

NO ASSOCIATION BETWEEN POLYMORPHISMS IN FOUR SEROTONIN RECEPTOR GENES, SEROTONIN TRANSPORTER GENE AND ALCOHOL-RELATED SUICIDE

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SUMMARY

Background: Serotonin (5-HT) is an important neurotransmitter with wide-ranging functions. Its dysfunction in the central nervous system seems to play an important role in many psychiatric disorders and suicidal behavior. The objective of this study was to examine the association between polymorphisms in different serotonin receptor genes (HTR): HTR1A (polymorphism -1019C>G), HTR1B (polymorphisms 861G>C and -161A>T), HTR1F (polymorphism -78C>T) and HTR2A (polymorphism -1420C>T), and serotonin transporter gene (5-HTT) (polymorphism LPR in promoter and VNTR in the second intron), and completed alcohol-related suicide, as well as between alcohol-dependent suicide victims.

Subjects and methods: The study subjects were 373 Slovenian suicide victims (mean age \pm SD: 48.8 \pm 17.7 years) autopsied in the years 2002 through 2005. During autopsy venous blood was drawn, and afterwards DNA extraction and alcoholimetric analysis were performed. Relatives of 79 suicide victims were interviewed using a semi-structured questionnaire designed according to Slovenian cultural and economic conditions. They provided information about the alcohol abuse of the suicide victims. Amongst the suicide victims were 25 alcohol misusers and 54 non-misusers.

Results: Association between polymorphisms in the selected serotonin receptor genes, transporter gene and completed alcohol-related suicide, as well as between alcohol-dependent suicide victims was not established.

Conclusions: Present results suggest that selected polymorphisms of the 5-HT receptor genes and transporter gene are not involved in genetic susceptibility to completed suicide under acute influence of alcohol or among alcohol-dependent individuals, but further studies in a larger sample are needed.

Key words: genetics – suicide – alcohol-dependency – candidate gene – serotonergic system – Slovenian population

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INTRODUCTION

According to the data of the World Health Organization approximately 1 million people every year commit suicide, and by the year 2020 the number of suicides will increase to 1.53 million people per year (World Health Organization 2003). In Europe, the countries with the highest suicide rates form a J-shaped area, which extends from the Scandinavian countries through countries of Eastern and Middle Europe down to Slovenia (Marusič 2005). A potent risk factor of suicide is alcohol dependence (Harris & Barraclough 1997, Pirkola et al. 2004) and among those who committed suicide 20 to 35% are alcohol-dependent individuals (Nörstrom & Ramstedt 2005). Data about the risk of alcohol use among light drinkers are not consistent and the role of alcohol in events leading to completed suicide is less clear (Nakaya et al. 2007, Crombie et al. 1998). Neurobiological evidence shows that the serotonergic dysfunction or other influences on the serotonergic system such as specific genetic and neurobiological factors might affect the risk for suicide (Joiner et al. 2005). A disruption in the serotonergic system is also associated with different psychiatric

disorders in addition to addiction and abuse disorders (Drago & Serretti 2009). Alcohol-dependence is also associated with alterations in the serotonergic signal transduction pathway and altered serotonin function is a genetic factor influencing suicidal behavior (Underwood et al. 2004). Further, impulsivity was associated with both suicidal behavior and alcohol misuse and is linked with serotonergic system dysfunction (Underwood et al. 2004).

At least 14 different receptors clustered into seven families are included in the serotonergic signaling system (Tohda et al. 2006). A number of these receptors may be implicated in the genesis of depression, suicidal behavior, aggression, impulsivity (Du et al. 2000) and alcohol-dependence (Parsian & Cloninger 2001). Neurotransmission over such a multitude of receptors permits great diversity in signaling and modulation of different central effects mediated over just a single neurotransmitter (Mann 2003).

