The Relationship Between Single Nucleotide Polymorphisms in 5-HT_{2A} Signal Transduction-Related Genes and the Response Efficacy to Selective Serotonin Reuptake Inhibitor Treatments in Chinese Patients with Major Depressive Disorder

Heng-Fen Li,^{1,*} Xue Yu,^{2,3,*} Kun Yang,^{2,3,†} Cha-Ye He,⁴ Shao-Jie Kou,⁵
Su-Xia Cao,¹ and Guang-Rong Xie^{6,†}

Objective: To explore the possible relationship between six single nucleotide polymorphisms (SNPs) (rs6311 and rs6305 of 5-HT_{2A}, rs5443 of *Gβ3*, rs2230739 of *ACDY9*, rs1549870 of *PDE1A* and rs255163 of *CREB1*, which are all related with 5-HT_{2A} the signal transduction pathway) and the response efficacy to selective serotonin reuptake inhibitor (SSRI) treatments in major depressive disorder (MDD) Chinese. **Methods:** This study included 194 depressed patients to investigate the influence of 6 polymorphisms in 5-HT_{2A} signal transduction-related genes on the efficacy of SSRIs assessed over 1 year. The efficacies of SSRIs on 194 MDD patients were evaluated in an 8-week open-trial study. Over 1 year, a follow-up study was completed for 174 of them to observe the long-term efficacy of SSRIs. The optimal-scaling regression analysis was used for testing the relationship between the different genotypes of five SNPs and the efficacy in MDD. **Results:** It showed that the patients with rs5443TT and rs2230739GG have a relatively good efficacy in response to short-term SSRIs. We also found that good efficacy appeared in depressed patients with rs2230739GG in response to long-term SSRIs. **Conclusions:** It suggested that different genotypes of rs5443 and rs2230739 might influence the signal transduction pathways of second message and affect therapeutic efficacy.

Introduction

DEPRESSIVE DISORDER is a common psychiatric disease, afflicting 3%–5% of the population worldwide. The etiologic foundations remain unknown, although decades of research on neurobiochemistry, neuropathology, and psychopharmacology have made great progress. Family, twin, and adoption studies on depression have provided evidence for the involvement of genetic factors, suggesting that heritable factors play an important role in the etiology of depression (Wender *et al.*, 1986; Sullivan *et al.*, 2000; Levinson, 2006; Lohoff, 2010).

The serotonin (5-HT) system plays an important role in the central nervous system, regulating mood, emotion, and stress.

Accumulating evidence from recent studies implicated 5-HT hypo-activity involved in the pathogenesis of depression and its normalization as a necessary predecessor of clinical response to antidepressant drugs (Bluer, 2001; Neumeister, 2003). Especially, the application of selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine and paroxetine has obtained a good effect in depression. Antidepressants quickly change the concentration of 5-HT neurotransmitter in the synapse region. However, the onset of their therapeutic action requires 2–4 weeks (Chen and Skolnick, 2007), and a first course of therapy provides symptom relief to only 60%–65% of patients. Due to the lag period between the initiation of antidepressant treatment and the onset of clinical effects, researchers inferred that the presynaptic and postsynaptic

¹Department of Psychiatry, the First Affiliated Hospital, Zhengzhou University, Zhengzhou, P.R. China.

²Basic Medical College, Tianjin Medical University, Tianjin, P.R. China.

³Department of Psychiatry, Tianjin Anding Hospital and Tianjin Mental Health Institute, Tianjin, P.R. China.

⁴Henan Institute of Science and Technology, Xinxiang, P.R. China.

⁵Department of Psychiatry, the Fifth Hospital of Kaifeng, Kaifeng, P.R. China.

⁶Mental Health Institute of the Second Xiangya Hospital, Central South University, Changsha, P.R. China.

*These two authors contributed equally to this work and should be considered co-first authors.

†These two authors contributed equally to this work and should be considered co-corresponding authors.

adaptive process was possibly involved in depression. 5-HT receptor gene polymorphisms have been the most studied candidates in explaining the onset of major depression and the treatment response to antidepressants. However, several other potential markers, including G β 3, adenylyl cyclase (ACDY9), phosphodiesterase (PDE1A), and CREB1, which are related to the 5-HT_{2A} signal transduction pathway investigated in this study, may also contribute.

