No Evidence for an Association of Polymorphisms of the Tryptophan Hydroxylase Gene With Affective Disorders or Attempted Suicide Among Japanese Patients

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Objective: Tryptophan hydroxylase is the rate-limiting enzyme in the biosynthesis of serotonin. The authors examined whether polymorphisms A218C and A779C in intron 7 of the tryptophan hydroxylase gene are associated with a risk for affective disorders or suicidal behavior. **Method:** Subjects were 141 patients with bipolar disorder and 73 patients with unipolar affective disorder, 46 of whom had a history of attempted suicide, and 208 healthy volunteers. All subjects were unrelated to each other, and all were Japanese. Genotyping was performed by polymerase chain reaction amplification followed by digestion by a restriction enzyme and single-strand conformational polymorphism analysis. **Results:** There was no significant genotypic or allelic association of the A218C polymorphism with bipolar disorder, unipolar depression, or history of attempted suicide. In nearly 100% of the subjects, genotypes for the A779C were identical to those for the A218C. **Conclusions:** The authors conclude that the examined polymorphisms are unlikely to have major relevance to the pathogenesis of affective disorders or suicidal behavior.

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Tryptophan hydroxylase (EC 1.14.16.4) is the rate-limiting enzyme in the biosynthesis of serotonin. Alterations in the serotonergic system have been implicated in affective disorders and suicidal behavior (1). A polymorphism (A218C) in intron 7 of the tryptophan hydroxylase gene was reported to be associated with bipolar affective disorder, but not with a history of attempted suicide (2). Another polymorphism (A779C) in intron 7 of the tryptophan hydroxylase gene, which is in tight linkage disequilibrium to the A218C polymorphism (3), was reported to be associated with attempted suicide (4, 5) and with 5-hydroxyindoleacetic acid concentration in CSF (4). However, the results were contradictory: the earlier study (4) reported an association between attempted suicide and the 779C allele, but the later study (5) reported a greater frequency of the 779A allele among suicide attempters. Abbar et al. (6) found no significant association between attempted suicide and the C2-38/AvaII polymorphism of the tryptophan hydroxylase gene. These contradictory results require further studies. The present study examined the polymorphisms in intron 7 of the tryptophan hydroxylase gene for genotypic and allelic association with bipolar disorder, unipolar depression, and attempted suicide.

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METHOD

The subjects were 141 patients with bipolar disorder (53 men; mean age=46.7 years, SD=13.8; 110 with bipolar I and 31 with bipolar II), 73 patients with unipolar major depression (25 men; mean age=55.0, SD=14.6), and 208 healthy volunteers (95 men; mean age=32.1, SD=13.3). These patients were recruited from the psychiatric clinics of Teikyo University Hospital, Shiga University of Medical Science Hospital, and Showa University Hospital from 1992 to 1997. Among the patients, 46 (seven men; mean age=45.7, SD=12.8) had a history of attempted suicide. Consensus diagnosis according to DSM-IV by at least two experienced psychiatrists was made for each patient on the basis of unstructured interviews and information from medical records.

Healthy volunteers were recruited from hospital staff and medical students, who were not assessed for psychiatric symptoms by any structured interview method but who showed good social functioning (i.e., no history of long-term absence from work or school) and reported themselves to be in good health. All patients and compari-

TABLE 1. Genotype and Allele Distributions for the A218C Polymorphism of the Tryptophan Hydroxylase Gene Among Patients With Bipolar Disorder, Patients With Unipolar Depression, Suicide Attempters, and Healthy Comparison Subjects

	Genotype Distribution[a]							Allele Frequency[b]				
		A/A		A/C		C/C			A		C	
Subjects	N	N	%	N	%	N	%	N	N	%	N	%
Comparison subjects	208	55	26.4	105	50.5	48	23.1	416	215	51.7	201	48.3
Patients												
Bipolar disorder	141	48	34.0	70	49.6	23	16.3	282	166	58.9	116	41.1
Unipolar depression	73	20	27.4	42	57.5	11	15.1	146	82	56.2	64	43.8
Suicide attempters	46	10	21.7	29	63.0	7	15.2	92	49	53.3	43	46.7

[a] Comparisons with the healthy subjects—bipolar group: χ^2=3.5, df=2, p=0.17; unipolar group: χ^2=2.2, df=2, p=0.34; suicide attempters: χ^2=2.5, df=2, p=0.28.
[b] Comparisons with the healthy subjects—bipolar group: χ^2=3.5, df=1, p=0.06; unipolar group: χ^2=0.9, df=1, p=0.35; suicide attempters: χ^2=0.1, df=1, p=0.78.

son subjects were unrelated to each other, and all were Japanese. Information on history of attempted suicide was obtained by scrutinizing medical records. Methods used for attempted suicide were overdose of medication for 13 individuals, cutting or stabbing for seven, hanging for five, jumping from high places for three, being run over by a train for three, intoxication for three, multiple methods for seven, and other for five.

