Possible Associations of NTRK2 Polymorphisms with Antidepressant Treatment Outcome: Findings from an Extended Tag SNP Approach

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Abstract

Background: Data from clinical studies and results from animal models suggest an involvement of the neurotrophin system in the pathology of depression and antidepressant treatment response. Genetic variations within the genes coding for the brain-derived neurotrophic factor (BDNF) and its key receptor TrkB (NTRK2) may therefore influence the response to antidepressant treatment.

Methods: We performed a single and multi-marker association study with antidepressant treatment outcome in 398 depressed Caucasian inpatients participating in the Munich Antidepressant Response Signature (MARS) project. Two Caucasian replication samples (N = 249 and N = 247) were investigated, resulting in a total number of 894 patients. 18 tagging SNPs in the BDNF gene region and 64 tagging SNPs in the NTRK2 gene region were genotyped in the discovery sample; 16 nominally associated SNPs were tested in two replication samples.

Results: In the discovery analysis, 7 BDNF SNPs and 9 NTRK2 SNPs were nominally associated with treatment response. Three NTRK2 SNPs (rs10868223, rs1659412 and rs11140778) also showed associations in at least one replication sample and in the combined sample with the same direction of effects (Pcorr = .018, Pcorr = .015 and Pcorr = .004, respectively). We observed an across-gene BDNF–NTRK2 SNP interaction for rs4923468 and rs1387926. No robust interaction of associated SNPs was found in an analysis of BDNF serum protein levels as a predictor for treatment outcome in a subset of 93 patients.

Conclusions/Limitations: Although not all associations in the discovery analysis could be unambiguously replicated, the findings of the present study identified single nucleotide variations in the BDNF and NTRK2 genes that might be involved in antidepressant treatment outcome and that have not been previously reported in this context. These new variants need further validation in future association studies.


Editor: Abraham A. Palmer, University of Chicago, United States of America

Received December 14, 2012; Accepted April 19, 2013; Published June 4, 2013

Competing Interests: The authors have the following interests. Arolt is member of advisory boards and/or gave presentations for the following companies: Astra-Zeneca, Janssen-Organon, Eli Lilly, Lundbeck, Pfizer, Servier, and Wyeth. He also received grants from Astra-Zeneca, Lundbeck, and Wyeth. He chaired the committee for the “Wyet Research Award Depression and Anxiety”. Czamara was funded by the Deutsche Forschungsgemeinschaft (German Research Foundation) within the framework of the Munich Cluster for Systems Neurology (EXC 1010 Synergy). Domschke has received speaker fees from Pfizer, Lilly and Bristol-Myers Squibb; she is a consultant for Johnson & Johnson and has received funding by Astra Zeneca. Hennings has received travel support to scientific congresses from Eli Lilly, Bayer Vital and Novartis. Menke, Holsboer inventors: Means and methods for diagnosing predisposition for treatment emergent suicidal ideation (TESI). European application number: 08016477.5. International application number: PCT/EP2009/061575. Uhr is patent holder of WO2005/108605A2 and WO2008/115180A2. Katharina Domschke is a PLOS ONE Editorial Board member. There are no further patents, products in development or marketed products to declare. This does not alter the authors’ adherence to all the PLOS ONE policies on sharing data and materials, as detailed online in the guide for authors.

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Introduction

Despite large efforts during the last decades, antidepressant treatment efficacy in depression is still unsatisfactory [1,2]. While antidepressants are generally effective and indispensable for the treatment of moderate and severe depression, up to two of three patients do not sufficiently respond to a first antidepressant treatment attempt [2]. Increased plasma corticosteroid levels, typically found in many patients with depression [3], may lead to a reduced trophic support of neurons and an impaired plasticity of critical brain structures involved in affective disorders [4,5]. Stress-induced perturbation of hormonal homeostasis, and specifically elevated corticosteroid concentrations decrease the expression of hippocampal brain-derived neurotrophic factor (BDNF) [6,7], a major mediator of neurogenesis and synaptic plasticity involved in learning and adaptive processes in the adult brain [8]. As antidepressants have been shown to reverse stress and depression induced BDNF downregulation and impaired neurogenesis [9,10], BDNF has been implicated in recovery mechanisms from depression, which resulted in the neurotrophin hypothesis of depression [4,5]. In several studies, reduced serum BDNF levels in depression and reduced hippocampal BDNF expression in mouse models of affective disorders could be reversed by various antidepressant interventions [4,5,11–13].

