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ARTICLE

Human 5-HT1A receptor C(−1019)G polymorphism and psychopathology

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Abstract

Dysfunction of the serotonin (5-HT1A) receptor (5-HTR1A) has been implicated in mood disorders, anxiety disorders, psychosis and the action of antidepressants. A common C(−1018)G [C(−1019)G] functional polymorphism in the promoter region of the human 5-HT1A receptor gene has been reported, which may be useful in identifying psychopathology associated with altered function of the human 5-HT1A receptor. We studied the relationship of this polymorphism to psychopathology and 5-HT1A binding in prefrontal cortex. The 5-HT1A receptor genotype for the C(−1019)G polymorphism was typed in 696 unrelated psychiatric subjects, 107 unrelated healthy volunteers, and in post-mortem brain samples from 241 cases. 5-HT1A receptor binding was assayed in post-mortem prefrontal cortex using [^{3}H]8-OH-DPAT, and specific binding determined by 1 μM 5-HT. An association of genotype distribution and allele frequency of the 5-HTR1A C(−1019)G locus was observed in schizophrenia ($\chi^2=9.51$, d.f.$=2$, $p=0.009$; $\chi^2=9.52$, d.f.$=1$, $p=0.002$; Armitage's trend test: $\chi^2=9.07$, d.f.$=1$, $p=0.003$), in substance use disorder ($\chi^2=8.41$, d.f.$=2$, $p=0.015$; $\chi^2=8.35$, d.f.$=1$, $p=0.004$; Armitage's trend test: $\chi^2=6.27$, d.f.$=1$, $p=0.0012$), and in panic attack ($\chi^2=6.31$, d.f.$=2$, $p=0.043$; $\chi^2=6.14$, d.f.$=1$, $p=0.013$; Armitage's trend test: $\chi^2=6.27$, d.f.$=1$, $p=0.012$). An association of the 5-HTR1A C(−1019)G locus with schizophrenia, substance use disorder, and panic attack was suggested by our results. In post-mortem brain samples, 5-HT1A receptor binding in prefrontal cortex and suicide were not associated with genotype. The relationship does not appear to be explained by binding differences, although we cannot rule out altered receptor affinity and transduction.

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Key words: 5-HT1A receptor, panic disorder, polymorphism, schizophrenia, substance use disorder, suicide.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) as a neurotransmitter is involved in physiological functions (pain, sleep, appetite), in psychiatric disorders (depression, schizophrenia, aggression, anxiety disorders) (Lucki, 1998), has been implicated in the diathesis for suicidal behaviour (Mann et al., 1989, 2001), and the action of antidepressants (Blier, 2001). There are 14 serotonin receptors (subdivided into seven classes according to both operational and structural characteristics) (Hoyer et al., 2002), including the 5-HT1A receptor. It is a somatodendritic autoreceptor and post-synaptic in cortical and subcortical terminal fields in the brain. The human 5-HT1A receptor gene has been cloned. The receptor has seven transmembrane domains and is coupled to a guanine nucleotide-binding protein that inhibits adenylate cyclase. The gene is located on chromosome 5 (5q11.2-13) (Fargin et al., 1988; Kobilka et al., 1987).

Post-mortem brain and in-vivo PET imaging studies of the 5-HT1A receptor report higher 5-HT1A receptor binding and altered gene expression in schizophrenia (Burnet et al., 1996, 1997; Gurevich and Joyce, 1997; Hashimoto et al., 1991; Simpson et al., 1996; Sumiyoshi et al., 1996; Tauscher et al., 2002). Joyce et al. (1993) and Arango et al. (1995) found more 5-HT1A binding sites in the ventral prefrontal cortex of suicides; although others using homogenized tissue

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from various brain regions found no difference (Arranz et al., 1994; Lowther et al., 1997; Matsubara et al., 1991). There are no studies of the 5-HT1A polymorphisms and human 5-HT1A brain binding. Very little is known of the relationship of functional variants of the 5-HT1A gene to suicidal behaviour.

Mice lacking the 5-HT1A receptor have higher anxiety levels compared to wild-type mice (Heisler et al., 1998; Parks et al., 1998; Ramboz et al., 1998; Zhuang et al., 1999). This behavioural phenotype can be reversed by a forebrain rescue early in development (Gross et al., 2002). Several PET-imaging studies report lower 5-HT1A binding in major depression in the hippocampus and other brain regions (Drevets et al., 2000; Sargent et al., 2000). Perhaps the 5-HT1A receptor gene plays a developmental role in the pathogenesis of human anxiety or mood disorders.

