Genetic association of the interaction between the *BDNF* and *GSK3B* genes and major depressive disorder in a Chinese population

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**Abstract** Alterations in brain-derived neurotrophic factor (*BDNF*)-signaling pathways may play an important role in the pathophysiology of major depressive disorder (MDD). Several lines of evidence have suggested that gene–gene interactions may confer susceptibility to MDD. The aim of this study was to analyze the single and combined effects of genes in the *BDNF* signal-transduction pathway on MDD in a Chinese population. We recruited 447 patients with MDD and 432 age- and gender-matched control subjects. Five SNPs in three *BDNF* signal-transduction pathway genes (*BDNF*, *GSK3B*, and *AKT1*) were used in association analyses. An allelic association between the *GSK3B* SNP rs6782799 and MDD was found in our sample (allelic: $\chi^2 = 5.24$, $P = 0.022$, corrected $P = 0.107$; genotypic: $\chi^2 = 5.55$, $P = 0.062$) with an odds ratio (OR) of 1.25 (95% confidence interval (CI) 1.03–1.52). Further gene–gene interaction analyses showed a significant effect of a two-locus *BDNF*/*GSK3B* interaction with MDD (*GSK3B* rs6782799 and *BDNF* rs7124442) (corrected $P = 0.011$), and also for a three-locus interaction (*GSK3B* rs6782799, *BDNF* rs6265 and *BDNF* rs7124442) (corrected $P = 0.019$).

Individuals carrying the combination of two risk alleles showed an OR value of 4.00 (95% CI 2.05–7.79), while those with the combination of three risk alleles gave the largest OR value of 4.46 (95% CI 2.15–9.24). Taken together, these findings support the assertion that the *GSK3B* gene is an important susceptibility factor for MDD in a Han Chinese population.

**Keywords** Major depressive disorder (MDD) · Brain-derived neurotrophic factor (*BDNF*) · Glycogen synthase kinase 3 beta (*GSK3B*) · Genetic association · Gene–gene interaction

**Introduction**

Major depressive disorder (MDD) is one of the most prevalent and costly neuropsychiatric diseases. Epidemiologic studies show that the lifetime prevalence of MDD is 12–17% (Wittchen et al. 1992). Although the etiology of MDD remains unclear, a genetic component is likely to contribute to its development, with heritability ranging from 40 to 50% (Bierut et al. 1999; Sullivan et al. 2000). Patients with a family history have a higher rate of recurrence and earlier age of onset than those without a family history (Tozzi et al. 2008). Thus, identification of MDD-susceptibility genes can contribute to the development of new treatment strategies and to the prevention of this illness as well as other mood disorders, including bipolar disorder.

Neuroimaging and postmortem studies in adult patients with depression have demonstrated that decreased hippocampal volume (an average of 9% across all studies), atrophy of existing neurons and decreased neurogenesis may contribute to the pathophysiology of MDD (Sheline et al. 2003; Neumeister et al. 2005; Janssen et al. 2007).
Decreased expression of brain-derived neurotrophic factor (BDNF) was observed in the hippocampus of suicidal patients with depression, while expression levels of BDNF were increased in patients medicated with antidepressants (Chen et al. 2001; Dwivedi et al. 2003; Karege et al. 2005). Furthermore, BDNF levels in the circulation were found to be significantly decreased in patients with depression (Karege et al. 2002; Aydemir et al. 2006; Kim et al. 2007), yet they returned to normal levels following treatment with antidepressants and electroconvulsive therapy (Aydemir et al. 2005; Zanardini et al. 2006). These findings suggest that alterations in the BDNF signaling pathway may underpin the pathophysiology of MDD. BDNF regulates neuronal survival in the central nervous system via the phosphatidylinositol 3-kinase (PI3-kinase)/protein kinase B (PKB, renamed AKT) pathway. Activated AKT regulates a number of cell survival-related proteins by phosphorylation, such as glycogen synthase kinase 3 beta (GSK3B) (Huang and Reichardt 2001). Thus, BDNF, AKT and GSK3B have become important candidate susceptibility genes for depression (Verhagen et al. 2008; Hsiung et al. 2003; Karege et al. 2007).