Apart from BDNF, the neurotrophin system comprises the nerve growth factor (NGF), neurotrophin 3 and 4/5, as well as their key receptors, the tropomyosin receptor kinase (Trk) family, including the major receptor for BDNF, TrkB, encoded by the NTRK2 gene [11]. Indeed, functional BDNF-TrkB signaling is required for behavioral effects induced by antidepressants [14] and increased NTRK2 mRNA expression [15] and signaling [14,16,17] upon antidepressive interventions have been recently reported. Thus, these two genes are in the focus of this study.

Up to now, pharmacogenetic studies on polymorphisms in the neurotrophin system nearly exclusively focused on the BDNF gene, especially the non-synonymous Val66Met (rs6265) polymorphism. Despite several attempts to replicate initial associations, many conflicting results have been reported on this polymorphism [18,19], which could at least in part be related to population effects [20]. Despite some positive associations reported for other BDNF and NTRK2 SNPs [21,22], previous pharmacogenetic studies did not find a major impact of BDNF and NTRK2 polymorphisms on antidepressant treatment including a German [23] and the STAR*D sample [24]. Presently there are no SNPs of the neurotrophin system that have been unambiguously shown to be associated with treatment response in depression.

We extended the previous approaches by testing pharmacogenetic associations not only for variants in the BDNF gene but also for variations in its main receptor gene, NTRK2, in depressed German inpatients participating in the Munich Antidepressant Response Signature (MARS) project, which is a naturalistic study dedicated to identify predictors of antidepressant treatment outcome. We selected 82 tagging SNPs covering 100% of BDNF variants with a minor allele frequency (MAF) ≥0.01 and 92.8% of NTRK2 variants with a MAF ≥0.1 based on HapMap project data. We tested all nominally significant SNPs out of the discovery analysis in 2 replication samples, resulting in a total sample size of 894 patients. In addition to our single-marker analysis we performed multi-marker approaches using a haplotype as well as a two-way interaction analysis.

Methods

Ethics Statement

The study was approved by the Ethics Committees of the Medical Facilities at the Ludwig Maximilians University, Munich, Germany, and at the University of Muenster, Muenster, Germany, respectively. Written informed consent was obtained from all subjects, and the study was carried out in accordance with the latest revision of the Declaration of Helsinki.

Sample Description

398 Caucasian inpatients (56.0% females, mean age 49.1±14.4 (SD) years) that participated in the MARS project (http://www.mars-depression.de) were included within 5 days after admission to the clinic for the treatment of an acute depressive episode, as described in detail previously [1]. Depressive symptoms were rated weekly with the 21-items version of the Hamilton Rating Scale for Depression (HAM-D) and all patients were at least moderately depressed at inclusion (HAM-D ≥14). Two replication samples were defined: (1) 249 newly recruited participants of the MARS project (MARS replication; 51.4% females, mean age 48.0±13.6 (SD) years), and (2) 247 of 340 Caucasian inpatients with an HAM-D ≥14 at inclusion recruited in a pharmacogenetic study at the University of Muenster, Westphalia, Germany [23,25] (Muenster replication; 59.1% females, mean age 49.2±14.9 (SD) years; see Table 1). MARS patients (N=647) suffered from a single major depressive episode (single MDE; 32.9%), recurrent depression (RD; 56.1%) or bipolar depression (BP; 11.0%). Among MARS bipolar patients, 42.6% (29 of 68) were subclassified as bipolar type I and 57.4% (39 of 68) as bipolar type 2. All patients received antidepressant treatment according to the choice of the attending doctor with antidepressant dosages adjusted according to therapeutic plasma level ranges. In accordance with previous studies from the MARS project [1,26–30], response was defined as an at least 50% reduction after five weeks of the HAM-D score at admission. Remission at discharge was defined as a HAM-D <10 (mean duration of hospital stay: 11.5±7.8 (SD) weeks).

DNA Sampling and SNP Genotyping

DNA was extracted from 30 ml of EDTA blood using Puregene whole-blood DNA extraction kits (Gentra Systems, Minneapolis, USA). Using the Tagger software implemented in the HapMap project browser (de Bakker, et al 2005), we retrieved tagging SNPs for the longest BDNF isoform (NM_170731, 66.86 kb; 18 SNPs) and the full-length NTRK2 gene (NM_006180, 355.04 kb; 64 SNPs), flanked by additional 20 kb of both 5’ and 3’ sequences according to Human HapMap Project Phase I and II data for the CEU population, leading to 100% and 92.8% SNP marker coverage, respectively. Pair-wise r² for a bin of linked SNPs was set to ≥0.8. The minor allele frequency (MAF) was set to ≥0.01 (BDNF) and ≥0.1 (NTRK2), respectively. MARS patients were genotyped using Sentrix Human-1 100 k, HumanHap300 k and Human610 Genotyping BeadChips (Illumina Inc., San Diego, USA) and MALDI-TOF mass-spectrometer (MassArray® system), as described previously [31]. Patients form the Muenster sample were genotyped using the MALDI-TOF mass-spectrometer. In case of insufficient genotyping quality (defined as an experiment-wise call rate <.97 or significant deviation from the Hardy-Weinberg equilibrium, HWE), melting curve analysis using real-time PCR was performed (rs11602246, rs16596412, rs20490406, rs1491850 in the Muenster replication sample; rs11140778 in the MARS replication sample). SNPs that entered analysis did not significantly deviate from HWE (Table 2, S1, S2) using a
Bonferroni-corrected. 05 level of significance (α = .05/82 = 6.1 × 10^{-4}).

