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Brain-Derived Neurotrophic Factor Serum Levels and Genotype: Association with Depression during Interferon-α Treatment

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Depression has been associated with inflammation, and inflammation may both influence and interact with growth factors such as brain-derived neurotrophic factor (BDNF). Both the functional Val66Met BDNF polymorphism (rs6265) and BDNF levels have been associated with depression. It is thus plausible that decreased BDNF could mediate and/or moderate cytokine-induced depression. We therefore prospectively employed the Beck Depression Inventory-II (BDI-II), the Hospital Anxiety and Depression Scale (HADS), and the Montgomery–Asberg Depression Rating Scale (MADRS) in 124 initially euthymic patients during treatment with interferon-alpha (IFN-α), assessing serum BDNF and rs6265. Using mixed-effect repeated measures, lower pretreatment BDNF was associated with higher depression symptoms during IFN-α treatment ($F_{144,17.2} = 6.8$; $P < 0.0001$). However, although the Met allele was associated with lower BDNF levels ($F_{1,83.0} = 5.0$; $P = 0.03$), it was only associated with increased MADRS scores ($F_{4,8.9} = 20.3$; $P < 0.001$), and not the BDI-II or HADS. An exploratory comparison of individual BDI-II items indicated that the Met allele was associated with suicidal ideation, sadness, and worthlessness, but not neurovegetative symptoms. Conversely, the serotonin transporter promoter polymorphism (5-HTTLPR) short allele was associated with neurovegetative symptoms such as insomnia, poor appetite and fatigue, but not sadness, worthlessness, or suicidal ideation. IFN-α therapy further lowered BDNF serum levels ($F_{4,37.7} = 5.0$; $P = 0.003$), but this decrease occurred regardless of depression development. The findings thus do not support the hypothesis that decreasing BDNF is the primary pathway by which IFN-α worsens depression. Nonetheless, the results support the hypothesis that BDNF levels influence resiliency against developing inflammatory cytokine-associated depression, and specifically to a subset of symptoms distinct from those influenced by 5-HTTLPR.
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INTRODUCTION

Major depressive disorder (MDD) is a common and heterogeneous syndrome. When comorbid with other chronic diseases, the adverse health effects are worse than any other combination of chronic diseases without depression (Evans *et al*, 2005; Moussavi *et al*, 2007). There is accumulating evidence that inflammatory cytokines have the capacity to induce depressive symptoms (Maes, 2011; Miller *et al*, 2009), and several pathways have been identified by which peripheral cytokines can influence the central nervous system (Quan and Banks, 2007), prompting the hypothesis that many instances of depression may have increased inflammatory cytokines as a critical element in their pathoetiology (Anisman, 2009; Dantzer *et al*, 2008; Lotrich, 2012; Raison *et al*, 2006). However, not every individual who is exposed to elevated inflammatory cytokines develops MDD, indicating a role for vulnerability and resiliency factors in moderating the adverse effects of inflammatory cytokines (Lotrich, 2011).

Iatrogenic MDD can be triggered by treatment with an exogenous inflammatory cytokine, interferon-alpha (IFN-α). This clinical situation has become a paradigmatic model for prospectively examining vulnerability/resilience to inflammatory cytokine-associated depression, as MDD develops in about 30% of non-depressed subjects within a few months of initiating IFN-α treatment (IFN-MDD) (Lotrich, 2009; Raison *et al*, 2005). A number of putative vulnerability factors for subsequent IFN-MDD have been identified ranging from a 'short' (S) low-expression allele in the promoter of the serotonin transporter (5-HTTLPR) (Bull *et al*, 2008; Lotrich *et al*, 2009), a polymorphism in the serotonin 1A receptor (Kraus *et al*, 2007), increased

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interleukin-6 (IL-6) serum levels and an IL-6 polymorphism (Bull *et al*, 2008; Prather *et al*, 2009), pre-existing poor sleep quality (Franzen *et al*, 2009), high neuroticism traits (Lotrich *et al*, 2007), increased hypothalamic-pituitary-adrenal axis sensitivity, (Raison *et al*, 2008), elevated ratio of omega-6 fatty acids to omega-3 fatty acids (Lotrich *et al*, 2012), and increased sensitivity to activating the p38 mitogen-activated protein kinase (Felger *et al*, 2011).