The human BDNF gene is located on chromosome 11p13 and consists of six 5' exons that are differentially spliced to a single 3' terminal exon (exon 7) that encodes the entire sequence of mature BDNF (GenBank accession AF411339). BDNF belongs to a family of closely related peptides that play a critical role in shaping plasticity in the mature nervous system (Thoenen 1995) and in promoting and modifying the growth, development and survival of neuronal populations. Recent evidence has suggested an association of BDNF polymorphisms with risk of MDD (Hwang et al. 2006; Ribeiro et al. 2007), although this has not been confirmed in other studies (Hong et al. 2003; Tsai et al. 2003; Choi et al. 2006). The major role of the critical enzyme V-akt murine thymoma viral oncogene homolog 1 (AKT1) is to facilitate growth factor-mediated cell survival and to block apoptotic cell death, suggesting that AKT1 may be involved in neurodevelopment (Wang et al. 2003). Toyota et al. (2003) screened all 11 exons and some flanking introns of the AKT1 gene in 22 unrelated patients with bipolar disorder and found a weak association between AKT1 haplotypes and the illness. The human GSK3B gene, consisting of 12 exons, is located on chromosome 3q13.33 and spans approximately 268 kb of DNA. GSK3B plays a key role in the phosphorylation and regulation of metabolic enzymes and many transcription factors, and it also modulates diverse cellular processes, such as embryogenesis, apoptosis and cell survival (Grimes and Jope 2001; Jope and Bijur 2002). Recently, Tsai et al. (2008) have reported an association between genetic variation in GSK3B and 4-week selective serotonin reuptake inhibitor antidepressant therapeutic response in Chinese individuals with MDD. Many genetic analyses in MDD and related psychiatric disorders have focused on the genes involved in the BDNF-functional pathway, especially those affecting synaptic transmission and plasticity.

To elucidate whether these genes in the BDNF signal-transduction pathway play a role in the development of MDD, we undertook a case–control study with a large sample size to examine the individual and combined effects of the BDNF, AKT1 and GSK3B genes on MDD in a Chinese population.

Materials and methods

Subjects

The participants were recruited from the Shanxi Province of China between March 2004 and February 2008. The patient group comprised 447 patients with MDD (225 males and 222 females; mean age 27.76 ± 7.98 years, range 18–60 years), who were recruited from clinical settings (98 inpatients and 349 outpatients). Clinical diagnosis was made by at least two consultant psychiatrists according to Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) criteria for MDD (American Psychiatric Association 1994). All patients recruited for this study were also assessed with the Chinese Version of the Modified Structured Clinical Interview for DSM-IV TR Axis I Disorders Patient Edition (SCID-I/P, 11/2002 revision). Among the patients, 80.8% were experiencing their first major depressive episode (n = 361), while the other 19.2% were in a relapse episode. The control group included 432 healthy volunteers (194 males and 238 females; mean age 28.32 ± 8.67 years, range 18–60 years). The details and processes of collection and diagnosis have been described elsewhere (Sun et al. 2008). All subjects were Chinese and of Han origin, who were from the same geographical area in Northern China and gave written informed consent. This study was approved by the Ethical Committee for Medicine of The First Hospital of Shanxi Medical University, China.

Single nucleotide polymorphism (SNP) selection

The SNPs were selected using the Hapview program (http://www.broad.mit.edu/mpg/ Haplovie) (Barrett et al. 2005) with Gabriel’s criteria (Gabriel et al. 2002), based on the HapMap database for the Chinese Han population of Beijing (CHB). The Haplovie program indicated two haplotype blocks within the BDNF gene (Fig. 1a). One tag SNP was selected from each haplotype block so that each SNP represented genetic information from its corresponding individual block. We selected two tag SNPs (rs6265 and rs7124442) from the BDNF gene for association analysis in this study.
There were 18 tag SNPs present in the chromosomal region harboring AKT1. Haploview indicated two haplotype blocks (Fig. 1b). Based on similar criteria, three tag SNPs, rs2494746 (block 1), rs3001371 (block 2) and rs2494738 (between these two blocks) were genotyped for the AKT1 gene.

