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Brain-Derived Neurotrophic Factor (BDNF) Val66Met Polymorphism Interacts with Gender to Influence Cortisol Responses to Mental Stress

Rong Jiang, PhD, Michael A. Babyak, PhD, Beverly H. Brummett, PhD, Ilene C. Siegler, PhD, MPH, Cynthia M. Kuhn, PhD, and Redford B. Williams, MD

Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, North Carolina, USA

Abstract

Brain-derived neurotrophic factor (BDNF) Val66Met polymorphism has been associated with cortisol responses to stress with gender differences reported, although the findings are not entirely consistent. To evaluate the role of Val66Met genotype and gender on cortisol responses to stress, we conducted a 45-min mental stress protocol including four tasks and four rest periods. Blood cortisol was collected for assay immediately before and after each task and rest period. A significant two-way interaction of Val66Met genotype $\times$ gender ($P = 0.022$) was observed on the total area under the curve (AUC), a total cortisol response over time, such that the Val/Val genotype was associated with a larger cortisol response to stress as compared to the Met group in women but not in men. Further contrast analyses between the Val/Val and Met group for each stress task showed a similar increased cortisol pattern among women Val/Val genotype but not among men. The present findings indicate the gender differences in the effect of Val66Met genotype on the cortisol responses to stress protocol, and extend the evidence for the importance of gender and the role of Val66Met in the modulation of stress reactivity and subsequent depression prevalence. Further studies and the underlying mechanism need to be investigated, which may provide an insight for prevention, intervention, and treatment strategies that target those at high risk.

Keywords

BDNF; Val66Met; rs6265; cortisol; HPA axis; gender differences

Corresponding author: Rong Jiang, Address: Room 47-118, 300 N. Duke St., Durham, NC 27701, USA, Tel: 919-684-0741, Fax: 919-681-8960, rong.jiang@duke.edu.

Conflict of interest

All authors declare no conflict of interest, except that Redford B. Williams is a founder and major stockholder in Williams LifeSkills, Inc.

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1. Introduction

Cortisol secretion during activation of the hypothalamic-pituitary-adrenal (HPA) axis is an important physiological process that occurs following exposure to psychological stress (Sapolsky et al., 2000). Enhanced HPA axis responses to stress have been associated with various stress-related disorders, including depression, anxiety, schizophrenia, substance use disorders, and a number of medical disorders (Herbert, 2013; McEwen, 2008). Brain-derived neurotrophic factor (BDNF), a critical regulator of the formation, plasticity, and integrity of neurons in brain, has been shown to be involved in regulating the HPA responses to stress (Naert et al., 2006; Taliaz et al., 2011) and in the neuronal repair from the stress-induced neuronal damage (Bergstrom et al., 2008). BDNF and its receptor, TrkB, are both expressed in the hypothalamic paraventricular nucleus (PVN) (Castren et al., 1995; Tapia-Arancibia et al., 2004) and many other brain regions. Although the precise mechanism linking BDNF to HPA axis activity remains unclear, animal studies have shown that HPA axis activity was maintained by both BDNF/TrkB and glucocorticoid signaling in the hypothalamic corticotropin releasing hormone (CRH) neurons (Jeanneteau et al., 2012). BDNF, through TrkB-cAMP response-element binding protein (TrkB-CREB) signaling, induces the expression of CRH, whereas glucocorticoids, via glucocorticoid receptor (GR) signaling, deactivate CREB-mediated CRH induction by neutralizing the function of the CREB coactivator CRTC2. Given similarities in mammalian neural function, in the present study we explore the possible association between BDNF variation and cortisol expression in humans under conditions of laboratory-induced psychological stress.

