Further evidence supporting the influence of brain-derived neurotrophic factor on the outcome of bipolar depression: independent effect of brain-derived neurotrophic factor and harm avoidance

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Abstract
Brain-derived neurotrophic factor is a candidate gene for response to antidepressant treatment. However, response to pharmacological treatments is moderated by both genetic and other factors within individuals. For example, there is evidence of an influence of the temperamental trait of harm avoidance on the outcome of depressive disorders. In the present study we aimed to investigate the effect of the brain-derived neurotrophic factor gene on medium-term outcome in a naturalistic sample of 86 depressed bipolar spectrum patients, taking into account harm avoidance.

Both single marker and haplotypes were significantly associated with severity of depression at month 6 after treatment initiation. The haplotype comprising the A-C alleles was associated with a poorer outcome. Harm avoidance maintained a significant effect on depressive outcome in bipolar disorder, independently from brain-derived neurotrophic factor genotypes. However, harm avoidance’s influence appeared to be more consistent in patients carrying the protective G-T combination of alleles.

Our results indicate brain-derived neurotrophic factor as involved in the outcome of depression in bipolar disorder. Harm avoidance did not interact with brain-derived neurotrophic factor genotypes, though its effect was still significant. Given that many factors may influence response to pharmacological treatments, studies that consider personality and other individual characteristics are warranted also in pharmacogenetic investigations.

Keywords
bipolar disorder, depression, BDNF gene, harm avoidance, outcome

Introduction
Much evidence exists of a genetic influence on the response to pharmacological treatments. The past few decades have witnessed much progress in the field of pharmacogenetics, and a number of interesting genes have been suggested as putative regulators of response to antidepressants (Drago et al., 2009).

Among these, recent attention has been focused on brain-derived neurotrophic factor (BDNF), a neurotrophin involved in the processes of differentiation and neuronal resilience. BDNF has been linked with severe psychiatric disorders, particularly schizophrenia (Ashe et al., 2001; Lu and Martinowich, 2008) and bipolar (BP) disorder (Fan and Sklar, 2008; Serretti and Mandelli, 2008). Of interest, in vitro and in vivo data provided direct evidence that BDNF is a key mediator of the therapeutic response to antidepressants (see D’Sa and Duman, 2002). Chronic, but not acute, selective serotonin reuptake inhibitor treatment has been associated with an increased hippocampal expression of BDNF in adult rats (Nibuya et al., 1995). A first clinical study in humans reported reduced BDNF protein levels in the brains of unmedicated depressed patients (Duman et al., 1999). It has been thus hypothesized that decreased levels of BDNF could participate in the hippocampal atrophy observed in depressed patients. Indeed, BDNF has also trophic effects on the serotoninergic neurons in the central nervous system (Mamounas et al., 2000). Further, antidepressant treatment has been shown to restore serum levels of BDNF in human patients with depression in several

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studies (Aydemir et al., 2005; Gervasoni et al., 2005; Gonul et al., 2005; Piccinni et al., 2008; Shimizu et al., 2003).

The serotonin transporter (SERT) is the major target of antidepressants. Recently, evidence has been reported that constitutive reduction in BDNF levels modulates SERT reuptake capacity in adult hippocampus, while induced higher levels of BDNF in the hippocampus enhances SERT function (Benmansour et al., 2008; Daws et al., 2007). Based on this evidence, a number of studies, though still preliminary, investigated BDNF genetic variants on the acute response to antidepressant treatment in human patients with depression. One of the most investigated genetic variations within the BDNF gene is rs6265 (196G/A), resulting in a Val66 to Met (V66M) change in the 50-pro-region of the protein. This variation has been associated with poorer episodic memory and abnormal hippocampal activation (Egan et al., 2003). After a first negative report (Tsai et al., 2003), the rs6265 A-allele has been associated with a better response to acute treatment (6–8 weeks) with antidepressants in two independent Asian populations (Choi et al., 2006; Yoshida et al., 2006), while negative findings were reported in Caucasians (Gratacos et al., 2008; Wilkie et al., 2007). However, Gratacos et al. found other polymorphisms (haplotype rs12273363-rs908867-rs1491850) associated with remission after one adequate antidepressant treatment (at least 6 weeks), and a recent meta-analysis confirmed the association between the rs6265 polymorphism and response to antidepressants (Kato and Serretti, in press). However, controversial data have been subsequently obtained in both Caucasians and Asians (Kang et al., in press, Licinio et al., 2009).

