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Relationship of Psychopathology to the Human Serotonin$_{1B}$ Genotype and Receptor Binding Kinetics in Postmortem Brain Tissue

Yung-yu Huang, M.S., Régis Grailhe, Ph.D., Victoria Arango, Ph.D., René Hen, Ph.D., and J. John Mann, M.D.

Knockout of the 5-HT$_{1B}$ gene in mice results in increased aggression, as well as alcohol and cocaine consumption. Given the clinical association of aggression, suicide, alcoholism, and substance abuse, we studied relationship of psychopathology to the human 5-HT$_{1B}$ receptor gene (N = 178) and postmortem human 5-HT$_{1B}$ receptor binding (N = 96) in the brain. The sample comprised: 71 suicide victims, 107 nonsuicides, 45 with a history of major depression and 79 without, 64 with a history of a alcoholism or substance abuse and 60 without, as well as 36 with a history of pathological aggression and 42 without. Single-strand conformational polymorphism (SSCP) analysis and DNA sequencing techniques were used to screen the coding region of the human 5-HT$_{1B}$ receptor gene in genomic DNA isolated from postmortem human brain tissue. Two common polymorphisms were identified in the 5-HT$_{1B}$ receptor gene, involving a silent C to T substitution at nucleotide 129 and a silent G to C substitution at nucleotide 861 of the coding region. These polymorphisms were found with the same frequency in the suicide and the nonsuicide groups and in those with and without a history of major depression, alcoholism, or pathological aggression. The binding indices (B_{max} and K_D of the 5-HT$_{1B}$ receptor in prefrontal cortex also did not differ in suicides and controls, major depression, alcoholism, and cases with a history of pathological aggression. The C129 or G861 allele had 20% fewer 5-HT$_{1B}$ receptor compared to the 129T or 861C allele. We did not identify a relationship between suicide, major depression, alcoholism, or pathological aggression with 5-HT$_{1B}$ receptor binding indices or genotype.

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KEY WORDS: *Serotonin; Human 5-HT$_{1B}$ gene; Suicide; Polymorphisms*

Several neuropsychiatric disorders such as mood disorders, substance abuse, or alcoholism have been associated with dysfunctions of the central serotonergic system (Coppen 1972; Goodwin and Post 1983; Ballenger et al. 1979; Virkkunen et al. 1994). The risk for suicidal behavior and aggressive acts has been suggested to be related to a common underlying predisposition to impulsive behavior that is modulated by serotonergic activity (Mann 1998). In rodents and primates, aggressiveness is increased after inhibition of serotonin synthesis or in association with lower serotonergic activity (Vergnes et al. 1986; Molina et al. 1987; Higley et al. 1992). Both suicidal acts and aggression have a genetic contribution in terms of cause or diathesis that is independent of the heritability of major psychiatric disorders (Brent et al.

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1996; Roy 1986; Roy et al. 1991; Schulsinger et al. 1979). The mechanism whereby genetics can affect aggressive and suicidal behavior is not known. The serotonergic system is one possibility because serotonergic activity is under substantial genetic control (Ishikawa et al. 1989; Higley et al. 1992; van Harten 1993). Patients at risk for suicidal acts not only have an increased level of lifetime aggression (Mann and Arango 1998), but also an increased rate of alcoholism and substance abuse. Alcoholism and substance abuse may facilitate suicidal acts, or may share a common predisposing factor with suicide and aggression, such as lower serotonergic activity. Genetic factors play a role in alcoholism (Schuckit et al. 1985) and major depression (Gershon et al. 1989) but their precise nature is unknown. Again, the serotonergic system may play a role.

Recently, aggressive behavior as well as increased alcohol and cocaine intake have been reported in 5-HT$_{1B}$ receptor gene knockout mice (Saudou et al. 1994; Ramboz et al. 1996; Crabbe et al. 1996; Rocha et al. 1998). This set of observations raises the possibility that abnormalities in the 5-HT$_{1B}$ receptor gene may contribute to human psychopathologies such as suicide, aggression, major depression, alcoholism, or substance abuse.

