REPEATED SUICIDAL BEHAVIOUR: STRESSFUL LIFE EVENTS AND GENETIC POLYMORPHISM OF SEROTONIN TRANSPORTER

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REPEATED SUICIDAL BEHAVIOUR: STRESSFUL LIFE EVENTS AND 5-HTTLPR GENETIC POLYMORPHISM

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SUMMARY

Stressful life events and dysregulated mono-aminergic neurotransmission have been associated with suicidal behaviour. The aim of this investigation was to analyze suicidal behaviour in multiple attempters in relation to the stressful life events, and to the polymorphism of the serotonin transporter (SERT) gene.

Multiple suicide attempters, admitted to the University Psychiatric Clinic, were interviewed for the number of previous suicide attempts and for the occurrence of stressful life events, recorded in a Life History Calendar. The patients were further genotyped for 5-HTTLPR polymorphism of SERT.

The number of suicide attempts was found to be significantly correlated with the number of negative life events experienced during the 6 months preceding each suicide attempt. The L/L genotype was associated with a reduced number of multiple suicide attempts.

These results should prompt future study with a larger number of subjects to further investigate the interaction of genetic and environmental factors in repeated suicidal behaviour.

KEYWORDS:

Repeated suicidal behaviour, stressful life events, serotonin transporter, genetic polymorphism

INTRODUCTION

Molecular genetic analysis of subjects with suicidal behaviour suggests that the underlying patho-physiological mechanism may involve dysfunction of genes related to serotoninergic neurotransmission, and its possible interaction with adverse environmental factors. The 5-HTTLPR (Serotonin Transporter Gene-linked Polymorphic Region) polymorphism consists of a long (L) or a short (S) allele, causing high (L/L) or low (S/S, S/L) functional activity respectively [1]. The function of this gene has been associated with psychiatric disorders [2], in particular depressive mood states associated with stressful life events [3]. Carriers of one or two S alleles are more frequently identified in suicide attempters [4-6] and these conclusions are further supported by the findings reported by Courtet [7].

Since previous studies have shown that a single (or clustered) major negative life event closely precedes a suicidal attempt [8-10], the aim of the present investigation has been to examine the stressful life events which occurred in multiple suicide attempters, their 5-HTTLPR genetic polymorphism of SERT, and the possible interaction of these factors.

METHOD

Sample characteristics

The study included 39 subjects (14 men, 25 women; average age 49.8 ± 11.8 SD) admitted to the Psychiatric Clinic of the University of Trieste for having committed

a suicide attempt. A suicide attempt was considered a self-injurious, or potentially self-injurious, act performed with the acknowledged intent to end one's life. The majority of the patients were diagnosed with major depression (n=22); four of these depressed patients had also alcohol abuse-dependence, or a borderline personality disorder (n=3) or an eating disorders (n=2). Four patients had bipolar disorder in a depressive phase which preceded the suicide attempt; twelve patients were schizophrenic-psychotic. At each suicide attempt, all patients had a Hamilton Depression Rating Scale score ≥ 22 (average 27.5 ± 1.64 SD).

Procedures

The subjects were informed about the research project and gave their written consent for their participation, according to the Declaration of Helsinki and Good Clinical Practice Guidelines. Anamnesis was obtained for their clinical and life history, particularly in relation to their suicidal behaviour. Axis I and II psychiatric diagnoses were assigned using a computerized version of the Diagnostic Interview Schedule (C-DIS) [11], which incorporated DSM-IV-TR criteria and the Hamilton Depression rating scale [12].

Measures

Stressful events were assessed with Paykel's Interview for Recent Life Events (IRLE) [13]. The validated Italian version of the IRLE [14] and of the Hamilton

Depression rating scale [12] were employed, and the data collected were used to prepare a life history calendar as reported by Caspi et al [15].

Genotyping

Genomic DNA was obtained from whole blood or buccal cells, using standard procedures (AasterAmp™ buccal swab brushes, Epicentre Technologies; GenElute™ blood Genomic DNA Kit, Sigma).

The genotyping was carried out by PCR, using the GC-RICH PCR System, (ROCHE), and the primers described by Gelernter [16]. The PCR products were separated by electrophoresis on a 2% agarose gel, and visualized by UV after ethidium bromide staining [17].

Statistical analysis

The relationship between the number of suicide attempts and the number of negative life events has been analyzed with a non parametric correlation test (Spearman's rho). The subjects were also classified as having reported a high ($n>2$) or low ($n\leq 2$) number of such life events in the 6 months preceding the suicide attempts, and as displaying a single or multiple number of suicide attempts. These data, stratified for functional activity of the SERT genotype, were analyzed in a multi-way contingency table using the Mantel-Haenszel analysis. Calculations were performed using Systat and SPSS 14 statistical software package.

RESULTS

The demographic and psychiatric characteristics of the subjects considered are illustrated in Table 1. Table 2 reports the number of stressful life events experienced in the 6 months preceding each suicide attempt, and the corresponding number of attempted suicides. The number of suicide attempts was not significantly different among groups of patients with different psychiatric diagnosis (Kruskall Wallis test); furthermore, a contingency table of the patients grouped as single or multiple attempters vs the psychiatric diagnosis provides non significant correlation (Pearson chi square test).

The number of suicide attempts was significantly correlated with the number of negative life events which occurred in the six months preceding each attempt (rho=0.711); any role played by events which had occurred earlier was less evident (rho= 0.497, p= 0.001, not shown in Table 2).