As shown in Fig. 1c, the HapMap CHB database contained 186 tag SNPs in the chromosomal region harboring the GSK3B gene. All tag SNPs were present in one haplotype block, so we selected only one SNP, rs6782799, for association analysis in this study.

### Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a phenol–chloroform extraction protocol. All six SNPs were detected by a polymerase chain reaction (PCR)-based genotyping protocol. The primers used for PCR amplification were designed using Primer 5.0 software, and their specificity was validated using NCBI BLASTN (http://www.ncbi.nlm.nih.gov/BLAST/). PCR amplification was performed in a 25 μl reaction volume containing 2.5 μl 10× buffer (Tiangen, Beijing, China), 200 μM of each dNTP, 0.4 μM of each primer, 1.0 unit of Taq DNA polymerase (Tiangen, Beijing, China) and 60 ng of genomic DNA. The conditions used for PCR amplification were denaturation at 95°C for 10 min, followed by 35 cycles of 95°C for 30 s, 54–63°C for 30 s and 72°C for 30 s, then a final extension at 72°C for 10 min. PCR products were purified using a MultiScreen-PCR plate (Millipore, MA, USA). The purified PCR products were bidirectionally sequenced using an ABI 3730 DNA sequencer (Perkin-Elmer, Applied Biosystems, Foster City, CA, USA), and the genotype of the SNPs was read by the Chromas program (version 2.31). The primer sequences and the length of the PCR products are given in Table 1.

As a genotyping quality control, 5% of the samples were randomly selected to repeat direct sequencing for all six SNPs and the results were 100% concordant.

### Statistical analysis

The Hardy–Weinberg equilibrium (HWE) was tested by the goodness-of-fit chi-squared ($\chi^2$) test for the genotypic distributions of the polymorphisms studied. Linkage disequilibrium (LD) blocks were defined using Haploview and the LD measurements were expressed as $D^0$ and $r^2$. Allelic, genotypic and haplotype associations were performed with the UNPHASED program (version 3.1.3) (Dudbridge 2003, 2008). Haplotypes with frequencies of $>3\%$ were considered for analyses. The UNPHASED program was also used to test allelic gene interaction association by considering the odds ratios (OR) of the conditioning haplotypes (in this model, it was assumed that the conditioning and test haplotypes were independent). A SNP positive by single locus analysis was used as a conditioning marker, while the others SNPs were the testing markers. The window size used was the number of test markers. If no window size was specified, the default value was 1. To circumvent the problem of multiple testing, we applied a permutation test.
for the global null hypothesis that all OR were equal. The permutation test is a built-in program of UNPHASED. It gave a significance level corrected for all SNPs tested; 10,000 permutations were used to obtain a global $P$ value in the present study. In addition, to clarify the allelic combination results, we computed the $\chi^2$ and OR (with 95% confidence interval (CI)) values using SPSS software for windows (version 13.0; SPSS, Chicago, IL) to determine the set of risk factors.

Power analysis for the case–control samples was carried out with PS Version 2.1.31 (Dupont and Plummer 1998), which indicated that this number of case–control samples had greater than 80% power to detect a genotype relative risk between 1.452 and 1.994 over a range of marker allele frequencies from 0.421 (rs3001371) to 0.058 (rs7124442).

### Results

The $\chi^2$ goodness-of-fit test showed that the genotypic distributions of all SNPs, except $AKT1$ rs2494738, were in HWE both in the case and in the control group (Table 2).

To test whether the violation of HWE at $AKT1$ rs2494738 was caused either by true association with MDD or other reasons, such as genotyping errors, we performed a test developed by Li (Li and Li 2008) to differentiate these two types of HWE deviation (data not shown). The result showed that the observed HWE deviation at rs2494738 was likely to be caused by an unknown reason instead of true association with MDD. We therefore removed it from further association analysis.

