Neuropsychopharmacology (2003) 28, 163–169

www.neuropsychopharmacology.org

Substance Abuse Disorder and Major Depression are Associated with the Human 5-HT_{1B} Receptor Gene (*HTR1B*) G861C Polymorphism

Yung-yu Huang[1], Maria A Oquendo[1], Jill M Harkavy Friedman[1], Lawrence L Greenhill[1], Beth Brodsky[1], Kevin M Malone[1], Vadim Khait[1] and J John Mann*,[1]

[1]*Department of Neuroscience, New York State Psychiatric Institute, Columbia University College of Physicians and Surgeons, New York, USA*

The 5-HT_{1B} receptor has been implicated in several psychopathologies, including pathological aggression, alcoholism and suicide. To test these and related potential genetic relationships in a single population, the human 5-HT_{1B} receptor (h5-HTR_{1B}) genotype for the G861C polymorphism was determined in 394 psychiatric patients and 96 healthy volunteers. Structured clinical interviews generated DSM III-R diagnoses. No significant association of the genotype or allele frequencies of the h5-HTR_{1B} G861C locus was observed with diagnoses of alcoholism, bipolar disorder, schizophrenia or a history of a suicide attempt. Exploratory analyses indicated an association of the genotype and allele frequencies of the h5-HTR_{1B} G861C locus with a history of substance abuse disorder ($\chi^2 = 9.51$, df = 2, $p = 0.009$; $\chi^2 = 7.31$, df = 1, $p = 0.007$, respectively) and with a diagnosis of a major depressive episode ($\chi^2 = 6.83$, df = 2, $p = 0.033$; $\chi^2 = 5.81$, df = 1, $p = 0.016$, respectively). Significant gene dose effects on the risk for substance abuse disorder and a major depressive episode were observed with the 861C allele (Armitage linearity tendency test: $\chi^2 = 7.20$, df = 1, $p = 0.008$; $\chi^2 = 6.80$, df = 1, $p = 0.009$, respectively). Substance abuse disorder and major depression appear to be associated with the h5-HTR_{1B} G861C locus in the patient population, but other psychopathologies such as bipolar disorder, schizophrenia, alcoholism, and suicide attempts were not found to be associated with this polymorphism. This preliminary result will need replication, given the limitations of association studies.
Neuropsychopharmacology (2003) **28,** 163–169. doi:10.1038/sj.npp.1300000

Keywords: human 5-HT_{1B} receptor gene; genes; *HTR1B*; substance abuse disorder; major depression; alcoholism; polymorphism

INTRODUCTION

Alcoholism, substance abuse disorder, suicidal acts, and mood disorders such as major depressive disorders and bipolar disorders are thought to involve dysfunctions of the brain serotonergic system (Ballenger *et al*, 1979; Goodwin and Post, 1983; Virkkunen *et al*, 1994; Mann and Arango, 1998; Mann, 1999). Comorbidity of these psychopathologies is common. Patients at risk for suicidal attempts most commonly have mood disorders and increased rates of alcoholism and substance abuse (Mann, 1998). Mood disorders are present in about 60% of all completed suicides and are frequently comorbid with alcoholism and substance abuse. Alcoholism, substance abuse, and major depression may facilitate suicidal acts partly because they share a common causal factor, such as lower serotonergic activity, which in turn influences behavioral inhibition (Mann, 1998). Patients with a history of suicidal acts are characteristically more impulsive and aggressive (Mann *et al*, 1996).

The human 5-HT_{1B} receptor is of interest because of several lines of evidence. It functions as a nerve terminal autoreceptor, regulating the release of 5-HT (Maura *et al*, 1993). Some, but not all, studies find that altered postmortem 5-HT_{1B} receptor binding is in association with suicide (Arranz *et al*, 1994; Lowther *et al*, 1997; Huang *et al*, 1999). Knockout of the 5-HT_{1B} gene in mice results in a phenotype characterized by increased aggression (Saudou *et al*, 1994), greater alcohol consumption (Crabbe *et al*, 1996), and cocaine consumption (Rocha *et al*, 1998). For a review, see Scearce-Levie *et al* (1999).

