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Possible Association of the *GSK3β* Gene with the Anxiety Symptoms of Major Depressive Disorder and P300 Waveform

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Glycogen synthase kinase-3β (GSK3β) may play an important role in the brain of patients with major depressive disorder (MDD); therefore, we investigated whether the *GSK3β* gene is involved in the etiology of MDD and whether it affects MDD endophenotypes. Three single-nucleotide polymorphisms (SNPs) (rs6438552, rs7633279, and rs334558) were genotyped in 559 MDD patients and 486 healthy controls. To explore quantitative traits of MDD, we analyzed the association of these SNPs with the factor scores of the 17-item Hamilton Depression Rating Scale (HAMD-17) and the Hamilton Anxiety Rating Scale (HAMA). We also determined the effects of these SNPs on the measurement of the P300 wave. Although no significant association between *GSK3β* SNPs and MDD was found, some genotypes and haplotypes were associated with anxiety symptoms in MDD. The three SNPs were associated with the HAMA total score and with the HAMD anxiety and somatization factor score ($p < 0.05$). Three-locus haplotype analysis showed the C-T-G carriers to have a strong association with the HAMA total score ($p = 0.032$). Moreover, the P300 latency and amplitude were also associated with *GSK3β* genotypes. The individuals with the T allele genotype, both in rs6438552 and rs7633279, have a longer P300 latency than those carrying the C/C ($p = 0.04$) and A/A genotype ($p = 0.013$). The individuals with the G/G genotype in rs334558 have a lower amplitude than those carrying the A allele genotype ($p = 0.007$). Our findings show, for the first time, that *GSK3β* polymorphisms may play an important role in MDD endophenotypes, especially in anxiety symptoms.

Introduction

Major depressive disorder (MDD) is one of the most prevalent and costly neuropsychiatric diseases, with a lifetime prevalence of 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 40–50% (Bierut *et al.*, 1999; Sullivan *et al.*, 2000). Thus, identification of MDD susceptibility genes can contribute, not only to understanding the etiology of the disease but also to the development of individualized prevention and treatment strategies. With the development of hypotheses concerning mental illness and neural plasticity, much interest has recently been shown in glycogen synthase kinase-3β (GSK3β), a key regulator of neuronal function (Lachman *et al.*, 2007; Meng *et al.*, 2008; Tsai *et al.*, 2008).

GSK3β was identified in the early 1980s as a key enzyme in the regulation of the glycogen synthesis. It is an ubiquitous cellular serine–threonine kinase that phosphorylates and inactivates glycogen synthase (Embi *et al.*, 1980; Chin *et al.*, 2005; Balaraman *et al.*, 2006). In addition to glucose metabolism, GSK3β is also involved in the regulation of critical intracellular signaling pathways that affect multiple processes, including embryogenesis, gene expression, cell cycle, apoptosis, and cell survival (Grimes and Jope, 2001; Jope and Bijur, 2002; Muyllaert *et al.*, 2008). GSK3β, an isoform of GSK3, is highly abundant in the brain and is highly expressed in neural tissue where its expression is regulated during development (Grimes and Jope, 2001). In addition to its function as a negative regulator of glycogen synthesis, GSK3β now has become recognized as an enzyme with broad influence. In neurons, GSK3β takes part in cytoskeletal organization and remodeling. It plays an important role in the BDNF pathway, and is

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thereby involved in mechanisms of synaptic plasticity, neurogenesis, and resilience to neuronal injury (Grimes and Jope, 2001; Gould and Manji, 2005; Muyllaert *et al.*, 2008). GSK3β may be implicated in the pathogenesis of MDD, and there are many studies showing that inhibition of GSK3β activity may affect the therapeutic effects of antidepressants. Treatment with antidepressants was found to inhibit GSK3β activity in the mouse brain (Li *et al.*, 2004; Roh *et al.*, 2005). Lithium, a mood stabilizer that enhances the action of antidepressants, was found to inhibit GSK3β *in vitro*, in the mouse brain (Klein and Melton, 1996; Stambolic *et al.*, 1996) and in human peripheral blood mononuclear cells (Li *et al.*, 2007). Furthermore, Tsai *et al.* (2008) have reported an association between genetic variation in *GSK3β* and response to 4 weeks of selective serotonin reuptake inhibitor antidepressant therapy in Chinese individuals with MDD.