One SNP variant, Val66Met (rs6265), producing an amino acid substitution (valine to methionine) at codon 66 on BDNF, has been shown to alter BDNF expression, processing, and BDNF-TrkB signaling (Chiaruttini et al., 2009; Sanchez et al., 2011), and thus may affect HPA axis function and cortisol levels after exposure to stress. Val66Met has been associated with cortisol responses to stress, although, as shown in Table 1, the risk allele is inconsistent. One study reported that persons with the Met/Met genotype exhibited significantly higher HPA axis activity (higher serum cortisol levels) during a drug challenge (dexamethasone/CRH test) than Val allele carriers (Schule et al., 2006). In contrast, another study found male carriers of Met allele had a significantly attenuated HPA axis and cardiovascular reactivity to a psychosocial stressor compared to subjects with Val/Val genotype (Alexander et al., 2010). Male Met allele carriers also reported lower levels of self-reported perceived stress and nervousness in this study. Gender differences have been studied with respect to Val66Met genotype on stress response, but the results are mixed. One study (Shalev et al., 2009) report that men with the Val/Val genotype had a larger rise in salivary cortisol during a public speaking stressor, but, in contrast, women with the Val/Val genotype showed a smaller cortisol rise. Another study found larger cortisol increases in Met carriers before a cold pressure stress, with men and women Met carriers showing similar cortisol increase (Colzato et al., 2011), indicating the cortisol levels increased in response to the anticipating stressful event, rather than to the physical stress procedure itself. In another study of *BDNF* Val66Met association with the salivary cortisol responses to laboratory induced social stress, both women and men with the Met/Met genotype showed a larger cortisol responses than those with Val variant (Tsuru et al., 2014). Gender differences were

also found in an animal study, in which a striking increase in depression-like behavior was observed in female conditional BDNF knockouts, but no effect was observed in male conditional knockouts (Monteggia et al., 2007).

The findings from research evaluating effects of the *BDNF* Val66Met polymorphism, as well as the gender differences, on both HPA axis to acute and chronic stress produce a mixed picture. In our prior research, we found, in two independent samples, that exposure to chronic stress was associated with a larger increase in depressive symptoms among persons with the Val/Val genotype compared to Met allele carriers (Jiang et al., 2013). Neither gender nor race moderated this effect of Val66Met genotype on depressive symptoms in persons exposed to high levels of chronic stress. In the present study, we tested the hypothesis that *BDNF* Val/Val was associated with larger cortisol responses to stress protocol, and this association may differ as a function of gender.

2. Materials and methods

2.1 Participants

The participants were recruited in a study designed to examine the effects of genetic, behavioral and environmental mechanisms on health disparities (Brummett et al., 2012). The study was conducted at Duke University Medical Center (DUMC). All subjects gave informed consent using a form approved by DUMC Institutional Review Board prior to their participation. All participants underwent a comprehensive psychological and medical examination to exclude subjects with current medical and psychiatric disorders. Those using prescribed (including contraceptives) and illegal drugs (as detected by a urine screen prior to entry into study) were also excluded. No pregnant women were included and all women were tested during the follicular phase of their menstrual cycle. The participants were randomized to either tryptophan enhancement or tryptophan depletion for the initial study purpose, with sham infusion (saline) or depletion (a “milkshake” fortified with a full complement of amino acids) on the first test day, followed by active infusion or depletion on the second test day. The present study focused on the first sham day, consisted of a sham tryptophan infusion or sham enhancement, followed by a mental stress protocol. The current study sample consisted of 156 (87 men, 69 whites) participants with a mean age of 34 years (range 18–49).

2.2 Study procedure and Mental stress protocol

Our detailed study procedure has been described elsewhere (Brummett et al., 2014; Brummett et al., 2012; Williams et al., 2001; Williams et al., 2008). On the sham day, beginning at 6:30 a.m. participants were not allowed food or liquids, nor were they allowed to smoke, until the completion of all study procedures at approximately 1:30 p.m. At 7:00a.m., for tryptophan sham infusion participants, an IV (D5 W/.5 N saline) was started and kept running at 50 cc per hour till 1:30 p.m. For sham infusion participants, they were required to consume a milkshake that consisted of all essential amino acids. All participants were seated in a reclined position and activity was limited to watching National Geographic videos or playing cards until noon. All participants had an indwelling IV catheter in place for drawing blood throughout the entire session. At noon, the baseline cortisol blood sample