Numerous neurobiological studies on suicide have been done on serotonin (5-HT) signal transduction-linked single nucleotide polymorphisms (SNPs) of serotonin receptor genes (HTR) 1A, 1B, 2A and in a lesser extent on 1F receptor. For example -1019C>G

polymorphism of the HTR1A promoter could be in association with psychosis, substance abuse, panic disorder, and suicide (Mann 1999, Lemonde et al. 2003, Wasserman et al. 2006) and allele G with Barbos type B alcoholism and numerous suicide attempts in the past (Koller et al. 2006). From various animal model studies and human genetics studies it appears that dysfunction of HTR1B could be implicated in pathogenesis of impulsive, aggressive, and sexual behavior, alcoholism, substance abuse and suicide (Mann 2003, Arango et al. 2003, Huang et al. 2003). Studies on polymorphisms of HTR1F and 2A, screened for association with migraine and suicide (Maassen Van Den Brink et al. 1998, Turecki et al. 2003, Correa et al. 2007).

Beside HTRs also serotonin transporter gene (5-HTT) seems to play an extremely important role in suicide, alcoholism and other disorders (Serretti et al., 2006). It was demonstrated that polymorphisms in the promoter region (5-HTTLPR) and in the second intron (5-HTTVNTR) have functional consequences affecting gene expression (Bondy et al. 2006). Studies investigating the association between the polymorphisms and suicidal behavior or suicide show inconsistent results (Akar et al. 2010, Courtet et al. 2001, Courtet et al. 2004, De Luca et al. 2005, Hranilovic et al. 2003, Shen et al. 2004, Wasserman et al. 2007, Yen et al. 2003). Meta-analysis have been carried out on alcohol-dependence, comorbid psychiatric condition and 5-HTTLPR to demonstrate an association with S allele (Feinn et al. 2005).

In our study we investigated the association of five polymorphisms in the four different serotonin receptor genes, namely HTR1A (polymorphism -1019C>G), HTR1B (polymorphisms 861G>C and -161A>T), HTR1F (polymorphism -78C>T) and HTR2A (polymorphism -1420C>T), two polymorphisms in serotonin transporter gene (5-HTTLPR and 5-HTTVNTR) with completed alcohol-related suicide, as well as between alcohol-dependent suicide victims. The study was conducted on the Slovenian population which is very prone to suicide, and has one of the highest suicidal rates in the world.

SUBJECTS AND METHODS

Subjects

The study was carried out at the Institute of Forensic Medicine and Institute of Biochemistry, Faculty of Medicine, University of Ljubljana. The study subjects were 373 suicide victims (283 (75.9%) men, 90 (24.1%) women) autopsied at the aforementioned institution. Consecutively in the years 2002 through 2005 during the course of autopsy blood from the femoral vein and urine were collected from all subjects. All subjects were unrelated Caucasians living in the extended Ljubljana region. The mean age \pm SD of the suicide victims was 48.8 ± 17.7 years. The male to female ratio was 3.1:1.

Suicide methods were distributed into groups: hanging (N=164, 40.0%), shooting (N=55, 14.7%), jumping from height (N=43, 11.5%), carbon monoxide/medication poisoning (N=43, 11.5%), drowning (N=27, 7.2%), cutting/stabbing (N=18, 4.8%), traffic accidents (N=18, 4.8%), and together self-burning, electric stress, other methods of suffocation than hanging and corrosive substances (N=5, 1.3%). The study was approved by the Slovenian National Medical Ethics Committee.

Alcoholimetric analysis

Specimens of venous blood and urine needed for analysis were preserved at 4°C until alcoholimetric analysis (Petković et al. 2005). Duplicate determinations of ethanol were performed by head-space gas chromatography (HSS-GC-FID), according to the Institute's routine laboratory protocol. An analytical cut-off in blood of 0.2 g/kg was used to report ingestion of alcohol before committed suicide (blood alcohol concentration (BAC) positive). Gender frequency distributions among BAC (blood alcohol concentration) negative and BAC positive suicide victims are shown in Table 1.

