



Potential Link between T102C Polymorphism in the Serotonin Receptors (5-HT2A) Gene and Treatment Response of Risperidone on Schizophrenia

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Abstract

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BACKGROUND: Schizophrenia affects 1% of population and its molecular etiology remains enigmatic despite enormous study. Approximately one-third of patients failed to respond with treatment. The 5-HT2A receptor appears to be one of important site of action of atypical antipsychotic drugs. It also has been suggested that the T102C gene polymorphism alters promoter activity and expression of 5-HT2A receptors and might be responsible for the associations with the efficacy of typical antipsychotics.

AIM: In this study, we aimed to evaluate the potential link between T102C polymorphism in the Serotonin Receptors (5-HT2A) and their response to risperidone.

METHODS: We studied 100 schizophrenia patients and 100 healthy volunteers as a comparison of 5-HT2A receptors gene polymorphism distribution, which were all Indonesian. The peripheral blood samples were obtained from all participants. The patients assessed by Positive and Negative Scale (PANSS) and Clinical Global Impression Scale (CGI) when admitted to the hospital. Clinical improvements then assessed 5 times in 4 weeks (when transferred to sub-acute ward, at the 1st, the 2nd, the 3rd, and the 4th week). To analyze the T102C polymorphism of 5-HT2A receptor gene, we used an allele specific polymerase chain reaction based restriction fragment length polymorphism (PCR-RFLP) method.

RESULTS: In this study, there were three various polymorphisms of T102C in the serotonin receptor (5-HT2A) gene: T/T, C/C, and T/C. The patients who had homozygous for T/T genotypes were found to give better improvement to risperidone than the patients who were C/C and T/C genotypes (P:0.001). Moreover, the patients with C allele genotype T/C and C/C had minimal response (P:0.001). We found no significant difference distribution of genotypes between schizophrenia patients and healthy volunteers (P:0.498).

CONCLUSION: Our result supported that there was a potential link between T102C polymorphism in the serotonin receptor (5-HT2A) gene and treatment response of risperidone. It suggested the importance of genetic screening such as examination of the Serotonin Receptor 5-HT2A (T102C) gene polymorphism to be performed to optimize therapeutic strategies with antipsychotic.

Background

Schizophrenia is a debilitating mental illness that affects 1% of the population, with an age of onset in the late 10 s or early 20 s. The disorder usually follows a chronic relapsing-remitting course, and severely limits the individual's ability to work and integrate into society. The overall cost of caring for people with schizophrenia is being one of the most expensive disorders across the adult lifespan [1]. Subsequent clinical experience with antipsychotic drugs indicated that approximately one-third of patients failed to response to treatment. However, the possibility of relapse in patients with good response still exists.

Risperidone, the most commonly prescribed atypical antipsychotic in Indonesia, has been reported to be favorable for treating both positive and negative symptoms of schizophrenia. Risperidone has moderate

to very high affinity for serotonin receptors (5-HT2A) and is also a potent inhibitor of dopamine-D2 receptors [2]. It has been hypothesized that clinical response to antipsychotic drugs may be determined by genetic variation in the neurotransmitter receptors to which the drug binds. In a way, alterations in genes coding for neuroleptics receptor proteins may affect their binding affinities for neuroleptics, the efficiency of signal transduction, or their level of expression, which, in turn, may alter the drug's therapeutic action [3].

The involvement of the serotonin (5-hydroxytryptamine; 5-HT) system in the etiology of schizophrenia has been hypothesized [4]. It has been shown that the number of 5-HT2A receptors is decreased in the brain prefrontal cortex of schizophrenia patients, as well as in relation to treatment with antipsychotic drugs [5]. Moreover, a positive association between different polymorphisms of the 5-HT2A receptors gene and schizophrenia has been reported [6]. 5-HT2A receptor is widely distributed in the brain, with

highest concentrations in the cortex, and is localized on cortical and hippocampal pyramidal glutamatergic neurons, as well as on g-aminobutyric acid (GABA) ergic interneurons, both of which play a key role in schizophrenia [6], [7], [8], [9]. 5-HT2A receptors is also located in the substantia nigra and ventral tegmentum, from which the nigrostriatal and mesocorticolimbic dopamine neurons, respectively, arise, as well as their terminal regions, indicating the possibility of a relevant role for these receptors in modulating many of the actions of antipsychotic drugs [9].