was drawn followed by a break. Then the 45-minute mental stress protocol was proceeded at approximately 12:45 p.m. as follows: 5-min baseline rest period, 5-min public speaking task (READ1, reading aloud an emotionally neutral text in the presence of the experimenter), 5-min rest period (RECOVER1), 5-min anger recall task (ANGER, recalling and describing an incident that made them extremely angry), 5-min rest period (RECOVER2), 5-min 2nd public speaking task (READ2), 5-min rest period (RECOVER3), 5-min sadness recall task (SAD, recalling and describing an incident made them extremely sad), and 5-min rest period (RECOVER4). Blood samples were drawn for cortisol assay immediately before the mental stress protocol started and after each task and rest period. Although various stress tasks, such as the Trier Social Stress Test (TSST), have been commonly used in research on physiological effects of acute stress, there has also been extensive use of anger recall as a stressor in studies of cardiovascular reactivity to acute stress (Gerin et al., 2006). The TSST is just one of many laboratory stressors that evoke a measurable HPA response in humans. There is some evidence that the TSST is superior to purely cognitive tasks or public speaking alone that activate a single stressor domain in terms of eliciting an HPA responses, but not tasks (e.g., Mannheim multicomponent stress test) that include negative emotion induction (Allen et al., 2014). It is shown that acute stressors that elicit increased anger are more likely to elicit increased cardiovascular reactivity in persons with high hostility than are stressors like mental arithmetic public speaking, TSST, etc. that do not elicit anger (Suarez and Williams, 1990). Our stress protocol provides for elicitation of negative emotions -- an effect that was confirmed in our prior publication (Brummett et al., 2014; Brummett et al., 2012) by increases in profile of mood states (POMS) anger and depression ratings, as well as having two research assistants present during the stress protocol, thereby “adding an element of public speaking stress” similar to that included in the TSST. Moreover, as Hu et al. (Hu et al., 2016) note, stressors that involve primarily cognitive challenge can affect autonomic reactivity in ways that differ markedly from stressors that elicit negative emotional activation. We have used the current stress protocol in several prior studies and there was a significant increase in plasma cortisol across the 45-min stress protocol (Brummett et al., 2014; Brummett et al., 2012) that is in the same range of the salivary cortisol responses to public speaking stress and TSST reported in the literature (Alexander et al., 2010; Shalev et al., 2009). In addition we have shown significant effects of this stress protocol on cardiovascular reactivity (Brummett et al., 2009; Williams et al., 2001) positive emotions (Brummett et al., 2009), and inflammatory function (Brummett et al., 2010) as well.

2.3 Cortisol

Blood samples were spun for 15 minutes in a refrigerated centrifuge and plasma was transferred into polypropylene tubes with 0.05 ml glutathione and then frozen at -70°C . The samples were processed at the Clinical Research Unit at DUMC. Cortisol was measured by specific radioimmunoassay (RIA) using Coat-A-Count kit from Diagnostic Products Inc. Inter-/Intra- assay coefficients of variation were less than 10% and 5%, respectively. As reviewed by Kuhn (Kuhn, 1989) and Hellhammer et al. (Hellhammer et al., 2009) a strong rationale for measuring blood rather than saliva in stress studies is the significant time lag in the increase in cortisol in saliva after induction of a stressor. One limitation of using saliva is that the magnitude of increases can be exaggerated once the capacity for binding to CBG is

exceeded, as the ratio of unbound to bound changes rapidly at that point. In addition, CBG-bound cortisol may play a bigger role in cortisol responses in tissues than previously thought (Hellhammer et al., 2009; Levine et al., 2007). Saliva measures are most useful for looking at responses that are not tightly time-synched like circadian rhythms, baseline levels, the awakening response (and even there it has its weaknesses) or in populations like children in which blood sampling is inadvisable. The advantage of using saliva is that is non-invasive, an element of experimental design that was not necessary in our current study, as blood was being collected for other purposes. There is no disadvantage to using blood in this experimental design, and decades of research against which to compare it to other studies.

2.4 Genotyping

Genomic DNA was extracted for genotyping by standard procedure using frozen blood samples from participants. The genotyping of *BDNF* Val66Met (rs6265) was conducted with 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 met the quality control established in the lab and the genotyping call rate was greater than 95%. The *BDNF* Val66Met genotype distribution was in Hardy-Weinberg equilibrium ($P = 0.11$).

