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Relationships between serotonin transporter promoter polymorphism, platelet serotonin transporter binding and clinical phenotype in suicidal and non-suicidal adolescent inpatients

Short Communication

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Summary. Relationships between the serotonin transporter promoter polymorphism (5-HTTLPR), platelet serotonin transporter (SERT) binding and clinical phenotype were examined in 32 suicidal and 28 non-suicidal Ashkenazi Israeli adolescent psychiatric inpatients. The 5-HTTLPR polymorphism was not associated with transporter binding or with suicidality or other clinical phenotypes. However, in the suicidal group, a significant positive correlation between platelet SERT density and anger scores ($n = 32$, $r = .40$; $p = .027$) and a negative correlation between platelet count and trait anxiety ($n = 32$, $r = -.42$; $p = .034$) were observed.

Keywords: Adolescent, serotonin, suicide, 5-HTTLPR, serotonin transporter, endophenotype.

Introduction

Suicidal behavior in adolescents has been found to have biochemical, genetic and psychological correlates (Apter et al., 1990; Brent et al., 2003; Mann, 2003). Liability to suicidal behavior might be familialy transmitted as a trait independent of axis I or II diagnoses (Brent et al., 1996). Association between serotonin (5-HT) dysfunction and suicide, as well as suicide-related behaviors such as violence, aggression, anger, anxiety and impulsivity, have been demonstrated in a number of studies (Coccaro et al., 1989; Mann et al., 1992). Thus, one candidate gene for suicidal behavior is the insertion/deletion polymorphism in the promoter region of the serotonin transporter promoter (5-HTTLPR) (Heils et al., 1996; Lesch et al., 1994).

Recently, numerous case-control studies (Bellivier et al., 2000; Courtet et al., 2004; Frisch et al., 1999; Geijer et al., 2000; Joiner Jr et al., 2002; Mann et al., 2000; Ohara et al., 1998), a large cohort study (Caspi et al., 2003), as well as a family-based study (Zalsman et al., 2001) have examined the possible association of 5-HTTLPR with suicidal behavior. In the latter study, a possible association of violent traits with this polymorphism was demonstrated in a subgroup of violent suicidal inpatient adolescents (Zalsman et al., 2001). A similar association was seen in two other studies (Bellivier et al., 2000; Courtet et al., 2001). The aim of the present pilot study was to investigate the relationships between the 5-HTTLPR genotype, the clinical phenotype of suicide-related traits and serotonin transporter (SERT) binding density as an endophenotype in a population of psychiatric inpatient adolescents.

Materials and methods

Patient sample

The sample consisted of 60 patients admitted over a period of two years to the adolescent psychiatric department of a university-affiliated psychiatric hospital in Israel. All patients were interviewed on admission and diagnosed using the K-SADS-PL (Kaufman et al., 1997), Hebrew version (Apter et al., 1989). All subjects were naïve to psychotropic drugs and had negative urine toxicology screens. Subjects were divided into those with recent suicide attempt ($n = 32$) defined as having a score ≥ 3 in the Suicide Potential Inventory (SPI) (Pfeffer, 1986), and non-suicidal control group ($n = 28$). All suicidal patients were assessed during the first week after the recent suicide attempt. All Subjects were of Jewish Ashkenazi origin (all four grandparents originating from Eastern Europe) to avoid ethnic genetic stratification (Gelernter et al., 1999). The mean ages of the suicidal and control groups (17.0 ± 2.1 and 16.8 ± 2.0 years, respectively; $t = .38$, $df = 58$, $p = .70$) were similar, as were the proportion of sexes in each group (male/female: 16/16 and 14/14, respectively). Exclusion criteria were diagnosis of mental retardation, organic brain syndrome and non-mastery of Hebrew. Diagnoses were established according to the DSM-IV criteria (APA, 1994). The main primary diagnoses were: schizophrenia ($n = 22$; 36.7%), conduct disorder ($n = 9$; 15%), bipolar I disorder ($n = 5$; 8.3%), major depression ($n = 2$ patients; 3.3%), eating disorder ($n = 2$; 3.3%), panic disorder ($n = 2$; 3.3%) and other axis I diagnoses ($n = 18$, 30%). Some patients in the two groups had comorbid diagnoses (not shown). The two groups did not differ in the frequency of diagnostic categories.

