ORIGINAL ARTICLE

A Serotonin Transporter Gene Promoter Polymorphism (5-HTTLPR) and Prefrontal Cortical Binding in Major Depression and Suicide

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Background: Major depression and suicide are associated with fewer serotonin transporter (5-HTT) sites. The 5′-flanking promoter region of the 5-HTT gene has a biallelic insertion/deletion (5-HTTLPR). We assayed prefrontal cortical (PFC) 5-HTT binding in major depression and suicide and examine the relationship to the 5-HTTLPR allele.

Methods: Postmortem brain samples from 220 individuals were genotyped for the 5-HTTLPR polymorphism. Binding of 5-HTT was assayed by quantitative autoradiography in the PFC of a subset of subjects (n=159). Clinical information, including *DSM-III-R* Axis I diagnoses, was obtained by psychological autopsy and medical chart review.

Results: Binding to 5-HTT was lower in the ventral PFC of suicides compared with nonsuicides and was lower throughout the PFC of subjects with a history of major depression. The 5-HTTLPR genotype was associated with major depression but not with suicide or 5-HTT binding.

Conclusions: A diffuse reduction of 5-HTT binding in the PFC of individuals with major depression may reflect a widespread impairment of serotonergic function consistent with the range of psychopathologic features in major depression. The localized reduction in 5-HTT binding in the ventral PFC of suicides may reflect reduced serotonin input to that brain region, underlying the predisposition to act on suicidal thoughts. The 5-HTTLPR genotype was not related to the level of 5-HTT binding and does not explain why 5-HTT binding is lower in major depression or suicide.

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MAJOR DEPRESSION[1-25] and suicidal behavior[16,26-46] are independently related to altered serotonergic system indices in the brain, cerebrospinal fluid (CSF), and platelets.[47-51] Two questions arise: Do serotonergic abnormalities in major depression involve different brain regions compared with the diathesis for suicide? Second, what factors cause these alterations in serotonergic function? Genetic factors partly explain the risks for major depression[52] and for suicide.[53,54] It is not known which genes are involved. Candidate genes that warrant investigation are those related to the serotonergic system. Genetic factors affect serotonergic activity, as indicated by the heritability of CSF levels of 5-hydroxyindoleacetic acid in nonhuman primates[55] and the reported association of suicidal acts and serotonergic indices, such as CSF levels of 5-hydroxyindoleacetic acid and the prolactin response to fenfluramine hydrochloride therapy, with an intronic polymorphism in the gene for tryptophan hydroxylase.[56-59] The most widely reported serotonergic abnormality in major depression and suicide involves the serotonin transporter (5-HTT).[51] Fewer platelet 5-HTT sites and reduced platelet serotonin uptake have been reported.[49,51] Both functional imaging and postmortem brain studies indicate less 5-HTT binding in the brain in depressed patients.[60,61] Because platelet 5-HTT binding seems to be unrelated to levels of brain 5-HTT binding,[51,61] direct studies of the brain are necessary to determine the state of 5-HTT binding in major depression.

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A preliminary study[46] of suicides reported that changes in 5-HTT and 5-HT_{1A} binding are localized to the ventral prefrontal cortex (PFC), an area involved in behavioral inhibition and, therefore, the potential location of a diathesis for suicidal behavior. Impulsive, aggressive behaviors are more common in suicide at-

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SUBJECTS, MATERIALS, AND METHODS

SUBJECTS

All cases (N=220) had a postmortem delay of less than 24 hours and had died without a prolonged agonal period. Next of kin consented to assay of the tissue and review of relevant medical and forensic records. With informed consent, 1 or more informants per case agreed to an interview for the purpose of a psychological autopsy. The protocol was approved by the relevant institutional review boards. Cause of death was determined by the Allegheny County, Pa, coroner (n=105) or the New York, NY, medical examiner (n=115). Suicide required evidence of intent and a self-inflicted fatal act. Nonsuicide controls were selected based on suicide not being the manner of death. Cases of uncertain manner of death were excluded. Subjects were diagnosed according to *DSM-III-R* criteria based on all available information from results of a psychological autopsy performed an average of 5.5 months after death according to a previously reported method,[79] coroner's or medical examiner's records, hospital records, and police reports. Psychological autopsy interviews were conducted by trained staff: a psychiatric social worker (T.M.K.) or a psychologist (S.O.). The diagnosis was made in conjunction with a psychiatrist (J.J.M.). We validated the psychological autopsy results for Axis I and Axis II disorders. We used the Structured Clinical Interview I and II for diagnosis, the Suicide History Form (Columbia University, New York, NY) for past suicidal acts, the Brown Goodwin Aggression History Scale for lifetime aggression, and a checklist for demographic data and other clinical details. The review of hospital records was done by an experienced psychiatrist (J.J.M.) using a checklist of criteria for *DSM-III-R* diagnoses. A diagnosis of alcoholism was based on the psychological autopsy and autopsy findings, such as cirrhosis combined with a blood or brain alcohol level greater than 0.15%. A history of pathological aggression was defined as a history of aggression resulting in arrest, major property damage, or injury to another individual that required medical attention or was fatal.