Accumulating evidence also supports an important role for decreased brain-derived neurotrophic factor (BDNF) activity in inflammatory cytokine-associated depression (Duman and Monteggia, 2006; Hashimoto, 2010; Kunugi *et al*, 2010; Numakawa *et al*, 2010). Inflammatory cytokines can decrease BDNF signaling (Cortese *et al*, 2011; Tong *et al*, 2008), as can lipopolysaccharide injections (Guan and Fang, 2006). Therefore, in addition to IFN-α's effects on serotonin (Raison *et al*, 2009), dopamine (Felger *et al*, 2007), glutamate (Raison *et al*, 2010b), and the hypothalamic-pituitary-adrenal axis (Raison *et al*, 2010a), a decrease in BDNF may ultimately be the reason for the development of depression during IFN-α treatment. Related to this, social isolation decreases central BDNF, an effect which is likely mediated by the inflammatory cytokine IL-1β (Barrientos *et al*, 2003; Ben Menachem-Zidon *et al*, 2008; Koo and Duman, 2008). IFN-α also appears to decrease cell proliferation in the hippocampus via increased IL-1β (Kaneko *et al*, 2006). In fact, the effects of both stress and inflammation may be mediated by impairments in growth factor function (Anisman, 2009; Koo *et al*, 2010; Peng *et al*, 2008). Moreover, many antidepressant effects likely occur through activation of BDNF's receptor (Saarelainen *et al*, 2003), and even the neuroprotective effect of a tricyclic antidepressant against lipopolysaccharide-induced apoptosis requires BDNF (Peng *et al*, 2008). In human bipolar populations, there is an inverse relationship between inflammatory cytokines and serum BDNF (Goldstein *et al*, 2011). Thus, it is feasible that cytokine-induced decreases in BDNF may result in the depressogenic effects of inflammatory cytokines such as IFN-α.

Consistent with this, several studies have associated low serum BDNF with MDD (Aydemir *et al*, 2006; Bocchio-Chiavetto *et al*, 2006; Gervasoni *et al*, 2005; Hashimoto, 2010; Sen *et al*, 2008; Shimizu *et al*, 2003; Verhagen *et al*, 2010), which subsequently normalizes with antidepressant treatment (Castren and Rantamaki, 2010; Chen *et al*, 2001). Also, a functional polymorphism causing a change from valine (Val) to methionine (Met) may result in diminished BDNF secretion (Egan *et al*, 2003). The Val to Met variant at amino acid 66 (Val66Met) results from a G758A polymorphism (rs6265) in BDNF's 11th exon. The Met allele has been associated with lower serum BDNF (Ozan *et al*, 2010), though this has not been consistently replicated (Duncan *et al*, 2009; Terracciano *et al*, 2010; Zhou *et al*, 2011). The Met allele has also been associated with increased suicide risk (Kanellopoulos *et al*, 2011; Pregelj *et al*, 2011; Sarchiapone *et al*, 2008), various depression-related traits (Beevers *et al*, 2009; Gatt *et al*, 2008; Gatt *et al*, 2010; Hayden *et al*, 2010; Jiang *et al*, 2005; Lau *et al*, 2010; Montag *et al*, 2010; Montag *et al*, 2008; Montag *et al*, 2009), and occasionally, a depression diagnosis (Aguilera *et al*, 2009; Borroni *et al*, 2009; Kim *et al*, 2008; Lavebratt *et al*, 2010). However, there are multiple studies in which this association with depression has not been replicated (Chen *et al*, 2008; Figueira *et al*, 2010; Middeldorp *et al*, 2010; Ribeiro *et al*, 2007; Suchanek *et al*, 2011; Wray *et al*, 2008).

There are therefore two potential non-mutually exclusive hypotheses that we examined. One is that IFN-α therapy decreases BDNF—but only in a subset of people who subsequently develop depression. That is, we examined whether decreased BDNF might mediate IFN-α's depressogenic effect. The second hypothesis is that pre-existing low BDNF increases subsequent risk for developing depression. Prior adversity could have lasting epigenetic effects on BDNF production (Roth *et al*, 2009) as could the Met allele, resulting in increased vulnerability to depression. Thus, we determined whether low BDNF and/or the BDNF Met allele enhances (ie moderates) the depressogenic effect of IFN-α.

Finally, different genetic regions have been associated with specific mood and anxiety traits in both mice (Henderson *et al*, 2004), and humans (Foley *et al*, 2003; Jang *et al*, 2004), which can affect genetic association study results (Lotrich, 2011). Consistent with this, we have found that polymorphisms affecting TNF-α and IL-28b are associated with specific mood-related symptom clusters in individuals receiving IFN-α (Lotrich *et al*, 2010; Lotrich *et al*, 2011). Also, we have found that a serotonin reuptake promoter polymorphism (5-HTTLPR) is associated with increased Beck Depression Inventory scores during IFN-α therapy (Lotrich *et al*, 2009), but another group did not find an association using the Hospital Anxiety Depression Scale (Kraus *et al*, 2007). Thus, we explored the possibility that either BDNF genotype and/or 5-HTTLPR may each influence a specific subset of depression symptoms.

MATERIALS AND METHODS

Participants

209 adult subjects with chronic HCV were screened using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-IV), as previously described in an overlapping cohort of subjects (Lotrich *et al*, 2009; Prather *et al*, 2009), and as approved by the University of Pittsburgh Institutional Review Board. Anyone taking antidepressants, anticonvulsants, or antipsychotics was excluded (none were taking steroids, although most took non-steroidal anti-inflammatory medications as needed for pain and fever during the course of IFN-α treatment). Those without active mood, anxiety, psychotic, or drug/alcohol abuse disorders were assessed for BDNF levels ($n = 156$). Of these, 124 provided blood for genotyping and subsequently started IFN-α treatment within 6 months—comprised weekly injections of pegylated IFN-α2 (PEG-IFN-α2a: 135 μg/week or PEG-IFN-α2b: 120 or 150 μg/week) augmented with oral ribavirin. No subjects were noted to develop incident MDD during the period between the initial baseline assessment and the start of IFN-α therapy (56 ± 55 days).