Protein Analysis

Morning fasting serum was available in 93 MARS patients at admission and after 6 weeks of treatment (mean ±2.1 weeks) as well in 97 age- and gender-matched healthy controls derived from a German sample recruited at the Max Planck Institute of Psychiatry, Munich. Among patients, 47 were classified as remitters and 65 as responders according to the above mentioned criteria. Serum total BDNF levels were assessed with an enzyme-linked immunoabsorbant assay (ELISA) kit (Promega BDNF Emax®, Madison, Wis.). All measurements were performed in

<table>
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<th>Genotype</th>
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*According to dbSNP build 132.

Uncorrected P values for the deviation from Hardy-Weinberg-Equilibrium; note that no P value exceeded the corrected (82 SNPs, Bonferroni) threshold of p<6.1×10^{-4}.

Nominal P values for associations with response after 5 weeks (strongest phenotype in the discovery sample).

doi:10.1371/journal.pone.0064947.t002
duplicates and mean values were taken for further analysis (mean difference between unicates was 6.5% ± 6.1 SD).

Power Calculation
The CaTS Power Calculator for Genetic Studies [32] was used for power calculation using a two-stage design. The experiment-wise alpha error was set to 6.1 x 10^-4 according to a Bonferroni-correction for 82 investigated SNPs. Given a predicted response rate to antidepressant treatment of 50% in an inpatient setting after 5 weeks [1], we calculated that a total sample size of N = 880 is sufficient to achieve a power of at least 90% (additive model) to detect genetic effects in a joint analysis of the discovery and replication samples assuming a relative risk of 1.40 or larger for SNPs with an allele frequency of at least 10%. If a multiplicative model is assumed, as it is proposed for complex diseases [33], a power of 93% can be expected. We can conclude from this power analysis that the combined sample size of the present study (N = 894) should be sufficient for detecting small to moderate effects of the investigated polymorphisms.

Statistical Analysis
Genetic association analysis with binary response variables (response at week 5 and remission at discharge) was performed by X^2 statistics using the WG-Permer software (http://www.mpipsykl.mpg.de/wg-permer) with allelic and genotypic models. For all association tests, the level of significance was set to 0.05, and P values were corrected for multiple comparisons using a resampling method as proposed by Westfall and Young [34] applying 10^4 permutations over all performed tests (i.e., for 82 SNPs analyzed in the discovery sample and for 16 SNPs analyzed in the replication or in the combined sample). Empirical P values were obtained using the Monte Carlo method implemented in the WG-Permer software to approximate the exact P value with the given standard distribution without relying on asymptotic distribution theory [35]. In case of a significant association, the Armitage’s test for trends [36] was calculated using the software provided by the Institute for Human Genetics, Munich (http://ihg2.helmholtz-muenchen.de) and confidence intervals were calculated using a log-normal distribution. Forest plots were drawn using the rmeta software package for R-2.5.0 (http://cran.r-project.org). In the replication study, we used Fisher products [37] corresponding to the geometric mean of the P values of the two response variables (i.e., response after 5 weeks and remission at discharge) to account for possible sample heterogeneity. We defined successful replication as a nominal association observed in one of the replication samples and same direction of the effect.

Group differences in demographic and baseline clinical data were compared using Pearson chi-square tests and t-tests for independent samples in case of quantitative data. Average change during treatment outcome was evaluated with t-tests for paired samples. Additionally, analysis of covariance was applied to evaluate the effects of treatment outcome (response, remission) on BDNF serum protein concentration with age, sex, and baseline BDNF as covariates. A logistic regression analysis was applied for evaluating SNP×protein interactions as a predictor for response or remission, respectively) using age and sex as covariates. All statistical analyses were conducted with SPSS for Windows (version 18.0, SPSS, Chicago, USA).

