



The Interaction of TPH2 and 5-HT2A Polymorphisms on Major Depressive Disorder Susceptibility in a Chinese Han Population: A Case-Control Study

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Purpose: TPH2 and 5-HT2A appear to play vital roles in the homeostatic regulation of serotonin levels in the brain, their genetic variations may lead to impaired homeostatic regulation of serotonin resulting in abnormal levels of serotonin in the brain, thus predisposing individuals to MDD. However, research studies have yet to confirm which gene-gene interaction effect between TPH2 and 5-HT2A polymorphisms results in increased susceptibility to MDD.

Methods: A total of 565 participants, consisting of 278 MDD patients and 287 healthy controls from the Chinese Han population, were recruited for the present study. Six single nucleotide polymorphisms (SNPs) of TPH2/5-HT2A were selected to assess their interaction by use of a generalized multifactor dimensionality reduction method.

Results: A-allele carriers of rs11178997 and rs120074175 were more likely to suffer from MDD than T-allele carriers of rs11178997, or G-allele carriers of rs120074175. The interaction between TPH2 (rs120074175, rs11178997) and 5-HT2A (rs7997012) was considered as the best multi-locus model upon the MDD susceptibility.

Conclusions: Our data identified an important effect of TPH2 genetic variants (rs11178997 and rs120074175) upon the risk of MDD, and suggested that the interaction of TPH2/5-HT2A polymorphism variants confer a greater susceptibility to MDD in Chinese Han population.

Keywords: major depressive disorder, TPH2, 5-HT2A, gene-gene interaction, polymorphisms

INTRODUCTION

Major depressive disorder (MDD) is a frequently occurring mental disorder with a strikingly high rate of relapses, and has become the leading cause of years lived with disability worldwide according to data compiled by the World Health Organization. Patients suffering with MDD struggle with severe role impairment (1) and most suffer from suicidal ideation, suicidal intent or suicidal attempts (2). Genetic factors have been found to be an important contributor to MDD (3), and inheritance can increase the risk of developing MDD by 40–70% (4).

Previous studies have provided strong evidence that serotonergic transmission is altered in MDD (5), and have identified polymorphism variants that are associated with deficits in the transmission of serotonin, which are believed to be involved in the pathophysiology of MDD (6). Polymorphism of the tryptophan hydroxylase-2 (TPH2) gene is one such example. TPH2 is the rate-limiting enzyme for the synthesis of serotonin in the brain (7). Functional polymorphisms of the TPH2 gene have been found to affect serotonin synthesis capacity and serotonin neurotransmission (8, 9), and several TPH2 gene polymorphisms have been confirmed to be associated with MDD. The first report of the association between TPH2 and MDD was published by Zill et al. (10). Subsequently, rs120074175 (1463G/A), a functional polymorphism variant of TPH2, which was shown to attenuate the synthesis of serotonin by ~80%, was also associated with MDD (8). A meta-analysis also showed that TPH2 rs4570625 (-703G/T) has strong epidemiological credibility for an involvement with MDD (11). In addition, the association of MDD and a haplotype block including rs4570625 and rs11178997 (473T/A) was confirmed (12). Collectively, these findings strongly indicated that TPH2 polymorphisms may play an important role in the development of MDD. In spite of the fact that TPH2 polymorphisms could result in the attenuation of serotonin synthesis, the genetic variation of TPH2 in isolation is unlikely to lead to a high risk of MDD (13, 14).

Interestingly, one research study suggested that the risk imposed by TPH2 may be modulated by the serotonin 2A receptor (5-HT2A) (15), an important regulator of the serotonin signaling (16). Multiple animal studies have confirmed that 5-HT2A binding is associated with serotonin levels (17, 18). In addition, changes in 5-HT2A binding levels have also been identified in different regions of the brain in depressed individuals (19, 20). Some genetic studies have also shown the association of 5-HT2A polymorphisms and MDD; for example, rs6311, rs6313, rs7997012 (21–23). 5-HT2A variants were identified as showing specific associations with MDD.

MDD is a polygenic disease; in other words, there are multiple and partially overlapping sets of MDD susceptibility genes which interact with each other, thus predisposing individuals to the development of MDD (24). Over recent years, genetic studies have identified numerous genetic variants implicated in MDD. However, results arising from the analyses of single markers have often been inconsistent, and for many candidate SNPs, results could not be replicated (25). Gene–gene interaction analysis is a promising method that can reveal susceptibility genes and their interaction, and has been confirmed as a particularly important method for revealing the molecular mechanisms of complex human diseases, such as MDD (26).

