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Association study between the novel functional polymorphism of the serotonin transporter gene and suicidal behaviour in schizophrenia

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Abstract A serotonin transporter gene linked polymorphic region (5-HTTLPR) has been investigated in several genetic association studies, including studies of schizophrenia and suicidality. The current study was designed to examine whether the new long (A/G) variant polymorphism of the 5-HTT gene may be associated with suicide attempts of 290 Caucasian schizophrenic patients. Among these patients, 92 had a history of suicide attempt. No association with history of suicide attempt was found in the multiallelic 5-HTTLPR ($p=0.305$), however we found significant association with the intron 2 VNTR polymorphism ($p=0.018$). When we performed a haplotype analysis, we found association between suicide attempt and haplotype distribution ($p=0.031$). These findings suggest that the intron 2 VNTR polymorphism in serotonin transporter gene may influence suicidal behaviour in schizophrenia.

1. Introduction

Suicide is the major contributor to the mortality of patients suffering from schizophrenia, accounting for about 10% of deaths in these patients (Caldwell and Gottesman, 1992). Also, the strongest risk factor for completed suicide is likely to be attributed to a history of previous attempts (Powell et al., 2000).

Genetic factors have been reported to modulate the risk for parasuicide, although the precise mechanism and amount of genetic contribution are still unknown (Roy, 1993). The serotonergic function is thought to be trait-dependent and associated with disturbances in the regulation of anxiety, impulsivity, and aggression (Mann, 1995).

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Table 1 Haplotype trend regression (Helix Tree) in attempters

Haplotype	Freq	Mean[a]	*P*-value
Long G, 10	0.013262	0.172472	0.251444
Long A, 10	0.251967	0.286484	0.259545
Short, 12	0.387774	0.295526	0.242935
Short, 10	0.059771	0.26005	0.28285
Long A, 12	0.250551	0.394828	0.026311
Long G, 12	0.02683	0.435092	0.271524
RareHap[b]	0.009845	0.680218	0.01

Furthermore, serotonergic alteration has been suggested in suicide by several studies. For example, low concentration of the metabolite of serotonin, 5-hdroxyl-indol-acetic-acid (5-HIAA), in the cerebrospinal fluid (CSF) has been associated with suicide attempts and suicide completions (Asberg et al., 1976), and low levels of 5-HT or 5-HIAA were also observed in suicide victims (Nordstrom et al., 1994). The serotonin transporter (5-HTT) is the primary target for selective serotonin reuptake inhibitors (SSRIs) and for some tricyclic antidepressants; the 5-HTT-linked polymorphic region (5-HTTLPR) polymorphism has been associated with violent suicide attempt in schizophrenia (Bayle et al., 2003). Furthermore, a link has been reported between suicide and 5-HT uptake binding sites in platelets and brain (Roy et al., 1999). The 5-HTT gene (also known as *SERT*, *SLC6A4*) is located on chromosome 17q11.1-q12. The 5-HTTLPR is described in the promoter of the 5-HTT gene, and has also been shown to be associated with suicidal behaviour in a meta-analysis of 2539 subjects (Anguelova et al., 2003). This meta-analysis pointed out the short allele's association with suicide attempt based on cases-control methods; no reports known to us have examined the haplotype combination of 5-HTTLPR with other polymorphisms regarding suicidality.

The 5-HTTLPR is a variable number tandem repeat (VNTR) polymorphism of a 14 to 16 copies of 22 bp imperfect repeat sequences, also known as 44 bp Ins/Del because the most common polymorphisms are 16 repeats (long) and 14 repeats (short). However, more rare variants of 20 copies have been identified (Delbruck et al., 1997).