A number of polymorphisms of the human 5-HT1A receptor gene have been identified. A *Ras*I polymorphism was first detected by DGGE and RFLP analysis and the variant identified as a G294A mutant (Warren et al., 1990) in a sample of unrelated African-Americans but not in Caucasians. Some other rare variants have been identified, including two amino-acid substitutes, Ser22Arg and Val28Ile (Bergen et al., 1996; Erdmann et al., 1995; Kawanishi et al., 1998; Lam et al., 1996; Nakhai et al., 1995). However, the gene frequencies in Caucasians are too low to be useful in an association study.

Wu and Comings (1999) reported a common C(−1018)G polymorphism in the promoter region of the 5-HT1A receptor gene based on the 5′ sequence reported by Parks and Shenk (1996). This locus was later identified as C(−1019)G polymorphism because of the presence of an extra base pair in the human genome sequence of the 5-HT1A receptor gene. This variant is associated with differences in gene expression in cell lines because the C/G polymorphic DNA–protein complex greatly reduces the β-galactosidase activity. The occurrence of a G allele at −1019 bp locus prevents binding of putative repressor proteins, leading to enhanced gene expression and reduced serotonergic neurotransmission (Lemonde et al., 2003). However, association studies of this C(−1019)G polymorphism with major depression (Arias et al., 2002; Lemonde et al., 2003; Zill et al., 2001) and panic disorder (Rothe et al., 2004) report mixed results. A significant effect of this locus on neuroticism scores was observed. Strobel et al. (2003) found that the G allele was associated with higher neuroticism scores. In a study of two structural polymorphisms of the 5-HT1A receptor gene (Pro161Leu and Gly272Asp), no difference in genotype distribution was found between suicide victims and controls in a Japanese population (Nishiguchi et al., 2002). The Pro161Leu polymorphism is not observed in a mostly Caucasian American population (Masellis et al., 2001) and so is not useful for studies in Caucasians.

There are no published studies of the relationship of genotype to 5-HT1A binding. More brain biological endophenotypes are more readily related to functional polymorphism and may explain the link to complex psychiatric disorders. We therefore conducted a study of the relationship of the 5-HTR1A C(−1019)G polymorphism to psychopathology in psychiatric subjects and healthy volunteers, as well as to post-mortem 5-HT1A binding in prefrontal cortex. We hypothesized that the higher expressing form of the gene would be associated with greater 5-HT1A binding to prefrontal cortex, and the low expressing variant with anxiety disorders, schizophrenia, mood disorders and suicidal behaviour.

Materials and methods

Subjects

The study population consisted of 803 living subjects (696 unrelated psychiatric subjects and 107 unrelated healthy volunteers as controls) and 241 post-mortem cases (85 suicide victims and 156 non-suicide controls). The 803 psychiatric subjects and healthy volunteers gave written consent as required by the Institutional Review Board. Next of kin of the deceased gave informed consent for assay of brain tissue and a clinical interview. Subjects and healthy volunteers were interviewed by psychiatrists or clinical psychologists and diagnosed according to DSM-IV criteria for Axis I and Axis II diagnoses using the SCID-I, SCID-II or SCID-NP structured clinical Interview as applicable. Suicidal behaviour was recorded on the Columbia Suicide History Form. Subjects were referred for treatment at a University teaching hospital. Some subjects and all volunteers had responded to advertisements. Clinical and demographic characteristics are listed in Table 1. There were no significant differences in age, sex and racial composition between subjects and healthy volunteers. Psychiatric subjects were divided clinically as follows: major depressive episode ($n=328$), ruled out a history of a major depressive episode ($n=339$), and indeterminate ($n=29$); a history of at least one suicide attempt ($n=214$) and ruled out a history of at least one suicide attempt ($n=480$); schizophrenia ($n=108$), ruled out schizophrenia ($n=582$); substance use disorder ($n=57$) and ruled out history of substance use disorder ($n=635$);

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Table 1. Demographic and clinical characteristics of living psychiatric subjects and healthy volunteers