Molecular cloning techniques have revealed the existence of two humans 5-HT$_{1D}$ receptor subtypes: 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ (Hamblin and Metcalf 1991; Demchyshyn et al. 1992; Weinshank et al. 1992; Levy et al. 1992). The mouse 5-HT$_{1B}$ receptor is the homologue of the human 5-HT$_{1D\beta}$. We have adopted the recommendation that the human 5-HT$_{1D\beta}$ receptor be renamed human 5-HT$_{1B}$ (h5-HT$_{1B}$) receptor (for a review see Hartig et al. 1996). The pattern of expression of this receptor is similar among mammalian species with a predominant expression in projections areas of raphe neurons as well as of striatal neurons (Boschert et al. 1994). This receptor inhibits transmitter release from nerve terminals (Hoyer and Middlemiss 1989; Martin and Humphrey 1994).

A common polymorphism at the human 5-HT$_{1B}$ receptor locus (G861C) was identified by Hinc II restriction enzyme (Sidenberg et al. 1993) and by SSCP and PCR-RFLP methods (Lappalainen et al. 1995). An uncommon molecular variant with the substitution of a cysteine for a phenylalanine residue (F124C) has been detected in the human 5-HT$_{1B}$ (5HT$_{1D\beta}$) receptor gene (Nöthen et al. 1994). Neither of these polymorphisms have been found to be associated with a phenotype. We decided to focus on the more common polymorphism (G861C). We have an extensive brain bank of cases with available clinical information. This has allowed us to evaluate the relationships of genotype to both neurochemical and psychopathological phenotype.

We studied the 5-HT$_{1B}$ receptor gene and receptor binding in postmortem brain samples from a series of cases subdivided into: 1) those who died by suicide versus other causes; 2) those with a history of alcoholism or substance abuse versus without; 3) those with a history of major depression versus those without; 4) and those with a history of pathological aggression versus those without such a history. We postulated a relationship between these clinical phenotypes, prefrontal or cortical 5-HT$_{1B}$ binding, and genotype.

MATERIALS AND METHODS

Brain Tissue

Human brain samples (N = 178) were collected at the autopsy from Medical Examiner's Office after consent was obtained from the next of kin, as required by the Institutional Review Board. Brain samples were free of neurological diseases and cases were negative for psychotropic drugs on toxicological analysis of body fluids. Clinical and demographic characteristics and cause of death in the suicides and nonsuicides are shown in Table 1. Prefrontal cortical tissue (Brodmann area 9) was dissected for the membrane binding studies because we have previously reported changes in levels of binding to the serotonin transporter and other serotonin receptors in suicide victims in this brain area (Arango et al. 1990; Stanley and Mann 1983; Stanley et al. 1982). The cerebellum was used for genomic DNA extraction. Tissue was stored at −70°C until the assay. A diagnosis of suicide was based on a consensus between the Medical Examiner and a clinical expert on suicide (JJM). Clinical and demographic information was obtained in most cases by our previously published psychological au-

Table 1. Demographic and Characteristics of Suicides and Nonsuicides

	Nonsuicides (N)	Suicides (N)
Sex		
Male	82	52
Female	25	19
Age (yr)		
Total	41.6 ± 15.6	42.0 ± 19.4
Female	44.3 ± 12.8	39.1 ± 16.0
Male	40.8 ± 16.3	43.0 ± 20.5
Race ratio (White:black:other)	55 : 35 : 17	51 : 8 : 12
Postmortem interval (hr)	12.6 ± 8.4	12.2 ± 8.8
Cause of death (%)		
Natural	55 (51)	–
Homicide	12 (11)	–
Motor vehicle accidents	23 (22)	–
Fall from height	–	18 (25)
Hanging	–	24 (34)
Gun shot wound	–	23 (32)
Other	17 (16)	6 (9)

topsy method (Kelly and Mann 1996), and in all the cases data were obtained from the Medical Examiners notes, police interviews, old hospital records, as well as toxicology and autopsy results. DSM-III-R criteria were used to make an Axis I diagnosis. Pathological aggression was defined as a history of aggression resulting in an injury to another person requiring medical attention or property damage resulting in legal consequences. We studied the human 5-HT_{1B} receptor gene (N = 178) and postmortem human 5-HT_{1B} receptor binding (N = 96) in the brain. The sample comprised: 71 suicide victims, 107 nonsuicides, 45 with a history of major depression and 79 without, 64 with a history of a alcoholism or substance abuse and 60 without, as well as 36 with a history of pathological aggression and 42 without.

