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Variant brain-derived neurotrophic factor Val66Met endophenotypes: implications for posttraumatic stress disorder

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Recently, a common single nucleotide polymorphism (SNP) has been identified in the gene encoding brain-derived neurotrophic factor (BDNF). The variant $BDNF_{Met}$ has been shown to have decreased activity-dependent BDNF secretion from neurons and to lead to impairments in specific forms of learning and altered susceptibility to stress. A mouse model containing $BDNF_{Met}$ has also been linked to increased anxiety-like behavior. In a translational study, mice and human carriers of the $BDNF_{Met}$ allele were compared in their ability to extinguish a learned fear memory. Both showed slower suppression of the learned fear response. In humans, the neural correlates of this behavior were validated using fMRI. As anxiety and fear extinction lie at the core of symptoms and therapeutic approaches to posttraumatic stress disorder (PTSD), we propose that BDNF genotype and neuroimaging may be useful as biomarkers to provide guidance for more customized therapeutic directions. The aim of this paper is to review the available knowledge on the BDNF Val66Met SNP, with emphasis on anxiety- and fear-related endophenotypes and its potential implications for PTSD.

Keywords: BDNF; Val66Met; anxiety; PTSD; fear extinction

Introduction

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of polypeptide growth factors, is widely expressed in the developing and adult mammalian brain and has been identified as a key regulator of neuronal development within the central nervous system.[1,2] In recent years, BDNF has been implicated in the development and treatment of a number of psychiatric disorders, including depression, anxiety, and eating disorders. In this review, we will focus on a recently identified single nucleotide polymorphism (SNP) that has been identified in the gene encoding BDNF. We specifically examine its role in the development of emotional and cognitive disorders. We will then expand upon this wealth of recent work to provide an argument for the potential role for this BDNF variant in additional forms of psychiatric disorders with their roots in emotional dysregulation, specifically posttraumatic stress disorder (PTSD).

BDNF is synthesized as a precursor protein (proBDNF) that is proteolytically cleaved to generate mature BDNF.[3] Throughout life, BDNF influences the proliferation, differentiation, morphology, and functional activity of neuronal cells. BDNF action is dictated by its binding to either of two functionally different classes of cell surface receptors, the TrkB receptor tyrosine kinase or the p75 neurotrophin receptor ($p75^{NTR}$), a member of the tumor necrosis factor receptor super family.[1] ProBDNF is preferentially bound by $p75^{NTR}$, whereas mature BDNF preferentially binds to the TrkB receptor.[4,5] BDNF binding to the TrkB receptor

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produces neurotrophic responses through rapid activation of the PI-3 kinase, Ras/MAPK, and PLC-γ pathways, thus influencing transcriptional events affecting the cell-cycle, neurite outgrowth, and synaptic plasticity.[6–9] ProBDNF binding to the $p75^{NTR}$ gives rise to an increase in JNK (c-Jun N-terminal kinase) and NF-κB (nuclear factor κB), which triggers apoptosis, axonal retraction, or the pruning of dendritic spines.[10]

As mentioned, an SNP in the gene encoding BDNF has recently been identified. This SNP results in an amino acid change from a valine (Val) to a methionine (Met) at position 66 (Val66Met) in the prodomain of BDNF ($BDNF_{Met}$). Thus far, this SNP in the BDNF gene has only been found in humans. The frequency of the $BDNF_{Met}$ allele is relatively common and is ethnically stratified. Approximately, 50% of Asians, 30% of Caucasians, and 4% of African Americans carry at least one $BDNF_{Met}$ allele.[11,12] In Asian and Caucasian populations, the incidence of homozygous $BDNF_{Met}$ allele carriers is around 20% and 4%, respectively.[13]

