International Journal of Neuropsychopharmacology (2010), **13**, 93–101. Copyright © 2009 CINP
doi:10.1017/S1461145709000030

ARTICLE

THEMATIC SECTION
New Aspects in the Treatment of Affective Disorders

CINP

Brain-derived neurotrophic factor (*BDNF*) gene: no major impact on antidepressant treatment response

Katharina Domschke[1], Bruce Lawford[2,3], Gonzalo Laje[4], Klaus Berger[5], Ross Young[3], Phillip Morris[3], Jürgen Deckert[6], Volker Arolt[1], Francis J. McMahon[4] and Bernhard T. Baune[7]

[1] *Department of Psychiatry, University of Muenster, Germany*
[2] *Division of Mental Health, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia*
[3] *Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia*
[4] *Genetic Basis of Mood and Anxiety Disorders Unit, NIMH, NIH, USDHHS*
[5] *Institute of Epidemiology and Social Medicine, University of Muenster, Germany*
[6] *Department of Psychiatry, University of Wuerzburg, Germany*
[7] *Department of Psychiatry, James Cook University, Queensland, Australia*

Abstract

The brain-derived neurotrophic factor (BDNF) has been suggested to play a pivotal role in the aetiology of affective disorders. In order to further clarify the impact of BDNF gene variation on major depression as well as antidepressant treatment response, association of three BDNF polymorphisms [rs7103411, Val66Met (rs6265) and rs7124442] with major depression and antidepressant treatment response was investigated in an overall sample of 268 German patients with major depression and 424 healthy controls. False discovery rate (FDR) was applied to control for multiple testing. Additionally, ten markers in BDNF were tested for association with citalopram outcome in the STAR*D sample. While BDNF was not associated with major depression as a categorical diagnosis, the BDNF rs7124442 TT genotype was significantly related to worse treatment outcome over 6 wk in major depression ($p=0.01$) particularly in anxious depression ($p=0.003$) in the German sample. However, BDNF rs7103411 and rs6265 similarly predicted worse treatment response over 6 wk in clinical subtypes of depression such as melancholic depression only (rs7103411: TT < CC, $p=0.003$; rs6265: GG < AA, $p=0.001$). All SNPs had main effects on antidepressant treatment response in ANOVA models when the remaining SNPs were considered as covariates. The STAR*D analyses did not yield significant results at any of the ten BDNF markers. Our results do not support an association between genetic variation in BDNF and antidepressant treatment response or remission. *Post-hoc* analyses provide some preliminary support for a potential minor role of genetic variation in BDNF and antidepressant treatment outcome in the context of melancholic depression.

Received 30 July 2008; Reviewed 3 September 2008; ; Revised 25 November 2008; Accepted 13 January 2009; First published online 23 February 2009

Key words: Antidepressant, BDNF, depression, neurotrophins, pharmacogenetics, STAR*D, treatment response.

Introduction

The brain-derived neurotrophic factor (BDNF) has attracted much research interest with respect to its role in the pathogenesis of affective disorders. BDNF, a member of the neurotrophin superfamily, is crucially involved in neuronal growth and in activity-dependent neuronal plasticity (Duman, 1999). Given the synaptic plasticity hypothesis of mood disorders, BDNF has been suggested to play a pivotal role in the aetiology of affective disorders as well as in the mediation of antidepressant treatment response (Green & Craddock, 2003). For instance, Chen *et al*. (2001) reported increased BDNF immunoreactivity in postmortem brains of patients under antidepressant medication, while depressive states in animal models have

Address for correspondence: Professor B. T. Baune, Ph.D., M.D., M.P.H., Department of Psychiatry and Psychiatric Neuroscience, School of Medicine and Dentistry, James Cook University, Queensland 4811, Australia.
Tel.: 0061 7 4781 6731 *Fax*: 0061 7 4781 6841
Email: bernhard.baune@jcu.edu.au

been found to be associated with reduced BDNF levels in the brain, reversible by central administration of BDNF (Nibuya *et al*. 1995; Shirayama *et al*. 2002; Siuciak *et al*. 1997; Smith *et al*. 1995). Furthermore, expression of BDNF has been shown to be modified by antidepressant treatment (Saarelainen *et al*. 2003).

The *BDNF* gene, located on chromosome 11p14 (MIM113505), encodes a precursor peptide (proBDNF), which is subsequently cleaved into its mature form (Seidah *et al*. 1996). A functional non-synonymous single nucleotide polymorphism (SNP) causing an amino-acid substitution of valine to methionine has been identified in codon 66 (Val66Met, rs6265) (Egan *et al*. 2003; Ventriglia *et al*. 2002).

In major depressive disorder (MDD), two Chinese studies failed to detect an effect of BDNF variation in relatively small samples of patients (Tsai *et al*. 2003: $n=152$; Hong *et al*. 2003: $n=84$). However, a significant association of a three-locus BDNF haplotype [rs988748, rs6265, (GT)n] was reported in a large case-control study analysing two independent samples of 465 and 312 patients with MDD (Schumacher *et al*. 2005). The BDNF 66Met allele was observed to be associated with harm avoidance as well as comorbid anxiety disorders and major depression (Jiang *et al*. 2005), major depression in Mexican-Americans (Ribeiro *et al*. 2007), geriatric depression (Hwang *et al*. 2006) and psychotic features and suicidal behaviour in Japanese patients with MDD (Iga *et al*. 2007). Association reports of the BDNF Val66Met polymorphism with four dimensions of neuroticism (depression, self-consciousness, anxiety, vulnerability) (Sen *et al*. 2003) as well as anxiety- and depression-related traits (Lang *et al*. 2005) further support a potential role of *BDNF* gene variation in the pathogenesis of major depression. However, a recent meta-analysis on genetic association studies of BDNF in depression did not reveal a major role of BDNF variation in major depression (Chen *et al*. 2008).