Membrane Preparation for Binding Study

A total of 200 mg of dissected brain tissue was homogenized in ice-cold 0.32 M sucrose and membrane homogenate centrifuged for 12 min at 4°C at 500g. The pellet was resuspended in 10 mM sucrose and centrifuged for 12 min at 500g. Resulting supernatants were combined and centrifuged at 35,0000g for 12 min. The membrane pellet (P_2) was resuspended with 50 mM Tris-HCl buffer (pH 7.7) and incubated at 37°C for 20 min to eliminate endogenous serotonin. At the end of pre-incubation, homogenates were immediately centrifuged at 4°C for 10 min at 35,000g. The P_2 pellet was resuspended in the incubation buffer (50 mM Tris-HCl, pH 7.7, containing 10 μM pargyline, 100 nM 8-OH-DPAT, 4 mM $CaCl_2$, and 0.1% ascorbic acid) and membrane fractions were diluted to a final concentration of 12 mg wet wt/ml for the binding assay. Four hundred microliters of membrane fraction were used in each assay tube. Total incubation volume was 500 μl and 100 nM 8-OH-DPAT were used to mask 5-HT_{1A} sites. 5-Hydroxy[^{3}H] tryptamine ([^{3}H]5-HT) was used as a radioligand to measure the 5-HT_{1D} binding sites (Peroutka and McCarthy 1989). Membranes were incubated at 37°C for 30 min with [^{3}H]5-HT at one of six concentrations between 0.25–4.0 nM. The reaction was terminated by addition of 4 ml of ice-cold 50 mM Tris-HCl buffer (pH 7.7). The membrane suspensions were then immediately filtered through Whatman GF/B filters using a 24-channel cell harvester (Brandel, Gaithersburg, MD, U.S.A.). The filters were further washed with two 4-ml rinses of ice-cold Tris-HCl buffer (pH 7.7). All filters had been soaked in 0.5% Polyethylenimine solution (Fluka Chemie) and dried prior to filtration. Nonspecific binding was determined in the presence of 5 μM sumatriptan. Binding indices, maximal number of binding sites (B_{max}), and equilibrium dissociation constant (K_D) were calculated by the EDBA/LIGAND program (Biosoft, UK). Protein content was determined by Folin-reagent procedure (Lowry et al. 1951).

DNA Isolation

Frozen brain tissue samples (150–200 mg) were placed into 1.5 ml microfuge tubes and thawed. A total of 0.5 ml of lysis buffer, containing 0.25 m NaCl, 1% SDS, 5 × TE buffer, pH 8.0, and 0.5 mg of proteinase K enzyme was added to each tube. Tubes were incubated in a water bath at 55°C for at least three hours with occasional shaking. When the brain samples were dissolved, tubes were removed, cooled, and equal volumes of phenol/chloroform/isoamyl alcohol mixture (25:24:1; Sigma) were added. Tubes were vortexed and centrifuged for 5 min in a microcentrifuge at 13,000g. An amount equal to 0.5 ml of the aqueous upper layer was transferred to clean tubes and DNA precipitated with equal volume of ice-cold absolute alcohol. After a further wash with 70% ice-cold alcohol, DNA filaments were dried *in vacuo*. Genomic DNA fractions were suspended in 1 × TE buffer.

Polymerase Chain Reaction (PCR)

Initially, a sense primer, 5′-CCTCTCCTTCGTCCGCTCCAT-3′, corresponding to coding region of the bases 1–21 of the human 5-HT_{1B} receptor gene sequence, and an antisense primer, 5′-TGGTCCCCAAAGGTCGCTTAG-3′, corresponding to bases 1253–1273, were use to produce a 1273 bp fragment. Standard PCR was carried out in a 100 μl volume, containing 1 μg genomic DNA, 5 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 2.5 U of AmpliTaq DNA Polymerase (Perkin-Elmer Cetus). Samples were processed in a GeneAmp PCR system 480 (Perkin-Elmer). After initial 5 min denaturation at 94°C, Taq polymerase and [α-^{32}P]dCTP (NEN; Du Pont) were added to the tubes to initiate PCR amplification. Forty temperature cycles were carried out, consisting of 1 min at 94°C, 1.5 min at 58°C, and 1.5 min at 72°C, followed by a final extension step of 72°C for 7 min. Another two sets of the primers were used to produce shorter fragments in order to heighten the sensitivity of the detection. Primers 3 and 4, 5′-CGGAGCTGGGGCGAGGAGA-3′ (corresponding to the bases 39–57) and 5′-GCTCATCACCTTGGCCACCA-3′ (bases 231–250);, respectively and primers 5 and 6, 5′-CCTGCTCTCCTCATCGCCCTCTA-3′ (bases 723–743) and 5′-GGGTTGATGAGGGAGTTGAGA-3′ (bases 1137–1157), respectively.