Haplotype Association Testing
The phenotype showing the strongest effect in the single-marker association analysis was further investigated in a haplotype analysis using the Haploview 4.1 software [38]. In order to detect informative associations complementary to our tagging SNP approach, haplotype analysis was performed using a D’-based linkage disequilibrium (LD) map based on haplotype blocks defined according to Gabriel et al. [39]. Analysis was performed in the discovery sample providing sufficient marker coverage. We used 10^4 random permutations implemented in Haploview to control for false-positive findings. Rare haplotypes (frequencies <0.01) were excluded from the analysis. We report P values for at least nominally significant haplotypes.

Interaction Analysis
In the combined sample, we analyzed all possible two-way interactions across both genes (BDNF and NTRK2 SNPs) and between the phenotype showing the strongest effect in the single-marker association analysis using a Bonferroni-corrected 0.05 level of significance (α = 0.05/63 = 7.9 x 10^-4). The interaction analysis was performed with a step-wise logistic regression using R-2.5.0 (http://cran.r-project.org). Age, sex and sample origin were included as covariates, and genotypes were coded following an allelic model to obtain maximal power.

Linkage Disequilibrium Mapping
We used the Haploview 4.1 software to map the r^2-based LD pattern from the CEU population (release 21). Haplotype blocks were defined according to the method of Gabriel et al. [39]. Using the SNP Annotation and Proxy Search (SNAP) program provided by the BROAD Institute (http://www.broadinstitute.org/mpg/snap), we further tested, whether the four SNPs that withstood correction for multiple testing in our combined analysis (rs2049046, rs10868223, rs1659412, rs11140778) were in common LD (within the BDNF and NTRK2 gene, respectively) with at least nominally associated SNPs reported in previous studies [21-23,40–42]. We used the 1000 Genomes Project Data set implemented in SNAP to retrieve LD informations of recently identified SNPs (e.g. rs61888800). We found only the BDNF SNP rs2030324, previously reported by Licinio [22], to be in high LD (r^2 = .90) with rs2049046 of the present study. Neither for any other BDNF (rs7124442, rs61888800, rs908367) nor NTRK2 SNP (rs1187362, rs1187327, rs2289656, rs2378672, rs7020204, rs2013566, rs11140793) annotated in the data set, we could identify proxies with an r^2 > .80.

Results
Association Analysis with Antidepressant Treatment
We tested the association between 18 BDNF and 64 NTRK2 tagging SNPs with response after week 5 and remission at discharge in the MARS study sample (N = 398). The strongest associations were found for response after 5 weeks and under an allelic model (Tables S1 and S2). Seven SNPs in the BDNF gene and nine SNPs in the NTRK2 gene were nominally associated with

Figure 1. Development of HAM-D values during antidepressive treatment depending on the rs10868223 (A), rs1659412 (B) and rs11140778 (C) genotype (combined sample). Repeated measurements (Greenhouse-Geisser, age and sex as covariates) revealed significant interaction effects for rs10868223 (P < .007) and rs1659412 (P = .012), but not for rs11140778 (P = .63). Stars indicate significant between-subjects differences at different time points (*, p < .05; **, p < .01; GLM with age and sex as covariates). Error bars are standard errors of the means.

doi:10.1371/journal.pone.0064947.g001
the strongest associated phenotype (response after 5 weeks). In the BDNF gene region, four SNPs were located in untranscribed regions (two 5' and two 3' UTRs), two within introns, and one SNP (rs6265) within an exon resulting in a valine-methionine amino acid exchange (Val66Met, Table 2 and Figure S2a,b). The strongest associations were found for rs2049046 (intronic; \( P = 4.9 \times 10^{-5} \) and rs11030094 (3' UTR; \( P = 1.5 \times 10^{-3} \)). In the sample analyzed, both SNPs were in strong LD \((r^2 = .92)\). Within the NTRK2 gene, the intronic SNP rs11140778 \((P = 4.12 \times 10^{-3})\) showed the strongest association with response after 5 weeks.

**Replication Studies**

In order to replicate these findings we genotyped the 16 SNPs showing nominally significant associations for the strongest phenotype (response after 5 weeks) in the MARS discovery sample in two replication samples. Although all patients fulfilled the same inclusion criteria (at least moderate depressive episode: HAM-D \( \geq 14 \)) and did not differ in baseline demographics, there were significant differences in baseline depression severity and response to antidepressant treatment between the three samples (Table 1).