Two studies so far have investigated the impact of the BDNF Val66Met polymorphism on antidepressant treatment response: a significantly beneficial effect of the Met allele on treatment response to citalopram was observed in a sample of Korean patients with MDD (Choi *et al*. 2006; $n=83$), while a Taiwanese study reported a trend towards association of the heterozygous Val66Met genotype with improved 4-wk fluoxetine antidepressant response (Tsai *et al*. 2003, $n=110$). However, there was no influence of BDNF Val66Met genotype on treatment-resistant depression as a categorical phenotype (Anttila *et al*. 2007).

Given these inconsistent findings available to date, we attempted to re-evaluate the role of *BDNF* gene variation in the aetiology of depression and in antidepressant treatment response investigating a set of three SNPs including the functional Val66Met polymorphism (rs6265), rs7124442, which has been shown to be functionally related to BDNF plasma levels in eating-disorder subjects (Mercader *et al*. 2007), as well as an intronic SNP rs7103411.

Material and methods

Sample

A sample of 268 unrelated Caucasian patients with a current major depression (mean age 49.7 ± 15.4 yr; 154 females, 114 males) admitted for in-patient treatment was consecutively recruited at the Department of Psychiatry, University of Muenster, Germany, between 2004 and 2006. For pharmacogenetic analyses, only patients with a HAMD admission score $\geqslant10$ and a treatment cycle of at least 6 wk from baseline were considered, leaving a sample of $n=256$ patients with major depression (mean age 50.4 ± 14.9 yr; 145 females, 111 males). The mean HAMD score at admission was 23.1 ± 7.3 and at discharge it was 6.0 ± 5.0, without showing any differences between gender and diagnostic subtypes of depression. Patients with schizoaffective disorders, bipolar disorder or comorbid substance abuse disorders, mental retardation, pregnancy and neurological, neurodegenerative disorders or other clinically unstable medical illnesses impairing psychiatric evaluation were not included in this analysis. In order to minimize the risk of ethnic stratification, Caucasian descent was ascertained by Caucasian background of both parents. The present sample has already been described in published genetic or pharmacogenetic studies targeting other gene systems (Baune *et al*. 2008*a*, *b*; Domschke *et al*. 2008*a*, *b*).

A control group of similar age and gender to the cases consisted of 424 [mean age 52.7 ± 7.2 yr; 214 females (mean age 52.1 ± 6.9 yr); 210 males (mean age 52.5 ± 7.8 yr)] healthy subjects of Caucasian German descent, where the presence of current clinically relevant depressive symptoms was excluded using the Centre for Epidemiological Studies Depression Scale (CES-D; Radloff, 1977).

Approval of the ethics committee of the University of Muenster, Muenster, Germany, and written informed consent from all subjects were obtained.

We extended our study to include samples from the Sequenced Treatment Alternatives to Relieve Depression study (STAR*D). This sample is described in detail in McMahon *et al*. (2006). In brief, 1953 outpatients (out of 4041) provided DNA for

pharmacogenetic studies. All patients were treated with citalopram at 'level 1' and were followed prospectively for up to 14 wk. Bi-weekly follow-up visits included assessments for severity and tolerability.

Assessment

Patients' diagnoses considering major depression and melancholic depression were ascertained by the use of a structured clinical interview (SCID-I) according to DSM-IV criteria. Anxious depression at admission was as defined recently by Fava *et al*. (2008). A combined anxiety/somatization factor $\geqslant 7$ on the $HAMD_{21}$ scale was regarded as a criterion for anxious depression ($n=81$ in the pharmacoresponse sample). Clinical course of depression was assessed using the Hamilton Depression ($HAMD_{21}$) scale, the Clinical Global Impression (CGI) scale and the Global Assessment of Functioning (GAF) scale. While scores on the $HAMD_{21}$ were obtained on a weekly basis, CGI and GAF scores were obtained at admission and discharge only. Adverse drug effects were monitored clinically but were not systematically assessed using standardized scales.

In the STAR*D sample, participants were required to have a HAMD score of $\geqslant 14$ to enter the study. The primary outcome measure for pharmacogenetics studies was the 16-item Quick Inventory for Depressive Symptomatology – Clinician-rated version ($QIDS\text{-}C_{16}$) taken at each visit.

Response

In the German sample, clinical response to treatment was measured by the relative intra-individual changes of $HAMD_{21}$ scores over the 6-wk study period as previously published (Baune *et al*. 2008*a*).

The phenotype definitions for the STAR*D sample included remission and response as categorical variables and change in $QIDS\text{-}C_{16}$ from baseline to endpoint as a continuous trait. To be included in the analyses, participants needed to complete at least 6 wk treatment and be able to tolerate citalopram. Those who had at least a 50% improvement in $QIDS\text{-}C_{16}$ score were considered responders and those who achieved a score of $\leqslant 5$ at endpoint were considered remitters. Additional details can be found in McMahon *et al*. (2006).