The role of BDNF in the treatment of BP depression, i.e. not restricted to antidepressants, is less clear. There is evidence of an involvement of BDNF in lithium prophylactic efficacy. Rybakowski et al. (2005) reported an association between the rs6265 A-allele and a better response to lithium prophylactic treatment for BP disorder, though no replication could be obtained on a Asiatic sample (Masui et al., 2006). Further, Rybakowski et al. found BDNF significantly interacting with SERT gene (5-HTTLPR polymorphism) in moderating the efficacy of lithium treatment (Rybakowski et al., 2007b). Recently, changes in serum BDNF levels have been also reported after treatment with both mood stabilizers and antipsychotics for an episode of acute mania (Tramontina et al., 2009).

Inconsistencies among studies may be due to a number of reasons, such as sample sizes, ethnicity, criteria for the selection of patients, and other across-study methodological disparities. Further, the contribution of genes to the risk of disorders and response to treatments is relatively small (Craddock and Jones, 1999) and individual and environmental factors may interfere, influencing the observed phenotype. It is commonly acknowledged that complex human behaviors result from the interplay between the individual genetic profile and other traits, both genetically and environmentally mediated. This has been recognized in many psychiatric disorders as well (see Jaffee and Price, 2007). However, psychopharmacological studies rarely take into account other clinical and individual features as predictors of response to treatments (Serretti et al., 2008).

Features consistently associated with the risk for mood disorder and response to treatment are ‘neurotic’ or ‘anxiety-related’ personality traits, also called neuroticism (Costa and McCrae, 1989) or harm avoidance (HA) (Cloninger, 1987). Studies have shown prevalent neurotic traits in patients with history of depressive disorders and resistant to treatments (Pelissolo and Corruble, 2002). In a previous investigation on a sample of BP spectrum patients, we found HA significantly influencing the outcome of a depressive episode over a follow-up period of six months (unpublished data). Further, we found HA to moderate the effect of a common polymorphism within SERT (5HTTLPR) on response to treatment (Mandelli et al., 2009).

In the present study, we aimed to investigate the effect of BDNF (rs6265 and rs11030104 polymorphisms) in the same sample of BP spectrum patients: a naturalistic sample of depressed BP patients followed up to six months. We hypothesized a role for BDNF in the outcome of BP depression after treatment with various agents (antidepressants, mood stabilizers and antipsychotics), according to previous but sparse evidence. Moreover, we hypothesized a modulating effect of personality traits, particularly HA, as previously observed in our sample.

Methods
Sample and evaluations
Sample and methods of evaluation have been previously described (Mandelli et al., 2009). Briefly, patients were included in the study if they met DSM-IV criteria for a BP spectrum disorder (Akiskal et al., 1977): Bipolar I (BP-I, n = 41, 47.7%), Bipolar II (BP-II, n = 20, 23.2%) or cyclothymic disorder (CtD, n = 25, 29.1%), satisfying criteria for a current major depression (by the SCID-I interview (First et al., 1995)), and aged between 18 and 80 years. Exclusion criteria were represented by mental retardation or documented IQ <70, cognitive impairment (evaluated by the Mini Mental State Examination (Crumm et al., 1993)), unstable or severe medical condition that could impair evaluations, pregnancy or breastfeeding.