Single-strand Conformational Polymorphism (SSCP) Analysis

SSCP analysis was based on the protocol of Orita et al. (1989). Radioisotopic PCR products were subjected to various digestions by the restriction enzymes *EaeI*, *StyI*, *BmyI*, and *SacII*, according to the manufacturer's in-

structions. Four microliters of the radioisotopic PCR product were mixed with 30 μl of SSCP gel-loading buffer containing 9.5 ml formamide (Fluka Chemie), 0.4 ml 0.5 M EDTA (pH 8.0), 0.05 ml 10% bromophenol blue (w/v), and 0.05 ml 10% xylene cyanol (w/v) and denatured for 5 min at 95°C. Samples were quickly chilled on ice water and then 5 μl of the samples were loaded on a 8% polyacrylamide gel (acrylamide:bisacrylamide 49:1). Electrophoresis was carried out in a cold room for six hours at 20 watts. The gels were dried and bands were visualized using autoradiography.

Statistical Analysis

Nominal variables, such as diagnosis were evaluated by the Chi^2 test with Yates' correction. Student's *t*-test was used for continuous variables. Separate effects or interactions among genotypes, 5-HT$_{1B}$ binding and phenotype subgroup variables were determined by the general linear models procedure. All tests were 2-tailed. Data were reported as mean ± standard deviation (SD) unless otherwise specified.

RESULTS

As shown in Table 1, there were no significant differences between suicides and nonsuicides in age, sex ratio, or postmortem interval (PMI). Cause of death was mostly an acute cardiac event in the nonsuicides.

The coding region of the 5-HT$_{1B}$ receptor gene was screened for the presence of DNA sequence variation in genomic DNA fractions isolated from postmortem brain tissues of 178 unrelated suicides and nonsuicides. The entire coding sequence of the intronless 5-HT$_{1B}$ receptor gene was obtained by PCR. Mutations were detected by SSCP analysis. Changes in the electrophoretic mobilities were observed for fragments digested by restriction enzyme EaeI on neutral polyacrylamide gels (Figure 1). Sequencing of this 1273 bp fragment revealed two common polymorphisms: a C to T substitution at nucleotide 129; and a G to C substitution at nucleotide 861 (Figure 2). To confirm these results, two sub-fragments containing the polymorphism sites were produced with PCR amplification. After digestion with *SacII* restriction endonuclease for the first sub-fragment (primers 3 and 4) and *StyI* restriction endonuclease for the second sub-fragment (primers 5 and 6), variations were easily detected by SSCP analysis (Figure 3) in each of the fragments. The two polymorphisms at nucleotide 129 and nucleotide 861 position of the coding region were in total linkage disequilibrium.

Genotype frequencies for both polymorphisms (nucleotides C129T and G861C) in human 5-HT$_{1B}$ receptor were 72%, 21%, and 7% in the suicide group and 70%, 27%, and 3% in the nonsuicide group. Frequencies for the C and T alleles were 82% and 18%, respectively, in the suicide, and 84% and 16%, respectively, in the nonsuicide groups (Table 2). There were no differences in genotype distribution or allelic frequency between suicide and nonsuicide groups (see Table 2). There was also no significant difference in the frequency of genotypes in the cases with or without major depression (Table 2). Moreover, no significant differences were found in the frequency of genotypes in those with versus without a history of pathological aggression, or those with versus without a history of alcoholism (Table 2).