The 220 cases (**Table 1**) included 82 people who had committed suicide and 138 nonsuicide controls. Subjects were divided clinically as follows: a definite lifetime history of major depressive episode either bipolar or unipolar (n=53), definitely no lifetime history of a major depressive episode (n=107), and indeterminate (n=60); a definite history of alcoholism or substance abuse (n=86), definitely no history of alcoholism or substance abuse (n=83), and indeterminate (n=51); and a definite history of pathological aggression (n=38), a definite history of no pathological aggression (n=92), and indeterminate (n=90). All indeterminate individuals were omitted from the applicable analyses. Other major diagnoses included schizophrenia (27%), schizophreniform reaction or schizoaffective disorder (12%), dysthymia (1%), and bipolar disorder (2%). All subjects had toxicologic screening of blood, urine, and bile and a clinical history. Most subjects had brain toxicologic screening for selected psychotropic drugs and alcohol (28% had positive brain screen results in the face of negative results of peripheral body fluid screening) to ensure drug-free status. Drugs screened for included benzodiazepines, tricyclics, antipsychotics, most drugs of abuse, lithium, trazodone hydrochloride, bupropion hydrochloride, selective serotonin reuptake inhibitors, amphetamine sulfate, caffeine, and alcohol. Other than drugs for medical conditions, results were as follows: positive screening results included benzodiazepines (n=6), cocaine/benzoylecognine (n=10), opiates (n=14), carbon monoxide (n=11), antipsychotics (n=2), caffeine (n=3), antidepressants (n=6), lithium (n=3), barbiturates (n=2), cannabis (n=5), and antihistamines (n=5). Some cases had more than 1 positive drug test result, and any cases with monoaminergic-related drugs did not have 5-HTT binding assayed. Subjects with evidence of a neurologic disorder on history or at autopsy were excluded.

We compared 162 cases who had complete psychological autopsies with 58 cases where sources of data were limited to the medical examiner's toxicologic and autopsy results, police reports, calls to physicians, and available hospital records. No differences were found in terms of genotype; age; postmortem interval to autopsy; 5-HTT binding; race, sex ratio; and rates of major depression, alcoholism, or pathological aggression. We also compared 159 cases who had 5-HTT binding assayed with 61 who did not. No differences were found except for a lower rate of major depression (112 of 159 vs 24 of 61; $\chi^2_3=18.8$, $P<.001$) and a higher rate of whites (28 of 103 vs 25 of 57; $\chi^2_1=4.6$, $P=.03$) in the group that had 5-HTT assayed. Therefore, it is likely that results found in cases with a complete psychological autopsy are relevant for cases without, and cases with 5-HTT binding closely resemble cases without 5-HTT binding.

Race distribution in the whole population was 136 whites, 28 Hispanics, 49 African Americans, and 7 Asians. Suicide cases differed from nonsuicide controls in race composition ($\chi^2_3=9.8$; $P=.02$) because of fewer African Americans (9 of 82 vs 40 of 138). There were more African Americans in the group without depression (30 of 107 compared with 5 of 53, $\chi^2_3=8.4$; $P=.04$).

tempters,[62-65] consistent with a common diathesis. We hypothesized that ventral PFC dysfunction underlies the diathesis for impulsive aggression and suicide.[64] Major depression involves more diverse psychopathologic features, suggesting involvement of more brain areas than only the ventral PFC.

A 44–base pair (bp) deletion/insertion polymorphism (5-HTTLPR) in the 5′ flanking regulatory region of the human 5-HTT gene results in differential expression of 5-HTT and serotonin reuptake in transformed lymphoblastoid cell lines.[66] The short form of the 5-HTTLPR locus is associated with 40% fewer binding sites in the homozygote (SS) and the heterozygote (SL) compared with the long form (LL). Therefore, fewer 5-HTT sites on platelets of depressed patients, and fewer 5-HTT sites in the PFC of suicides, may be because of an association of the short 5-HTTLPR allele with major depression or suicide.

Results of studies[67-71] disagree regarding an association of the 5-HTTLPR genotype with mood disorders.