Finally, 86 unrelated Italian individuals, with Italian antecedents of at least three generations, were included in the sample after they accepted the study procedures and signed the informed consent as approved by the local ethical committee. Patients were treated with conventional antidepressants and/or mood stabilizers at standard therapeutic doses for at least three months. Concomitant treatment with antipsychotics and sedative/anxiolytic drugs did not represent an exclusion criterion (Table 1). Treatment was maintained for six months when possible (drop out and switches to mania listed in Table 1); whereas dosages of drugs could be adjusted on the basis of the clinician’s judgment. At intake, patients were evaluated for Axis II personality disorders by the SCID-II (First et al., 1990) and for HA by the Italian version of the TCI-R (Martinotti et al., 2008). Depressive symptoms’ severity was evaluated by the Hamilton Depression Rating Scale (HDRS, or HAMD) (Hamilton, 1960) at intake and after one, three and six months of treatment.
Genomic DNA was extracted from a sample (200 μl) of peripheral blood using High Pure PCR Template Preparation Kit (Roche Diagnostic GmbH, Mannheim, Germany) according to the manufacturer’s instructions. Genotyping was performed by a duplex PCR followed by a double digestion. A total of 50 ng of genomic DNA was amplified in a total volume of 25 μl containing GoTaq® 5X Flexi Buffer (1X; Fermentas Inc., Burlington, ON), MgCl2 (2 mM), dNTP (4 mM), the oligonucleotide primers for the two BDNF fragments encompassing the rs6265 and rs11030104 loci (1 μM each; Table 1), and GoTaq® DNA polymerase (1 U; Promega Co., Madison, WI). Thermal cycling (PTC-200; MJ Research Inc., Waltham, MA) consisted of 5 min of initial denaturation at 95°C followed by 6 cycles of 94°C (30 s), 68°C (30 s) and 72°C (30 s), and 30 cycles of 94°C (30 s), 62°C (30 s) and 72°C (30 s), with a final extension step at 72°C (5 min). Subsequently, 5 μl of PCR product was simultaneously digested with Eco72I and Rsal (2 U each; Fermentas). Digestion was performed in a 20 μl reaction assay (Tango® buffer, Fermentas; 37°C; 4 h). For rs6265, two fragments (351 and 212 bp) were obtained in the case of the G allele and just one (563 bp) in the case of the A allele, while for rs11030104 two fragments (398 and 115 bp) were obtained for the C allele and a single fragment (513 bp) for the T allele. Digestion products were separated by electrophoresis on 3% agarose gel containing ethidium bromide.

Statistical analysis

‘Haploview’ was employed to generate a map of linkage disequilibrium (LD) among markers and to calculate the Hardy-Weinberg equilibrium (HWE). Univariate analyses were performed by standard tests (r-Student, one-way analysis of variance – ANOVA – $\chi^2$) when appropriate. Analyses were systematically controlled for duration of current episode prior intake, baseline severity and treatment by the one-way or repeated-measures analysis of covariance (ANCOVA). Mean treatment dosages were recorded according the ‘Antidepressant Treatment History Form’ (Sackeim, 2001). The last observation carried forward (LOCF) method was employed for missing data for dropped-out patients, while no LOCF was applied in the case of switch to mania (only one subject). The ‘R’ environment (The R Project for Statistical Computing) was employed to analyse haplotype association (haplo.stat package) with both discrete and continuous variables and controlling for covariates. Permutation analysis (10,000) was performed in order to have a better estimation of the significance of results. The effect of HA on genetic association was analysed by the general linear model (GLM) and haplo.glm command in ‘R’ for haplotypes, which allows both the inclusion of variables as covariates and as factors to be tested for interactions.

With a conservative significance of 0.01, in our sample we had a sufficient power ($> 0.80$) to detect only medium effect sizes of $f = 0.37$ in ANCOVA analyses, corresponding to a difference of approximately 1.8 points on HDRS scores between genotypes (Faul, 2007).

Results

Clinical and demographic features of the sample were previously presented and analysed (Mandelli et al., unpublished data; Mandelli et al., 2009). Briefly, the sample comprised 44 females (51%) and 42 males (49%). Mean age at intake was 46 ± 11 years (range: 22–76), mean age at onset was 30 ± 7 years (range: 18–51) and mean depressive severity, according to HDRS, was 18.4 ± 4.5 (range 12–36). A personality disorder was present in 36% of the individuals and half of the subjects had a lifetime history of substance use disorder (SUD), though all subjects were detoxified and abstinent at intake. This high rate of lifetime SUD was due to our intentional inclusion of this kind of patients.