Characteristics	Psychiatric subjects	Healthy volunteers
Sex (M/F), *n* (%)	308/388 (44/66)	59/48 (55/45)
Age (yr), mean ± S.D.	38 ± 13	39 ± 15
Race ratio (Caucasian/African-Hispanic/Asian/other), *n* (%)	479/113/86/10/8 (69/16/12/1.4/1)	74/12/14/6/1 (69/11/13/6/1)
Lifetime diagnoses, *n* (%)		
Major depressive episode	328 (47)	0 (0)
Bipolar disorder	88 (13)	0 (0)
Schizophrenia	108 (15)	0 (0)
Other disorders	172 (25)	0 (0)
Total	696	107
Cormorbid diagnoses or psychopathology		
Alcoholism	94 (14)	0
Substance use disorder	57 (8)	0
Suicide attempt	214 (31)	0
Panic disorder	87 (13)	0

bipolar disorder ($n=88$), ruled out bipolar disorder ($n=579$); and alcohol abuse ($n=94$), ruled out alcoholism ($n=598$). In some cases the presence or absence of a specific diagnosis could not be determined. Comorbid diagnoses of alcoholism (14%), substance use disorder (8%) or panic disorder (13%) were found in the psychiatric subjects (Table 1). Because of the high rate of comorbidities in the patient population, the comparisons were first done between the healthy volunteer group and the sample of patients and then with different diagnostic categories. Indeterminate cases were omitted from the applicable analyses.

Demographic variables from suicide victims ($n=85$) and non-suicide post-mortem cases ($n=156$) were comparable. The mean age of the suicide group was 43.3 ± 20 yr compared with 43.0 ± 15.5 yr in the non-suicide group. The post-mortem interval (PMI) of the suicide group was 16.4 ± 6.0 h compared to 15.5 ± 5.6 h for the non-suicide group. The male/female ratio was 2.7:1 in the suicide group and 3.5:1 in the non-suicide controls. A DSM-III-R Axis I and Axis II diagnosis was made on all post-mortem brain cases using our validated psychological autopsy method (Kelly and Mann, 1996). Post-mortem cases were divided as follows: a history of major depressive episode ($n=54$), ruled out history of a major depressive episode ($n=112$), and indeterminate ($n=75$); a history of alcoholism ($n=73$), ruled out history of alcoholism ($n=113$), and indeterminate ($n=131$). Demographic features of the post-mortem study population were listed in Table 2. Drugs that may affect 5-HT1A binding were an exclusion criterion and also screening by toxicological analysis.

Extraction of DNA from buffy coat fraction and post-mortem brain tissue

Buccal mucosa cheek swabs (Puregene Kit, Gentra Systems, Minneapolis, MN, USA or BuccalAmp DNA Extraction Kit, Epicentre, Madison, WI, USA) were also collected when blood collection was not possible. DNA was extracted from lymphocytes and epithelial cells following the standard procedure. 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 500:1 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% NP-40, 0.45% Tween-20] containing 12 g Proteinase K. After incubation at 50–60 °C for 1 h, heating at 95 °C for 10 min inactivated the Proteinase K enzyme. The samples were diluted with 10 mM Tris–EDTA buffer at 1:5 dilution for PCR.

DNA isolation from post-mortem brain tissues has been previously described (Huang et al., 1999). In brief, frozen brain tissue samples (150–200 mg) were placed into 1.5 ml microcentrifuge tubes and thawed. A total of 0.5 ml of lysis buffer, containing

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Table 2. Demographic and clinical features of the study population in post-mortem cases ($n = 241$)

Characteristics	Suicides	Non-suicides
Sex (M/F), *n* (%)	62/23 (73/27)	121/35 (78/22)
Age (yr), mean ± S.D.	43 ± 20	43 ± 17
Race ratio (Caucasian/African-American/Hispanic/Asian), *n* (%)	61/9/12/3 (71/11/14/3)	86/46/20/4 (55/29/13/3)
Diagnoses, yes/no, *n* (%)		
Major depression history	43 (51)	11 (7)
Alcoholism history	19 (22)	54 (35)
Total	85	156

0.25 M NaCl, 1% SDS, 5× TE buffer (pH 8.0), and 0.5 mg Proteinase K enzyme was added to each tube. Tubes were incubated in a water bath at 55 °C for at least 3 h 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, St. Louis, MO, USA) added. Tubes were vortexed and centrifuged at 13 000 *g*. A total of 0.5 ml of the aqueous upper layer was transferred to clean tubes and DNA was precipitated with an equal volume of ice-cold 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 and were stored at −20 °C.