2.5 Statistical Analyses

All statistical tests were performed using SAS 9.3 (SAS, Cary, NC). Cortisol was operationalized as the area under the curve (AUC) to the ground, a reliable index of total cortisol production across the time (Khoury et al., 2015), from the baseline assessment to the end of the stress protocol using the trapezoid method (Pruessner et al., 2003). The genotypic data were collapsed into Val/Val vs. Val/Met plus Met/Met due to the small cell size of Met/Met. The primary analysis was carried out with the general linear model, in which cortisol AUC was the response variable. Initial models included predictors of age, gender, race, sham tryptophan enhancement or sham depletion status, Val66Met genotype (Val/Val vs. Val/Met + Met/Met), and baseline cortisol level, a three-way interaction term of Val66Met, race, and gender, and the three subordinate two-way interaction terms with Val66Met, race, and gender. If the three-way interaction term was not significant, we re-estimated the model without that term, including only the subordinate two-way terms. We supplemented the AUC analysis by also estimating a repeated measures mixed model (PROC MIXED with REPEATED statement) as a second analysis to examine the association of *BDNF* Val66Met genotype and gender with cortisol levels over the course of mental stress protocol. Cortisol was natural logarithm transformed in order to normalize the distribution of model residuals. In the mixed model, we focused our tests on gender and genotype differences of cortisol levels measured at the occasions of the stress tasks. Therefore, the CONTRAST statement in PROC MIXED was used to look at the group difference between each genotype $\times$ gender group at the each task time point. The mixed models used the UNSTRUCTURED covariance type. The level of significance was at 0.05 (two tailed) for all statistical analyses.

3. Results

3.1. Demographic statistical characteristics stratified by Val66Met genotypes

The demographic statistical characteristics of the sample are shown in Table 2 stratified by Val/Val ($n = 131$) and Met carriers (Met/Met: $n = 2$ and Val/Met: $n = 23$). Demographics were similar ($P_s > 0.16$) between Val66Met genotypes except race. The Met genotype frequency in African Americans was smaller than in Caucasians ($n = 6$, 6.9% in African American vs. $n = 19$, 27.5% in Caucasian; $P < 0.001$). The overall baseline of cortisol level was 87.3 ± 37.5 ng/ml with the range from 30.7 – 234.6 ng/ml.

3.2 Impact of Val66Met $\times$ gender on the AUC

We observed non-significant three-way interaction for the race, gender, and genotype ($P=0.152$). After eliminating the three-way interaction term, the linear model with subordinate two-way interaction results show a significant gender by Val66Met genotype interaction on cortisol AUC ($P=0.013$) over the mental stress protocol, while the other two interactions were not significant (race by genotype $P=0.953$; race by gender $P=0.233$). After removing the two non-significant two-way interactions in the linear model, the gender by Val66Met genotype interaction on cortisol AUC was still significant ($P=0.022$). Figure 1 shows the adjusted mean cortisol AUC for each Val66Met genotype in each gender. In women, the adjusted mean cortisol AUC was significantly higher in those with Val/Val genotype (mean AUC=184.4, 95% CI=181.9, 187.0) than in Met carriers (mean AUC=178.4, 95% CI=173.4, 183.3). The difference between the two groups was 6.1 (95% CI=0.4, 11.7), which was statistically significant ($P = 0.03$); while there was no significant genotype effect in men.

3.3 Group contrasts between Val66Met genotypes after each task

Further contrast analyses in the mixed model were consistent with the AUC result, showing a similar pattern (Figure 2): among women Val/Val genotype was associated with higher cortisol levels across the stress protocol, while no association was seen among men. The adjusted means and 95% confidence intervals (CIs) of the contrasts of group difference (Val/Val versus Met) for cortisol level in response to each task, stratified by female and male, are presented in Table 3. In women, the cortisol levels in Val/Val carriers after anger recall (ANGER, $P=0.018$), the following rest (RECOVER2, $P=0.041$), the 2nd public reading (READ2, $P=0.017$), the following rest (RECOVER3, $P=0.036$), and the rest following sad recall (RECOVER4, $P=0.043$) were significantly higher than those in Met carriers. The cortisol level was marginally higher in Val/Val women after sad recall task (SAD, $P=0.066$) compared to Met women. The contrasts after the 1st 5-minute public reading task (READ1, $P=0.265$) and the 1st 5-minute rest (RECOVER1, $P=0.162$) were not statistically significant. We also looked at the contrast of the average across the eight stress tasks between Val/Val and Met groups (Table 3, last row), and the cortisol level in female Val/Val group was significantly higher than female Met group (log transformed difference = 0.16, $P = 0.026$). In contrast, as shown in Table 3, among men there was no significant difference between Val66Met genotypes in cortisol levels in response to any tasks ($P > 0.20$). Calculating the geometric mean cortisol level from the log-transformed values, the mean cortisol across the stress protocol, adjusted for race, age, tryptophan enhancement or depletion status, and