Treatments administered to the patients are listed in Table 1. While neither SUD nor Axis II personality disorders influenced response to treatment for current episode, a result of interest was the significant effect of the temperamental trait of HA: subjects with high scores had a poorer outcome as compared with those characterized by low HA scores. Other demographic and clinical features were not different from those previously observed in the same sample (Mandelli et al., 2009) or in the larger one, which included non-clinically depressed BP patients, apart from severity of depressive symptoms (Mazza et al., 2009).

BDNF polymorphisms

Both polymorphisms were in HWE (rs6265 $p = 0.9$, rs11030104 $p = 0.41$). Genotype frequencies were as follows: rs6265 GG 0.72, GA 0.26, AA 0.02; rs11030104 TT 0.65, TC 0.29, CC 0.06, in line with those observed in Caucasian subjects (NCBI Entrez SNP database). Single nucleotide polymorphisms (SNPs) were in reciprocal full LD ($D’ = 1$, LOD = 45.6,

Table 1. Rates of drugs administered to patients, rates of drop out and switches to mania

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antidepressant only</td>
<td>26.4</td>
</tr>
<tr>
<td>Antidepressant plus a mood stabilizer</td>
<td>16.7</td>
</tr>
<tr>
<td>Antidepressant plus an antipsychotic</td>
<td>11.1</td>
</tr>
<tr>
<td>Antidepressant plus a mood stabilizer, plus an antipsychotic</td>
<td>8.3</td>
</tr>
<tr>
<td>Mood stabilizer only</td>
<td>13.9</td>
</tr>
<tr>
<td>Mood stabilizer plus an antipsychotic</td>
<td>13.9</td>
</tr>
<tr>
<td>Antipsychotic only</td>
<td>9.7</td>
</tr>
<tr>
<td>Sedative/anxiolytic drugs</td>
<td>26.4</td>
</tr>
<tr>
<td>Drop out</td>
<td>30.2</td>
</tr>
<tr>
<td>Drop out at month 3</td>
<td>5.8</td>
</tr>
<tr>
<td>Drop out at month 6</td>
<td>24.4</td>
</tr>
<tr>
<td>Drop out for side effects</td>
<td>5.8</td>
</tr>
<tr>
<td>Drop out for lack of efficacy</td>
<td>5.8</td>
</tr>
<tr>
<td>Drop out for lack of compliance</td>
<td>18.6</td>
</tr>
<tr>
<td>Switched to mania</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Mean treatment dosages were recorded according the ‘Antidepressant Treatment History Form’ (Sackeim, 2001). The last observation carried forward (LOCF) method was employed for missing data for dropped-out patients, while no LOCF was applied in the case of switch to mania (only one subject). The ‘R’ environment (The R Project for Statistical Computing) was employed to analyse haplotype association (haplo.stat package) with both discrete and continuous variables and controlling for covariates. Permutation analysis (10,000) was performed in order to have a better estimation of the significance of results. The effect of HA on genetic association was analysed by the general linear model (GLM) and haplo.glm command in ‘R’ for haplotypes, which allows both the inclusion of variables as covariates and as factors to be tested for interactions.

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BDNF and demographic/clinical variables

BDNF genotypes were not differently stratified for sex, age, marital status and educational level. BDNF genotypes were not associated with family history for psychiatric disorders, BP sub-diagnosis, age at onset, lifetime SUD, Axis II personality disorder, comorbidity for anxiety disorders, HA scores, number of previous illness episodes, duration of current episode prior to intake, treatment administered and doses. Haplotype analyses did not reveal any association with clinical and demographic variables. Similarly, BDNF genotypes and haplotypes were not associated with baseline total depressive scores (Table 2) (data not shown, all p values > 0.01).

However, as shown in Table 2, rs6265 and rs11030104 genotypes were significantly associated with HDRS scores after six months of treatment. Associations were independent from baseline HDRS scores, treatment dosage, sex and age. Further, the results were not influenced by HA scores, though, as expected, HA significantly influenced HDRS scores at month 6 (p = 0.006). HA was not influenced by comorbidity for anxiety disorders, as its effect on outcome was independent from anxiety disorders.

