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Serotonin receptor 2C gene polymorphism associated with post-stroke depression in Chinese patients

W.K. Tang[1], N. Tang[2], C.D. Liao[2], H.J. Liang[1], V.C.T. Mok[3], G.S. Ungvari[4] and K.S. Wong[3]

[1]Department of Psychiatry, Chinese University of Hong Kong, Hong Kong, China
[2]Department of Chemical Pathology, Chinese University of Hong Kong, Hong Kong, China
[3]Department of Medicine and Therapeutics, Chinese University of Hong Kong, Hong Kong, China
[4]University of Notre Dame Australia, Marian Centre, Perth, Australia

Corresponding author: W.K. Tang
E-mail: tangwk@cuhk.edu.hk

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ABSTRACT. The serotonin receptor 2C (HTR2C) gene has been shown to play a pivotal role in major depression. We examined the association between post-stroke depression (PSD) and polymorphism in HTR2C. A cohort of 223 patients with acute lacunar stroke admitted to the stroke unit of a university-affiliated regional hospital in Hong Kong was recruited. Three months after the onset of the index stroke, a research assistant administered the locally validated 15-item Geriatric Depression Scale. PSD was defined as a geriatric depression scale score of 7 or above. Possible confounding factors, including previous history of stroke, severity of stroke, level of social support, and recent life events, were investigated. All patients were genotyped for polymorphisms of HTR2C. Separate analyses were performed for males and females. Sixty-one patients were found to have

PSD. There were significant associations between the HTR2C gene and PSD status in the male patients, but not in the female ones. After adjusting for possible confounders, the rs12837651 T allele (odds ratio = 4.020) and the rs2192371 G allele (odds ratio = 2.866) were found to be significantly associated with PSD in males. Genetic variation in HTR2C receptors appears to be involved in the pathogenesis of PSD in Chinese males.

Key words: Stroke; Depression; HTR2C; SNP; Disease association

INTRODUCTION

Depression is the most common and serious affective disorder following stroke (Hackett and Anderson, 2005), although the neuroanatomical model of post-stroke depression (PSD), a depressive illness in patients with well-established cerebrovascular disease, remains unclear.

Serotonin receptor 2C (HTR2C) is one of the most relevant and investigated serotonin receptors in both human and animal studies (Drago and Serretti, 2009). It has been shown to play a pivotal role in many different psychiatric behaviors, including major depression, bipolar disorder, suicide, and schizophrenia (Lerer et al., 2001; Iwamoto et al., 2009). HTR2C receptors are implicated in the neuroendocrine changes observed in depression (Newman et al., 1998; Heisler et al., 2007). HTR2C receptors are also involved in the actions of several classes of antidepressants (Millan, 2005), and HTR2C antagonists may have antidepressant activity (Barabanova et al., 2007).

No previous study has examined the relationship between the HTR2C polymorphism and PSD. This study was conducted to determine the relationship between HTR2C polymorphisms and PSD in stroke survivors.

MATERIAL AND METHODS

Study sample

Among the 2369 patients with acute ischemic stroke admitted to the acute stroke unit of a university-affiliated regional hospital in Hong Kong between June 2006 and May 2009, 223 patients with first-ever or recurrent acute ischemic stroke were recruited (Figure 1). The inclusion criteria were: 1) Chinese ethnicity; 2) Cantonese as the primary language; 3) age 18 or above; 4) well-documented (clinical presentation and a CT brain scan) first or recurrent acute stroke within the 7 days prior to admission; 5) an MRI scan; 6) Mini-Mental State Examination (MMSE) (Chiu et al., 1994) score of 20 or above; and 7) ability and willingness to give informed consent. The exclusion criteria included: 1) transient ischemic attack, cerebral hemorrhage, subdural hematoma or subarachnoid hemorrhage; 2) history of central nervous system disease such as dementia, tumor, trauma, or hydrocephalus; 3) history of depression or other psychiatric disorders before the index stroke; 4) severe aphasia [best language score of the National Institute of Heath Stroke Scale (NIHSS) on admission ≥2]; 5) severe visual or auditory impairment; 6) physically unfit for interview; and 7) recurrent stroke before a 3-month interview.