In a previous study, we had observed a weak potential association between a *GSK3β*-associated single-nucleotide polymorphism (SNP), rs6782799, and MDD (Yang *et al.*, 2010; Zhang *et al.*, 2010). For this reason, we have further investigated the association between the *GSK3β* and MDD in a Chinese population using an enlarged sample size and an increased number of SNPs. In addition, we analyzed the association between the *GSK3β* polymorphisms and endophenotypes in MDD patients.

Materials and Methods

Subjects

The patient group comprised 581 MDD patients (264 men and 317 women), with a mean age of 32.0 ± 9.8 years, ranging from 18 to 65 years. Clinical diagnosis was made by at least two consultant psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for MD (American Psychiatric Association, 2000). 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). We excluded potential participants who were pregnant or had significant medical conditions, unstable psychiatric features (e.g., suicidal), or a history of substance abuse or drug addiction within the previous 6 months, with the exception of nicotine dependence. Other Axis I comorbid disorders were not excluded.

The control group consisted of 486 healthy volunteers (217 men and 269 women), with a mean age of 32.8 ± 8.6 years, ranging from 18 to 65 years. They were recruited from local communities or were people undergoing routine health check-ups. All control subjects were assessed using the SCID. Subjects with relevant physical diseases or a history of major psychiatric disorders or suicidal behavior were excluded, and those who had a first-degree relative with a history of severe mental disorder or suicidal behavior were also excluded.

The patients in our study were not receiving medical treatment currently. They were all on the first episode of disease. In addition, the 17-item Hamilton Depression Rating Scale (HAMD-17) and the Hamilton Anxiety Rating Scale (HAMA) were used to assess clinical characteristics. For exploratory purposes, we examined the factor scores of HAMD-17, including the anxiety/somatization factor (items 10, 11, 12, 13, 15, and 17), the dysgnosia factor (items 2, 3, and 9), the blocking factor (items1, 7, 8, and 14), and the sleep factor (items 4, 5, and 6). HAMA factor scores were also analyzed, including the somatic anxious factor (items 7, 8, 9, 10, 11, 12, and 13) and the mental anxious factor (items 1, 2, 3, 4, 5, 6, and 14). All subjects were of Han Chinese origin, came from the same geographical area in Northern China, and gave written informed consent. This study was approved by the Ethics Committee for Medicine of the First Hospital of Shanxi Medical University, China.

P300 wave event-related potential assessments

Of the subjects genotyped, 267 patients and 248 controls underwent P300 assessments. They sat alert with eyes closed while listening to stimuli presented biaurally through ear plugs. The P300 event-related potential component was assessed by a standard auditory odd-ball task. The stimuli were four hundred 80-dB tones with 20-ms duration; 20% were target stimuli (1500 Hz), and 80% were standard stimuli (1000 Hz). The participants were instructed to press a button in response to the target stimuli. Electroencephalogram (EEG) data were collected from the central electrode according to the 10/20 International System, referenced to the left ear. Eye movements were recorded from the outer canthus of each eye, above and below the left eye. Electron impedances were kept below 5Ω. For the recording of EEG activity, the analog/digital rate was 500 Hz, and the filter setting was 0.03 Hz (high pass) and 120 Hz (low pass). The EEG was segmented in epochs of 900-ms duration (−100 to 800 ms relative to stimulus onset). The P300 peaks were defined as the most positive point constituting a peak between 250 and 650 ms after stimulus by use of maximum-likelihood estimation. The measurement has been described previously (Xu *et al.*, 2010).

SNP selection and genotyping

Three *GSK3β* SNPs were selected to test their disease association in the Chinese Han population, two of which are tag SNPs based on the information of the HapMap for the Han Chinese in a Beijing (CHB) population (www.hapmap.org). rs6438552 in intron 5 and rs7633279 in intron 8 have been reported as risk loci for MDD in other studies (Kwok *et al.*, 2005). Further, we also investigated rs334558 located in promoter 5 for genetic analysis.

