INFLUENCE OF THE SEROTONIN TRANSPORTER PROMOTER GENE POLYMORPHISM ON SUSCEPTIBILITY TO POSTTRAUMATIC STRESS DISORDER

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Posttraumatic stress disorder (PTSD) is a prevalent anxiety disorder marked by behavioral, physiologic, and hormonal alterations. The etiology of PTSD is unknown, although exposure to a traumatic event constitutes a necessary, but not sufficient, factor. Serotonergic dysfunction has been implicated in PTSD. The present study examined the possible association between the serotonin-transporter-linked polymorphic region (SERTPR) and PTSD. The genotype and allele frequencies of the SERTPR were analyzed in 100 PTSD patients and 197 unrelated healthy controls using a case–control design. The frequency of the s/s genotype was significantly higher in PTSD patients than in normal controls. These findings suggest that the SERTPR s/s genotype is one of the genetic factors for the susceptibility to PTSD. Further investigations are required into the influence of gene polymorphisms on the biological mechanisms of PTSD, its clinical expression, and its response to treatment. Depression and Anxiety 21:135–139, 2005. © 2005 Wiley-Liss, Inc.

Key words: PTSD; serotonin transporter; polymorphism

INTRODUCTION

Posttraumatic stress disorder (PTSD) is an often-disabling psychiatric disorder that can result from exposure to trauma. PTSD develops in only a subset of persons exposed to traumatic stress, suggesting individual difference may influence the susceptibility to PTSD. Although previous studies have demonstrated that the lifetime prevalence of exposure to traumatic events is 40 to 80% [Breslau et al., 1991; Kessler et al.,

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PTSD, posttraumatic stress disorder.

The present study examined the possible association between the SERTPR and vulnerability to PTSD, as suggested by the association of SERTPR with differences in brain serotonergic function and the effects of serotonergic drugs.

### METHODS

#### PARTICIPANTS

PTSD patients were recruited from existing clinical populations visiting 11 collaborating centers. A total of 100 patients with PTSD were included in this study. Demographic and clinical characteristics for the sample are shown in Table 1. Trained psychiatrists examined all subjects using the Structured Clinical Interview for DSM-IV–Korean version [Han and Hong, 2000], leading to diagnoses according to DSM-IV criteria. Patients with a history of alcohol or drug dependence, neurological disorders, or any concomitant DSM-IV Axis I and prominent Axis II psychiatric disorders were excluded. Patients who had suffered from major depression before the traumatic event also were excluded; however, PTSD patients who developed major depression within 9 months after the trauma were included in the present study.

The normal control group included 197 randomly selected individuals visiting the hospital for regular health screening (age = 34.76±11.06 years; males/females = 76/121). A total of 224 physically healthy participants were interviewed by trained psychiatrists and were ascertained to be free of major psychiatric problems. This process led to the exclusion of 27 patients due to a past history or family history of substance abuse/dependence or major psychiatric disorders (e.g., schizophrenia, mood disorder, or anxiety disorders). Those in the control and patient groups were all unrelated Korean individuals. Venous blood was drawn from each individual. The study was approved by the institutional review board at the collaborating center. The benefits and risks of study

| TABLE 1. Demographic and clinical characteristics of PTSD patients |
|---------------------------------|-------------------------|
| Age, yr (mean±SD)              | 35.29±10.29            |
| Gender (male/female)           | 43/57                  |
| Duration of illness, yr (mean±SD) | 7.4±6.3               |
| Trauma type (%)                |                        |
| Serious accident/fire/injury   | 47                      |
| Physical assault               | 25                      |
| Natural disaster               | 6                       |
| War or combat                  | 4                       |
| Sexual abuse                   | 3                       |
| Witness violent death          | 3                       |
| Other event                    | 12                      |

PTSD, posttraumatic stress disorder.
participation were fully explained to each participant, and their written informed consent was obtained.

