



Lack of an association between 5-HT6 receptor gene polymorphisms and suicide victims

Okamura, Kenji ; Shirakawa, Osamu ; Nishiguchi, Naoki ; Ono, Hisae ;
Nushida, Hideyuki ; Ueno, Yasuhiro ; Maeda, Kiyoshi

(Citation)

Psychiatry and Clinical Neurosciences, 59(3):345-349

(Issue Date)

2005-06

(Resource Type)

journal article

(Version)

Accepted Manuscript

(Rights)

The definitive version is available at www.blackwell-synergy.com.

(URL)

<https://hdl.handle.net/20.500.14094/90000359>



Lack of an Association Between 5-HT₆ Receptor Gene Polymorphisms and Suicide Victims

KENJI OKAMURA, MD,¹ OSAMU SHIRAKAWA, MD, PhD,¹

NAOKI NISHIGUCHI, MD, PhD,¹ HISAE ONO, MD, PhD,¹

HIDEYUKI NUSHIDA, MD, PhD,² YASUHIRO UENO, MD, PhD²

AND KIYOSHI MAEDA, MD, PhD¹

¹Division of Psychiatry and Neurology, Department of Environmental Health and Safety,
Faculty of Medical Sciences, Kobe University Graduate School of Medicine, Kobe,
Japan

²Division of Legal Medicine, Department of Environment Health and Safety, Faculty of
Medical Sciences, Kobe University Graduate School of Medicine, Kobe, Japan

*Correspondence and proof should be sent to: Osamu Shirakawa, M.D., Ph.D.

Division of Psychiatry and Neurology, Department of Environmental Health and Safety,
Faculty of Medical Sciences, Kobe University Graduate School of Medicine

7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

TEL: +81-78-382-6065

FAX: +81-78-382-6079

E-mail: sirakawa@kobe-u.ac.jp

Running title: 5-HT₆ receptor polymorphism and suicide

Our manuscript consists of 2945 words and includes 2 tables.

Specification of field : Molecular psychiatry

Abstract

An association between serotonergic dysfunction in the brain and suicidal behavior has previously been suggested. The high affinity of some antipsychotic and antidepressant drugs to serotonin 6 (5-HT₆) receptors, and the predominant localization of 5-HT₆ receptors in some limbic regions, suggest that 5-HT₆ receptors play a role in the pathogenesis of suicide. The objective of the present study was to examine the association between suicide victims and two polymorphisms of the 5-HT₆ receptor gene: a biallelic polymorphism (267C/T) in exon 1 and a trinucleotide repeat polymorphism ((GCC)_{2/3}) in the 5'-upstream region of the gene. We genotyped the two polymorphisms in 163 suicide victims and 166 controls, and compared the distribution of genotype and allele frequencies between the two groups. Haplotype frequencies of these two polymorphisms were estimated from genotypic data by the maximum-likelihood method. In both polymorphisms, there were no significant differences in genotype or allele frequencies between the suicide victims and the controls. Moreover, there were no significant differences in the haplotype distributions of these polymorphisms between the two groups. These findings suggest that it is unlikely that the 5-HT₆ receptor gene is involved in the susceptibility to suicide.

Key words: suicide victims, serotonin 6 receptor gene, association study, polymorphism, haplotype analysis.

INTRODUCTION

Several lines of evidence suggest that serotonergic dysfunction is involved in the susceptibility to suicide. A low level of 5-hydroxyindoleacetic acid, the principal metabolite of serotonin, in cerebrospinal fluid and a blunted prolactin response to fenfluramine, which indicates serotonergic hypofunction in the brain, have been found in subjects with suicidal behavior.^{1,2} These findings seem to be independent of psychiatric diagnoses and to correlate with the lethality of suicide attempts. Thus, suicide is explained by factors other than the presence of a specific psychiatric disorder.³

Family, twin, and adoption studies have shown that genetic factors partly explain the risks for suicide.⁴ Genetic variations in the serotonergic system are thought to affect serotonin turnover,⁵ the density of serotonin transporters (5-HTT),⁶ and the levels of tryptophan hydroxylase (TPH),⁷ which is the rate-limiting enzyme in serotonin biosynthesis. Therefore, a functional alteration of serotonergic neurotransmission due to gene polymorphisms might lead to serotonergic dysfunction, which could contribute to suicidal behavior. Over the last years, a growing number of molecular genetic studies carried out investigating an association between suicidal behavior and a genetic variant in serotonergic candidate genes, particularly TPH, 5-HTT, monoamine oxidase A, serotonin 2A, 1A and 1B receptor genes.⁷⁻¹³ Studies on the

association of serotonergic candidate gene polymorphism with suicidal behavior have so far not been conclusive.¹⁴

