Original Article

DOI 10.3349/ymj.2010.51.1.27
pISSN: 0513-5796, eISSN: 1976-2437

Yonsei Med J 51(1): 27-32, 2010

YMJ

No Evidence of an Association between A218C Polymorphism of the Tryptophan Hydroxylase 1 Gene and Aggression in Schizophrenia in a Korean Population

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Purpose: We investigated the association between the tryptohan hydroxylase 1 (*TPH1*) gene and aggression in schizophrenia in a Korean population. **Materials and Methods:** The sample included 61 aggressive patients as well as 104 non-aggressive patients from psychiatric hospitals and 335 healthy volunteers in Korea. Blood samples were collected from all participants for *TPH1* A218C genotyping. The patients were administered standard psychiatric interviews as well as a self-report questionnaire for anger-related traits. **Results:** In the case-control phenotypic comparisons, there was no significant association between the aggressive patients and the *TPH1* A218C polymorphism. There was no significant effect of the *TPH1* genotype on the anger-related traits, or no significant interaction between the genotype and group (aggressive and non-aggressive patients). **Conclusion:** These findings suggest that *TPH1* does not play a major role in aggressive behavior via anger in schizophrenic patients.

Key Words : Tryptophan hydroxylase, aggression, schizophrenia, anger

Received: February 18, 2009
Revised: April 8, 2009
Accepted: April 8, 2009

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· The authors have no financial conflicts of interest.

INTRODUCTION

Aggressive behavior is frequently reported in patients with schizophrenia, and its prevalence is estimated to be 2 to 10 times that of the general population.[1,2] The causes of aggressive behavior in schizophrenia are complex and multifactorial, but a genetic contribution to aggressive behavior has been demonstrated in twin and adoption studies in community sample.[3-5] There is a large body of evidence to suggest that disturbances of central serotonin (5-HT) functions play an important role in aggressive behavior.[6]

The gene responsible for 5-HT synthesis, the tryptophan hydroxylase (*TPH*) gene, is one of the candidate genes associated with aggression. Several separate lines of biological evidence which suggest its role in modulating serotonergic function in the brain[7] together with genetic association studies point to the involvement of *TPH1* in aggression or anger-related traits within various psychiatric conditions.[8,9] The A218C polymorphism in intron 7 is one of the most widely studied polymorphisms in this gene and could affect the expression of the *TPH1* gene by causing a strong linkage disequilibrium with other functional polymorphisms in the promoter region.[10] In the previous case-control genetic study by Nolan, et al.,[11] a marginal association between *TPH1* A218C and

aggressive schizophrenia was found, only in male patients, in spite of the small sample size.

Based on the above findings, we investigated whether the genetic variant of *TPH1* A218C is associated with aggression in schizophrenic patients. To this end, a case-control phenotypic comparison was carried out, followed by an investigation of anger-related traits as traits affecting aggressive behavior in schizophrenic patients.

