

Suicidal Behavior and the Serotonin Transporter Gene Polymorphism (5-HTTLPR) with Novel Subtypes, in Danish Schizophrenic Patients

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Abstract: *Background:* Literature reports a genetic component for suicidal behavior, especially of determinant/violent type. One of the candidates has been the polymorphism 5-HTTLPR in the serotonin promoter. Employing a between group design, we wished to test the association between suicidal behavior and serotonin-related polymorphisms.

Method: 350 Danish patients with average 14 years' duration of illness and with well researched history of suicidal behavior participated. Three groups were identified: 1. without suicidal behavior, 2. with suicidal behavior of non-determinant/non-violent methods, and 3. suicidal behavior with determinant/violent methods. We used the common alleles S and L as well as the new aspect with allelic subtypes S_A , S_G , L_A , L_G to constitute 3 functional genotypes: SS, SL and LL. We also included duration of illness, age at onset and sex in our study as potential covariates.

Results: We tested suicidal behavior types 2 and 3 versus type 1 for distribution differences as well as for possible trend. We did not find any statistical significant relations.

Conclusions: We could not find support for a relevant relation between the polymorphisms in the serotonin promoter and suicidal behavior in our schizophrenic patient sample.

Keywords: Suicidal behavior, genetics, schizophrenia, serotonin transporter.

INTRODUCTION

Psychiatric disorders, such as schizophrenia [1] are well-known risk factors for suicidal behavior. Whether the causes of suicide are identical in different psychiatric diseases is unknown, but suicidal behavior is known to be particularly frequent at certain stages of each disorders. In schizophrenia most suicides occur within the first years after the onset of disease [2,3].

There is evidence that suicidal behavior is influenced by biological and genetic factors, especially suicidal behavior by violent /determinant means [4-6]. Of importance, this form of suicidal behavior has been associated with low concentrations of the serotonin metabolite 5-HIAA in the cerebrospinal fluid [7]. Several studies have attempted to identify genes involved in suicidal behavior in psychiatric disorders, with particular focus on the role of the serotonergic pathway in affective disorders [8-11]. In a recent meta-analysis, the authors found association between variations in the 5-HTT gene (serotonin transporter gene) and suicidal behavior in general [12]. To date, only a limited

number of studies have been conducted with the aim of identifying genetic risk factors of suicidality in schizophrenia, and they rarely distinguish between different forms of suicidal behavior [13]. However, it is likely that the 5-HTT gene is associated with violent, as opposed to non-violent, suicidal behaviors. For example, Bayle *et al.* reported an association between violent suicidal behavior among schizophrenic patients and the polymorphism designated 5-HTTLPR in the promoter of the gene encoding for the serotonin (5-hydroxytryptamine, 5-HTT) transporter SERT [5]. The serotonin transporter gene is located on chromosome 17. This gene has a polymorphic repetitive element, 5-HTTLPR, with two common alleles, designated "L" for long or "S" for short. The uptake of 5-HT is approximately two-fold higher in cells containing the homozygous LL form [10]. As a new aspect, a single nucleotide polymorphism, *rs25531* (A > G), in one of the repetitive elements of 5-HTTLPR leads to the appearance of the allelic subtypes designated S_A , S_G , L_A and L_G [14-16]. Of these alleles S_A , L_A and L_G are common among *Caucasians* while S_G is a rare allele. Applying this new knowledge, a replication study could not confirm the findings by Bayle *et al.* [6].

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Based on the potential link between lower levels of 5-HTT and suicidal behavior, we hypothesized that genotypes with the S allele would show an association with lifetime suicidal behavior types more specifically than the SS genotype would show a special relation to the violent/determinant type of lifetime suicidal behavior. Also we would include the new aspect of the allelic subtypes.

MATERIALS AND METHODS

Sample

A total of 350 schizophrenic patients were included in the study. Patients had previously been recruited to the Danish Psychiatric Biobank from six psychiatric hospitals and centers in the Copenhagen area. All patients were ethnically Caucasians and Danes, i.e. the patient and both parents were born in Denmark. Baseline data were obtained on sex, age at onset (defined as age at first psychiatric contact) and illness duration (defined as recruitment age minus age at onset).