Impact of BDNF Val66Met on BDNF availability

The molecular and cellular effects of BDNF Val66Met have been studied using a number of model systems, including cell culture and animals models. The initial work was carried out in *in vitro* cell culture systems. Transfection of $BDNF_{Met}$ into neurons does not alter total levels of BDNF.[14,15] This was shown in neuronal cultures from mice in which $BDNF_{Met}$ was genetically knocked into the endogenous BDNF locus. Specifically, $BDNF_{Met}$ was less efficiently trafficked to neuronal processes and 20–30% less BDNF was released under depolarizing conditions in cells from hetero- and homozygous $BDNF_{Met}$ carriers, respectively.[14] A number of groups hypothesized that $BDNF_{Met}$ could lead to less efficient sorting of BDNF into secretory granules. Subsequently, it was demonstrated that $BDNF_{Met}$ bound less efficiently to the protein sortilin, a molecule implicated both in the trafficking of BDNF in the biosynthetic pathway and as a coreceptor with $p75^{NTR}$ that binds proBDNF.[5,16]

Impact of BDNF Val66Met on neuronal survival and morphology

As mentioned previously, BDNF plays a significant role in the development of neuronal cells. To study the potential role of BDNF Val66Met on neuronal development, we and another lab developed lines of mice in which the BDNF Val66Met allele was genetically knocked into mice in a targeted fashion. In our lab, the endogenous mouse BDNF gene was replaced with a modified version of the mouse BDNF gene containing the Val66Met SNP.[14] In a second lab, Cao and colleagues developed a mouse in which the human version of the BDNF gene containing the Val66Met SNP was genetically knocked into the mouse genome.[17] To study the impact of BDNF Val66Met on neuronal birth, we carried out a series of studies in which we tracked rates of proliferation of new cells within the subventricular zone and their eventual migration and survival within the olfactory bulb (OB) in wild-type and $BDNF_{Met}$ homozygous mice. Interestingly, we found that $BDNF_{Met}$ led to a significant reduction in the survival of newly born cells in the OB, suggesting that altered BDNF availability as a result of the Val66Met SNP could lead to a reduction in the birth of new cells in the adult brain.[18] In a separate series of studies, Cao and colleagues demonstrated that, during development, $BDNF_{Met}$ alters the ability of axons to survive within the developing olfactory system, indicative of potentially significant effects on axonal development throughout the brain.[17] Finally, using Golgi impregnation, we have demonstrated that the complexity of dendritic arbors of neurons in the hippocampus is significantly less elaborate in $BDNF_{Met}$ homozygous mice compared to wild-type mice.[14] This reduction in dendritic complexity mirrors that of mice that have been exposed to chronic stress regimens.[19] In these same $BDNF_{Met}$ mice, we found that in the hippocampus, a BDNF rich region, there was a significant reduction in volume. These data replicated findings from human imaging studies in which $BDNF_{Met}$ carriers were found to have reduced hippocampal volume compared to age- and sex-matched controls.

BDNF Val66Met leads to altered neuronal function

BDNF has been implicated in the electrical plasticity of neurons, specifically in the processes of long-term potentiation (LTP) and long-term depression (LTD). In a recent series of experiments, we examined whether and how the BDNF Val66Met polymorphism affects hippocampal neurotransmission and synaptic plasticity using mice homozygous for the $BDNF_{Met}$ allele. We found

that both young and adult $BDNF_{Met}$ homozygous mice exhibited a decrease in TBS (theta-burst stimulation)-induced LTP at the CA3-CA1 synapses. We also observed a decrease in N-methyl-D-aspartate (NMDA) receptor-dependent LTD in these mice. These data suggest that in human $BDNF_{Met}$ carriers, electrophysiological processes associated with memory function could be disrupted as a result of the BDNF Val66Met SNP.[20]

BDNF Val66Met is associated with altered hippocampal memory function

$BDNF_{Met}$ has been associated with alterations in hippocampal plasticity and morphology. In a study of human schizophrenic patients and age-matched controls, Egan and colleagues demonstrated that $BDNF_{Met}$ allele carriers have impairments in an episodic memory task.[15] Subsequently, these findings were in part replicated by Dempster *et al.*[21] using a healthy control group. This same task, when tested using functional magnetic resonance imaging (fMRI), demonstrated that $BDNF_{Met}$ homozygous individuals had reduced hippocampal activation compared with controls and had a gene-dose-dependent reduction in *n*-acetyl aspartate, an intracellular marker of neuronal activity, indicating potential hippocampal dysregulation. These findings were later confirmed in another fMRI study by the same group.[22] They found again that $BDNF_{Met}$ carriers showed a relative decrease in hippocampal activation during encoding and retrieval of a declarative memory task. The $BDNF_{Met}$ allele carriers also made more errors on a retrieval memory task. A separate group in Australia found a similar decrease in hippocampal gray matter in $BDNF_{Met}$ carriers and also found that $BDNF_{Met}$ homozygous individuals make more errors on a verbal recall task.[23] Finally, in a fMRI study, Sambataro and colleagues found that $BDNF_{Met}$ allele carriers show a steeper decline in age-related hippocampal activation during a declarative memory task.[24]