MATERIALS AND METHODS

Study population

The sample included 61 aggressive schizophrenic patients (39 men, 22 women) as well as 104 non-aggressive patients (57 men, 47 women) and 335 healthy volunteers (204 men, 131 women) recruited from the Korean population. The DSM-IV diagnosed schizophrenic patients were recruited to the study between 2004 and 2007 from the inpatients population in Severance hospital, Seoul, and the Chookryoung Evangelical Mental Hospital, Gyeonggi, South Korea. Patients with other comorbid major Axis I psychiatric disorders were not included in this study. The aggressive patients were selected according to rigorous criteria for identifying both a current and past history of aggressive behavior. As described in previous study,[12] patients were included in the aggressive group if they had significant episodes of violence, resulting in repeated confinement at least twice per week in the two weeks prior to study inclusion, and a past history of aggressive behaviour defined as a documented history of at least two (and frequently many more) serious assaults against others. Non-aggressive patients were selected for the study if they had no history of significant assaultive or threatening behavior, reported by family members or staff, and if no such behavior was observed during the interview. All of the patients were assessed within 2 weeks after admission and underwent a psychiatric interview conducted by two independent clinicians, based on all available clinical information, examination of case records, and information provided by relatives and mental health professionals. Patients were assessed by the Modified Over Aggression Scale (MOAS)[13] for aggressive behavior. We could obtain the data for anger-related traits by the State-Trait Anger Expression Inventory (STAXI)[14] in 45 aggressive patients and 84 non-aggressive patients. Of patients, 52 patients in the aggressive group and 82 patients in the non-aggressive group were taking neuroleptics at the time of the study. When the doses of neuroleptics were converted into chlorpromazine equivalent doses,[15] there was no significant difference in the dose of neuroleptics between the aggressive and non-aggressive patients. A subgroup consisting of about 30% of the patients within the schizophrenia sample (13 aggressive patients, 32 non-aggressive patients) also completed the assessment of symptoms using the Positive and Negative Syndrome Scale (PANSS) as part of another ongoing study by our group. There was no significant difference between the patients who completed PANSS and those who did not, in terms of age, MOAS or illness variables (onset, duration, hospitalisation and dose of neuroleptics) (data available on request). The exclusion criteria were a history of neurological disorders, substance abuse in the previous 3 months and an estimated intelligence quotient (IQ) of less than 70.

Healthy unrelated Korean volunteers with a mean age of 24.5 years (SD 5.7, range 19-57) were recruited from the residents of Seoul by advertisement. They had no reported current or past history of psychiatric disorders, including significant violent episodes. The Institutional Review Boards of both Severance Hospital and Chookryoung Evangelical Mental Hospital approved the study, and written informed consent was obtained from all participants or their legal guardians after the nature of the study was fully explained to them.

State-trait anger expression inventory

The STAXI[14] is a 44-item self-report questionnaire that is divided into seven scales. It measures the current intensity of anger (State anger) and disposition toward anger as a personality trait (Trait anger) using two subscales that measure the subject's general disposition toward angry feelings (Angry temperament) and the tendency to express anger when criticized (Angry reaction). The other scales measure the frequency with which provoked anger is suppressed (Anger-in), the frequency of the expression of anger toward other people or objects (Anger-out), and the frequency at which the expression of anger is controlled (Anger control). The Korean version of the STAXI was used in this study.[16] The STAXI has demonstrated satisfactory reliability in schizophrenic patients with Cronbach's α = 0.82-0.85.[17]

Genotyping

Genotyping was performed blind to history of aggressive behavior. Genomic DNA was extracted from blood leukocytes by using a commercial DNA extraction kit (ABI, Foster City, CA, USA). The genotyping of *TPH* polymorphism (A218, rs1800532) was carried out using ABI PRISM SNaPShot Multiplex kit (ABI, Foster City, CA, USA) according to manufacturer's recommendation. Briefly, the genomic DNA flanking the SNP for rs1800532 was amplified by polymerase chain reaction (PCR) with the primers 5'-CATGTT CCATGCTCTATATGTGT-3' and 5'-TGTCTGATT TTTTTCAGTGTTACATT-3'. The

PCR conditions were as follows: 10 minutes at 95˚C for 1 cycle, and 30 cycles on 95˚C for 30s, 55˚C for 1 min, 72˚C for 1 min followed by 1 cycle of 72˚C for 7 minutes. After amplification, the PCR products were treated with 1 unit each of shrimp alkaline phosphatase (Roche) and exonuclease I (USB Corporation) at 37˚C for 60 minutes and 72˚C for 15 minutes. One μL of the PCR products was added to a SNaPshot Multiplex Ready reaction mixture containing 0.15 pmols of genotyping primer of *TPH* (5'-TTAT TAATTGACAACCTATTAGGTG-3') for primer extension reaction. The primer extension reaction was carried out for 25 cycles of 96˚C for 10 seconds, 50˚C for 5 seconds, and 60˚C for 30 seconds. The reaction products were treated with 1 unit of SAP at 37˚C for 1 hour and 72˚C for 15 minutes to remove excess fluorescent dye terminators. One μL of the final reaction sample containing the extension products was added to 9 μL of Hi-Di formamide (ABI, Foster City, CA, USA). The mixture was incubated at 95˚C for 5 minutes, followed by 5 minutes on ice and then analyzed by electrophoresis in ABI Prism 3730xl DNA analyzer. Results were analyzed using GeneScan analysis software (ABI, Foster City, CA, USA). Deviation from Hardy-Weinberg equilibrium was assessed as described by Wigginton, et al.[18]

