

Escitalopram Response in Panic Disorder is Not Associated with Three 5-HT_{1A} Receptor Gene Polymorphisms (Rs6295, Rs10042486, or Rs1364043) in Chinese-Han Patients

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Abstract

Background: Prior studies have implicated 5-HT_{1A} receptor gene (*HTR1A*) polymorphisms in the etiology of panic disorder (PD) as well as in the therapeutic response to selective serotonin reuptake inhibitors (SSRIs). A functional *HTR1A* C-1019G polymorphism (rs6295) in the promoter region and two non-synonymous polymorphisms (rs10042486 and rs1364043) were previously found to be associated with SSRI pharmacogenetics. However, no studies have examined this relationship in Chinese-Han PD patients.

Methods: Seventy-seven patients with PD according to DSM-IV criteria were recruited. All of them with a minimum score of 8 on the seven item Panic Disorder Severity Scale, Chinese version (PDSS-CV). In this study, we evaluated the association between three single nucleotide polymorphisms (SNPs) of *HTR1A* (rs6295, rs10042486, and rs1364043) and the clinical effects of escitalopram in Chinese-Han PD patients.

Results: The C/C genotype of C-1019G carriers showed better responses to escitalopram compared to the C/G or G/G genotypes. However, after Bonferroni correction, the effect was not statistically significant. Similarly, no significant associations were noted between rs10042486 or rs1364043 and the escitalopram clinical response.

Conclusion: Our results suggest that these three single nucleotide polymorphisms (SNPs) in 5-HT_{1A} genes examined here may not influence the escitalopram response in Chinese-Han PD patients.

Keywords: 5-HT_{1A} receptor gene; Single nucleotide polymorphism; Panic disorder; Escitalopram

Introduction

Panic disorder (PD) is a common anxiety disorder that affects up to 5% of the population over their lifetime [1]. In addition, PD has been linked to increased cardiovascular morbidity and mortality, which typically increases the burden of suffering as well as economic costs [2,3]. Therefore, it is valuable to determine its etiology and provide targeted treatment methods for this disorder.

Positron emission tomography (PET) analyses have found significantly reduced 5-HT_{1A} radio ligand binding in the raphe nuclei, orbitofrontal cortex, temporal cortex, and amygdala regions of PD patients compared to control subjects [4]. Moreover, genetic studies suggest the involvement of the 5-HT_{1A} receptor gene in panic disorder [5]. Animal studies have demonstrated that 5-HT_{1A} receptor knockout mice show increased anxiety-like behavior [6]. Thus, based on neuroimaging data, animal models, and genetic studies, the 5-HT_{1A} receptor gene, located on chromosome 5 (5q11.2–13), is a putative candidate gene for PD [7]. Of several gene polymorphisms, studies have focused on a functional C-1019G single nucleotide polymorphism (SNP rs6295) located in the promoter of *HTR1A*, which regulates *HTR1A* transcription, and is associated with schizophrenia, bipolar disorder, anxiety, depression-related personality traits, and antidepressant responses [8–10]. In particular, two very recent studies reported the C-1019G polymorphism was associated with clinical responses in Japanese and Caucasian PD patients [11,12]. Additionally, this polymorphism and a nearby functional polymorphism are in strong linkage disequilibrium [13]. Based on these results, we hypothesized that other *HTR1A* polymorphisms in strong linkage disequilibrium with rs6295 might also influence the clinical response. Two polymorphisms of *HTR1A* (rs10042486 and rs1364043) were previously found to be

associated with SSRI pharmacogenetics. However, no association study of *HTR1A* gene polymorphisms (rs6295, rs10042486, and rs1364043) and escitalopram clinical response in Chinese-Han PD patients has been reported.

Therefore, we conducted an association study with three *HTR1A* gene SNPs (rs6295, rs10042486, and rs1364043) to determine the best predictive value for clinical responses in Chinese-Han PD patients.

Methods

Subjects

This study was performed at Shenyang Mental Health Center. The Ethics Committee of Shenyang Mental Health Center approved this study, and each subject provided written informed consent before enrollment. Seventy-seven patients (24 males, 53 females) with PD according to DSM-IV criteria were recruited.