Genetic factors are likely to play an etiological role in alcoholism (Schuckit *et al*, 1985), mood disorders (Gershon *et al*, 1989), and psychoactive substance abuse disorders (Bierut *et al*, 1998; Merikangas *et al*, 1998; Tsuang *et al*, 1998). Addictions are genetically influenced complex disorders (Goldman and Bergen, 1998). Suicidal behavior has a genetic contribution to its diathesis, independently of the heritability of major psychiatric disorders associated with suicide risk (Schulsinger *et al*, 1979; Roy, 1986; Roy *et al*, 1991; Brent *et al*, 1996). The mechanisms whereby genetics can affect mood, alcoholism, substance abuse, and

*Correspondence: J John Mann, Department of Neuroscience, New York State Psychiatric Institute, 1051 Riverside Drive, Unit 42, New York, NY 10032, USA. Tel: +1 212 543 5571, Fax: +1 212 543 6017, E-mail: jjm@columbia.edu
Received 26 November 2001; revised 20 May 2002; accepted 29 May 2002

suicidal behaviors are not known, but the serotonergic system is one possibility.

A common polymorphism of the h5-HTR_{1B} receptor gene was first identified by the *Hinc*II restriction enzyme (Sidenberg *et al*, 1993) and later identified by secondary structural content prediction (SSCP) and polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) methods at the G861C locus in the coding region (Lappalainen *et al*, 1995). Aside from this one, three other common polymorphisms of the 5-HT_{1B} serotonin receptor gene have been identified. We previously identified a polymorphism at nucleotide 129 of the coding region by the SSCP method (Huang *et al*, 1999). This h5-HTR C129T polymorphism is in absolute linkage disequilibrium with the polymorphism at nucleotide 861 of the coding region identified by Lappalainen *et al* (1995). The other two polymorphisms are D6S284, a flanking dinucleotide repeat sequence (Weissenbach *et al*, 1992), and T-261G, a mutation in a 5′-flanking region (Nöthen *et al*, 1994). An uncommon molecular variant with substitution of a cysteine for a phenylalanine residue (F124C) has also been detected (Nöthen *et al*, 1994).

Lappalainen *et al* (1998) first reported in a Finnish population that antisocial alcoholics had a higher 861C allele frequency at the h5-HTR_{1B} G861C locus. We have reported an h5-HTR_{1B} allelic association with 5-HT_{1B} receptor binding in the postmortem brain prefrontal cortex, namely, lower B_{max} values for 5-HT_{1B} binding in association with the 861C allele (Huang *et al*, 1999) and Arango *et al* (1995) reported that less postmortem 5-HT_{1B} binding may be present in alcoholism, consistent with the 5-HT_{1B} knockout mouse phenotype (Crabbe *et al*, 1996). Recently, Fehr *et al* (2000) reported a higher frequency of the h5-HTR_{1B} 861G allele, not the 861C allele found by Lappalainen *et al* (1998), among alcohol-dependent patients as compared with control subjects. The different results may reflect population differences, including rates of comorbid psychopathology.

Since alcoholism is often comorbid with substance abuse, suicidal behavior, psychoses, and mood disorders, we assessed the possible association between the h5-HTR_{1B} gene *HTR1B* and a range of psychopathologies in a clinical sample. Considering previously reported results, we hypothesized that the 861C allele would be associated with alcoholism, substance abuse, and suicidal behavior. Other analyses would be exploratory.

MATERIALS AND METHODS

Subjects

DNA samples ($n = 490$) for genotyping were obtained from patients ($n = 394$) and healthy volunteers ($n = 96$) who gave written consent as required by the Institutional Review Board. Patients and healthy volunteers were interviewed by psychiatrists or clinical psychologists and diagnosed according to DSM III-R criteria for Axis I and Axis II diagnoses using the SCID-I, SCID-II or SCID-NP as applicable. Clinical and demographic characteristics are listed in Table 1. Suicidal behavior was recorded on the Columbia Suicide History Form. Patients presented were referred for treatment at a university teaching hospital.