Leukocyte DNA was extracted using a standard phenol–chloroform method and quantified using a microspectrophotometer (NaNoDropND-1000; Thermo Scientific). Real-time quantitative polymerase chain reaction was performed using a TaqMan minor groove binder (MGB) probe and appropriate primers (Applied Biosystems). TaqMan MGB probes are oligonucleotides capable of binding to specific allelic loci. They have different fluorescent labels, denoted by VIC and FAM. On 384-well plates, the wet DNA method was used to manually add sample to the reaction plate. The reaction system is a 5-μL system, with each test well containing 2.5 μL of 2× TaqMan Master Mix (Applied Biosystems), 0.25 μL of 20× TaqMan genotyping assay mix (Applied Biosystems), 1.25 μL of ddH_2O, and 1 μL of DNA at 50 ng/μL. A reference reaction was also run containing 1 μL of ddH_2O instead of DNA. Reaction conditions were 95°C for 10 min, 95°C for 15 s, and 60°C for 60 s. Steps two and three were repeated 50 times. According to the PCR results, 5 to 10 additional assays were performed using different probes as necessary.

Statistical analysis

The Haploview program (version 4.1; Broad Institute of MIT and Harvard) (www.broad.mit.edu/mpg/haploview) was applied to test the genotypic distributions of SNPs for the Hardy–Weinberg equilibrium (HWE) and to estimate linkage disequilibrium (LD) between these three SNPs in which the LD strength was expressed by measurements D' and r^2. Allelic, genotypic, and haplotypic associations were analyzed using the UNPHASED program (version 3.1.5) (www.mrcbsu.cam.ac.uk/personal/frank/software/unphased). SPSS for Windows (version 15.0; SPSS) was used to perform data analyses. The effects of individual genotypes on P300 amplitude and P300 latency were also analyzed by Student's *t*-test (two-tailed). The significance level was set at a *p*-value of 0.05.

Results

The genotypic distributions of the three *GSK3β* SNPs did not deviate from the HWE in the control group. The LD analysis was showed in Figure 1.

Association with MDD risk

The mean age ($t = -1.357$, $df = 1062.8$, $p = 0.175$) and sex distributions ($\chi^2 = 0.066$, $df = 1$, $p = 0.797$) of the MDD patients and the healthy controls were similar. The genotype and allele distributions of the *GSK3β* SNPs (compared between the patient and control groups) are summarized in Table 1. As shown in Table 1, the analysis for single-locus effects showed no significant association between these SNPs and MDD (all *p* after permutation > 0.05). The results of global case–control haplotypic analysis and comparisons of individual haplotypes between groups are presented in Table 2. However, there was no significant association between haplotypes and the risk of MDD ($\chi^2 = 11.75$, $p = 0.068$, adjusted $p = 0.166$).

Association with MDD endophenotypes

To explore endophenotypes of MDD, we analyzed SNP association with subfactors of HAMD-17 and HAMA in MDD patients (Table 3). As shown in Table 3, *GSK3β* genotypes

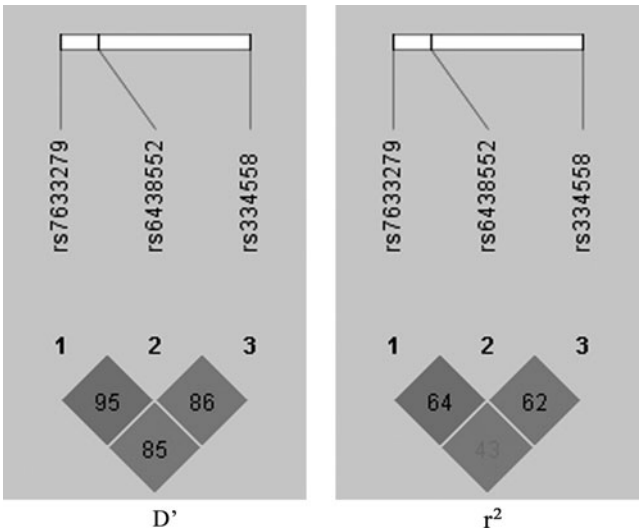

FIG. 1. The linkage disequilibrium (LD) patterns of the three markers in the control group: D′ and r^2.