In particular, patients in the Muenster replication sample had a lower HAM-D at baseline compared to the discovery sample \((P < .001; \text{Bonferroni post-hoc test})\). Both replication samples had lower HAM-D scores at discharge than the discovery sample \((P < .001, \text{each}; \text{Bonferroni post-hoc test})\). Highest remission rates at discharge were observed in the Muenster sample, whereas highest response rates after week 5 were found in the MARS replication sample. To better account for this sample heterogeneity, we used Fisher Products [37] of these two phenotypes \((i.e. \text{response after 5 weeks and remission at discharge})\) in subsequent analyses of the replication samples \(\text{referred to as ‘outcome’ if not otherwise specified.}\)

Treatment outcome was tested in replication samples under the allelic model, which showed the strongest associations in the discovery sample. Of the 16 nominally associated SNPs in the discovery sample, we found one BDNF SNP \((rs11602246; P = .01)\) and one NTRK2 SNP \((rs10868223; P = .009)\) to be nominally associated with outcome in the MARS replication sample \((\chi^2 = 249)\). In the Muenster replication sample, the three NTRK2 SNPs \(rs1659412, rs1662695\) and \(rs11140778\) showed nominal associations \((P = .01; P = .03; P = .003, \text{respectively})\). In the combined analysis of all patients across the three samples we found the SNPs \(rs2049046 (BDNF; P_{corr} = .021), rs10868223 (NTRK2; P_{corr} = .018), rs1659412 (NTRK2; P_{corr} = .015)\) and \(rs11140778 (NTRK2; P_{corr} = .004)\) to be significantly associated with response after correction for multiple testing (Table 3, Figure 1). According to our definition of a positive replication, we could replicate the three NTRK2 SNPs \((rs10868223, rs1659412\) and \(rs11140778\) as they showed associations in at least one replication sample and had a lower \(P\) value in the combined analysis compared to any single sample of the study, withstanding correction for multiple testing. For these three markers, we calculated the Armitage test of trends as a measure of the effect size separately in the three different samples and for both phenotypes (Figure 2). Under the definition for a replication, the most significantly associated BDNF marker in the discovery sample, \(rs2049046\) could not be replicated, although the association in the combined analysis still withstood correction for multiple testing \((P_{corr} = .02)\).

As the results of single SNP associations might be confounded by combining patients with bipolar and unipolar depression, we reanalyzed the combined sample for unipolar depressed patients only \((\chi^2 = 780; 16\text{ SNPs})\). Again, \(rs10868223, rs1659412\) and \(rs11140778\) showed the strongest association with outcome withstanding correction for multiple testing \((P_{corr} = .009, P_{corr} = .04\) and \(P_{corr} = .012, \text{respectively})\).

**Medication- and Gender-specific Associations**

Stratifying the patients in the combined sample according to their antidepressant medication (tricyclic antidepressants, TCA; serotonin-noradrenaline reuptake inhibitors, SNRI; noradrenergic specific-serotonergic antidepressants, NASSA; noradrenaline reuptake inhibitors, NARI; monoamineoxidase inhibitors, MAOI and others) revealed SSRI-specific associations for \(rs2049046 (BDNF)\) and \(rs11140778 (NTRK2)\). Co-medication of mood stabilizers, benzodiazepines and lithium did not reveal genotype-dependent differences in prescription rates for these SNPs. Gender-specific effects were observed for \(rs11140778 (male\) and \(rs1639412 (female)\) (Tables S3–S5; Document S1).

**Haplotype Association Testing**

We performed a haplotype-based association analysis to test for informative associations complementary to our single marker approach using the discovery sample providing sufficient marker coverage \((82\text{ SNPs, best phenotype of the single marker analysis in this sample (response after week 5), allelic model})\). None of twenty D’-based haplotype blocks \((2\text{ in the BDNF and 7 in the NTRK2 gene, respectively; Figure S1})\) contained nominally significant haplotypes, most of them including SNPs that had been nominally associated in the single marker analysis of both, the discovery as well as the combined sample \((Figure 3, Table S6).\) The GC haplotype of block one \((rs1030094, rs11602246)\) and the GGGACT haplotype of block 3 \((rs6265, rs11030109, rs10355211, rs2049046, rs4923468\) and \(rs12273363)\), both within the BDNF gene, showed a significant association withstanding a permutation-based correction for multiple testing \((P = 7.4 \times 10^{-5}\) and \(P = 7.3 \times 10^{-3}\), respectively).

**Interaction Analysis**

We performed gene-wise logistic regression for each pair of \(7\text{ BDNF and 9\text{ NTRK2 SNPs genotyped in the combined sample, including age, sex and sample origin as covariates. Given a Bonferroni-based threshold of significance for }7\times9 = 63\text{ statistical tests }\(\chi^2 = 7.9 \times 10^{-5}\), the interaction of the BDNF SNP rs4923468 with the NTRK2 SNP rs1387926 showed a significant association with outcome \((P = 4.6 \times 10^{-5})\). No other pairs of BDNF and NTRK2 SNPs showed associations.