The study protocol was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong. All participants signed a consent form.

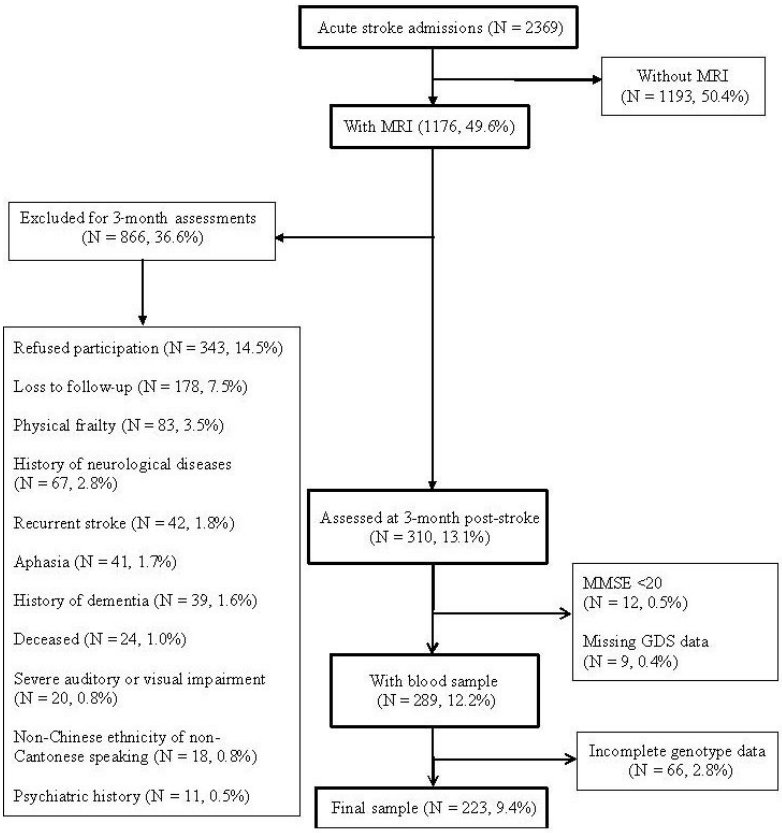

Figure 1. Recruitment profile of the study. MRI = magnetic resonance image; MMSE = Mini-Mental State Examination; GDS = Geriatric Depression Scale.

Psychiatric and behavioral assessments

The extent of neurological impairment in terms of the NIHSS (Brott et al., 1989) total score on admission was measured by a research nurse. Three months after the onset of the index stroke, a research assistant who was blind to subjects' radiological data administered the validated 15-item Geriatric Depression Scale (GDS) (Lim et al., 2000). The timing of the assessment was chosen to avoid the period of transient emotional adjustment to the disability caused by the stroke. PSD was defined as a GDS score of 7 or above (Cheng et al., 2010). All of the participants were assessed with the MMSE, the Lubben Social Network Scale (LSNS) (Lubben, 1998) and the Modified Life Event Scale (MLES) (Paykel et al., 1971). The LSNS is a composite social network scale specifically designed for use in the elderly. It measures the level of social support that patients receive and their social interaction with relatives and friends. The LSNS has been translated into Chinese and validated in Hong Kong elderly (Chi and Boey, 1994). The MLES records the presence of 18 adverse and distressing life events in the past 6 months. Its score indicates the total number of adverse events encountered by patients.

Genetic analysis

At the time of the psychiatric assessment, 9 mL blood was collected from each participant. Genomic DNA was extracted from peripheral blood samples using a commercial DNA extraction kit (Qiagen, USA). PCR was prepared by a Biomek robotic station and carried out under standard conditions. Genotyping was performed using the Sequenom MassArray platform (Sequenom, San Diego, CA, USA). The iPLEX assay was performed in accordance with manufacturer instructions.