TABLE 1. GENOTYPE DISTRIBUTIONS AND ALLELE FREQUENCIES OF SINGLE-NUCLEOTIDE POLYMORPHISMS IN MAJOR DEPRESSIVE DISORDER PATIENTS AND HEALTHY CONTROLS

SNP	*Group*	n	*Genotype (%)*			χ^2	p	*Allele (%)*		χ^2	p	*OR (95% CI)*	*HWE*-p
rs6438552			C/C	C/T	T/T			C	T				
	Patients	496	155 (31.3)	240 (48.4)	101 (20.4)	4.232	0.121	550 (55.4)	442 (44.6)	3.905	0.048[a]	1.199 (1.001–1.436)	0.646
	Controls	476	178 (37.4)	214 (45.0)	84 (17.6)			570 (60.0)	382 (40.1)				0.160
rs7633279			T/T	T/A	A/A			T	A				
	Patients	526	138 (26.2)	250 (47.5)	138 (26.2)	1.405	0.495	526 (50)	526 (50)	0.486	0.486	0.940 (0.789–1.119)	0.257
	Controls	483	112 (23.1)	244 (50.5)	127 (26.3)			468 (48.4)	498 (51.6)				0.803
rs334558			G/G	G/A	A/A			G	A				
	Patients	489	180 (36.8)	222 (45.4)	87 (17.8)	2.678	0.262	582 (59.5)	396 (40.5)	2.805	0.094	1.173 (0.973–1.414)	0.200
	Controls	444	185 (41.7)	192 (43.2)	67 (15.1)			562 (63.3)	326 (36.7)				0.144

[a]Adjusted *p* is 0.144 after 10000 permutation tests.
CI, confidence interval; HWE, Hardy–Weinberg equilibrium; OR, odds ratio; SNP, single-nucleotide polymorphism.

TABLE 2. ESTIMATED HAPLOTYPE FREQUENCIES AND ASSOCIATION SIGNIFICANCE

Haplotype	rs6438552 *C/T*	rs7633279 *T/A*	rs334558 *G/A*	*Frequency (%)*		χ^2	p	*OR (95% CI)*	*Global* p (χ^2)
				Patients n = *581*	*Controls* n = *486*				
1	C	T	G	49.79	77.01	5.342	0.021	1 (1–1)	0.068 (11.75)
2	C	T	A	8.579	5.484	0.549	0.459	2.419 (0.686–8.539)	
3	C	A	G	387.5	414.4	0.643	0.423	1.446 (0.978–2.137)	
4	C	A	A	35.18	20.11	4.267	0.039	2.706 (1.376–5.32)	
5	T	T	G	70.38	57.1	1.368	0.242	1.906 (1.139–3.19)	
6	T	T	A	295.2	292.4	0.365	0.546	1.562 (1.049–2.324)	
7	T	A	G	5.373	5.49	0.005	0.945	1.513 (0.419–5.467)	

were associated with the HAMA total score and two of the factor scores (all $p < 0.05$). The T/T genotype in rs6438552 and the A/A genotype in both rs7633279 and rs334558 all associate with a higher anxiety score than other genotypes. Similarly, we also found an association between the anxiety/somatization factor score in HAMD and the genotypes of rs6438552: $p = 0.002$, rs7633279: $p = 0.027$, but not of rs334558 ($p > 0.05$). The three-locus haplotype analysis showed the C-T-G carriers to have a strong association with the HAMA total score ($p = 0.002$, adjusted $p = 0.032$) (Table 4). In addition, we also found a strong association between the rs334558 genotype and the sleeping factor of HAMD ($p = 0.047$, adjusted $p = 0.009$) (Table 3), and with the haplotype (global $p < 0.001$, adjusted $p = 0.008$) (Table 4).

Association with the P300 wave event-related potential

Compared with control subjects, patients with MDD displayed significantly longer P300 latency (controls = 299.0 ± 32.6 ms, patients = 307.1 ± 41.9 ms, $p < 0.001$) and lower P300 amplitude (controls = 8.4 ± 4.1 μV, patients = 7.9 ± 3.8 μV, $p < 0.001$).

We also estimated genotype association with P300 latency and amplitude (Table 5). When subjects were grouped according to *GSK3β* genotypes, the P300 latency and amplitude were also associated with genotypes of the three SNPs. The individuals with the T genotype in rs6438552 (C/T + T/T genotype) and rs7633279 (A/T + T/T genotype) have a longer P300 latency than those carrying the C/C ($p = 0.040$) and A/A genotypes ($p = 0.013$). The individuals with the G/G genotype in rs334558 showed a lower amplitude than those carrying the A genotype ($p = 0.007$).

