Serotonin Transporter Gene May Be Involved in Short-Term Risk of Subsequent Suicide Attempts

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Background: In the first year following a suicide attempt, patients are at high risk for reattempt and for completed suicide. We aim to determine the predictive value of two serotonin-related genes, the tryptophan hydroxylase (TPH) and serotonin transporter (5-HTTLPR) genes that have been involved in the susceptibility to suicidal behavior.

Methods: After a one-year follow-up study of 103 patients hospitalized after a suicide attempt, patients have been genotyped for both the A218C TPH and the functional S/L 5-HTTLPR polymorphisms.

Results: Patients who reattempted suicide during the follow-up period had significantly higher frequencies of the S allele and the SS genotype. The odds ratio for the SS genotype vs. the LL genotype was 6.5 (95% CI [1.18–35.84]). No difference was observed for TPH gene. Patients carrying the SS genotype were more impulsive. However, multivariate analysis suggested an independent effect of both the SS genotype and impulsivity on the risk of repeated suicide attempts.

Conclusions: These results suggest that the 5-HTTLPR SS genotype is associated with further suicide attempts among patients who have previously attempted suicide.

Key Words: Serotonin transporter, tryptophan hydroxylase, suicidal behavior, repetition, polymorphism, association study, impulsivity

The prevention of suicide and the reduction of nonfatal suicidal behavior is part of the World Health Organization’s Health-for-All Policy (World Health Organization 1999). Thus, suicide prevention strategies should focus in priority on high-risk subjects, which means that we need to improve their recognition. A previous suicide attempt is the best predictor of a future suicide or suicide attempt (Beck et al 1975; Beck and Steer 1989; Leon et al 1990; Hawton et al 1998), and subsequent attempts are generally more severe (Malone et al 1995). Indeed, in a recent systematic literature review, Owens et al (2002) estimated that the median rates of nonfatal repetition were about 16% at 1 year and 25% in studies lasting longer than 4 years. The rates of repeated suicide attempts were even higher in several studies, reaching 40% during follow-up periods of 5 to 8 years (Skogstad 1988; Brauns and Berzewski 1988; Johnsson-Fridell et al 1996). For subsequent suicide, the median rates were about 2% at 1-year follow-up, increasing to 7% in the studies lasting over 9 years. Patients who attempted suicide are well-known consumers of psychiatric resources and are at a high risk of actually committing suicide (Rissmiller et al 1994).

The lack of information concerning which preventive strategies are effective is probably related at least partly to the difficulty involved in identifying the subjects who should benefit from them. In a large-scale survey in the United Kingdom, where 24% of all suicide cases had been in contact with the mental health services within the 12 months before their death, most people who committed suicide had been estimated by the clinicians to be at no or low risk at the time of the final service contact (Appleby et al 1999). Despite the substantial risks of eventual suicide in subjects who have already attempted to commit suicide, clinicians lack robust predictors that can be used to quantify this risk. Although further efforts may identify clinical profiles of more value as predictors, a search for relevant biological measures is clearly warranted. A large amount of data from studies in biological psychiatry led Mann (1998) to suggest that the serotonin (5-HT) dysfunction is related to the diathesis for suicidal behavior. Since the seminal studies by Asberg et al (1976), follow-up studies have suggested that low cerebrospinal fluid (CSF) 5-hydroxyindole acetic acid (5-HIAA) levels are a predictor of future suicide attempts and suicide completion and are associated with a substantial increase in short-term suicide risk in suicide attempters (Traskman et al 1981; Roy et al 1989; Nordström et al 1994). Roy (1999) reported that the low affinity of the platelets serotonin uptake protein for serotonin has a predictive value for subsequent suicidal behavior during a 5-year follow-up period.

Impulsive aggression is closely related to lifetime suicidal behavior and is also associated with a serotonergic dysfunction (Mann et al 1999; Placidi et al 2001). People who make repetitive suicide attempts often have dramatic or impulsive personality traits (Paris 1996), and impulsivity is the component of borderline personality disorder that best correlates with repetitive suicide attempts (Brodsky et al 1997).