**BDNF Protein Analysis**

In order to evaluate the potential functional impact of the associated polymorphisms, we measured BDNF serum protein concentration in 93 unselected MARS patients with available blood samples at admission and after antidepressant treatment. Compared to 97 age- and sex-matched healthy controls, no difference in baseline BDNF concentration could be observed \((22.13\text{ ng/ml }+/−9.98\text{ SD (patients)} vs. 22.49\text{ ng/ml }+/−9.74\text{ SD (controls); }P = .803)\). Also during antidepressant treatment, no change in average BDNF levels was found \((22.13\text{ ng/ml }+/−9.98\text{ SD vs. }23.19\text{ ng/ml }+/−10.00\text{ SD; }P = .258)\). However, when considering antidepressant treatment outcome in terms of remission status, we observed a significant effect on the BDNF concentration after treatment \((P = .024)\) with higher BDNF concentration levels in remitters. No effects were detected for response status \((P = .279)\). When testing the moderating effects of NTRK2 SNPs showing a replicated effect on treatment outcome, we observed a genotype x protein \(after\ treatment\) interaction for \(rs10868223\) with remission \((P = .019)\) and for \(rs11140778\) with
response ($P = .038$) (see Figure S3). These effects were significant at the nominal level, not surviving correction for multiple testing.

Further, interrogating a publicly available expression quantitative trait loci (eQTL) database (GENEVAR; [43]) for the three NTRK2 SNPs in the combined analysis with transcripts of either BDNF or NTRK2, we found nominal associations for rs11140778 and rs10868223 with two BDNF transcripts (GI_34106709-A, and...
GI\_34106708-I; P = .03 and P = .03) not withstanding correction for multiple testing.

Discussion

This pharmacogenetic study investigated the association of 18 \textit{BDNF} and 64 \textit{NTRK2} tagging SNPs with antidepressant treatment outcome in a representative sample of Caucasian inpatients. We tested all 16 nominally significant SNPs of the discovery analysis in 2 replication samples, resulting in a total sample size of 894 patients.

We found 3 \textit{NTRK2} SNPs (rs10868223, rs1659412 and rs11140778) showing associations in the discovery and at least one replication sample as well as in the combined sample of 894 patients with a lower P value as compared to the discovery sample and withstanding correction for multiple testing. None of these \textit{NTRK2} markers has been investigated in pharmacogenetic studies so far. Nevertheless, the functional impact of these non-coding SNPs remains unclear as robust association could neither be found in our protein analysis nor in a publicly available eQTL database.

\textit{NTRK2} polymorphisms have so far been studied for association with childhood-onset mood disorders [40,44], Alzheimer’s disease [45], suicidality [31,46] and antidepressant response [21,24,47]. Dong and colleagues [21] found two \textit{NTRK2} coding SNPs (rs2289657 and rs56142442) to be associated with response in a Mexican American MDD sample (N = 272), whereas association of \textit{NTRK2} SNPs was neither found in the GENDEP [47] nor the STAR*D sample [24]. The significant \textit{NTRK2} SNPs found in the present study are located 5' of the transcriptional unit (rs10868223 and rs1659412) and in an intron (rs11140778). They are not in LD with previously reported \textit{NTRK2} SNPs. In addition, as previous studies did not tag regions far outside the coding regions, rs10868223 and rs1659412 could not have been detected so far.

Similarly to previous studies that report interactive \textit{BDNF}×\textit{NTRK2} interaction on the SNP level for geriatric depression [48] and suicidality [31,46], we found a \textit{BDNF}×\textit{NTRK2} SNP interaction for antidepressant treatment outcome in the present study. The findings of our interaction study have to be interpreted cautiously as only nominally associated SNPs from the

Table 3. Association with Treatment Outcome.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>NARS Discovery</th>
<th>MARS Replication</th>
<th>Muenster Replication</th>
<th>Combined Sample</th>
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</table>

\(^a\)Empirical P values for the associations with treatment outcome (FPM analysis) under an allelic model. 
\(^b\)P value, permutation-based correction for multiple testing (16 SNPs).
\(^c\)Not polymorphic.
\(^d\)Note that this P value would not fulfill a more conservative threshold of alpha = .025 correcting for the fact that two replication samples have been analyzed.

doi:10.1371/journal.pone.0064947.t003

Figure 3. Significant haplotype blocks, both located in the \textit{BDNF} gene, which showed association with response after 5 weeks (best phenotype of the single marker analysis). Note, that for optimal gene coverage, haplotype analysis was performed in the discovery sample only (N = 398, 82 SNPs).
doi:10.1371/journal.pone.0064947.g003
initial sample entered the analysis and we corrected the p-value only for this subset of SNPs. Future studies, including SNP×SNP and SNP×protein analyses, need to substantiate these initial findings and may help elucidating the complex interplay of BDNF and its main receptor TrkB.