Based on the earlier observations, TPH2 and 5-HT2A appear to play vital roles in the homeostatic regulation of serotonin levels in the brain. Furthermore, TPH2 and 5-HT2A genetic variations may lead to impaired homeostatic regulation of serotonin resulting in abnormal levels of serotonin in the brain for long periods of time, thus predisposing individuals to MDD. However, research studies have yet to confirm what gene–gene interaction effect between TPH2 and 5-HT2A results in increased susceptibility to MDD.

The purpose of the present study was thus to investigate the specific interaction between the TPH2 and 5-HT2A genes and whether this mechanism contributes to susceptibility for MDD. SNPs selected in this study are not only associated with MDD but also associated with its gene expression (8, 27–29). Resultant data will contribute to a better understanding of MDD and genetic predisposition, and will assist in further interpreting the role of serotonin in susceptibility for MDD.

MATERIALS AND METHODS

Subjects

In total, 278 MDD patients, and 287 healthy controls, were recruited for the present study. All subjects were from the Chinese Han population, and were living in the same geographical area in the north of China. MDD patients were diagnosed according to the criteria for MDD described in the Fourth Edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). The 24-item Hamilton Rating Scale for Depression (HAM-D) was used to evaluate all patients. Our inclusion criteria required that subjects had a HAM-D score of 21 or higher, and had not received antidepressant treatment for 1 month preceding assessment. Our exclusion criteria included: (1) a history of brain organic mental disorders or other mental disorders; (2) a family history of genetic disorders; (3) individuals with mental retardation or dementia; and (4) individuals recently receiving blood transfusion treatment. All psychiatrists involved in patient diagnosis were specifically trained using the Structured Clinical Interview for DSM-IV disorders (SCID-I). Each patient was interviewed independently by at least two psychiatrists. Written informed consent was obtained from all participants and the study was approved by the Research Ethics Committee of Harbin Medical University, China.

DNA Isolation and Genotyping

Venous blood was taken from all participants and DNA isolated from EDTA-anticoagulated blood samples using the AxyPrep™ Blood Genomic DNA Minprep Kit (Axygen, Union City, CA, USA). Primer Premier 5.0 software was used to design the primers for polymerase chain reaction (PCR) amplification and final primers were evaluated using NCBI-BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Primer sequences are given in

Table 1.

DNA samples (TPH2: rs4570625, rs120074175, rs11178997; 5-HT2A: rs7997012, rs6311, rs6313) were genotyped using PCR TaqMan assays and read on an ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). PCR amplicons were sequenced and purified using an ABI 3730 DNA Sequencer.

Statistical Analysis

The Hardy–Weinberg equilibrium test was carried out for all SNP loci and an independent-samples *t*-test was used to estimate differences in age distribution between case and control groups. The chi-square test was used to investigate differences in categorical variables, such as gender or differences in the distribution of genotype. A $p < 0.05$ (two-tailed) was considered

TABLE 1 | The primers sequence for SNPs of TPH2 and 5-HT2A.

Gene	SNP ID	Polymorphisms	Location	Primer sequence (5' → 3')
TPH2	rs4570625	G/T	5'Regulatory region	F: 5'-AGTAGAGAGAAAAACCACAAGAGTATT-3' R: 5'-CATTGCCTCAAGCATTATCA-3'
	rs11178997	A/T	5'Regulatory region	F: 5'-TCCTTTTATCTATCCCTCGTACCAA-3' R: 5'-GGTCCTGCACCACATTTTCA-3'
	rs120074175	A/G	Coding region	F: 5'-TGAGGCAATGGATATCTTGATTACC-3' R: 5'-GCACTGCTGAATGCTTAAACCA-3'
5-HT2A	rs7997012	A/G	Intron region	F: 5'-CTAACCTCATGTCACCTCACA-3' R: 5'-ATGTTGCAAACAATGGGCCAG-3'
	rs6311	T/C	5'Regulatory region	F: 5'-ATGGCCTTTTGTGCAGATTCC-3' R: 5'-AGAGTTATCACCACAGACGTG-3'
	rs6313	T/C	Intron region	F: 5'-GCTACAAGTTCTGGCTTAGAC-3' R: 5'-TGAAGTAAGGAGAGACACGAC-3'

as statistically significant. SPSS 19.0 software was used for all statistical analysis.