Recent studies have found different results of potential link between T102C polymorphism in the 5HT2A receptors gene and treatment response in schizophrenia. Investigation in Chinese schizophrenia subjects with C/C genotype predicted better response to risperidone than T/T and T/C genotype as well as in Korean people [10], [11]. One of the investigation in Turkey origin showed that the subjects who had homozygous for T/T genotype was found to give better response to risperidone treatment than the patients who had C/C and T/C genotypes with respect to SANS and BPRS score [12]. In contrast, there was a study in Japanese people reported no association between T102C polymorphism in the 5-HT2A receptors and response therapy of risperidone [13].

With these distinctive findings obtained from studies in several populations, there is a need to determine the potential link between T102C polymorphism in the 5-HT2A receptors gene and treatment response of schizophrenia patients in Indonesia who received risperidone. Since Indonesia has numbers of ethnic groups, therefore in the present study, we were focusing in the genotype of Makassar ethnic group.

Methods

This prospective cohort observational study involved 200 subjects. We divided the subjects into two groups. First group was a hundred schizophrenic inpatients in Prefecture Mental Hospital of South Sulawesi (Makassar, Indonesia). The patients would be included if: (1) They were diagnosed as schizophrenia according to DSM-V by psychiatrist who was in charge in the ward at that time, (2) no significant organic or neurologic disorders, (3) the patients were treated with risperidone in therapeutic dose from 2 to 4 mg by psychiatric who was in charge in the ward at that time, and (4) they had no history of alcohol or drug abuse in the previous 12 months, (5) they were ranging from 18 to 40 years of age [14]. The second group was a hundred of healthy volunteers ranging from 18 to 40 years of age with no history of psychiatric disorder

or family history as a comparison of 5-HT2A receptors gene polymorphism distribution. All subjects provided written informed consent.

Clinical improvement of schizophrenia group assessed 6 times in 4 weeks (when admitted to the hospital; transferred to sub-acute ward; the 1st; the 2nd; the 3rd; and the 4th weeks) using Positive and Negative Symptom Scale (PANSS). Clinical Global Impression (CGI) scale were used to measure illness severity when admitted to the hospital (CGI-S); global improvement on the 4th week (CGI-I); and therapeutic response on the 4th week (CGI-E). This research study obtained approval from the Ethics Committee of Hasanuddin University.

Molecular analysis

Venous blood samples (3 ml) were collected in ethylenediaminetetraacetic acid (EDTA) containing tubes. We conducted an allele specific polymerase chain reaction (PCR) based restriction fragment length polymorphism (PCR-RFLP) to genotype for serotonin receptors (5-HT2A). The primers 5-HT2A-F5'-CTGTCTGCTACAAGTTCTGGCTTT'-3 and 5-Ht2A-R5'-CTGCAGCTTTTTCTCTAGGG-3 were used in this study. PCR tubes were inserted into the PCR machine and set up the machine by 35 cycles with a temperature of 95° for 30 s, 60° for 30 s, and 72° for 30 s. The Msp1 enzyme was used to obtain a 5-HT2A receptor T102C gene polymorphism. Samples were incubated at 37° for 15 min. Then, an electrophoresis of 2% agarose was performed with ethidium bromide. Allele 1 (T102C allele) was represented by the uncut 342 bp PCR product, and allele 2 (C102) allele consisted of two fragments 215 and 126 bp (Figure 1).

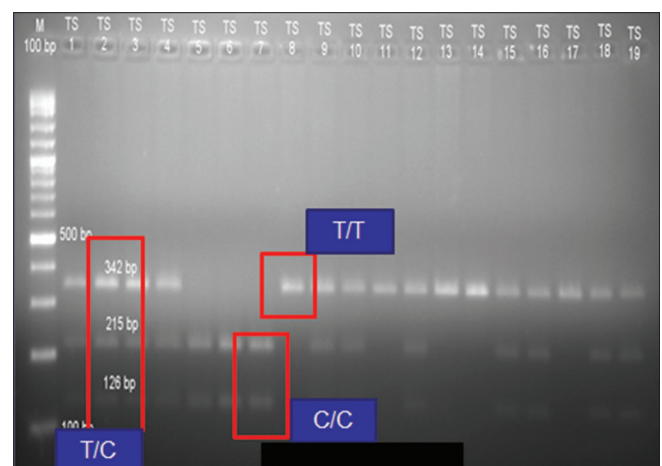


Figure 1: PCR-RFLP results of T102C polymorphism in the 5-HT2A Receptors Gene in Schizophrenia Subjects. PCR Products of 5-HT2A and Msp1 restriction enzyme: 342 bp: T/T, 215 and 126 bp: C/C, 342, 215 and 126 bp: T/C. Lane 1, 2, 4, 5, 8, 9, 14, 15, 16, 17, 18: T/C Genotype, Lane 3, 6, 7, 10, 12, 13, 19: T/T Genotype, Lane 11: C/C Genotype

Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) 22.0 program for windows. Chi-square and one-way ANOVA test were used for the statistical analysis of the data.