Medication

Patients were treated in a naturalistic setting with a variety of antidepressant medications [mirtazapine ($n=28$, 10.9%), citalopram/escitalopram ($n=38$, 14.8%), venlafaxine ($n=45$, 17.6%), mirtazapine + citalopram/escitalopram ($n=38$, 14.8%), mirtazapine + venlafaxine ($n=63$, 24.6%), other (TCA, MAO inhibitors (MAOIs), lithium; $n=44$, 17.2%)]. As comedication atypical antipsychotics (quetiapine, olanzapine, risperidone; $n=121$, 47.3%) as well as mood stabilizers (lithium, valproic acid; $n=60$, 23.4%) were used in addition to antidepressant treatment. Benzodiazepines were used in three cases only. None of the included patients had received electroconvulsive therapy within the 6 months preceding the present investigation.

Genotyping

In the German sample, genotyping of rs7103411 (position: chr11:27,656,701) rs6265 (position: chr11:27,636,492) and rs7124442 (position: chr11:27,633,617) was carried out following published protocols applying the multiplex genotyping assay iPLEX™ for use with the MassARRAY platform (Oeth *et al*. 2007), yielding a genotyping completion rate of 93.7–96.6% for all included patients and controls. While two genotyping failures occurred in the overall depression sample concerning each SNP (rs7103411/rs6265/rs7124442, $n=266$), genotyping failures in the control group resulted in a genotype availability of $n=415$ for rs7103411, $n=415$ for rs6265, and $n=410$ for rs7124442. Genotypes were determined by investigators blinded for clinical diagnoses.

In the STAR*D sample, ten SNPs in BDNF were tested as a part of a larger list of 768 markers in 68 genes. The markers tested were: rs1519479, rs2203877, rs1519480, rs6265 (Val66Met), rs11030104, rs12273363, rs7931247, rs1491850, rs7119334 and rs10742184. These SNPs were genotyped at Illumina Inc. (USA) with a 99% success rate.

Statistical analysis

The categorical association analysis of allele and genotype distribution across patients with major depression ($n=268$) and age- and gender-matched healthy controls was performed by means of Armitage's trend test as provided by the DeFinetti program available as an online source (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl; T. F. Wienker & T. M. Strom).

Comparisons of HAMD, CGI and GAF baseline scores across genotype groups were carried out with one-way ANOVA (>2 categories). The pharmacogenetic investigation of BDNF variant effects on HAMD change scores over 6 wk antidepressant treatment was performed using an ANOVA with repeated

Table 1. Overall reduction of % HAMD scores across BDNF rs7103411, rs6265, rs7124442 genotypes in the pharmacogenetic sample of patients with major depression ($n=254$) stratified for gender, anxious and melancholic depression

	Major depression (% HAMD reduction)			
BDNF genotypes	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	*p* value[a,b]
Rs7103411				
HAMD change weeks 2–6[c,e]	CC ($n=14$)	CT ($n=85$)	TT ($n=155$)	
MDD ($n=254$)	−68.0 ± 18.2	−33.0 ± 7.0	−20.0 ± 5.2	0.034
Female ($n=144$)[d]	−50.1 ± 25.3	−23.0 ± 11.0	−23.5 ± 6.8	0.082
Male ($n=110$)[d]	−75.4 ± 26.5	−40.1 ± 9.0	−17.0 ± 8.3	***0.009***
Anxious depression ($n=81$)	−93.0 ± 28.3	−52.4 ± 10.1	−22.2 ± 7.9	***0.005***
Melancholic depression ($n=95$)	−97.4 ± 28.8	−52.7 ± 10.1	−1.1 ± 10.5	***0.003***
Rs6265				
HAMD change weeks 2–6[c,e]	AA ($n=11$)	AG ($n=80$)	GG ($n=163$)	
MDD ($n=254$)	−16.0 ± 20.0	−10.6 ± 7.4	−35.1 ± 5.0	0.411
Female ($n=144$)[d]	−32.7 ± 28.5	−16.6 ± 10.9	−27.8 ± 6.5	0.88
Male ($n=110$)[d]	−2.9 ± 27.2	−8.6 ± 9.9	−42.7 ± 7.7	0.23
Anxious depression ($n=81$)	−33.6 ± 29.6	−6.9 ± 11.3	−53.2 ± 7.1	0.27
Melancholic depression ($n=95$)	55.7 ± 30.5	2.7 ± 10.9	−58.5 ± 10.1	***0.001***
Rs7124442				
HAMD change weeks 2–6[c,e]	CC ($n=31$)	CT ($n=97$)	TT ($n=126$)	
MDD ($n=254$)	−44.4 ± 6.6	−23.4 ± 3.7	−24.7 ± 3.3	***0.01***
Female ($n=144$)[d]	−47.2 ± 9.4	−23.1 ± 5.3	−21.1 ± 4.4	0.06
Male ($n=110$)[d]	−41.1 ± 9.1	−23.1 ± 5.1	−30.7 ± 4.9	0.34
Anxious depression ($n=81$)	−66.1 ± 12.1	−37.2 ± 6.6	−33.6 ± 5.5	***0.003***
Melancholic depression ($n=95$)	−20.9 ± 14.1	−38.5 ± 5.5	−22.3 ± 4.8	0.70

HAMD, Hamilton Depression Rating Scale; MDD, major depressive disorder.

p value = ANOVA with repeated measures [covariates: age, gender, polypharmacy (treatment with various antidepressants) and treatment with antidepressants + atypical antipsychotics, HAMD score at admission] for the comparison of HAMD change score between homozygous genotypes.