Haplotype analysis was performed using Haploview software (version 4.2) and analyzed differences in haplotype frequencies between case and control groups. Gene-gene interactions were tested using generalized multifactor dimensionality reduction (GMDR) software (version v0.7) and default parameters. In the configuration file, 10-fold cross-validation (CV) was defined and threshold ratio set at 1.0. The analysis was performed ten times using ten different random number seeds. The results were averaged to avoid spurious outcomes due to chance division of the data. Age and gender were regarded as covariates. The best gene-gene interaction model was selected based upon the values arising from CV consistency and accuracy testing. Interaction graphs and dendrograms were used to help interpret a multi-locus model of disease susceptibility.

RESULTS

Participant characteristics are shown in **Table 2**. In total, our study incorporated 565 participants, including 278 MDD patients and 287 healthy controls. There were 74 males (26.6%) and 204 females (73.4%) in the MDD group, and 83 males (28.9%) and 204 females (71.1%) in the control group. The mean age of MDD cases and controls was 42.79 years and 41.90 years, respectively. There was no statistically significant difference between cases and controls in terms of either gender or age distribution (gender: $p = 0.378$, age: $p = 0.542$). The HAMD score of the MDD group ranged from 21 to 58 points.

The genotype distributions of TPH2 and 5-HT2A polymorphisms conformed to the Hardy-Weinberg equilibrium and the results of our single marker analysis are presented in **Table 3**. Significant differences in genotypic and allelic distributions between cases and controls were confirmed at locus rs11178997 ($p = 0.000$ for both genotype and allele) and rs120074175 ($p = 0.000$ for both genotype and allele) of the TPH2 gene; after Bonferroni correction, these differences remained significant. Odds ratio analysis showed that A-allele carriers of rs11178997 and rs120074175 were more likely to suffer from MDD than T-allele carriers of rs11178997, or

TABLE 2 | The characteristics of participants.

Variables	MDD ($n = 278$)	Control ($n = 287$)	p -value
Sex(males/females)	74/204	83/204	0.542
Age(mean \pm SD)	42.79 \pm 12.21	41.90 \pm 11.93	0.378
HAMD score(mean \pm SD)	30.38 \pm 6.58	5.93 \pm 5.76	

G-allele carriers of rs120074175 (rs11178997: OR = 1.637, 95%CI: 1.290–2.078; rs120074175: OR = 7.909, 95%CI: 5.987–10.447). Differences in the distribution of rs6311 and rs6313 of the 5-HT2A gene were observed only in terms of genotype (rs6311: $p = 0.029$; rs6313: $p = 0.049$), but after Bonferroni correction, these differences were not significant. We did not identify an association between MDD and the other SNPs tested (TPH2:rs4570625, 5-HT2A: rs7997012).

Haplotype frequencies in case and control groups were estimated by Haploview software (version 4.2) and our results are shown in **Table 4**. Strong Linkage Disequilibrium (LD) was observed between the 5-HT2A rs6311 and rs6313 ($D' = 1.0$, $r^2 = 0.979$), but the 5-HT2A rs6311-rs6313 haplotypes were not associated with MDD.

GMDR was used to detect the interaction effects of six TPH2/5-HT2A SNPs in MDD susceptibility. The results of gene-gene interaction models, using age and gender as covariates, are given in **Table 5**. In total, 1,000 replications were used to determine the empirical p -value of prediction accuracy using the permutation test. Significant 2-locus to 6-locus gene-gene interaction models were observed ($p < 0.001$). The interaction between TPH2 (rs120074175, rs11178997) and 5-HT2A (rs7997012) showed a CV consistency of 10/10 and a testing accuracy of 81.86%, and this was therefore considered as the best multi-locus model. The testing accuracy of all multi-locus models was higher than the accuracy of the best single locus model. This result suggested there was an interaction effect of TPH2 and 5-HT2A upon MDD susceptibility.

An interaction graph and an interaction dendrogram were created in order to interpret the relationship between the six SNPs tested. As shown in **Figure 1**, we found that rs11178997 and rs120074175 both had strong independent

TABLE 3 | Genotypic and allelic distributions of TPH2 and 5-HT2A polymorphisms of MDD patients and controls.