Table 1 Demographic and Clinical Characteristics

Characteristic	Patients	Healthy volunteers
Sex (M/F)	179/215	51/45
Age (y), mean ± SD	38.9 ± 13.6	40.9 ± 15.9
Race ratio (Caucasians/African–Americans/ Hispanics/Asians/Others)	280/45/55/7/7	70/8/12/5/1
Lifetime diagnoses, N (%)*		
Major depression	208 (61)	0 (0)
Bipolar disorder	52 (15)	0 (0)
Schizophrenia	83 (24)	0 (0)
Comorbid diagnoses, N (%)		
Alcoholism	97 (32)	0 (0)
Substance abuse disorder	83 (26)	0 (0)
At least 1 suicide attempt	132 (42)	0 (0)

*Multiple diagnoses applied in the patient population.

Volunteers had responded to advertisements. Psychiatric subjects were divided clinically as follows: a definite history of major depressive episode ($n = 208$), definitely no such history ($n = 183$) or indeterminate ($n = 3$); a definite history of alcohol abuse ($n = 97$) or definitely no such history ($n = 297$); a definite history of substance abuse disorder ($n = 83$) or definitely no such history ($n = 311$); a definite history of bipolar or unipolar depression ($n = 52$), definitely no such history ($n = 339$) or indeterminate ($n = 3$); a definite history of at least one suicide attempt ($n = 132$) or definitely no such history ($n = 262$); and, last, a definite history of schizophrenia ($n = 83$), definitely no such history ($n = 311$) or indeterminate ($n = 3$). Because of the high rate of comorbidities in the patient population, the comparisons were done both with the entire sample of patients and between the different diagnostic categories. Data for those with indeterminate history were omitted from the applicable analyses. There were no significant differences in demographics between patients and healthy volunteers for age and sex.

DNA Isolation from Blood Samples

Venous blood samples were collected in EDTA Vacutainers (Becton Dickinson, Franklin Lakes, NJ). The blood samples were first centrifuged at low speed (150*g*) for 15 min at room temperature to obtain platelet-rich plasma (PRP) fractions. After removal of PRP layers, the remaining blood fractions were centrifuged at 750*g* for 15 min to obtain the buffy coat fractions, which were further purified by layering the individual fraction on top of a 4-ml Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient. The tubes were centrifuged at 750*g* for 15 min in a Sorvall GLC-2B centrifuge (Kendro Laboratory Products, Asheville, NC). The white cell interface layers were transferred into new plastic tubes, and 3 ml of phosphate-buffered saline (PBS) buffer was added into each fraction. The fractions were then further centrifuged at 11 000*g* in a Sorvall RC-5B centrifuge for 5 min at 4°C. PBS buffer (3 ml) was used to wash the white cell pellets, which were further centrifuged at 11 000*g* for 5 min. The supernatants were discarded and the pellets were stored at −20°C for DNA extraction.

Extraction of DNA from Buffy Coat Fraction

DNA extraction from lymphocyte pellets was performed as described by Higuchi (1992). In brief, thawed lymphocyte pellets were resuspended in 3 ml PBS buffer. The suspensions were centrifuged at 11 000*g* for 5 min at 4°C and the supernatants were discarded. Pellets were resuspended in 500 µl of PCR buffer (50 mM KCl, 10 mM Tris HCl, pH 8.3, 2.5 mM $MgCl_2$, 0.1 mg/ml gelatin, 0.45% NP40, 0.45% Tween 20) containing 12 µg proteinase K. After incubation at 50–60°C for 1 h, the proteinase K was inactivated by heating at 95°C for 10 min. The samples were diluted with 10 mM Tris-EDTA buffer at 1:5 dilution for PCR. Genomic DNA fractions were stored at −20°C.