The results from single-marker analysis of the five SNPs are given in Table 2. An allelic association between $GSK3B$ rs6782799 and MDD was found in our sample (allelic: $\chi^2 = 5.24, \ P = 0.022$; genotypic: $\chi^2 = 5.55, \ P = 0.062$) with an OR of 1.25 (95% CI 1.03–1.52). The C-allele of the $GSK3B$ gene polymorphism was more common in the patient group than in the control group. Following adjustment for multiple testing by permutation analysis (10,000 permutations), the global $P$ value was 0.107 for allelic association.

Moreover, MDD risk estimates associated with different genetic models are shown in the Tables 3 and 4. The recessive models showed no association between the SNPs genotype and the risk of MDD (all $P \geq 0.05$). In case of the genotypic association of rs6782799, analyses using the dominant model showed association between the SNP and the risk of MDD ($\chi^2 = 5.19, \ P = 0.023$). Individuals with the c-allele were more likely to have MDD (OR 1.39, 95% CI 1.05–1.84 for the dominant model).

The haplotype blocks were defined using the default CI settings in HAPLOVIEW (Dudbridge 2003, 2008), and no haplotypic association was observed in the genes encoding $AKT1$ or $BDNF$ (data not shown).

To assess further the combined effects of these genetic variants on MDD risk, we performed gene–gene interaction analysis for the five SNPs using UNPHASED. The allele-based analysis of the gene–gene interactions or combinations is shown in Table 5. Because $GSK3B$ rs6782799 showed a weak allelic association in our sample ($P = 0.022$; corrected $P = 0.107$), we used this locus as a conditioning marker for analyses of gene–gene interaction. Significant allelic gene–gene interaction effects were observed between $BDNF$ and $GSK3B$, including the combination between $BDNF$ rs6265 and $GSK3B$ rs6782799 ($\chi^2 = 6.028, \ df = 2, \ P = 0.049$, corrected $P = 0.134$), between $BDNF$ rs7124442 and $GSK3B$ rs6782799 ($\chi^2 = 11.92, \ df = 2, \ P = 0.003$, corrected $P = 0.011$) and

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### Table 1 Primer sequences and length of PCR products

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>SNP ID</th>
<th>Location</th>
<th>Polymorphisms (major allele &gt; minor allele)</th>
<th>Primer sequence ($3'\rightarrow 5'$)</th>
<th>$T_m$ (°C)</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF</td>
<td>rs6265</td>
<td>Exon 5</td>
<td>G &gt; A</td>
<td>F: GCTTTTCCTACAGTTCCAC R: TCTGCTGGCGTTACCCCAC</td>
<td>56</td>
<td>491</td>
</tr>
<tr>
<td></td>
<td>rs7124442</td>
<td>Exon 2</td>
<td>T &gt; C</td>
<td>F: ACCATAACCCAGGAATGAGA R: GTCAACATAAACCATACAGC</td>
<td>56</td>
<td>398</td>
</tr>
<tr>
<td>AKT1</td>
<td>rs2494746</td>
<td>Intron 2</td>
<td>G &gt; C</td>
<td>F: TAGCAGATGGTTACCA R: CAGGCAGCGACTATGTTG</td>
<td>54</td>
<td>516</td>
</tr>
<tr>
<td></td>
<td>rs2494738</td>
<td>Intron 2</td>
<td>G &gt; A</td>
<td>F: CATTCTTGAGGAGGAGTACG R: GGCCGACCTGTGTTGATT</td>
<td>63</td>
<td>494</td>
</tr>
<tr>
<td></td>
<td>rs3001371</td>
<td>Intron 4</td>
<td>A &gt; G</td>
<td>F: GGACCAGCTGATCGAGCCAGC R: TCACGAACCTCCTGGAC</td>
<td>58</td>
<td>740</td>
</tr>
<tr>
<td>GSK3B</td>
<td>rs6782799</td>
<td>Intron 7</td>
<td>T &gt; C</td>
<td>F: TTTGTGAGGTGCTTCTG R: AGGAATGTAGCAGCAAT</td>
<td>58</td>
<td>883</td>
</tr>
</tbody>
</table>