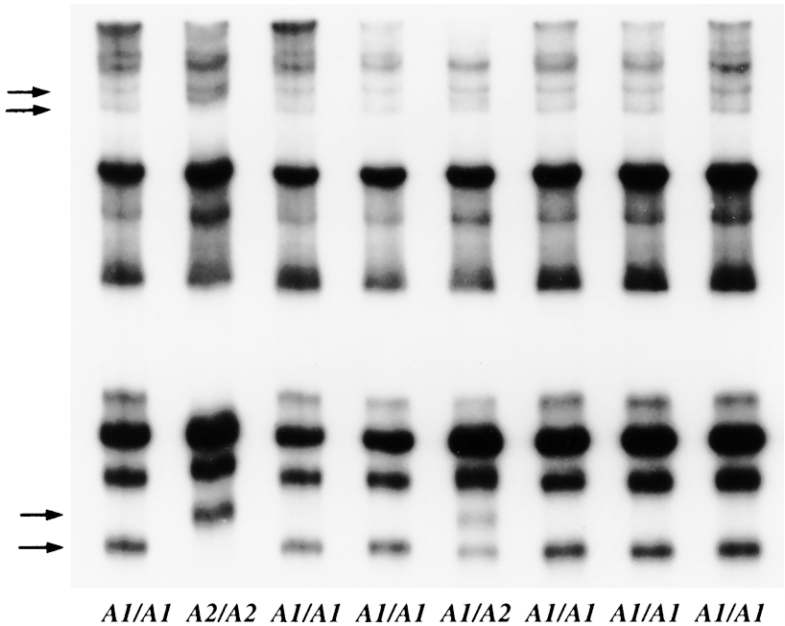

Figure 1. SSCP analysis patterns of PCR fragment (1273 bp) after digestion with *EaeI* restriction endonuclease. Homozygote or mutant variants are denoted with signs A1/A1 or A2/A2; heterozygote is denoted with a sign A1/A2.

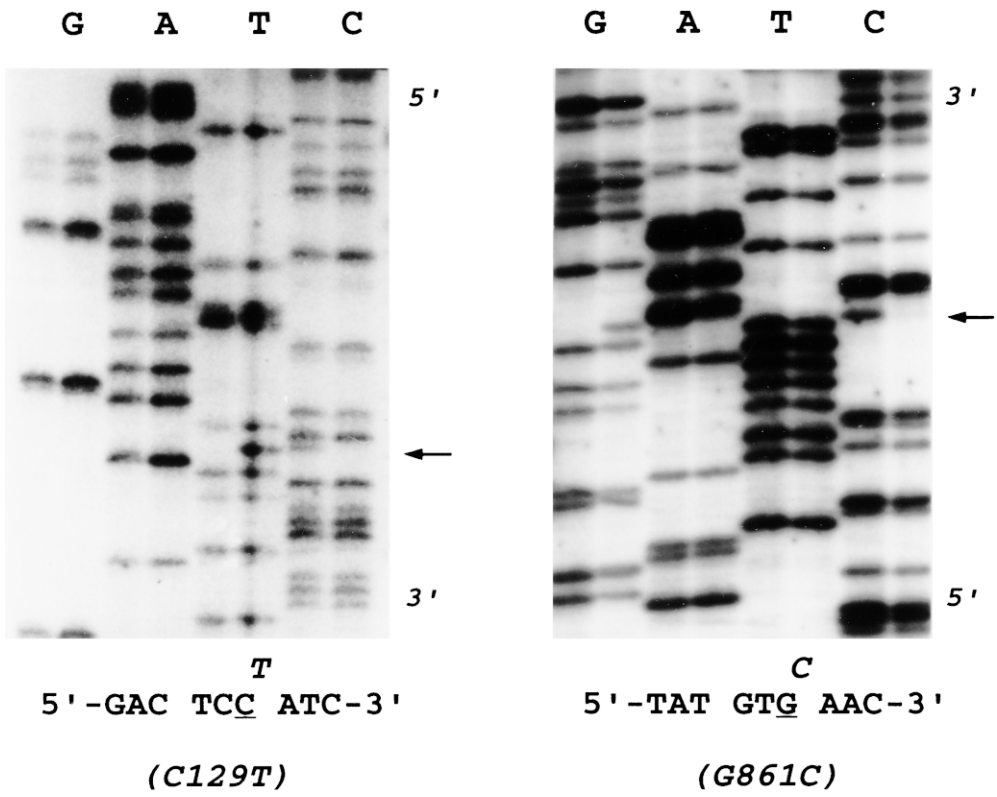

Figure 2. DNA sequence analysis revealing two polymorphic sites in the human 5-HT_{1B} receptor gene. Following the separation of DNA strands, single-stranded DNA was sequenced by the dideoxy nucleotide chain terminal reaction method (*fmol*® DNA sequencing system, Promega). The C to T substitution at nucleotide 129 and G to C substitution at nucleotide 861 of the coding region are marked with arrows.

