Relationship between 5-HTTLPR polymorphism and post-stroke depression

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ABSTRACT. Post-stroke depression (PSD) is a mental illness characterized by subjective feelings of depression, cognitive dysfunction, and decreased interest. The serotoninergic system is involved in the pathogenesis of depressive disorders and is regulated by the serotonin transporter gene. The serotonin transporter-linked polymorphic region (5-HTTLPR) has been examined as a factor associated with depression and other mental disorders. This study was performed to explore the relationship between 5-HTTLPR and PSD in a Han Chinese population. In total, 199 patients with PSD and 202 unrelated non-PSD patients were recruited from psychiatric hospitals. Depression was diagnosed using the Diagnostic
and Statistical Manual of Mental Disorders-Fourth Edition. Blood samples were collected from all patients for 5-HTTLPR genotyping. Genotype and allele frequencies were compared between the two groups. SS genotype frequency was significantly higher in the PSD group than in the non-PSD group. LL genotype frequency was significantly higher in the non-PSD group than in the PSD group (P < 0.01). This study describes a positive association between 5-HTTLPR and PSD in a Han Chinese population and provides genetic evidence to support the genetic susceptibility of PSD.

**Key words:** 5-HT transporter gene-linked polymorphic region; Gene polymorphism; Post-stroke depression; Stroke

**INTRODUCTION**

Post-stroke depression (PSD) is a common complication in stroke patients. It is characterized by a depressed mood, decreased interest, and sleep disorders, and has an adverse effect on the rehabilitation and outcome of patients with the primary disease (Bilge et al., 2008). One epidemiological survey showed that 30-50% of stroke patients develops depression (Stefano, 2008). PSD leads to a diminished quality of life, delayed recovery of neurological function, increased mortality, and a greater burden on families and society (Hackett et al., 2010). In recent years, much attention has been paid to the serotonin (5-hydroxytryptamine; 5-HT) transporter gene-linked polymorphic region (5-HTTLPR) genotype in PSD patients and the neurotransmitter serotonin hypothesis, but current studies show that the correlation between 5-HTTLPR and PSD is controversial (Fang and Cheng, 2009; Choi-Kwon et al., 2012; Mak et al., 2013). The present study further explores the association between the 5-HTTLPR and PSD from a biological viewpoint in order to provide a theoretical basis for the etiology of PSD.

**MATERIAL AND METHODS**

**Subjects**

The studied sample group consisted of 199 patients with PSD (101 men and 98 women) and 202 patients with acute stroke without depression (named non-PSD) (102 men and 100 women). Sample characteristics and demographics are shown in Table 1. All individuals were unrelated, living in the North Henan Province of China, and all had biological grandparents of Chinese-Han ancestry. The protocol was approved by the Ethical Committee of the Second Affiliated Hospital of Xinxiang Medical University. Written informed consent was obtained from all participants after the objectives and procedures of the study were fully explained.

**Diagnosis and inclusion criteria**

All patients with acute stroke hospitalized at the Second Affiliated Hospital of Xinxiang Medical University were approached regarding participation. Inclusion criteria were as follows: confirmed ischemic stroke by brain computed tomography; ability to complete the necessary investigations and questionnaires; and capacity to understand the objective of the study and
provide informed consent. Exclusion criteria were: severe physical illnesses that interfered with recovery from stroke; communication difficulties due to dysphasia or dysarthria precluding giving informed consent and questionnaire completion; neuropsychiatric comorbidities including dementia, Parkinson’s disease, brain tumor, epilepsy, psychoses, and alcohol or substance dependence; and severe physical illnesses limiting movement prior to stroke.

PSD was diagnosed by at least two experienced psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV; 1994), and a score ≥21 points on the Hamilton Depression Rating Scale (HAM-D-24).

**DNA extraction and genotyping**

Peripheral blood samples from the subjects were drawn into vacutainer tubes containing the anticoagulant ethylenediaminetetraacetic acid and were stored at -70°C until use. Genomic DNA was extracted from leukocytes using the RelaxGene Blood DNA System (Tiangen Biotech, Beijing, China). DNA was suspended in TE-buffer for use in genotyping. The primers for amplification of 5-HTTLPR have been described previously (Goenjian et al., 2012). The upstream primer was 5’-ATGCCAGCACCTAACCCCTAATGT-3’ and the downstream primer was 5’-GGACCGCAAGGTGGGCGGGA-3’.

PCR was performed in a 25-µL reaction volume containing 2.5 µL 10X PCR buffer, 0.5 µL dNTP mix (10 mM), 1 µL each primer (10 µM), 100 ng genomic DNA, 1 U Taq DNA polymerase (Tiangen Biotech, Beijing, China), and sterile deionized water. PCR conditions were: pre-denaturation at 95°C for 10 min; 35 cycles of denaturation at 95°C for 1 min, annealing at 69°C for 1 min, and extension at 72°C for 1 min; and final extension at 72°C for 20 min. Amplification products were separated using 2% agarose gel electrophoresis and images were obtained using an ultraviolet gel imaging system (Uvitec, Cambridge, UK) after ethidium bromide staining. The amplification fragment was 419 or 376 bp. For the purpose of verification, genomic DNA samples were sent for direct sequencing and all showed results consistent with our enzymatic genotyping.

**Statistical analysis**

Demographic characteristics were compared between PSD patients and non-PSD patients using Student t-tests, χ² tests, or Fisher exact tests. Deviations in the genotype counts from Hardy-Weinberg equilibrium were tested using the χ² test. Statistical differences in genotypic and allelic distributions between two groups were evaluated by the χ² test with a significance level of 0.05.
Odds ratios (OR) and 95% confidence interval (95%CI) were calculated to evaluate the effects of different alleles on PSD risk.