Serotonin 6 (5-HT₆) receptors, which are predominantly expressed in the limbic and cortical regions of the human brain,¹⁵⁻¹⁷ may have a role in the biological susceptibility to suicidal behavior. The evidence for this, although indirect, is that two antipsychotic drugs (clozapine and olanzapine) that strongly interact with 5-HT₆ receptors^{15,17} or affect their expression¹⁸ were also found to reduce the rates of attempted and/or completed suicides in patients with schizophrenia.¹⁹⁻²²

If 5-HT₆ receptors have a role in the biological susceptibility to suicidal behavior, it may be due to polymorphisms in the 5-HT₆ receptor gene. The gene has been mapped to chromosome 1p35-p36 and the receptor is positively linked to adenylyl cyclase.²³ The open reading frame of the receptor cDNA is 1,320 base pairs (bp) long, encoding a protein of 440 amino acids. Two polymorphisms have been detected in the human 5-HT₆ receptors. A silent *Rsa* I restriction fragment length polymorphism has been detected at bp 267 C/T in exon 1,²³ and a trinucleotide repeat polymorphism, (GCC)_{2/3}, has been detected at nucleotide positions between –1093 and –1085 bp upstream from the translation start site.²⁴ Five other variants of the 5-HT₆ receptor gene have been described in a German population, but they were not examined in this study because one of the five variants was in complete linkage disequilibrium with

267C/T polymorphism, the other four variants had very low allele frequencies, and none involved changes in the amino acid sequence.²⁵

To explore the hypothesis that the 5-HT₆ receptor gene is involved in the susceptibility to suicide, we examined whether 267C/T and (GCC)_{2/3} polymorphisms are associated with suicide victims.

METHODS

The study population consisted of 163 suicide victims (113 males and 50 females; mean age $\pm$ SD, 47.6 ± 17.9 years) who completed suicide and on whom autopsies were conducted in the Department of Legal Medicine, Kobe University School of Medicine. In most cases, accurate information about the clinical backgrounds of the suicide completers could not be obtained under our ethical code for genetic studies. The controls consisted of 166 unrelated volunteers (114 males and 52 females; mean age $\pm$ SD, 45.7 ± 16.4 years) who were recruited from the general population of the Kobe city area in Japan. All were healthy and of Japanese descent and none manifested psychiatric problems in brief interviews by psychiatrists. This study was approved by the Ethics Committee of Kobe University Graduate School of Medicine. All control subjects and the families of all suicides in this study gave informed consent.

Peripheral blood was obtained from suicide victims and controls, and leukocyte DNA was extracted for genotype determination. The genotypes of the 5-HT₆ receptor gene 267C/T polymorphisms were determined by the method of Kohen et al.²³ Target sequences were amplified by the polymerase chain reaction (PCR) using primers 5'-AAC TTC TTC CTG GTG TCG CTC TTC-3' and 5'-ATG AGC AGG TAG CGG TCC AGG C-3'. The PCR products were digested with *Rsa* I at 37°C for 4 to 12 hr and then electrophoresed on 3% agarose gels. The PCR product was visualized by ethidium bromide staining and UV transillumination. The 267C allele showed DNA fragments of 371 bp and 126 bp, whereas the 267T allele PCR product remained uncut with a PCR fragment size of 497 bp. The (GCC)_{2/3} polymorphism was genotyped as described by Ohmori et al.²⁴ Target sequences were amplified by PCR using primers 5'-CCC GTT GTG AGT GGG CAG CAC C-3' and 5'-CCT CCC AAC CCA CAC GTG GCT GC-3' (a mismatched nucleotide is underlined). PCR products were digested with *Fsp* I at 37°C for 4 hr and then electrophoresed on 4% agarose gels. The (GCC)₂ allele showed PCR fragments of 84 bp and 23 bp, whereas the (GCC)₃ allele PCR product remained uncut with a PCR fragment size of 110 bp.