We consulted the HapMap database data (March 2008 release, www.hapmap.org, population Han Chinese). Tag SNPs of HTR2C were selected by the LD-based approach implemented in Haploview/HapMap. In brief, the algorithm is a pairwise tagging method, using a threshold of $r^2 = 0.8$ (Stram, 2004) and a minor allele frequency of 5%. Initially, there were 8 tag SNPs, 2 of which (rs6579495 and rs2316100) could not be incorporated into the multiplex assay design, so 6 tagging SNPs were chosen for genotyping. One tag SNP was subsequently rejected because of missing values (rs5988072). Therefore, 5 tag SNPs, namely HTR2C rs12833104 (A/G), rs498177 (T/C), rs12837651 (T/C), rs2192371 (A/G), and rs6643897 (T/G), were analyzed. The genotyping rate for these 5 SNPs were 98.6, 96.4, 94.4, 72.2, and 91.3%, respectively.

Statistical analysis

To control for other covariates, demographic (age, education) and clinical variables (previous stroke, hypertension, diabetes mellitus, NIHSS, LSNS, MLES, and MMSE scores) between PSD and non-PSD groups were compared using chi-square, Student *t*, and Mann-Whitney U-tests.

The Hardy-Weinberg equilibrium (HWE) test for genotype distribution was performed using the chi-square test with 1 degree of freedom. Separate analyses were performed for males and females, since the HTR2C gene is located on the X chromosome. Univariate association between PSD status and genotype and demographic and clinical characteristics was determined by the chi-square test, the *t*-test, and the Mann-Whitney U-test. To determine the risk adjusted to demographic and clinical characteristics (odds ratio), a stepwise logistic regression was performed to weigh the importance of individual associated SNP identified. For each SNP, a single regression model was constructed where significant demographic and clinical variables in the univariate analyses were also entered. Haplotype evaluation was performed with the Haploview program (Barrett et al., 2005), based on the X-linked additive mode of inheritance. The significance level for statistical tests was 0.05.

RESULTS

A total of 223 patients met the entry criteria and formed the study sample. Patients who were excluded from the study had a higher NIHSS score (5.6 ± 4.9 *vs* 4.0 ± 2.8; $P < 0.001$). The age (67.3 ± 10.1 *vs* 67.7 ± 12.0; $P < 0.614$) and male (57.0 *vs* 55.3%; $P < 0.709$) distribution of the excluded and included groups were similar.

Of the 223 patients screened, 61 (27.4%; 28 men, 33 women) had PSD. The demographic and stroke-related data are shown in Table 1. There was no significant difference between the PSD and non-PSD groups in terms of age, education, hypertension, diabetes mellitus, and the MMSE score. Among the men, the PSD group was more likely to have had a previous stroke, and to have a high degree of neurological deficits (NIHSS score)

and a lower level of social support (LSNS score). Among the women, the PSD group had a lower level of social support (LSNS score) and more life events (MLES score) (Table 1).

The genotype frequencies of all SNPs were in HWE. There were significant associations between the HTR2C gene and PSD status in the male but not female subjects in the allele/genotype analysis. After adjusting for previous stroke, NIHSS and LSNS scores, the rs12837651 T allele (odds ratio = 4.020, 95%CI = 1.160-13.929) and the rs2192371 G allele (odds ratio = 2.866, 95%CI = 1.060-7.748) were significantly associated with PSD (Table 2). We did not find any association between the HTR2C gene and PSD in the haplotype analysis (Table 3).

Table 1. Demographic characteristics, psychosocial risk factors, and stroke severity by post-stroke depression (PSD) status.