The study was approved by the Geha Hospital Review Board and written informed consent was obtained from all subjects and their parents prior to entry to the study.

Clinical assessment

The clinical phenotype of all subjects was determined by the following rating scales:

1. *The Suicide Potential Inventory* (SPI). The SPI is a semi-structured interview used to assess suicidal behavior in children (Pfeffer, 1986). The SPI has been translated into Hebrew an has been shown to be reliable and valid for this population (Ofek et al., 1998).
2. *The Beck Depression Inventory* (BDI). The BDI is a self-report instrument designed to measure the severity of depression in adults and adolescents (Beck and Steer, 1987).
3. *The Multidimensional Anger Inventory* (MAI). The MAI consists of 24 items measured on a five-point scale, assessing different dimensions of anger such as frequency, duration, magnitude, mode of expression, hostile outlook and anger-eliciting situations (Siegel, 1986).
4. *The State-Trait Anxiety Inventory* (STAI). The STAI is a 40-item self-report that measures state and trait anxiety using two 20-item sub-scales (Spilberger et al., 1970).

Sample handling and analyses

Within one week of hospitalization, fifty milliliters of venous blood were drawn from each subject into EDTA-containing tubes. Platelets isolation and membrane preparation were performed as previously described (Anderson et al., 2002). Transporter binding densities for a frozen pooled platelet quality assessment sample were determined with a day-to-day CV of 8.6% ($N = 3$) during the period that study samples were analyzed. DNA isolation was performed as described by Miller et al. (1988). The insertion/deletion polymorphism in the regulatory region of the serotonin transporter (5-HTTLPR) gene was genotyped as previously described (Lesch et al., 1996).

Statistical analysis

Pearson's correlation was calculated between the continuous variables (e.g. anger, serotonin binding, platelet count). Analyses of Variance (ANOVA) or t-test were employed to test the dependence of the continuous variables (rating scales) on the categorical variables (SS, SL, LL genotype, suicidal/non-suicidal). χ^2 analysis was performed to analyze the association of the genotype to suicidality (as a categorical variable). Given the exploratory nature of the study, all tests were two-tailed and uncorrected for multiple testing.

Results

5-HTTLPR genotype and clinical phenotype

The observed distribution of LL, LS and SS genotypes (8, 26 and 19, respectively) was consistent with Hardy-Weinberg equilibrium. Genotype frequencies were similar in suicidal and non-suicidal adolescent patients ($\chi^2 = .96$; $df = 2$; $p = .619$). Behavioral rating scores for suicidality (SPI scores), depression (BDI), anger (MAI) and state and trait anxiety (STAI) did not differ across genotypes in the suicidal and non-suicidal subgroups, or in the total group. The suicidal group had higher scores in all the clinical measures (data not shown).

Platelet SERT binding and clinical phenotype

The SERT binding densities and number of platelets did not differ significantly between the suicidal ($n = 32$) and non-suicidal ($n = 28$) groups ($t = -.68$, $df = 58$,

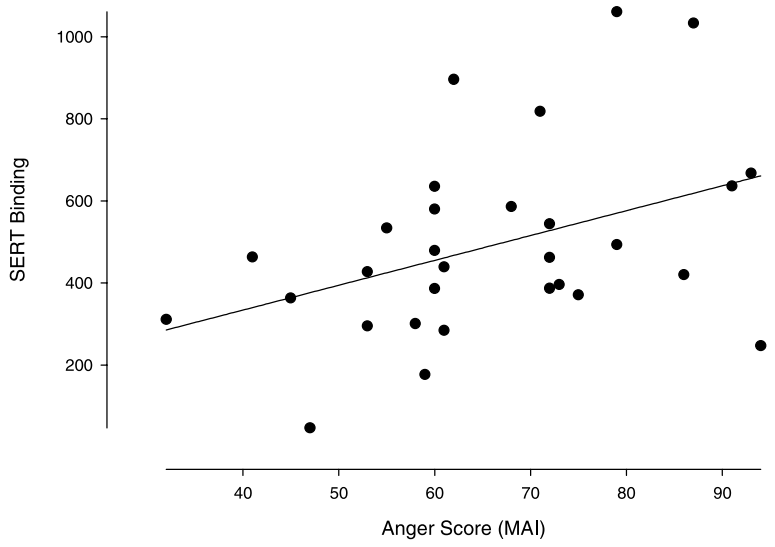

Fig. 1. Correlation of anger scores and SERT binding. $r = 0.40$; $p = 0.027$. *MAI* The Multidimensional Anger Inventory (Siegel, 1986), *SERT* Serotonin Transporter Receptor