The level of serotonergic system activity is probably a genetically determined trait, and one mechanism by which genetic factors may affect the risk of suicide (Brent et al 1996) is by reducing serotonergic function (Mann 1998). Personality traits related to impulsive aggression are probably caused by genetic factors (Coccaro et al 1994, 1997) and involved in the transmission of suicidal behavior in families (Brent et al 1996). Thus, impulsivity may be an intermediate phenotype associated with the serotonergic genes involved in vulnerability to suicidal behavior and to repetitive suicide attempts.

We showed that two major serotonin-related candidate genes, one encoding tryptophan hydroxylase (TPH), the rate-limiting enzyme of serotonin synthesis, and the other encoding the
functional polymorphism in the serotonin transporter gene promoter region (5-HTTLPR), are associated with suicidal behavior (Abbar et al 2001; Courret et al 2001). In both studies, where suicide attempters have been compared with subjects without personal or family history of suicidal behavior, we reported a dominant effect of both the TPH A218 and 5-HTTLPR S alleles. For the TPH gene, using bearers of the C allele as the reference group, the odds ratios (ORs) for the S allele and the SS genotype were 1.54 [95% confidence interval (CI) (1.19–1.98)] and 2.49 [95% CI (1.43–4.33)], respectively (Abbar et al 2001). For the 5-HTTLPR, using bearers of the L allele as the reference group, the ORs for the S allele and the SS genotype were 1.72 [95% CI (1.09–2.71)] and 3.63 [95% CI (1.27–10.40)], respectively (Courret et al 2001).

Recently, it has been shown that TPH is encoded by two different genes, TPH2 being 150 times more expressed in brainstem than TPH1 (Walther et al 2003); however, the scientific rationale for testing the implication of TPH1 in reattempt is different genes, TPH2 being 150 times more expressed in brainstem than TPH1 (Walther et al 2003); however, the scientific rationale for testing the implication of TPH1 in reattempt is independent from those included in our prior studies (Abbar et al 2001; Courtet et al 2001). We also looked at whether impulsivity in suicide attempters was associated with the investigated genes in relation to the risk of future suicide attempts.

### Methods and Materials

#### Sample

The follow-up study included 103 consecutive patients who were hospitalized in our unit after a suicide attempt. Written informed consent was obtained from all subjects. DSM-IV axis I psychiatric disorders were diagnosed by experienced psychiatrists (PC, ST) following psychiatric evaluations and by using the Mini International Neuropsychiatric Interview version 5.0.0 (Sheehan et al 1998). A suicide attempt was defined as intentional self-harm that was not self-mutilatory in nature and required medical evaluation and treatment in an emergency or intensive unit (Mann 1998). Suicide attempts were classified as violent according to the criteria proposed by Asberg et al (1976). Serious attempts were violent or nonviolent attempts that justified admission to an intensive unit. Lifetime impulsivity history was rated using the French version of the Barratt Impulsivity Scale (BIS) (Bayle et al 2000).

After the index admission, all patients were discharged back to the care of their referring psychiatrist. After 1 year, all patients and current psychiatrists were interviewed to determine if there had been any suicidal behavior since the index admission. At 1 year, 20 patients had made a subsequent suicide attempt.

To minimize population heterogeneity, patients were all of West European Caucasian origin. The study was approved by the institutional ethics committee.

#### Laboratory Methods

Blood samples (15 mL) were collected into ethylenediaminetetraacetic acid (EDTA)-treated tubes, and genomic DNA was extracted from white blood cells (DNA extraction kit from Amersham-Pharmacia Biotech, Dübendorf, Switzerland). Polymorphisms in the promoter region of the serotonin transporter (5-HTT) gene and intron 7 of the TPH gene were genotyped as previously described (Abbar et al 2001; Courtet et al 2001).