Life Events-Clinical Features

Relatives of suicide victims were interviewed by two investigators using a semi-structured questionnaire designed according to Slovenian cultural and economic conditions. After invitation of two hundred twelve relatives, relatives of seventy nine suicide victims provided information according to a set of three questions about alcohol abuse. Seventy nine suicide victims (mean age $48.1 \pm$ SD: 17.7 years) were included in the subjects group on whom a psychological autopsy was performed, and amongst them were 25 alcohol misusers (mean age $51.9 \pm$ SD: 12.1 years) and 54 non-misusers (mean age $46.3 \pm$ SD: 19.6). The male to female ratio was 1.9:1 and amongst the alcohol misusers 5.3:1 (Table 2). Suicide methods were distributed as follows: hanging (N=27, 34.2%), shooting (N=16, 20.3%), carbon monoxide/medication poisoning (N=15, 19.0%), drowning (N=6, 7.6%), cutting/stabbing (N=5, 6.3%), jumping from height (N=4, 5.1%), traffic accident (N=4, 5.1%), and corrosive substances (N=1, 1.3%). In the group of alcohol misusers the leading methods were: hanging (N=9, 36.0%) and shooting (N=8, 32.0%)

Genotyping

Venous blood was drawn and immediately stored at -70°C until DNA extraction was performed with Wizard® Genomic DNA Purification Kit (Promega, USA). The polymorphic loci were genotyped as it was previously described (Pungercic et al. 2006, Videtič et al. 2006, Videtič et al. 2009).

Statistical analysis

All statistical analyses were carried out using SPSS v.14.0 (Statistical Package for the Social Sciences, Chicago, IL, USA) and Microsoft Excel. Mean and median were used as descriptive statistics. Percentages, t-test and Pearson chi-square or Fisher exact test (FET) were used for comparison between groups. Hardy-Weinberg equilibrium was calculated with an on-line program (<http://krunch.med.yale.edu/hwsim/>; Yale University, New Haven, CT, USA). The results were considered statistically significant if $P < 0.05$.

Haplotype analysis for the selected markers was performed with THESIAS program v. 3.1 (Tregouet & Garelle 2007).

RESULTS

The results for genotype counts and frequency distributions are shown in Table 3. Genotype distributions were in Hardy-Weinberg equilibrium (data not shown). The suicide victims were arranged into two subgroups on the basis of presence of alcohol in their blood at the time of the suicide. After comparing genotype distributions between subgroup with blood alcohol concentra-

tion under and over analytical cut-off value, these did not differ between subgroups. The acute use of alcohol before accomplished suicide was more frequent among male than female (83.7% vs. 16.3%, $P < 0.05$) (Table 1). Results of the special questionnaire about alcohol misuse and dependence were available only for a small proportion of the suicide victims ($n=79$, 21.2% of the total) and are shown in Table 2. Relatives of the deceased in the subgroup of male suicide victims more frequently reported alcohol-dependence or misuse (84.0% vs. 16.0%, $P < 0.05$) (Table 2). All suicide victims in the alcohol misusers subgroup had BAC equal or above 0.2 g/kg at time of suicide.

We checked for differences in genotype frequency distributions among the suicide victims with BAC above and below 0.2 g/kg. Comparison of these two subgroups did not show significant differences. Further we checked for differences in genotype frequency distribution among suicide victims reported to misuse alcohol and those who did not. Again, comparison of these two subgroups did not show significant differences (Table 3).

Haplotype analysis did not reveal a haplotype that would have statistically significant distributions between the studied (sub)groups.

Table 1. Gender frequency distributions among BAC-negative and -positive suicide victims. P values for differences in gender frequency distributions among BAC-negative and -positive suicide victims were calculated with t-test

Number of suicide victims	Mean age (±SD)	Gender		Blood alcohol concentration by gender				
		Female n (%)	Male n (%)	<0.2 g/kg		≥0.2 g/kg		P value (BAC ≥ 0.2 g/kg)
				n (%)	n (%)	n (%)	n (%)	
373	48.8 (±17.7)	90 (24.1)	283 (75.9)	70 (77.8)	20 (22.2)	180 (63.3)	103 (36.4)	<0.05

Table 2. Differences in gender frequency distributions among alcohol misusers. P values for differences in gender frequency distributions among alcohol misusers were calculated with t-test