Recent pharmacogenetic investigations have focused on the genes in the serotonergic pathway, including G protein and effective apparatus in its downstream, considered as the therapeutic target in depression (Cheng *et al.*, 2007). The genes of 5-HT_{2A} (McMahon *et al.*, 2006), ACDY9 (Mato *et al.*, 2010), G β 3 (Lee *et al.*, 2004; Lin *et al.*, 2009), and PDEs (Gould and Manji, 2002; Hines and Tabakoff, 2005; Hines *et al.*, 2006) are strongly associated with treatment response to antidepressants in major depressive disorder (MDD). Therefore, such genes are considered candidates for susceptibility to depression.

In this article, we will investigate the relationship between six single nucleotide polymorphisms (SNPs) of five genes (5-HT_{2A}, G β 3, AC₉, PDE_{1A}, and CREB₁) and the efficacy in MDD by the optimal-scaling regression analysis.

Methods

Subjects

A total of 194 subjects with recurrent major depressive episodes were recruited, who were thoroughly informed about the nature and aims of the examinations and were included in the study only after giving written informed consent to a protocol approved by the local ethics committee of the Second Xiangya Hospital in Central South University. The patients were especially informed that the rejection of participation in the study would not affect future treatment. Complete physical, neurological, and laboratory examinations showed that all the subjects were free of significant physical or neurological illnesses. All patients were Chinese Han.

The MDD subjects were diagnosed after a semi-structured interview by two experienced psychiatrists according to DSM-IV criteria, and had a 17-item Hamilton Depression Rating Scale total score ≥ 21 . All of them were outpatients and inpatients of the Mental Hospital of Henan Province and Mental Health Institute of the Second Xiangya Hospital from March 2005 to May 2007. Patients who got the same diagnosis from the two psychiatrists were included in the study. Patients with schizophrenia or other psychoses, organic mental disorders, substance-related disorder, anxiety disorder, or any other primary psychiatric disorders were excluded.

All patients were treated daily with the SSRI antidepressants such as citalopram (20 mg), paroxetine (20 mg), and

sertraline (50 mg) for at least 8 weeks. The efficacy of SSRIs on 194 patients with MDD was evaluated in an open-trial study. A 1-year follow-up study was performed for 174 of the patients to observe the long-term efficacy of SSRIs.

Selecting candidate genes

The candidate genes were chosen from the published papers and fulfilled the following criteria: (1) The gene expression of related products was involved in the onset or correlated with the treatment of mood disorder; and (2) genes were possible candidates for mood disorder.

Selecting of SNPs

The SNPs were chosen according to the criteria as follows: (1) They were located around the candidate genes and potentially associated with behavior and/or mental disorders by the published papers; and (2) the mRNA expression of the genes were potentially influenced by them. Six SNPs were selected from the database of SNPs of the National Center for Biotechnology Information and the National Human Genome Research Institute (See Table 1).

Genotyping methods

DNA was extracted from EDTA-containing venous-blood samples by using QIAmap blood kits (Tiagen) and then frozen at -70°C until analysis at the Central Lab of Second Affiliated Hospital in Xinxiang Medical College. SNPs were examined by polymerase chain reaction (PCR)-ligase detection reaction (LDR) as reported by Girigoswami *et al.* (2008) at the Laboratory of Molecular Biology of Donghua University in Shanghai. Briefly, the primers and probes were designed (Yihe) for detection of mutant genes (See Table 2).

PCR amplifications were carried out in 20 μL buffer containing 10 mmol Tris-HCl (pH 8.0), 50 mM KCl, 2 mM MgCl₂, 200 μM dNTP, 300 nM each primer, 1.5 units of Gold DNA polymerase (Qiagen), and 5 μM of DNA template. The amplification was performed on a PE 9600 thermal cycler (ABI PRISM) by heating to 95°C for 15 min and cycling for 35 cycles at 94°C for 30 s, 59°C for 1 min, 72°C for 1 min, and 72°C for 7 min for a final extension.