#### Statistical Analysis

Means and standard deviations were used to describe quantitative variables, and proportions were used to describe categorical variables. The distributions of continuous variables (age, scores) were tested with the Shapiro-Wilk test and were not normal. Therefore, the Mann–Whitney rank sum test and Kruskall–Wallis tests were used to compare the means of more than two groups. For categorical variables, the percentages according to relapse or genotype were compared with the χ² analysis (2-tailed) or Fisher exact test if χ² was not valid. The global score of the BIS was transformed in dichotomous variables, using a receiver operating characteristic (ROC) curve to determine the cutoff point that gave the best number of correctly predicted event responses (reAttempts). We obtained a threshold of 66. Odds ratios with 95% confidence intervals were calculated by Woolf's method. The Armitage linearity tendency test was used to identify dose effects of susceptibility alleles (Armitage and Berry 1987). Logistic regression models were used to study the multivariate relationships of potential predictors with reattempt status and their relative importance. Predictors in these models were selected a priori on the basis of previous research and on the results of the univariate analysis. Variables were selected in a stepwise manner. Age at inclusion in years was included in the model as a continuous variable. The other dichotomous variables entered in the model were gender and depressive disorder. The categories with the most favorable outcome were taken as the reference level. All of the other categories were included in the model as dummy variables. Odds ratios and their confidence intervals were calculated. The α-to-enter and α-to-exit were set, respectively, at .20 and .10. To assess the predictive ability of the model, we calculated the concordance rate between the predicted and observed responses. The goodness-of-fit of the logistic regression model was assessed using the likelihood ratio test and the Hosmer and Lemeshow test (Hosmer and Lemeshow 1989).

The SAS version 6.12/UNIX (PROC FREQ, PROC UNIVARIATE, PROC NPAR1WAY, PROC LOGISTIC) software was used for statistical analysis (SAS Institute 1989).

#### Results

The clinical data concerning the 103 suicide attempters at their index admission are shown in Table 1. Twenty-seven (26%) of the 103 patients admitted to our unit after a suicide attempt dropped out of the study during the 1-year follow-up period, mostly after the third month and without any further attempts during this period. These patients did not differ from the 76 remaining patients for the suicidal history (number and severity of attempts) and for DSM-IV Axis I diagnosis (Table 1).

Seventy-six patients completed the follow-up study: 20 patients reattempted suicide and 56 did not reattempt suicide during the follow-up period. The gender distribution, the psychiatric diagnoses, and the suicidal behavior history were similar in the two groups on index admission: there were no differences for the rates of repeaters, violent attempters, and serious attempters; for the age at onset; or for the family history of suicidal behavior (Table 1).

Concerning the 5 HTTLPR polymorphism, the frequency of the S allele and the short/short (SS) genotype were significantly
higher in the group of patients who reattempted suicide during the follow-up period than in individuals who did not (72.5% vs. 48%, \( \chi^2 = 7.01, df = 1, p = .008 \); 55% vs. 20%, \( \chi^2 = 9.08, df = 2, p = .01 \), respectively) (Table 2).

When using subjects bearing the L allele and the long/long (LL) genotype as the reference group, the odds ratios for the S allele and the SS genotype were 2.83 (95% CI (1.29–6.22)) and 6.5 (95% CI (1.18–35.84)), respectively.

This suggests that patients who carry the S allele or the SS genotype are at high risk of short-term subsequent suicide attempts; however, most subjects from both groups had a history of previous suicide attempts at index admission. We investigated whether the S allele or the SS genotype were also associated with a higher risk of repeated suicide attempts before and/or during the follow-up. For this, we compared the 17 subjects who had attempted suicide only once with the 59 patients who had attempted suicide at least twice. The SS genotype frequencies were higher in patients who made several suicide attempts (31%) than in patients who had only made one suicide attempt (24%) and than in controls with no history of suicide attempts we published previously (16%) (Courtet et al 2001). The frequency of the SS genotype increased with the repetition of suicide attempts (Armitage linearity tendency test: 5.59, \( df = 1, p = .01 \)).

No difference was found for intron 7 of the TPH gene (Table 3).