	Male			Female		
	PSD (N = 28)	Non-PSD (N = 99)	P	PSD (N = 33)	Non-PSD (N = 63)	P
Age (years)	66.4 ± 10.1	67.0 ± 8.9	0.774†	67.0 ± 11.9	68.3 ± 11.1	0.617†
Education level (years)	6.7 ± 3.7	7.3 ± 4.8	0.715§	5.0 ± 4.8	4.3 ± 4.4	0.447§
Previous stroke	11 (40.7)	20 (20.8)	0.035‡	5 (15.2)	10 (15.9)	0.926‡
Hypertension	18 (66.7)	58 (60.4)	0.555‡	22 (66.7)	49 (77.8)	0.239‡
Diabetes mellitus	8 (29.6)	20 (20.8)	0.336‡	13 (39.4)	22 (34.9)	0.665‡
MMSE score	27.0 ± 2.5	27.5 ± 2.3	0.291§	25.8 ± 2.8	25.5 ± 3.1	0.684§
NIHSS total score	4.7 ± 2.5	3.8 ± 2.6	0.058§	4.3 ± 2.9	3.9 ± 3.1	0.378§
LSNS score	25.4 ± 9.0	29.8 ± 7.7	0.034§	28.7 ± 8.6	33.4 ± 7.4	0.020§
MLES score	2.5 ± 1.5	1.9 ± 0.9	0.567§	2.5 ± 1.5	1.8 ± 0.9	0.033§

Data are reported as means ± SD or as number with percent in parentheses. LSNS = Lubben Social Network Scale; MLES = Modified Life Event Scale; MMSE = Mini-Mental State Examination; NIHSS = National Institute of Health Stroke Scale; SD = standard deviation; †*t*-test; ‡chi-square test; §Mann-Whitney U-test.

Table 2. Genotype and allele distributions by post-stroke depression (PSD) status.

Male						
SNP ID	Phenotype	MAF	N	Allele distribution		P value
				M	m	Allele‖
rs12833104	PSD	0.250	28	21	7	0.161
G > A	Non-PSD	0.173	98	81	17	
rs498177	PSD	0.286	28	20	8	0.217
T > C	Non-PSD	0.194	98	79	19	
rs12837651	PSD	0.286	28	20	8	0.026
C > T	Non-PSD	0.124	97	85	12	
rs2192371	PSD	0.571	28	12	16	0.038
A > G	Non-PSD	0.343	99	65	34	
rs6643897	PSD	0.164	25	21	4	0.116
G > T	Non-PSD	0.110	91	81	10	

Female									
SNP ID	Phenotype	MAF	N	Genotype distribution§			P value		
				M/M	M/m	m/m	HWE	Genotype#	Allele#
rs12833104	PSD	0.182	33	21	12	0	0.200	0.205	0.117
G > A	Non-PSD	0.105	62	50	11	1	0.667		
rs498177	PSD	0.234	32	19	11	2	0.811	0.370	0.181
T > C	Non-PSD	0.361	61	42	16	3	0.379		
rs12837651	PSD	0.089	28	23	5	0	0.604	0.572	0.762
C > T	Non-PSD	0.183	60	50	9	1	0.442		
rs2192371	PSD	0.470	33	9	17	7	0.845	0.993	0.888
A > G	Non-PSD	0.493	62	15	36	12	0.248		
rs6643897	PSD	0.134	29	22	6	1	0.484	0.211	0.100
G > T	Non-PSD	0.068	59	52	6	1	0.133		

Major (M) allele > minor (m) allele; MAF = minor allele frequency. ‖Adjusted for previous stroke, NIHSS and LSNS scores; HWE = Hardy-Weinberg equilibrium. #Adjusted for LSNS and MLES scores.

Table 3. Haplotype analysis by post-stroke depression (PSD) status.

HTR2C common haplotypes	Phenotype	Individual haplotype frequency	P
Male rs498177- rs12837651- rs2192371- rs12833104- rs6643897			
TCAGG	PSD	0.429	0.295
	Non-PSD	0.541	
TCGGG	PSD	0.286	0.428
	Non-PSD	0.214	
CTGAT	PSD	0.207	0.211
	Non-PSD	0.115	
CTGAG	PSD	0.000	0.180
	Non-PSD	0.061	
TCAAT	PSD	0.000	0.223
	Non-PSD	0.051	
CTGAG	PSD	0.043	0.171
	Non-PSD	0.007	
Female rs498177- rs12837651- rs2192371- rs12833104- rs6643897			
TCAGG	PSD	0.442	0.848
	Non-PSD	0.457	
TCGGG	PSD	0.290	
	Non-PSD	0.356	0.367
CTGAT	PSD	0.102	
	Non-PSD	0.080	0.615
CCAGG	PSD	0.072	
	Non-PSD	0.059	0.732
CTGAG	PSD	0.035	
	Non-PSD	0.023	0.639
CCGGG	PSD	0.007	
	Non-PSD	0.015	0.627
TCAGT	PSD	0.017	
	Non-PSD	0.009	0.598