Allele-specific PCR amplification

Allele-specific PCR amplification (ASA) was performed in 20 μl of reaction mixture containing 1× PCR buffer, 40–100 ng DNA, 2 mm $MgCl_2$, 4% of DMSO (dimethyl sulphoxide), 50 nM of each dNTP, 0.8 U Red *Taq* polymerase (Sigma), and 40 ng of each primer. The primer sets were originally designed by Wu and Comings (1999). Duplicate samples of genomic DNA from each individual were subjected to PCR with either the 5′-CTGAGGGAGTAAGGCTGGAC-3′ (A) and 5′-GAAGACCGAGTGTGTCTTC**G**-3′ (B) primer set specific for the 'G' allele or the 5′-CTGAGGGAGTAAGGCTGGAC-3′ (A) and 5′-GAAGACCGAGTGTGTCTTC**C**-3′ (C) primer set specific for the 'C' allele. The reaction conditions were as follows: 94 °C for 5 min, 30 cycles at 94 °C for 30 s, 55 °C for 30 s and a final extension step at 72 °C for 4 min. All PCR amplifications were performed in a DNA Robocycler (Stratagene, La Jolla, CA, USA). Electrophoresis was performed in 1.5% agarose and the PCR amplification products visualized with UV light.

[^{3}H]8-OH-DPAT binding and quantitative autoradiograms

Protocols for binding and quantitation of autoradiograms had been published previously (Arango et al., 1995). In brief, tissue sections were preincubated (30 min, 23 °C) in 170 mM Tris–HCl buffer containing 4 mM $CaCl_2$ and 138 mM sucrose (pH 7.6). Incubations were carried out in the same buffer containing 2 nM [^{3}H]8-OH-DPAT (Burnet et al., 1996) and 0.01% ascorbic acid at 21 °C for 60 min. We used a 2 nM concentration of [^{3}H]8-OH-DPAT which is close to the reported K_D in human brain (Cheetham et al., 1990; Dillon et al., 1991). Non-specific binding was determined by parallel incubations of adjacent sections with 1 μM 5-HT (oxalate salt). Non-specific binding was less than 10% of total binding. Ten nM of sertraline masked binding to serotonin transporter sites (Frazer and Hensler, 1990). Sections were washed for 20 min (2 × 5 min) in Tris–HCl buffer at 4 °C. Autoradiograms were analysed using a PC-based image analysis system (MCID, Imaging Research Inc., Canada).

Statistical analysis

Data analysis was performed using SPSS for Windows, Version 10.1 (SPSS Inc., Chicago, IL, USA) and EasyStat (Nee, 2001) for binding and allelic frequencies. Genotype and allele frequencies were contrasted in subjects and healthy volunteers or suicide victims and non-suicide controls using a contingency table χ^2 test with Yates' correction or Fisher's exact test when indicated. Armitage's trend test was also applied to test the significance of the genotypic data (Armitage, 1955; Sasieni, 1997). This test corresponds to the score test in the logistic regression model (Devlin and Roeder, 1999). A multivariate regression model was employed in the analysis of binding data

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Table 3. Comparisons of genotype distributions and allele frequencies for the human 5-HT1A receptor gene C(−1019)G locus between the healthy volunteers and the subjects with history of major depression, bipolar disorders, schizophrenia and other psychiatric disorders such as alcoholism, substance use disorder, suicide attempt, panic disorder and panic attack

		Genotype distribution (%)					Allele frequency (%)			
Clinical characteristics	*n*	C/C	C/G	G/G	χ^2	*p*	C	G	χ^2	*p*
Healthy volunteers[a]	107	34 (32)	50 (47)	23 (23)			118 (55)	96 (45)		
Patients with history of										
Major depressive episode	328	80 (24)	156 (48)	92 (28)	3.00	0.223	316 (48)	340 (52)	2.86	0.091
Bipolar disorders	88	23 (26)	35 (40)	30 (34)	3.88	0.144	81 (46)	95 (54)	2.86	0.091
Schizophrenia[b]	108	21 (19)	44 (41)	43 (40)	9.51	0.009	86 (40)	130 (60)	9.52	0.002
Other disorders	172	40 (23)	88 (51)	44 (26)	2.57	0.283	168 (49)	176 (51)	1.85	0.173
All patients	696									
Cormobidity or psychopathology										
Alcoholism	94	20 (21)	47 (50)	27 (29)	3.22	0.200	87 (46)	101 (54)	2.80	0.094
Substance use disorder[c]	57	9 (16)	25 (44)	23 (40)	8.41	0.015	43 (38)	71 (62)	8.35	0.004
Suicide attempt	214	53 (28)	97 (45)	64 (30)	3.19	0.203	203 (47)	225 (53)	3.09	0.079
Panic disorder	87	20 (23)	43 (49)	24 (28)	2.14	0.343	83 (48)	91 (52)	1.84	0.175
Panic attack[d]	54	9 (17)	25 (46)	20 (37)	6.31	0.043	43 (40)	65 (60)	6.14	0.013