baseline cortisol level, was 101.7 ng/ml (log-transformed value = 4.62, standard error = 0.08) in the Val/Val female group, compared to 86.5 ng/ml (log-transformed value = 4.46, standard error = 0.05) in the Met female group. The increase in cortisol from the baseline to the average level during the stress protocol was 25.2 ng/ml in the Val/Val group, compared to the decrease of 8.7 ng/ml in the Met group.

4. Discussion

The results in this study showed that compared to Met carriers, the Val/Val genotype was associated with a larger cortisol responses to acute mental stress in women but not in men, indicating the gender differences in the effect of Val66Met polymorphism on cortisol reactivity to stress. The greater adverse effect of stress on cortisol responses to stress in women Val/Val genotype was consistent with our previous finding in two independent samples that chronic stress was associated with a larger increase in depressive symptoms in both women and men with Val/Val genotype compared to Met carriers (Jiang et al., 2013). The association found in the current study of Val/Val genotype with larger cortisol during stress exposure could be one mechanism underlying the observed association of the Val/Val genotype with higher levels of negative mood and depressive symptoms in persons exposed to acute (Alexander et al., 2010) and chronic stress (Jiang et al., 2013) compared to Met carriers. Studies have shown, for example, that a higher level of cortisol is a risk factor for subsequent depressive disorder in both adolescents and adults (Goodyer et al., 2000; Harris et al., 2000). Furthermore, increased risk for subsequent onset of depression was found only in carriers of the Val/Val genotype who also had higher morning waking cortisol (Goodyer et al., 2010). Both subscales of POMS anger and depression ratings were increased after the stress protocol, but no significant difference between Val/Val and Met carriers (data not shown). The POMS depression subscale change before and after stress protocol was also correlated with cortisol change between before and after stress protocol ($P = 0.026$), adjusted for age and race. In our previous study, we also reported that the increases in POMS anger and depression ratings were mediated by the larger cortisol responses associated with another gene variant in men (Brummett et al., 2012). Based on the present finding of higher cortisol levels in Val/Val women during mental stress protocol, along with our prior findings of higher depression in persons with Val/Val genotype exposed to higher levels of chronic stress (Jiang et al., 2013), and the POMS depression increase correlated with cortisol increase, it is possible to speculate that one potential mechanism underlying the association between stress and depression may be due to larger HPA axis responses to stress with the Val66Met Val/Val genotype, at least in women.

The present findings show that the Val/Val was associated with increased cortisol levels during acute mental stress only in women, but not in men. Some studies have shown that females exhibit higher levels of glucocorticoids (cortisol or corticosterone) as compared to males (Lightman et al., 2008; Netherton et al., 2004; Seale et al., 2004). Postpubertal women show an increase of major depressive disorder which could be partly resulting from their increased cortisol levels (Herbert, 2013). Furthermore, gender differences have been documented as early as the neonatal period, at all individual levels of the HPA axis, as well as in the conglomerate function of the axis (Panagiotakopoulos and Neigh, 2014). Sex hormones, such as estrogen, have been demonstrated to modulate BDNF expression in brain

in animal studies (Hill et al., 2013; Sasahara et al., 2007; Sohrabji et al., 1995). In vivo, BDNF mRNA was rapidly up-regulated in the cerebral cortex in ovariectomized animals exposed to estrogen (Sohrabji et al., 1995). Estrogen administration to neonatal mice increased the BDNF level in the cerebellum, whereas tamoxifen (estrogen antagonist) decreased the BDNF level (Sasahara et al., 2007). It is possible, therefore, that the effect of Val/Val genotype on increased cortisol responses to mental stress only found in women might be due to BDNF expression increased by estrogen in women.