$T_m$ melting temperature
between BDNF rs6265, BDNF rs7124442 and GSK3B rs6782799 ($\chi^2 = 14.45$, $df = 4$, $P = 0.006$, corrected $P = 0.019$). In contrast, no genetic interactions between the AKT1 and GSK3B loci influenced MDD risk in our Chinese sample.

In addition, we computed the $\chi^2$ and OR (with 95% CI) to determine the set of risk factors selected by gene–gene interaction (data not shown). Subjects with the T allele of both SNPs BDNF rs7124442 and GSK3B rs6782799 were used as the reference group. Gradually draw into both C-allele of two SNPs, the OR value increased dramatically. The OR value scaled up from 1.00 to 4.00 (95% CI 2.05–7.79; $P < 0.001$; assuming OR T–T = 1.00) in the C–C group. Similarly, the OR value also increased when combinations of three alleles were assessed: the largest OR value was for BDNF rs6265 (A-allele), BDNF rs7124442 (T-allele) and GSK3B rs6782799 (C-allele), at 4.46 (95% CI 2.15–9.24; $P = 0.002$; assuming OR G–T–T = 1.00). Because the frequencies of the GCC and ACC combinations were $<$3%, they were not considered for OR analysis.

**Discussion**

In this study, we evaluated the individual and joint effects of seven polymorphisms in three genes on MDD risk in a Chinese population. The results suggested that the SNP rs6782799 in the GSK3B gene not only had a major effect on MDD, but also when combined with the BDNF locus, the risk could be further increased.

Several lines of evidence indicate that GSK3B is a good candidate molecule for MDD susceptibility. However, to date, not all studies have found GSK3B to be associated with MDD itself, but rather with particular clinical features (Benedetti et al. 2004; Serretti et al. 2008) or antidepressant usage (Adli et al. 2007; Tsai et al. 2008). In the current study, our analyses revealed a weak association (allelic: $P = 0.022$; genotypic: $P = 0.062$) between the GSK3B rs6782799 polymorphism and MDD, although this association did not reach statistical significance after correction for multiple testing (allelic: corrected $P = 0.107$). This result is not consistent with previously reported findings in another Chinese Han population (Tsai et al. 2008). The different SNPs selection in the two studies may be a major reason. Four SNPs (rs3345585 rs13321783, rs2319398 and rs6808874) and one SNP (rs6782799) in GSK3B gene were selected in Tsai’s and our studies, respectively. Except for SNP rs3345585 (located in promoter region), others four SNPs are located in intron region in GSK3B gene and they are not the risk factor but just serve as a DNA marker. In the current study, the positive GSK3B SNP rs6782799 may capture a LD signal from a disease-causing variant and cislink to this variant. Another
possible reason for this conflicting result is the sample size. The sample from Tsai’s study comprised 230 Chinese MDD patients, while our study recruited 447. Compared with the study by Tsai et al. (2008), we used a relatively large sample size and had more power to detect associations. The present finding may be more reliable although it still should be interpreted with caution.

A large body of literature has focused on studying the genetic association between the BDNF gene and depression. However, genetic association studies of the SNP