DISCUSSION

To the best of our knowledge, this is the first study to report an association between polymorphisms of HTR2C and the risk of depression in stroke survivors. The results suggest that the rs12837651 T allele and the rs2192371 G allele are associated with PSD in patients with well-established cerebrovascular disease. Carriers of these potential risk alleles had 3-4 times higher odds of depression compared with individuals with other genotypes.

Certain HTR2C polymorphisms have previously been linked to unipolar depression (Vimaleswaran et al., 2010), suicide (Videtic et al., 2009), and bipolar disorders (Lerer et al., 2001), but do not seem to influence antidepressant response (Peters et al., 2004). Cys23Ser is one of the most frequently investigated polymorphisms, but occurs in only 2.9% of Chinese stroke survivors (Zhang et al., 2008), and thus, it was not included in this study. We could not identify any published report on the effect of rs12837651 and rs2192371 polymorphisms on depression or other psychiatric disorders.

To date, few studies have investigated the role of the serotonin gene polymorphisms

in PSD. A single study comparing 75 depressed and 75 nondepressed stroke survivors investigated only serotonin transporter gene polymorphisms and found that 5-HTTLPR and STin2 VNTR polymorphisms are associated with PSD in stroke survivors (Kohen et al., 2008). The association between PSD and 5-HTTLPR polymorphisms has also been reported by other investigators (Ramasubbu et al., 2006, 2008; Fang et al., 2011).

It is still uncertain how HTR2C polymorphisms may affect the risk of PSD. Neuroendocrine challenge studies with fenfluramine have consistently shown the blunted release of prolactin in depressed patients (Newman et al., 1998), and this fenfluramine-induced prolactin release has been shown to be mediated by HTR2C receptors (Coccaro et al., 1996). Similarly, the chronic activation of the hypothalamic-pituitary-adrenal axis, a frequent characteristic of depressive episodes, is dependent on HTR2C activity (Heisler et al., 2007). Finally, the administration of HTR2C antagonist generates antidepressant activity in animal models (Barabanova et al., 2007).

This study has a number of limitations. First, it had a small sample size, which reduces its statistical power. Second, stroke severity in the sample was mild and PSD assessment was made only once, at the 3-month follow-up. Third, patients with more severe stroke, those who died before the 3-month follow-up, and those who became depressed later were excluded, as were those unable to give their consent due to dementia or aphasia. These selection biases may limit the generalizability of the findings.

In conclusion, the results of this study suggest a possible role for genetic variation in HTR2C receptors in the pathogenesis of PSD. The replication of these findings in a larger sample is warranted.

REFERENCES

Barabanova SV, Fedotova I and Sapronov NS (2007). Effects of antagonists of 1A and 2A/2C subtypes of serotonin receptors on the depressive behavior and expression of c-Fos protein in hypothalamus of ovariectomized rats. *Eksp. Klin. Farmakol.* 70: 3-7.

Barrett JC, Fry B, Maller J and Daly MJ (2005). Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263-265.

Brott T, Adams HP Jr, Olinger CP, Marler JR, et al. (1989). Measurements of acute cerebral infarction: a clinical examination scale. *Stroke* 20: 864-870.

Cheng ST, Yu EC, Lee SY, Wong JY, et al. (2010). The geriatric depression scale as a screening tool for depression and suicide ideation: a replication and extention. *Am. J. Geriatr. Psychiatry* 18: 256-265.

Chi I and Boey KW (1994). A Mental Health and Social Support Study of the Old-Old in Hong Kong Department of Social Work and Social Administration. Resource Paper Series, University of Hong Kong, Hong Kong.

Chiu HFK, Lee HC, Chung WS and Kwong PK (1994). Reliability and validity of the Cantonese version of mini-mental state examination - preliminary study. *Hong Kong J. Psychiatry* 4: 25-28.