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TISSUE COLLECTION AND PREPARATION

Autoradiographic receptor assays of coronal sections of PFC were conducted on 159 cases selected based on toxicologic findings and tissue availability. Our tissue collection and preparation procedures have been detailed previously.[46,80] Coronal serial sections (20 µm) (n=150) were taken from generally the right hemicerebrum, just anterior to the genu of the corpus callosum, and thaw-mounted onto 7.6×12.7-cm gelatin-subbed, acid-cleaned glass slides. An intercalating set (every 200 µm) was stained for Nissl substance for anatomical reference. Two adjacent sections (every 1000 µm) (n=6) per brain were used for each receptor assay. Tissue sections were desiccated and stored in sealed boxes at –20°C for 24 hours and at –80°C thereafter.

AUTORADIOGRAPHIC BINDING ASSAY

Details of this assay are provided in Arango et al.[46]

^{3}H-Cyanoimipramine Binding

Sections were preincubated for 30 minutes at 23°C in 50-mmol/L Tris-hydrochloride buffer with 130-mmol/L sodium chloride and 5-mmol/L potassium chloride (pH 7.4). Total binding was determined by incubation (4°C) in the same buffer containing 0.4-nmol/L ^{3}H-cyanoimipramine for 24 hours. Nonspecific binding was defined by coincubation of adjacent sections with 1-µmol/L sertraline hydrochloride (modification of Kovachich et al[81]). Nonspecific binding was less than 20% of total binding. Sections were washed in cold buffer for 1 hour (3×20 minutes). Following the washes, the slides were dipped in distilled water (4°C) and the tissue was dried with cold, filtered air and vacuum desiccated overnight at 4°C. Dried sections were arranged in x-ray cassettes along with tritium standards and exposed to ^{3}H-sensitive film for 17 to 20 weeks. At the end of the exposure period, the films were developed.

Quantitation of Autoradiograms

Autoradiograms were analyzed using a PC-based image analysis system (MCID; Imaging Research Inc, Hamilton, Ontario). Films were digitized, shade-corrected, and calibrated against tritium standards. Binding to the 5-HTT in the cortical gray matter was mostly bilaminar. We report specific binding over the entire thickness of the gray matter (layers I-VI). Brodmann areas 8, 9, 46, 45, 47, 11, 12, 32, and 24 were identified using gyral and sulcal landmarks, cytoarchitecture, and a standardized coronal atlas (Robert Perry, MD, and Edward Bird, MD, written communication, 1985; available on request). We selected areas to analyze for this study as representative of lateral (46), dorsal (9), and ventral (45 and 47) PFC. We used subtracted images to measure specific binding, in femtomoles per milligram of tissue, in gyrus and sulcus separately for each Brodmann area. Standards were corrected daily for radioactive decay. All samples were coded so that laboratory staff were masked to the diagnosis during assays and analyses.

GENOTYPING

All subjects included in the study underwent genotyping. DNA was extracted from frozen brain tissue (150-200 mg) according to a previously published method.[82] We used a modification of the amplification protocol of Lesch et al.[66] Briefly, oligonucleotide primers flanking the 5-HTPPLPR 5′GGCGTTGCCGCTCTGAATGC-3′ (–1416 to –1397) and 5′-GAGGGGACTGAGCTGGACAACCAC-3′ (–910 to –888) were used to produce 484- or 528-bp fragments. Polymerase chain reaction was carried out in a final volume of 25 µL containing 50 ng of genomic DNA, 100 ng of each primer, 50-mmol/L potassium chloride, 10-mmol/L Tris-hydrochloride (pH 8.3), 1.5-mmol/L magnesium chloride, 0.01% gelatin, 2.5 mmol/L of each deoxynucleotide triphosphate (deoxyguanosine triphosphate [dGTP]/7-deaza-2′-dGTP and dGTP equal amount), and 0.8 U of AmpliTaq DNA polymerase (Promega, Madison, Wis). Two microliters of α-phosphorus 32–labeled deoxycytidine triphosphate (111 TBq/mmol New England Nuclear, Du Pont, Boston, Mass) was added to the cocktail mixture. Samples were processed in a polymerase chain reaction system (GeneAmp PCR System 2400; Perkin-Elmer PE-Biosystems, Foster City, Calif). A modified quick hot start was devised by initial heating of the instrument to 94°C. After placement of the tubes into the thermoblock, the reaction was proceeded to denature for an additional 5 minutes. Thirty-five temperature cycles were carried out, consisting of 30 seconds at 94°C, 30 seconds at 61°C, and 40 seconds at 72°C, followed by a final extension step of 72°C for 7 minutes. Electrophoresis was carried out in a 6% nondenatured polyacrylamide gel (19:1) at room temperature for 16 hours at 100 V. The gels were dried and bands were visualized by autoradiography.

STATISTICS

For continuous variables we used analysis of variance and multiple regression analyses. For nominal variables such as genotype and diagnosis, we used contingency tables; where both types of variables were used to predict group membership, logistic regression was used. Results are reported as mean±SD. Differences were considered significant at $P<.05$ (2 tailed).