Bold and italics = significant *p* value at significance after false discovery rate (FDR) correction for multiple hypotheses testing.

[a] In this analysis: FDR *p* value cut-off = 0.014; [b] FDR *p* value cut-off = 0.01, obtained from the combination of this and previous analyses as published by our group (Baune *et al*. 2008*b*; Domschke *et al*. 2008*a*, *b*).

[c] *p* values from ANOVA models with covariates age, gender, polypharmacy (treatment with various antidepressants), treatment with antidepressants + atypical antipsychotics, HAMD score at admission.

[d] Covariates: age, polypharmacy (treatment with various antidepressants), treatment with antidepressants + atypical antipsychotics, HAMD score at admission.

[e] Intra-individual relative change scores of the $HAMD_{21}$ scale between weeks 2 and 6: multivariable ANOVA [covariates: age, gender polypharmacy (treatment with various antidepressants), treatment with antidepressants + atypical antipsychotics, HAMD score at admission] with repeated measures independent of change in week 1.

measures (genotype as fixed factor and time-point as repeated measure), and number of covariates (see below) (Table 1). False discovery rate (FDR; Benjamini & Hochberg, 1995) was applied to control for multiple testing and prevent Type I error. FDR was applied in two different ways. First, FDR was calculated for the number of hypotheses tested in the present study. The resulting FDR (labelled as 'within-sample FDR') has a corrected *p* value of $\leqslant 0.014$. Second, FDR was calculated based on the current BDNF analysis plus pharmacogenetic analyses of other neurotransmitter systems in this sample of MDD patients as previously published by our group (Baune *et al*. 2008*b*; Domschke *et al*. 2008*a*, *b*). The resulting FDR is labelled 'between-samples FDR' and shows a corrected *p* value of $\leqslant 0.01$.

The ANOVA analyses were stratified for gender, anxious and melancholic depression. Covariates (e.g. age, gender, duration of depressive illness, lifetime number of depressive episodes, number of hospitalizations due to depression, class of antidepressant, i.e. SSRIs, SNRA, NaSSRA, TCAs, MAOIs, polypharmacy,

treatment with antidepressants + atypical antipsychotics, antidepressants + mood stabilizer or family history of mental disorders) were included in these multivariable ANOVA models, if they showed an impact on treatment response over 6 wk. In order to test whether the three investigated SNPs affect treatment response independently of each other, the BDNF SNP of interest was entered as a fixed factor while the respective other two BDNF SNPs were included as covariates in the ANOVA models.

In addition, we performed separate multiple ANOVAs (for included covariates see above; applying FDR cut-off of $p \leqslant 0.014$) to obtain results on the weekly intra-individual HAMD change scores across genotype groups. In addition, separate ANOVAs were performed to investigate if the effects of the BDNF genotypes on treatment response were mediated through covariates (dummy variables) such as class of antidepressant, i.e. SSRIs, SNRA, NaSSRA, TCAs, MAOIs (SSRIs *vs*. TCAs, MAOIs), treatment with antidepressants + atypical antipsychotics (antidepressant alone *vs*. antidepressant + atypical antipsychotics) or antidepressants + mood stabilizer (antidepressant alone *vs*. antidepressant + mood stabilizer). With these parameters, for continuous measurements, our sample had a high power (80%) to detect a difference of at least 3.6% change on the HAMD scale between two genotypes. Hardy–Weinberg equilibrium was examined using the DeFinetti program

For haplotype analyses, the most frequent 'TGT' haplotype (rs7103411/rs6265/rs7124442) (46.9%, determined by means of Haploview 4.0) containing the associated rs7124442 T allele was analysed against the remaining haplotypes for association with depression and for pharmacoresponse over 6 wk antidepressant treatment.

The association analyses in STAR*D were performed using PLINK. To account for potential racial stratification, given the racial heterogeneity of STAR*D participants that could cause an inflation of association results, we used genomic control. *Post-hoc* tests included stratification based on gender and depression subtype (anxious and melancholic).

Results

In the German sample, the distribution of rs7103411, rs6265 and rs7124442 genotypes did not significantly differ from the expected numbers calculated on the basis of observed allele frequencies according to the Hardy–Weinberg equilibrium for the overall patient sample (rs7103411: $p = 0.75$; rs6265: $p = 0.95$; rs7124442: $p = 0.14$), the pharmacogenetic sample (rs7103411: $p = 0.59$; rs6265: $p = 0.77$; rs7124442: $p = 0.074$) as well as for the control sample (rs7103411: $p = 0.31$; rs6265: $p = 0.24$; rs7124442: $p = 0.48$). Linkage disequilibrium between the three SNPs was moderately high between rs6265 and rs7103411 ($D' = 0.976$, $r^2 = 0.8252$) and low between SNPs rs6265 and rs7124442 ($D' = -0.973$, $r^2 = 0.0948$) and SNPs rs7103411 and rs7124442 ($D' = -0.937$, $r^2 = 0.1$).