Only subjects with a minimum score of 8 on the seven item Panic Disorder Severity Scale, Chinese version (PDSS-CV) qualified for the

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Received October 04, 2017; **Accepted** October 25, 2017; **Published** October 27, 2017

Citation: Wenhui C, Qian L, Suxia C, Jianyue P, Hengfen L (2017) Escitalopram Response in Panic Disorder is Not Associated with Three 5-HT_{1A} Receptor Gene Polymorphisms (Rs6295, Rs10042486, or Rs1364043) in Chinese-Han Patients. J Depress Anxiety S12: 006. doi:10.4172/2167-1044.S12-006

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study. Patients with substance abuse, severe organic mental disorders, or concomitant major psychiatric disorders were excluded. The mean age of patients was 30.61±6.51 years (± SD) and ranged from 18 to 60 years. The clinical response to treatment was assessed using the PDSS-CV at the beginning of treatment and every two weeks thereafter. It took 5 to 7 days before patients received the full therapeutic escitalopram dose (10-20 mg/day). A significant clinical response was defined as a 50% decrease in PDSS-CV total score within 4 weeks of escitalopram treatment.

Polymorphism genotyping

Genomic DNA was extracted from EDTA-treated venous blood using standard techniques and DNA extraction kits (Wizard Genomic DNA purification kit; Promega, USA). Genotyping for the three SNPs (rs6295, rs10042486, and rs1364043) was performed using polymerase chain reaction (PCR) and post-PCR ligase detection reaction (LDR) assays. Genomic DNA was amplified using the following primers: rs6295 (sense: 5'-ACGGAGGTACCGTTTTGTTG-3', antisense: 3'-CCCAGAGTG-GCAATAGGAGA-5'), rs10042486 (sense: 5'-GGTTGGAGTTTCT-CAGTAATTGG-3', antisense: 3'-TGGGGATTGGGAAGAATTA-AA-5'), rs1364043 (sense: 5'-AAGCGAACTCAAACAGCAAAA-3', antisense: 3'-TTTGAAGAGGGCACAAATTC-5'). PCR reactions had a final volume of 20 µl containing 100 ng genomic DNA, dNTPs (0.2 mM of each), 1.0 ng/l template, 2.0 mM PCR buffer, 0.6 mM Mg²⁺, 0.2 U/ml HOT Start Taq DNA polymerase, 9.8 µl H₂O, 4 µl Q-solution, and 0.4 pM primer mix. After an initial denaturation step of 15 min at 95°C, DNA was amplified by 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 1 min, and extension at 72°C for 1 min, followed by a final extension step at 72°C for 10 min. Successful amplification was confirmed by running 2 µl of the PCR product mixture on agarose gels stained with ethidium bromide.

LDR assays were performed in a final volume of 10 µl containing ≥1 µl PCR products, 6.95 µl H₂O, 0.05 µl ligase, 1 µl probe mix, and 1 µl buffer. The initial denaturation for 2 min at 95°C was followed by 35 cycles of denaturation at 94°C for 30 s and annealing and extension at 60°C for 2 min. LDR products were used for allele discrimination. LDR products were denatured at 95°C for 2 min and mixed with 1 µl of the size standard ABI GS-500 ROX (Applied Biosystems Inc., USA). The products were electrophoresed in 10% polyacrylamide with 5 M carbamide at 3000 V for approximately 2.5 hours. GENESCAN TM 672 software was used to collect data, correct the line of electrophoresis, and measure different fragments. Genemapper software was used to analyze the genotype distributions.

Statistical analysis

We used chi-square analyses or Fisher's tests to compare escitalopram clinical responses with genotype frequencies. Fisher's exact tests were substituted for chi-square tests when sample size was smaller than five subjects. Independent-sample t-tests were used to evaluate differences in PDSS-CV scores, age, and escitalopram doses between the response and non-response groups during the 4 weeks of treatment. For multiple comparisons, Bonferroni correction was applied using the formula: $a' = a/k$. Where a' is the corrected significance level for multiple comparisons, a is the significance level (0.05) and k is the number of independent significance tests. Statistical analyses were performed using the SPSS 17.0 software package.