We then investigated whether impulsivity was associated with the risk of subsequent suicide attempts and the 5-HTTLPR SS genotype. Impulsivity tended to be slightly higher in reattempters than in patients who did not reattempt: 65.35 ± 12.59 versus 60.43 ± 10.50 (analysis of variance [ANOVA], \( F = 2.74, p = .10 \)). When compared with the data reported in the validation study of the Barratt Impulsivity Scale (Bayle et al 2000), patients in the present study showed similar impulsivity scores to those of multiple suicide attempters (63.3 ± 15.6) and higher impulsivity scores than subjects from the general population (55.8 ± 17.5). As we hypothesized that a high level of impulsivity might be associated with a higher risk of reattempt in suicide attempters, we sought a threshold for the impulsivity score associated with better sensitivity and specificity. The cutoff was obtained for a score of 66 on the Barratt Impulsivity Scale. The risk of reattempt was higher in patients with an impulsivity score above 66 than in those with lower scores (40% vs. 20% respectively, \( p = .058 \)).

Impulsivity score was significantly higher in patients carrying the SS genotype than in patients carrying the short/long (SL) and LL genotypes (65.58, 63.53, and 57.24, respectively; ANOVA, \( F = 3.79, p = .026 \)). Classical suicidal behavior risk factors (gender, age, history of major depressive disorder, history of substance or alcohol abuse or dependence, and history of multiple attempts at inclusion), 5-HTTLPR genotype, and impulsivity scores were

<table>
<thead>
<tr>
<th>Table 1. Characteristics and Lifetime Psychiatric Diagnoses of Suicide Attempters at Index Admission</th>
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<tr>
<td>Characteristic</td>
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<tr>
<td>Women, No (%)</td>
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<td>Age, y (mean ± SD)</td>
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<tr>
<td>History of Suicide Attempts (SA) at Index Admission</td>
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<tr>
<td>At least 1 violent SA, no (%)</td>
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<td>At least 1 serious SA, no (%)</td>
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<td>Age at onset &lt; 20 years</td>
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<td>At least 2 previous SA, no (%)</td>
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<tr>
<td>Number of SA (mean ± SD)</td>
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<tr>
<td>Family History (first-degree relatives) of Suicidal Behavior, No (%)</td>
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<tr>
<td>MDD</td>
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<td>Bipolar disorders</td>
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<td>Eating disorders</td>
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<td>Alcohol and substance abuse/dependence</td>
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<td>GAD</td>
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<td>Agoraphobia</td>
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<td>Social phobia</td>
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<td>Panic disorder</td>
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<tr>
<th>Table 2. Population Association Between 5-HTTLPR and Repeated Suicide Attempt at 1 Year</th>
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<tr>
<td>Genotypes</td>
</tr>
<tr>
<td>Reattempt during Follow-Up (n = 20)</td>
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<tr>
<td>No Reattempt during Follow-Up (n = 56)</td>
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</tbody>
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5-HTTLPR, serotonin transporter gene; SS, short/short; SL, short/long; LL, long/long; S, short; L, long.
Table 3. Population Association Between Introns 7 of TPH and Repeated Suicide Attempt at 1 Year

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Reattempt during Follow-Up</th>
<th>No Reattempt during Follow-Up</th>
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<tr>
<td></td>
<td>AA (%)</td>
<td>AC (%)</td>
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<tr>
<td>n = 20</td>
<td>3 (15)</td>
<td>9 (45)</td>
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<tr>
<td>n = 56</td>
<td>12 (21)</td>
<td>26 (46)</td>
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TPH, tryptophan hydroxylase.

Table 4. Multivariate Analysis (Logistic Regression) of Prognostic Factors of Subsequent Suicide Attempt

<table>
<thead>
<tr>
<th>Variables</th>
<th>Adjusted ORs</th>
<th>95% CI</th>
<th>p (Wald $\chi^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (SS vs. SL or LL)</td>
<td>5.4</td>
<td>1.6–20.8</td>
<td>.009</td>
</tr>
<tr>
<td>BIS Score ($\geq$ 66 vs. &gt; 66)</td>
<td>3.9</td>
<td>1.1–14.4</td>
<td>.03</td>
</tr>
<tr>
<td>Age of Inclusion (OR for 10-Year decrease)</td>
<td>1.6</td>
<td>1.0–2.9</td>
<td>.05</td>
</tr>
<tr>
<td>Depressive Disorder (with vs. without)</td>
<td>5.3</td>
<td>1.4–26.7</td>
<td>.02</td>
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OR, odds ratio; CI, confidence interval; SS, short/short; SL, short/long; LL, long/long; BIS, Barratt Impulsivity Scale.