[a] Hardy–Weinberg equilibrium (HWE) test in the healthy volunteer group was performed and no deviation was found ($\chi^2=0.329$, d.f. = 1, $p=0.566$).
[b] Armitage's trend test $\chi^2=9.07$, d.f. = 1, $p=0.003$.
[c] Armitage's trend test $\chi^2=8.29$, d.f. = 1, $p=0.004$.
[d] Armitage's trend test $\chi^2=6.27$, d.f. = 1, $p=0.012$.

from the autoradiograms (Arango et al., 1995). The significance level was $p<0.05$ and all tests were two-tailed. Data are reported as mean ± S.D. unless indicated otherwise.

Results

Living psychiatric subjects and healthy volunteer analyses

Psychiatric patients and unrelated healthy volunteers did not differ in age, sex or racial composition (Table 1). Genotype distribution and allele frequency in healthy volunteers and psychiatric subjects are compared in Table 3. Genotype frequencies for all psychiatric subjects were 24% (C/C), 46% (C/G) and 30% (G/G), and the frequencies for the C(−1019) and −1019G alleles were 47% and 53% respectively. Genotype frequencies for the healthy volunteers were 31% (C/C), 47% (C/G) and 22% (G/G) respectively; the allele frequencies were 55% (C) and 45% (G). Because there were no differences in genotype distribution and allele frequencies between males and females in either patient or control groups ($\chi^2=2.01$, d.f. = 2, $p=0.366$; $\chi^2=1.52$, d.f. = 1, $p=0.217$; $\chi^2=0.67$, d.f. = 2, $p=0.715$; $\chi^2=0.16$, d.f. = 1, $p=0.692$ respectively) (data not shown), males and females were combined for subsequent analysis. The genotype distribution in the healthy volunteer group was in Hardy–Weinberg Equilibrium (HWE) ($\chi^2=0.329$, d.f. = 1, $p=0.566$).

In the psychiatric subjects, the frequency of the −1019G allele for African-Americans, Hispanics, Caucasians and Asians/Others was 57, 64, 51 and 42% respectively, and in the healthy volunteer group 54, 39, 43 and 56%, respectively. Allele frequencies in Caucasian and Asian/Other subgroups in the entire study population were not different from each other ($\chi^2=0.01$, d.f. = 1, $p=0.925$). The combined Caucasian/Asian/Other subgroups differed from the Hispanic ($\chi^2=7.44$, d.f. = 1, $p=0.006$) and the African-American ($\chi^2=3.80$, d.f. = 1, $p=0.051$) subgroups. No difference in allelic frequency was found between the African-American and Hispanic subgroups ($\chi^2=0.48$, d.f. = 1, $p=0.487$) in the entire study population.

Statistically significant differences were observed both in genotype distributions and allelic frequencies between healthy volunteers and schizophrenic subjects ($\chi^2=9.51$, d.f. = 2, $p=0.009$; $\chi^2=9.52$, d.f. = 1, $p=0.002$ respectively), and between healthy volunteers and

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Table 4. Comparisons of genotype distributions and allele frequencies for the human 5-HT1A receptor gene C(−1019)G locus between suicides and non-suicide groups, with or without major depressive episode (MDE) groups

		Genotype distribution (%)					Allele frequency (%)			
Clinical characteristics	*n*	C/C	C/G	G/G	χ^2	*p*	C	G	χ^2	*p*
Suicide	85	25 (29)	40 (47)	20 (24)			90 (53)	80 (47)		
Non-suicide	156	43 (28)	63 (40)	50 (32)	2.02	0.365	149 (48)	163 (52)	1.18	0.277
MDE	54	12 (22)	27 (50)	15 (28)			51 (47)	57 (53)		
Confirmed non-MDE	112	37 (33)	50 (45)	25 (22)	2.12	0.347	124 (55)	100 (45)	1.62	0.203