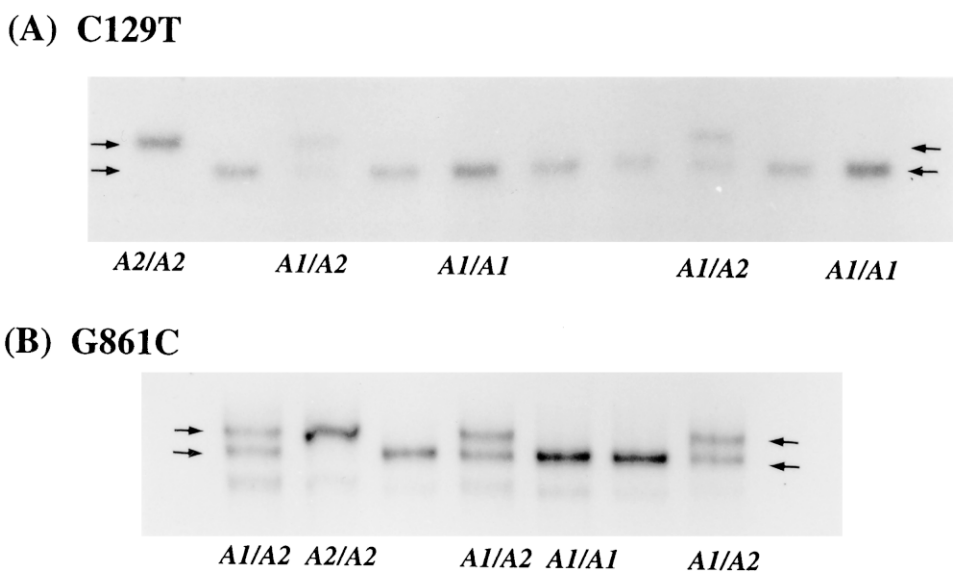

Figure 3. SSCP analysis patterns of PCR sub-fragments. After digestion with *SacII* restriction endonuclease for the first sub-fragment **(A)** and *StyI* restriction endonuclease for the second sub-fragment **(B)**, variations were observed by SSCP analysis. The variations in electrophoresis patterns are due to conformational changes of C to T substitution at nt129 in the first sub-fragment (primers 3 and 4) and G to C substitution at nt 861 in the second sub-fragment (primers 5 and 6). Homozygote or mutant variants are denoted with signs A1/A1 or A2/A2; heterozygote is denoted with the sign A1/A2. Differences in the migration pattern are marked with arrows.

Table 2. Genotype Distribution and Allele Frequencies for the Human 5-HT1$_B$ Receptor Gene and Psychopathology: A Comparison of Suicides Versus Nonsuicides, Subjects with Major Depression Versus Without, Subjects with Alcoholism Versus Without and Subjects with a History of Pathological Aggression Versus Without

		Genotype Distribution (%)						Allele Frequency (%)			
		N	G861G	G861C	C861C	χ^2	*p*	G861	861C	χ^2	*p*
Suicide	Yes	71	51 (72)	15 (21)	5 (7)			117 (82)	25 (18)		
	No	107	75 (70)	29 (27)	3 (3)	2.34	.310	179 (84)	35 (16)	0.03	.870
Major depression	Yes	45	32 (71)	12 (23)	1 (2)			76 (84)	14 (16)		
	No	79	57 (72)	18 (23)	4 (5)	0.76	.685	132 (84)	26 (16)	0.00	.995
Alcoholism	Yes	64	47 (73)	16 (25)	1 (2)			110 (86)	18 (14)		
	No	60	40 (67)	16 (27)	4 (7)	2.24	.327	96 (80)	24 (20)	1.16	.281
Pathological aggression	Yes	36	25 (72)	10 (21)	1 (7)			60 (83)	12 (17)		
	No	42	28 (70)	9 (27)	5 (3)	2.44	.290	65 (77)	19 (23)	0.53	.467

The mean B_{max} values for 5-HT$_{1B}$ binding sites were 58.2 ± 32.2 fmol/ml protein (N = 42) in the suicides and 63.1 ± 29.1 fmol/mg protein (N = 54) in nonsuicides. The mean K_D was 1.2 ± 0.7 nM in the suicides (N = 42) and 1.2 ± 0.6 nM in nonsuicides (N = 54) (Table 3). No significant differences were found in B_{max} (t = −.66, df = 94, p = .508) or K_D (t = .50, df = 94, p = .618) in suicides and nonsuicides. In the entire series of 96 cases where binding data and genotyping was available, the mean B_{max} values for 5-HT$_{1B}$ binding sites differed across genotype (see Table 3). Using general linear models procedure to control for other effects (race and suicide), there was a significant different association between B_{max} values for 5-HT$_{1B}$ receptor binding and G861G and G861C genotypes (N = 91, F = 5.29, df = 1, p = .024). Variables such as race or suicide alone did not influence B_{max} values. The G861C genotype (N = 23) had 20% fewer binding sites than the G861G genotype (N = 69) (t = 1.86, df = 90, p = .067) in the overall sample (Table 3).