Polymerase Chain Reaction

The h5-HTR_{1B} G861C polymorphism was typed by PCR and *Hinc*II restriction enzyme digestion, as described previously (Sidenberg *et al*, 1993). Briefly, the oligonucleotide primers sense Mann22 (5′-TGAACACCGACCACATCCT-3′) and antisense Mann23 (5′-CTCAGGATCCATTGCCCTT-3′) were used to amplify a PCR fragment of 712 bp length. PCR was carried out in a 25-µl volume, containing 100 ng DNA, 1 pmol of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2 mM $MgCl_2$, 0.01% gelatin, 200 µM of each dNTP and 1 U of Red Tag DNA Polymerase (Fisher Scientific, Hampton, NH). Samples were processed in a GeneAmp PCR System 2400 (Perkin-Elmer, Norwalk, CT). A total of 30 temperature cycles were carried out, consisting of 30 s at 94°C, 40 s at 60°C and 30 s at 72°C, followed by a final extension step of 72°C for 4 min. The PCR fragments were digested with *Hinc*II restriction enzyme (Gibco BRL, Rockville, MD), which cuts them into two fragments of 570 and 142 bp when guanine is present at nucleotide 861 and into three fragments of 310, 260 and 142 bp if cytosine is present at this position. The digested PCR products were separated on a 1.0% agarose gel.

Statistical Analysis

Statistical analyses were performed using SPSS software (SPSS, Chicago, IL; 1999 edition), GB-STAT School Pak software (Dynamic Microsystems, Silver Spring, MD; 1997 edition) and SAS software (SAS Institute, Cary, NC). Genotype and allele frequencies were contrasted in patients and healthy volunteers using a contingency table χ^2 statistic with Yates's correction whenever indicated. Hardy–Weinberg equilibrium was determined in each diagnostic and demographic categories by comparing the genotype frequencies with expected ones by two-tailed χ^2 tests and Fisher's exact test. An Armitage linearity tendency test (Armitage 1955) was applied to detect the gene dose effects and trends for the susceptible alleles. The effects of gender and race on the clinical variables were determined by odds ratio on the EasyStat software (Nee 2001). The significance level was $p < 0.05$ for two-tailed tests. Data are reported as mean ± SD unless otherwise indicated.

RESULTS

Genotype and allele frequencies of the human 5-HTR_{1B} G861C polymorphisms were determined from DNA samples of 394 unrelated psychiatric patients with or without a history of mood disorder, major depression, suicide attempt, schizophrenia, alcoholism, or substance abuse disorder and 96 unrelated healthy volunteers. Groups did not differ significantly in age and sex distribution (Table 1). Comparisons were between all patients and healthy volunteers as well as between the different diagnostic categories.

The h5-HTR_{1B} G861C genotype distributions were in Hardy–Weinberg equilibrium as assessed by the χ^2 test (data not shown). In the combined groups of patients and healthy volunteers, no differences of genotype distribution and allele frequencies were observed between males and females ($\chi^2 = 0.99$, df = 2, $p = 0.610$; $\chi^2 = 0.42$, df = 1, $p = 0.520$, respectively; data not shown). There were also no differences in the genotype distribution and allele frequencies between male and female patients ($\chi^2 = 1.71$, df = 2, $p = 0.425$; $\chi^2 = 1.26$, df = 1, $p = 0.261$, respectively). Thus, males and females were combined for subsequent analysis.

Genotype frequencies for the G861C polymorphism were 56, 39 and 5%, respectively, for all subjects studied, and the frequencies for the 861G and 861C alleles were 76 and 24%. No differences in genotype distribution and allele frequencies were observed between the entire patient group and the healthy volunteer group ($\chi^2 = 0.51$, df = 2, $p = 0.774$; $\chi^2 = 0.02$, df = 1, $p = 0.899$, respectively; Table 2). Comparison of genotype distribution and allele frequency between healthy volunteers and patients with specific psychiatric disorders are listed in Table 2. No statistically significant differences were observed compared with healthy volunteers.

Within the patient group (see Table 3), statistically significant differences of genotype distributions and allele frequencies were observed in the patients with, compared to without, a history of substance abuse ($\chi^2 = 9.51$, df = 2, $p = 0.009$, Fisher's exact $p = 0.009$; $\chi^2 = 7.31$, df = 1, $p = 0.007$, respectively), and in patients with, compared to without, a history of major depression ($\chi^2 = 6.83$, df = 2, $p = 0.033$, Fisher's exact $p = 0.034$; $\chi^2 = 5.81$, df = 1, $p = 0.016$, respectively). There was a significant dose effect for the risk associated with the 861C allele for substance abuse and major depression as assessed by the Armitage linearity tendency test ($\chi^2 = 7.20$, df = 1, $p = 0.008$; $\chi^2 = 6.80$, df = 1, $p = 0.009$, respectively).