However, none of these studies measured 5-HTT binding in the same subjects. Aggressive and impulsive traits, alcoholism, and substance abuse all carry an elevated risk of suicide and are also associated with serotonergic abnormalities.[72,73] There is disagreement about whether the 5-HTTLPR genotype is associated with harm avoidance temperament,[66] alcoholism,[74-76] anxiety, or impulsive traits.[77,78]

We report the first study to examine the relationship of the 5-HTTLPR genotype to PFC 5-HTT binding, major depression, and suicide. Antemortem psychiatric state was assessed and 5-HTT binding was assayed in multiple areas of the PFC by autoradiography on slide-mounted sections.

RESULTS

CLINICAL AND DEMOGRAPHIC FEATURES

Age and postmortem delay did not differ significantly between suicides and nonsuicide controls or between those

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Table 1. Demographic and Clinical Features of the Study Population (N = 220)

	Lifetime Major Depression Status Known (n = 160)*			
	History (n = 53)	No History (n = 107)	Suicides (n = 82)	Nonsuicide Controls (n = 138)
Age, mean ± SD, y	44 ± 20	42 ± 17	43 ± 20	42 ± 16
Sex, M/F, No.	37/16†	91/16	61/21	107/31
Race, white/Hispanic/black/Asian, No.	40/7/5/1‡	58/16/30/3	59/11/9/3§	77/17/40/4
Postmortem delay, mean ± SD, h	17 ± 6	16 ± 6	16 ± 6	16 ± 6
Major depression history, yes/no, No.	53	107	43/20‖	10/87
Alcoholism or substance abuse history, yes/no/indeterminate, No.	21/31¶/1	58/48/1	29/35/18	58/48/32
History of assaultive aggression, yes/no/indeterminate, No.	14/32/7	31/69/7	23/36#/23	25/67/46

*Lifetime major depressive status was indeterminate for 60 individuals.
†Sex ratio in major depression: $\chi^2_1 = 5.1$; P = .02 (relatively more women with major depression).
‡Major depression vs no major depression: $\chi^2_3 = 8.4$; P = .04.
§Suicide vs nonsuicide: $\chi^2_3 = 9.8$; P = .02.
‖ Rate of major depression in suicides vs nonsuicide controls: $\chi^2_1 = 57.9$; P<.001. The denominator is less than the total for either suicides or nonsuicide controls because we could not rule in or rule out depression in all cases. The same applies to alcoholism and substance abuse or aggression.
¶Rate of alcoholism in suicides vs nonsuicide controls ($\chi^2_1 = 1.4$; P = .23) and in individuals with vs without major depression ($\chi^2_1 = 2.9$; P = .09).
#Rate of pathological aggression in suicides vs nonsuicide controls: $\chi^2_1 = 2.3$; P = .13.

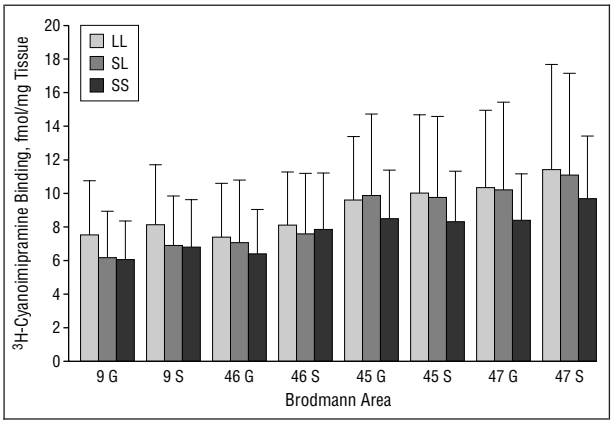

Figure 1. *Mean±SD serotonin transporter (5-HTT) binding in Brodmann areas in the prefrontal cortex subdivided by sulcus (S) and gyrus (G): comparison of 5-HTTLPR genotypes. SS indicates short form of the 5-HTTLPR locus in the homozygote (n=33); SL, short and long form of the 5-HTTLPR locus in the heterozygote (n=74); and LL, long form of the 5-HTTLPR locus (n=48).*

with vs without a history of major depression (Table 1). Men outnumbered women in the overall sample by about 3:1 (168 men and 52 women), but the proportions were similar across the subgroups of those with and without suicide, alcoholism, substance abuse, or pathological aggression. However, there were relatively more women in the major depression group (χ^2_1=5.1; *P*=.02). Rates of a history of pathological aggression or alcoholism or substance abuse did not distinguish suicides or the group with a history of major depression. There was a strong association of suicide and major depression. In a logistic regression in which suicide was the dependent variable and sex, major depression, and pathological aggression were independent variables, major depression correlated with suicide (χ^2_1=39.2; *P*<.001). None of the other variables were independently significant correlates of suicide, although aggression showed a trend (χ^2_1=2.77; *P*=.10). Aggression correlated with alcoholism and substance abuse (χ^2_1=22.5; *P*<.001).