The short allele predicts lower levels of 5-HTT mRNA and HTT activity in vitro (Heils et al., 1996; Lesch et al., 1996) and has been correlated in vivo with higher amygdala activation (Hariri et al., 2002) as well as functional coupling between prefrontal cortex and amygdala (Heinz et al., 2005); both anatomical regions are thought to be involved in the patophysiology of schizophrenia, mood regulation and impulsivity. A single nucleotide variant (A to G) was detected in the 6th motif, present only in long variant of HTTLPR, creating an AP2 binding site. In European American and African American populations, this polymorphism is informative enough to be used in population based genetic association. The Long G and Long A alleles are functionally distinct, in fact only the Long A is associated with high levels of 5HTT mRNA expression (Goldman et al., 1994). The function of the long variant when the G nucleotide is present is more similar to the short variant, with a low level of serotonin transporter (5-HTT) mRNA.

5-HTT also has another VNTR of 17 bp in intron 2, near the 5-HTTLPR, that seems to modulate the gene's transcription in an allele-dependent manner with three different number repeat (9, 10, 12) (Hranilovic et al., 2004). We were interested in investigating the putative role of the new functional allele Long G in suicide attempters using a large and well characterized sample of patients with schizophrenia. Furthermore, we were interested in the Linkage Disequilibrium (LD) allele relationship between these two VNTRs and haplotype analysis using the two polymorphisms, based on their combined effect on gene expression (Hranilovic et al., 2004).

2. Experimental procedures

The study population consists of 290 patients with schizophrenia who were recruited in the Toronto area for participation in our molecular genetic study program. Subjects ranged in age from 20 to 68 (41 ± 10.7 SD). The mean age at onset for schizophrenia was 19.8 ± 5.5 SD. Female subjects comprised 25% of the sample. Ninety-two of the patients had attempted suicide at least once. Written informed consent was obtained from all participants prior to examination. The Structured Interview for Psychiatric Diagnosis (SCID-I) was administered to all subjects by trained clinical interviewers. According to the Mood module of SCID (First et al., 1997) suicidality was assessed on a quantitative scale, with the following order: 1 = thoughts of death, 2 = suicide ideation, 3 = suicide plan, 4 = suicide attempt. Furthermore we coded 5 to equal violent attempt based on the different psychopathological valence compared with non-violent attempts. According to the SCID-I criteria among the schizophrenia patients, 179 patients had history of suicide ideas or attempt and the mean score for all 290 suicide ideation/behaviour scores was 1.92 ± 1.89. Attempters and non-attempters were group-matched for ethnicity and gender.

Genomic DNA was obtained from peripheral leukocytes, using high salt extraction methods (Lahiri and Nurnberger, 1991). Genotypes of the 5-HTTLPR and 5-HTT VNTR polymorphisms were determined by PCR and electrophoresis on 2.5% agarose gel. The 5HTTLPR A/G polymorphism was detected by MspI restriction enzyme digestion, followed by electrophoresis. Product sizes for the digest were: Long A = 307 bp; Short = 263 bp; Long G = 154 + 153. Allelic and haplotype association tests in suicide attempters were performed using Haplotype trend regression analysis (Zaykin et al., 2002), by Helix Tree (Lambert, 2004). Suicide ideation/behaviour scores were used for analyses of quantitative traits.

3. Results

The main result for single marker trend regression in the attempter group was significant for the VNTR-2 ($p = 0.0188$), however the 5-HTTLPR was negative ($p = 0.305$).

The quantitative analysis of suicide specifiers with Helix Tree showed significant association with alleles in VNTR-2 ($p = 0.050$) but not with 5-HTLPR ($p = 0.187$). When we looked at the individual allele test we found that the 10-repeat allele was protective ($p = 0.05$) and slightly associated with less severity in suicidality score ($p = 0.09$). Both markers were found to be in the Hardy–Weinberg Equilibrium (VNTR-2 $p = 0.46$; 5-HTLPR $p = 0.38$). We calculated the multiallelic D' and r using Helix Tree. The linkage between the two VNTRs was significant but not strong in this study population ($D' = 0.55$; $r = 0.38$; $\chi^2 = 51.97$; $p < 0.01$).

For the haplotypes of these two polymorphisms in the attempter population, the analysis showed a significant global χ^2 ($p = 0.0312$). The combination of Long A and 12 repeat alleles was at risk for suicide attempt (Table 1).