Statistical analyses

The differences in the genotype and allele frequencies between the affected (aggressive patients) and unaffected individuals (non-aggressive patients or healthy volunteers) were evaluated using a χ^2 test. To test the interaction between the genotypes and group (aggressive patients and non-aggressive patients) on anger-related traits, multivariate analysis of variance (MANOVA) was performed, followed by univariate analysis. Gender was entered as a covariate in all analyses to control the possible gender effect on the genotype.[19] *p*-values < 0.05 were considered as significant. All statistical tests were performed using SPSS version 11.0.

RESULTS

The demographic and clinical characteristics of the patients are shown in Table 1. As shown, the aggressive and non-aggressive patients did not differ in age, age at onset or educational level. As expected, the aggressive patients had

Table 1. The Epidemiological and Clinical Data of the Schizophrenic Patients

Characteristics	Aggressive patients (n = 61)	Non-aggressive patients (n = 104)	Analysis		
			t	df	*p*
Age (yrs)	36.8 (9.4)	39.4 (10.4)	1.55	163	0.124
Age at onset (yrs)	25.1 (6.8)	24.2 (7.0)	0.71	139	0.479
Education (yrs)	12.2 (3.4)	11.9 (3.0)	0.41	120	0.680
Chlorpromazine equivalent doses (mg)	527.7 (394.5)	425.3 (324.1)	1.64	133	0.103
MOAS, total score	19.1 (7.4)	6.2 (8.2)	9.03	133	< 0.001
STAXI, total score	106.7 (20.5)	90.9 (20.3)	4.21	127	< 0.001

Data are mean (SD).
MOAS, modified over aggressionscales; STAXI, state-trait anger expression inventory.

Table 2. Frequency of Genotypes and Alleles for the *TPH1* A218C Gene in Aggressive Schizophrenic Patients, Non-Aggressive Schizophrenic Patients and Healthy Volunteers, Stratified by Gender

	Genotype								Allele					
	A/A		A/C		C/C		Analysis*		A		C		Analysis*	
	n	%	*n*	%	*n*	%	χ^2_2	*p*	*n*	%	*n*	%	χ^2_1	*p*
Aggressive patients (n = 61)	15	25	30	49	16	26			60	49	62	51		
Male (n = 39)	8	21	19	49	12	31			35	45	43	55		
Female (n = 22)	7	32	11	50	4	18			25	57	19	43		
Non-aggressive patients (n = 104)	28	27	49	47	27	26	0.12	0.944	105	50	103	50	0.05	0.820
Male (n = 57)	13	23	30	53	14	25	0.46	0.797	56	49	58	51	0.34	0.562
Female (n = 47)	15	32	19	40	13	28	0.86	0.650	49	52	45	48	0.27	0.607
Healthy controls (n = 335)	66	20	180	54	89	27	0.81	0.668	312	47	358	53	0.28	0.595
Male (n = 204)	43	21	107	53	54	27	0.32	0.854	193	47	215	53	0.16	0.693
Female (n = 131)	23	18	73	56	35	27	2.60	0.272	119	45	143	55	1.96	0.161

*Comparisons of aggressive patients with non-aggressive patients or healthy volunteers.