LDR reactions were carried out in 20 μL in a buffer containing 20 mM Tris-HCl (pH 7.6), 10 mM MgCl₂, 100 mM KCl, 10 mM DTT, 1 mM EDTA, 1 mM NAD⁺, 12.5 nM of each probe, 3 μL PCR product, and 0.1 mM DNA ligase. The LDR was performed on a PE 9600 thermal cycler by incubating at 95°C for 2 min and cycling for 35 cycles at 95°C for 30 s and 65°C for 4 min. The reaction was stopped by adding 0.5 μL of 0.5 mM EDTA. 1 μL of the LDR products were mixed with an equal volume of ABI GS-500 ROX (fluorescent labeling

TABLE 1. THE INFORMATION OF FIVE SINGLE NUCLEOTIDE POLYMORPHISMS

Gene	dbSNPID	Region	Allele	Function
5-HT _{2A}	rs6311	13q14	C/T	Promoter region
5-HT _{2A}	rs6305	13q14	C/T	Promoter region
G β 3	rs5443	12p13	C/T	Exon10 synonymous
ACDY9	rs2230739	1p36.2—1p36.3	A/G	Exon 6 synonymous
PDE1A	rs1549870	2q31—2q32	A/G	Intron 3
CREB1	rs2551638	2q33—2q35	A/G	promoter region

TABLE 2. PRIMERS AND PROBES USED IN THE POLYMERASE CHAIN REACTION–LIGASE DETECTION REACTION PROTOCOL

Primers and probes	Sequences	Length of products (bp)
PCR primers		
rs6311-up	AAATAAGGCTAGAAAACAGTATGTCC	88
rs6311-low	CCACTCTGGACACAAACACTG	
rs6305-up	CTGGACGTGCTCTTCTCCAC	320
rs6305-low	TTCTTTCCTGAAGCGAATCTG	
rs5443-up	GTCAGGTGGGAGGCAGAG	283
rs5443-low	TCATGGAGTCCCAGACATTG	
rs2551638-up	TGAGGAAAGAAAGCGACACA	238
rs2551638-low	GGGTTTTCTTTCCGAGACT	
rs2230739-up	GGGGTAGTAGAGGGAGACAGC	206
rs2230739-low	AGCTGAAGGTGGGACTAGCA	
rs1549870-up	AGGTCCCTGTGTGGAAGTGA	410
rs1549870-low	TCCCAGGAGTCACTTTACCC	
Probe		
rs6311_MODIFY	P-GACACTCACAGCACTCCGAGGACATTTTTTTTTTTTTTTTTT-FAM	
rs6311_C	TTTTTTTTTTTTTTTTTCTGTGGCTTTGGATGGAAGTGCCG	82
rs6311_T	TTTTTTTTTTTTTTTTTCTGTGTGGCTTTGGATGGAAGTGCCA	84
rs5443_MODIFY	P-GACGTGATGCCGAGATGATGCTCTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs5443_C	TTTTTTTTTTTTTTTTTTTTTTTTTTTTCGCGCCACTGAGGGAGAAGGCCACG	102
rs5443_T	TTTTTTTTTTTTTTTTTTTTTTTTTTTTCGCGCCACTGAGGGAGAAGGCCACA	104
rs2551638_MODIFY	P-GCGGGGCTCTGCCCGGACCATGGCTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs2551638_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTGCTGGCCCCGATACTGTGGCACCTT	98
rs2551638_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTGCTGGCCCCGATACTGTGGCACCTC	100
rs2230739_MODIFY	P-ATGACCTGTGTGGAGCAGGAGTGGATTTTTTTTTTTTTTTTTTTTTT-FAM	
rs2230739_A	TTTTTTTTTTTTTTTTTTTTTTTAAACGCTCTTCACGGGGGAGTTCCTT	94
rs2230739_G	TTTTTTTTTTTTTTTTTTTTTTTAAACGCTCTTCACGGGGGAGTTCCTC	96
rs1549870_MODIFY	P-TCCTTTATCTCATTTCATCCCATTTTTTTTTTTTTTTTTTTTTTTT-FAM	
rs1549870_A	TTTTTTTTTTTTTTTTTTTTTTTTTTTATTTTCTCTGTCTACTCTCCATT	114
rs1549870_G	TTTTTTTTTTTTTTTTTTTTTTTTTTTATTTTCTCTGTCTACTCTCCATC	116

PCR, polymerase chain reaction.

molecular weight standard) and deionization formamide. The mixture was denatured at 94°C for 2 min, chilled rapidly on ice before loading on a 5% polyacrylamide and 5 M urea gel, and electrophoresed for 2.5 h at 3000V (377 DNA Prism Sequencer; Applied Biosystems). Genemapper software was used for the analysis ligation products.

Statistical methods

The relationships between genotypes of six SNPs and efficacy were measured by the optimal-scaling regression analysis.