After description of the study, written informed consent was obtained from each subject. Genomic DNA was extracted from whole blood according to standard procedures. Genotyping for the A218C polymorphism was performed according to the procedure of Bellivier et al. (2). Genotyping for the A779C was done by polymerase chain reaction with primers of CTTATATGTGTGAGTCTGAGTGGC (forward) and AAGAGTTCATGGCAGGTATCTCTG (reverse), which give a 218 base-pair polymerase chain reaction product encompassing the A779C site, followed by single-strand conformational polymorphism analysis with 20% polyacrylamide gel electrophoresis with silver staining. Genotypic data were read blind to the subject's status as patient or comparison subject.

The presence of Hardy-Weinberg equilibrium was tested by the chi-square test for goodness of fit. The genotype and allele distributions between the patients and comparison subjects were compared by using the chi-square test for independence. We performed a logistic regression analysis within the patient group to examine the effect of the tryptophan hydroxylase A218C genotype on suicidal behavior; in this analysis, history of attempted suicide was a dependent variable, and age, sex, diagnosis, and genotype were independent variables. All p values are two-tailed.

RESULTS

Table 1 shows the genotype and allele distributions for the A218C polymorphism of the tryptophan hydroxylase gene. The genotype distributions were in Hardy-Weinberg equilibrium for all of the groups (for the comparison group, χ^2=0.0, df=1, p=0.88; for the bipolar group, χ^2=0.1, df=1, p=0.77; for the unipolar group, χ^2=2.1, df=1, p=0.15; and for the suicide attempters, χ^2=3.3, df=1, p=0.07). There was no significant difference in genotype or allele distribution between any of the patient groups and the comparison group. Even when homozygosity for either allele was assumed to be a risk factor, no significant difference was found for any patient group compared with the healthy subjects. The logistic regression analysis within the patient group did not reveal any significant effect of the tryptophan hydroxylase genotype (homozygosity for either allele) on suicide attempt (homozygosity for the 218A allele: Wald statistic=1.1, df=1, p=0.28; homozygosity for the 218C allele: Wald statistic=1.4, df=1, p=0.24).

For the A779C polymorphism, we genotyped 118 subjects (75 patients and 43 comparison subjects) and confirmed the previous finding of tight linkage disequilibrium between the A218C and the A779C polymorphisms (3). Observed genotypes for the A779C in all but three of the subjects (97%) were identical to those for the A218C polymorphism. Subsequent sequencing analysis revealed that the 218A and 218C alleles were linked to the 779A and the 779C alleles, respectively. All three of the exceptional individuals were heterozygous for the A218C and homozygous for the 779A allele.

DISCUSSION

We failed to find any significant association of the A218C polymorphism of the tryptophan hydroxylase gene with bipolar disorder, unipolar depression, or attempted suicide in our group of 214 patients, suggesting that this polymorphism has no major effect on the pathogenesis of these psychiatric conditions. We demonstrated a tight linkage disequilibrium between the A218C and the A779C polymorphisms (genotypes were identical for 115 of 118 subjects), which is consistent with a previous report (3). Therefore, it is also unlikely that the A779C polymorphism has a major effect on susceptibility to affective disorders or suicidal behavior. However, the finding of Nielsen et al. (4) was obtained in a sample of alcoholic violent offenders. It is possible that the discrepancies between our study and the previous studies (4, 5) may be attributable in part to differential patient profiles, including personality pathology.

The number of subjects in this study provides a power of 90% with a 5% significance level to detect an odds ratio of 1.7 for allelic association for bipolar disorder, 1.9 for unipolar depression, and 2.2 for attempted suicide. There is only a small chance that a clinically meaningful difference would have been missed with the data.

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