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ORIGINAL PAPER

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Suicide, stress and serotonin receptor 1A promoter polymorphism -1019C>G in Slovenian suicide victims

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Abstract Implication of serotonergic system in suicide and suicide attempts has been discussed for several years. One of the most abundant serotonin receptors in the mammalian brain is the receptor 1A (5-HT1A); studies of its polymorphisms and suicide have provided very inconsistent results so far. The suggestion that the G allele depresses HTR1A autoreceptor expression, and therefore reduces serotonergic neurotransmission that might predispose to depression and suicide, made the promoter polymorphism -1019C>G a very promising candidate gene. In our study we analyzed promoter polymorphism -1019C>G on 323 suicide victims and 190 controls (all of Slovenian origin), taking into account sex, suicide method, and in case of suicide victims also stressful life events. Differences in the distributions of genotype and allele frequencies were not statistically significant between suicide victims and control group, and the same was found for distributions according to sex and suicide method. For 62 suicide victims information about stressful life events in the month prior to the suicide and in childhood was provided. For analysis we combined CG/GG genotypes and compared them to the CC genotype. More stressful life events in the month prior to the suicide were reported for the subgroup with CC genotype (mean number of events = 2.53; SD = 1.50) in comparison to subgroup with CG/GG genotypes (mean number of events = 1.58; SD = 1.32; $P < 0.05$). However, subgroups of suicide victims with CC or CG/GG genotypes did not differ regarding numbers of reported stressful life events in childhood ($P > 0.05$). Our study provides no evidence for the implication of HTR1A promoter polymorphism in suicide in general, but it suggests further studies that would take into account the interconnected network of suicide completion, genetic background and stress, beside other risk factors.

Key words serotonin receptor 1A · polymorphism · Slavic/Slovenian population · suicide · stressful life events

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Introduction

The possible implication of serotonergic system in suicide and attempted suicide has been discussed for several years. An early publication of findings on the subject was by Asberg et al. [5] who reported low 5-hydroxyindolacetic acid (5-HIAA) concentrations in the cerebrospinal fluid of suicide victims [6].

Through the 30 years since then, numerous studies of comprehensive serotonergic system have been performed but searches for candidates in the largest group of serotonergic genes, the serotonin (5-hydroxytryptamine, 5-HT) receptors, have produced very inconsistent results [8]. One of the most abundant of the serotonin receptors in the mammalian brain is the receptor 1A (5-HT1A) [1, 17]. A member of the G-protein coupled receptor superfamily, this acts as a postsynaptic receptor as well as a major somatodendritic autoreceptor on serotonergic Raphe neurons

[1, 17]. The gene for the 5-HT1A receptor (HTR1A) lies on chromosome 5q11.2-13, is intronless, and has a number of single nucleotide polymorphisms (SNPs) [12]. First who studied two functional polymorphisms, Pro16Leu (47C>T) and Gly272Asp (815G>A), in connection with suicide was Nishiguchi et al. [16]. He did not find any statistically important differences in genotype or allele frequency distributions between suicide victims and control group [16]. We did the first study of this particular polymorphism on a population of Slovenian Caucasians, and failed to find our population polymorphic [21]. However, the studies of promoter polymorphism -1019C>G [25] in the HTR1A so far have been more incentive [2, 15]. The polymorphism -1019C>G is a part of an imperfect palindrome sequence of 26 bp that bounds a single repressor, nuclear DEAF-1-related (NUDR) protein [15]. Since this SNP lies in the promoter region that has been associated with a significant repressor activity, it has been hypothesized that variation in the sequence of the repressor binding region could lead to impaired repression of the HTR1A. Consequently it could enhance the negative feedback inhibition of serotonergic Raphe neurons and lead to a lower serotonergic neurotransmission, causing a predisposition to depression and suicide [15, 23]. It was also reported that allele G is associated with Barbos type B alcoholism and number of suicide attempts in history [14].

