Abstract

Introduction:
In humans, opioidergic neurotransmission appears to modulate a variety of behaviors, including the stress response and cognitive processes, as well as anxiety and psychosis. One neurobiological process which may be modified by the polymorphism of the μ opioid receptor gene is the HPA axis response to stress. Hypothalamic corticotropin-releasing hormone (CRH) neurons, which affect glucocorticoid release by stimulating pituitary adenocorticotropic (ACTH) secretion, are directly and indirectly inhibited by β-endorphin-producing neurons via the μ opioid receptor. Both exaggerated and blunted HPA responses to stress have been associated with high risk for psychosis. Many studies have suggested that the μ opioid receptor gene plays an important role in response to stress, motivation, and numerous psychiatric entities. The present association study tested the hypothesis that the μ opioid receptor confers susceptibility to schizophrenia.

Method:
After informed consent was obtained, 100 schizophrenia patients and 100 control subjects were enrolled in this study. The genotypes of the μ opioid receptor (OPRM1) using 3 common SNPs rs1799971, rs2075572, and rs648893, were assessed by allele-specific PCR and restriction enzyme digestion. The PCR products were digested by restriction enzyme.

Results:
The frequency of the rs1799971 and rs2075572 of the μ opioid receptor was significantly increased in all schizophrenia patients (rs1799971 Fisher’s Exact Test P = 0.0118 and rs2075572 Fisher’s Exact Test P = 0.044782). There were no associations of rs1799971 and rs2075572 polymorphism of the μ opioid receptor with substance dependence among schizophrenia patients and normal control.

Conclusion:
This allelic association suggests that the functional polymorphism rs1799971 and rs2075572 of the μ opioid receptor may play a role in susceptibility to schizophrenia.

Discussion
The opioidergic neurotransmitter system plays an important role in regulating activation of the hypothalamic-pituitary-adrenal (HPA) axis. Initial activation of the HPA axis occurs at the level of the paraventricular nucleus of the hypothalamus, where neurons that produce corticotropin-releasing factor (CRF) are located. CRF receptors in this area express μ opioid receptors and are under tonic inhibition by neurons of the arcuate nucleus that contain δ opioid (Bond et al., 1998). CRF receptor occupancy in this area suppresses μ opioid receptors and are under tonic inhibition by neurons of the arcuate nucleus that contain δ opioid (Bond et al., 1998). Genetic factors appear to be important modulators of HPA axis activation. The HPA axis appears to be involved, including the normal stress response (Bond et al., 1998; LaForge et al., 2000) and psychosis in which HPA axis dynamics appear to be abnormal. Similarly, there is growing evidence that altered opioidergic neurotransmission and HPA axis dynamics may affect alcohol- and drug-seeking behaviors (Piazza and Le Moal, 1997; Kreek and Koob, 1998).

Various investigations have evaluated the μ opioid receptor polymorphism with regard to drug abuse vulnerability. However, after careful repeat checking with two times PCR and 2 times sequences, we found significant differences in rs1799971 and rs2075572 between schizophrenic and control groups for the μ opioid receptor in the whole sample. The frequency of the rs1799971 and rs2075572 of the μ opioid receptor was significantly increased in all schizophrenia patients (rs1799971 Fisher’s Exact Test P = 0.0118 and rs2075572 Fisher’s Exact Test P = 0.044782). There were no associations of rs1799971 and rs2075572 polymorphism of the μ opioid receptor with substance dependence among schizophrenia patients and normal control. Although the sample size is small, we observed highly significant differences of the distribution of the rs1799971 and rs2075572 of μ opioid receptor among schizophrenia patients and normal control. These allelic associations suggest that the functional polymorphism rs1799971 and rs2075572 of the μ opioid receptor may play a role in susceptibility to schizophrenia. Further replication studies are necessary to confirm the present tentative allelic association.

Conclusion:
Our study suggests that the functional polymorphism rs1799971 and rs2075572 of μ opioid receptor may play a role in susceptibility to schizophrenia.

Table 1. Genotyping of the μ opioid receptor gene polymorphisms

<table>
<thead>
<tr>
<th>SNP</th>
<th>5’primer sequence</th>
<th>3’primer sequence</th>
<th>T (°C)</th>
<th>Genotype identification method</th>
<th>Chi-Square Test P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1799971</td>
<td>5’GTCTCGGTGCTCCTGGCTACCTC</td>
<td>5’TTCGGACCGCATGGGTCGGACCG</td>
<td>65</td>
<td>PCR-RFLP (BsiE1)</td>
<td>0.0118</td>
</tr>
<tr>
<td>rs2075572</td>
<td>5’AACAGATTAGGTCATTCTCACTTTA</td>
<td>5’GT3’(R)</td>
<td>60</td>
<td>PCR-RFLP (MboI)</td>
<td>0.044782</td>
</tr>
<tr>
<td>rs648893</td>
<td>5’TTCGGACCGCATGGGTCGGACCG</td>
<td>5’GT3’(R)</td>
<td>60</td>
<td>PCR-RFLP (MboI)</td>
<td>0.044782</td>
</tr>
</tbody>
</table>

Extraction of genomic DNA:
Blood samples were collected in anonymously identified 15 ml Vacutainer tubes (Becton Dickinson). DNA was prepared by a modified SDS/Polyethylen K procedure (Guin et al., 1979). About 10 ml of diluted blood was kept on ice for 30 min and centrifuged at 2,500 rpm for 15 min at 4°C. The pellet was resuspended in 30 ml of cold miller’s RBC buffer and centrifuged at the same speed. The pellet was well resuspended in 5 ml of 8% buffer (150 mM NaCl, 25mM NaEDTA). Stof of proteinase K (20mg/ml), 50% of 20% SDS was added, and the suspension was mixed until it became clear and viscous, the tube was incubated overnight at 42°C with gentle rotation. Next the suspension was added to 3.4 ml of 8% ammonium acetate and centrifuged at 3500 rpm for 15 min. The supernatant was transferred to a new Falcon tube, and 2 volumes of ice-cold absolute ethanol were added. The DNA was precipitated twice in 70% ethanol, partially dried, and slowly solubilized in 1xTE with RNase at 42°C. DNA purity and quantity was evaluated by a spectrophotometer. A 260/280 nm ratio of 1.9 to 1.8 was usually obtained and the final concentration was adjusted to 100 μg/ml.

Genotyping:
The genotypes of the μ opioid receptor (OPRM1) using 3 common SNPs rs1799971, rs2075572, and rs648893, were assessed by the PCR-RFLP methods.

References