subjects with a history of substance use disorder ($\chi^2=8.41$, d.f. = 2, $p=0.015$; $\chi^2=8.35$, d.f. = 1, $p=0.004$ respectively) (Table 3). There were also significant dose effects for the risk associated with the C(−1019)G allele for schizophrenia and substance use disorder as assessed by Armitage's trend test ($\chi^2=9.07$, d.f. = 1, $p=0.003$; $\chi^2=8.29$, d.f. = 1, $p=0.004$ respectively). In order to minimize a possible confounding bias due to potential population stratification, we compared the healthy volunteer ($n=74$) and patient groups in Caucasian cases only. Significant association in genotype distribution and allelic frequency were confirmed in schizophrenia ($n=45$; $\chi^2=8.97$, d.f. = 2, $p=0.011$; $\chi^2=6.80$, d.f. = 1, $p=0.009$ respectively). Too few subjects in each subgroup prevented an analysis of sex and ethnicity effects on the findings in substance use disorder. No associations were observed for genotype distribution and allelic frequency in panic disorder ($\chi^2=2.14$, d.f. = 2, $p=0.343$; $\chi^2=1.84$, d.f. = 1, $p=0.175$ respectively) (Table 3). However, there were associations for genotype and allelic frequencies in panic attack ($\chi^2=6.31$, d.f. = 2, $p=0.043$; $\chi^2=6.14$, d.f. = 1, $p=0.013$ respectively). No association was found with major depression ($n=232$; $\chi^2=1.84$, d.f. = 2, $p=0.398$; $\chi^2=1.35$, d.f. = 1, $p=0.245$ respectively) (data not shown).

The relationship of schizophrenia to the 5-HTR1A C(−1019)G locus [OR (odds ratio) = 0.652, 95% CI (confidence interval) = 0.486–0.875; $\chi^2=8.12$, $p=0.004$) was independent of sex, which was not associated with genotype ($\chi^2=0.30$, $p=0.585$). Controlling for gender, a significant association with schizophrenia was confirmed with the Cochran–Mantel–Haenszel statistic ($\chi^2=7.80$, d.f. = 1, $p=0.005$). Moreover, after combining psychiatric subjects and healthy volunteer controls, the association of genotype and allelic frequency with schizophrenia was still significant ($\chi^2=7.68$, d.f. = 2, $p=0.022$; $\chi^2=6.42$, d.f. = 1, $p=0.011$) (data not shown).

No significant association of allelic frequencies was observed with major depression ($\chi^2=3.00$, d.f. = 1, $p=0.223$), bipolar disorder ($\chi^2=3.88$, d.f. = 1, $p=0.144$), past alcoholism ($\chi^2=3.22$, d.f. = 1, $p=0.200$), or a history of suicide attempts ($\chi^2=3.19$, d.f. = 1, $p=0.203$) (Table 3). Direct comparisons of genotype distributions and allele frequencies for the 5-HT1A receptor gene C(−1019)G locus between suicide and non-suicide groups, with or without major depressive episode groups are shown in Table 4.

Analysis of post-mortem cases

Genotype frequencies for the C(−1019)G polymorphism for all post-mortem cases were 28% (C/C), 43% (C/G) and 29% (G/G) respectively. Frequencies for the C(−1019) and the –1019G alleles were both 50%. The genotype and allelic frequency distributions were comparable to those of the living subjects and healthy volunteers ($\chi^2=1.52$, d.f. = 2, $p=0.457$; $\chi^2=0.39$, d.f. = 1, $p=0.531$ respectively). In agreement with the living subjects study, the genotype distribution and allele frequencies between male and female post-mortem groups were not different ($\chi^2=1.20$, d.f. = 2, $p=0.548$; $\chi^2=0.41$ d.f. = 1, $p=0.520$ respectively). No associations of genotype and allelic frequencies were observed with suicide ($\chi^2=2.02$, d.f. = 2, $p=0.365$; $\chi^2=1.18$, d.f. = 2, $p=0.277$ respectively) or with a history of a major depressive episode in the combined group of suicide and non-suicide ($\chi^2=2.12$, d.f. = 2, $p=0.347$; $\chi^2=1.62$, d.f. = 1, $p=0.203$ respectively) (Table 3). Insufficient data and cases were available for analyses of associations with substance use disorder, panic disorder or schizophrenia in the post-mortem cases.