Gene	SNP	Sample	Genotype (%)			p	Allele (%)		p	Odds ratio (95%CI)
			GG	TG	TT		G	T		
TPH2	rs4570625	Case	73 (26.3)	131 (47.1)	74 (26.6)	0.129	277 (49.8)	279 (50.2)	0.079	1.233 (0.976–1.558)
		Control	55 (19.2)	146 (50.9)	86 (29.9)		256 (44.6)	318 (55.4)		
	rs11178997	Case	93 (33.5)	170 (61.1)	15 (5.4)	0.000*	356 (64.0)	200 (36.0)	0.000*	1.637 (1.290–2.078)
		Control	135 (47.0)	29 (10.1)	123 (42.9)		299 (52.1)	275 (47.9)		
	rs120074175	Case	207 (74.5)	48 (17.2)	23 (8.3)	0.000*	462 (83.1)	94 (16.9)	0.000*	7.909 (5.987–10.447)
		Control	108 (37.6)	4 (1.4)	175 (61.0)		220 (38.3)	354 (61.7)		
5-HT2A	rs7997012	Case	22 (7.9)	106 (38.1)	150 (54.0)	0.247	150 (27.0)	406 (73.0)	0.257	1.167 (0.893–1.526)
		Control	13 (4.5)	112 (39.0)	162 (56.5)		138 (24.0)	436 (76.0)		
	rs6311	Case	82 (29.5)	128 (46.0)	68 (24.5)	0.029	292 (52.5)	264 (47.5)	0.662	1.053 (0.834–1.330)
		Control	65 (22.6)	164 (57.2)	58 (20.2)		294 (51.2)	280 (48.8)		
	rs6313	Case	79 (28.4)	129 (46.4)	70 (25.2)	0.049	287 (51.6)	269 (48.4)	0.847	1.023 (0.810–1.292)
		Control	65 (22.6)	163 (56.8)	59 (20.6)		293 (51.0)	281 (49.0)		

Bolded indicates statistically significant ($p < 0.05$).

* indicates the difference was still significant after Bonferroni correction.

TABLE 4 | Haplotype-based association analysis results.

Gene	Haplotype	Case ratios	Control ratios	χ^2	p
5-HT2A	TT	0.516	0.510	0.037	0.847
	CC	0.475	0.488	0.191	0.662

effects (rs11178997, 27.41%; rs120074175, 26.51%). The strongest redundant interaction occurred between rs120074175 and rs11178997 in the TPH2 gene with an entropy of –15.00%. Rs120074175 also had a redundant interaction with rs6311 and rs6313, while rs11178997 had a redundant interaction with the other four SNPs. The interactions between other SNPs were relatively independent.

DISCUSSION

Serotonin has been proven to be involved in the pathogenesis of MDD (6), although the precise role of the serotonin system in MDD is still under debate (5, 30). TPH2 and 5-HT2A are key regulators of central serotonin transmission, and are considered as potential risk genes for MDD. Aberrant genes are known to predispose subjects to depression (31), although the validation of MDD susceptibility genes, including TPH2 and 5-HT2A, its interaction is unclear leading to the need for further investigations of the potential gene-gene interaction effects played by the key regulators of serotonin system in MDD susceptibility.

In the present study, we attempted to verify the relationship of TPH2 variants (rs4570625, rs120074175, and rs11178997) and 5-HT2A variants (rs7997012, rs6311, and rs6313) with MDD by evaluating their single and interaction effects upon MDD susceptibility in a Chinese Han population. The results of the present study not only suggested the important effect of the TPH2 gene upon the risk of MDD, but also provided preliminary evidence that the interaction between TPH2 and 5-HT2A polymorphism variants may influence MDD susceptibility. To the best of our knowledge, this is the first report to investigate TPH2/5-HT2A interaction effects upon the risk of MDD. In addition, our results suggested that the impaired homeostatic regulation of serotonin may predispose individuals to MDD.