To get a clearer picture of the potential effects of the $BDNF_{Met}$ allele on memory function, we used our knockin BDNF Val66Met mouse model. This manipulation allows us to assay memory function on genetically homogenous background as well as control many of the potential environmental factors that may impact gene function and neural development. Using a contextual fear-conditioning task, we tested mice on their ability to recall and generate a fear response to a context in which they previously received a series of three footshocks. This task has previously been shown to rely on the hippocampus. We found that contextual fear memory was significantly impaired in $BDNF_{Met}$ homozygous mice. These data provide additional convergent evidence for a role for BDNF Val66Met and alterations in hippocampal memory function.[14]

BDNF Val66Met and affective disorders

Human studies attempting to link the Val66Met SNP with affective/anxiety disorders have resulted in mixed results. A 2008 meta-analysis focusing on the Val66Met polymorphism and anxiety-related traits reported no significant association between the SNP and anxiety disorder or with harm avoidance, a trait that is thought to be closely associated with anxiety and depression.[25] They found that healthy $BDNF_{Met}$ carrying subjects had significantly lower neuroticism scores than noncarriers. However, in these studies the sample and effect sizes were small. Another recent meta-analysis found no overall association between carrying the $BDNF_{Met}$ allele and diagnosis with major depressive disorder (MDD),[26] an effect that remained when stratified for ethnicity (Caucasian or Asian). Interestingly, when gender was taken into account, male homozygous carriers of the $BDNF_{Met}$ allele were significantly more likely to be diagnosed with MDD than noncarriers. These results are consistent with another recent study in which $BDNF_{Met}$ homozygous subjects exhibited significantly increased anxiety-related traits (e.g., harm avoidance, fear of uncertainty, and anticipatory worry) compared with noncarriers.[27]

Other studies have begun to investigate the response to stress in $BDNF_{Met}$ allele carriers. In one such study, an interaction between early life stress and Val66Met status was found for measures of anxiety and depression.[23] Individuals carrying the $BDNF_{Met}$ allele who had been exposed to early life stress were found to have reduced hippocampal volume compared to noncarriers. The size of the hippocampi of these subjects was correlated with reduced lateral prefrontal cortex (PFC) volume and higher depression scores. The interaction between BDNF genotype and early life stress also indirectly predicted higher scores on neuroticism and anxiety, albeit with modest effect sizes. In a separate study of healthy twins with either high or low

familial risk for affective disorder, an interaction was found between risk level and stress response. Individuals in the high-risk group and carrying the $BDNF_{Met}$ allele were found to have higher levels of evening cortisol, suggesting that familial risk of affective disorders in combination with carrying the $BDNF_{Met}$ allele may impact stress responsiveness.[28] This finding is consistent with another recent study of subjects admitted with major depression in which $BDNF_{Met}$ homozygous individuals were found to have a greater hypothalamus-pituitary-adrenal response to dexamethasone challenge compared with $BDNF_{Met}$ heterozygotes and noncarriers.[29]

Because of its relative scarcity in the population, most human studies have difficulties reaching statistical power for groups of $BDNF_{Met}$ homozygous subjects. In the mouse model, this problem can be avoided. In the studies by Chen *et al.*[14] only homozygous $BDNF^{Met/Met}$ mice showed significantly increased anxiety-related behavior. The reported behaviors included less spontaneous exploratory behavior of the center of the open field and less time and fewer entries into the open arms of the elevated plus maze compared with littermate control $BDNF^{Val/Val}$ mice. Furthermore, the $BDNF^{Met/Met}$ mice had greater latency to consume sweetened milk in the novelty-induced hypophagia task, a test that is regarded especially sensitive to chronic antidepressant-induced modulation of anxiety-like behavior.[30] Interestingly, the $BDNF^{Met/Met}$ mice did not respond with decreased anxiety-like behavior to chronic (21 days) treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine. In the experiments performed by Chen *et al.*,[14] only male $BDNF_{Met}$ mice were used; however, the finding of increased anxiety-related behavior in the open field task has also been replicated in female $BDNF^{Met/Met}$ mice.[31]

