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The roles of brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) in predicting treatment remission in a Chinese Han population with generalized anxiety disorder

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ABSTRACT

Neurotrophic factors, particularly brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF), are involved in neuroplasticity in the nervous system. To explore the characteristics of BDNF and GDNF and their roles in predicting treatment remission in a Chinese Han population with generalized anxiety disorder (GAD), 85 GAD patients were treated with escitalopram or venlafaxine randomly for 8 weeks. The serum BDNF/GDNF levels were detected, while Hamilton Anxiety Rating Scale (HAMA) scores were measured at baseline and after 8 weeks of treatment. The differences in serum BDNF/GDNF levels between GAD patients (n = 85) and healthy controls (n = 73) and between remission and nonremission were then compared. The serum BDNF levels were lower in GAD patients (1197.24 ± 367.41 μg/L) than in healthy controls (1378.09 ± 382.46 μg/L) ($P < 0.05$). The serum GDNF levels were also lower in GAD patients (10.19 ± 9.86 μg/L) than in healthy controls (13.73 ± 9.44 μg/L) ($P < 0.05$). The BDNF level was negatively correlated with baseline HAMA score ($P < 0.05$). The GDNF level was negatively correlated with baseline HAMA score ($P < 0.05$). The BDNF level was positively correlated with GDNF level ($P < 0.05$). Both baseline BDNF/GDNF levels in remission and nonremission showed no statistically significant differences. No significant correlation was found between baseline BDNF level and the HAMA reduction rate or between baseline GDNF levels and the HAMA reduction rate. Both serum BDNF and GDNF were demonstrated to be potential biomarkers of GAD, it seems that serum BDNF and GDNF levels can be used to assess the baseline anxiety severity of GAD but cannot serve as a factor to predict treatment remission.

1. Introduction

Generalized anxiety disorder (GAD) is a psychiatric disorder characterized by pervasive anxiety, dysfunctional worries, nervous and motor strain, autonomic hyperactivity and hyper arousal (American Psychiatric Association, 2013). Excessive, uncontrollable worry about a variety of topics in the absence of respective stimuli or in a manner disproportionate to their potentially posed risk is the key diagnostic criterion of GAD (American Psychiatric Association). GAD usually begins during adolescence and affects approximately 5.7% of the general population across their lifespan, with the highest incidence among women (Kessler et al., 2005; Tempesta et al., 2013). GAD patients experience a considerable degree of impairment and disability in family, social and educational functions. The physiopathology of GAD is unknown, but plenty of evidence suggests a complex interaction between susceptibility genes, environmental stressors, and biochemical mechanisms (Enoch et al., 2010; Gatt et al., 2009). Its etiological interrelatedness with dimensional measures of trait anxiety, such as pathological worry, fear of uncertainty, or neuroticism, and its high rate of treatment resistance have attracted the attention of psychiatrists aiming to identify biomarkers of disease risk and treatment response (Gottschalk et al., 2017).

Brain derived neurotrophic factor (BDNF) is a member of the neurotrophin family involved in several forms of plasticity in the nervous system, including neuronal maturation and synaptic remodeling (Yoshii et al., 2010). BDNF is involved in anxiety-like behaviors in

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preclinical models, and it is well established that BDNF is a key mediator of synaptic plasticity in fear circuits. Recent research suggested that pathological anxiety may be modulated by genetic variation in BDNF (Andero and Ressler, 2012; Fullana et al., 2012). Many studies suggest that BDNF could be involved in the neurobiology of GAD and might represent a useful marker (Carlino et al., 2015; Pallanti et al., 2014; Yuan et al., 2015). Unlike the relatively consistent results of BDNF in major depressive disorder, the results of BDNF in GAD are controversial. No significant difference was found in serum BDNF levels for GAD in Italy (Carlino et al., 2015), but in the same study, comparison showed a statistically significant reduction of serum BDNF level in female GAD subjects with respect to healthy controls. Yuan et al. (2015) found that BDNF plasma levels in GAD patients were significantly lower than those in healthy controls, but the results of Pallanti et al. (2014) showed an unexpected increase in levels of both BDNF and ARTN in patients with GAD. Our previous study also found the serum level was elevated in GAD compared to healthy controls and returned to normal after effective treatment (Shen et al., 2011).