Diagnosis and Phenotypic Rating

All patients were diagnosed as having schizophrenia (F20) according to ICD-10 Research Criteria. The reliability of the clinical diagnosis was confirmed by a semi-structured interview using the OPCRIT instrument by an experienced consultant psychiatrist [17]. Comprehensive lifetime clinical data and socio-demographic information were collected from medical records covering numerous hospital admissions and through interviews with patients and medical staff. Suicidal behavior was scored in an operational manner and distinction was made to rate suicidal intent, which has proven unreliable [18]. Patients were classified into the following categories: Type 1: no suicidal behavior, Type 2: suicidal behavior without violent means, and Type 3 suicidal behavior with violent/determinant means [4,5]. Type 2 and Type 3 patient groups were separately compared with the Type 1 patient group. We also combined Types 1 and 2 groups in order to contrast them with the Type 3 group.

Genotyping

Genotyping of 5-HTTLPR including *rs25531* was done using a novel procedure which permits detection of all the major alleles, namely S_A , L_A and L_G , in addition to S_G [19]. Analysis of 5-HTTLPR sequences by the NEBcutter V2.0 program revealed that digestion with *NotI* would allow determination of all major alleles in addition to some of the rare alleles. Using this program we also found that digestion with *NlaIII* in a separate reaction could improve the ability to discriminate the S_G alleles 14-B and 14-D from 16-D(L_G) and permit identification of the rare 14-C allele. Consequently, we used both restriction enzymes. Amplified products in volumes of 25 μ l were mixed with 2.5 μ l of 10x restriction enzyme buffer (200 mmol/l Tris-acetate, 500 mmol/l potassium acetate, 100 mmol/l magnesium acetate, 10 mmol/l dithiothreitol, pH 7.9) and 0.25 μ l of 10 mg/ml bovine serum albumin. Subsequently, this mixture was divided into two portions of equal volumes. To the first of these two portions we added 10 units of *NotI*; 5 units of *NlaIII* were added to the other. Both digestion mixtures were incubated overnight at 37°C. Fragments of DNAs were

subjected to electrophoresis in gels composed of 3.5% MetaPhor (Cambrex Bio Science Rockland Inc., Rockland, Maine, USA) and stained with ethidium bromide.

The short allele (S) predicts lower levels of 5-HTT and HTT activity *in vitro*. The long G and long A alleles are functionally distinct, so the G nucleotide is more similar to the short (S) variant [20]. In this study, the long variant G and the short variant (S) are therefore grouped together and designated S, while L_A is designated L.

In addition to the three major alleles, S_A (14 repeat units), L_A and L_G (both composed of 16 repeat units) a longer variant composed of 21 repeat units was detected. We classified the latter as L.

Risk Factors

We considered that the schizophrenia was the precipitating factor and the duration of schizophrenic illness was the risk period. The genotypes LL, LS and SS were risk factors, and sex and age at onset were studied as potential covariates.

Data Analysis

Linkage equilibrium was tested according to Hardy-Weinberg's law by Chi-Square test for deviation from Hardy-Weinberg Equilibrium.

Comparability of sex for the three Lifetime suicidal behavior types was tested by Chi-square-test. Comparability of age at onset and for duration of illness was tested by One-Way ANOVA-test.

Testing of 2x3 tables was performed by Kruskal-Wallis test and testing for trends in 2x3 tables was performed by Armitage test for trend. The 2x2 matrix was tested by Chi-square-test. Logistic regression was applied to adjust for covariates.

We used a significance level at 0.05, and in general, testing was 2-sided. The calculations were performed by SPSS, version 16.

Ethics

The study was approved by the Danish Scientific-Ethical Committees and the Danish Data Protection Agency. All patients had given written informed consent prior to inclusion into the project.

RESULTS

Data were derived from 350 patients. Table 1 shows data on lifetime suicidal behavior types and original genotypes as well as functional genotypes. The distribution in Type 2 versus Type 1 was not deviant from Hardy-Weinberg equilibrium ($p = 0.18$), nor the distribution in Type 3 versus Type 1 ($p = 0.59$).