BDNF Val66Met and fear-related behavior

Models of fear memory assess the response to fearful or neutral stimuli. The most commonly used fear conditioning, Pavlovian classical conditioning, consists of a form of learning in which a neutral stimulus and/or context is associated with an aversive one, resulting in a fear response to the originally neutral stimulus/context. The term fear extinction is used to describe the process of gradual attenuation of a fear response to that stimulus after it is no longer associated with danger.

As already mentioned, Chen *et al.*[14] showed that contextual fear memory (i.e., fear response to the environment, where the aversive stimulus was delivered) was attenuated in homozygous $BDNF^{Met/Met}$ mice. However, in that same study, they noted no difference between genotypes for cued-dependent fear memory.[14]

The first association between the Val66Met polymorphism and ability to extinguish fearful memories in the Val66Met transgenic mouse model was described by Yu *et al.*,[32] using a conditioned taste aversion task, in which mice were conditioned to associate sucrose water with LiCl-induced nausea. The authors reported no effect of genotype on the acquisition or retention of the aversive memory. However, mice homozygous for the $BDNF_{Met}$ allele showed a marked decrease in the rate of extinction for this learned aversion. The slower extinction in $BDNF_{Met}$ homozygous mice was accompanied with lower levels of c-Fos expression in the ventromedial PFC (vmPFC), an area implicated in the extinction of aversive memories.[33–35] In addition, compared to wild-type, littermate controls, naïve $BDNF_{Met}$ homozygous mice had diminished dendritic arborization as well as a 17% volume reduction of the vmPFC. Finally, the authors found that the impairment in fear extinction could be rescued by a single injection of the partial NMDA-receptor agonist D-cycloserine (DCS) during extinction training. In a cohort of healthy human subjects, Hajcak *et al.*[36] studied the relationship between the $BDNF_{Met}$ allele and generalization of fear conditioned startle using a paradigm similar to that originally developed by Lissek *et al.*[37] In this study, a paradigm where the danger and safety cues consisted of rectangles of different sizes and the aversive stimulus, a mild shock to the triceps was always paired with the middle-sized rectangle. It was found that $BDNF_{Met}$ allele carriers showed a specific deficit in the startle response to the medium-sized rectangle (danger cue) but not to the rectangle most similar in size. However, the sample size was small (44 noncarriers, and 10 $BDNF_{Met}$ heterozyotes and three $BDNF_{Met}$ homozygous carriers grouped together). In another study investigating fear potentiated startle, where a picture was paired with a mild shock to the ankle, the authors reported that $BDNF_{Met}$ allele carriers showed a reduced startle response to the aversive stimulus in late acquisition and early extinction blocks, but no effect on skin conductance. Again, the sample size was small

(39 noncarriers, and seven $BDNF_{Met}$ heterozygotes and two $BDNF_{Met}$ homozygotes).

In a recent translational study, we found that human $BDNF_{Met}$ allele carriers have intact fear conditioning but impairments in the extinction of a learned fear response, as measured by skin conductance.[38] This finding was replicated in a parallel study conducted in BDNF Val66Met knockin mice. In mice that were conditioned to anticipate a mild footshock following a tone, $BDNF_{Met}$ mice failed to suppress the learned fear response over the course of 30 extinction trials. This impairment was dose-dependent, such that mice homozygous for $BDNF_{Met}$ were significantly slower than heterozygous mice. The parallel human experiments involved 36 healthy young adults (noncarriers) and 36 $BDNF_{Met}$ allele carriers (31 heterozygotes and five BDNF homozygotes). While undergoing fMRI, participants were fear conditioned by presentation of two different colored squares, one of which was paired with an aversive sound. Once an association was formed, extinction was carried out by multiple presentations of both squares in the absence of the aversive sound. The $BDNF_{Met}$ human subjects were found to have decreased activation of the vmPFC compared with noncarriers, a finding consistent with a failure to effectively engage circuitry implicated in fear extinction. This was consistent with findings in $BDNF_{Met}$ mice, in which lower levels of c-Fos were found in the vmPFC of $BDNF_{Met}$ mice following extinction training.