**RESULTS**

After PCR amplification, genotyping was performed by agarose gel electrophoresis. Length variation in the 5-HTTLPR was defined by two variable nucleotide tandem repeat elements. The short variation (S) allele of the 5-HTTLPR polymorphism led to 14 repeats of base pair sequences. In contrast, a long variation (L) allele led to 16 repeats of base pair sequences (Mak et al., 2013). The LL genotype had a band of 419 bp; the LS genotype had bands of 419 and 376 bp; and the SS genotype had a band of 376 bp. The genotype concordance between the BeadStation 500G PCR (Illumina, Inc., San Diego, CA, USA) and DNA sequencing was 100%.

There was no deviation from the Hardy-Weinberg equilibrium of 5-HTTLPR genotype in the PSD group ($\chi^2 = 2.71$, $P > 0.05$) and non-PSD group ($\chi^2 = 2.86$, $P > 0.05$). Significant differences were found in genotype ($\chi^2 = 16.75$, $P = 0.002$) and allele ($\chi^2 = 15.12$, $P = 0.001$) frequencies between the groups. The frequency of the L allele was significantly lower in PSD patients (35.7%) than in non-PSD patients (49.3%; OR = 0.53, 95%CI = 0.39-0.72). The results are shown in Table 2.

**Table 2.** 5-HTTLPR genotype and allele frequency distribution.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>Allele frequency</th>
<th>P value</th>
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<tbody>
<tr>
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<tr>
<td></td>
<td>LL</td>
<td>LS</td>
<td>SS</td>
</tr>
<tr>
<td>PSD (N = 199)</td>
<td>20 (10.1)</td>
<td>102 (51.2)</td>
<td>77 (38.6)</td>
</tr>
<tr>
<td>Non-PSD (N = 202)</td>
<td>43 (21.3)</td>
<td>113 (55.9)</td>
<td>46 (22.8)</td>
</tr>
<tr>
<td>OR (95%CI)</td>
<td>0.28 (0.15-0.53)</td>
<td>0.54 (0.34-0.85)</td>
<td>1</td>
</tr>
</tbody>
</table>

PSD = post-stroke depression; OR = odds ratio; HWE = Hardy-Weinberg equilibrium.

**DISCUSSION**

A reduced level of 5-HT neurotransmitters is one of the most accepted hypotheses in the pathogenesis of depression (Marazziti et al., 2013). Studies have shown that the 5-HTTLPR short (S) allele is associated with emotion and cognition (Lee et al., 2003). Whether the pathogenesis and predisposing factors for PSD are the same as those for depression itself has been a research focus in recent years. The pathogenesis of PSD is complicated and related to biopsychosocial factors. It is reported that selective serotonin reuptake inhibitors (SSRIs) are effective in treating PSD (Rasmussen et al., 2003; Niedermaier et al., 2004; Robinson et al., 2008), indicating that PSD and depression result in the disruption of 5-HT neurotransmitters. The 5-HT transporter plays a key role during the metabolism of 5-HT and is the drug target of SSRI antidepressants. Its activity is regulated by 5-HTT transcription regulation (Iversen, 2006; Cole et al., 2011). Therefore, the 5-HTT is considered an important candidate in the genetic research of PSD.

It has been shown that 5-HTT transcription is regulated by 5-HTTLPR. 5-HTTLPR is composed of long (L) and short (S) allele insertion/deletion polymorphisms, and this change influences gene transcription. The transcription ability of the S allele is inferior to that of the L allele (Gonda et al., 2009), causing low expression of 5-HT at the site of action. Some studies of PSD and 5-HTTLPR have demonstrated that the SS genotype and S allele are associated with the pathogenesis of PSD (Ramasubbu et al., 2008; Kim et al., 2012). Other studies have
shown that an increase in the S allele or deletion of the L allele in 5-HTTLPR is correlated with the occurrence of major PSD (Fang and Cheng, 2009). 5-HTTLPR is also associated with alterations in vasoreactivity. There are no significant differences in hippocampus volume and neuronal structure between S and L allele carriers (Cole et al., 2011).

Our findings suggest that genotype distribution and allele frequencies of 5-HTTLPR differ significantly between PSD and non-PSD patients. SS genotype distribution and S allele frequencies in PSD patients are higher than in non-PSD patients (Table 1). LL genotype distribution and L allele frequencies are lower in PSD patients than in non-PSD patients (Table 1). This is consistent with previous studies showing that the S allele of 5-HTTLPR may be an independent risk factor for depression (Lee et al., 2005). The S allele is also positively correlated with post-traumatic stress disorder incidence rate (Kolassa et al., 2010). The L allele may be a protective factor and can reduce the incidence of depression (Grabe et al., 2009). The LL genotype may also increase vascular tension during the stressful event, which may cause ischemia, and is associated with the incidence of vascular events (Brummett et al., 2011). Our results show that SS gene carriers may be prone to development of PSD and the S allele may be a susceptibility factor for PSD. Other studies have suggested that 5-HTTLPR is not associated with PSD or depression (Lasky-Su et al., 2005; Choi-Kwon et al., 2012). These inconsistencies may be explained by different group criteria, sample size, diagnostic criteria, geographic and ethnic background, and different 5-HTTLPR distribution (Fang et al., 2011).

In summary, our results suggest that PSD may be due to genetic susceptibility. Stroke patients with the SS genotype may be more prone to develop depression. This study was limited by failure to explore the correlation between 5-HTTLPR and SSRIs and their effects after stroke. We only studied the gene polymorphisms of 5-HTT without analysis of linkage equilibrium with other polymorphisms. Therefore, a more in-depth subsequent study is required to validate these results.

Conflicts of interest

The authors declare no conflict of interest.

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