Depression Assessment

Depression symptoms were assessed at baseline and then monthly during IFN-α treatment (for up to 4 months) using the Beck Depression Inventory-II (BDI-II) (Beck *et al*, 1988)

Table 1 Baseline Characteristics of Subjects in this Study: Relationship with Either the Val/Met Polymorphism or Correlation with Baseline BDNF Levels

	Val/Met and Met/Met	Val/Val	Correlation with BDNF
Age (years)	50.8 ± 11.7	47.1 ± 11.5	r = 0.17; *P* = 0.09
Gender (% female)	35%	26.5%	r = 0.08; ns
Race (% Caucasian)	87.8%	87.4%	r = 0.06; ns
Weight (kg)	82.5 ± 18.1	86.6 ± 16.5	r = 0.08; ns
Sustained Viral Response	47%	45%	r = 0.2; *P* = 0.1
History of MDD	19.5%	18.8%	r = 0.1; ns
C-Reactive Protein (ng/ml)	2.4 ± 3.7	1.9 ± 2.3	r = 0.1; ns
BDNF (ng/ml)	17.0 ± 10.5	19.3 ± 10.0	
CIRS-G	4.5 ± 2.4	3.7 ± 1.6	r = 0.1; ns
BDI	8.1 ± 6.1	8.6 ± 9.0	r = 0.1; ns
HADS	8.0 ± 5.7	8.2 ± 6.4	r = 0.1; ns
MADRS	3.4 ± 4.0	3.2 ± 3.7	r = 0.06; ns
PSQI	6.9 ± 4.2	6.8 ± 4.7	r = 0.01; ns
AIAQ	36.9 ± 18.7	37.2 ± 20.2	r = 0.1; ns

Prior to IFN-α treatment, the demographics, serum brain-derived neurotrophic factor (BDNF), Cumulative Illness Rating Scale-Geriatric (CIRS-G), Beck Depression Inventory (BDI-II), Hospital Anxiety and Depression Scale (HADS), Montgomery–Asberg Depression Rating Scale (MADRS), Pittsburgh Sleep Quality Index (PSQI), and Anger Irritability and Assault Questionnaire (AIAQ). There were no significant differences in these measures comparing those homozygous for the valine (Val) allele and those with a methionine (Met) allele. Subjects with lower BDNF levels trended towards being older and trended towards having a lesser chance of achieving a sustained clearance of their HCV infection—but otherwise there were no significant (ns, $p > 0.15$) correlations (r) at baseline.

as well as the Montgomery–Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979) and Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983). Several quantitative assessments, rather than a categorical outcome of MDD, were selected because of increasing evidence that many genetic vulnerabilities may be associated with specific symptom clusters during IFN-α therapy (Lotrich *et al*, 2010; Lotrich *et al*, 2011; Su *et al*, 2010). Subjects were given the opportunity to either mail in questionnaire results or complete them on routine clinic days. We did not observe an effect of date between last injection and date of questionnaire completion (ranging from 1 to 6 days) on depression symptoms—consistent with the very long half-life of pegylated IFN-α and serum levels that remain at a plateau during treatment (Jen *et al*, 2001).

However, participants who did develop DSM-IV defined MDD (SCID-IV assessed and confirmed by a psychiatrist) during the course of treatment—or where concerns about lethality arose—were typically started on an antidepressant. Moreover, if not already requested by the subject or their clinicians, an abbreviated SCID-IV interview that was focused specifically on mood disorders was employed at months 2 and 4. Data from individuals on antidepressant medications or no longer taking IFN-α are censored from the analyses.

BDNF, CRP, and Polymorphism Assessment

Blood samples were obtained between 1000 and 1600 hours, and serum (which does not contain platelets) was stored at -80C (between 5 and 60 months with no freeze-thaw cycles) until serum BDNF levels were measured using a high-sensitivity (<20 pg/mL) and specific (no crossreactivity with other growth factors, except 13% crossreactivity with pro-BDNF) quantitative enzyme immunoassay (ELISA) (R&D Systems, Minneapolis, MN). There was no relationship between storage time and BDNF measures. All samples were measured in duplicate and the average intra-assay and inter-assay coefficients of variation were 6.2% and 11.3%, respectively. Of note, BDNF serum levels are not greatly associated with diurnal circadian rhythms (Choi *et al*, 2011) nor with platelet counts (Trajkovska *et al*, 2007). Serum CRP levels were determined using ELISA (Diaclone, Besancon, France) as previously described (Prather *et al*, 2009).