None of the three BDNF SNPs was associated with severity of depression as measured by HAMD score at baseline, number of lifetime episodes of depression, suicide attempts and duration of illness in years.

Categorical association results in the German sample

No association of any of the three investigated BDNF polymorphisms with major depression was observed (rs7103411: $p = 0.79$; rs6265: $p = 0.51$; rs7124442: $p = 0.16$) in the samples of 268 patients with major depression and 424 controls. The case and control samples showed similar distributions for age and gender. A diagnostic subtyping into anxious depression ($n = 81$) and melancholic depression ($n = 95$) also revealed no association with any of the BDNF SNPs. No significant allele frequency differences between cases and controls were observed for any of the three SNPs. In addition, dichotomous haplotype analyses for the rs7103411/rs6265/rs7124442 TGT haplotype (*vs*. rest) showed no associations with depression or diagnostic subtypes.

Pharmacogenetic results in the German sample

Within the pharmacogenetic sample comprising 254 patients (admission HAMD score $\geqslant 10$) with major depression, the subsamples stratified for gender, anxious depression and melancholic depression did not significantly differ for age, education, marital status or comorbidity with anxious depression. Since treatment response over 6 wk (expressed through the overall HAMD % change) was influenced by gender, age, polypharmacy (treatment with various antidepressants) and treatment with antidepressants + atypical antipsychotics, these variables were considered as covariates in multivariate ANOVA procedures. Conversely, treatment response over 6 wk was not influenced by duration of depressive illness, lifetime number of depressive episodes, number of hospitalizations, class of antidepressant (i.e. SSRIs, SNRAs, NaSSRAs, TCAs, MAOIs), antidepressants + mood stabilizer or family history of mental disorders (data not shown).

Table 1 presents results from ANOVA and the BDNF SNP of interest as fixed factor (covariates age,

gender, polypharmacy and treatment with antidepressants combined with atypical antipsychotics, remaining two BDNF SNPs) with repeated measures for the intra-individual % HAMD reduction over the course of 6 wk antidepressant treatment across genotypes of the three investigated BDNF SNPs. The TT genotype of BDNF rs7124442 was significantly related to worse treatment outcome over 6 wk in MDD ($p=0.01$), particularly in anxious depression ($p=0.003$). The other two investigated BDNF SNPs similarly predicted worse treatment response over 6 wk in the melancholic subtype of depression (rs7103411: TT < CC genotype, $p=0.003$; rs6265: GG < AA genotype, $p=0.001$) and partly in anxious depression (rs7103411: TT < CC genotype, $p=0.005$) and male gender (rs7103411: TT < CC genotype, $p=0.009$) (see Table 1). However, analyses across all three genotypes or applying a recessive model did not reveal significant results. All of these results remain significant after applying the within-sample FDR (cut-off $p \leqslant 0.014$) and between analyses FDR (cut-off $p \leqslant 0.01$).

Furthermore, haplotype analyses for the rs7103411/rs6265/rs7124442 TGT haplotype containing the associated rs7124442 T allele (*vs.* rest) showed an association with overall worse treatment response over 6 wk in MDD (HAMD reduction −22.9% *vs.* −39.4%, $p=0.005$), in anxious depression (HAMD reduction −31.5% *vs.* −62.8%, $p=0.004$) and in female patients (HAMD reduction −25.7% *vs.* −37.1%, $p=0.01$).

Regarding the course of *BDNF* gene variation impacting on treatment response (HAMD reduction), BDNF rs7124442 (comparison of homozygous genotypes CC *vs.* TT) showed an early effect after 2 wk antidepressant treatment which was maintained up to week 6 in the overall group of patients with major depression (HAMD at week 2: −28.8% *vs.* −11.0%, $p=0.027$; HAMD at week 3: −41.2% *vs.* −17.1%, $p=0.007$; HAMD at week 4: −44.9% *vs.* −28.9.0%, $p=0.12$; HAMD at week 5: −57.1% *vs.* −30.2.0%, $p=0.009$; HAMD at week 6: −59.1% *vs.* −37.1%, $p=0.049$) as well as in the subgroup of anxious depression showing early effects after 3 wk treatment (HAMD at week 2: −45.6% *vs.* −19.8%, $p=0.11$; HAMD at week 3: −67.8% *vs.* −22.5%, $p=0.012$; HAMD at week 4: −73.4% *vs.* −34.9%, $p=0.027$; HAMD at week 5: −90.3% *vs.* −38.5%, $p=0.008$; HAMD at week 6: −98.4% *vs.* −44.8%, $p=0.004$). When applying the within-sample FDR cut-off p value (0.014), the results for pharmacoresponse in the overall MDD sample remain significant for weeks 3 and 5, whereas the anxious depression subgroup shows significant results for weeks 3, 5 and 6.