GENOTYPING

Blood samples (5–10 ml) were collected into ethylenediaminetetraacetic acid, and genomic DNA was extracted from white blood cells. The polymerase chain reaction (PCR) analysis used the following primers: forward, 5'-GGC GTT GCC GCT CTG AAT TGC-3'; reverse, 5'-GGA CTG AGC TGG ACA ACC CAC-3'. PCR analyses (on 25-μl samples) were performed according to a previously described protocol [Heils et al., 1996]. PCR products were separated on 3% agarose gels supplemented with ethidium bromide to allow identification of the long (528 bp) and short (484 bp) variants.

STATISTICAL ANALYSIS

The presence of Hardy-Weinberg equilibrium was tested with the χ² test for goodness of fit. The participants were divided into two groups according to the genotype present: l/l+s/s and s/s. For continuous data, differences between two groups were assessed by t test, and the chi-square test was used for categorical data. All analyses were performed using standard software (SPSS for Windows), and P values smaller than 0.05 were considered statistically significant. A correction for multiple testing was not performed because an explorative approach to a genetically complex trait in which the relationship between genotype and phenotype has not been established, and thus, such corrections might inappropriately increase the likelihood that real effects will be missed (type II error rates) [Rothman, 1990].

RESULTS

The genotypes, allele, and allele carrier frequencies of SERTPR in the patients with PTSD and normal controls are given in Table 2. The sex and age distributions were not significantly different between these two groups, χ² = 0.54, df(1), P = .46, and t = 0.402, df(295), P = .69, respectively. The genotypes of SERTPR were classified into two groups, carriers of the l allele (l/l and l/s genotypes) and carriers of the s/s genotype, because the l allele has been reported to have a higher SERT density and activity than the s allele [Heils et al., 1996; Lesch et al., 1996]. There were marginal differences in genotypes between the two groups, χ² = 4.18, df(2), P = .124, and the allele frequency was significantly different, χ² = 4.05, df(1), P = .044, OR = 1.65, 95% confidence interval (CI) 1.01–2.68. When the genotypes of SERTPR were dichotomized into s/s and l/s+l/l groups, the frequency of the s/s genotype was significantly higher in patients with PTSD, χ² = 4.14, df(1), P = .042, OR = 1.77, 95% CI 1.02–3.06. The distributions of the SERTPR genotype in both groups were in Hardy-Weinberg equilibrium, PTSD: χ² = 0.16, P = 0.69; controls: χ² = 0.0041, P = .95.

DISCUSSION

In the present study, we found that the allele and allele carrier frequencies of SERTPR polymorphism differed significantly between patients with PTSD and normal controls. To the best of our knowledge, this is the first report of a SERTPR allelic variation affecting the vulnerability to PTSD.

Several biological abnormalities have been associated with PTSD and may constitute a basis for searches for specific hypothesis-driven candidate genes. A small number of genetic quantitative-trait loci were shown to explain the majority of variance in various relevant PTSD animal paradigms, including habituation of auditory startle, behavioral measures of rodent anxious reactivity to novel environment, contextual and auditory-cued fear conditioning, and avoidance learning in inbred mouse strains [Caldarone et al., 1997; Owen et al., 1997]. In this context, a single gene may contribute additively and interchangeably to vulnerability to PTSD, but its contribution is neither necessary nor sufficient for manifesting the expression of the phenotype of PTSD.

The genes associated with the serotonergic system are potential candidates for explaining the susceptibility to PTSD. There are previous reports of changes in serotonergic activity following severe stress or trauma in animals [Dunn, 1989; Shimizu et al., 1992].

<table>
<thead>
<tr>
<th>Genotype</th>
<th>PTSD (n = 100)</th>
<th>Controls (n = 197)</th>
</tr>
</thead>
<tbody>
<tr>
<td>s/s</td>
<td>77 (77%)</td>
<td>129 (65%)</td>
</tr>
<tr>
<td>s/l</td>
<td>21 (21%)</td>
<td>61 (31%)</td>
</tr>
<tr>
<td>l/l</td>
<td>2 (2%)</td>
<td>7 (4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele frequency</th>
<th>Allele carrier frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>s</td>
<td>0.87</td>
</tr>
<tr>
<td>l</td>
<td>0.13</td>
</tr>
<tr>
<td>l- (s/s)</td>
<td>0.81</td>
</tr>
<tr>
<td>l+ (s/l+l/l)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Genotypes: χ² = 4.18, df = 2, P = .124; alleles: χ² = 4.05, df = 1, P = .044 (OR = 1.65, 95% CI = 1.01–2.68); allele carriers: χ² = 4.14, df = 1, P = .042 (OR = 1.77, 95% CI = 1.02–3.06).