Genomic DNA isolated from lymphocytes (QuickGene-Mini-80 kit; Fujifilm Life Science; www.autogen.com) was assessed using the 5'-nuclease Taqman assay (ABI 7900 DNA detection system), employing Assays-on-Demand and Assays-by-Design (Applied Biosystems, Foster City, CA) with >95% accuracy. Although the BDNF polymorphism was in Harvey–Weinberg equilibrium, only three subjects were homozygous for Met/Met, which were therefore combined with the 38 Met/Val heterozygotes. Val/Val homozygotes were thus compared with any subject carrying the 'lower secreted' Met allele. The serotonin transporter length polymorphic region (5-HTTLPR), including the G/A variant in the Long allele, was determined as previously described (Lotrich *et al*, 2009), and Long/Long (La/La) homozygotes were compared with any subjects carrying either low expressing polymorphism (the Short (S) or the Lg allele).

Statistical Analyses

All statistics employed SPSS 18.0, and results are reported as mean ± SD, and in graphs as mean ± SE of the mean. Repeated-measure mixed-effect analyses, robust to randomly missing data (many subjects did not complete all assessments at all time points), were used to compare symptom changes over time. For these mixed-effect models, we first examined repeated covariance structures and selected analyses that provided the smallest Aikake Information Criteria (typically this was an unstructured covariance).

RESULTS

As seen in Table 1, subjects in this study were primarily middle aged (but ranged from 18 to 72 years), about 2/3 were male and mostly Caucasian, and almost 20% had a prior history of MDD in remission but with some sleep quality problems. All subjects starting IFN-α therapy had a Cumulative Illness Rating Scale-Geriatric (CIRS-G) (Miller *et al*, 1992) score of at least 2 (because of HCV infection). Most subjects had only a few other medical problems diagnosed and treated such as hypertension and hyperlipidemia (eg, <2% were being treated with statins) and 64% had CIRS-G scores of ⩽4. There was a trend for older

subjects to have lower BDNF levels but other demographics were not correlated with BDNF levels (Table 1). Unless stated otherwise, we therefore did not include these variables as covariates in the analyses below.

Lower baseline BDNF was associated with increasing BDI-II symptoms over time during IFN-α therapy ($F_{144,17.2} = 6.8$; $P < 0.0001$). This supports the hypothesis that baseline BDNF may be inversely related to subsequent depression vulnerability. We next included baseline BDI-II as a covariate because of its known association with subsequent depression risk. The association between BDNF and subsequent BDI-II over time was still significant ($F_{118,17.4} = 2.0$; $P = 0.05$). Likewise, when we covaried for baseline PSQI because of its known strong association with subsequent depression (Franzen *et al*, 2009; Prather *et al*, 2009), BDNF continued to be predictive of increased depression symptoms ($F_{97,20.9} = 3.7$; $P = 0.001$). Thus, neither baseline BDI-II nor PSQI mediates the relationship between BDNF and depression risk during IFN-α therapy.

To better characterize and illustrate these findings, we divided baseline BDNF levels by a median split (below or above 17 ng/ml). Compared with higher baseline BDNF levels, lower baseline levels (BDNF <17 ng/ml) were associated with higher BDI-II scores ($F_{1,98.9} = 4.7$; $P = 0.03$) as well as MADRS ($F_{1,138.1} = 4.2$; $P = 0.04$) and HADS scores ($F_{1,187.0} = 5.9$; $P = 0.02$) (Figure 1) though the interaction with time for these three questionnaires was lost ($F_{4,89.7} = 1.1$; $P = 0.3$; $F_{4,198.5} = 0.6$; $P = 0.7$; $F_{4,200.6} = 1.3$; $P = 0.3$, respectively). However, when covarying for baseline BDI-II, dichotomized baseline BDNF was associated with increasing MADRS and BDI-II scores over time ($F_{4,46.2} = 2.7$; $P = 0.04$). These prospective results are consistent with BDNF levels being a moderator of subsequent vulnerability to depression.

During IFN-α treatment, serum BDNF levels lessened over time in most subjects ($F_{4,37.7} = 5.0$; $P = 0.003$) (Figure 2). Nonetheless, BDNF levels went down similarly in the subjects who developed MDD and in those who completed treatment ($F_{4,47.7} = 0.6$; $P = 0.6$) (Figure 2). Therefore, although IFN-α treatment appears to decrease BDNF, these findings do not support the hypothesis that simply decreasing peripheral BDNF during IFN-α therapy is necessarily associated with the emergence of depression. However, as seen in Figure 2, those developing MDD started and ended with somewhat lower BDNF levels. Thus, we specifically examined the lowest BDNF level during IFN-α treatment for a subject ('BDNF nadir'), and found that those who ultimately developed MDD had the lowest BDNF nadir (9.6 ± 0 0.8 ng/ml) *vs* the other subjects (14.0 ± 1.2 ng/ml; $P = 0.001$); and the area under the curve (AUC) in a receiver operating curve (ROC) for 'lowest BDNF' being associated with depression was 0.69. This is similar in effect size to the prediction of MDD by baseline BDI-II scores, where the AUC = 0.67.

It is therefore possible that low BDNF nadirs achieved during treatment could be responsible for the development of depression. However, pretreatment BDNF was strongly associated with the BDNF nadir ($r = 0.57$ $P < 0.0001$). Moreover, although pretreatment BDNF was associated with depression during IFN-α therapy as noted above, the 'BDNF nadir' was not statistically associated with increasing depression symptoms (eg, for MADRS, $F_{60,13.1} = 1.7$; $P = 0.15$). This later finding further weakens the hypothesis that BDNF mediates rather than moderates the depressive effects of IFN-α therapy—though the mediation hypothesis is not completely ruled out.