Beside genetic studies of 5-HT1A there have been some protein studies. The results that they have provided are suggesting the implication of 5-HT1A in suicide in different manners. Namely, in a study of Arango et al. [3] a reduction in the 5-HT1A distribution volume, a decrease of an index of the total number of 5-HT1A receptors in the dorsal Raphe nucleus and lower receptor binding capacity in the median Raphe nucleus were found when comparing suicide victims with a control group [3]. Reduction of 5-HT1A autoreceptors as an adaptive mechanism, whose function is to increase serotonin activity, was suggested as an explanation of these results [3]. On the other hand, studies of Joyce et al. [13] and Arango et al. [4] determined an increase in the number of binding sites in the ventral prefrontal cortex of suicide victims. Huang et al. [12] replicated this result in a *post mortem* study on a much larger sample and found the binding capacity of 5-HT1A to be increased. However, this finding was not related to the genotype of HTR1A polymorphism -1019C>G nor was the polymorphism -1019G overrepresented in the group of suicide victims. This latter result was replicated by Wasserman et al. [23], who found an indication of a possible role of the G allele in those attempting suicide when exposed to traumatic and/or stressful life events [23]. The importance of environmental stress on various components of the serotonergic system has been supported by studies performed in the past 40 years [10]. Specific combinations of stressors with specific genotypes/biological stress markers that could provoke suicidal behavior or suicide are in progress [10, 20, 22].

Since there is some evidence suggesting a possible implication of 5-HT1A in suicide as well as polymorphism, -1019C>G of 5-HT1A, we wanted to investigate this genetic marker in the Slovenian population which has one of the highest suicide rates (26.3 suicide victims per 100,000 citizens) in the world [24], and thus contribute views supportive or contrary to the hypothesis which at present has a major role in the research of molecular genetics of suicide. We also wished to evaluate stressful life events in the month before suicide completion and in childhood in relation to HTR1A genotype of suicide victims.

Materials and methods

Research subjects

The study, approved by the Slovenian Medical Ethics Committee, enrolled 323 suicide victims and 190 control subjects from autopsies at the Institute for Forensic Medicine, Faculty of Medicine of the University of Ljubljana. Venous blood used for DNA extraction was collected from all subjects in the course of autopsy.

The mean age ± SD of the group of suicide victims was 48.3 ± 18.3 and of control group 49 ± 17.8 years. The male to female ratio in the control group was 1.9:1 and in the suicide group 2.9:1 which was in accordance with the historical suicide ratio between Slovenian men and women. Suicide methods were distributed as follows: hanging (n = 140, 43.3%), shooting (n = 49, 15.2%), jumping from height (n = 36, 11.1%), CO/medication poisoning (n = 34, 10.5%), drowning (n = 26, 8.0%), cutting superficial wounds (n = 15, 4.6%), lying under train (n = 15, 4.6%), and taken together, self-burning, suffocation, electrocution and planned car accident (n = 8, 2.7%).

Stressful life events

One hundred relatives of suicide victims were invited to provide information about stressful life events of suicide victims in a month prior to suicide and in childhood. Thirty-eight of the 100 relatives declined to participate but relatives of the remaining 62 victims provided information about stressful life events of the suicide victims who included 40 males and 22 females with mean age ± SD of 50 ± 17 and 48.0 ± 17 years, respectively. Information about stressful life events in a month prior to suicide was collected according to a subset of 12 life event categories with considerable long-term contextual threat as published by Brugha et al. [9]. The items were modified according to our understanding of Slovenian culture. Information about stressful life events in childhood was collected according to a set of seven life event categories encompassing items regarding physical, emotional and sexual abuse, general traumatic experience, parent's death, divorce or mental disorder. Also these items were designed according to our understanding of Slovenian culture. Structured questions were asked by two investigators. The number of all stressful life events in a month prior to suicide and in childhood was gathered separately for each suicide victim.