Discussion

Several lines of evidence indicate that *GSK3β* is a good candidate for MDD susceptibility. In this study, we investigated whether common SNPs in the *GSK3β* gene were associated with MDD and its endophenotypes. Significant association with MDD was not shown for alleles and genotypes of a single locus. Analysis of three-locus *GSK3β* haplotypes was subsequently performed, but still no meaningful association was found. We therefore investigated whether the *GSK3β* genotypes and haplotypes were associated with endophenotypes of MDD. Our findings showed that three *GSK3β* SNPs are strongly associated with anxiety symptoms and with the P300 measures, both in genotypes and haplotypes. In particular, the T allele of rs6438552 may be a risk allele, because it was associated not only with the anxiety symptoms of MDD but also with the delayed P300 latency. Moreover, there was a risk trend in the case–control study of rs6438552, although it was not statistically significant ($p = 0.048$, adjusted $p = 0.144$).

In a recent study, Tsai *et al.* (2008) recruited 230 Chinese MDD patients and also found no significant association of MDD with the alleles and genotypes of single locus or four-locus haplotypes (rs334558, rs13321783, rs2319398, and rs6808874). However, three of the four polymorphisms investigated were significantly associated with an antidepressant therapeutic effect ($p = 0.002$–0.011). Compared with the study of Tsai *et al.*, we used a relatively large sample size and had more power to detect associations. The present findings may be more reliable, although they should still be interpreted with caution. As mentioned above, in a previous study, we

Table 3. *GSK3β* Genotype Associations with Factor Scores in HAMA and HAMD-17

	rs6438552					rs7633279					rs334558					
	Mean score					*Mean score*					*Mean score*					
Factors	*CC (154)*	*CT (238)*	*TT (99)*	χ^2	p	*TT (136)*	*TA (247)*	*AA (138)*	χ^2	p	*GG (179)*	*GA (220)*	*AA (85)*	χ^2	p	*Adjusted* p
HAMA-T	17.96	16.98	19.35	11.13	0.004	18.46	16.86	18.99	12.75	0.002	17.78	17.66	19.65	7.083	0.029	0.010
HAMA-1	6.20	5.58	6.95	10.25	0.006	6.34	5.66	6.66	7.017	0.030	6.15	5.92	7.11	6.449	0.040	0.018
HAMA-2	11.76	11.40	12.40	7.121	0.028	12.13	11.20	12.34	14.12	0.001	11.63	11.75	12.54	5.13	0.077	0.003
HAMD-T	22.61	22.02	22.79	3.436	0.179	22.45	21.93	23.02	7.017	0.058	22.74	22.45	23.11	0.804	0.669	0.138
HAMD-1	7.06	6.42	7.39	12.82	0.002	6.85	6.53	7.28	7.203	0.027	7.13	6.81	7.18	2.286	0.319	0.007
HAMD-2	4.01	4.09	3.74	3.436	0.179	3.79	4.16	3.86	4.13	0.127	4.17	4.01	3.71	7.239	0.027	0.060
HAMD-3	7.39	7.58	7.75	2.038	0.361	7.71	7.51	7.42	1.013	0.603	7.45	7.64	7.80	0.834	0.659	0.615
HAMD-4	3.46	3.32	3.49	1.03	0.598	3.53	3.20	3.71	5.838	0.054	3.31	3.46	3.84	9.323	0.047	0.009

$GSK_3\beta$, glycogen synthase kinase-3β; HAMD, Hamilton depression rating scale; HAMA, Hamilton anxiety rating scale; HAMA-T, HAMA total; HAMA-1, HAMA somatic anxious factor; HAMA-2, HAMA mental anxious factor; HAMD-T, HAMD Total; HAMD-1, HAMD-17 anxiety and somatization factor; HAMD-2, HAMD-17 dysgnosia factor; HAMD-3, HAMD-17 blocking factor; HAMD-4, HAMD-17 sleep factor.