To date there is conflicting evidence regarding gender differences in cortisol response to stress in human, with some studies showing no difference, with others reporting higher cortisol in either gender. The larger increase in cortisol levels found in this study among Val/Val women only, for example, contrasts with the finding by Shalev et al. in which male Val/Val homozygotes showed a greater rise in salivary cortisol than Val/Met during the Trier Social Stress Test, while female Val/Vals showed a smaller response (Shalev et al., 2009). The equivocal evidence in human studies may in part result from methodological difference, for instances, the participant tasks or stimulus characteristics. The stressors used to test the HPA axis have a wide diversity from psychological stress to pharmacological means. Stround et al. showed that women had greater cortisol elevations to social rejection challenges, while men had higher cortisol responses to achievement challenges (Stround et al., 2002). Other studies have shown higher elevations of cortisol in women, especially when the stressor is pharmacologic in nature (Born et al., 1995; Greenspan et al., 1993). Furthermore, BDNF levels fluctuate through the lifespan (lower in childhood and rise in early adulthood) and have different functions throughout development that could result in varying response to stressor (Perea et al., 2012). The serotonin transporter polymorphism 5HTTLPR has been shown to interact with BDNF Val66Met in affecting HPA axis function (Dougherty et al., 2010), an epistasis effect could influence the sexual dimorphism as well. Other environmental factors like smoking may influence the stress response as Jamal et al. (Jamal et al., 2015) reported that Val/Val nicotine-dependence smokers had more severe symptoms of depression and anxiety than the other groups, but no difference in Met carriers. In this study sample, the participants were not allowed to smoke during the stress protocol, but the smoking status in the screening procedure measured as who had been smoking ever or not without nicotine dependent smoker information. The smoker frequency was not different between women (47.8%) and men (47.1%) ($P = 0.93$) or between Val/Val (47.3%) and Met (48.0%) genotype ($P = 0.95$). There was no significant genotype $\times$ smoking on any POMS anxiety or POMS depression at any stage. Differences in the factors reviewed above could be playing a role findings of gender differences in the effect of Val66Met genotype on the HPA reactivity to stress and future studies are need to understand the effects of these factors.

The present study has several limitations. First, the modest sample size raises the possibility of unstable estimates of association, and thus requires replication in additional samples. Second, our sample comprised of 18–49 years old participants. The observed associations may not be generalizable to age groups outside of this range. Third, we did not have ancestry markers available to account for possible population stratification. Again, gene-gene interaction effect in the HPA axis function was not accounted for, and, therefore, the current

findings can only be interpreted a SNP-specific genetic association without any influences of other genetic variations.

Prior research has shown that cognitive behavioral stress management (CBSM) training produces reductions in cortisol responses to TSST as well as reductions in both negative emotions and afternoon serum cortisol levels in women undergoing treatment for breast cancer (Antoni et al., 2009). Group-based CBSM has also been shown to reduce mortality in CHD patients (Gulliksson et al., 2011; Orth-Gomer et al., 2009). Higher cortisol responses to acute stress (Stroop and mirror tracing) was associated with greater extent of coronary artery calcification in healthy men and women (Hamer et al., 2010), therefore, the present findings that Val/Val women had higher cortisol responses to stress suggests they might also be at higher risk of developing CHD, which could be reduced by CBSM effects to reduce their elevated cortisol reactivity to stress. For example, a clinical trial of CBSM in CHD patients with Val/Val genotype women could result in improved prognosis.

To summarize, the present results indicate there are gender differences in the effect of Val66Met genotype on the cortisol responses to a stress protocol that increases negative affects. The higher cortisol level only in Val/Val women during the stress protocol, together with our previous findings, suggests a potential mechanism that the association between stress and depression may be mediated by increased HPA axis activity in response to stress, at least in women. The present findings extend the evidence for the importance of gender and the role of Val66Met in the modulation of stress reactivity. It is imperative to further understand the mechanisms underlying gender differences in BDNF Val66Met effects on HPA responses stress that could provide an insight for prevention, intervention, and treatment strategies that target those at high risk.

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Highlights

- The role of Val66Met genotype and gender on cortisol response to stress was evaluated.
- A 45-min stress protocol with four tasks and four rest periods was conducted.
- Significant Val66Met genotype $\times$ gender on total cortisol was observed.
- Val/Val carriers had a larger cortisol to stress than Met group in women, not in men.
- Provide an insight for prevention and treatment strategies that target those at high risk.

Figure 1.
BDNF Val66Met genotype in relation to cortisol area under the curve (AUC, mean and SE) in women and men.