Genotyping

Genomic DNA was extracted from blood samples with a Wizard® Genomic DNA Purification Kit (Promega, USA). Genotyping with PCR was carried out in a total volume of 25 μL, containing

Table 1 Genotype and allele counts and frequency distributions for polymorphism -1019C>G for serotonin 1A receptor gene in suicide victims and control groups

	Genotype			Allele	
	CC	CG	GG	C	G
Control group, *n* (%)	49 (25.8)	84 (44.2)	57 (30)	182 (47.9)	198 (52.1)
Suicide victims, *n* (%)	72 (22.3)	160 (49.5)	91 (28.2)	304 (47.1)	342 (52.9)
	$\chi^2 = 1.47$, $df = 2$, $P = 0.482$			$\chi^2 = 0.07$, $df = 1$, $P = 0.846$	

P values for differences in genotype and allele frequency distributions were calculated with Pearson Chi-square test

30–100 ng of DNA, 0.3–0.4 μM primers and 0.625 U *Taq* DNA polymerase in PCR Master Mix (Promega), in a GeneAmp® PCR System 2700 amplificator (Applied Biosystems, USA). Samples were amplified with PCR by initial denaturation for 10 min at 95°C and 35 cycles of denaturation at 95°C, annealing at 54°C and elongation at 72°C, each for 45 s, and completed with final extension step at 72°C for 7 min. Primers for the PCR designed previously by Hong et al. [11] were used. PCR products were digested with *Hpy*CH4 IV (New England Biolabs® Inc.). One uncut band of 182 bp corresponded to the C allele, while two bands of 158 and 24 bp corresponded to G allele. After digestion, the PCR products were separated on 2.5% agarose gel and visualized with ethidium bromide staining and UV light.

Statistical analysis

Hardy–Weinberg (HW) equilibrium for both sample groups was calculated with an on-line program (http://www.kursus.kvl.dk/shares/vetgen/_Popgen/genetik/applets/kitest.htm).

Since the group of suicide victims with data about stressful life events was relatively small and because the G allele leads to higher expression of HTR1A, we combined the genotypes CG/GG, and compared them to the CC, to gain some more statistical power. The mean numbers ± SD of stressful life events in a month before suicide were calculated for each subgroup and *t* test was performed for comparison. The same procedure was used for subgroup comparison regarding stressful life events in childhood.

Statistical computation of *P* value, Pearson Chi-Square and *t* test were carried out using the Statistical Package for Social Studies SPSS v.13.0.

Results

Genotype frequencies in suicide victims and in the control group were in HW equilibrium (control group $\chi = 2.48$, $df = 1$, $P = 0.12$, suicide victims $\chi^2 = 0.01$, $df = 1$, $P = 0.92$). The difference in the distributions of genotype and allele frequencies, presented in Table 1 was not found to be statistically significant between suicide victims and control group. We checked for differences in genotype and allele frequency distributions according to sex in both groups, but we did not find any (data not shown). We further combined GG and CG genotypes and compared them to the CC genotype, but this combination also failed to reveal statistically important differences between the two groups studied (data not shown). Since the method of suicide may be important, the suicide victims were placed into one of two groups as proposed by Asberg et al. [5]: violent and non-violent. However, no significant result for any of the two groups (violent $\chi^2 = 1.13$, $df = 2$, $P = 0.568$, non-violent $\chi^2 = 1.22$, $df = 2$, $P = 0.542$) was observed.

Results of the special questionnaire about stressful life events of the suicide victims were available only for a small proportion of suicide victims ($n = 62$, 20% of the total) and are shown in Table 2. For the analysis we combined CG and GG genotypes, and compared them to the CC group. More stressful life events in the month prior to the suicide were reported for the subgroup with CC genotype (mean number of events = 2.53; SD = 1.50) in comparison to subgroup with CG/GG genotypes (mean number of events = 1.58; SD = 1.32; $P < 0.05$). However, subgroups of suicide victims with CC or CG/GG genotypes did not differ regarding numbers of reported stressful life events in childhood ($P > 0.05$).