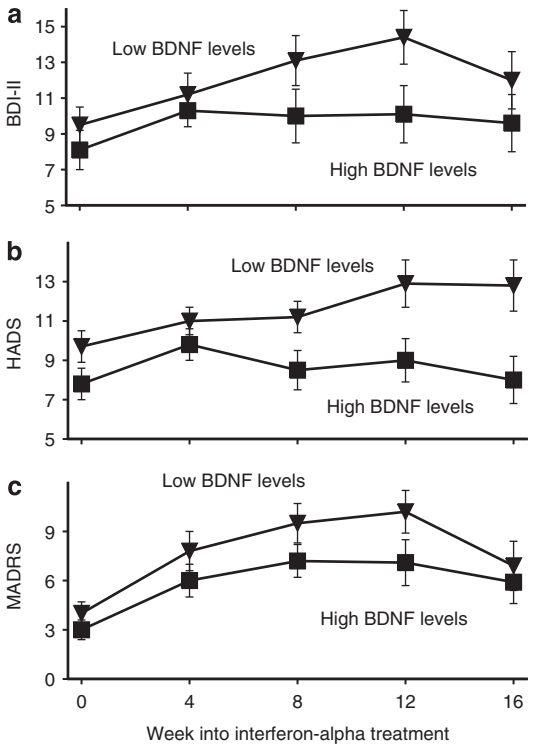

Figure 1 Subjects with below the median BDNF levels (<17 ng/ml) demonstrate elevated BDI-II (a), MADRS (b) and HADS (c) scores during IFN-α therapy.

Throughout IFN-α treatment, those with the Val/Val genotype had higher BDNF levels than subjects carrying the Met allele ($F_{1,83.0} = 5.0$; $P = 0.03$), but BDNF levels went down similarly in both genetic groups ($F_{4,42.9} = 0.3$; $P = 0.9$) (Figure 2). Consistent with the findings above, the BDNF Met allele was associated with increased MADRS scores over time ($F_{4,8.9} = 20.3$; $P < 0.001$)—even when covarying for baseline BDI-II ($F_{4,10.2} = 20.0$; $P < 0.001$). As baseline BDNF was also associated with increased MADRS scores over time, we next covaried for baseline BDNF to examine whether low BDNF might mediate the relationship between the Met allele and increased MADRS scores—and the Met allele was no longer associated with increased MADRS over time ($F_{4,24.7} = 1.9$; $P = 0.14$). This suggests that lower BDNF levels potentially mediate the genetic effect of the Met allele on depression risk.

Despite these findings using MADRS, the Val/Met genotype was not associated with total BDI-II ($F_{4,32.4} = 0.7$; $P = 0.6$) nor the total HADS score ($F_{4,30.5} = 0.7$; $P = 0.6$) during IFN-α therapy—whether we controlled for baseline BDI-II or not. To help clarify this discrepancy, we therefore further explored specific questions in these questionnaires (with no correction for multiple testing). The Met allele was specifically associated with increased emergence of only a subset of symptoms during IFN-α therapy including suicidal ideation (Q9 of the BDI-II; $F_{4,112.2} = 2.5$; $P < 0.05$) (Figure 3). Notably, suicidal thoughts were uncommon—only 6.3% of

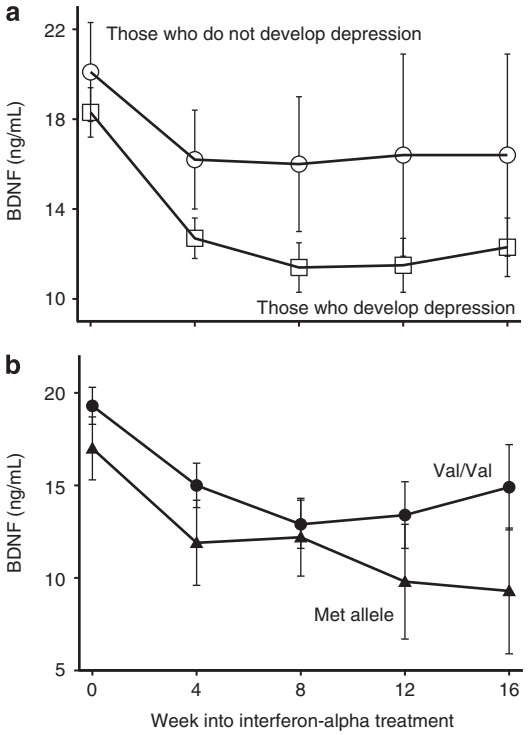

Figure 2 Serum brain-derived neurotrophic factor decreases during IFN-α therapy ($F_{4,37.7} = 5.0$; $P = 0.003$), but similarly whether patients develop MDD (open squares) or not (open circles) ($F_{4,47.7} = 0.6$; $P = 0.6$). Those with the Met allele typically had lower BDNF throughout IFN-α therapy ($F_{1,83.0} = 5.0$; $P = 0.03$), but serum BDNF levels decreased similarly in both genetic groups (Val/Val = black circles; Met allele = triangles) over time ($F_{4,42.9} = 0.3$; $P = 0.9$).