Discussion

This is the first follow-up cohort study of at-risk subjects to investigate whether two serotoninergic genes involved in vulnerability to suicidal behavior are associated with an increased risk of subsequent suicidal behavior. Our results suggest that the S allele of 5-HTTLPR is involved in the propensity to repeated suicidal behavior. At the functional level, the 5-HTTLPR S allele is associated with reduced serotoninergic activity (Lesh et al 1996; Reist et al 2001) and with a lower level of CSF 5-HIAA in monkeys with deleterious rearing experiences (Bennett et al 2002). Thus, our results are concordant with the findings of early biochemical studies, suggesting a correlation between low levels of 5-HT transmission and the risk of future suicide attempts (Roy et al 1989; Roy 1999).

The lack of association between the short-term risk of reattempt and the TPH gene suggest that the serotoninergic genes encoding for TPH and the serotonin transporter have different effects on susceptibility to suicidal behavior. It may also reflect a lack of power in our study.

Our results may help to refine the phenotype associated with genetic susceptibility to suicidal behavior, as well as the phenotype associated with the 5-HTTLPR. Previous studies suggested that the 5-HTTLPR was associated with violent suicidal behavior (Bondy et al 2000; Courtet et al 2001) but not with nonviolent suicidal behavior (Courtet et al 2003). In a new sample of patients included in the present study, despite the small size of each subgroup, we observed that the frequencies of the S allele and SS genotype in violent suicidal attempters and in nonviolent attempters were similar to the frequencies reported previously in these groups (data not shown) (Courtet et al 2001, 2003). The heterogeneity of suicide attempters according to the characteristics of the suicidal acts (violence, repetition) may explain discrepancies in the results of association studies. Indeed, when considering our whole sample, we observed a trend for an association between suicide attempt and the SS genotype ($p = .07$) and a marginal association with the S allele ($p < .05$). This trend may be explained by the high frequency of multiple suicide attempters (73% of whom 22% have a history of violent suicide attempts) in the population studied.

The suicidal phenotype may include different phenomena and types, each with distinct biology bases. Thus, refining the phenotype may be useful to reduce the amount of etiologic and genetic heterogeneity and thus help us to identify susceptibility genes. Taken together with the previously published association studies, our data suggest that the low-activity allele of the 5-HTTLPR is associated with a psychobiological trait involved both in the risk of violent suicidal behavior and in the propensity to repeated suicidal behavior.

Our data support the hypothesis that such a trait is related to impulsivity. We observed an association between the 5-HTTLPR and impulsivity; however, multivariate analysis showed that both 5-HTTLPR and impulsivity significantly influenced the risk of reattempt. We observed that six of the eight patients with both the SS genotype and a high impulsivity score (above the defined threshold of 66) reattempted suicide ($p = .001$). About 50% of patients carrying the SS genotype, 40% of patients with an impulsivity score above 66, and 75% of patients with both reattempted suicide. This is consistent with the notion that other genetic and/or developmental factors interact with genes in the constitution of psychobiological traits and that environmental triggers interplay to increase the risk of subsequent suicide attempts. Indeed, impulsivity seems to be associated preferentially with low lethality suicidal behavior (Oquendo et al 2003), as was the case in most of our patients. Nevertheless, we cannot exclude the possibility that some impulsive patients may attempt suicide using a violent means. This would explain the role of the availability of the suicidal means as a well-known risk factor.