Table 2 Negative stressful life events in a month before suicide and in childhood of suicide victims according to genotypes for polymorphism -1019C>G of serotonin 1A receptor gene

Characteristics	Genotype		*P* (for CC vs. CG + GG)
	CC (%) *n* = 17	CG + GG (%) *n* = 45	
Sex, *n*			
Male, 40	10 (58.8)	30 (66.7)	NA
Female, 22	7 (41.2)	15 (33.3)	NA
Age (years), mean ± SD			
Male 48 ± 17	NA	NA	NA
Female 50 ± 17	NA	NA	NA
Life events			
In a month before suicide[a]	2.53 (1.50)	1.58 (1.32)	0.018 ($t = 2.43$; $df = 60$)
In childhood[a]	1.82 (1.38)	1.80 (1.53)	0.406 ($t = 0.055$; $df = 60$)

NA not applicable
[a]Mean no. of occurrences (SD)

Discussion

The results of the present study do not support the implication of serotonin receptor 1A promoter polymorphism -1019C>G in suicide in general. Neither distinct gender nor suicide methods showed any difference in genotype distributions between the groups investigated. However, more stressful life events in the month prior to the suicide were reported for the subgroup of suicide victims with CC genotype (mean number of events = 2.53; SD = 1.50) in comparison to subgroup with CG/GG genotypes (mean number of events = 1.58; SD = 1.32; $P < 0.05$), suggesting that some of the suicide victims might be more dependent on environment stressors while others on genotype.

Implication of serotonin receptor 1A and its promoter polymorphism in suicide and suicidal behavior has produced several contradictory opinions resulting from either supporting [15, 18] or opposing studies [12, 19, 23]. In our investigation we failed to replicate the results from a previous study of Lemonde et al. [15] who studied suicide victims of French–Canadian origin. Allele G was enriched fourfold in their cohort of suicide victims and the frequency distributions of CC/CG/GG genotypes were as follows: control group 0.73/0.22/0.05 and suicide victims 0.54/0.30/0.16. Genotype frequencies in both our groups departed significantly from Lemonde's results, since we had the prevalence of the heterozygots in both groups. On the other hand, we observed genotype frequency distributions similar to those reported by Huang et al. [12]: CC/CG/GG: 0.29/0.47/0.24 and 0.28/0.40/0.32 for suicide victims and non-suicide, respectively. Similar results were obtained in a study of Serretti et al. [19] who studied suicide in German and Italian populations. Genotype frequency distributions in the ethnical groups were 0.25/0.51/0.24 and 0.27/0.54/0.19, and were not statistically significant in any of them.

Since stress is one of the environmental factors that may predispose individuals to suicide we focused on this specific point. The results of our study of the questionnaire data about the suicide victims provided some interesting findings concerning the environment–genotype interaction influence on suicide completion. This has been indirectly confirmed by several twin, adoption and family studies which estimated that 43% of the variability in suicidal behavior may be explained by genetics, and the remaining 57% by environmental factors [8]. Both factors are important and dependent on one another. In our group of suicide victims there were cases where environmental factors may play some more evident role in the suicide and cases where a greater contribution to suicide might come from hereditary factors. We observed a tendency for suicide victims with the CC genotype to have more stressful life events prior to suicide completion. In such cases the decision to commit suicide came probably more from the environment than from the genetic background, since the C allele does not influence HTR1A expression. However, we could only speculate that in the group of suicide victims with CG/GG genotype, where higher expression of the HTR1A mRNA could lead to lower serotonergic neurotransmission [15], a higher proportion of genetic background underlies the suicide. However, there are some limitations in this connection. One must take into consideration that our group of suicide victims with certain stressful life events was small, not homogenous, and did not have enough statistical power. More psychological autopsies will be necessary to further investigate and support these preliminary findings.

Our results support the hypothesis that environmental and genetic factors together influence suicidal behavior [7, 22] but in individual suicide victims different extents of each genetic or environmental influence could be involved. In conclusion, we have to stress that allelic variation of promoter polymorphism -1019C>G in HTR1A might only contribute to suicide completion and susceptibility to stressful life events that could trigger suicide, but further investigations with more homogenous samples and data are needed to buttress this hypothesis. However, the present study is one of the first that takes into consideration suicide completion, genetic background and stress at the same time. Further studies will be very welcome to help elucidate this potentially interconnected network of polygenic and multi factorial disorder of suicide.

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