It has been suggested that various antidepressants may act differentially on the neurotrophin system [49] and pharmacogenetic associations may be restricted to specific drug treatments [22]. In the BDNF gene, Dwivedi et al. found a desipramine-specific increase in exons I and III mRNA in both the frontal cortex and hippocampus, whereas fluoxetine increased only exon II mRNA in the hippocampus [49]. Similarly, we found a differential pattern of associated SNPs dependent on the type of antidepressant in our study. In particular, the intronic BDNF SNP rs2049046 and the NTRK2 SNP rs11140778, both significantly associated in the combined sample, may exert their effects on response via SSRI-mediated mechanisms. We found that the strong association of rs2049046 in the discovery sample is related to SSRI treated patients only. Interestingly, Domshike et al. [29] described a SSRI-specific association for the BDNF SNP rs7124442, and most previous studies showing pharmacogenetic associations with BDNF included SSRI treated patients only [19,30–52]. However, Lúcio et al. [22] described an intronic BDNF SNP to be nominally associated with response in a desipramine treated, but not a fluoxetine treated subgroup of Mexican Americans. Our own results do not contradict the hypothesis of medication-specific effects of BDNF polymorphisms upon treatment outcome. To further substantiate this assumption, larger samples with a randomized parallel group design are necessary.

Although our study was sufficiently powered to detect small to moderate genetic effects, we could not resolve the ambiguity of pharmacogenetic findings for BDNF. Several clinical and preclinical studies have assigned a major impact of BDNF in the pathophysiology of depression and its recovery mechanisms, while pharmacogenetic and case-control studies on BDNF polymorphisms have produced conflicting results [19,22–24,41,47,50–56]. In particular, a recent meta-analysis including a total of 2912 MDD patients and 10843 controls did not find an association of the functional Val66Met (rs202535) polymorphism with unipolar depression in the total sample, but in a separate analysis of male participants [57]. Similarly, no consistent case-control association could be demonstrated in bipolar depression [42,50–61]. Considerable global population diversity of BDNF allele and haplotype frequencies have been held responsible for these inconsistencies across studies [20].

In an extensive exonic analysis including novel sequence variants in a Mexican American sample, Licinio et al. [22] detected eight nominally significant markers associated with response. Interestingly, one of them, rs2030324 is located in the same LD block as rs2049046 in the CEU population (SNP’s pairwise $r^2 = 0.9$) that showed significant association in the combined sample mainly originates from the male subgroup of patients. On the other hand, rs1659412, also significantly associated in the combined sample, appears to moderate antidepressant response predominantly in female patients. Of note, both SNPs did not even show nominal associations in the opposing gender. Gender-specific effects have been reported for BDNF associations with depression and antidepressant treatment [23,57], as well as for NTRK2 with obsessive-compulsive disorder [44]. Nevertheless, results have to be interpreted cautiously and subsamples might still have been underpowered to rule out false-negative associations.

Several limitations of the present study should be mentioned. First, although our power analysis suggested being sufficient to detect moderate genetic effects in the combined sample, the study might still have been underpowered for statistical effects in complex phenotypes like antidepressant response possibly accounting for the lack of replication of BDNF associations found in the discovery sample. Another issue are the multiple statistical tests performed in this study including several post-hoc analyses. Thus, although having corrected for the number of SNPs genotyped, reported association may still have been found just by chance. In addition, at least part of the patients have been also analyzed on genome-wide level [29] without significant association of BDNF and NTRK2 polymorphisms. Nevertheless, the current study follows a candidate gene approach with selectively genotyped SNPs. Conversely, a random selection of 82 SNPs genotyped in 925 patients in the discovery sample using Human610 Genotyping BeadChips (165 responder, 160 non-responder) resulted in only two nominal significant associations ($P = .003$ and $P = .026$, respectively), not withstanding correction for 82 SNPs investigated ($P = .110$ and $P = .874$; Westfall-Young; data not shown).

Further, although statistically significant, the clinical relevance of observed genetic effects can be questioned. According to recent approaches, combining multiple genetic markers could provide more reliable and meaningful prediction of antidepressant response [29]. Nevertheless, several clinical and preclinical studies implicated pathophysiological involvement of the neurotrophins in antidepressant response [4,5,11–13]. We therefore argue, that a thorough genetic approach with extended gene coverage like in the present study can add to the understanding and characterization of the neurotrophin system in antidepressant response mechanisms.