Table 4. *GSK3β* Haplotype Associations with Factor Scores in HAMA and HAMD-17

	p (χ^2)									
Factors	*CTG*	*CTA*	*CAG*	*CAA*	*TTG*	*TTA*	*TAG*	χ^2	*Global* p	*Adjusted* p
HAMA-T	0.002 (9.149)	0.386 (0.751)	0.460 (0.547)	0.053 (3.757)	0.283 (1.155)	0.027 (4.891)	0.881 (0.022)	17.15	0.009	0.032
HAMA-1	0.004 (8.201)	0.76 (0.093)	0.402 (0.704)	0.032 (4.575)	0.648 (0.209)	0.141 (2.168)	0.398 (0.713)	14.89	0.021	0.052
HAMA-2	0.014 (6.027)	0.040 (4.217)	0.658 (0.195)	0.225 (1.472)	0.130 (2.289)	0.012 (6.27)	0.499 (0.458)	17.65	0.007	0.133
HAMD-T	0.120 (2.412)	0.671 (0.181)	0.804 (0.061)	0.826 (0.048)	0.467 (0.530)	0.277 (1.182)	0.252 (1.31)	5.211	0.517	0.601
HAMD-1	0.043 (4.087)	0.478 (0.503)	0.959 (0.003)	0.720 (0.128)	0.319 (0.994)	0.628 (0.236)	0.140 (2.181)	7.324	0.292	0.295
HAMD-2	0.058 (3.593)	0.069 (3.303)	0.027 (4.887)	0.020 (5.413)	0.909 (0.013)	0.067 (3.351)	0.292 (1.111)	18.2	0.006	0.170
HAMD-3	0.731 (0.119)	0.134 (2.247)	0.072 (3.245)	0.327 (0.962)	0.260 (1.27)	0.026 (4.986)	0.116 (2.473)	12.72	0.048	0.193
HAMD-4	0.009 (6.807)	0.475 (0.510)	0.368 (0.810)	0.001 (10.98)	0.144 (2.136)	0.044 (4.043)	0.008 (6.948)	29.96	0.000	0.008

Table 5. *GSK3β* Genotype Associations with P300 Latency and Amplitude

Measures	*Genotype*	n	*Mean* ($\overline{X} \pm S$)	*95% CI*	F	p	*Genotype*	n	*Mean* ($\overline{X} \pm S$)	t	p
rs6438552											
LAT	CC	65	296.00 ± 37.28	286.76–305.24	3.558	0.030	CC	65	296.00 ± 37.28	−2.067	0.040
	CT	106	304.17 ± 41.71	296.14–312.20			CT + TT	157	307.72 ± 38.90		
	TT	51	315.10 ± 31.38	306.27–323.92							
	Total	222	304.29 ± 38.72	299.17–309.41							
AMP	CC	65	8.00 ± 3.61	7.10–8.89	1.527	0.220	CC	65	8.00 ± 3.61	−1.662	0.098
	CT	106	9.19 ± 4.74	8.27–10.10			CT + TT	157	8.97 ± 4.68		
	TT	51	8.51 ± 4.54	7.23–9.79							
	Total	222	8.68 ± 4.40	8.10–9.26							
rs7633279											
LAT	TT	65	308.31 ± 32.24	300.32–316.30	3.196	0.043	AA	56	292.36 ± 40.62	2.516	0.013
	TA	113	306.57 ± 40.81	298.96–314.17			TT + TA	178	307.20 ± 37.82		
	AA	56	292.36 ± 40.62	281.48–303.24							
	Total	234	303.65 ± 38.94	298.63–308.67							
AMP	TT	65	9.03 ± 4.07	8.03–1.04	1.617	0.201	AA	56	292.36 ± 40.62	1.798	0.074
	TA	106	9.12 ± 4.88	8.21–10.03			TT + TA	178	307.20 ± 37.82		
	AA	51	7.87 ± 3.88	6.83–8.91							
	Total	222	8.80 ± 4.45	8.229.37							
rs334558											
LAT	TT	65	308.31 ± 32.24	300.32–316.30	3.196	0.043	GG	77	298.34 ± 39.76	−1.523	0.129
	TA	113	306.57 ± 40.81	298.96–314.17			GA + AA	151	306.82 ± 39.78		
	AA	56	292.36 ± 40.62	281.48–303.24							
	Total	234	303.65 ± 38.94	298.63–308.67							
AMP	TT	65	9.03 ± 4.07	8.03–1.04	1.617	0.201	GG	77	7.82 ± 3.78	−2.731	0.007
	TA	106	9.12 ± 4.88	8.21–10.03			GA + AA	151	9.40 ± 4.80		
	AA	51	7.87 ± 3.88	6.83–8.91							
	Total	222	8.80 ± 4.45	8.229.37							

LAT, latency; AMP, amplitude.

observed a weak potential association between a *GSK3β*-associated SNP and MDD, while the combined effects of the *BDNF* and *GSK3β* genes were associated with MDD (Zhang *et al.*, 2010). Furthermore, an interaction between *BDNF* and *GSK3β* may modify the relationship between negative life events and MDD in Chinese individuals (Yang *et al.*, 2010). This was concordant with our present findings, because no strong association between a *GSK3β*-associated SNP and MDD was found. Moreover, we could infer that the *GSK3β* gene may not participate in the etiology of MDD on its own, but that the gene–gene interactions of *GSK3β* and other genes may play an important role.