Results

Baseline characteristics on patients in the schizophrenia group and healthy volunteers group

Demographic characteristics of subjects were revealed and compared in Table 1. The schizophrenia group included a total of 100 (65 males and 35 females) and the healthy volunteers group included a total 100 (39 males and 61 females). The presentation of subjects with ≥30 years old was significantly higher in Schizophrenia Group; nevertheless, subjects below 30 years old significantly higher in Healthy Volunteers Group. There was a significant difference in age and gender existed in the Schizophrenia Group and Healthy Volunteers Group with $p < 0.001$ ($p < 0.005$).

Table 1: The clinical baseline characteristics

Characteristics	Schizophrenia group (n = 100) (%)	Healthy volunteers group (n = 100) (%)	p-value (p < 0.05)
Age (years)			
<20 years	1 (1)	19 (19)	<0.001
20–29 years	27 (27)	70 (70)	
30–39 years	39 (39)	11 (11)	
≥40	33 (33)	0 (0)	
Gender			
Male	65 (65)	39 (39)	<0.001
Female	35 (35)	61 (61)	

Minimal Improvement: ±25% reduction of the total scales compared with baseline on the PANSS scores.
 Much Improvement: ±50% reduction of the total scales compared with baseline on the PANSS scores [17].
 OR: Odds Ratio ; CI: Confidence Interval

Genotype distribution of 5-HT2A receptors in the schizophrenia group and healthy volunteers group

We found no significant difference in genotype distribution between schizophrenic group (n TT = 36, TC = 58, CC = 6) and healthy volunteers group (n TT = 44, TC = 50, CC = 6) with $p = 0,498$ ($p < 0.05$) (Figure 2).

Association of PANSS score with T102C polymorphism in the 5-HT2A receptors gene

There was no significant difference improvement rates in PANSS 1 to PANSS 5 between T/T, T,C and C/C genotype with $p < 0.001$ ($p > 0.05$) (Figure 3). PANSS 6 displayed a significant improvement rates ($p < 0.05$) in T/T genotype compared to T/C and C/C genotype.

T102C polymorphism in the 5-HT2A receptors displayed a significant association with PANSS total improvement rates (Table 2). Our findings showed that the subjects who had homozygous for T/T genotypes were found to give much improvement to risperidone than the subjects who were C/C and T/C genotypes with $p < 0.001$ ($p < 0.05$). In addition, subjects with T/C and C/C genotype appeared to confer higher possibility

Table 2: Association of PANSS score with T102C polymorphism in the 5-HT2A receptors gene

Genotype	Treatment response		Total	p-value	OR (95% CI)*
	Minimal improvement	Much improvement			
T/T	3	33	36	<0.001	1.0
	8.3%	91.7%	100%		
T/C	35	23	58		16.7
	60.3%	39.7%	100%		(4.6–61.0)
C/C	3	3	6		11,0 (1.5–80.4)
	50%	50%	100%		
Total	41	59	100		
	41%	59%	100%		