A large number of association studies have shown that the 5-HTTLPR is involved in susceptibility to affective disorders, in the pharmacological response to antidepressants, in the risk of antidepressant-induced mania in bipolar patients, and in susceptibility to rapid cycling, suggesting that the associated trait is related to mood instability (Bellivier et al 2002). Together with these data, our results suggest that the serotonin transporter gene is associated with an instability pattern involved in a propensity to recurrence of mood states, as well as suicidal acts. One may hypothesize that the 5-HTTLPR S allele is associated with a common phenotype characterized by an affective and behavioral
instability. Other studies in nonclinical populations indicated that this polymorphism may be associated with anxiety-related traits and with a greater neuronal activity of the amygdala in response to fearful stimuli (Lesch et al 1996; Hariri et al 2002). These results suggest that genes coding for key proteins of the serotonergic system are associated with traits involved in several psychiatric disorders and/or acting at different stages of the suicidal process, from the negative thoughts to the suicidal act itself (Marusic and Farmer 2001). It is possible that the variants of the serotonin transporter gene have differential effects, depending on their expression levels in specific brain areas (prefrontal cortex, amygdala) and in combination with other genetic and nongenetic factors, leading to different patterns of emotional or behavioral instability.

The serotonin transporter is the initial target for specific serotonin reuptake inhibitors (SSRI). In a recent controlled trial, Verkes et al (1998) reported that paroxetine, an SSRI, effectively reduced the recurrence of suicide attempts in subjects with personality disorders, independently of its antidepressant effect; however, the treatment only reduced the recurrence of suicide attempts in patients who had made less than five suicide attempts at inclusion. Specific pharmacogenomic studies would be useful to investigate whether patients carrying the S allele are less responsive to SSRI treatments, leading to a lack of preventive effect for subsequent suicide attempts.

The main limitation in our prospective study is the number of patients lost during the 1-year follow-up period, which was about one quarter (27/103). The failure for outpatients to comply with aftercare is a well-documented problem among attempted suicide patients hospitalized after a suicide attempt (Isacsson and Rich 2001). In clinical trials of psychosocial interventions after a suicide attempt, as few as 40% to 50% of patients could be contacted directly after 1 year (Hawton et al 1981; Van Heeringen et al 1995; van der Sande et al 1997). Although we cannot be sure that the patients that dropped out made further suicide attempts or committed suicide, some data suggest that this is not the case. First, the patients that dropped out tended to present, while not significantly different, fewer psychiatric conditions and a less severe suicidal history than the patients who completed the follow-up, suggesting that they would present a lower risk of reattempt. Furthermore, Rossi et al (2002) suggested that patients who drop out of care have fewer psychiatric conditions and that their global functioning is better. Second, as suicide attempters from our geographic area are mainly admitted to our university hospital, it is unlikely that most of these patients reattempt suicide without us finding out. Lastly, it is unlikely that a significant number of the 27 patients committed suicide based on the estimates of the number of completed suicides at 1 year, which is about 2% of suicide attempters (Owens et al 2002).

Interestingly, we did not find any difference for the serotonin transporter genotypes and allele frequencies between the patients that dropped out and the patients who completed the study (genotypes: $\chi^2 = 2.52, p = .28$; alleles: $\chi^2 = 2.30, p = .13$). Moreover, the patients that dropped out had significantly lower S allele and SS genotype frequencies than patients who reattempted suicide (genotypes: $\chi^2 = 8.93, p = .01$; alleles: $\chi^2 = 8.30, p = .003$).

In summary, we carried out a 1-year follow-up study of suicidal behavior among suicide attempters. Our results suggest that the serotonin transporter gene, but not the TPH gene, is associated with a propensity toward repeated suicidal behavior. Our results also suggest that among patients who have previously attempted suicide, this genotype may be a predictive marker of an increased risk of further suicidal behavior. The serotonin transporter gene may be associated with impulsivity. Before drawing definitive conclusions, replication is required in larger samples, especially with a higher number of males. If confirmed, the SS genotype may be predictive of an increased risk of further suicidal behavior in suicide attempters, and impulsivity may be a psychobiological trait associated with the serotonin transporter gene. The identification of polymorphisms in serotonin-related candidate genes may provide a more powerful tool for the prediction and prevention of suicidal behavior in patients who have already attempted to commit suicide.

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