Association between toll-like receptor 4 expression and symptoms of major depressive disorder

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Background: In our previous study, toll-like receptor 4 (TLR4) mRNA expression level was associated with severity of major depressive disorder (MDD) evaluated with the 17-item Hamilton Depression Rating Scale (HAMD-17). However, there are few studies that have investigated the relationship between symptoms of MDD and changes in TLR4 expression. Therefore, the aim of the present study was to further analyze the association between subscales of HAMD-17 and TLR4.

Methods: Fifty-one patients with MDD (15 male and 36 female) participated in this study. HAMD-17 was used to assess the symptoms of major depression. The mRNA expression levels of TLR4 were examined in parallel with a housekeeping gene, using real-time polymerase chain reaction. A stepwise linear regression forward model was used to evaluate the relationships between items of HAMD-17 and TLR4 expression.

Results: Some sickness behavior-associated symptoms, including suicide, somatic symptoms of anxiety, or performance of work and activities, were not associated with TLR4 expression. However, psychological signs of anxiety and loss of weight in HAMD-17 can predict the expression level of TLR4.

Conclusion: Our results suggest a significant association between anxiety, body weight loss, and TLR4 mRNA levels in patients with MDD. Larger longitudinal studies combining both subjective and objective measures of depression are needed to clarify the link between TLR4 and symptoms of depression.

Keywords: major depressive disorder, innate immune, toll-like receptor, anxiety

Introduction

Major depressive disorder (MDD) is one of the most serious psychiatric diseases throughout the world, and causes numerous socioeconomic burdens. The most serious consequence of MDD is increased risk of suicide. The etiology and pathophysiology of MDD still remains unclear at the present time. There is evidence from numerous studies suggesting that the etiology of MDD is heterogeneous. Despite the hypothesis of disturbances in neurotransmitter systems and alterations in levels of neurotrophic factors, there are an increasing number of reports to suggest that immune abnormalities also play a role in MDD. This is not only supported by animal studies, but has also been shown in human investigations. In a recent review, whereby the literature on this subject was summarized, it was suggested that inflammatory activity can increase anhedonia-like behavior in animal models. Similarly, in humans, another meta-analysis (conducted by Dowlati et al.) found that the proinflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), were significantly higher in depressed than
in control subjects. Similar changes in C-reactive protein in depressive patients were also found in another meta-analysis. Moreover, psychosocial and physiological stressors—which are two of the most frequently mentioned etiological factors in MDD—can induce the production of proinflammatory mediators, such as IL-1 and reactive oxygen species, and hypothalamic–hypophysal–adrenal axis disturbances in the brain. Moreover, disturbances of the immune system impaired neurotransmitter metabolism and neural circuits that regulate mood. For example, increases in cytokine and inflammatory mediators decrease the production of serotonin. All the evidence indicates that the alterations—either primary or secondary to outside stimulation—affect internal regulation in the brain and result in mood abnormalities.

The toll-like receptors (TLRs) are one of the protein types expressed in the circumventricular organs and the choroid plexus. TLRs are considered one of the pattern recognition proteins of the innate immune response, which can defend against external microorganisms and recognize endogenous ligands, to mediate the inflammatory response to stress. The TLR family contains various subtypes, including TLR2 (which binds with bacterial lipoproteins and lipoteichoic acid), TLR3 (which recognizes double-stranded RNA), and TLR7 (which recognizes single-stranded DNA). Because of their potential roles in neuropsychiatric disease, TLRs are attracting increasing attention. Alterations in TLRs have been found in psychiatric disorders, such as increased expression in TLR3, TLR4, TLR5, and TLR7 in patients with MDD, reduced mRNA expression in schizophrenia, and enhanced response to ligand in psychosis. Moreover, in addition to alterations in TLRs, changes in adaptor proteins of TLRs have also been found. Indeed, in a report by Hajebrami et al the adaptor proteins of TLRs were found to be significantly elevated in depressed patients. In addition, genetic polymorphism of TLR4 has been investigated in bipolar disorder, which revealed that the rs1927914 AA and rs11536891 TT genotypes were more frequent in bipolar disorder patients than in controls.

The TLR family member, TLR4, has received the most attention in recent decades. TLR4, initially discovered for its role in the recognition of bacterial lipopolysaccharide (LPS), has been found to have a regulatory role in the adrenomedullary response to stressful inflammatory stimuli. Recently, researchers have started trying to link TLR4 with neuropsychiatric disorders. For example, McKerman et al pointed out that there is an enhanced response in TLR2 and TLR4 activation in schizophrenia and bipolar disorder.