An effect of BDNF could be observed also on response (% reduction from baseline) at month 3 (rs6265: F = 4.3, df = 2, p = 0.016, A-dominant model: t = 2.72, df = 80 p = 0.008; rs11030104 co-dominant model: F = 3.9, df = 2, p = 0.024, C-dominant model: t = 2.6, df = 80, p = 0.01), other than at month 6 (rs6265 co-dominant model: F = 6.0, df = 2, p = 0.0038, A-dominant model: t = 3.4, df = 80, p = 0.00098; rs11030104 co-dominant model: F = 7.3, df = 2, p = 0.0012, C-dominant model: t = 3.8, df = 80, p = 0.0026). Again, the effects were neither influenced by HA scores, nor by treatment dosage, sex and age.

By repeated measures ANOVA, both polymorphisms were significantly associated with response to antidepressant treatment over the medium term (3-6 months) (rs6265: F = 7.91 df = 3, 240, p = 0.0005, Figure 1; rs11030104: F = 8.32, df = 3, 240, p = 0.0003, Figure 2). Again, results were not influenced by HA scores.

The analysis of haplotypes confirmed a significant association with severity at month 6 (Global stat = 10.86, df = 2, p = 0.004, sim p = 0.003). Carriers of the A-C haplotype (frequency 15%) were significantly more severe than other patients at 6 months after intake (Table 3). Including HA

Table 2. BDNF genotypes and severity of depressive symptoms at intake (baseline) and at each following evaluation (month 1, 3 and 6)

<table>
<thead>
<tr>
<th>BDNF genotypes</th>
<th>HDRS scores Mean ± SD</th>
<th>rs6265</th>
<th>N = 62</th>
<th>N = 22</th>
<th>N = 2</th>
<th>F, p</th>
<th>rs6265 A_dom</th>
<th>t, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6265</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>Baseline</td>
<td>18.8 ± 4.9</td>
<td>17.1 ± 2.5</td>
<td>21.5 ± 6.4</td>
<td>1.58, 0.21</td>
<td>1.18, 0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Month 1</td>
<td>15.3 ± 2.7</td>
<td>14.9 ± 2.2</td>
<td>17.5 ± 4.9</td>
<td>0.59, 0.55</td>
<td>0.25, 0.80</td>
<td></td>
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<tr>
<td></td>
<td>Month 3</td>
<td>12.7 ± 4.9</td>
<td>14.5 ± 4.9</td>
<td>22.5 ± 2.1</td>
<td>4.67, 0.012</td>
<td>2.04, 0.04</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Month 6</td>
<td>11.1 ± 4.7</td>
<td>15.1 ± 5.2</td>
<td>16.5 ± 6.4</td>
<td>6.19, 0.003</td>
<td>3.52, 0.0007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs11030104</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>Baseline</td>
<td>19.0 ± 4.9</td>
<td>17.3 ± 2.8</td>
<td>17.8 ± 5.9</td>
<td>1.30, 0.28</td>
<td>1.6, 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Month 1</td>
<td>15.4 ± 3.8</td>
<td>15.0 ± 2.1</td>
<td>15.2 ± 3.6</td>
<td>0.12, 0.89</td>
<td>0.48, 0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Month 3</td>
<td>12.8 ± 5.0</td>
<td>14.2 ± 4.7</td>
<td>16.5 ± 4.6</td>
<td>1.42, 0.25</td>
<td>1.45, 0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Month 6</td>
<td>11.0 ± 4.7</td>
<td>14.6 ± 5.0</td>
<td>16.7 ± 6.4</td>
<td>5.20, 0.007</td>
<td>3.25, 0.002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HDRS: Hamilton depressive rating scale; rs6265 A_dom: dominant model for allele A; rs11030104 C_dom: dominant model for allele C.
scores as a covariate or a factor (high/low HA) in GLM, both DBNF haplotypes and HA showed a significant effect on HDRS scores at month 6, but no interaction could be observed between the two factors (Table 4).

Finally, although no significant interaction was observed between DBNF and HA on course of BP depression, HA did however have a significant influence on course of symptoms in subjects carriers of the ‘protective’ G-T haplotype \((F = 5.03, \text{df} = 3, 312, p = 0.002)\) (Figure 3, C).

