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ORIGINAL PAPER

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5HT-2C receptor polymorphism in suicide victims

Association studies in German and Slavic populations

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Abstract Sustainable observations suggest that suicidal behaviour by itself may have biological correlates, among which those related to the serotonergic synapse hold the key position. Based on the association of suicide and serotonergic dysfunction, it was proposed that genetic mechanisms affecting suicidal behaviour could be related to the alterations of the genes encoding the elements of 5HT synapse. The present study tested the association of the polymorphism in the serotonin 2C (5HT-2C) receptor coding region (Cys23Ser) with suicide commitment. Study was based on two independent samples, one of German (284 suicide victims *versus* 297 controls) and other of Slavic/Croatian (118 suicide victims *versus* 275 controls) ethnicity. No significant differences in allele or genotype frequencies between victims and controls were demonstrated. Results did not provide supporting evidence for the potential involvement of the investigated variants of 5HT-2C receptor in the susceptibility to suicide.

Key words 5HT-2C receptor · gene · suicidal behaviour · ethnicity

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Introduction

Suicide is a serious public and mental health problem, which in many countries ranks among the top ten causes of mortality (Diekstra 1996). It was proposed that suicidality could be delineated on a phenomenological level and regarded as a syndrome with the status of the disorder itself, independent of the co-occurring mental illnesses (Ahrens and Linden 1996). Epidemiological studies suggest that suicidal behaviour has a genetic contribution that is independent of the heritability for commonly associated psychiatric disorders (Brenet 1996; Roy 2001; Turecki 2001). One mechanism by which genetic factors might affect the risk for suicide is by the control of serotonergic neurotransmission. Namely, extensive neurochemical research has implicated serotonin (5-hydroxytryptamine, 5HT) as the main neurotransmitter involved in suicidal behaviour (for review see Asberg and Forslund 2000; Mann et al. 2001). The link between serotonergic dysfunction and suicide seems to be sustainable across different psychiatric diagnoses.

The serotonin 2C (5HT-2C) receptor, a functional element of the 5HT synapse, is involved in numerous brain functions, including regulation of mood, neuroendocrine response and appetite (Barnes and Sharp 1999). Pharmacological studies have also indicated its role in different pathological conditions, like depression (Moreau et al. 1996), alcoholism (George et al. 1997), and anxiety (Kennett et al. 1997). The human gene encoding the 5HT-2C receptor is placed on the X chromosome and

contains a single nucleotide polymorphism in the coding region (C/G at the position 68), which results with the substitution of cysteine for serine at position 23 (Cys23Ser) in the extracellular N-terminal domain (Lappalainen et al. 1995). The initial study by Lappalainen et al. (1995) provided no evidence for the functional consequences of this polymorphism, while the subsequent study (Lappalainen et al. 1999) reported on the different functional properties of the Cys and Ser variants. The association of the Cys23Ser polymorphism with various conditions characterised by serotonergic dysfunction (for example, migraine, affective disorders, alcohol dependence) has been investigated, but to our knowledge, there are no data on its role in suicidal behaviour.

Serotonergic neurotransmission is mediated by at least 14 types of 5HT receptors (Barnes and Sharp 1999). The importance of the 5HT-2C receptor gene as a candidate in the genetic research of suicide could be supported by the following lines of evidence. Binding studies demonstrated increased densities of the 5HT-2 receptor sites in brains of suicide victims (Arango et al. 1990). The 5HT-2C receptor was shown to mediate the prolactin response to fenfluramine (Albinsson et al. 1994; Coccaro et al. 1996). A series of studies demonstrated that suicidal behaviour is linked with the blunted prolactin response to fenfluramine (Asberg and Forslund 2000; Mann et al. 2001) and, moreover, that the increase in reduction of prolactin response correlates with the lethality and the intent of the suicidal act (Malone et al. 1996). Furthermore, gender specific differences of suicidal behaviour suggest an X-linked genetic factor. Namely, it was observed that women attempt suicide four times as often as men, while men commit suicide three times as often as women (Kaplan and Sadock 1998). Finally, the role of the 5HT-2C receptor gene was demonstrated in impulsivity (Evans et al. 2000), the trait thought to be relevant to suicidal behaviour.