Table 3. Multivariate Analysis of Variances (MANOVA) for *TPH1* A218C in the Aggressive and the Non-Aggressive Schizophrenic Patients

	F	df	*p*
Genotype*	0.76	12 / 226	0.690
Group†	3.02	6 / 112	0.009
Gender†	1.78	6 / 112	0.110
Genotype × Group	0.75	12 / 226	0.699
Genotype × Gender	0.88	12 / 226	0.568
Group × Gender	1.99	6 / 112	0.074
Genotype × Group × Gender	0.84	12 / 226	0.614

General Linear Model Multivariate Statistics, Pillai-Spur, variables; State-Trait Anger Expression Inventory, factors; genotype, group, gender.
*AA (n = 31) / AC (n = 68) / CC (n = 30).
† Aggressive patients (n = 45) / Non-aggressive patients (n = 84).
† Males (n = 71) / Females (n = 58).

Table 4. Mean Scores for Subscales of the State and Traits Anger Expression Inventory (STAXI) as a Function of *TPH* Genotype in Schizophrenic Patients

	Aggressive patients (n = 45)					Non-aggressive patients (n = 84)				
	Genotypic distribution			Analysis		Genotypic distribution			Analysis	
	AA	AC	CC	*F*	*p*	AA	AC	CC	*F*	*p*
	(n = 10)	(n = 24)	(n = 11)	(df = 2 / 41)		(n = 21)	(n = 44)	(n = 19)	(df = 2 / 80)	
State anger	16.0 (6.8)	15.6 (6.5)	16.4 (9.0)	0.03	0.970	13.0 (3.8)	13.4 (4.6)	14.6 (6.2)	0.60	0.554
Trait anger	18.8 (5.6)	20.0 (5.2)	19.1 (6.5)	0.23	0.796	14.7 (3.7)	16.4 (4.6)	17.4 (5.4)	1.80	0.172
Angry reaction	9.4 (3.2)	10.3 (3.3)	9.4 (3.4)	0.43	0.652	7.2 (2.2)	8.7 (2.8)	9.3 (3.3)	2.79	0.067
Angry temperament	9.4 (2.9)	9.7 (3.4)	9.7 (3.6)	0.03	0.974	7.5 (2.2)	7.7 (2.2)	8.2 (2.7)	0.47	0.626
Anger-in	15.5 (5.6)	17.4 (4.7)	14.7 (3.9)	1.59	0.217	14.5 (4.7)	14.8 (3.7)	14.2 (4.4)	0.13	0.875
Anger-out	14.6 (4.6)	15.9 (5.4)	14.7 (4.3)	0.50	0.609	11.2 (3.2)	12.6 (3.7)	13.4 (4.3)	0.98	0.145
Anger control	18.8 (6.3)	21.0 (5.4)	19.6 (4.5)	0.69	0.509	21.2 (5.7)	21.5 (4.8)	23.2 (7.5)	0.70	0.500

Values were mean (SD).
Analysis by univariate ANOVA for the effect of genotype on subscales of STAXI with gender as a covariate.

significantly higher scores in MOAS and STAXI than the non-aggressive patients. In addition, the positive scale [24.7 (9.8) vs. 14.5 (8.2); t = 3.7, df = 43, $p = 0.001$], negative scale [19.5 (7.0) vs. 13.2 (7.1); t = 2.8, df = 43, $p = 0.007$], general subscale [48.4 (16.7) vs. 28.3 (12.4); t = 4.5, df = 43, $p < 0.001$], and total PANSS scores [92.0 (30.8) vs. 56.7 (25.2); t = 4.0, df = 43, $p < 0.001$] of the aggressive patients were significantly higher than those of the non-aggressive patients.