The genotype distribution and Hardy-Weinberg equilibrium were tested by chi-square test for goodness of fit. Comparisons of the genotype frequencies and allele frequencies between the two groups were performed using a chi-square test and a two-

tailed Fisher's exact test, respectively. Haplotype frequencies were estimated from genotypic data by the maximum-likelihood method using the LDSUPPORT program.²⁶ The *P* level of significance retained was 0.05.

RESULTS

The samples of 163 suicide victims and 166 controls were genotyped for 267C/T and (GCC)_{2/3} polymorphisms of the 5-HT₆ receptor gene. The distributions of genotype and allele frequencies of both polymorphisms in suicide victims and controls are shown in Table 1. The genotype distributions of the two polymorphisms in both groups were in Hardy-Weinberg equilibrium. In the 267C/T polymorphism, no significant difference was found in the genotype distribution ($\chi^2 = 0.130$, d.f. = 2, *P* = 0.937) or allele frequencies ($\chi^2 = 0.100$, d.f. = 1, *P* = 0.751) between the suicide victims and the controls. Similarly, no significant difference was found in the genotype distribution ($\chi^2 = 1.089$, d.f. = 2, *P* = 0.580) or allele frequencies ($\chi^2 = 0.305$, d.f. = 1, *P* = 0.581) of the (GCC)_{2/3} polymorphism between the two groups. There was also no difference in genotype distribution or allele frequency of the two polymorphisms after stratification by gender (data not shown).

Maximum likelihood analysis of haplotype distributions (Table 2) demonstrated the presence of tight linkage disequilibrium between the two

polymorphisms in both groups ($P < 0.0001$). No significant difference was found in the haplotype distributions between the two groups ($\chi^2 = 0.738$, d.f. = 3, $P = 0.864$).

DISCUSSION

In this study, no association was found between the 267C/T polymorphism and suicide victims. Recently, we learned of a similar study by Turecki et al.,²⁷ in which no association was found between the 267C/T polymorphism and suicide completion in a Caucasian population. Our result is consistent with the results of Turecki et al.²⁷ To our knowledge, our study is the first association study of the trinucleotide repeat polymorphism, (GCC)_{2/3}, in the 5'-upstream region of the 5-HT₆ receptor gene in suicide completers. Although it is possible that the (GCC)_{2/3} polymorphism affects transcriptional regulation, the physiological function of this polymorphism remains unclear. In this study, no association was found between the (GCC)_{2/3} polymorphism and suicide victims. In the 267C/T and (GCC)_{2/3} polymorphisms, the genotype and allele distributions in this study were almost consistent with those previously reported by Shinkai et al.²⁸ and Ohmori et al.²⁴ respectively. Moreover, the two polymorphisms were in tight linkage disequilibrium and the haplotype distribution of the 5-HT₆ receptor gene in suicide victims was not different from that of the controls. The present findings in a Japanese population could

not support our hypothesis that the 5-HT₆ receptor gene is involved in the susceptibility to suicide.

Positive associations were found between the 267T allele and schizophrenia,²⁹ and between the 267C allele and bipolar affective disorder,²⁵ but other studies^{28,30} did not find an association between the 5-HT₆ receptor gene and either schizophrenia or affective disorder. Thus, the results are still far inconclusive about the association of 5-HT₆ receptor gene with specific psychiatric disorders.

This study has some limitations. First, psychiatric diagnoses were not available in this study under our ethical code for genetic studies. We cannot exclude the possibility that psychiatric diagnoses of suicide completers would have affected our results. Second, the sample size of the subjects enrolled may be insufficient. The sample has a power of 1.00 to detect a medium effect size ($w = 0.30$) and of 0.73 to detect a small effect size ($w = 0.10$) at the $P < 0.05$ level for allele comparison. This means that the present study may fail to detect small effects of these polymorphisms on suicide due to a type II error. Third, haplotype map approaches were not applied.³¹

In the present study, our results do not provide any evidence that the 5-HT₆ receptor gene is involved in the susceptibility to suicide. To clarify the serotonergic dysfunction in suicide by molecular genetic approaches, further studies should plan for larger samples, multi-SNP haplotype analysis and association studies of other

serotonergic candidate genes, such as 5-HT₄ and 5-HT₇ receptor genes.