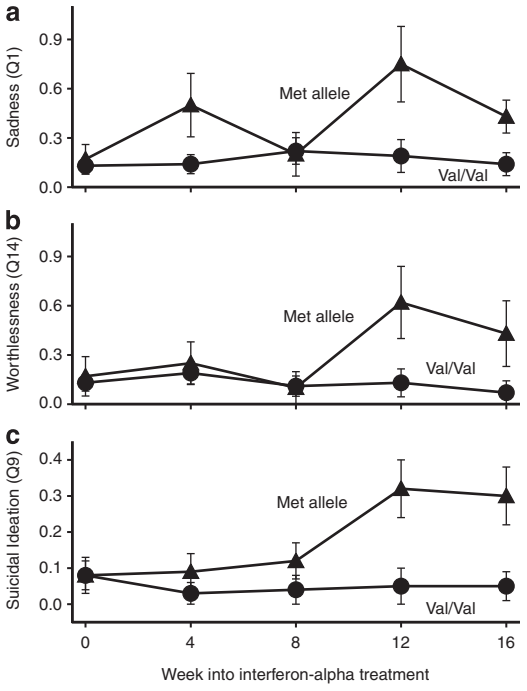

Figure 3 During IFN-α therapy, worsening of psychological symptoms such as 'sadness' (a) and 'worthlessness' (b) increased more in those with the Met allele ($F_{4,21.2} = 3.2$; $P = 0.03$ and $F_{4,56.1} = 2.7$; $P = 0.04$), most prominently by month 3. Those with the Met allele also had increases in their response to question 9 (c) of the BDI-II regarding suicidal ideation ($F_{4,112.2} = 2.5$; $P < 0.05$).

people with Met allele and 3.2% of those with Val/Val answered 'I have thoughts of killing myself, but I would not carry them out' on BDI-II question 9 during IFN-α treatment (and there were no suicide attempts by any participants during this study). The Met allele was also associated with increased psychological symptoms on the BDI-II, including sadness (Q1; $F_{4,21.2} = 3.2$; $P = 0.03$), and worthlessness (Q14; $F_{4,56.1} = 2.7$; $P = 0.04$) (Figure 3). Conversely, there was no BDNF genetic association with emergence of any neurovegetative symptoms such as insomnia (Q16; $F_{4,47.1} = 0.7$; $P = 0.6$), fatigue (Q20; $F_{4,44.2} = 0.4$; $P = 0.8$), nor appetite (Q18; $F_{4,39.9} = 1.8$; $P = 0.14$).

As we have previously found an association between the 5-HTTLPR polymorphism and risk for MDD during IFN-α treatment (Lotrich *et al*, 2009), we therefore similarly explored individual BDI-II questions for this polymorphism. During IFN-α therapy, the S allele was associated with the emergence of worsening insomnia (Q16; $F_{4,24.4} = 4.0$; $P = 0.01$) and appetite (Q18; $F_{4,16.9} = 3.2$; $P = 0.04$). Fatigue was also worse throughout treatment with IFN-α in those with the S allele (Q20; $F_{1,38.2} = 4.1$; $P = 0.049$), although it worsened equally for both genotypes ($F_{4,25.8} = 0.4$; $P = 0.8$) (Figure 4). Conversely, there was absolutely no association of the 5-HTTLPR polymorphism and worsening sadness ($F_{4,16.9} = 0.1$; $P = 0.98$), suicidal thoughts ($F_{4,59.7} = 0.5$; $P = 0.7$) nor feelings of worthlessness ($F_{4,44.8} = 0.2$; $P = 0.9$). We also replicated a prior negative finding of no association between 5-HTTLPR and HADS scores during IFN-α treatment (Kraus *et al*, 2007)—though there was a trend for subjects with the S allele to have greater increase in HADS scores during IFN-α therapy ($F_{4,17.8} = 2.6$; $P = 0.07$).

As the BDNF Val/Met polymorphism was associated with a subset of depression symptoms, and 5-HTTLPR may be associated with a different cluster of symptoms, we finally explored whether there might be an interaction with 5-HTTLPR. We did not observe this (Figure 5). The BDNF Val/Met polymorphism ($F_{4,69.3} = 2.7$; $P = 0.02$) and 5-HTTLPR ($F_{4,69.3} = 3.1$; $P = 0.01$) were both additively associated with increased depression over time during INF-α therapy, but there was no interaction ($F_{4,69.3} = 1.5$; $P = 0.21$). Those patients with both the Val/Val and L/L genotype had essentially no worsening of depression symptoms, and those with both the Met allele and S allele had the greatest increase in symptoms (Figure 5).