[^{3}H]8-OH-DPAT binding in cortical layers throughout the prefrontal cortex in post-mortem brain samples was not associated with genotype in either suicides or non-suicides (Figure 1). As we previously reported, 5-HT1A binding in males was lower

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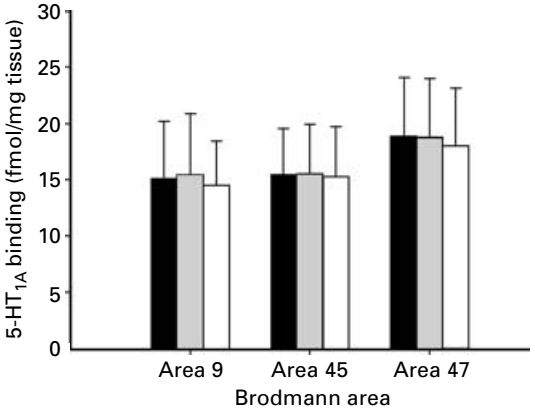

Figure 1. Relationship between post-mortem 5-HT_{1A} receptor binding in prefrontal cortex and C(−1019)G polymorphism in suicides and non-suicide. Genotype: ■, C/C; ▨, C/G; □, G/G.

compared to females, and ventral prefrontal cortical binding was higher in the suicide group compared to the non-suicide group (data not shown). Neither effect was explained by genotype.

Discussion

The C(−1019)G polymorphism in the promoter region of the human 5-HT1A receptor gene was found to have a significant association with schizophrenia and substance use disorder. No significant associations were found with major depression, bipolar disorder, alcoholism, panic disorder or suicide attempt. Our findings lend further support to a role for the 5-HT1A receptor gene in the pathophysiology of schizophrenia and substance use disorder, but the mechanisms are unclear. The G allele is associated with greater expression in cell lines and we found a higher frequency of the G allele in schizophrenia. Several studies have reported 15–80% greater post-mortem and in-vivo 5-HT1A receptor binding in schizophrenia. Thus, this allele could explain reports of higher 5-HT1A binding in the brain in schizophrenia (Bantick et al., 2001; Burnet et al., 1996, 1997; Gurevich and Joyce, 1997; Hashimoto et al., 1991; Simpson et al., 1996; Sumiyoshi et al., 1996; Tauscher et al., 2002). On the other hand, we did not find more 5-HT1A binding associated with the G allele in our post-mortem study, but did not have enough cases to analyse binding in schizophrenia separately. How this genotype or more 5-HT1A binding was related to schizophrenia remains to be determined. There is an interaction between the serotonergic and dopaminergic systems in the midbrain and striatum via the 5-HT2 receptor and 5-HT1A receptor modulating dopamine release that may underlie an effect in schizophrenia (for a review, see Kapur and Remington, 1996).

Our results for major depression are consistent with the finding by Zill et al. (2001) of no significant association between 5-HTR1A C(−1019)G polymorphism and major depression, but differ from Lemonde et al. (2000, 2003). We also did not find an association of the 5-HT1A receptor polymorphism and either suicide attempts in the patient sample, or suicide in the post-mortem brain cases. Nonetheless, we have previously reported greater 5-HT1A receptor binding localized to the ventral prefrontal cortex in suicides (Arango et al., 1995), and now confirm that result in this much larger sample, but find that the greater 5-HT1A binding is unrelated to the genotype of the 5-HTR1A C(−1019)G promoter locus. The concentration of ligand we used in the binding study was close to the K_D and, therefore, sensitive to altered number of binding sites or altered affinity. Lemonde et al. (2003) suggested that the C(−1019)G polymorphism might regulate 5-HT1A gene expression in vivo through depression of the 5-HT1A promoter in pre-synaptic raphe neurons leading to reduced serotonergic transmission due to impaired binding of a nuclear deformed epidermal autoregulatory factor 1 (DEAF-1)-related (NUDR) transcriptional regulator protein that acts as a repressor. The function of the 5-HT1A receptor is abrogated by the 5-HTR1A C(−1019)G polymorphism. The G allele is associated with gene expression.

When we compared our results to those of Lemonde et al. (2003), there were no differences in genotype distribution and allelic frequencies between depressed patients from their Ontario cohort and our New York cohort ($\chi^2 = 0.08$, d.f. = 2, $p = 0.960$; $\chi^2 = 1.29$, d.f. = 1, $p = 0.256$ respectively). Genotype distributions are almost identical (C/C:C/G:G/G: 23, 47 and 28% vs. 24, 48 and 28% in Lemonde et al. and our study respectively). The different findings can be explained by different genotype distributions in the control cohorts from Ontario and New York (C/C:C/G:G/G: 37, 51 and 12% vs. 31, 47 and 21% respectively). If we compare the Ontario control cohort and our New York subjects with major depression, there is an apparent association with this polymorphism ($\chi^2 = 16.41$, d.f. = 2, $p \leqslant 0.001$). Thus, the identification by Lemonde et al. (2003) of an association between subjects with major depression and the C(−1019)G polymorphism appears to be mainly due to the characteristics of their control group and not the disease sample. That may reflect a stratification effect.

