsistency could stem from the difference in the importance and effects of chronic stress between childhood and adulthood. As previously noted, significant childhood adversity has been differentially related to juvenile vs. adult-onset depression (Jaffee et al., 2002). In addition, most individuals who experience depression in adulthood do not report depression during childhood (Klein et al., 1999) although a significant proportion of depressed children become depressed adults (Harrington et al., 1990; Lewinsohn et al., 2000); and children and adults responded differently to anti-depressant drugs indicating a difference in their biological response to chronic stress (Bylund and Reed, 2007). Also, BDNF levels fluctuate through the lifespan (lower in childhood and rise in early adulthood) and have different functions throughout development, particularly during development, that could result in varying behavioral outcomes (Perea et al., 2012). Czira et al. (2011) reported the association between *BDNF* Val66Met genotype and depression symptoms was restricted to older individuals (> 74 years) and men, suggesting age and gender effects in this association. Retrospective reports of childhood stress are subject to recall bias that was associated with genotype in the studies that found Met carriers more sensitive to effects of childhood stress (Wichers et al., 2008). In our study the *BDNF* Val66Met genotype was not associated with chronic stress in adulthood ($p > 0.241$) in both Duke-CG and MESA samples. The stronger association between stressful life events and prevalence of depression in Met carriers than those with the Val/Val genotype in an elderly Korean sample (Kim et al., 2007) is at variance with our findings in the present

study. The frequency of the Met allele was much higher in this Korean sample (47%) than the frequencies of 17–21% that have been observed in western samples (Kim et al., 2007), suggesting the possibility that effects of the *BDNF* Val66-Met variants on gene function could be different in Asian populations.

The present study had certain limitations. Depressive symptoms were assessed using the CES-D scores, rather than a clinical diagnosis and thus the results may not reflect observations in clinical samples. The measure of chronic stress was different across the two samples and the stress indexed by the measure of chronic burden in MESA was likely to be less severe than that of being a caregiver for a relative with major dementia. Although we adjusted for the use of anti-depressant medications in both samples, it is still possible that the use of anti-depressants may have resulted in an underestimate of depressive symptoms. The evaluation of gene–environment interactions is not a simple matter (Munafo and Flint, 2009; Zammit, Owen, & Lewis, 2010a; Zammit, Wiles, & Lewis, 2010b), and we cannot be certain what mechanism is responsible for our finding that Val/Val genotype is associated with a larger impact of chronic stress on depressive symptoms in our two samples. One possibility is that the effect of Val/Val genotype on *BDNF* expression is different in older persons exposed to current stress than in younger persons exposed to stress in childhood. It is also possible that chronic stress exerts epigenetic effects that modify the influence of Val/Val genotype on *BDNF* expression. More research will be required to identify the responsible mechanism(s). Further research is needed to better understand the biological mechanisms of *BDNF* polymorphisms in brain functioning and in response to chronic stress, and may help us explain the inconsistency of the risk alleles of *BDNF* Val66Met on depressive symptoms.

In conclusion, this study's findings in two independent samples of increased depressive symptoms in persons with the Val/Val genotype who are exposed to chronic stress in adulthood make a strong case for moderation of the association between *BDNF* Val66Met genotype and depression by exposure to chronic stress in adulthood. The fact that both samples in the present study were older also suggests the importance of this gene with regard to symptoms of depression in late life. More research will be required to determine which *BDNF* Val66Met variants are associated with increased depression risk in persons exposed to childhood trauma and whether there are population differences in not only allele frequency but also allele effects on gene function. If the present findings are confirmed in further research, intervention studies could be undertaken to determine whether behavioral interventions that have been found effective in reducing depression symptom levels in persons exposed to chronic stress (Bishop et al., 2005; Williams et al., 2010) will be effective in preventing depression in persons with the Val/Val genotype who are living under conditions of chronic stress. Table 2

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Fig.1.

The mean of SQRT (CES-D) by BDNF Val66Met genotype and caregiving status in Duke-CG, interaction $p = 0.039$.

Fig. 2.
The mean of SQRT (CES-D) by BNDF Val66Met genotype and chronic burden in MESA, interaction $p = 0.005$.

Table 1
Study sample characteristics

	Duke-CG	MESA
Number of samples	238	5524
Age (years, mean $\pm$ SD)	61.7 $\pm$ 13.6	62.2 $\pm$ 10.2
BMI (kg/m ² , mean $\pm$ SD) ^a	26.5 $\pm$ 5.1	28.9 $\pm$ 5.4
Anti-depressant Medication use (%) ^a	43 (18.1)	459 (8.3)
CES-D (mean $\pm$ SD) ^a	10.0 $\pm$ 9.5	7.7 $\pm$ 7.7
<i>Gender</i>		
Men (%)	65 (27.3)	2618 (47.4)
Women (%)	173 (72.7)	2906 (52.6)
<i>BDNF Val66Met genotype</i>		
Val/Val (%)	162 (68.1)	4117 (74.5)
Val/Met (%)	67 (28.1)	1246 (22.6)
Met/Met (%)	9 (3.8)	161 (2.9)
<i>Caregiving stress</i>		
Caregiver (%)	122 (51.3)	
Control (%)	116 (48.7)	
<i>Chronic burden</i>		
0 (%)		1753 (31.7)
1 (%)		1782 (32.3)
2 (%)		1149 (20.8)
3 (%)		516 (9.4)
4 (%)		240 (4.3)
5 (%)		84 (1.5)

^aThe variable was significant different between Duke-CG and MESA samples

Table 2
Model estimates, standard errors (SE) and *p*-values on square root of CES-D scores in both samples

Effect	Duke-CG			MESA		
	Estimate	SE	<i>P</i>	Estimate	SE	<i>P</i>
Intercept	1.854	0.675	0.007	1.887	0.170	<0.0001
Age	-0.008	0.007	0.289	0.003	0.002	0.045
Gender (female as reference)	-0.039	0.223	0.860	-0.243	0.035	<0.0001
Anti-depressant medication	0.329	0.231	0.156	0.409	0.063	<0.0001
BMI	0.038	0.017	0.027	0.004	0.003	0.167
Race = Caucasian				-0.319	0.051	<0.0001
Race = African American				-0.313	0.057	<0.0001
Site = 3				-0.061	0.068	0.376
Site = 4				0.347	0.061	<0.0001
Site = 5				0.078	0.068	0.255
Site = 6				0.191	0.062	0.002
Site = 7				0.084	0.072	0.243
<i>BDNF</i> Val66Met	0.320	0.277	0.250	0.097	0.057	0.088
Chronic Stress	1.402	0.217	<0.0001	0.430	0.017	<0.0001
<i>BDNF</i> Val66Met × Chronic Stress	-0.788	0.379	0.039	-0.091	0.032	0.005

Reference group: no anti-depressant medication use, Val/Val genotype, no chronic stress exposure and females in Duke-CG; no anti-depressant medication use, Val/Val genotype, no chronic stress exposure, female Hispanic at site = 8 in MESA.