DISCUSSION

IFN-α therapy resulted in decreasing BDNF levels along with worsening depression scores, similar to a prior report of 17 patients in the Netherlands (Kenis *et al*, 2010). In support of a moderator hypothesis, lower BDNF levels prior to IFN-α therapy were predictive of greater depression symptoms during IFN-α treatment, even when controlling for baseline BDI-II scores and sleep quality. However, conclusions regarding a mediator hypothesis were more equivocal. BDNF decreased both in those who developed

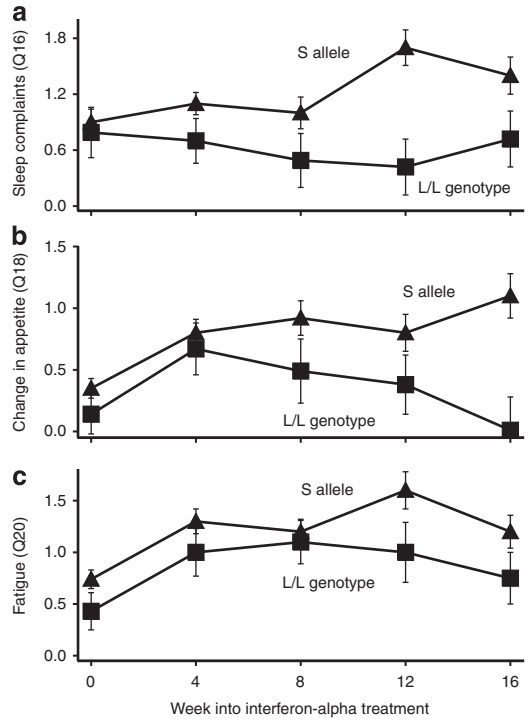

Figure 4 During IFN-α therapy, neurovegetative symptoms such as sleep problems (Panel a. $F_{4,24.4} = 4.0$; $P = 0.01$) and appetite changes (Panel b. $F_{4,16.9} = 3.2$; $P = 0.04$) increased more in those with the Short allele compared to the Long/Long genotype. Those with the Short allele also had worse fatigue throughout treatment (Panel c. $F_{1,38.2} = 4.1$; $P = 0.049$), though both genotypes experienced this side effect.

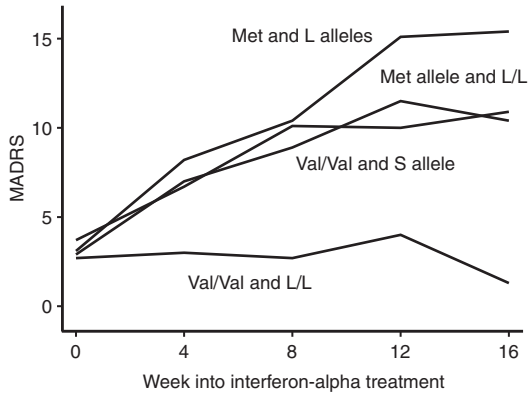

Figure 5 During IFN-α therapy, those with both BDNF Val/Val and 5-HTTLPR L/L genotypes had essentially no change in Montgomery–Asperg Depression Rating Scales symptoms, while those with both the Met allele and S allele had the greatest increase in symptoms.

depression and those who did not—consistent with IFN-α having behavioral effects in rodents without affecting cortical BDNF levels (Fahey *et al*, 2007).

Other studies also support a moderating effect of BDNF on depression risk. Lower BDNF (and/or the BDNF Met allele) increases risk for depression symptoms in rhesus macaques exposed to early adversity (Kunugi *et al*, 2010), and in humans exposed to stress (Aguilera *et al*, 2009; Cirulli *et al*, 2011; Drachmann *et al*, 2009), with alcohol dependence (Su *et al*, 2011) or with Alzheimers disease (Zhang *et al*, 2011).

There are several plausible pathways by which BDNF could moderate inflammatory cytokine effects. First, inflammation can influence phosphorylation of BDNF's receptor (TrkB), interfering with BDNF signaling (Cortese *et al*, 2011); and subsequent intracellular signal transduction can be impaired by inflammatory cytokines such as IL-1 (Tong *et al*, 2008). Both processes could impact depression more in those who start out with low BDNF. Third, BDNF and inflammatory cytokines both influence serotonin transporter transcription and function (Mossner *et al*, 2000; Mossner *et al*, 1998; Zhu *et al*, 2006; Zhu *et al*, 2010). IFN-α increases serotonin transporter transcription via the MAP kinase intracellular signaling pathway (Tsao *et al*, 2008), and both BDNF and inflammatory cytokines share overlapping intracellular signal transduction pathways including MAP kinases (Duman *et al*, 2007; Zhu *et al*, 2010) and NF-kappaB (Kairisalo *et al*, 2009). Fourth, the BDNF Met allele has been associated with an elevated cortical response to a dexamethasone/corticosterone releasing hormone challenge (Schule *et al*, 2006), which is notable given that there is a greater cortical response to the initial injection of IFN-α in those at increased risk for subsequent depression (Raison *et al*, 2008).

Likely mediated by low BDNF levels, the Met allele was also predictive of depression symptoms. Moreover, similar to prior reports (Kanellopoulos *et al*, 2011; Pregelj *et al*, 2011; Sarchiapone *et al*, 2008), we specifically found that the Met allele was associated with increased suicide ideation, increased sadness, and a sense of worthlessness. The Met allele was not associated with enhanced fatigue, insomnia, or appetite complaints. Thus, how one measures depression may matter for genetic studies.