$p = .496$; $t = -1.17$, $df = 58$, $p = 0.246$, respectively). Pearson's correlation tests were calculated for SERT binding and the psychometric measures and showed a significant positive correlation between MAI scores and binding in the suicidal group ($n = 32$, $r = .40$; $p = .027$), but not in the non-suicidal group ($n = 28$, $r = -.12$; $p = .589$) (Fig. 1). No other significant behavioral correlations were found with SERT binding.

No significant correlations (Pearson's r) were observed between platelet count and the psychometric measures. However, based on our previous finding of a correlation between trait and not state anxiety and suicidality, a partial correlation was performed between SERT binding and trait anxiety with controlling for the effects of state anxiety. The results showed a significant negative correlation between trait anxiety and number of platelets ($n = 32$; $r = -.42$; $p = .034$) in the suicidal group and no such correlation in the non-suicidal group ($n = 28$; $r = -.01$; $p = .970$). In addition, in the whole group, a significant negative correlation was found between number of platelets and the SPI score, controlling for the effects of state anxiety ($n = 60$; $r = -.23$; $p = .040$). These findings were not significant if corrected for multiple comparisons.

5-HTTLPR genotype and platelet SERT binding

Platelet SERT binding did not differ between the three genotype groups of the total ($n = 60$) study population (SS, LS and LL means of 481 ± 260, 609 ± 119 and 526 ± 279 fmol/10^9 platelets, respectively; $F = .316$, $df = 2,58$, $p = .730$). Genotype differences in SERT binding were also not seen when genotype subgroups were compared within the suicidal and non-suicidal groups and when analyzed as two genotypic groups (SS + SL versus LL, see Caspi et al., 2003) (data not given).

Discussion

In general, we did not find strong relationships between the transporter genotype, clinical phenotype and platelet transporter (SERT) binding. The lack of a relationship between genotype and platelet SERT binding is not unexpected. Although a number of studies have found the 5-HTTLPR to influence expression and activity of the transporter, all studies examining platelet SERT binding and 5-HTTLPR genotype have not found a strong association between the two variables (Anderson et al., 2002).

It is of interest that higher levels of anger in the suicidal group, but not in the non-suicidal group, were correlated with increased platelet SERT binding (Fig. 1). This finding offers some (albeit limited) support for the hypothesis that anger in suicidal adolescents may be related to altered serotonergic functioning. This is in agreement with previous findings regarding a possible association of the 5-HTTLPR polymorphism with aggression and violence among suicidal subjects (Bellivier et al., 2000; Courtet et al., 2001; Zalsman et al., 2001).

The finding of a correlation between levels of trait anxiety and platelet count in adolescent suicidal inpatients is worth noting. There is scarce data in the literature on the relationship between trait anxiety and platelet count. However, it has been reported that persistent anxiety is associated with reduced platelet count in subjects exposed to stressful stimuli (Liesse et al., 1980) and reduced stress-induced increases in platelet count have been observed in subjects exposed to persistent stress (Mundal and Rostrup, 1996). We have previously reported that anxiety is a risk factor for suicidal behavior in adolescents (Ohring et al., 1996) and it has been observed that adolescents with anxiety disorders developing major depression are at a high risk for suicide (Pawlak et al., 1999). It is worth noting that recent meta-analysis of the FDA database suggests that suicide risk in patients with anxiety disorders is higher than previously thought (Khan et al., 2002). However, these findings should be judged with caution since the sample is small and the statistical significance is lost after Bonferroni correction and might represent a type 1 error.

As noted, a major limitation of this pilot study is the small number of patients studied and multiple comparisons. The complex relationships between suicidal behavior, anxiety, serotonin transporter genotype, transporter expression, and platelet count merit further investigation in a larger sample of adolescents and adults.

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