RELATIONSHIP BETWEEN REGIONAL PFC 5-HTT BINDING AND GENOTYPE

No effect of genotype on 5-HTT binding in the PFC was apparent in any of the Brodmann areas (**Figure 1**). Lesch et al[66] reported that the LL genotype differs from the SL and SS genotypes in having 40% more 5-HTT binding sites in transformed lymphoblastoid cells. However, we found that the LL group did not differ significantly in 5-HTT binding from the combined SL and SS groups, and the SL and SS genotypes did not differ in 5-HTT binding from each other. Moreover, controlling for a history of major depression or sex made no difference to the result.

SEX EFFECTS AND PFC 5-HTT BINDING AND GENOTYPE

Women had lower 5-HTT binding compared with men in most Brodmann areas studied (**Figure 2**). The mean difference was 25.4% (range, 18.0%-34.0%). This effect was confirmed in a logistic regression model in which all brain regions were compacted as repeated measures into a single independent variable and sex was a dependent variable (χ^2=46.8; *P*<.001). The sex difference was statistically significant in all Brodmann areas within the LL genotype (women<men) (data not shown). Although sex contrasts were not statistically significant within the other genotypes, this was possibly because of the smaller sample sizes. No association of genotype and sex was found (χ^2_2=1.96; *P*=.38). We found a significant effect for sex on 5-HTT binding to the gyrus of Brodmann area 9 ($F_{149,2}$=8.341; *P*<.001), but no effect of genotype (*P*=.80) and no significant interaction effect (*P*=.54). Results in other Brodmann areas were similar. Postmortem delay and age did not correlate with 5-HTT binding (data not shown).

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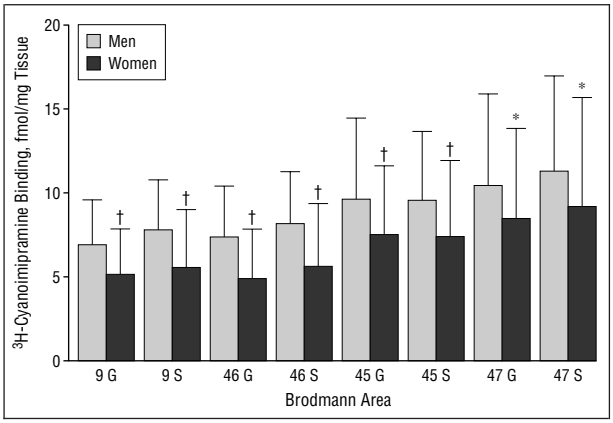

Figure 2. *Mean ± SD serotonin transporter (5-HTT) binding in Brodmann areas in the prefrontal cortex subdivided by sulcus (S) and gyrus (G): comparison of men (n=124) and women (n=35). Asterisk indicates* P<*.1; dagger,* P<*.01 (both by analysis of variance).*

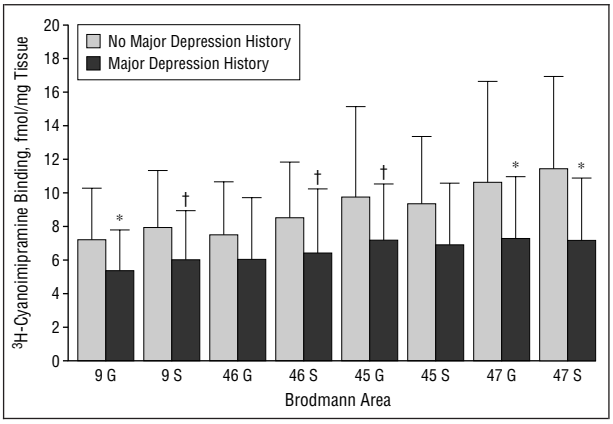

Figure 3. *Mean ± SD serotonin transporter (5-HTT) binding in Brodmann areas in the prefrontal cortex subdivided by sulcus (S) and gyrus (G): comparison of 5-HTT binding in subjects with (n=29) vs without (n=71) a history of major depression. Asterisk indicates* P<*.01; dagger,* P<*.05 (both by analysis of variance).*