Discussion: relevance for PTSD

PTSD is a condition characterized by increased anxiety as well as a reduced ability to extinguish fearful memories after exposure to one or more traumatic events. There is also a significant comorbidity with MDD. To date, only one publication provides data on a possible link between Val66Met status and PTSD. In that study, no overrepresentation of $BDNF_{Met}$ allele carriers was seen in a cohort of 96 war veterans diagnosed with PTSD.[39] However, this result was inconclusive because of low statistical power, and further studies are warranted. In the largest study focusing on the acquisition of fear-related behavior, neither humans nor mice carrying the $BDNF_{Met}$ allele showed any difference in the ability to learn to generate a conditioned fear response to a cue.[38] $BDNF_{Met}$ mice had decreased expression of a contextual fear memory,[14] which in theory could partially be a protective factor for the development of PTSD symptoms. However, that protective effect would likely be overshadowed by the impaired or slowed ability to extinguish fearful memories.

We concluded, based on the currently available literature, that the variant BDNF Val66Met polymorphism does not consistently confer an increased overall risk for affective- or anxiety-related disorders or traits. The only correlation found in a large meta-analysis suggested that males homozygous for the $BDNF_{Met}$ allele have an increased risk of developing MDD.[26]

Although most of the human studies to date have not found a conclusive relationship between a single $BDNF_{Met}$ allele and anxiety-related traits, it is still unclear whether the same is true for homozygous $BDNF_{Met}$ allele carriers. This is in part due to low statistical power given the low percentage of homozygous individuals in the population. By contrast, the mouse model clearly indicates there is a dose–response relationship between the number of alleles and anxiety-related behavior.[14] Hence, the mouse model provides robust evidence for an anxiety-like endophenotype that has been replicated in both male and female homozygous mice. In support of these findings, a recent study on healthy volunteers with a large number of homozygotes found that only homozygote carriers of the $BDNF_{Met}$ allele scored significantly higher on measures of anxiety compared to noncarriers. This effect replicated across both sexes.[27]

Both human and rodent $BDNF_{Met}$ allele carriers, after being exposed to an aversive event, are slower to show an attenuated response to a cue that has previously been associated with that aversive event, even after that cue no longer represents danger, that is, impaired fear extinction.[32,38] The study by Soliman *et al.*[38] found a significant impairment in fear extinction in heterozygous $BDNF^{Val/Met}$ individuals. In addition to the behavioral findings, Soliman *et al.*[38] also show that $BDNF_{Met}$ allele carriers recruit the amygdala and vmPFC differently than noncarriers. This is a similar pattern of increased amygdala activation and decreased medial PFC (mPFC) activation to what has been observed in fMRI studies of PTSD patients.[40,41] In addition, the relative extent of decrease in amygdala activation during fear extinction has been correlated with the degree of extinction success in healthy volunteers.[35]

Extinction of fearful memories is thought to be brought on by the formation of a new memory, one that associates the stimulus previously paired with a fearful event with one that signals safety. This is in contrast to the notion that fear extinction is in fact an erasure of the original memory. Recent findings suggest that BDNF availability in the infralimbic mPFC from hippocampal projections is critical for the formation of such new memories.[42] It is therefore conceivable that the reduced activity-dependent secretion of BDNF associated with the $BDNF_{Met}$ allele leads to decreased synaptic plasticity in the mPFC and thereby deficiency in the formation of the new memory required for fear extinction.