Number of suicide victims with psychological autopsy	Mean age (±SD)	Gender		Alcohol misusers		P value (alcohol misusers by gender)
		Female n (%)	Male n (%)	Female n (%)	Male n (%)	
79	48.1 (±17.7)	27 (34.2)	52 (65.8)	4 (14.8)	21 (40.4)	< 0.05

DISCUSSION

Our findings suggest that men are more prone to the risk of completed suicide under acute influence of alcohol, what is in accordance with previous studies (Crombie et al. 1998, Bilban & Skibin 2005). This significant difference could be in accordance with reports that in Slovenia men abuse alcohol more often than women. Alcohol was present in all suicide victims reported for alcohol-dependence and its presence could be explained as a reflection of usual behavior than an indication of a contributory role in the decision to commit suicide (Crombie et al. 1998).

The results of the present study do not support the implication of serotonin receptor 1A promoter

polymorphism -1019C>G in alcohol-related suicide; neither do they support it in the subgroup with reported alcohol-dependence. Our results are accordant with results from a previous study of Koller et al. (2006) who studied alcohol-dependent suicide attempters and did not find an association between the two. The plausible role of 1A receptors in suicide among alcohol-dependent individuals was indicated in the study of Underwood et al. (2004), where in suicide cases among alcohol-dependent individuals up-regulation of HTR1A in the ventral prefrontal cortex may fail and so did not mitigate the impact of decreased serotonergic transmission in suicidal alcohol-dependent individuals (Underwood et al. 2004).

Table 3. Genotype counts and frequency distributions. P values for differences in genotype frequency distributions were calculated with Pearson Chi-square test or Fischer's exact test. All samples were in Hardy-Weinberg equilibrium. Genotype frequency distributions were compared between suicide victims with BAC under or over 0.2 g/kg and all P values were above 0.05.

Locus/ Polymorphism	Genotype	BAC		Alcohol	
		<0.2g/kg n (%)	≥0.2g/kg n (%)	Non-misusers n (%)	Misusers n (%)
5-HT 1A -1019C>G	CC	44 (21.9)	21 (25.6)	8 (25.0)	5 (25.0)
	CG	96 (47.8)	42 (51.2)	14 (43.8)	9 (45.0)
	GG	61 (30.3)	19 (23.2)	10 (31.2)	6 (30.0)
		$\chi^2 = 1.555$, df = 2, P = 0.467		$\chi^2 = 0.011$, df = 2, P = 0.995	
5-HT 1B 861G>C	GG	95 (62.1)	41 (67.2)	25 (65.8)	11 (55.0)
	GC	51 (33.3)	19 (31.1)	12 (31.6)	9 (45.0)
	CC	7 (4.6)	1 (1.7)	1 (2.6)	0 (0.0)
		P(FET) = 0.645		P(FET) = 0.605	
5-HT 1B -161TA>T	AA	46 (31.3)	19 (33.4)	11 (30.6)	5 (25.0)
	AT	79 (53.7)	30 (52.6)	21 (58.3)	13 (65.0)
	TT	22 (15.0)	8 (14.0)	4 (11.1)	2 (10.0)
		$\chi^2 = 0.087$, df = 2, P = 0.953		P(FET) = 0.916	
5-HT 1F -78TC>T	CC	152 (99.3)	59 (96.7)	38 (100)	18 (45.0)
	CT	1 (0.7)	2 (3.3)	0 (0.0)	2 (5.0)
	TT	0 (0.0)	0 (0.0)	0 (0.0)	20 (50.0)
		P(FET) = 0.196		P(FET) = 0.115	
5-HT 2A -1420C>T	CC	126 (82.9)	53 (86.9)	29 (78.4)	16 (40.0)
	CT	22 (14.5)	8 (13.1)	8 (21.6)	4 (10.0)
	TT	4 (2.6)	0 (0.0)	0 (0.0)	0 (0.0)
		P(FET) = 0.654		P(FET) = 1.000	
5-HTT* LPR	SS	29 (19.0)	9 (15.0)	9 (23.7)	3 (17.6)
	LS	64 (41.8)	33 (55.0)	19 (50.0)	9 (52.9)
	LL	60 (39.2)	18 (30.0)	10 (26.3)	5 (29.5)
		$\chi^2 = 3.019$, df = 2, P = 0.231		P(FET) = 0.864	
5-HTT** VNTR	9,10	2 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
	9,12	3 (2.0)	1 (1.6)	0 (0.0)	0 (0.0)
	10,10	19 (12.4)	7 (11.5)	6 (15.8)	2 (11.8)
	10,12	66 (43.1)	25 (41.0)	18 (47.4)	7 (41.2)
	12,12	63 (41.2)	28 (45.9)	14 (36.8)	8 (47.0)
		P(FET) = 0.976		$\chi^2 = 0.536$, df = 2, P = 0.791	