Results

The clinical features in major depressive disorder

The demographic data and main characteristics of the 194 study subjects were listed in Table 3. The results showed that 57.7% patients had a good response to 8-week SSRIs (See Table 3).

Relationship between five SNPs and the efficacy of SSRIs

Except that all the genotypes of rs2551638 in the CREB1 gene were GG, the genotypes of other SNPs showed polymorphisms. For the efficacy response to short-term SSRIs, we classified HRSD subtraction mark rate $\geq 50\%$ as effective, others as noneffective, and analyzed the relationships between the different genotypes of five SNPs and the efficacy by

the optimal-scaling regression analysis suited for categorical variables in MDD. Table 4 showed standard partial regression coefficients and significance test results. We found that rs5443 was related with the efficacy of response to short-term SSRI treatments ($\text{Beta} = 0.181$, $p = 0.03$). We also found that there was a correlation relationship between rs2230739 and the efficacy of response to short-term SSRIs ($\text{Beta} = 0.283$, $p = 0.000$), although the coefficient was thought to be small. By analyzing

TABLE 3. DEMOGRAPHIC DATA OF THE STUDY SUBJECTS

	Depressed patients (n = 194)
Sex (male/female)	77/117
Age (years)	38.12 $\pm$ 2.36
BMI (kg/m ²)	23.11 $\pm$ 0.19
Ages of received education (years)	8.72 $\pm$ 0.55
Female menostasis (yes/no)	39/78
Age range	18–60 years
Age of onset (years)	36.26 $\pm$ 1.58
Duration of total illness (months)	19.34 $\pm$ 2.45
Duration of current episode (months)	2.17 $\pm$ 0.26
Numbers of episodes (n)	1.87 $\pm$ 0.56
Family history (yes/no)	14/180
HDRS at admission	32.51 $\pm$ 1.27
HDRS at 8-week treatment	11.32 $\pm$ 1.66

Data were generally reported as mean $\pm$ SE.
HDRS, Hamilton Depression Rating Scale.

TABLE 4. STANDARD PARTIAL REGRESSION COEFFICIENTS AND SIGNIFICANCE TEST IN 194 MAJOR DEPRESSIVE DISORDER (THE EFFICACY RESPONSE TO SHORT-TERM SELECTIVE SEROTONIN REUPTAKE INHIBITORS)

	Standardized coefficients		df	F	Sig.
	Beta	Std. Error			
rs6311	0.081	0.075	2	1.190	0.307
rs6305	-0.053	0.75	2	-831	0.407
rs2230739	0.283	0.075	2	14.221	0.000
rs5443	0.181	0.075	2	5.897	0.003
rs1549870	-0.088	0.074	1	1.387	0.241

Dependent variable: short-term efficacy of SSRI.
SSRI, selective serotonin reuptake inhibitor.

the samples further, we found that the TT genotype had better short-term efficacy than TC, CC of rs5443, and GG genotype better than AG, AA of rs2230739. Other SNPs such as rs6311, rs1549870 was not related with the efficacy response to short-term SSRIs ($p > 0.05$) (See Table 4).

For the efficacy of response to long-term SSRIs, we classified HRSD subtraction mark rate $\geq 50\%$ as effective, others as noneffective, and analyzed the relationships between the different genotypes of five SNPs and the efficacy by the optimal-scaling regression analysis suited for categorical variables in MDD. We found that rs2230739 was related to the efficacy response to long-term SSRIs treatments (Beta = 0.400, $p = 0.000$). By analyzing the samples further, we found that the GG genotype had better long-term efficacy than AG, AA of rs2230739. Other SNPs such as rs6311, rs5443, and rs1549870 were not related to the efficacy response to long-term SSRIs ($p > 0.05$) (See Table 5).

Discussion

To our knowledge, this is the first study looking at the relationship between the SNPs of five genes that are related with 5-HT signal transduction pathway and the efficacy of response to SSRI treatment by the optimal-scaling regression analysis. The optimal-scaling regression analysis differed from the general regression analysis. An important development in multidimensional data analysis has been the optimal assignment of quantitative values to such qualitative scales. This form of optimal quantification (scaling, scoring) is a general approach that treats multivariate (categorical) data.