The genotype and allele frequencies of the variants for the aggressive and non-aggressive patients with schizophrenia are shown in Table 2, along with those for the healthy controls. The *TPH1* A218C genotype frequencies in both the healthy controls and schizophrenic patients did not deviate from Hardy-Weinberg equilibrium. There were no significant differences in the genotype or allele frequencies between the aggressive patients and controls (non-aggressive patients or healthy volunteers). When the analysis was stratified by gender, the genotype and allele frequencies in the aggressive, non-aggressive patients groups and healthy volunteers were not significantly different.

A MANOVA was computed for patients with schizophrenia by integrating the seven STAXI subscales as well as three factors: the genotype, group and gender. We could not find any effect of genotype on the anger-related traits or any interaction among the genotype, groups, and gender (Table 3). In further detailed univariate analysis followed for genotype effects, there was no effect of genotype on the STAXI subscales in aggressive or non-aggressive patients (Table 4).

DISCUSSION

In the present study, tests of association with a case-control phenotypic comparison and continuous outcome for anger-related traits revealed no evidence of an association between the *TPH1* A218C gene and aggression in schizophrenic patients.

To the best of our knowledge, this is the first study to systematically investigate the association between the tryptophan hydroxylase gene and aggressive schizophrenia.

It is interesting to note that our findings are incongruent with those of a previous study in a Caucasian sample.[11] One explanation for the discrepancy between our findings and others may be the small sample sizes of aggressive patients in both studies: 61 and 42, respectively, coupled with the fact that the previous positive finding was found in the sample of 34 male patients. Since it is beyond the means of most investigators to gather an adequate sample size of aggressive schizophrenic patients, meta-analyses of well-prepared small studies should be conducted in the future.

In this study, the *TPH1* genotype had no effect on anger-related traits in either the aggressive or non-aggressive schizophrenic patients. In our previous study, aggressive behavior was more frequent in schizophrenic patients who were prone to becoming angry.[20] Based on our findings, it is suspected that other serotonin-related genes, such as serotonin transporter-related gene,[21] or other *TPH* gene, *TPH2*, which is considered to control serotonin synthesis in the brain,[22] may play a role in aggressive behavior via anger-related traits in schizophrenic patients. On the other hand, as the A218C single nucleotide polymorphism is not considered to be the sole determinant of the functionality of the *TPH1* gene,[10,23] an analysis of the effect of each haplotype on the expression of the *TPH1* gene is also required, in order to clarify the involvement of the *TPH* gene in aggressive schizophrenia.

The major strength of the current study is its use of rigorous criteria to define the phenotype. Thus, although the recruitment period was twice long as initially planned because aggressive patients who met the criteria were rare, the relatively homogenous phenotype of the patients adds strength to the findings in this study. This study has a few limitations which need to be taken into consideration, however. The first limitation is the relatively small sample size, as already pointed out above. Small sample sizes can lead to both type 1 and type 2 errors. The power of our sample was 53% between aggressive patients and healthy controls, and 42% between aggressive patients and non-aggressive patients, to detect an allele frequency difference of 10% at alpha = 0.05 (two-tailed). The second major limitation is that a self-rating instrument was used to measure anger-related traits. However, we did not think that this led to biased results, because the STAXI has demonstrated high reliability in schizophrenic patients in other studies.[15] A third limitation is that the severity of illness could have affected the genotype findings. However, it seems unlikely to be a confounding factor, as there was no association between the PANSS score and *TPH* genotype in our subsidiary analysis. A fourth is that ages of patients with schizophrenia and control subjects were not matched.

In conclusion, we could not find any association between the genetic variations of *TPH1* A218C and aggression in Korean schizophrenia population in the present study. Despite the present findings, there is no doubt that genetic variants remain an important factor in aggressive behavior. Further studies are needed to identify the susceptibility gene in patients with schizophrenia and other clinical or non-clinical samples using well-characterized aggression phenotypes.

ACKNOWLEDGEMENTS

This work was supported by the Biomedical Brain Research Center, Ministry of Health & Welfare, Republic of Korea (01-PJ8-PG6-01NE01-0003) and a 2007 Inje University research grant.

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