Table 2 shows baseline distribution according to lifetime suicidal behavior types and genotypes (functional), sex, age at onset and duration of illness. For sex differences the distribution related to suicidal types was not significant by Chi-square ($p = 0.43$). For age at onset the distribution was tested with One-Way ANOVA-test and found significant ($p =$

Table 1. Suicidality Type and Original Data on Genotypes

Functional Genotypes	Genotypes	Lifetime Suicidal Behavior		
		Type 1: No Suicidal Behavior	Type 2: Non-Violent/ Non-Determinant Suicidal Behavior	Type3: Violent/Determinant Behavior
SS	SS	29	18	9
	SLg	13	5	2
	LgLg	1	1	0
SL	SLa	97	56	11
	LgLa	15	13	2
LL	LaLa	44	25	8
	LaL21	1	0	0

0.047). Pair wise testing showed significant difference between Type 1 and Type 2 ($p = 0.001$), while Type 1 versus Type 3 only differed marginally ($p = 0.06$). Type 2 and 3 had exactly equal mean of age at onset. Duration of illness was tested with One-Way ANOVA-test and found not significant ($p = 0.28$).

Calculations for genotypes and Lifetime suicidal behavior Type 2 versus Type 1: Kruskal-Wallis test for this 2x3 table was not significant ($p = 0.91$), nor was Armitage test for trend ($p = 0.98$). Calculations for Type 3 versus Type 1: Kruskal-Wallis test for this 2x3 table was not significant ($p = 0.21$), nor was Armitage test for trend ($p = 0.44$). Both these calculations were repeated through logistic regression with suicidal type as dependent variable and genotypes as independent variable and age-at-onset as a covariate. This only changed the results marginally.

We amalgamated genotypes and Lifetime suicidal behavior types in order to gain statistical power. In a 2x2 matrix the SS genotype was tested versus SL plus LL and Lifetime suicidal behavior Type 3 versus Types 1 plus 2. In this way the difference was not significant ($p = 0.08$). This testing was repeated through logistic regression with suicidal type as dependent variable and genotypes as independent variable and age-at-onset as a covariate. This only changed the results marginally.

DISCUSSION

As the duration of illness was high, and the suicidal behavior among schizophrenic patients mostly occurs in the first years of illness, we considered our concept and patient recruitment a strong basis for the study. However, a methodological concern is the completeness of hospital records as a tool of assessing lifetime suicidal behavior. It is possible that information about earlier suicide attempts may have been missed over the years, and that the use of all available current hospital records could have resulted in under-ascertainment of lifetime suicidal behavior. However, this possibility of misclassification of the outcome would be independent of the serotonin genotypes and would therefore not bias our findings. Other studies [21] have used interviews of patients to examine occurrence of attempted suicide and found that approximately one third of persons with schizophrenia or related disorder had attempted suicide. The proportion with any suicidal behavior throughout lifetime was higher in our sample, and we believe that differences in methods of assessments and sample selection characteristics provide likely explanations for this difference.

Conceptually, the phenotype under study is non-fatal suicidal conduct in patients with a diagnosis of schizophrenia. The study does not include individuals who committed suicide. It could be argued that those who actually commit suicide represent an extreme phenotype of

Table 2. Suicidality Types and Independent Variables

Independent Variables		Lifetime Suicidal Behavior		
		Type 1: Non Suicidal Behavior (%)	Type 2: Non-Violent/Non-Determinant Suicidal Behavior (%)	Type3: Violent/Determinant Behavior (%)
Genotype	SS	43(21)	24(20)	11(34)
	SL	112(56)	69(59)	13(41)
	LL	45(23)	25(21)	8(25)
Sex	Male	115(58)	59(50)	18(56)
	Female	85(42)	59(50)	14(44)
Age at Onset (SD)		28.56 (SD 10.24)	24.80 (SD 7.36)	24.81 (SD 11.48)
Duration (SD)		13.63 (SD 9.85)	15.13 (SD 10.08)	15.79 (SD 10.08)

suicidal conduct of the violent/determinant type. However it is possible that these potential participants could add a slight effect, if they were included.

We think that our study with this enriched design (cases and controls from the same diagnostic group) had a reasonable power to detect a relevant difference in suicidal behavior risk genotype. Generally, we found the lifetime suicidal behavior types comparable. The finding that lower age at onset carries higher risk for suicidal behavior is well-known, but adjustment for this did not change the overall results. Bayle *et al.* reported an association between violent suicidal behavior in 185 schizophrenic patients carrying the SS genotype [5]. We could not confirm this finding in our study with 350 schizophrenic patients.

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