BDNF-related pharmacoresponse specific for type of antidepressant treatment in the German sample

When investigating patients with major depression stratified for type of antidepressant medication, a significantly negative influence of the BDNF rs7124442 TT genotype on treatment response was observed for SSRI antidepressants over 6 wk treatment as opposed to the CC genotype (HAMD reduction for TT *vs.* CC genotype: −22.6% *vs.* −59.8%, $p=0.002$). The effect was first visible after 3 wk antidepressant treatment (mean difference between CC and TT genotypes: −38.1%, $p=0.002$) and became most prominent after 5 and 6 wk treatment (week 5: mean difference between CC and TT genotypes −47.4%, $p=0.002$; week 6: mean difference between CC and TT genotypes −48.4%, $p=0.007$). These p values are lower than the FDR cut-off of $p \leqslant 0.014$ and therefore remain statistically significant. This could not be observed for any other class of antidepressant. Moreover, BDNF rs7124442 did not have any influence on treatment response under polypharmacy, treatment with antidepressants + atypical antipsychotics or antidepressants + mood stabilizers.

Applying multivariable ANOVA (covariates: age, gender, class of antidepressants) for the influence of the three BDNF SNPs on GAF and CGI change scores between admission and discharge, no significant pharmacogenetic treatment effects for either of the global scales was detected in patients with MDD or anxious depression.

*Pharmacogenetic results in STAR*D sample*

The STAR*D analyses including remission, response and change in QIDS-C_{16} score did not yield any significant results at any of the ten markers reported in the present study. *Post-hoc* tests, stratifying on depression subtype showed no associations after correction for multiple testing.

Discussion

Despite no overall association finding of BDNF variants with major depression, the present study investigating *BDNF* gene variants in major depression supports a potential role of BDNF rs7124442 as well as a rs7103411/rs6265/rs7124442 haplotype containing the associated rs7124442 T allele in treatment response to antidepressants in the German sample. BDNF rs7124442 has previously been analysed with respect to Alzheimer's disease (Huang *et al.* 2007), but has not been examined in depression to the best of our

knowledge. BDNF rs7124442 is located in exon VIII after the polyadenylation site in the 3′ untranslated region of the *BDNF* gene (LocusLink. National Center for Biotechnology Information: http://www.ncbi.nih.gov). Given the converging evidence for a reduced BDNF state in depression (see above), the rs7124442 T allele associated with impaired treatment response to antidepressants might confer a decreased BDNF activity or expression by as yet unknown mechanisms of alternative splicing for example. However, this hypothesis has to be considered speculative at this stage, since no molecular genetic functional data on this polymorphism are yet available and Mercader and colleagues found an increase in plasma BDNF levels associated with the rs7124442 T allele (Mercader *et al*. 2007).

The observed potential impact of BDNF rs7124442 on antidepressant treatment response particularly under SSRI treatment fits well with studies in the rat model, where administration of SSRIs has been reported to significantly increase BDNF levels (Altar *et al*. 2003; Neeper *et al*. 1996). The interaction between serotonin and BDNF has been described as being reciprocal, with BDNF on the one hand promoting development and function of serotonergic neurons and serotonin as amplified by SSRIs on the other hand increasing BDNF transcription via cAMP responsive element binding protein (CREB) phosphorylation (as reviewed by Martinowich & Lu, 2008).

Furthermore, the present association of BDNF rs7124442 seems to originate solely from the female subgroup of patients. This is in line with previous observations of gender-specific pharmacogenetic effects such as in MAOA (Domschke *et al*. 2008*b*). Major depression is approximately twice as frequent in women as in men (Weissman *et al*. 1993) with a significantly higher heritability in women (42%) than in men (29%) (Jansson *et al*. 2004; Kendler *et al*. 2006), which suggests a sexually dimorphic pattern of genetic susceptibility to the disorder potentially in part being conferred by *BDNF* gene variation.

However, apart from a potential effect in the subgroup of melancholic and partly anxious depression we did not detect any effect of the BDNF Val66Met polymorphism or rs7103411 on overall antidepressant treatment response in patients with major depression.

This is in line with the failure to observe any association of any of the ten investigated BDNF markers in the STAR*D sample including BDNF Val66Met with citalopram treatment. Although a false-positive finding in the German sample cannot be ruled out, SNP rs7124442 was not genotyped in STAR*D. Furthermore, none of the markers included are in significant linkage disequilibrium with rs7124442, therefore Type II error cannot be excluded either.

The following limitations have to be considered while interpreting the present results. In the German sample, patients were recruited in a naturalistic setting allowing for a large sample size; however, this implies treatment with a variety of antidepressants, no standardized dosage regime and no standardized control of plasma drug levels. Thus, treatment compliance could only be controlled for by routine nurse observations lacking objective measures of compliance, which has to be considered a possible major confounding factor. Furthermore, none of the patients was drug naive with respect to antidepressant medication; however, no detailed data on the type of antidepressant pre-medication was available. Antidepressant treatment prior to the present investigation might have influenced the presently evaluated treatment response which could not be controlled for in detail. In addition, since *a priori* only patients with a treatment cycle of at least 6 wk from baseline were included in the present study, no drop-outs due to non-response could be accounted for. Moreover, comorbidity with personality disorders could not be controlled for, which might have confounded the present pharmacogenetic finding. Furthermore, the relatively small sample sizes of patients in the diagnostic subgroups (stratification for gender, melancholic and anxious depression, subgroups receiving a single antidepressant medication, three-way interactions) might have increased the risk for a false-negative as well as a false-positive result. Thus, the present observations regarding depression subtype or pharmacotherapy type cannot be conclusively evaluated and have to be considered as exploratory results. Since a number of tests were carried out here, Type I error might have occurred in the pharmacogenetic analyses despite the introduction of FDR-corrected p values for this study and in comparison to other pharmacogenetic studies published by our group (Baune *et al*. 2008*b*; Domschke *et al*. 2008*a*, *b*). Finally, grouping of genotypes underlying the present results has to be considered arbitrarily, since with the exception of BDNF Val66Met (rs6265) no functional data on the selected BDNF variants are currently available.