Table 3 MDD risk estimates associated with dominant genetic model

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Dominant model</th>
<th>Case</th>
<th>Control</th>
<th>$\chi^2$</th>
<th>$p$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF</td>
<td>rs6265</td>
<td>G/G</td>
<td>121</td>
<td>118</td>
<td>0.05</td>
<td>0.819</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>A/G + A/A</td>
<td>302</td>
<td>305</td>
<td>0.97</td>
<td>0.72–1.30</td>
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</tr>
<tr>
<td>BDNF</td>
<td>rs7124442</td>
<td>T/T</td>
<td>372</td>
<td>376</td>
<td>2.18</td>
<td>0.140</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/T + C/C</td>
<td>63</td>
<td>47</td>
<td>1.35</td>
<td>0.90–2.03</td>
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</tr>
<tr>
<td>AKT1</td>
<td>rs2494746</td>
<td>G/G</td>
<td>178</td>
<td>176</td>
<td>0.42</td>
<td>0.518</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/G + C/C</td>
<td>259</td>
<td>234</td>
<td>1.09</td>
<td>0.83–1.44</td>
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</tr>
<tr>
<td>AKT1</td>
<td>rs3001371</td>
<td>A/A</td>
<td>141</td>
<td>135</td>
<td>0.00</td>
<td>0.957</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>A/G + G/G</td>
<td>300</td>
<td>285</td>
<td>1.01</td>
<td>0.76–1.34</td>
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<tr>
<td>GSK3B</td>
<td>rs6782799</td>
<td>T/T</td>
<td>135</td>
<td>163</td>
<td>5.19</td>
<td>0.023</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>C/T + C/C</td>
<td>301</td>
<td>262</td>
<td>1.39</td>
<td>1.05–1.84</td>
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</tr>
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</table>

CI confidence interval, OR odds ratio, SNP single nucleotide polymorphisms

Table 4 MDD risk estimates associated with recessive genetic model

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Recessive model</th>
<th>Case</th>
<th>Control</th>
<th>$\chi^2$</th>
<th>$p$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF</td>
<td>rs6265</td>
<td>A/A</td>
<td>89</td>
<td>95</td>
<td>0.25</td>
<td>0.617</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>A/G + G/G</td>
<td>334</td>
<td>328</td>
<td>1.09</td>
<td>0.78–1.51</td>
<td></td>
</tr>
<tr>
<td>BDNF</td>
<td>rs7124442</td>
<td>C/C</td>
<td>3</td>
<td>2</td>
<td>0.17</td>
<td>0.677</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>C/T + T/T</td>
<td>432</td>
<td>421</td>
<td>0.68</td>
<td>0.11–4.11</td>
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<tr>
<td>AKT1</td>
<td>rs2494746</td>
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<td>0.58</td>
<td>0.447</td>
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<tr>
<td></td>
<td></td>
<td>C/G + G/G</td>
<td>376</td>
<td>360</td>
<td>0.86</td>
<td>0.57–1.28</td>
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<tr>
<td>AKT1</td>
<td>rs3001371</td>
<td>G/G</td>
<td>78</td>
<td>69</td>
<td>0.24</td>
<td>0.624</td>
<td>1</td>
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<td></td>
<td></td>
<td>A/G + A/A</td>
<td>363</td>
<td>351</td>
<td>0.91</td>
<td>0.64–1.31</td>
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<tr>
<td>GSK3B</td>
<td>rs6782799</td>
<td>C/C</td>
<td>86</td>
<td>69</td>
<td>1.78</td>
<td>0.183</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>C/T + T/T</td>
<td>350</td>
<td>356</td>
<td>0.79</td>
<td>0.56–1.12</td>
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</tbody>
</table>

CI confidence interval, OR odds ratio, SNP single nucleotide polymorphisms

Table 5 The conditional test for a combined effect of paired SNPs

<table>
<thead>
<tr>
<th>Makers to analyses</th>
<th>Conditioning markers</th>
<th>Genotype</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\chi^2$</td>
<td>df</td>
</tr>
<tr>
<td>rs2494746</td>
<td>rs6782799</td>
<td>6.23</td>
<td>6</td>
</tr>
<tr>
<td>rs3001371</td>
<td>rs6782799</td>
<td>5.17</td>
<td>6</td>
</tr>
<tr>
<td>rs2494746-rs3001371</td>
<td>rs6782799</td>
<td>15.38</td>
<td>16</td>
</tr>
<tr>
<td>rs6265</td>
<td>rs6782799</td>
<td>7.48</td>
<td>6</td>
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<tr>
<td>rs7124442</td>
<td>rs6782799</td>
<td>7.59</td>
<td>3</td>
</tr>
<tr>
<td>rs6265-rs7124442</td>
<td>rs6782799</td>
<td>10.61</td>
<td>10</td>
</tr>
</tbody>
</table>