In single-locus association analysis, we observed significant differences in genotypic and allelic distribution with the rs11178997 and rs120074175 TPH2 variants. The number of A-allele individuals for rs11178997/rs120074175 was significantly greater in the MDD group than in the control group, which revealed that A-allele carriers of rs11178997 and rs120074175 were more likely to suffer from MDD with a 1.637-fold and a 7.909-fold increased risk, respectively, compared to non-carriers. This result suggested that the TPH2 gene might play a major role in MDD, particularly the rs11178997 TPH2 polymorphism, which had the most independent effect. Previous studies have found that both the rs11178997 (27) and the rs120074175 (8) polymorphism affected the transcriptional activity of TPH2, thereby influencing serotonin production. Papers by Van Den Bogaert et al. and Cichonet al. have, respectively reported an association between rs11178997 and unipolar or bipolar affective disorder (32, 33), while Zhang et al.

TABLE 5 | The best gene-gene interaction models obtained by GMDR.

Locus no.	Best model	Testing accuracy (%)	CV consistency	p value
1	TPH2(rs120074175)	74.91	9/10	<0.001
2	TPH2(rs120074175,rs11178997)	81.18	10/10	<0.001
3	TPH2(rs120074175,rs11178997), 5-HT2A(rs7997012)	81.86	10/10	<0.001
4	TPH2(rs4570625,rs120074175,rs11178997), 5-HT2A(rs6311)	77.10	5/10	<0.001
5	TPH2(rs4570625,rs120074175,rs11178997), 5-HT2A(rs7997012, rs6311)	77.93	10/10	<0.001
6	TPH2(rs4570625,rs120074175,rs11178997), 5-HT2A(rs7997012, rs6311,rs6313)	77.90	10/10	<0.001

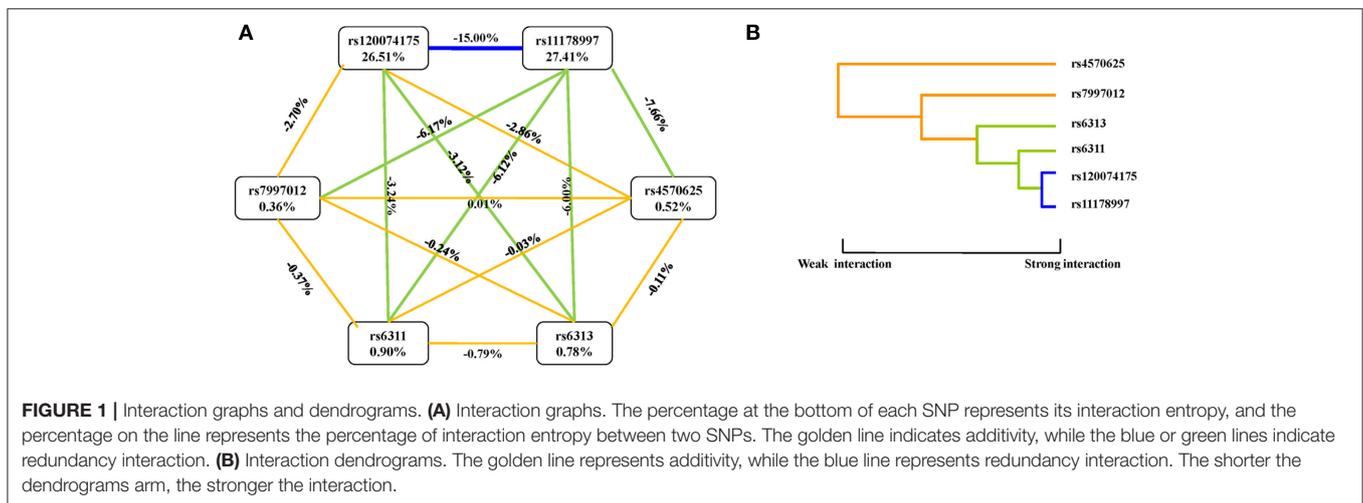


FIGURE 1 | Interaction graphs and dendrograms. **(A)** Interaction graphs. The percentage at the bottom of each SNP represents its interaction entropy, and the percentage on the line represents the percentage of interaction entropy between two SNPs. The golden line indicates additivity, while the blue or green lines indicate redundancy interaction. **(B)** Interaction dendrograms. The golden line represents additivity, while the blue line represents redundancy interaction. The shorter the dendrograms arm, the stronger the interaction.

reported an association of rs120074175 with unipolar major depression (8).