No significant association of genotype or allele frequencies was observed within the patient group with alcoholism, bipolar disorders, schizophrenia, or a history of suicide attempts (see Table 3).

In the subgroup of patients with a major depressive episode, significant differences of genotype distribution and allele frequencies were confirmed between the patients with and without a history of substance abuse disorder ($\chi^2 = 13.20$, df = 2, $p = 0.001$; $\chi^2 = 7.01$, df = 1, $p = 0.008$, respectively; Table 4). Effects of gender and race on the genotype distribution and allele frequencies were also assessed. To determine whether the relationship between substance abuse disorder and the h5-HTR_{1B} G861C locus

Table 2 Genotype Distributions and Allele Frequencies for the Human 5-HT$_{1B}$ Receptor Gene G861C Locus in Healthy Volunteers and Psychiatric Patients with Major Depression, Alcoholism, Substance Abuse Disorder, Bipolar Disorders, Schizophrenia, and Suicide Attempt

	Genotype distribution (%)						Allele frequency (%)			
	N	GG	GC	CC	χ^2	*p*	G	C	χ^2	*p*
Healthy volunteers	96	54 (56)	36 (38)	6 (6)		144 (75)	48 (25)			
Patients with history of										
Major depressive episode	208	104 (50)	92 (44)	12 (6)	1.23	0.542	300 (72)	116 (28)	0.42	0.518
Bipolar disorders	52	33 (63)	17 (33)	2 (4)	0.88	0.645	83 (80)	21 (20)	0.62	0.430
Schizophrenia	83	50 (60)	30 (36)	3 (4)	0.76	0.684	130 (78)	36 (22)	0.38	0.528
Alcoholism	97	48 (50)	44 (45)	5 (5)	1.24	0.538	140 (72)	54 (28)	0.27	0.606
Substance abuse disorder	83	37 (44)	38 (46)	8 (10)	2.59	0.275	112 (67)	54 (33)	2.12	0.145
Suicide attempt	132	77 (58)	47 (36)	8 (6)	0.10	0.951	201 (76)	63 (24)	0.03	0.866
Patients (all)	394	221 (56)	155(39)	18 (5)	0.51	0.774	597 (76)	191 (24)	0.02	0.899

Two-tailed χ^2 tests, compared with the healthy volunteer group.

Table 3 Genotype Distributions and Allele Frequencies for the Human 5-HT$_{1B}$ Receptor Gene G861C Locus Among the Psychiatric Patients who have Different Comorbid Psychopathology, and Suicide Attempts

	Genotype distribution (%)						Allele frequency (%)			
	N	GG	GC	CC	χ^2	*p*	G	C	χ^2	*p*
Major depressive episode										
With	208	104 (50)	92 (44)	12 (6)			300 (72)	116 (28)		
Without	183	115 (63)	62 (34)	6 (3)	**6.83**	**0.033**[a]	292 (80)	74 (20)	**5.81**	**0.016**
Bipolar disorders										
With	52	33 (63)	17 (33)	2 (4)			83 (80)	21 (20)		
Without	339	186 (55)	137 (40)	16 (5)	1.35	0.509	509 (75)	169 (25)	0.86	0.355
Schizophrenia										
With	83	50 (60)	30 (36)	3 (4)			130 (78)	36 (22)		
Without	308	169 (55)	124 (40)	15 (5)	0.84	0.656	462 (75)	154 (25)	0.61	0.435
Alcoholism										
With	97	48 (50)	44 (45)	5 (5)			140 (72)	54 (28)		
Without	297	173 (58)	111 (37)	13 (5)	2.28	0.319	457 (77)	137 (23)	1.56	0.211
Substance abuse disorder										
With	83	37 (44)	38 (46)	8 (10)			112 (67)	54 (33)		
Without	311	184 (59)	117 (38)	10 (3)	**9.51**	**0.009**[b]	485 (78)	137 (22)	**7.31**	**0.007**
Suicide attempt										
With	132	77 (58)	47 (36)	8 (6)			201 (76)	63 (24)		
Without	262	144 (55)	108 (41)	10 (4)	1.85	0.397	396 (76)	128 (24)	0.01	0.931