Coccaro EF, Kavoussi RJ, Oakes M, Cooper TB, et al. (1996). 5-HT2a/2c receptor blockade by amesergide fully attenuates prolactin response to d-fenfluramine challenge in physically healthy human subjects. *Psychopharmacology* 126: 24-30.

Drago A and Serretti A (2009). Focus on HTR2C: a possible suggestion for genetic studies of complex disorders. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 150B: 601-637.

Fang J, Yan W, Jiang GX, Li W, et al. (2011). Serotonin transporter gene polymorphism in Chinese patients with poststroke depression: a case-control study. *Stroke* 42: 1461-1463.

Hackett ML and Anderson CS (2005). Predictors of depression after stroke: a systematic review of observational studies. *Stroke* 36: 2296-2301.

Heisler LK, Pronchuk N, Nonogaki K, Zhou L, et al. (2007). Serotonin activates the hypothalamic-pituitary-adrenal axis via serotonin 2C receptor stimulation. *J. Neurosci.* 27: 6956-6964.

Iwamoto K, Bundo M and Kato T (2009). Serotonin receptor 2C and mental disorders: genetic, expression and RNA editing studies. *RNA. Biol.* 6: 248-253.

Kohen R, Cain KC, Mitchell PH, Becker K, et al. (2008). Association of serotonin transporter gene polymorphisms with poststroke depression. *Arch. Gen. Psychiatry* 65: 1296-1302.

Lerer B, Macciardi F, Segman RH, Adolfsson R, et al. (2001). Variability of 5-HT2C receptor cys23ser polymorphism among European populations and vulnerability to affective disorder. *Mol. Psychiatry* 6: 579-585.

Lim PP, Ng LL, Chiam PC, Ong PS, et al. (2000). Validation and comparison of three brief depression scales in an elderly Chinese population. *Int. J. Geriatr. Psychiatry* 15: 824-830.

Lubben JE (1998). Assessing social networks among elderly populations. *Fam. Community Health* 11: 42-52.

Millan MJ (2005). Serotonin 5-HT2C receptors as a target for the treatment of depressive and anxious states: focus on novel therapeutic strategies. *Therapie* 60: 441-460.

Newman ME, Shapira B and Lerer B (1998). Evaluation of central serotonergic function in affective and related disorders by the fenfluramine challenge test: a critical review. *Int. J. Neuropsychopharmacol.* 1: 49-69.

Paykel ES, Prusoff BA and Uhlenhuth EH (1971). Scaling of life events. *Arch. Gen. Psychiatry* 25: 340-347.

Peters EJ, Slager SL, McGrath PJ, Knowles JA, et al. (2004). Investigation of serotonin-related genes in antidepressant response. *Mol. Psychiatry* 9: 879-889.

Ramasubbu R, Tobias R, Buchan AM and Bech-Hansen NT (2006). Serotonin transporter gene promoter region polymorphism associated with poststroke major depression. *J. Neuropsychiatry Clin. Neurosci.* 18: 96-99.

Ramasubbu R, Tobias R and Bech-Hansen NT (2008). Extended evaluation of serotonin transporter gene functional polymorphisms in subjects with post-stroke depression. *Can. J. Psychiatry* 53: 197-201.

Stram DO (2004). Tag SNP selection for association studies. *Genet. Epidemiol.* 27: 365-374.

Videtic A, Peternelj TT, Zupanc T, Balazic J, et al. (2009). Promoter and functional polymorphisms of HTR2C and suicide victims. *Genes Brain Behav.* 8: 541-545.

Vimaleswaran KS, Zhao JH, Wainwright NW, Surtees PG, et al. (2010). Association between serotonin 5-HT-2C receptor gene (HTR2C) polymorphisms and obesity- and mental health-related phenotypes in a large population-based cohort. *Int. J. Obes.* 34: 1028-1033.

Zhang J, Shen Y, He G, Li X, et al. (2008). Lack of association between three serotonin genes and suicidal behavior in Chinese psychiatric patients. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 32: 467-471.