CI confidence interval, OR odds ratio, SNP single nucleotide polymorphisms

<sup>a</sup> The adjusted $P$ value was 0.134 after 10,000 permutation tests
<sup>b</sup> The adjusted $P$ value was 0.011 after 10,000 permutation tests
<sup>c</sup> The adjusted $P$ value was 0.019 after 10,000 permutation tests
BDNF rs6265 in MDD have produced inconsistent results. Two meta-analyses have suggested that BDNF is associated with depression or the response to antidepressants (Verhagen et al. 2008; Kato and Serretti 2008), while another meta-analysis showed no association (Chen et al. 2008). In the current study, using analysis of single loci, we did not find either of the two BDNF polymorphisms (rs6265 and rs7124442) to be associated with MDD in a Chinese sample. It is difficult to interpret these controversial results. There may be several interpretations for the discrepancy. For one thing, ethnic differences in polymorphism frequencies might contribute to inconsistent results in genetic association studies. Shimizu et al. (2004) pointed that the frequencies of healthy individuals who carried the BDNF rs6265 G/G genotype were significantly decreased in Japanese (33.8%) than in Italians (48.7%) or in Americans (68.4%). For another, MDD is a complex disease that is thought to be caused by multiple genetic factors each of small effect. Gene–gene interactions are likely to contribute to the pathophysiology of illness.

The results of our gene–gene interactions, as analyzed by UNPHASED, showed that a two-locus interaction (GSK3B rs6782799 and BDNF rs7124442) and a three-locus interaction (GSK3B rs6782799, BDNF rs6265 and BDNF rs7124442) confer an increased risk for MDD. Further analyses showed that the combination of two risk alleles gave an OR value of 4.00, while the combination of three risk alleles gave the largest OR value of 4.46. The finding of GSK3B–BDNF interactions provides a better understanding of the genetic mechanisms of MDD. Of note, GSK3B–BDNF gene interactions confer the risk of disease have been found in schizophrenia without tardive dyskinesia (Park et al. 2009), although the particular SNPs selected from the two genes were different from those used in this study. In addition, the biological link between BDNF and GSK3B has been established. GSK3B may be a negative regulator of BDNF. For example, Foulstone et al. (1999) reported BDNF inhibited GSK3B in cerebellar granule cells by increasing serine-9 phosphorylation. Further study showed overexpression of GSK3B can abolish the cyclic AMP response element binding protein (CREB) phosphorylation induced by BDNF and this inhibition of BDNF-induced CREB phosphorylation in GSK3B-overexpressing SH-SY5Y cells was blocked by treatment with lithium (Ma et al. 2002).

Our study has several strengths. We restricted our analysis to the Northern Chinese population to reduce the possible effects of population stratification. Moreover, the structured diagnostic interview tools—SCID-I/P—were applied in the diagnostic process both for MDD patients and for controls. It has been reported that a strict diagnostic approach is more powerful than using a less restrictive definition of depression in detecting genetic association between polymorphisms and disease (Cervilla et al. 2007). We also note, however, that we selected very few tag SNPs from these three genes; indeed, only one from GSK3B. In future studies we need to examine more SNPs, including those affecting function.

In another study, we have found a significant interaction between the BDNF and GSK3B genes and negative life events and the risk of MDD in another independent Chinese sample set (manuscript submitted). Taken together, our two findings suggest that the BDNF and GSK3B genes are involved in MDD in the Chinese population with complex pathogenic mechanisms, including effects of the gene itself, gene–gene interactions and/or gene–environment interactions. Further research should be conducted to replicate this finding using independent samples from other ethnic populations.

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Conflict of interest statement The authors declare that they have no conflicts of interest.

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