Two-tailed χ^2 tests, comparisons between the patients with and without a history of major depression, alcoholism, substance abuse disorder, bipolar disorders, schizophrenia, and suicide attempts. [a]Fisher's exact test (one-tail): $p = 0.034$; Armitage linearity tendency test: $\chi^2 = 6.80$, df = 1, $p = 0.009$; boldface signifies statistically significant. [b]Fisher's Exact test (one-tail): $p = 0.009$; Armitage linearity tendency test: $\chi^2 = 7.20$, df = 1, $p = 0.008$; boldface signifies statistically significant.

was explained by gender, we examined the relationships of sex and substance abuse disorder to the h5-HTR$_{1B}$ G861C locus. The effect of substance abuse disorder on the h5-HTR$_{1B}$ G861C locus (odds ratio, OR = 1.67; 95% confidence intervals CI = 1.14–2.44; $\chi^2 = 6.96$, $p = 0.008$) was independent of sex ($\chi^2 = 0.04$, $p = 0.837$). Including sex in the model, an association was significant between major depression and the G861C locus (OR = 1.64, CI = 1.16–2.31; $\chi^2 = 7.81$, $p = 0.005$), whereas that of sex was not ($\chi^2 = 0.23$, $p = 0.630$). Including sex in the model confirmed the absence of a relationship of genotype to bipolar disorder, alcoholism or suicide attempt history.

The frequency of the 861C allele for African–Americans, Caucasians, Hispanics and Asians/Others was 20, 22, 36 and 38%, respectively. We determined that allele frequencies in African–Americans and Caucasians in the entire study population were not statistically different ($\chi^2 = 0.173$, df = 1, $p = 0.678$), but there was a significant difference between Hispanics and Caucasians ($\chi^2 = 10.70$, df = 1, $p = 0.001$) and between Asians/Others and Caucasians ($\chi^2 = 4.21$, df = 1, $p = 0.040$). Thus, the possibility of race influence on the relationship between substance abuse and G861C locus was examined. Limitations of sample size and distribution of allele frequency required us to subdivide our study population into two subgroups (African–Americans/Caucasians *vs* Hispanics/Asians/Others). We determined that the relationship of substance abuse disorder to the h5-HTR$_{1B}$ G861C locus (OR = 1.79, CI = 1.23–2.60; $\chi^2 = 9.08$, $p = 0.003$) was significant, whereas its relationship to race was not ($\chi^2 = 0.53$, $p = 0.471$). We also found that major depression is associated with the h5-HTR$_{1B}$ G861C locus (OR = 0.46, CI = 0.31–0.68; $\chi^2 = 14.93$, $p = 0.001$), independent of race, which has no association with major depression ($\chi^2 = 0.32$, df = 1, $p = 0.574$). Thus, after includ-

Table 4 Genotype Distributions and Allele Frequencies for the Human 5-HT_{1B} Receptor Gene G861C Locus in the Major Depressive Patients with or without History of Alcoholism, Substance Abuse Disorder, and Suicide Attempt

	Genotype distribution (%)						Allele frequency (%)			
	N	**GG**	**GC**	**CC**	χ^2	***p***	**G**	**C**	χ^2	***p***
Sex										
M	71	33 (46)	32 (45)	6 (8)			98 (69)	44 (31)		
F	137	71 (52)	60 (44)	6 (4)	1.63	0.443	202 (74)	72 (26)	0.17	0.683
Alcoholism										
With	56	27 (48)	26 (46)	3 (5)			80 (71)	32 (29)		
Without	152	77 (51)	66 (43)	9 (6)	0.16	0.925	220 (72)	84 (28)	0.00	0.947
Substance abuse disorder										
With	41	15 (42)	19 (50)	7 (8)			49 (60)	33 (40)		
Without	167	89 (53)	73 (44)	5 (3)	**13.20**	**0.001**[a]	251 (75)	83 (25)	**7.01**	**0.008**
Suicide attempt										
With	77	46 (60)	26 (33)	5 (7)			118 (77)	36 (23)		
Without	131	58 (44)	66 (50)	7 (5)	5.46	0.065	182 (69)	80 (31)	2.13	0.145