An earlier review suggested that the pathophysiology of depression might result, at least in part, from the direct dysregulation of brain 5-HT2A neurotransmission or indirectly from the dysfunction of other neurotransmitter systems that are under the control of 5-HT2A (34). Some existing studies have attempted to explore the association between the 5-HT2A SNPs and MDD (35–37), but few of these have met with success (38, 39). After Bonferroni correction, our current data showed no association of three 5-HT2A SNPs (rs7997012, rs6311, rs6313) with MDD at either the single-locus or haplotypic level. The inconsistency in our data may have arisen due to sample sizes, different ethnicities, and different definitions of disease; however, the negative results of the single marker analyses do not preclude the fact that the 5-HT2A gene variation has a minor effect upon MDD susceptibility (40). A recent study found that 5-HT2A mRNA levels in the peripheral blood mononuclear cells of MDD patients may have been associated with the severity of depression and the duration of illness in an Iranian population (41).

GMDR analysis can improve the predictive power of genetic association with MDD (42), and has been used to investigate gene-gene interaction in other biological pathways. GMDR is a non-parametric and genetic model for detecting and

characterizing non-linear interactions among discrete genetic attributes that are sensitive to the detection of high-order interactions (43). Our GMDR results indicated potential gene-gene interactions between TPH2 and 5-HT2A with significant 2-locus to 6-locus interaction models, which confer a greater susceptibility to MDD. The best TPH2/5-HT2A interaction model was a 3-locus model (TPH2: rs120074175, rs11178997; 5-HT2A: rs7997012) using age and gender as covariates, which had a higher testing accuracy than the multi-locus interaction model of the single TPH2 gene. Identifying interaction between TPH2 and 5-HT2A is likely to contribute to a better understanding of genetic predisposition to MDD. In addition, we also found that both rs11178997 and rs120074175 have greater independent effects in terms of TPH2/5-HT2A interaction, which was far stronger than some other MDD susceptibility genes (44), and there was a strong redundant interaction between these variants, suggesting that TPH2 polymorphism may play vital roles in interaction.

Furthermore, previous research has suggested that the biological interaction between TPH2 and 5-HT2A is involved in the development of MDD. An animal study showed, using a TPH2 decrease-of-function model, gave rise to increased 5-HT2A binding in some brain regions (18). Another animal study observed that long-term activation of the 5-HT2A

receptor induced an increase in TPH2 gene expression, TPH2 activity, and serotonin levels (15). These findings suggest that concurrent TPH2 and 5-HT2A variations may develop into long-term abnormal serotonin levels, thus predisposing individuals to MDD.

In interpreting the results of the current study, it is important to consider the following limitations. Firstly, in the current study, the geographic region and ethnic origin were strictly controlled to reduce the potential effects of population stratification; consequently, our data need to be further verified in other ethnicities and geographical regions. Secondly, our sample size was relatively small with only 565 subjects (case: 278, control: 287), although our study still possessed a *post-hoc* power of 0.99 to detect a moderate (0.5) effect size at the 0.05 significance level (two-tailed). Thirdly, SNPs were chosen in this study with more evidence that they are associated with MDD. The interaction of other TPH2/5-HT2A SNPs, which are potentially associated with MDD, was not investigated in the present study. It is therefore necessary to perform further investigations to better understand the role of interaction between TPH2 and 5-HT2A polymorphisms in MDD susceptibility.

CONCLUSION

For the first time, this study reports the interaction of serotonin-related genes, TPH2 and 5-HT2A, upon MDD susceptibility in a Chinese Han population. Our data identified an important effect of TPH2 gene variants (rs11178997 and rs120074175) upon the risk of MDD, and suggested that the interaction

of TPH2/5-HT2A polymorphism variants confer a greater susceptibility to MDD. The present study also provided preliminary evidence that the interaction of TPH2 and 5-HT2A polymorphism variants may influence the susceptibility to MDD in a Chinese Han population, and suggested that impaired serotonin homeostatic regulation may be a risk factor for MDD.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Ethics Committee of Harbin Medical University with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of Harbin Medical University.

AUTHOR CONTRIBUTIONS

JY and YY designed the study. XQ, XY, and ZQ participated in the acquisition of data, which were analyzed by XuZ and JM. JY and XuZ wrote the article. BB, XiZ, and DC critically reviewed it. All authors assisted in the revision process, and gave approval for the final version of the article to be published.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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