Figure 2. Blood cortisol level (geometric means and 95% CIs) after each task during the mental stress protocol from the repeated mixed model adjusted for race, age, tryptophan enhancement or depletion status, and baseline cortisol level.

Table 1

Literature summary on BDNF genotype and stress on cortisol levels.

Literature	Genotype Frequency	Gender	Ethnicity	Stress	Cortisol	Finding
Schüle et al., 2006	Val/Val:Val/Met: Met/Met = 10:65:12	86 men, 101 women	not specified	Dexamethasone/CRH drug challenge	Blood	Met/Met carriers showed a higher cortisol levels than Val/Val or Val/Met genotype carriers. This pattern was shown in both women and men.
Shalev et al., 2009	Val/Val:Val/Met = 68:29	46 men, 51 women	European Caucasian/Israeli	Tier Social Stress Test	Salivary	In males, Val/Val carriers showed a greater rise in cortisol than Val/Met, while in females, the opposite trend was observed. The same pattern was observed for blood pressure and heart rate.
Alexander et al., 2010	Val/Val:Val/Met: Met/Met = 66:30:4	Only in men	European Caucasian/German	Public speaking	Salivary	Met carriers (Val/Met and Met/Met) had a significantly attenuated cortisol response and heart rate reactivity to stressor compared to Val/Val genotype.
Colzato et al., 2011	Val/Val: Met = 59: 39	44 men, 54 women	European Caucasian/Netherlander	Cold pressure test for one hand	Salivary	Met carriers showed more anxious, nervous and insecure during the cold pressure test than Val/Val, and higher anticipatory cortisol in response to the painful experience before cold test, not to the cold test. No gender difference.
Tsuru et al., 2014	Val/Val:Val/Met: Met/Met = 46:85:97	138 men, 88 women	Asian/Japanese	Tier Social Stress Test	Salivary	Met/Met women and men had stronger cortisol responses than Val/Met and Val/Val individuals in the TSST.

Table 2

Statistic characteristics stratified by Val66Met genotype.

	Val/Val (n =131)	Met (Val/Met =23) (Met/Met=2) (n =25)	P
Women (%)	54 (41.2)	15 (60.0)	0.162
Caucasian (%)	50 (38.2)	19 (76.0)	0.001
Age, years (SD)	33.6 (8.2)	35.3 (9.5)	0.233
Baseline cortisol, ng/ml, (SD)	86.6 (37.2)	90.9 (40.0)	0.609
Sham Tryptophan Enhancement (%)	61 (46.6)	15 (60.0)	0.260

Table 3

The means and 95% confidence intervals (CIs) of the contrasts for log-transformed cortisol level between Val/Val and Met genotypes after each task during the mental stress protocol stratified by women and men.

Contrast (Val/Val - Met)	Women			Men		
	Mean (Incortisol)	95% CI	P	Mean (Incortisol)	95% CI	P
Read1	-0.066	-0.183, 0.051	0.265	-0.079	-0.200, 0.041	0.195
Recover1	0.103	-0.042, 0.247	0.162	0.055	-0.103, 0.213	0.495
Anger	0.208	0.036, 0.380	0.018	0.043	-0.165, 0.252	0.683
Recover2	0.206	0.009, 0.403	0.041	0.003	-0.217, 0.222	0.981
Read2	0.224	0.040, 0.408	0.017	-0.011	-0.216, 0.195	0.919
Recover3	0.208	0.013, 0.402	0.036	0.037	-0.178, 0.252	0.735
Sad	0.183	-0.012, 0.378	0.066	0.035	-0.192, 0.262	0.762
Recover4	0.214	0.007, 0.421	0.043	0.052	-0.186, 0.291	0.666
Average	0.160	0.019, 0.300	0.026	0.067	-0.578, 0.713	0.837

95%CI = 95% confidence interval; P = p value; READ1 = 1st 5-min public speaking task; RECOVER1 = 1st 5-min rest period; ANGER = 5-min anger recall task; RECOVER2 = 5-min rest period after anger recall; READ2 = 2nd 5-min public speaking task; RECOVER3 = 5-min rest period after 2nd public speaking task; SAD = 5-min sadness recall task; RECOVER4 = 5-min rest period after sad recall task.