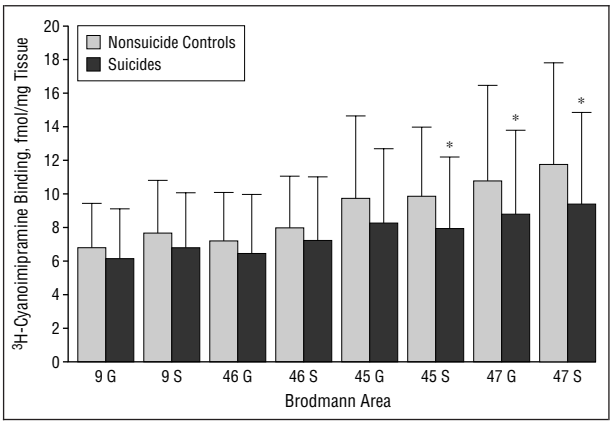

Figure 4. *Mean ± SD serotonin transporter (5-HTT) binding in Brodmann areas in the prefrontal cortex subdivided by sulcus (S) and gyrus (G): comparison of suicides (n=60) and those dying of other causes (n=99). Asterisk indicates* P<*.05 by analysis of variance.*

RELATIONSHIP OF PFC 5-HTT BINDING TO MAJOR DEPRESSION, SUICIDE, AND OTHER PSYCHOPATHOLOGIC DISORDERS

Binding of 5-HTT to the PFC of patients with a history of major depression was significantly lower in the gyrus and sulcus of most Brodmann areas compared with those without a history of major depression (**Figure 3**). The mean difference across brain regions was 35% (range, 23%-63%). Mean reduction in binding in men with vs without depression was 33% (range, 17%-65%) in contrast to depressed vs nondepressed women (19%; range, 10%-26%). More women had a history of major depression (Table 1), and women had lower 5-HTT binding than men. Moreover, suicide was associated with major depression (Table 1) and lower 5-HTT binding. Therefore, to determine whether lower 5-HTT binding was associated with major depression, independently of sex and suicide, we included sex, suicide status, and binding together in a single logistic regression model. All were independent significant correlates of major depression ($P<.001$ for 5-HTT binding to all brain regions as a single compact variable, suicide, and sex), and the overall model was highly significant ($\chi^2=14.1$, $P<.001$).

In contrast to major depression, 5-HTT binding to the PFC of suicides was lower only in the orbital or ventral PFC (18% lower in Brodmann areas 45 and 47) (**Figure 4**) compared with nonsuicide controls. Because of the higher rate of suicide in cases with a history of major depression (Table 1), and the association of lower 5-HTT binding to major depression, we examined the interrelationship of major depression and suicide to 5-HTT binding in the PFC. Within the group with a history of major depression, suicides had lower 5-HTT binding than nonsuicide controls (6.5±3.5 vs 8.4±1.2, $F=4.88$; $P=.03$). In the group without major depression, suicides did not differ from nonsuicides ($F=1.93$; $P=.17$). An analysis of variance that included sex and suicide groups as independent variables and 5-HTT binding to the ventral PFC (a single compacted variable based on data from Brodmann areas 45 and 47) as the dependent variable confirmed that the effect of suicide ($F_{151,1}=19.3$; $P<.001$) on binding was independent of sex ($F=18.9$; $P<.001$). Binding of 5-HTT was not different in those with vs without a history of pathological aggression or alcoholism or substance abuse (data not shown).

RELATIONSHIP OF 5-HTTLPR GENOTYPE TO SUICIDE, MAJOR DEPRESSION, AND OTHER PSYCHOPATHOLOGIC DISORDERS

Allelic frequencies for the L allele were .580 and .570, and for the S allele .420 and .430 in the subjects with and without major depression, respectively, and did not significantly differ ($\chi^2_1=0.02$; $P=.87$). A statistically significant association of genotype with a history of major depression was found ($\chi^2_2=9.6$; $P=.008$) (**Table 2**), with a relatively higher rate of heterozygotes in the group with a history of major depression. However, departure from Hardy-Weinberg Equilibrium examined by a χ^2 test indicated that there was no difference in the observed frequencies of genotypes compared with predicted frequency in the groups with and without major depression. Allelic frequencies for L were .524 and .582, and for the S allele .476 and .418 in the subjects with and without a

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Table 2. Observed Frequencies for Serotonin Transporter Promoter (5-HTTLPR) Genotypes in Subjects With vs Without Major Depression*

	Subjects, No.		
Genotype	Without Depression	With Depression	Total
LL	44	15	**59**
SL	32	30	**62**
SS	27	8	**35**
Total	**103**	**53**	**156**

*χ^2_2 = 9.6; P = .008 (data are missing for 5 individuals for technical reasons, including 4 with known data on the presence or absence of major depression).