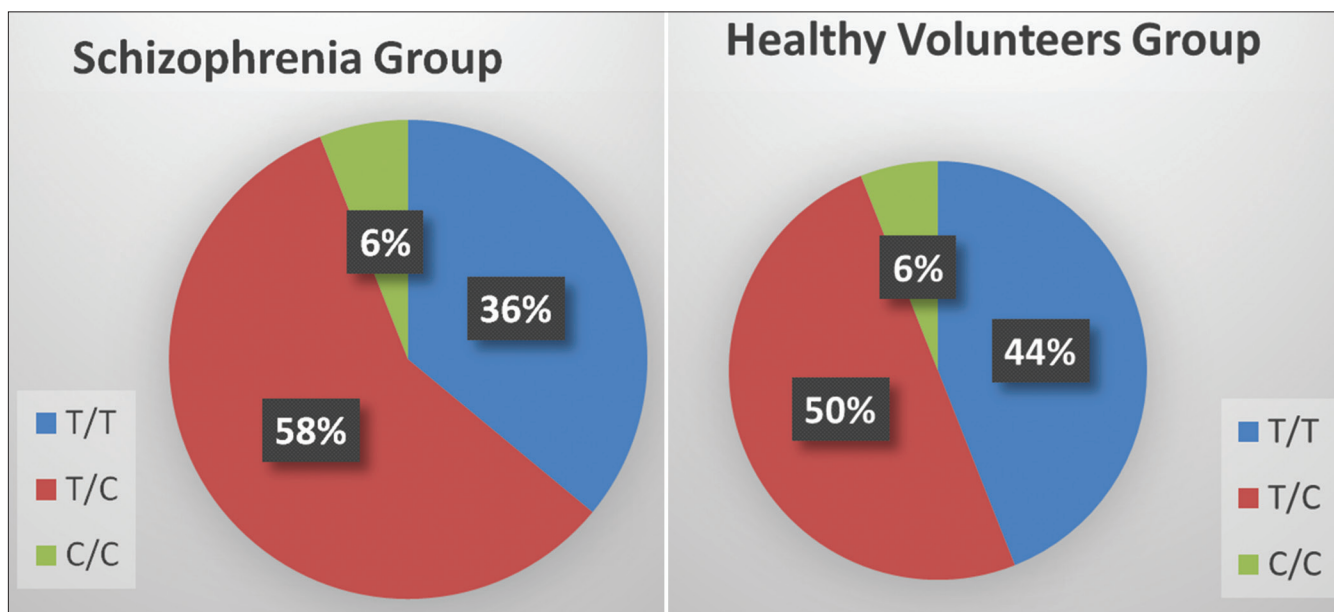


Figure 2: T102C Polymorphism in the 5-HT2A Receptors Genotype Distribution In Schizophrenia Group and Healthy Volunteers Group. No significant difference in genotype distribution between schizophrenic group (n TT = 36, TC = 58, CC = 6) and healthy volunteers group (n TT = 44, TC = 50, CC = 6)

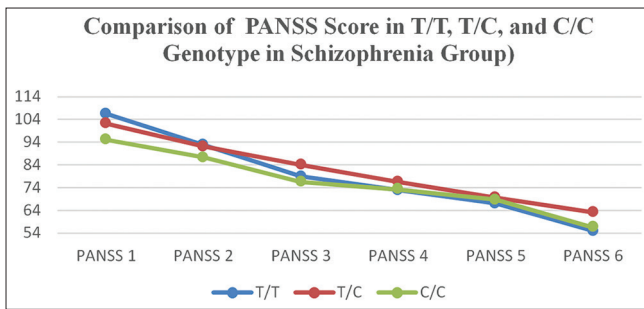


Figure 3: Comparison of Risperidone Response Therapy Evaluated by PANSS Score in 4 Weeks in Schizophrenia Group. PANSS 1: Evaluated when admitted to the hospital; PANSS 2: Evaluated when transferred to sub-acute ward; PANSS 3: Evaluated after 1 week given risperidone; PANSS 4: evaluated after 2 weeks given risperidone; PANSS 5: Evaluated after 3 weeks given risperidone; PANSS 6: evaluated after 4 weeks given risperidone. No significant difference improvement rates in PANSS 1 to PANSS 5 between T/T, T, C, and C/C genotype. PANSS 6 displayed a significant improvement rates ($p < 0.05$) in T/T genotype compared to T/C and C/C genotype.

than subjects with T/T genotype to sustain minimal improvement in risperidone treatment response 16.7 times and 11 times, respectively.

Association of CGI score with T102C polymorphism in the 5-HT2A receptors

Similarly for CGI-I (Table 3) and CGI-E scale (Table 4), the subjects with T/T genotype showed a better respond to risperidone treatment ($p < 0.05$) compared to subjects with T/C and C/C genotype. For the assessment of side effects on the CGI-E scale, only four subjects (4%) experienced extra pyramidal syndrome (EPS) side effects that significantly interferes with patient’s function therefore trihexyphenidyl 2 mg was added, 1 tablet per 12 h. The CGI-S was unable to be linked with the polymorphism because the results of the assessment were all considerable “severely ill.”

Discussion

There were two major findings in the presence study. First, there was no significant difference in T102C polymorphism in the 5-HT2A receptors gene distribution between Schizophrenia group and Healthy Volunteers Group. Second, there was a potential link between T102C polymorphism in the 5-HT2A receptors gene and risperidone treatment response on schizophrenia subjects.