In summary, we did not find an association between genetic variation in BDNF and antidepressant treatment response and remission. *Post-hoc* analyses provide some preliminary support for a potential minor role of genetic variation in BDNF and antidepressant treatment outcome in the context of melancholic depression.

Acknowledgements

We gratefully acknowledge the skilful technical support of Kathrin Schwarte, Chris Swagell and Ian Hughes. This research was supported in part by the Intramural Research Program of the National Institute of Mental Health, National Institutes of Health, USDHHS.

Statement of Interest

None.

References

Altar CA, Whitehead RE, Chen G, Wörtwein G, Madsen TM (2003). Effects of electroconvulsive seizures and antidepressant drugs on brain-derived neurotrophic factor protein in rat brain. *Biological Psychiatry* **54**, 703–709.

Anttila S, Huuhka K, Huuhka M, Rontu R, Hurme M, Leinonen E, Lehtimäki T (2007). Interaction between 5-HT1A and BDNF genotypes increases the risk of treatment-resistant depression. *Journal of Neural Transmission* **114**, 1065–1068.

Baune BT, Hohoff C, Berger K, Neumann A, Mortensen S, Roehrs T, Deckert J, Arolt V, Domschke K (2008*a*). Association of the COMT val158met variant with antidepressant treatment response in major depression. *Neuropsychopharmacology* **33**, 924–932.

Baune BT, Hohoff C, Roehrs T, Deckert J, Arolt V, Domschke K (2008*b*). Serotonin receptor 1A -1019C/G variant: impact on antidepressant pharmacoresponse in melancholic depression? *Neuroscience Letters* **436**, 111–115.

Benjamini Y, Hochberg Y (1995). Controlling the false discovery rate – a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society B* **57**, 289–300.

Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT (2001). Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biological Psychiatry* **50**, 260–265.

Chen L, Lawlor D, Lewis S, Yuan W, Abdollahi M, Timpson N, Day I, Ebrahim S, Smith G, Shugart Y (2008). Genetic association study of BDNF in depression: finding from two cohort studies and a meta-analysis. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics* **147B**, 814–821.

Choi M, Kang R, Lim S, Oh K, Lee M (2006). Brain-derived neurotrophic factor gene polymorphism (Val66Met) and citalopram response in major depressive disorder. *Brain Research* **1118**, 176–182.

Domschke K, Dannlowski U, Ohrmann P, Lawford B, Bauer J, Kugel H, Heindel W, Young R, Morris P, Arolt V, *et al.* (2008*a*). Cannabinoid receptor 1 (CNR1) gene: impact on antidepressant treatment response and emotion processing in major depression. *European Journal of Neuropsychopharmacology* **18**, 751–759.

Domschke K, Hohoff C, Mortensen L, Roehrs T, Deckert J, Arolt V, Baune B (2008*b*). Monoamine oxidase A variant influences antidepressant treatment response in female patients with major depression. *Progress in Neuropsychopharmacology & Biological Psychiatry* **32**, 224–228.

Duman R (1999). The neurochemistry of mood disorders: preclinical studies. In: Charney D, Nestler E, Bunney B (Eds), *The Neurobiology of Mental Illness* (pp. 333–347). New York: Oxford University Press.

Egan M, Kojima M, Callicott J, Goldberg T, Kolachana B, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger D (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* **112**, 257–269.

Fava M, Rush A, Alpert J, Balasubramani G, Wisniewski S, Carmin C, Biggs M, Zisook S, Leuchter A, Howland R, Warden D, Trivedi M (2008). Difference in treatment outcome in outpatients with anxious versus nonanxious depression: a STAR*D report. *American Journal of Psychiatry* **165**, 342–351.

Green E Craddock N (2003). Brain-derived neurotrophic factor as a potential risk locus for bipolar disorder: evidence, limitations, and implications. *Current Psychiatry Reports* **5**, 469–476.

Hong C, Huo S, Yen F, Tung C, Pan G, Tsai S (2003). Association study of a brain-derived neurotrophic-factor genetic polymorphism and mood disorders, age of onset and suicidal behavior. *Neuropsychobiology* **48**, 186–189.

Huang R, Huang J, Cathcart H, Smith S, Poduslo S (2007). Genetic variants in brain-derived neurotrophic factor associated with Alzheimer's disease. *Journal of Medical Genetics* **44**, 66–71.

Hwang J, Tsai S, Hong C, Yang C, Lirng J, Yang Y (2006). The Val66Met polymorphism of the brain-derived neurotrophic-factor gene is associated with geriatric depression. *Neurobiology of Aging* **27**, 1834–1837.