TABLE 5. STANDARD PARTIAL REGRESSION COEFFICIENTS AND SIGNIFICANCE TEST IN 194 MAJOR DEPRESSIVE DISORDER (THE EFFICACY RESPONSE TO LONG-TERM SELECTIVE SEROTONIN REUPTAKE INHIBITORS)

	Standardized coefficients		df	F	Sig.
	Beta	Std. Error			
rs 6311	-0.070	0.072	2	0.932	0.396
Rs6305	0.012	0.073	2	0.047	0.828
rs 230739	0.400	0.073	2	29.942	0.000
rs 5443	0.060	0.074	1	0.672	0.414
rs 549870	0.077	0.073	2	1.097	0.336

Dependent variable: long-term efficacy.

Optimization is a relative notion, because it is always obtained with respect to the particular data set that is analyzed and the particular criterion that is optimized. The results suggested that the TT genotype had better short-term efficacy than TC, CC of rs5443 in SSRI 8-week treatments. This demonstrated that rs5443 played an important role in the efficacy response to short-term SSRIs in MDD. There were many reports about the association between the polymorphism of *Gβ3* 825C/T (rs5443) (Wilkie *et al.*, 2007) and major depression. This study was similar to a foreign one which found that the T allelic gene of *Gβ3* 825C/T (rs5443) was related with depression. Compared with those who did not carry a T allelic gene (rs5443), the depression patients who carried a T allelic gene have a better response to SSRIs.

The results also suggested that the GG genotype has better efficacy response to short-term and long-term SSRIs than AG, AA of rs2230739. *ADCY9* was a subunit recently found. Although its physiological significance is not completely understood, much attention to its effects on psychological activities has been recently paid by scholars (Iwatsubo *et al.*, 2006). We presumed that although the study did not discover that the SNPs of rs2230739 A/G in *ADCY9* were associated with major depression, SSRIs may alter the pathologic signal transduction pathway via the regulation of G protein, *ADCY9*, and related enzymes so that when an exogenous substance that influences the level of 5-HT is provided, good therapeutical effects ensue. *ADCY9* has an effect on enzymatic activity and cAMP, and it can mediate cell growth via cAMP (Gros *et al.*, 2005); thus, *ADCY9* plays an important role in the process of signal transduction via 5-HT.

In clinical aspects, we observed that lithium carbonate as a mood stabilizer had a certain effect on the treatment of treatment-resistant depression. Moreover, many researchers have proved the efficacy of lithium, which has a close relationship with G protein and *ADCY9* (Crossley and Bauer, 2007; Guzzetta *et al.*, 2007).

The study also showed that there was no clear evidence between the polymorphism of rs6311 and SSRIs efficacy, compared with rs5443 and rs2230739. The study prompted us to consider that the different genotypes of G protein and *ADCY9* might influence the neurobiological effect of SSRIs. After the patients with the different genotypes of rs5443 and rs2230739 are treated with SSRIs, second messenger transduction pathway may be mediated, leading to an antidepressant effect. Since the transduction process of second messenger is complex in cells, the definitive mechanism needs further study to be confirmed.

Long-term follow-up studies showed that 63.2% exhibited recurrent episodes. Only 41.9% of depressed patients had significant efficacy in a 1-year follow-up. It is similar to the study of Demyttenaere *et al.* (2008). According to optimal scaling, we found that a long-term effect might be related to the polymorphism of rs2230739. The patients with the GG genotype of rs2230739 had a good long-term effect. Long-term SSRI treatment may have been expressed via third messenger and CREB (Chen *et al.*, 2001), which then influenced the repairing function of nerve cells. Further studies are needed to prove whether the patients with GG types are much better than others in repairing the function of central nerve cells.

However, there were some limitations. First, the sample size is not enough. Second, these SNPs could not represent all genes correlated with 5-HT2A. Therefore, we will enlarge the

sample size and further increase the genes to research, and will regard 5-HT_{2A} rs6311C/T polymorphism as a biological endo-phenotype to be explored in future.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:

Guang-Rong Xie, M.D.

Mental Health Institute of the Second Xiangya Hospital

Central South University

No. 139 Renmin Middle Road

Changsha 410011

P.R. China

E-mail: guangrong-xie@163.com

Kun Yang, M.D.

Department of Psychiatry

Tianjin Anding Hospital and Tianjin Mental Health Institute

No.13 Liulin Road

Tianjin 300222

P.R. China

E-mail: yangkun8124@yahoo.com.cn