The clinical symptoms of MDD vary greatly among individuals, so a single genetic association may not fully explain its complexity. Therefore, in future studies, it will be necessary to pay close attention to the endophenotypes of MDD. To our knowledge, this is the first report of evidence that the *GSK3β* gene is associated with the risk of anxiety symptoms in MDD in a Chinese population sample. However, in recent years, many studies have shown that a brain-derived neurotrophic factor (*BDNF*) gene polymorphism (rs6265) is associated with the anxiety symptoms of MDD (Jiang *et al.*, 2005; Lang *et al.*, 2005; Chen *et al.*, 2006; Hunnerkopf *et al.*, 2007; Enoch *et al.*, 2008). BDNF regulates neuronal survival in the central nervous system via the phosphatidylinositol 3-kinase (PI3-kinase)/protein kinase B (PKB) pathway, and activated PKB regulates a number of cell survival-related proteins, such as GSK3β (Huang and Reichardt, 2001). As a component in the final steps of the BDNF pathway, GSK3β may be involved in the same pathophysiological mechanism of MDD as BDNF. This association is consistent with previous findings concerning BDNF and suggests that the whole BDNF system may be associated with the anxiety symptoms in MDD.

The current data and previously published reports support the assertion that *GSK3β* is involved in the development of MDD in a Han Chinese population, but that it is not directly involved in the pathomechanism. This is likely to reflect the complexity of psychiatric disorders, which are thought to be caused by multiple genetic factors, each providing a small effect. Thus, rs6438552 in *GSK3β* may be directly involved in one of the MDD endophenotypes, the anxiety symptom.

To clarify the role of *GSK3β* in disease components or endophenotypes and to understand the function of *GSK3β* in MDD, we further examined the association between *GSK3β* and the P300 waveform of the MDD endophenotype. Generally, quantitative trait association studies (based on phenotypic or endophenotypic subgroups) offer several advantages over case–control studies, one of which is a 4-fold to 8-fold increase in statistical power, thus greatly decreasing the required sample size to achieve sufficient statistical power (Wang *et al.*, 2005; Potkin *et al.*, 2009). Our results of longer P300 latency in MDD patients compared to healthy controls confirm those of previous reports (Bruder *et al.*, 1991; Himani *et al.*, 1999; Karaaslan *et al.*, 2003). Moreover, individuals with the T allele genotype, both in rs6438552 (C/T + T/T) and rs7633279 (A/T + T/T), have a longer P300 latency than those with the C/C or A/A genotype. The latency of P300 has been considered a measure of attentional resource allocation (Coull, 1998), and its prolongation has been discussed as an index of neurodegenerative processes (O'Donnell *et al.*,

1995) affecting the callosal size and the efficiency of interhemispheric transmission (Johnson, 1993). On the other hand, the decrease in the P300 amplitude may also represent a neurocognitive vulnerability marker for the development of depression (Urretavizcaya *et al.*, 2003; Zhang *et al.*, 2007). In our study, the individuals with the G/G genotype in rs334558, which is associated with a decreased P300 amplitude, may have more difficulty in neurocognition.

Our results suggest the possibility that MDD patients carrying the T allele genotype in rs6438552 might have more difficulties with the speed of perception and cognitive processing of auditory stimuli, possibly due to a neurodegenerative process leading to impaired interhemispheric transmission. This is in accordance with the presumed function of *GSK3β* in neural conduction. Overall, these findings suggest that the *GSK3β* SNP, rs6438552, may convey risk for MDD by disrupting neural connectivity, possibly white-matter integrity, leading to slower cognitive processing. This is speculative at present and needs to be confirmed by further neurobiological experiments.

In summary, *GSK3β* may be a susceptibility factor for MDD, and a significant association locus may be rs6438552; it was associated with the anxiety symptoms of MDD, and its genotypes were related to P300 latency. Further study of this association is needed to replicate this finding in ethnically different samples and in larger sample sizes.

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