Glial cell line-derived neurotrophic factor (GDNF) is a member of the transforming growth factor-β(TGF-β) superfamily, along with neurturin (NRTN), artemin (ARTN) and persephin (PSPN). It is extensively distributed in hypothalamus, substantia nigra, and thalamus (Golden et al., 2015; Skibinska et al., 2017). GDNF has been associated with the modulation of synaptic plasticity and the formation of neural circuits (Skibinska et al., 2017). GDNF promotes the differentiation of dopaminergic neurons (Christophersen et al., 2007) and serotonergic neurons as well as GABAergic neurons (Ducray et al., 2006), and it also protects neurons and glial cells against oxidative stress (Golan, 2011). GDNF has been proven to be involved in the pathogenesis of depressive disorders and other psychiatric disorders, and a reduction in GDNF levels has been found in patients with MDD (Lin et al., 2015; Takebayashi et al., 2006). Furthermore, anti-depression treatments can elevate the GDNF levels in depression (Ibáñez and Andressoo, 2017). So far, there are few studies on the association of GDNF with anxiety symptoms (De et al., 2014), but the changes of GDNF in GAD and whether GDNF can predict the treatment outcome are still unknown.

The dysregulation of the neurotransmitters serotonin (5-HT), noradrenaline (NA), dopamine (DA) and γ-aminobutyric acid (GABA) is commonly accepted to be involved in the etiology of GAD (Nikolaus et al., 2010). BDNF and GDNF have complex interactions with 5HT/DA systems, which are closely involved in the pathology and the treatment response of GAD (Baldwin et al., 2017; Popova et al., 2017), and as neurotrophic factors, BDNF and GDNF, interact with each other too through the pathway of 5-HT system (Popova et al., 2017).

Taken together, the existing research suggests that GAD patients have abnormal BDNF levels, and considering the changes of GDNF levels in MDD, GAD patients may also have abnormal GDNF levels. BDNF and GDNF levels may predict the response to treatment with antidepressants. In this study, we compared the serum BDNF and GDNF levels of GAD patients to those of healthy controls. We also compared the serum BDNF and GDNF levels of antidepressant remission to those of nonremission. By doing so, we sought to determine the changes of BDNF levels in GAD and explore the changes in GDNF levels; we also sought to explore the possibility of BDNF and GDNF levels in predicting treatment response in Chinese GAD patients.

2. Materials and methods

2.1. Participants

Initially, 183 patients were screened from the Huzhou Third Hospital between December 2014 and June 2016. Sixty-two cases failed to meet the inclusion and exclusion criteria and 36 patients refused to participate this study. Eighty-five GAD patients who met the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria for generalized anxiety disorder were included in the research. Inclusion criteria included all patients with scores ≥17 on the Hamilton Rating Scale for Anxiety (HAMA) and ≤14 on the 17-item Hamilton Rating Scale for Depression (HAM-D17) (Qian et al., 2017). Exclusion criteria included any illness requiring medical intervention, epilepsy, dementia, substance abuse disorders, schizophrenia, delusional disorder, psychotic disorders not elsewhere classified, bipolar disorder, major depression disorder, and personality disorders diagnosed by DSM-IV. Chronic or acute somatic or neurological diseases requiring medical intervention were also excluded. At the time of testing, all subjects were free of major psychotropic drugs for at least 2 weeks. In total, 73 healthy controls recruited from the local community were assessed by a psychiatrist using the Structural Clinical Interview for DSM-IV Disorders (SCID). The HAMA scores of all control subjects were <7. Those with a history of any psychiatric disorder were excluded. The study was approved by the Ethics Committee of Huzhou Third Hospital. Written informed consent was obtained from all participants after a complete and extensive description of the study. All participants were enrolled by clinicians. Based on our pilot study (comparison of BDNF between GAD and healthy controls), the sample size of GAD was approximately 90 cases with $\alpha = 0.05$, $\beta = 0.10$.

2.2. Treatment

After screening, eligible patients were randomly assigned to receive a flexible dose of escitalopram or venlafaxine for 8 weeks by a random number table. The drug was single blinded to the scale raters. At the end of the study, the dosage of escitalopram ranged from 10 to 20 mg/d, with a mean dose of 16.15 ± 3.52 mg/d; the dosage of venlafaxine ranged from 150 to 225 mg/d, with a mean dose of 184.26 ± 25.72 mg/d. Zolpidem was used to treat the insomnia syndrome with a dosage of 10 mg/d if needed.