ACKNOWLEDGMENTS

This research was supported by a Research Grant for Nervous and Mental Disorders from the Ministry of Health, Labour and Welfare Japan, by Grants from the Ministry of Education, Science, Culture and Sports, and by Health Sciences Research Grants from the Ministry of Health, Labour and Welfare Japan.

The authors wish to thank Dr Naoyuki Kamatani and Dr Hyosuke Inoue from the Division of Statistical Genetics, Institute of Rheumatology, Tokyo Women's Medical University (Tokyo, Japan) for haplotype analysis.

REFERENCES

1. Asberg M. Neurotransmitters and suicidal behavior. The evidence from cerebrospinal fluid studies. *Ann. N. Y. Acad. Sci.* 1997; **836**: 158-181.
2. Malone KM, Corbitt EM, Li S, Mann JJ. Prolactin response to fenfluramine and suicidal attempt lethality in major depression. *Br. J. Psychiatry* 1996; **168**: 324-329.
3. Mann JJ, Oquendo M, Underwood MD, Arango V. The neurobiology of suicide risk: a review for the clinicians. *J. Clin. Psychiatry* 1999; **60(Suppl. 2)**: 7–11.
4. Roy A, Rylander G, Sarchiapone M. Genetics of suicides: family studies and molecular genetics. *Ann. N. Y. Acad. Sci.* 1997; **836**: 135-157.
5. Highly JD, Thompson WW, Champoux M et al. Paternal and maternal genetic and environmental contributions to cerebrospinal fluid monoamine metabolites in rhesus monkeys (*Macaca mulatta*). *Arch. Gen. Psychiatry* 1993; **50**: 615-623.
6. Du L, Faludi G, Palkovits M et al. Frequency of long allele in serotonin transporter gene is increased in depressed suicide victims. *Biol. Psychiatry* 1999; **46**: 196-201.

7. Ono H, Shirakawa O, Kitamura N et al. Tryptophan hydroxylase immunoreactivity is altered by the genetic variation in postmortem brain samples of both suicide victims and controls. *Mol. Psychiatry* 2002; **7**: 1127-1132.
8. Ono H, Shirakawa O, Nishiguchi N et al. Tryptophan hydroxylase gene polymorphisms are not associated with suicide. *Am. J. Med. Genet.* 2000; **96**: 861-863.
9. Courtet P, Baud P, Abbar M et al. Association between violent suicidal behavior and the low activity allele of the serotonin transporter gene. *Mol. Psychiatry* 2001; **6**: 338-341.
10. Ono H, Shirakawa O, Nishiguchi N et al. No evidence of an association between a functional monoamine oxidase a gene polymorphism and completed suicides. *Am. J. Med. Genet.* 2002; **114**: 340-342.
11. Ono H, Shirakawa O, Nishiguchi N et al. Serotonin 2A receptor gene polymorphism is not associated with completed suicide. *J. Psychiatr. Res.* 2001; **35**: 173-176.

12. Nishiguchi N, Shirakawa O, Ono H et al. Lack of an association between 5-HT_{1A} receptor gene structural polymorphisms and suicide victims. *Am. J. Med. Genet.* 2002; **114**: 423-425.
13. Nishiguchi N, Shirakawa O, Ono H, et al. No evidence of an association between 5HT_{1B} receptor gene polymorphism and suicide victims in a Japanese population. *Am. J. Med. Genet.* 2001; **105**: 343-345.
14. Arango V, Huang YY, Underwood MD, Mann JJ. Genetics of the serotonergic system in suicidal behavior. *J. Psychiatr. Res.* 2003; **37**: 375-386.
15. Monsma FJ Jr, Shen Y, Ward RP, Hamblin MW, Sibley DR. Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol. Pharmacol.* 1993; **43**: 320-327.
16. Gerard C, Martres MP, Lefevre K et al. Immuno-localization of serotonin 5-HT₆ receptor-like material in the rat central nervous system. *Brain Res.* 1997; **746**: 207-219.
17. Roth BL, Craigo SC, Choudhary MS. Binding of typical and atypical antipsychotic

agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J. Pharmacol.*

Exp. Ther. 1994; **268**: 1403-1410.

18. Zhukovskaya NL, Neumaier JF. Clozapine downregulates 5-hydroxytryptamine₆ (5-HT₆) and upregulates 5-HT₇ receptors in HeLa cells. *Neurosci. Lett.* 2000; **288**: 236-240.