The current association study evaluates, for the first time, the role of the Cys23Ser polymorphism in the susceptibility to suicide. The study was based on the most lethal form of suicidal behaviour, i. e. suicide completion, and included a large number of suicide victims (N = 402) and control subjects (N = 572) belonging to two ethnically different, but homogenous samples, one of German (German) and another of Slavic (Croatian) origin.

Subjects and methods

Subjects

The case sample consisted of 402 individuals who committed suicide (284 of German and 118 of Croatian ethnicity, Table 1). Suicide methods included: hanging (41 %), shooting (22.9 %), CO and medication poisoning (16.2 %), penetrating lesions (5.2 %), jumping from height, drowning, lying under a train and others (14.7 %). Diagnostic information was available for a proportion of subjects. Blood for DNA extraction was obtained in the course of autopsy at the Institute for Legal Medicine of the Ludwig-Maximilians University Munich (German sample) and at the Department of Forensic Medicine, University of Zagreb (Croatian sample).

Control group consisted of 572 individuals (297 of German and 275 of Croatian ethnicity, Table 1) without personal or family history of neuropsychiatric disorders, including suicidal attempt. Informed written consent was obtained from control subjects and from victims' relatives. The study was approved by the Ethical Committee of the Ludwig-Maximilians University, Munich, and by the Ethical Committee of Medical Faculty, University of Zagreb.

Genotyping

Genomic DNA was isolated from the blood using standard procedures. For 5HT-2C Cys23Ser genotyping, the target DNA sequence was amplified by polymerase chain reaction (PCR) throughout 35 cycles (94 °C for 25 s, 56 °C for 30 s, 72 °C for 20 s) followed by final extension at 72 °C for 7 min. Reaction was carried out in a total volume of 12 µl containing 50 ng of DNA, 200 µM dNTP, 1.5 mM $MgCl_2$, 0.4 U of Taq DNA polymerase and 0.4 µM primers. Primer sequences were taken from the original protocol (Lappalainen et al. 1995). 6 µL aliquots of the PCR products were digested overnight with 15 U of *Hinf I* in a total volume of 20 µL. Digests were electrophoresed on 10 % polyacrylamide gels and visualised by silver staining. The uncut amplicon of 104 bp corresponded to Cys23 allele, while Ser23 allele gave two fragments of 86 and 18 bp.

Statistical analysis

Statistical analyses were carried out using GraphPad InStat (version 3.01) software. Due to the X-chromosomal location of the investigated gene, males and females were considered separately. Differences in genotype distributions between case and control groups, as well as the presence of Hardy-Weinberg equilibrium, were tested by the χ^2 test for independence. Comparisons of allele frequencies were performed by the two-sided Fisher's Exact Test (FET).

Table 1 Demographic characteristics of subjects

Samples	German		Croatian		Combined	
	Controls	Victims	Controls	Victims	Controls	Victims
Males						
N (%)	151 (50.8)	203 (71.5)	163 (59.3)	86 (72.9)	314 (54.9)	289 (71.9)
Mean age ± SD	40 ± 15	46 ± 17	42 ± 12	49 ± 20	42 ± 14	47 ± 18
Females						
N (%)	146 (49.2)	81 (28.5)	112 (40.7)	32 (27.1)	258 (45.1)	113 (28.1)
Mean age ± SD	42 ± 14	49 ± 19	43 ± 12	59 ± 18	42 ± 13	52 ± 19
Total						
N (%)	297 (100)	284 (100)	275 (100)	118 (100)	572 (100)	402 (100)
Mean age ± SD	41 ± 15	47 ± 18	43 ± 12	52 ± 20	42 ± 14	48 ± 19

Results and discussion

The allele and genotype frequencies of the Cys23Ser polymorphism in suicide victims and control subjects are presented in Table 2 (females) and Table 3 (males).

In contrast to the previous report on the variation in the allele frequencies among different populations (Lerer et al. 2001), which are based, however, on the far smaller samples (mainly below 100 subjects per ethnicity), we did not find significant differences between German and Croatian groups (FET, $p = 0.1448$ and 0.7996 for controls and victims, respectively). All subsequent analyses were, thus, performed in both ways: separately for each population (in order to detect potential ethnical differences in regard to association with suicide), and also in the combined German/Croatian sample (which considerably increased the statistical power).