DISCUSSION

Two polymorphisms of the 5-HT$_{1B}$ receptor gene were identified in the genomic DNA samples obtained from 178 unrelated subjects. One of these polymorphisms had been previously reported by Lappalainen et al. (1995) at nucleotide 861 of the coding region. We identified a new one at nucleotide 129 of the coding region. This polymorphism was in absolute linkage disequilibrium with the polymorphism at nucleotide 861 identified by Lappalainen et al. (1995). Across our entire study population we found similar allelic frequencies (83% and 17%) to those reported by Lappalainen (72% and 28%). These two polymorphic sites are silent and do not change primary amino acid structure composition of the receptor. Since the two polymorphisms are in total linkage disequilibrium, they may have appeared simultaneously during evolution.

We found no association of suicide, major depression, alcoholism, or history of pathological aggression

Table 3. Relationship of B_{max} and Kd in a postmortem human 5-HT$_{1B}$ receptor to 5-HT$_{1B}$ genotypes, and to a history of suicide, or major depression, or alcoholism and/or pathological aggression

		N	Bmax (fmol/mg protein)				Kd (nM)			
Genotype[a,b]	G861G	69	64.3 ± 30.6				1.2 ± 0.5			
	G861C	23	50.9 ± 28.0				1.4 ± 0.8			
	C861C	4	68.7 ± 52.1				0.9 ± 0.1			
		N		*t*	*df*	*p*		*t*	*df*	*p*
Suicide	Yes	42	58.9 ± 34.0				1.2 ± 0.7			
	No	54	63.2 ± 29.1	−0.66	94	.51	1.2 ± 0.6	0.50	94	.62
Major depression	Yes	19	62.5 ± 30.5				1.2 ± 0.6			
	No	27	57.7 ± 25.0	0.60	44	.55	1.2 ± 0.6	−0.18	18	.86
Alcoholism	Yes	26	56.2 ± 22.3				1.3 ± 0.7			
	No	20	62.9 ± 33.9	0.81	44	.42	1.3 ± 0.7	−0.15	19	.74
Pathological aggression	Yes	20	61.0 ± 27.2				1.4 ± 0.8			
	No	8	58.8 ± 33.8	−0.18	26	.86	1.1 ± 0.5	0.91	7	.39

[a] B_{max}: t = 1.86. df = 90, p = .067 (G861G vs. G861C), t = −0.27, df = 71, p = .790 (G861G vs. C861C), t = −0.67, df = 3, p = .549 (G861C vs. C861C).
[b] K_D: t = −0.918, df = 28, p = .366, t = 0.960, df = 71, p = .340, t = 0.986, df = 25, p = .334.

with 5-HT_{1B} genotypes or allelic frequency for the two polymorphism. However, these findings do not imply that 5-HT_{1B} receptor gene has no role in neuropsychiatric disorders. Our study population was small and could have missed an uncommon association. While this manuscript was under review, a study was published (Lappalainen et al. 1998) reporting an association of alcoholism with the G861C locus; alcoholics had a higher 861C allele frequency. In a separate group of Southwestern American Indians, a condition described as antisocial alcoholism (implies a combination of aggressive traits and alcoholism) was also linked to G861C (Lappalainen et al. 1998). Our sample included only 64 alcoholics and further study of this possible association in a larger sample would be of interest. The fact that homozygotes were found in our entire study population in a Mendelian ratio suggests that there is no selection pressure against that mutation, therefore, no major detrimental consequence of that mutation.

Our present findings show an allelic association of the 5-HT_{1B} receptor gene with 5-HT_{1B} binding kinetics in prefrontal cortex in the entire study population. There was a significant association between B_{max} values for 5-HT_{1B} receptor binding and both G861G and G861C genotypes when race and suicide were considered in the general linear model ($N = 91$, $F = 5.29$, $df = 1$, $p = .024$). It is possible that the less frequent alleles (129T and 861C) may be associated with decreased 5-HT_{1B} binding. At the least, heterozygotes had less binding than G861 homozygotes ($t = 1.86$, $df = 90$, $p = .067$; Table 3). The reduced receptor levels associated with G861C genotypes is interesting in the light of the association found between this genotype and antisocial alcoholism (Lappalainen et al. 1998).