Iga J, Ueno S, Yamauchi K, Numata S, Tayoshi-Shibuya S, Kinouchi S, Nakataki M, Song H, Hokoishi K, Tanabe H, Sano A, Ohmori T (2007). The Val66Met polymorphism of the brain-derived neurotrophic factor gene is associated with psychotic feature and suicidal behavior in Japanese major depressive patients. *American Journal of Medical Genetics, Part B Neuropsychiatric Genetics* **144**, 1003–1006.

Jansson M, Gatz M, Berg S, Johansson B, Malmberg B, McClearn G, Schalling M, Pedersen N (2004). Gender differences in heritability of depressive symptoms in the elderly. *Psychological Medicine* **34**, 471–479.

Jiang X, Xu K, Hoberman J, Tian F, Marko AJ, Waheed JF, Harris CR, Marini AM, Enoch MA, Lipsky RH (2005). BDNF variation and mood disorders: a novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. *Neuropsychopharmacology* **30**, 1353–1361.

Kendler K, Gatz M, Gardner C, Pedersen N (2006). A Swedish national twin study of lifetime major depression. *American Journal of Psychiatry* **163**, 109–114.

Lang U, Hellweg R, Kalus P, Bajbouj M, Lenzen K, Sander T, Kunz D, Gallinat J (2005). Association of a functional BDNF polymorphism and anxiety-related personality traits. *Psychopharmacology* **180**, 95–99.

Martinowich K, Lu B (2008). Interaction between BDNF and serotonin: role in mood disorders. *Neuropsychopharmacology* **33**, 73–83.

McMahon FJ, Buervenich S, Charney D, Lipsky R, Rush AJ, Wilson AF, Sorant AJ, Papanicolaou GJ, Laje G, Fava M, *et al*. (2006). Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. *American Journal of Human Genetics* **78**, 804–814.

Mercader J, Ribasés M, Gratacòs M, González J, Bayés M, de Cid R, Badía A, Fernández-Aranda F, Estivill X (2007). Altered brain-derived neurotrophic factor blood levels and gene variability are associated with anorexia and bulimia. *Genes Brain Behavior* **6**, 706–716.

Neeper S, Gomez-Pinilla F, Choi J, Cotman C (1996). Physical activity increases mRA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Research* **726**, 49–56.

Nibuya M, Morinobu S, Duman R (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *Journal of Neuroscience* **15**, 7539–7547.

Oeth P, Beaulieu M, Park C, Kosman D, del Mistro G, van den Boom D, Jurinke C (2007). 'iPLEX™ assay: increased plexing efficiency and flexibility for MassARRAY system through single base primer extension with mass-modified terminators (http://www.agrf.org.au/docstore/snp/iPlex.pdf). Accessed 21 June 2008.

Radloff L (1977). The CES-D Scale: a self-report depression scale for research in the general population. *Applied Psychological Measurement* **1**, 385–401.

Ribeiro L, Busnello J, Cantor R, Whelan F, Whittaker P, Deloukas P, Wong M, Licinio J (2007). The brain-derived neurotrophic factor rs6265 (Val66Met) polymorphism and depression in Mexican-Americans. *Neuroreport* **18**, 1291–1293.

Saarelainen T, Hendolin P, Lucas G, Koponen E, Sairanen M, MacDonald E, Agerman K, Haapasalo A, Nawa H, Aloyz R, Ernfors P, Castrén E (2003). Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *Journal of Neuroscience* **23**, 349–357.

Schumacher J, Jamra R, Becker T, Ohlraun S, Klopp N, Binder E, Schulze T, Deschner M, Schmäl C, Höfels S, *et al*. (2005). Evidence for a relationship between genetic variants at the brain-derived neurotrophic factor (BDNF) locus and major depression. *Biological Psychiatry* **58**, 307–314.

Seidah N, Benjannet S, Pareek S, Chrétien M, Murphy R (1996). Cellular processing of the neurotrophin precursors of NT3 and BDNF by the mammalian proprotein convertases. *FEBS Letters* **379**, 247–250.

Sen S, Nesse R, Stoltenberg S, Li S, Gleiberman L, Chakravarti A, Weder A, Burmeister M (2003). A BDNF coding variant is associated with the NEO personality inventory domain neuroticism, a risk factor for depression. *Neuropsychopharmacology* **28**, 397–401.

Shirayama Y, Chen A, Nakagawa S, Russell D, Duman R (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *Journal of Neuroscience* **22**, 3251–3261.

Siuciak J, Lewis D, Wiegand S, Lindsay R (1997). Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). *Pharmacology Biochemistry Behavior* **56**, 131–137.

Smith M, Makino S, Kvetnansky R, Post R (1995). Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *Journal of Neuroscience* **15**, 1768–1777.

Tsai S, Cheng C, Yu Y, Chen T, Hong C (2003). Association study of a brain-derived neurotrophic-factor genetic polymorphism and major depressive disorders, symptomatology, and antidepressant response. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics* **123**, 19–22.

Ventriglia M, Bocchio Chiavetto L, Benussi L, Binetti G, Zanetti O, Riva MA, Gennarelli M (2002). Association between the BDNF 196 A/G polymorphism and sporadic Alzheimer's disease. *Molecular Psychiatry* **7**, 136–137.

Weissman M, Bland R, Joyce P, Newman S, Wells J, Wittchen H (1993). Sex differences in rates of depression: cross-national perspectives. *Journal of Affective Disorder* **29**, 77–84.