Two-tailed χ^2 tests, comparisons between the major depressive patients with or without a history of alcoholism or substance abuse disorder or suicide attempts.
[a]Fisher's exact test (one-tail): $p = 0.003$; boldface signifies statistically significant.

ing race in the model, an association was present between both substance disorder and major depression with the h5-HTR_{1B} G861C locus. Similar analyses found no relationship of the h5-HTR_{1B} G861C locus to alcoholism, bipolar disorder or suicide attempt history. Using the general linear model, with genotype as the dependent variable and substance abuse, alcoholism, major depressive episode, suicide attempt history, bipolar disorder, and schizophrenia all included in the model, only relationships of substance abuse and major depression to genotype were statistically significant ($F = 7.54$, $p = 0.006$; $F = 8.87$, $p = 0.003$, respectively).

DISCUSSION

We found a statistically significant association of substance abuse and major depression with the h5-HTR_{1B} genotype in the patient population (Table 3). Substance abusers had a higher 861C allele frequency than nonabusers in the patient group (33 *vs* 22%). We did not find an association between the h5-HTR_{1B} gene and alcoholism. The present results are not directly comparable to findings by either Lappalainen *et al* (1998) or Fehr *et al* (2000), who did not separate substance abuse from alcoholism. Lappalainen *et al* reported a linkage and significant association of the G861C locus in Finnish subjects with antisocial alcoholism (which implies a combination of aggressive traits and alcoholism) who had a higher 861C allele frequency of 32% compared with unaffected subjects (26%). In a separate group of Southwestern American Indians, antisocial alcoholism was also linked to the G861C polymorphism but not linked with alcoholics without antisocial personality feature. It is possible that the results of Lappalainen *et al* (1998) reflect an association with other types of substance abuse and not alcoholism since they did not examine substance abuse apart from alcoholism.

Fehr *et al* (2000) reported results that differ from those of Lappalainen *et al* (1998): namely, a lower frequency of the 861C allele (26%) in nonantisocial alcohol-dependent patients compared with 34% in a normal healthy volunteer group in a German population. Lappalainen reported an even higher 861C allele frequency in male alcohol-dependent patients, but no significant association in females. Our population of alcoholic abusers was mostly nonantisocial (96%) and had an 861C allele frequency of 28%, compared with 23% in our nonalcoholic patient population. Perhaps with a larger sample we would confirm the findings of Lappalainen *et al* (1998). Across our entire study population we also found allele frequencies (76 and 24%) similar to those reported by Lappalainen *et al* (1995) in their diverse populations of Finns, American–Indians and Caucasians (72 and 28%), and similar to those reported by Fehr *et al* (2000) in their German population (73 and 27%). Thus, it is not clear why the results of Fehr *et al* (2000) differ from Lappalainen *et al* (1998), but other comorbid psychopathology in the study population is one possibility. Our reported allele frequencies are slightly different from those of our previous, postmortem study, 83 and 17% (Huang *et al*, 1999), probably because the postmortem population was selected on the basis of brain availability and negative toxicology for drugs and CNS medications.

Consistent with our results, Kranzler *et al* (2002) reported that there was no evidence of allelic association of the *HTR1B* gene with alcoholism and antisocial substance dependence in their study population of European–Americans and African–Americans. They genotyped three polymorphisms (namely G861C, C129T and G-261T) and found a near-complete disequilibrium between G861C and C129T loci, as we had previously suggested (Huang *et al*, 1999). In agreement with our conclusion, they also noticed that genotyping of the T129C locus is unlikely to provide any additional useful information. The finding of no association between antisocial substance dependence and the 5-HTR_{1B} G861C locus is not inconsistent with our finding regarding nonantisocial patients with substance abuse. We observed a statistically significant association between genotype and substance abuse. Our patients with substance abuse were mostly nonantisocial. Differences in the phenotypes in the populations may have contributed to the different findings.