In fact, different specific depression symptoms may be influenced by different genes (Jang *et al*, 2004), a phenomenon long noted in mice where different chromosomal regions are implicated in anxiety—depending on what behavioral test is used (Henderson *et al*, 2004). The possibility that the Met allele is only associated with risk for a subset of symptoms may be one plausible reason that some studies do not replicate an association between depression risk and the BDNF Val/Met polymorphism (Chen *et al*, 2008; Figueira *et al*, 2010; Middeldorp *et al*, 2010; Ribeiro *et al*, 2007; Suchanek *et al*, 2011; Wray *et al*, 2008). There may also be treatment implications. The BDNF Met allele could be associated with better response to SSRIs (Su *et al*, 2011; Taylor *et al*, 2011; Zhang *et al*, 2011), and suicidal ideation is the least common residual symptom following SSRI treatment (McClintock *et al*, 2011).

Conversely for the 5-HTTLPR insertion/deletion polymorphism, the S allele was specifically associated with increased fatigue, insomnia, and appetite complaints during IFN-α therapy—but not with suicidal ideation, sadness, or worthlessness. Consistent with this, we replicated a prior negative finding that the S allele was not strongly associated with increased HADS scores during IFN-α treatment (Kraus *et al*, 2007), despite being associated with categorical DSM-IV-defined MDD (Bull *et al*, 2008; Lotrich *et al*, 2009). This may have treatment implications—(i) emergent neurovegetative symptoms during IFN-α therapy are less responsive to

SSRI treatment (Capuron *et al*, 2002); (ii) neurovegetative symptoms are less response to SSRI treatment of MDD in general (McClintock *et al*, 2011; Morrow *et al*, 2003; Nierenberg *et al*, 2010; Targum and Fava, 2011); and (iii)the S allele (which increases risk for neurovegetative symptoms) has been associated with a poor response to SSRI treatment (Lotrich *et al*, 2008; Porcelli *et al*, 2012).

Finally, we found that the effects of the two polymorphisms examined were additive, consistent with the non-overlapping influence on specific depression symptoms. Those who had both risk alleles (the Met and the 5-HTTLPR S alleles) had the greatest increase in total symptoms, whereas those with the two resilient genotypes (Val/Val and L/L) had essentially no increase in symptoms. This latter finding is exploratory and will need to be replicated.

One limitation to these findings is that generalization is minimized by studying resilient subjects who were not depressed despite their chronic hepatitis C infection. A second caveat is that conclusions are limited by the use of a single homogeneous inflammatory cytokine, IFN-α. Thirdly, we did not directly assess cortical BDNF. Given that IFN-α therapy is associated with higher IL-6 in those who develop MDD (Prather *et al*, 2009) and IL-6 can induce lymphocytes to increase BDNF production (Kunz *et al*, 2009), the parsimonious explanation for lowering serum BDNF during IFN-α therapy is that it is a consequence of decreased CNS BDNF (rather than decreased peripheral synthesis) (Lisak *et al*, 2007). In support of this, BDNF can readily cross the blood–brain barrier via a high-capacity transport system (Pan *et al*, 1998; Poduslo and Curran, 1996), serum BDNF correlates with cortical BDNF mRNA expression (Gervasoni *et al*, 2005), and serum BDNF may be affected by changes in cortical BDNF. For example, electroconvulsive treatments result in increased cortical BDNF followed by a rise in serum BDNF (Sartorius *et al*, 2009).

However, many immune and vascular endothelial cells also produce BNDF (Besser and Wank, 1999; Furuno and Nakanishi, 2006; Kerschensteiner *et al*, 1999; Kimata, 2005; Nakahashi *et al*, 2000; Prakash *et al*, 2009; Rezaee *et al*, 2010; Ziemssen *et al*, 2002); serum BDNF also correlates with leukocyte BDNF mRNA (Cattaneo *et al*, 2009), and systemic levels of BDNF are actually increased during attacks of multiple sclerosis (Liguori *et al*, 2009). Thus, there may be a complicated relationship between cortical and serum BDNF (Gass and Hellweg, 2010).

Regardless, accruing data indicate a critical role of BDNF and neuroplasticity in the vulnerability to depression (Calabrese *et al*, 2009; Castren *et al*, 2010; Sen *et al*, 2008); many instances of depression are strongly linked with increased inflammatory cytokines (Haroon *et al*, 2012; Miller *et al*, 2009), and there is a likely interaction between BDNF and inflammatory cytokines (Lotrich, 2012). Herein, we report that lower BDNF levels (and the Val/Met polymorphisms rs6265) can increase susceptibility to subsequent inflammatory cytokine-associated depression. Rs6265, which is associated with lower BDNF levels, appears to act in conjunction with 5-HTTLPR to increase the risk of total depression symptoms whereby each polymorphism may be influential on distinct sets of depression symptoms. Both BDNF and 5-HT are therefore viable targets for improving resiliency against developing inflammatory cytokine-associated depression.

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DISCLOSURE

The authors declare no conflict of interest.

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