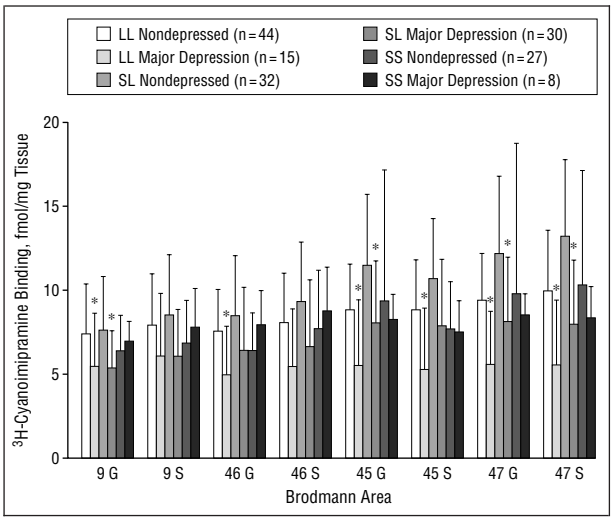

Figure 5. *Mean±SD serotonin transporter (5-HTT) binding in Brodmann areas in prefrontal cortex subdivided by sulcus (S) and gyrus (G): comparison of those with (n=29) vs without (n=71) a history of major depression within subgroups defined by 5-HTTLPR genotype. Asterisk indicates* P<.05 *by analysis of variance; SS, short form of the 5-HTTLPR locus in the homozygote; SL, short and long form of the 5-HTTLPR locus in the heterozygote; and LL, long form of the 5-HTTLPR locus.*

history of suicide, respectively (χ^2_1=1.15; *P*=.28). Genotype was not associated with suicide (χ^2_1=; *P*=.53). Genotype distribution and allelic frequency also did not differ depending on a history of alcoholism, substance abuse, or pathological aggression (data not shown).

5-HTTLPR GENOTYPE AND LOWER 5-HTT BINDING IN MAJOR DEPRESSION AND SUICIDE

To determine whether the lower 5-HTT binding in the group with a history of major depression is explained by genotype, we used dummy variables for nominal variables in the regression analysis: for genotype, SS=2, SL=1, and LL=0; and presence of major depressive episode=1 and absence of major depressive episode=0. A diagnosis of major depressive episode was a significant correlate of binding (*P*=.002), whereas genotype was not (*P*=.24). **Figure 5** illustrates how 5-HTT binding is lower in major depression independent of genotype. Suicide was associated with lower 5-HTT binding in Brodmann area 47 of the ventral PFC ($F_{1,146}$=4.9; *P*=.03), and in the same region 5-HTT binding was independent of 5-HTT genotype ($F_{2,146}$=1.131; *P*=.33). There was no significant interaction.

COMMENT

This study, the most comprehensive postmortem study of major depression and suicide thus far, to our knowledge, found that postmortem 5-HTT binding is lower throughout the dorsal-ventral extent of the PFC in individuals with a history of major depression. Previous studies report less 5-HTT binding in the occipital cortex of depressed patients dying of causes other than suicide[60] and less 5-HTT binding in vivo in the midbrain of depressed patients.[61] Hence, the reduction in 5-HTT binding associated with major depression involves the brainstem and several cortical regions. Negative results were reported by Ferrier et al[83] in a small series of elderly depressed patients but with a long postmortem delay.

We also replicated a previous study[46] of lower 5-HTT binding localized to the ventral PFC of suicides. Most, but not all, studies[31-35,40-43,46,84,85] of suicides found a reduction in 5-HTT binding, although 1 study[36] found an increase and 4 studies[37-39,86] found no change in the PFC. Negative findings might be a consequence of looking at the "wrong" brain region. Moreover, previous studies lacked information as to which suicide or nonsuicides had a history of major depression and could not distinguish effects of the two disorders.

Because we used 0.4-nmol/L ^{3}H-cyanoimipramine, approximately 3½ times its reported K_D in rat brain,[81] our results most likely reflect differences in the number of binding sites and not affinity. Previous postmortem and platelet studies[35,40,46,51] have reported that differences in 5-HTT binding in depression or suicide are because of fewer binding sites or B_{max}, and not altered K_D.

Alterations in 5-HTT binding specifically related to suicide risk, as opposed to major depression, were concentrated in the ventral PFC.[46] Acquired ventral PFC injuries are associated with disinhibition and an increase in impulsive behaviors, including aggression and suicide attempts.[87,88] Therefore, reduced serotonergic input into this brain region might underlie an impairment of behavioral inhibition or restraint and an increased propensity for suicidal acts in patients who feel depressed or hopeless.

We found that the short 5-HTTLPR allele was not more frequent in major depression, and unlike studies[66] in transformed lymphoblastoid cell lines, the S allele was not associated with less 5-HTT binding in the brain. Hence, it does not seem to explain why 5-HTT binding is lower in major depression. Perhaps regulatory mechanisms governing gene expression and 5-HTT binding sites in intact brain differ from transformed cell lines and dampen potential differences in the number of 5-HTT binding sites in the brain.