Since the “leaky gut” hypothesis was put forward, the link between TLR4 and MDD is starting to become more well established. The “leaky gut” hypothesis states that the translocation of enterobacteria induces and activates the inflammatory and oxidative/nitrosative stress pathways through TLR4 signaling. In a recent report by Garate et al it was suggested that LPS from Gram-negative bacteria translocating through the gastrointestinal tract due to an increase in its permeability are responsible for TLR4 activation found in the brain. This reaction would result in increases in inflammatory mediators in the central nervous system. Moreover, in humans, another study attempted to investigate the differences in TLR4 in MDD patients, and to assess alterations with cognitive behavioral therapy alone. In this report, it was found that the expression of TLR4 was significantly higher in MDD patients than in healthy controls, and that expression was decreased with a course of cognitive behavioral therapy alone.

In our previous report, TLR4 was shown to be the only member of this protein family to have significant association with MDD severity. Here, we performed further investigations to determine the associations between items of the 17-item Hamilton Depression Rating Scale (HAMD-17) and TLR4.

**Methods**

**Subjects**

In an extension of our previously published study, we recruited MDD patients through the Structured Clinical Interview for DSM-IV Axis I Disorders, conducted by a senior psychiatrist (Hung YY) from August 2013 to December 2014, at Kaohsiung Chang Gung Memorial Hospital Medical Center in Taiwan. Institutional Review Board approval was obtained from the hospital ethics committee (101-5012A3, 103-5114B) and informed consent was obtained for each participant. We used the HAMD-17 to evaluate the symptoms and severity of MDD. The exclusion criteria were 1) alcohol dependence and 2) any history of immune inflammatory disease. In order to exclude any chronic physical illness, all patients received routine physical examination, chest X-rays, electrocardiogram, and routine blood tests. At the same time, patients were checked for acute infections or allergic reactions at the time of entering this study.

**Sample preparation and analysis**

Blood samples were collected through venous drawing (5 mL) and stored in PAXgene blood RNA tubes in the early morning, after patients had fasted for 9 hours. The tubes were stored in a −80°C freezer immediately after blood was drawn, and the samples remained in the freezer until they were assayed. We used TRIzol reagent to isolate the RNA and performed real-time polymerase chain reaction (RT-PCR) using a set of primers.
for TLR4 (sense 5′-TGAGCAGCTGCTGCTGATC-3′; anti-
sense 5′-CAGGGCTTTTCTGAGTCTGC-3′) (Lopez et al). The QuantiTect Reverse Transcription Kit (Qiagen, Venlo, the Netherlands) was used for the reverse transcription of RNA. The PTC-200 apparatus (Bio-Rad Laboratories, Hercules, CA, USA) and the QuantiTect SYBR Green PCR kit (Qiagen) were applied for the complete analysis. We used glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a housekeeping gene to assess the relative abundance of mRNA.

**Statistical procedure**

A stepwise multiple linear regression forward model was used to judge the subscores of HAMD-17 that correlated with the TLR4 levels. All results are presented as mean ± standard deviation. Statistical significance was set at P<0.05. All statistical analyses were performed through the Statistical Product and Service Solutions (SPSS, IBM Corporation, Armonk, NY, USA), version 22.

**Results**

**Epidemiologic data**

Altogether, 51 patients with MDD were recruited. Table 1 shows baseline characteristics regarding participants’ sex, age, and body mass index (BMI) (15 male/36 female, mean age, 46.25±10.04 years; BMI, 23.90±4.96 kg/m²). Smoking (17 patients), education duration (11.24±2.96 years), HAMD-17 score (27.20±5.40), and TLR4 relative expression (1.50±1.82) are also shown in Table 1. Table 2 shows the average scores of different HAMD-17 items.

**Table 1 Demographic findings and clinical data of patients with major depressive disorder**

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46±10.04</td>
</tr>
<tr>
<td>Male</td>
<td>45.67±9.53</td>
</tr>
<tr>
<td>Female</td>
<td>46.50±10.37</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>15/36</td>
</tr>
<tr>
<td>BMI</td>
<td>23.90±4.96</td>
</tr>
<tr>
<td>Male</td>
<td>22.91±4.04</td>
</tr>
<tr>
<td>Female</td>
<td>24.32±5.29</td>
</tr>
<tr>
<td>Smoking (yes/no)</td>
<td>17/34</td>
</tr>
<tr>
<td>Education duration (years)</td>
<td>11.24±2.96</td>
</tr>
<tr>
<td>Male</td>
<td>10.80±3.32</td>
</tr>
<tr>
<td>Female</td>
<td>11.41±2.82</td>
</tr>
<tr>
<td>TLR4 (relative fold difference)</td>
<td>1.50±1.82</td>
</tr>
<tr>
<td>HAMD-17</td>
<td>27.20±5.40</td>
</tr>
<tr>
<td>Male</td>
<td>30.20±6.11</td>
</tr>
<tr>
<td>Female</td>
<td>25.94±4.60</td>
</tr>
</tbody>
</table>