*Genotypes for 5-HTTLPR: SS, short/short; SL, short/long; LL, long/long; S = short allele; L = long allele.

**Genotypes for 5-HTTVNTR: alleles: 9, 10, 12 are numbers of variable repeats; combinations of repeats are: 9,10; 9,12; 10,10; 10,12; 12,12.

In our study we further looked for implication of serotonin receptor 1B and its polymorphisms 861G>C and -161A>T in alcohol-related suicide and suicide among alcohol-dependent individuals. We observed no association between serotonergic receptors gene variants and suicide. The same was truth for suicide victims and controls from general Slovenian population (Videtič et al. 2006) and is in line with previous results on polymorphism 861G>C HTR1B on the Croatian population (Stefulj et al. 2004) and in studies by Nishiguchi et al. (2001) and Rujescu et al. (2003).

Further, no significant association was found between polymorphism -78C>T in the serotonin receptor 1F gene and (sub)groups of suicide victims. Previous

study by Videtič et al. (2006) showed no differences in studied polymorphism in promoter region -78C>T among Slovenian suicide victims and control groups.

As the last of the HTR polymorphisms in the present study, we tested polymorphism -1420C>T of serotonin receptor gene 2A, which has been reported to be altered in subjects with suicidal behavior, mood disorders, and aggressive-impulsive traits (Khait et al. 2005). As in the cases of prior investigated polymorphisms in our study we did not find a significant difference between investigated (sub)groups of suicide victims. A previous study on the same population sample by Videtič et al. (2006) revealed for -1420C>T in HTR2A a tendency for association with suicide.

Our results on the 5-HT transporter polymorphisms LPR and VNTR showed no association with alcohol-related suicide, which is in accordance with the results of the study on Slovenian suicide victims (Pungercic et al. 2006) and also meta-analysis performed by Lin & Tsai (2004). Meta-analyses on alcohol-dependence by Feinn et al. (2005) and McHugh et al. (2010), and the analysis of Florez et al. (2008), however, showed association with LPR and VNTR. Furthermore was the S allele of LPR more frequently observed in suicidal compared to non-suicidal alcohol-dependent subjects (Preuss et al. 2001). Despite some positive association results we have to keep in mind that the results are very conflicting although the number of studies is very large.

In order to upgrade our study we calculated different combinations of haplotype effects and risky alleles in the haplotypes, but their distributions in alcohol-related suicide did not show any association.

In our study the sample in subgroups of suicide victims was small, especially the sample of suicide victims with a performed psychological autopsy. This is main limitation of our study. We were not able, despite the relatively high number of candidate genes studied, to find any statistically significant association. Also haplotype analysis and subgrouping with respect to more homogeneous endophenotypes, and inclusion of quantifiable characteristics were not sensible enough to help untangle the genetic background of such a complex disorder as alcohol-related suicide.

CONCLUSIONS

The results we have presented suggest that selected polymorphisms of 5-HT receptor genes and transporter gene are not involved in genetic susceptibility to completed suicide under the acute influence of alcohol or among alcohol-dependent individuals in the Slovenian population, however further studies on a larger sample are needed.

However, because the study was accomplished in a country with a small population which is very prone to suicide and with high per capita alcohol consumption, it can be informative and could contribute to the answer about the implication of the serotonergic signaling pathway in alcohol-related suicide.

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