Our study found no difference in 5-HT_{1B} binding parameters between suicide, major depression, alcoholism, or individual with pathological aggression and nonsuicides (Figure 4). Arranz et al. (1994) reported that there was no difference in B_{max} and K_D between the suicide and nonsuicide groups for the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} binding sites in postmortem frontal cortex samples. However, they found a significant decrease in the number of 5-HT_{1B} binding sites and in the K_D in nondepressed suicides. We found that the presence or absence of major depression made no detectable difference to the results. On the other hand, Lowther et al. (1997) reported that there were significantly more 5-HT_{1B} receptor binding sites in globus pallidus, but not in the frontal or parietal cortices from the antidepressant-free suicides who died by violent means. We did not examine the globus pallidus of suicides who died by violent means and therefore cannot comment on this result. The absence of altered binding in prefrontal cortex in their study is in agreement with our results.

Although 5-HT_{1B} receptor appears to play a role in the modulation of aggressive behavior and alcohol or cocaine consumption in animal models (Olivier and Most 1990; Saudou et al. 1994; Crabbe et al., 1996; Rocha et al., 1998), we found no differences in 5-HT_{1B} binding kinetics in association with alcoholism or pathological aggression. The mean number of 5-HT_{1B} receptor binding sites appeared to be about 10% lower in cases who were suicides, or with major depression and alcoholism. Although these differences were not statistically significant, they were in the hypothesized direction. Given the retrospective data gathering method involving a psychological autopsy, we may have missed relevant clinical data and underestimated rates of substance abuse and pathological aggression. Further studies are needed in larger samples of pathologically aggressive patients as well as alcoholics.

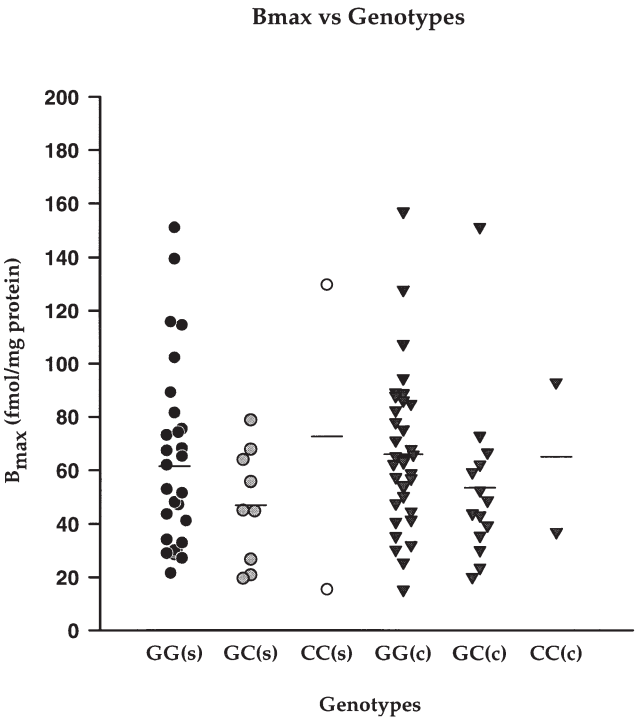

Figure 4. Human 5-HT_{1B} receptor number (B_{max}, fmol/mg protein) in cerebral cortex and 5-HT_{1B} genotype in suicides and nonsuicides [s = suicide group (circles), c = nonsuicide group (triangles)].

A difference in B_{max} values between suicide and nonsuicide groups, or in major depression, alcoholism, or aggression would suggest the possibility that the level of expression of 5-HT_{1B} receptors may be altered. That would indicate the need to seek mutations in the promoter region that may be associated with psychopathology.

In summary, the PCR product of the 5-HT_{1B} receptor gene was digested with several restriction enzymes and evaluated by SSCP analysis. Two polymorphisms, one not previously reported, were identified in the human 5-HT_{1B} receptor gene that did not alter amino acid com-

position. We were unable to identify a relationship among 5-HT$_{1B}$ genotypes binding indices and psychopathology. A weak correlation between 5-HT$_{1B}$ receptor number and 5-HT$_{1B}$ genotypes was observed. Further studies in a larger population are needed, as well as studies towards the identification of other polymorphisms in the 5-HT$_{1B}$ gene that may modulate the risk for suicide, aggression, depression, and alcoholism.

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