Panic disorder has been the subject of several genetic studies with inconclusive results (Gorman et al.,

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2000; Smoller and Tsuang, 1998; Tsuang et al., 2001; Weissman, 1993). 5-HT1A-deficient mice show an increasing anxiety and stress response (Gross et al., 2002; Parks et al., 1998; Parks and Shenk, 1996; Ramboz et al., 1998; Zhuang et al., 1999). Partial agonists of 5-HT1A, such as buspirone and gespirone, are anxiolytic drugs but produce variable results as anxiolytics in different rodent behavioural models, indicating different genetic models for the pharmacological study (Heisler et al., 1998). In our study, we found no association of panic disorder with the G allele. Frequency of the higher expressing –1019G allele (52%) was similar compared to the healthy volunteer group (45%). Recently, Rothe et al. (2004) also found no association between this locus and panic disorder but found that the G allele is associated with panic disorder with agoraphobia. Strobel et al. (2003) found the G allele to be associated with depression, and a trend for an association with anxiety neuroticism-related traits. Since the numbers of our subjects with panic disorder with agoraphobia are few, we did not perform such an association analysis. However, we found there is an association between panic attack and allelic frequency ($\chi^2 = 6.14$, d.f. $= 1$, $p = 0.012$). These findings suggest a role of allelic variation in 5-HT1A receptor expression in the development of anxiety-related disorders. Different roles in anxiety for the autoreceptor and the terminal field post-synaptic receptor are suggested by prevention of the anxiety phenotype by hippocampal expression rescue in the 5-HT1A knockout mouse model (Gross et al., 2002), indicating that the pathophysiology leading to anxiety disorders is dependent on 5-HT1A expression in early development.

The risk for substance use disorder is significantly influenced by familial, genetic and environmental factors (Kendler, 2001; Kendler and Gardner, 1998; Merikangas et al., 1998; Pickens et al., 1991; Tsuang et al., 1998). No association of the functional 5-HTR1A polymorphism with substance use disorder has been previously reported. However, involvement of 5-HT1A receptor in the potentiation of cocaine reinforcement is reported (Parsons et al., 1998). A reciprocal facilitatory/inhibitory influence of 5-HT1A agonist/antagonists upon cocaine-induced locomotion has been found (Carey et al., 2002). 5-HT1A and 5-HT1B receptors modulate behaviours in stressful and anxiety-arousing situations (Weller et al., 2003). Interestingly, we previously reported a significant association between substance use disorder and human 5-HT1B receptor genotype for the G861C polymorphism (Huang et al., 2003). The present finding of the association of the 5-HT1A receptor gene with substance use disorder provides further support for the relationship of substance use disorder to the serotonin system.

Limitations

Although the sample size is relatively large compared with previous studies, the relatively small subgroup samples in some instances require replication of the findings. However, there was no deviation from HWE in the healthy volunteers, reducing the chance of a false-positive association due to a population effect (Schaid and Jacobsen, 1999). Care was taken to eliminate potential spurious results due to race or gender. The method of genomic control is reported to be useful for determining appropriate corrections for population-based association tests, however, it requires additional genotyping of many markers or samples to detect subtle population difference and confounding factors (Wacholder et al., 2000). We used instead Armitage's trend test (Armitage, 1955) that corresponds to the additive genetic model adopted in the genomic control method (Devlin and Roeder, 1999) to assess a trend in genetic relative risks and reduce type I error (Schaid and Jacobsen, 1999). Nevertheless, we could not completely rule out population stratification effects or sampling deficiency due to the sample size and local recruitment effect, a potential concern in any association study.

The findings that this 5-HT1A promoter polymorphism contributes to dysfunction in the regulation of 5-HT1A receptor and, thereby, to the pathogenesis of schizophrenia and substance use disorder, and perhaps panic attacks, needs to be replicated in studies of larger sample sizes, and the relationship of genotype to 5-HT1A receptor signal transduction and binding needs to be determined in these disorders.

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Statement of Interest

None.

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