### Table 3. Haplotype association with depressive severity at month 6 after intake

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
<th>Stat-value</th>
<th>p-value</th>
<th>sim p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-T</td>
<td>0.805</td>
<td>-2.89</td>
<td>0.004</td>
<td>0.003</td>
</tr>
<tr>
<td>G-C</td>
<td>0.043</td>
<td>0.028</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>A-C</td>
<td>0.152</td>
<td>3.26</td>
<td>0.001</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

### Table 4. Analysis of haplotypes on severity at month 6 including harm avoidance (HA) as a covariate or as an independent factor in general linear model

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>t-stat</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HA as a covariate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(intercept)*</td>
<td>5.42</td>
<td>1.53</td>
<td>3.53</td>
<td>0.0008</td>
</tr>
<tr>
<td>HA</td>
<td>3.27</td>
<td>1.06</td>
<td>3.09</td>
<td>0.003</td>
</tr>
<tr>
<td>G-C</td>
<td>2.21</td>
<td>1.59</td>
<td>1.39</td>
<td>0.17</td>
</tr>
<tr>
<td>A-C</td>
<td>3.92</td>
<td>0.99</td>
<td>3.97</td>
<td>0.0002</td>
</tr>
<tr>
<td><strong>HA as an interaction factor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(intercept)*</td>
<td>4.70</td>
<td>1.83</td>
<td>2.56</td>
<td>0.013</td>
</tr>
<tr>
<td>G-C x HA</td>
<td>-0.55</td>
<td>4.45</td>
<td>-0.12</td>
<td>0.90</td>
</tr>
<tr>
<td>A-C x HA</td>
<td>-1.64</td>
<td>2.12</td>
<td>-0.77</td>
<td>0.44</td>
</tr>
</tbody>
</table>

*G-T haplotype as baseline category for the design matrix.

A similar trend, though not significant, could be observed for single markers (Figure 3, A, B).

### Discussion

The present study confirms a significant influence of the BDNF gene on efficacy of treatment in bipolar depression. According to preclinical studies, BDNF may be a key mediator of the therapeutic response to drugs (D’Sa and Duman, 2002). BDNF protein levels have been associated with SERT reuptake capacity (Daws et al., 2007), thus BDNF may indirectly interfere with the action of antidepressants. There is indeed evidence that BDNF variants may interfere with the efficacy of fluoxetine in normalizing anxiety behaviours in mice (Chen et al., 2006). Further, levels of BDNF have been associated with abnormal hippocampal activation (Egan et al., 2003) and hypothesized as a factor involved in the hippocampal atrophy observed in depressed patients. Accordingly, reduced levels of BDNF have been observed in unmedicated depressed patients (Duman et al., 1999).

Previous studies reported BDNF involved in the response to acute antidepressant treatment (Choi et al., 2006; Gratacos et al., 2008; Licinio et al., 2009; Yoshida et al., 2006), though negative reports have been published as well (Kang et al., in press; Tsai et al., 2003; Wilkie et al., 2007). BDNF has been also been found to be associated with efficacy of treatments for acute mania (Tramontina et al., 2009) and lithium prophylactic efficacy in BP patients (Rybakowski et al., 2005). Studies have mainly focused the rs6265 polymorphism (Val66Met), which reported positive associations, though not univocally. To our knowledge, this is the first study confirming rs6265 as involved in the efficacy of different agents in treating BP depression.

Nevertheless, which allele has to be considered the risk variant remains unclear. We found the rs6265 A-allele as associated with a poorer outcome, while Choi et al. (2006) reported it associated with a better response in a Korean population. Yoshida et al. (2006) reported evidence for a heterosis effect in Japanese. This discrepancy may be explained by the genetic diversity between ethnicities. Indeed, in Caucasians the rs6265 A-allele has a frequency of about 0.17, while in Asian populations it is more frequent, ranging from 0.34 to 0.63. Rs6265 may be further in different LD with other functional variants (the flip-flop hypothesis of Lin et al., 2007). A similar case has already been reported: the short-allele of 5HTTLPR (SERT gene) is more frequent in Asian populations and associated with a better response to antidepressants, in contrast to the observations in Caucasian populations, where the short allele is less frequent and associated with a poor response (Kato and Serretti, in press). On the other hand, Rybakowski et al. reported the same rs6265 A allele associated with a better efficacy of long-term efficacy of lithium as a prophylactic agent in a Caucasian population (Rybakowski et al., 2005). Thus, further studies are needed to clarify the role of BDNF rs6265 polymorphism in the treatment of BP depression.