Twenty subjects were carriers of the (S/S) and (S/L) genotypes, while fourteen were carriers of the (L/L) 5-HTTLPR allelic variant. Within this cohort the distribution of allelic variants did not significantly differ from the one provided by Lesch et al. in a Caucasian population [1], and follows the Hardy Weinberg equilibrium.

Table 3 illustrates the results of the analysis of the frequencies in a multi-way contingency table, in which the subjects were classified as having committed one or multiple suicide attempts. The number of life events was considered to be "low" when this value was equal to (or lower than) the currently observed median of 2. "High"

number of events exceeded 2. Data were stratified for SERT polymorphism. For patients carrying the L/L allelic variant, the results obtained were not statistically significant. Patients carrying at least one S allele (S/L or S/S) displayed a number of multiple attempts significantly larger after the occurrence of a greater number of life events (Pearson chi-square = 4.001, p = 0.045, odds ratio = 6.5). The Mantel-Haenszel statistic indicates a statistically significant effect when these data are stratified on the genotype of the subjects (Table 3).

DISCUSSION

The present study was performed to explore the role played by environmental and genetic factor in subjects of both sexes who had committed multiple suicide attempts. The number of the repeated suicidal attempts appeared to be significantly correlated to the number of stressful life events experienced in the 6 months preceding each suicide attempt; the role played by earlier events was less pronounced. These findings are consistent with those reported by Heikkinen at al [9] and by Maris [10].

Our data suggest that multiple suicidal behaviour was significantly associated with the 5-HTTLPR genetic polymorphism, as L/L genotype was related to a reduced number of suicidal attempts.

This observation seems to differ from those by Du et al. [18] and Hranilovic et al. [19] showing that the 5-HTTLPR L/L genotype was present with high frequency in depressed subjects with suicidal behaviour. On the other hand, the present results accord with the findings by Courtet et al [20] who investigated patients reattempting suicide during a 1-year follow up, and showed that the presence of at least one S-allele might be a predictor of a second suicide attempt. Moreover, the present findings are consistent with those of Wasserman et al [21] reporting a significantly higher prevalence of the S/S genotype in suicide attempters with high medical damage scores resulting from the attempted suicide.

The limited size of our sample of subjects who could be included in the present study restricts the power of data analysis. The examination of a larger number subjects may allow to better clarify the role played by the interaction of environmental factors, such as stressful life events, with constitutive genetic ones, such as SERT polymorphism, in multiple suicide behaviour.

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KEY POINTS

- Stressful life events are reported as significant factors contributing to suicidal behavior: the factors responsible for repeated suicidal behavior have received limited attention.
- 5-HTTLPR polymorphism has been characterized in the promoter region of the serotonin transporter gene possessing high penetrance, and allelic variants with different function and high penetrance.
- The 5-HTTLPR haplotype has been found to be associated with an increase in depressive mood disorders in subjects exposed to stressful life events, with a significant interaction between these genetic and environmental factors.
- The present investigation shows multiple suicidal behavior to be associated with recent stressful life events experienced by the subjects, and with the 5-HTTLPR genetic polymorphism of serotonin transporter, L/L haplotype being related to a reduced number of multiple suicide attempts.

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TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE SUBJECTS

Variables		
Age *:	49.8 ± 11.8	
Gender:	Female	25 (64.1 %)
	Male	14 (35.9 %)
Marital status:	Single	12 (30.8 %)
	Married	10 (25.6 %)
	Divorced	15 (38.5 %)
	Widow/er	2 (5.1 %)
Educational level:	Primary	29 (74.4 %)
	High school	9 (23.1 %)
	College	1 (2.6 %)
Occupation:	Unemployed	17 (43.6 %)
	Employed	8 (20.7 %)
	Student	-
	Pensioner	14 (35.9 %)
Axis-I diagnosis		
Alcohol and drug abuse-dependence		10 (25.7 %)
Schizophrenia and other psychosis		12 (30.8 %)
Major depression		22 (56.4 %)
Bipolar disorder		4 (10.3 %)

The values in the table are the numbers (percentage in parenthesis) observed in the cohort of 39 subjects considered (* age = mean ± standard deviation); some of the subjects received the diagnosis of more than one disorder related to the Axis-I (see Methods section).

TABLE 2. NUMBER OF LIFE EVENTS AND NUMBER OF REPEATED SUICIDE ATTEMPTS

N° of suicide attempts	N° of suicide attempters	N° of life events
1	12	1.0±1.1
2	8	1.7±0.9
3	6	3.8±2,5
4	6	5.8±3.6
5	7	8.4±5.5

The number of negative life-events which occurred in the six months preceding each suicide attempt is reported as the mean ± standard deviation. The Spearman coefficient of the correlation between the number of suicide attempts and the life events is rho= 0.711, $p<0.001$.

TABLE 3. MULTIWAY TABLE ANALYSIS OF THE NUMBER OF SUICIDE ATTEMPTS AS A FUNCTION OF LIFE EVENTS, STRATIFIED FOR THE GENETIC POLYMORPHISM 5-HTTLPR

Allelic variant	N° of life events #	Subjects with 1 attempt	Subjects with more than 1 attempt	p $	Odds ratio	p *
L/L	Low	5	6	0.145		0.041
	High	0	3			
S/S, S/L	Low	5	5	0.045	6.5	
	High	2	13			

$: Pearson chi-square

*: Mantel-Haenszel statistics

: high means greater than the median value of 2 life events per subject