Our data showed same distribution of T102C polymorphism in the 5-HT2A receptors gene in Schizophrenia Group and Healthy Volunteers Group. T/C genotype is mainly found amongst the subjects with a prevalence of 58% in Schizophrenia Group and 50% in the Healthy Volunteers Group. T/T genotype

Table 3: Association of CGI-I score with T102C polymorphism in the 5-HT2A receptors gene

Genotype	CGI-I		Total	p-value
	Much improved	Very much improved		
T/T	3 8.3%	33 91.7%	36 100%	<0.001
T/C	35 60.3%	23 39.7%	58 100%	
C/C	3 50%	3 50%	6 100%	
Total	41 41%	59 59%	100 100%	

was found in 36% subjects in Schizophrenia Group and in 44% subjects in Healthy Volunteers Group. For C/C genotype, the prevalence was similar 6% of subjects in both groups. Based on these findings, we suspected T/C genotype may become dominant genotype of T102C polymorphism in the 5-HT2A receptors gene in our population. Despite of that, we assumed T102C polymorphism in the serotonin receptors gene unable to be a predictor for schizophrenia susceptibility in this population.

The results suggested a potential link of T102C polymorphism in the 5-HT2A receptors gene on risperidone treatment response evaluated in PANSS and CGI scale. In this study, subjects with T/T genotype showed better improvement in treatment compared to subjects with T/C and C/C genotype, suggested that T102C polymorphism in the 5-HT2A receptors gene played an important role in therapeutic response to risperidone. There needs to be an explanation of biological why T102C polymorphism in the 5-HT2A receptor gene gave different responses in the treatment of schizophrenia, but there was possibility that binding affinity of T/T genotype in 5-HT2A receptor T102C was higher than the other genotypes such as

Table 4: Association of CGI-E score with T102C polymorphism in the 5-HT2A receptors gene

Genotype	CGI-E		Total	p-value
	Marked improvement	Moderate improvement		
T/T	32 88.9%	4 11.1%	36 100%	<0.001
T/C	23 39.7%	35 60.3%	58 100%	
C/C	3 50%	3 50%	6 100%	
Total	58 58%	42 42%	100 100%	

Marked improvement: Vast improvement. Complete or nearly complete remission of all symptoms; Moderate Improvement: Decided improvement. Partial remission of symptoms [18].

C/C and T/C in serotonergic system [15]. Furthermore, a functional promoter’s variant of 5-HT2A receptor might differentially alter transcription, thereby affecting the number of receptors. These results in line with Herken *et al.* investigated in Turkey origin showed that there was an association between T102C polymorphism in the 5-HT2A receptors gene and risperidone response therapy in Schizophrenia showed that subjects with homozygous T/T genotype were found to give better response to risperidone treatment than subjects with C allele C/C and T/C genotype. Therefore, we assumed that T102C polymorphism in the 5-HT2A receptors gene could be used to predict treatment response of risperidone or

other antipsychotic which has moderate to very high affinity for the serotonergic receptors [12].

Recent studies have found different results of potential link between T102C polymorphism in the 5HT2A receptors gene and treatment response in schizophrenia. Investigation in Chinese schizophrenia subjects with C/C genotype predicted better response to risperidone than T/T and T/C genotype as well as in Korean people. Ethnic diversity may be one of the factors that affected the outcome of this study [10], [11].

According to our findings, subjects with T/T genotype will give better response if treated by antipsychotic with moderate to very high affinity for the serotonergic receptors. Whereas subjects with T/C and C/C genotype will give better response, if treated by antipsychotic with low-to-moderate affinity for the serotonergic receptors or higher affinity to other receptor.

The correlation between age and gender with treatment response was not examined. This might be a limitation of present study. Although gender is a predictor of clinical response to antipsychotic treatment, its influence is not the same for all antipsychotic. There was another study found that risperidone had no difference in gender for clinical response [16]. In the present study, to elude the confounding factors of treatment response, we excluded younger (<18 years of age) and elder (≥40 years of age) population.

Conclusion

There were polymorphisms of the 5-HT_{2A} Receptor (T102C) gene in people with schizophrenia and healthy volunteers (T/T, T/C and C/C). Our result supported that there was a potential link between T102C polymorphism in the 5HT2A receptors gene and treatment response of risperidone. T/T genotype was found to give better improvement than C/C and T/C genotypes. Our findings suggested that genetic screening needs to be performed to optimize therapeutic strategies of schizophrenia patients. Further studies are needed to find the possible impacts of genes interaction in the occurrence and response therapy of schizophrenia patients.

Declarations

Authors' contributions

All the authors were involved in the conception of this study SS, FI, AFY, and STL to interpretation of the research findings and contributed to the drafting of

the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

The research has been permitted and acknowledged by Hasanuddin University Ethic Medical Committee. Before each interview, each participant was given written information on the study. Each participant was also informed that his or her participant was voluntary. Before each interview, we emphasized the importance of maintaining confidentiality in relation to patient cases. All participants provided written consent to participate in this study.

Availability of data and materials

The datasets used and analyzed for this study are available from the corresponding author on reasonable request.

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