**Note**: Data are presented as mean ± standard deviation.

**Abbreviations**: BMI, body mass index; HAMD-17, 17-item Hamilton Depression Rating Scale; TLR4, toll-like receptor 4.

**Table 2 Scores of different items in HAMD-17**

<table>
<thead>
<tr>
<th>Items in HAMD-17</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depressed mood</td>
<td>3.10±0.94</td>
</tr>
<tr>
<td>Feelings of guilt</td>
<td>1.49±1.07</td>
</tr>
<tr>
<td>Suicide</td>
<td>2.39±1.23</td>
</tr>
<tr>
<td>Insomnia: early in the night</td>
<td>1.31±0.81</td>
</tr>
<tr>
<td>Insomnia: middle of the night</td>
<td>1.31±0.81</td>
</tr>
<tr>
<td>Insomnia: early hours of the morning</td>
<td>1.20±0.94</td>
</tr>
<tr>
<td>Work and activities</td>
<td>3.06±0.90</td>
</tr>
<tr>
<td>Retardation</td>
<td>1.33±0.89</td>
</tr>
<tr>
<td>Agitation</td>
<td>1.86±1.13</td>
</tr>
<tr>
<td>Anxiety (psychic)</td>
<td>2.25±1.04</td>
</tr>
<tr>
<td>Anxiety (somatic)</td>
<td>1.88±1.19</td>
</tr>
<tr>
<td>Gastrointestinal somatic symptoms</td>
<td>1.00±0.63</td>
</tr>
<tr>
<td>General somatic symptoms</td>
<td>1.10±0.54</td>
</tr>
<tr>
<td>Genital symptoms</td>
<td>0.67±0.82</td>
</tr>
<tr>
<td>Hypochondriasis</td>
<td>1.29±1.24</td>
</tr>
<tr>
<td>Loss of weight</td>
<td>0.94±0.90</td>
</tr>
<tr>
<td>Insight</td>
<td>0.86±0.78</td>
</tr>
</tbody>
</table>

**Note**: Data are presented as mean ± standard deviation.

**Abbreviation**: HAMD-17, 17-item Hamilton Depression Rating Scale.

**Cox regression of TLR4**

Through the cox regression model, we were able to determine whether the subscores of HAMD-17 had significant associations with the TLR4 mRNA levels (Table 3). Within the subscores of HAMD-17, the scores for psychological anxiety and loss of weight showed significant associations with peripheral TLR4 mRNA levels (P=0.01 in the model for combination of psychological anxiety and loss of weight).

**Discussion**

To our knowledge, this is the first study to investigate the association with peripheral TLR4 mRNA levels and specific HAMD-17 subscores. The main results of our study are that psychological anxiety and loss of weight predict peripheral TLR4 mRNA expression.

In the present study, we show that anxiety has a significant association with peripheral TLR4 mRNA expression. The immune system in the gastrointestinal environment plays an important role in body weight and psychological anxiety. Gastric and duodenal ulcers were found to have a correlation with the Hamilton anxiety scale in a cross-sectional study, in a population of 2,000 students at Belgrade University. In recent reviews, exposure to physical, physiological, as well as psychological, stressors was found to have numerous impacts on homeostasis of the intestinal microbiologic environment. Moreover, disruption of the intestinal microbiologic environment resulted in overproduction of the TLR4 system.
There are several limitations of the present study. Firstly, the effects of distribution of different types of leukocytes on TLR4 mRNA expression were unclear based on the present work, as TLR4 mRNA expression was summed for the different cell types in the blood. The decreased percentages of lymphocytes reported in a previous study may cause deviation. Further studies with cell-type specific designs are therefore necessary in the future. Secondly, cytokines were not analyzed in this study. There is evidence that sickness behaviors, including weight loss and anxiety, are mediated through the effects of proinflammatory cytokines, such as IL-1, TNFα, and IL-6. The association between TLR4 and weight loss or anxiety may be mediated by cytokines. Thirdly, an increase in the content of TLR4-specific mRNA may not, as a necessary consequence, cause an increase in TLR-specific surface expression of proteins. The dissociation between the protein and mRNA for receptors was noted in patients with sepsis, and in some cell lines. Finally, the sample size of the current study was very small; thus, further analyses using larger sample sizes and healthy controls are needed to avoid type I error and confirm these results.

In conclusion, our report highlights the significant association between anxiety, weight loss, and TLR4 mRNA levels in patients with MDD. These findings contribute to evidence on the important role of TLR4 in anxiety-related behavior. However, further investigations and carefully controlled studies are still needed, to provide more evidence on the mechanism of TLR4-associated sickness behavior in patients with MDD.

**Acknowledgment**

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**Disclosure**

The authors report no conflicts of interest in this work.

**References**


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