There is a significant need for new therapeutic directions for many psychiatric disorders. In addition to new therapies, a lot would be gained if the use of currently available pharmacological and psychotherapeutic therapies could be more effectively used. In the case of PTSD, a recent meta-analysis showed that, on average, 80% of PTSD patients improved by 70% using different therapeutic approaches.[43] With today's diagnostic tools, it is very difficult to predict who will benefit the most from a particular therapy. Furthermore, many patients go through a painful "trial and error" phase before the most effective therapeutic combination for that particular individual is established. Hence, biomarkers that predict treatment response would be very beneficial.

In the mouse model, adult $BDNF_{Met}$ mice show blunted alteration in anxiety-like behaviors after chronic SSRI treatment,[14] the most commonly used pharmacological treatment for PTSD. The recent study by Soliman *et al.*[38] also validates that both humans and mice carrying the Met allele are worse at, or at least take longer, to extinguish fearful memories. This finding implies that they may not respond as readily to exposure therapy, which is one of the best documented and most widely used psychotherapeutic approaches for PTSD.[44] The fact that exposure therapy relies on intact ability to extinguish fearful memories suggests that it could be informative to genotype PTSD patients with regard to BDNF Val66Met in order to be able to offer modified or alternative treatment strategies.

Therapies aimed at normalizing BDNF function could be divided into at least two different categories: (i) therapies aimed at normalizing BDNF secretion during development in order to rescue structural changes caused by BDNF Val66Met and (ii) therapies aimed at normalizing BDNF secretion in adult life in order to ensure intact moment to moment BDNF-dependent synaptic plasticity and related functions. Drug discovery strategies to increase BDNF release from synapses or to prolong the half-life of secreted BDNF may improve therapeutic responses for humans with this common BDNF polymorphism.

Alternatively, therapies that do not involve BDNF signaling could be used to circumvent the deficit in BDNF signaling as well as the decreased response seen after SSRI treatment. As BDNF Val66Met status is suggested to be associated with an impaired ability to extinguish fearful memories, therapies aimed at enhancing fear extinction could be extremely useful. DCS, a partial NMDA receptor agonist, has emerged as one of the most promising pharmacological therapies aimed at enhancing fear extinction, that is, the efficacy of exposure therapy.[45,46] Several studies report beneficial effects of DCS administered before early sessions of exposure therapy for patients diagnosed with acrophobia and social phobia.[46,47] Clinical studies evaluating the effects of DCS in enhancing exposure therapy for PTSD are currently under way. As NMDA receptors are necessary for BDNF-induced fear extinction,[42] it is plausible that NMDA receptor modulation could be an efficient means of bypassing the deficit in fear extinction supposedly caused by disrupted BDNF secretion in $BDNF_{Met}$ allele carriers. In the mouse model, Yu *et al.*[32] demonstrated that one dose of DCS at a critical time during fear extinction can rescue the impairment associated with the Val66Met status. Future studies are needed to confirm whether exposure therapy combined with NMDA receptor modulators could be of similar value for PTSD patients carrying the $BDNF_{Met}$ allele.

Propranolol, a nonselective β-blocker, has also been proposed to attenuate subsequent fear response when administered directly after retrieval of a previously experienced traumatic event.[48] However, preliminary results from clinical trials using propranolol in the immediate aftermath of a traumatic event to prevent development of PTSD have failed to show a significant effect of the treatment.[49,50]

Recent findings from both rodent and human studies also suggest that fear memories can be disrupted without the use of any drugs by performing

fear extinction during reconsolidation within a limited time window after reexposure to a cue that predicts the aversive event,[51,52] an approach that may be beneficial in $BDNF_{Met}$ allele carriers.

In conclusion, several new therapies are emerging that may be used alone or in concert for PTSD patients who do not successfully respond to SSRI and conventional exposure therapy alone. Further studies are needed to clarify whether Val66Met genotype confers an increased risk for the development of affective disorders, including PTSD. Independent of that, recent studies suggest that BDNF genotype based therapy could be applicable for PTSD as well as other affective- and anxiety-related disorders. The study by Soliman *et al.*[38] also implicates that, aside from genotype, behavioral tests of extinction capacity and neuroimaging could also serve as biomarkers to direct more personalized psychiatric treatment. Future studies on patient cohorts will elucidate whether these biomarkers prove to be useful in a clinical setting.

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Conflicts of interest

The authors declare no conflicts of interest.

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