Results

The genotype distributions of all *5-HT1A* gene polymorphisms were in Hardy-Weinberg equilibrium ($p > 0.05$). The mean age, escitalopram

Variables	Responders	Non-responders	t	p
Age	29.03 ± 6.79	31.56 ± 6.40	-1.67	0.10
Escitalopram dose	16.55 ± 3.80	17.50 ± 2.73	-1.27	0.21
PDSS score (baseline)	20.97 ± 2.21	21.08 ± 2.00	-0.24	0.81
PDSS score (week 2)	17.07 ± 1.91	17.48 ± 2.05	-0.87	0.39
PDSS score (week 4)	10.24 ± 1.02	12.00 ± 1.41	-5.83	<0.01

Table 1: The mean age, escitalopram dose, and PDSS score between responders and non-responders in PD patients.

Genotype	Responders [n (%)]	Non-responders [n (%)]	χ ²	p
rs6295				
GG	13 (30.2)	30 (69.8)	6.79	0.04
CG	9 (36.0)	16 (64.0)		
CC	7 (77.8)	2 (22.2)		
rs10042486				
CC	1 (33.3)	2 (66.7)	0.54	0.83
CT	11 (42.3)	15 (57.7)		
TT	17 (35.4)	31 (64.6)		
rs1364043				
GG	10 (35.7)	18 (64.3)	0.78	0.70
GT	17 (41.5)	24 (58.5)		
TT	2 (25.0)	6 (75.0)		

Table 2: Genotype distributions in the responders and non-responders after escitalopram treatment for 4 weeks.

dose, and PDSS-CV score of the responders and non-responders are presented in (Table 1) These two groups showed no significant differences in PDSS-CV scores at baseline, age, or escitalopram dose ($p > 0.05$).

The genotype distributions for the three SNPs in the responders and non-responders after 4 weeks of escitalopram treatment are shown in (Table 2) The C/C genotype of C-1019G showed better response to escitalopram, compared to the C/G and G/G genotypes ($\chi^2=6.79$, $p=0.04$). However, this difference was not significant after adjusting the significance level for multiple tests (for $k=3$ groups and $k \times (k-1)/2=3$ comparisons, the corrected significance level is 0.017). Similarly, no statistically significant differences in rs10042486 and rs1364043 genotype distributions were noted between the two groups ($\chi^2=0.54$, $p=0.83$ for rs10042486, $\chi^2=0.78$, $p=0.70$ for rs1364043).

Discussion

To the best of our knowledge, this is the first study to evaluate the relationship between the three *HTR1A* gene SNPs and SSRI clinical response in Chinese-Han PD patients. In the present study, we not only compare the genotypes distribution in responders and non-responders group, but also adopt genotype-time interaction model to further explore the differences in PDSS Scale changes according to *5-HT1A* polymorphisms over time. Our finding failed to detect any association either rs6295, rs10042486 and rs1364043 of the *HTR1A* gene polymorphism and antidepressant response or differences in PDSS-CV Scale changes according to *5-HT1A* polymorphisms after 4 weeks of escitalopram treatment in Chinese-Han PD patients.

The *5-HT1A* receptor gene is located both presynaptically (as an auto receptor) and post-synaptically (as a heteroreceptor). The auto receptor exerts a negative feedback function and when stimulated by 5-HT, induces hyperpolarization of 5-HT neurons and decreases the neuronal firing rate, thereby resulting in decreased 5-HT synthesis and release. The *5-HT1A* heteroreceptor is involved in mediating 5-HT actions on target neurons receiving serotonergic innervation. Thus, it has the dual ability to modulate global serotonin levels as well as local responses to released serotonin [7,14].