There are 16 studies[67-71,85,89-98] examining linkage or association between the 5-HTTLPR locus and mood disorders in a variety of unipolar and bipolar samples from several ethnic groups. Only 4 of 16 studies are positive,[70,71,85,90] and 1 of the positive studies[85] found an association between depressed suicides and the long form

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of the 5-HTTLPR polymorphism (the opposite of the hypothesized relationship). We did find an association, explained by a higher rate of heterozygotes in the major depressive disorder group. Therefore, it is unclear whether this locus is associated with mood disorders. Our postmortem brain results suggest that even if this locus were associated with mood disorders, it does not directly explain the difference in PFC 5-HTT binding. Other brain[85] and platelet[98] studies have produced conflicting results. In vivo studies[51,61] suggest a systemic reduction in 5-HTT sites in major depression, a finding consistent with a genetic or systemic effect. Increased corticosteroid secretion is associated with more severe depression,[99-101] but animal studies[102] do not suggest an effect on 5-HTT gene expression or binding. Other promoter region variants, alterations in transcription factors, and possible functional variation of different 5-HTT transcripts await further study.[103-105]

We did not find an association of the 5-HTTLPR polymorphism and suicide, and it has been little studied in the literature. Nevertheless, the reduction in 5-HTT binding localized to the orbital PFC in suicides in our study seems unrelated to the 5-HTTLPR locus.

Women have less 5-HTT binding in the PFC than men,[46] and we replicated this finding. Reduced gene expression in association with the S allele does not explain fewer binding sites in women. Our finding of no association of 5-HTTLPR genotype with sex agrees with results of other studies.[92,106-108] Paradoxically, estradiol therapy increases the expression of the 5-HTT gene and the number of 5-HTT binding sites in several brain regions.[109-111] However, in a postmortem human brain study, Hernandez and Sokolov[112] found no association between 5-HTT messenger RNA levels and sex. Therefore, the mechanism underlying the sex differences in 5-HTT binding remains unknown. Less 5-HTT binding in the PFC of women is in the same direction, and has the same brain distribution, as lower 5-HTT binding in major depression and therefore may be related to the higher rate of major depression in women.[113]

Binding of 5-HTT might be affected by psychotropics, particularly antidepressants, administered ante mortem. Our study, and at least 2 others,[43,86] excluded individuals with recent antemortem antidepressant use. Other studies did not exclude such individuals. Results of human platelet and rodent studies[51,114] disagree as to whether tricyclic antidepressants or selective serotonin reuptake inhibitors alter transporter binding or gene expression in an enduring fashion after long-term administration. Nemeroff et al[115] found that platelet 5-HTT binding was reduced in never-medicated depressed patients, evidence that past medication does not explain these results.

We did not find an alteration in 5-HTT binding to the PFC or an association of genotype with alcoholism or substance abuse or with a history of pathological aggression. Alcoholism, impulsive homicide, and arson are associated with lower CSF 5-hydroxyindoleacetic acid levels.[116-120] Less 5-HTT binding is reported in the brainstem of impulsively violent offenders[121] and alcoholics.[122] Little et al[123] found higher 5-HTT binding in postmortem tissue of individuals with the LL genotype but also higher 5-HTT binding in alcoholics with the S allele. These results seem contradictory and might be an artifact of a small sample size. We did not study brainstem 5-HTT binding, and CSF 5-hydroxyindoleacetic acid is a measure of overall serotonin turnover and not 5-HTT binding. Therefore, different results might reflect use of different indices of serotonergic function. Alternatively, the psychological autopsy method may be insufficiently sensitive for diagnoses such as alcoholism, substance abuse, or a lifetime history of pathological aggression.

Results of several studies[66,74-77] disagree regarding the relationship of the 5-HTTLPR locus to alcoholism or harm avoidance, but data are lacking regarding criminal, impulsive aggression. Studies of impulsive, pathological, or criminal aggression and the 5-HTTLPR locus would be of interest.

This study has limitations. The sample size is large relative to previous studies, but, unavoidably, subsamples were small, increasing the risk of type I and type II errors. Replication of these results is needed. The psychological autopsy method could not always determine whether subjects had major depression at the time of death. This might not matter because a serotonergic abnormality seems to be present in patients between episodes of major depression.[25,124] We had insufficient data to distinguish impulsive from nonimpulsive aggression. That distinction might be important because studies[125] suggest that only impulsive violence is associated with reduced serotonergic function.

This study found lower 5-HTT binding in the dorsal-ventral extent of the PFC of deceased individuals with a history of major depression and confirmed a reduction in 5-HTT binding involving the orbital PFC of suicides. A promoter region insertion/deletion in the gene for the 5-HTT (5-HTTLPR) did not explain lower PFC 5-HTT binding in major depression, suicide, or women.

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