SERTPR, serotonin-transporter-linked polymorphine region; PTSD, posttraumatic stress disorder.
Some investigators have found low platelet serotonin concentrations in patients with PTSD, especially those with depressive moods [Muck-Seler et al., 2003; Spivak et al., 1999]. These results suggest that a dysregulation in serotonergic activity plays a significant role in the pathophysiology and clinical manifestations of PTSD. The therapeutic effects of serotonin reuptake inhibitors also support a role for alterations in the serotonergic system in the pathophysiology of PTSD [Ballenger et al., 2000; Hidalgo and Davidson, 2000].

From the results of a human study, Kirschbaum and colleagues [1992] suggested considerable heritable contributions to the set point of the hypothalamic–pituitary–adrenal (HPA) axis, and cortisol responses to corticotropin-releasing factor and psychological stress. Individuals with hyperregulation of the HPA axis may have a higher risk of developing PTSD. Some researchers have reported that serotonin plays an important role in the functional and structural changes in the hippocampal formation induced by steroid hormones and traumatic stress [Karten et al., 1996; Korte et al., 1996; Mueller and Beck, 2000; van Riel et al., 2002]. The evidence for serotonin involvement in hippocampal changes comes from studies of psychosocial stress in rats, in which remodeling of dendrites is accompanied by the downregulation of SERT expression in the hippocampus, indicating either a reduced density of serotonin terminals or a reduced expression of the SERT [McKittrick et al., 2000]. Moreover, repeated restraint stress and psychosocial stress in rats suppresses the expression of the inhibitory 5-HT1A receptor in the hippocampus [Flugge, 1995; McKittrick et al., 2000]. These animal studies convincingly demonstrate bidirectional interactions between the HPA axis and the serotonin system.

Our demonstration that the s/s genotype is correlated with vulnerability to PTSD can be explained by an association between the s allele of SERTPR and a reduction in the transcriptional efficiency of the SERT gene promoter, leading to lower SERT expression or lower cellular uptake of serotonin [Heils et al., 1996; Lesch et al., 1996]. The higher frequency of the s/s genotype in PTSD patients might be related to the decreased expression of SERT in the brain.

Note that personality factors also can influence exposure to certain PTSD-inducing environments [Jang et al., 2003]. Previous studies have shown that the s allele of SERTPR is associated with antisocial/aggressive traits, habitual violent behavior, the temperament profile of high novelty seeking and low harm avoidance, and type II alcoholics [Hallikainen et al., 1999; Sander et al., 1998], suggesting the involvement of this genetic variant in the psychobiological vulnerability for exposure to trauma.

Our study is subject to some limitations. First, normal controls in our study were not selected for trauma exposure, and hence a proportion of the controls may have carried a genetic vulnerability to PTSD that remained unexpressed due to the absence of trauma exposure. Therefore, some individuals in the control group may develop PTSD after trauma exposure, which would lessen the difference between the two groups. Second, we cannot exclude the presence of a population-stratification bias [Gorwood, 1999], although the relatively high degree of genetic homogeneity of the Korean population [Kim, 2003] makes stratification bias unlikely in our sample. Third, the relatively small sample size limits the generalizability of our findings.

Future studies should extensively investigate the genetic vulnerability to PTSD, especially using larger samples. In particular, the possible risk due to multiple-gene polymorphisms—occurring in the same individuals and affecting simultaneously different neurotransmission systems and their behavioral correlates—should be investigated further.

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Association Between SERTPR and PTSD

139


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