Heterogeneity of patients is an important issue in genetic association studies and may have limited the replication of initial findings in the present study. In particular, we observed marked differences in depression severity and response rates among samples. In addition, besides unipolar depression, patients with a bipolar disorder were included, which might have impaired the homogeneity of our sample. Nevertheless, during a depressive episode, there are no pathognomonic characteristics that can reliably differentiate the two disorders and misdiagnosis of bipolar disorder as unipolar depression occurs frequently [62,63]. Given a relatively high annual conversion rate of recurrent unipolar to bipolar disorder [64], these entities may indeed share common disease mechanisms, too. Further, neither clinical data [1,64] nor genetic analyses [29,65] support the view that these clinical entities must be considered separately and a re-analysis with unipolar patients only confirmed the initial findings. All patients fulfilled the same inclusion criteria and did not differ in baseline demographic, duration of hospital stay and other characteristics of the
current episode. In addition, adjusting quantitative phenotype variables for sample origin did not alter the SNP associations.

Four SNPs (rs2049046, rs6265, rs9969765 and rs11140793) showed nominal deviations from the HWE (DHW) including the most significantly associated SNP in the discovery analysis (rs2049046). Using a Bonferroni-based threshold for multiple testing of the HWE can be questioned and may not be conservative enough. However, given that 82 SNPs have been genotyped in the initial sample, false positive deviations of HWE can be expected in about 4 cases for an alpha set to .05, which may have been the case in the current analysis. DHW may also rely on the underlying disease or phenotypic model itself and may thus be regarded as an indicator of a true association (discussed in [66]). Further, there was no indication for insufficient genotyping quality as a possible explanation for DHW and deviations were only slight (minimal $P = .02$) which has been reported in previous studies with associated variants (e.g. [67]).

The current study does not have a placebo arm, making it impossible to disentangle placebo-related and real pharmacological effects, and only a part of our patients was treated with only one class of antidepressant agent, increasing the risk of false-positive and false-negative findings in the medication-specific analyses. On the other hand, due to the naturalistic design of the present study allowing medication of doctor’s choice, our results are probably more generalizable and more relevant for clinical practice. In addition, still a considerable number of patients entered the medication-specific analysis and our findings are in line with previous studies that reported $BDNF$ SNP associations in SSRI treated patients [19,23,50–52].

In summary, in an extended gene coverage approach, we found variants in the $NTRK2$ gene that may potentially predict antidepressant treatment outcome. Consistent with previous reports, no major effect of the extensively studied $BDNF$ Val66Met polymorphism with antidepressant response could be found. The functional impact of associated variants remains unclear and replications of associations in subsequent studies including functional assessments like RNA and protein measurements are warranted to further substantiate initial findings of the present study.

**Supporting Information**

**Figure S1** $D'$-based linkage disequilibrium (LD) of the $BDNF$ (left panel) and $NTRK2$ (right panel) gene regions retrieved from HapMap for the CEU population (release 21). Haplotype blocks were defined according to Gabriel et al. [39].

**Figure S2** $R^2$-based linkage disequilibrium (LD) of the $BDNF$ (A) and $NTRK2$ (B) gene regions retrieved from HapMap for the CEU population (release 21). Diamonds indicate genotyped SNPs in the discovery sample. Red diamonds represent nominally associated SNPs in the discovery sample. The exon (black boxes)-intron structures of the longest isoforms of $BDNF$ (NM_170751) and $NTRK2$ (NM_006180) are depicted according to dbSNP build 132. In case of $BDNF$, black boxes indicate alternative exons (I-IX) as proposed by Pruunsild, et al [Pruunsild, et al 2007], and are approximated relative to the SNPs.

**References**


**Table S1** $BDNF$ SNPs in the MARS discovery sample.

**Table S2** $NTRK2$ SNPs in the MARS discovery sample.

**Table S3** Effect of SSRI on SNP association.

**Table S4** Association with response depending on specific medication.

**Table S5** Effect of gender on SNP association.

**Table S6** Haplotypes Associated with Response after 5 Weeks.

**Document S1** Medication- and gender-specific association.

**Acknowledgments**

We thank Florian Holsboer for fruitful discussion and many helpful comments during the course of the project. We are grateful to Gertraud Ernst-Jansen, Beate Siegel, Melanie Huber, Elisabeth Kappellmann, Maik Kœdel and Susann Sauer for excellent technical assistance. Excellent support in data bank management was provided by Hildegard Pfister and Benno Pütz.

**Author Contributions**

Conceived and designed the experiments: JH MK SL. Performed the experiments: JH TB SH TK SL AM MG AE MU. Analyzed the data: JH DC CW AH MI BM. Contributed reagents/materials/analysis tools: KD ex vivo experiments: JH TB SH TK SL AM MG AE MU. Analyzed the data: JH DC CW AH MI BM. Contributed reagents/materials/analysis tools: KD VA BB MG AE. Wrote the paper: JH SL.