The functional C-1019G SNP rs6295, located in the 26 bp palindromic sequence of the *HTR1A* promoter, may influence *HTR1A* transcription activity by binding two transcription factors: deformed epidermal auto regulatory factor-1 (Deaf-1) and hairy enhancer of split 5 (Hes5). It was reported that the transcription factors Deaf-1 and Hes5 can repress expression of the *5-HT1A* auto receptor by binding the C-allele but not the G-allele [15-18]. Therefore, the G-1019 allele correlates with increased levels of *5-HT1A* auto receptors. A brain imaging study also found that the G allele and *5-HT1A* receptor binding increases in both the raphe nuclei and other brain regions [19]. Therefore, this could cause a greater extent of desensitization of *5-HT1A* auto receptors, reduce raphe firing, decrease serotonin release, and confer resistance to SSRIs [20,21]. In contrast, in cells expressing *5-HT1A* post synoptically, Deaf-1 activity is opposed in presynaptic versus postsynaptic neuronal cells. Thus, Deaf-1 activates *5-HT1A* transcription by binding to the C allele but not the G allele [20,21]. Therefore, the G-1019 allele might reduce Deaf-1-enhanced postsynaptic *5-HT1A* transcription as well as enhance resistance to antidepressant-induced increases in 5-HT [15,16]. Thus, it is reasonable to speculate that the G/G genotype could result in worse clinical responses unless SSRI treatment activates 5-HT neurons more strongly in these patients due to extensive desensitization of overexpressed autoreceptors. In agreement with this, Yevtushenko and co-authors found that the C allele of rs6295 showed substantial symptom improvement in Caucasian PD patients [12]. Ishiguro et al. also found that Japanese PD patients who were rs6295C/C carriers showed improved clinical response to paroxetine pharmacotherapy compared to non-carriers [11].

Several factors could account for these discrepancies. First, the rs6295 polymorphism may be in linkage disequilibrium with another genetic variation polymorphisms within the *5-HT1A* promoter region that are causally related to antidepressant response, and it can also be speculated that the linkage disequilibrium of rs6295 with other polymorphisms within the *5-HT1A* gene may not be same for all ethnic populations, thus may help explain the previous inconsistent results. Second, the GG genotype distributions can differ markedly between populations and this difference can influence the statistical power. For example, in the study of Ishiguro, rs6295G/G genotype distributions were 3.1%, while the distributions were 19.6% in the study of Yevtushenko [11,15]. Third, the promoter region sequences of *5-HT1A* have not been fully elucidated, the DNA sequences of promoter region may also differ by ethnic background, this influence the interaction of multiple cis and trans elements controlling *5-HT1A* transcription result in the different expressions of *5-HT1A* either in auto receptor or hetero receptor result in contributing to influence clinical response.

We did not observe an association between rs10042486 or rs1364043 and escitalopram response. One reason may be because the sequences of the *5-HT1A* gene promoter region may differ in ethnic populations. Thus, the linkage disequilibrium of rs6295, rs10042486, and rs1364043 may not be the same in all ethnic backgrounds. Consequently, rs10042486 and rs1364043 may not be involved in regulating *HTR1A* transcription, and subsequently do not influence the clinical response. In addition, even if rs10042486 and rs1364043 are in fact involved in the development of PD, these polymorphisms may not affect the size of the clinical effect.

One limitation of this study is the rather small sample size. Thus, we cannot evaluate sex and subclinical factors to elucidate their role in clinical responses. Further studies should examine sex and sub clinical interactions to fully explore their role in clinical responses. A second limitation is the absence of a placebo control, it is generally accepted

that the placebo response plays a significantly role in the therapeutic response to antidepressant agents and thus we cannot exclude the possibility that some PD patients in the responder group responded to the placebo response alone [22]. The lack of a placebo control limits whether the observed associations were directly attributed to drug-related genetic or other biological factors [23-26].

Conclusion

In summary, our study indicates that *5-HT1A* genetic variation isn't associated with escitalopram response. However, studies with larger sample sizes in different ethnic backgrounds may be necessary to fully elucidate the nature of this relationship.

Acknowledgment

This study was supported by the National Natural Science Foundation of China (81371494).

Conflict of Interest

All authors declare that they have no conflicts of interest.

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This article was originally published in a special issue, **Mixed symptoms & Syndromes of Anxiety & Depression** handled by Editor(s). Dr. Domenico Chirchiglia, Chair of Neurosurgery, University of Catanzaro, Italy