19. Meltzer HY, Okayli G. Reduction of suicidality during clozapine treatment of neuroleptic-resistant schizophrenia: impact on risk-benefit assessment. *Am. J. Psychiatry* 1995; **152**: 183-190.

20. Meltzer HY, Alphs L, Green AI et al. International Suicide Prevention Trial Study Group, Clozapine treatment for suicidality in schizophrenia: international suicide prevention trial (InterSePT). *Arch. Gen. Psychiatry* 2003; **60**: 82-91.

21. Walker AM, Lanza LL, Arellano F, Rothman KJ. Mortality in current and former uses of clozapine. *Epidemiology* 1997; **8**: 671-677.

22. Glazer WM. Formulary decisions and health economics. *J. Clin. Psychiatry* 1998; **59**(suppl. 19): 23-29.

23. Kohen R, Metcalf MA, Khan N et al. Cloning, characterization, and chromosomal localization of a human 5-HT₆ serotonin receptor. *J. Neurochem.* 1996; **66**: 47-56.
24. Ohmori O, Shinkai T, Hori H, Nakamura J. Novel polymorphism in the 5'-upstream region of the human 5-HT₆ receptor gene and schizophrenia. *Neurosci. Lett.* 2001; **310**: 17-20.
25. Vogt IR, Shimron-Abarbanell D, Neidt H et al. Investigation of the human serotonin 6 (5-HT₆) receptor gene in bipolar affective disorder and schizophrenia. *Am. J. Med. Genet.* 2000; **96**: 217-221.
26. Kitamura Y, Moriguchi M, Kaneko H et al. Determination of probability distribution of diplotype configuration (diplotype distribution) for each subject from genotypic data using the EM algorithm. *Ann. Hum. Genet.* 2002; **66**: 183-193.
27. Turecki G, Sequeira A, Gingras Y et al. Suicide and serotonin: study of variation at seven serotonin receptor genes in suicide completers. *Am. J. Med. Genet.* 2003; **118B**: 36-40.

28. Shinkai T, Ohmori O, Kojima H, Terao T, Suzuki T, Abe K. Association study of the 5-HT₆ receptor gene in schizophrenia. *Am. J. Med. Genet.* 1999; **88**: 120-122.
29. Tsai SJ, Chiu HJ, Wang YC, Hong CJ. Association study of serotonin-6 receptor variant (C267T) with schizophrenia and aggressive behavior. *Neurosci. Lett.* 1999; **271**: 135-137.
30. Hong CJ, Tsai SJ, Cheng CY, Liao WY, Song HL, Lai HC. Association analysis of the 5-HT₆ receptor polymorphism (C267T) in mood disorders. *Am. J. Med. Genet.* 1999; **88**: 601-602.
31. van den Oord EJ, Neale BM. Will haplotype maps be useful for finding genes? *Mol. Psychiatry* 2004; **9**: 227-236

Table 1. Genotype and Allele Frequencies of the 267C/T and (GCC)_{2/3} Polymorphisms of the 5HT₆ Receptor Gene in Suicide Victims and Controls

	Genotype			Allele	
	<i>n</i> (%)			<i>n</i> (%)	
267C/T (<i>n</i>)	C/C	C/T	T/T	C	T
Control (166)	65 (39.2)	83 (50.0)	18 (10.8)	213 (64.2)	119 (35.8)
Suicide (163)	67 (41.1)	79 (48.5)	17 (10.4)	213 (65.3)	113 (34.7)
(GCC) _{2/3} (<i>n</i>)	(GCC) ₃ /(GCC) ₃	(GCC) ₃ /(GCC) ₂	(GCC) ₂ /(GCC) ₂	(GCC) ₃	(GCC) ₂
Control (166)	85 (51.2)	67 (40.4)	14 (8.4)	237 (71.4)	95 (28.6)
Suicide (163)	85 (52.2)	69 (42.3)	9 (5.5)	239 (73.3)	87 (26.7)

Table 2. Estimated Haplotype Frequencies of the 267C/T and (GCC)_{2/3}Polymorphisms of the 5HT₆ Receptor Gene in Suicide Victims and Controls

	Haplotype			
	C-(GCC) ₃	T-(GCC) ₂	T-(GCC) ₃	C-(GCC) ₂
Control	0.618	0.263	0.096	0.024
Suicide	0.643	0.257	0.090	0.010