Our finding is quite robust because it was independent of sex and race and, even in the subgroup of patients with

major depression, statistically significant differences of genotype distribution and allele frequencies were confirmed in patients with or without a history of substance abuse. No association was observed with a history of alcohol abuse, regardless of the presence of a major depressive episode. Our patients are mostly not antisocial. In fact, Lappalainen *et al* (1998) did not find an association between this G861C locus with nonantisocial alcoholism in the population of Southwestern American–Indians, raising the possibility that the antisocial group may be important to their finding.

We did not find an association with suicide attempts, although there was such a trend in the major depressive patients with a history of suicide attempts ($\chi^2 = 5.46$, df = 2, $p = 0.065$; Table 4). Nishiguchi *et al* (2001) found no relationship between the h5-HTR_{1B} gene G861C polymorphism and suicide victims in a Japanese population. The possibility of the association between the h5-HTR_{1B} G861C locus and suicide requires further study where allowance can be made for the effect of associated psychiatric illness and for gender and ethnicity.

We found that allele frequencies varied widely among our ethnic groups. The frequencies of the allele 861C range from 20% in African–Americans to 36% in Hispanics and 42% in Asians. In our combined population of African–American and Caucasian patients, a significant relationship was observed between substance abuse disorder and the genotype distribution of the h5-HTR_{1B} G861C locus, even when we included race in the model. Interestingly, a stronger association was found between substance abuse disorder and the h5-HTR_{1B} G861C genotype ($\chi^2 = 14.72$, df = 2, $p = 0.001$) in the subpopulation of Caucasian patients with a history of major depression.

We found a significant association between the G861C locus and a major depressive disorder that was independent of race and gender. In contrast, no association was found with bipolar disorder. This agrees with a report of a negative linkage finding (Mundo *et al*, 2001).

The present findings are consistent with some, but not all, of the results of our postmortem study of alcoholics (Huang *et al*, 1999). In that study, no association of suicide, alcoholism, major depression, or a history of pathological aggression was found with 5-HTR_{1B} genotypes or allele frequencies for the G861C and C129T polymorphisms. Although we did not find an association of the 861C allele with alcoholism, the frequency of the 861C allele in the postmortem study was lower than that reported by Lappalainen *et al* (1998) or in the present study. We found, however, that people with the 5-HT_{1B} 861C allele had 20% fewer 5-HT_{1B} binding sites in the prefrontal cortex. In our previous postmortem study, using a matched pair design to control for variance owing to age, sex and postmortem delay, 25% fewer 5-HT_{1B} binding sites were found in the group with alcoholism (Arango *et al*, 1995). Thus, that study suggested that there may be an association of fewer 5-HT_{1B} receptors with both the 861C allele and alcoholism. Like Lappalainen *et al* (1998), we did not find an association with alcoholism without antisocial personality disorder in either our previous study or the present one.

Although the 5-HT_{1B} receptor has been identified in many regions of the human central nervous system, its role in psychopathology is still being determined. The observation of increased aggressive behavior as well as increased alcohol and cocaine intake in 5-HT_{1B} receptor gene knockout mice (Saudou *et al*, 1994; Ramboz *et al*, 1996; Crabbe *et al*, 1996; Rocha *et al*, 1998) and the findings of an increased level of lifetime aggression and an increased rate of alcoholism and substance abuse in patients who are at risk for suicidal acts (Mann and Arango, 1998) led us to hypothesize that there is a possibility that abnormalities in the h5-HTR_{1B} receptor gene (*HTR1B*) may contribute to human psychopathologies such as suicide, aggressive behavior, major depression and alcoholism or substance abuse disorder. This study did not support an association with alcoholism or suicidal behavior. Whether the h5-HTR_{1B} gene is associated with depression and substance abuse disorders remains to be tested in larger samples. Consistent confirmations by other independent investigators are required to verify the present finding.

In conclusion, we found a significant association of the h5-HTR_{1B} gene G861C locus with substance abuse disorders and a major depressive disorder. Further studies are needed in a larger number of patients with subtypes of mood disorders as well as substance abuse to replicate our results and to evaluate independent associations with alcoholism, antisocial personality features and pathological aggression.

ACKNOWLEDGEMENTS

This work was partly supported by Public Health Service grants MH40210 and MH46745 and grants from The Diane Goldberg Foundation and The St Louis Community Foundation.

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