In our study, we also found a second SNP, rs11030104, associated with depressive outcome and a significant effect of the combination of alleles in the two markers. To our knowledge, only Licinio et al. (Licinio et al., 2009) investigated...
rs11030104 in response to antidepressant treatment, but no significant association was observed.

Other than investigating the influence of BDNF variants on depressive outcome, a secondary aim of this study was to test a potential interaction with the trait of HA, which is strongly associated with response to treatments (Pelissolo and Corruble, 2002). We did not find a direct interaction between BDNF variants and HA levels. Multivariate analyses should be taken with caution in this study, because of the limited power to detect true positive associations and the low number of subjects in groups of genotypes and levels of HA. Nevertheless, the influence of HA remained statistically significant, independently from the genetic profile of individuals. Of interest is the possible differential impact of HA levels in subjects carrying the genetic variants associated with a better outcome, which was not evident in subjects carrying the risk variants. However, larger studies are required to confirm this preliminary observation.

In a previous analysis performed on this sample of subjects, we found a significant effect of the 5-HTTLPR polymorphism on outcome of depression as well (Mandelli et al., 2009). There is evidence of a potential interaction between the two genes, SERT and BDNF, on the risk for depressive disorders (for example, Kaufman et al., 2006), as well as on response to psychotropic treatments (Rybakowski et al., 2007a). Unfortunately, we could not test for the interaction between BDNF variants and the 5-HTTLPR polymorphism, because the small size of our sample strongly limited the possibility to detect true associations.

The present study is different from those previously reported for two main reasons. First, the length of follow-up was considerably longer than most of previous studies, which were mainly limited to the acute treatment over 6–8 weeks. Thus, our results are not completely in disagreement with negative studies in Caucasians, since we did not observe a significant effect of BDNF on acute treatment (8–12 weeks), but only at 6 months after treatment initiation. Thus, it is possible that BDNF variants influence the medium-term outcome of BP depression instead of acute response. Second, we employed only few exclusion criteria while collecting subjects, and the inclusion of comorbid disorders such as lifetime SUD, personality disorders (36.0%) and anxiety disorders (11.6%) was allowed. We did not put a narrow diagnostic criterion, including BP-I, BP-II and CtxM patients, and neither did we include a cut-off of depressive severity for inclusion (for example HDRS > 15). Patients were included if they satisfied DSM-IV criteria for a major depressive episode independently from severity. Further, patients did not receive a single standardized treatment as is often done in clinical trials, and only 55% of subjects were treated with antidepressants. These choices were made in order to obtain a ‘naturalistic’ sample of BP patients, representative of real clinical settings. Indeed, the inclusion of heterogeneous patients could allow the testing of more clinical variables that could potentially

![Figure 3. Interaction between HA and BDNF genotypes and haplotypes.](https://example.com/figure3.png)
influence the individual response to treatments independently or in interaction with genetic variations. On the other hand, unipolar major depressive patients were not included in our sample, introducing a further discrepancy with respect to the large part of samples investigated so far for BDNF. Thus, although our sample may be to some extent representative of a general population of BP patients, comparison with previous investigations may be problematical. Moreover, the naturalistic characterization of the sample may have introduced a number of confounding factors, although we systematically controlled for some important features. On the other hand, a number of variables potentially influencing the outcome of BP depression were not taken into account, such as developmental history, social support and current stressors. Moreover, a major limitation of the present study is represented by the small sample size, which may have lead to false positives and reduced the reliability of multivariate analyses. We applied a correction to the level of significance, but this does not completely ensure the reliability of results. Thus, findings should be taken with caution and further studies on larger samples are required.

In conclusion, despite many critical points, results of our investigation support the involvement of BDNF in the outcome of BP depression, in accordance with some previously published reports, though in disagreement with others. In particular, BDNF seem to influence the medium-term response to treatments rather than the acute one. The influence of HA is independent of BDNF genotypes, though further research is required to confirm this finding.

References


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