Because of the X-chromosomal location of the 5HT-2C receptor gene, males and females were first analysed separately. Genotype frequencies in females accorded with Hardy-Weinberg equilibrium in both, German (controls: $\chi^2 = 0.1660$, d. f. = 2, $p = 0.9203$; victims: $\chi^2 = 0.3879$, d. f. = 2, $p = 0.8237$) and Croatian (controls: $\chi^2 = 0.0.$ 2181, d. f. = 2, $p = 0.8967$; victims: $\chi^2 = 2.091$, d. f. = 2, $p = 0.3515$) samples. Female controls and victims did not differ in genotype or allele frequencies in the Croatian, German or combined sample (Table 2). Similarly, male victims and controls did not show differences in allele frequencies in any of the samples (Table 3).

No statistically significant differences in allele frequencies between male and female subjects, as contrasted to some previous reports (Fehr et al. 2000), were observed either in the control (FET, $p = 0.0577$, 0.7770 and 0.3014 for German, Croatian and combined samples, respectively) or victim (FET, $p = 0.8907$, 0.6589 and 0.9075 for German, Croatian and combined samples, respectively) sample. This allowed the fusion of male and female samples, providing considerably high statistical power for detecting potential association. Allele frequencies of the overall (male and female) samples, again, did not differ between controls and victims (FET, $p = 0.5871$, 0.2037 and 1.0000 for German, Croatian and combined samples, respectively).

Taking all together, we did not find any significant differences in the distribution of the Cys23Ser polymorphism between control subjects and suicide victims, regardless of the gender- or ethnicity-related subanalyses. Our results together with the recent report on the lack of association between the polymorphism in the promoter region of the 5HT-2C receptor gene and suicide in French-Canadian population (Turecki et al. 2003), speak against the major role of the 5HT-2C receptor gene in the susceptibility to suicide, although they do not completely exclude the possibility that polymorphisms in other regions of the gene could still be involved in the disorder.

In conclusion, the presented results, obtained on a large number of suicide victims and controls, and based on two ethnically homogenous samples, did not provide the evidence for the role of the Cys23Ser variants of the 5HT-2C receptor in the susceptibility to suicide. The control data, not confirming previously reported ethnic (Lerer et al. 2001) and gender (Fehr et al. 2000) differences extend the insight into the gene variability and could serve as a respective reference for the subsequent association studies on this polymorphism.

Table 2 Allele and genotype counts and frequencies of the Cys23Ser polymorphism of the 5HT-2C receptor in female control subjects and suicide completers. *p* values for differences in genotype and allele distributions are calculated by Chi-square and Fisher's Exact Test, respectively

Samples	German		Croatian		Combined	
	Controls (N = 146)	Victims (N = 81)	Controls (N = 112)	Victims (N = 32)	Controls (N = 258)	Victims (N = 113)
Genotypes N (%)						
Cys/Cys	102 (69.9)	57 (70.4)	80 (71.4)	23 (71.9)	182 (70.5)	80 (70.8)
Cys/Ser	39 (26.7)	20 (24.7)	28 (25.0)	6 (18.8)	67 (26.0)	26 (23.0)
Ser/Ser	5 (3.4)	4 (4.9)	4 (3.6)	3 (9.4)	9 (3.5)	7 (6.2)
p	**0.8250**		**0.3435**		**0.4471**	
Alleles N (%)						
Cys	243 (83.2)	134 (82.7)	188 (83.9)	52 (81.3)	431 (83.5)	186 (82.3)
Ser	49 (16.8)	28 (17.3)	36 (16.1)	12 (18.7)	85 (16.5)	40 (17.7)
p	**0.8967**		**0.5751**		**0.6714**	

Table 3 Allele counts and frequencies of the Cys23Ser polymorphism of the 5HT-2C receptor in male control subjects and suicide completers. *p* values for differences in allele distributions are calculated by Fisher's Exact Test

Samples	German		Croatian		Combined	
	Controls (N = 151)	Victims (N = 203)	Controls (N = 163)	Victims (N = 86)	Controls (N = 314)	Victims (N = 289)
Alleles N (%)						
Cys	114 (75.5)	166 (81.8)	139 (85.3)	73 (84.9)	253 (80.6)	239 (82.7)
Ser	37 (24.5)	37 (18.2)	24 (14.7)	13 (15.1)	61 (19.4)	50 (17.3)
p	**0.1861**		**1.0000**		**0.5290**	

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