2.3. Rating symptoms and efficacy

The severity of the GAD symptoms was rated using HAMA, which was administered by a single trained rater. The primary efficacy endpoint was change from the patient's HAMA baseline. The HAMA reduction rate was defined as (baseline HAMA - endpoint HAMA)/baseline HAMA × 100%. A remission was defined as HAMA ≤ 7 after 8 weeks' treatment, otherwise, they were classified as nonremissions (Shen et al., 2011). The rater and laboratory workers were blind to the purpose of the study. In addition, the rater was blind to the BDNF/GDNF data, and the HAMA scores were not disclosed to the laboratory workers. The internal consistency index was kappa = 0.82. To maintain blindness, a trained research coordinator managed all data and schedules. The clinician's drug choice was based on the anticipated side effects and the symptomatic characteristics of the patients. The dosing protocol was flexible and conducted according to the clinician's assessment of the symptoms and side effects.

2.4. Determination of serum BDNF/GDNF level

Ten-milliliter blood samples were collected into anticoagulant-free tubes between 7 and 8 AM and immediately transferred to the laboratory for serum preparation. Blood samples were split into two aliquots, one for BDNF and one for GDNF. After 1 h incubation, serum was separated by centrifugation and stored at −70 °C for further analyses. The BDNF concentration was determined by a human BDNF Kit (ELISA method, Wuhan doctor Germany) according to the manufacturer's instructions. Determination of optical density by each hole of the microtiter plate reader (OD) was performed. Then, the standard curve was calculated with the standard concentration and the OD value, and finally the sample concentration of BDNF was calculated. The concentrations were expressed as μg/L. The assay's threshold for detecting GDNF is 0.3 μg/L-10 μg/L. Intra-assay and interassay variability were <5% coefficient of variation (CV) and <10% CV, respectively.

Serum concentrations of GDNF were measured using ELISA kits (human GDNF ELISA kit, Wuhan doctor Germany) according to the manufacturer's instructions. Then, the standard curve was calculated with the standard concentration and the OD value, and finally the sample concentration of GDNF was calculated. The assay's threshold for detecting GDNF is 0.3 μg/L-9 μg/L. Intra-assay and interassay variability were <5% coefficient of variation (CV) and <10% CV, respectively.

To minimize interassay variations, BDNF/GDNF was determined after all samples were collected. All assays were carried out by the same operator using the same instrument and the same recommended buffers, diluents, and substrates.

2.5. Statistical analysis

The study endpoint for all analyses was the 8th week, and the last-observation-carried-forward (LOCF) method was used to account for missing data. SPSS 19.0 for Windows was used to analyze the collected data. Data were generally reported as the mean ± SD. Student's *t*-tests were performed for the comparisons of some demographic data, including the serum BDNF/GDNF levels of male and female patients as well as comparisons between remission and nonremission. Relationships between BDNF/GDNF and clinical variables were evaluated using Pearson correlations. A difference was considered significant at two tailed $P < 0.05$.

3. Results

3.1. Demographic and clinical characteristics of GAD patients and control subjects

Eighty-five GAD cases were enrolled at baseline, and 1 case was lost after 8 weeks of treatment. We analyzed the data of all 85 GAD cases and 73 healthy controls. There was no significant difference in the male/female ratio ($\chi^2 = 2.23$, $P = 0.14$), age ($t = 1.52$, $P = 0.13$), education status (t = 1.46, $P = 0.15$), and family history ($\chi^2 = 3.35$, $P = 0.07$) between the GAD subjects and healthy controls (see Table 1).

3.2. Characteristics of serum BDNF/GDNF in GAD patients and comparison with healthy controls

The serum BDNF levels were lower in GAD patients (1197.24 ± 367.41 μg/L) than in healthy controls (1378.09 ± 382.46 μg/L) (t = −3.02, df = 150, $P = 0.003$). The serum GDNF levels were also lower in GAD patients (10.19 ± 9.86 μg/L) than in healthy controls (13.73 ± 9.44 μg/L) (t = −2.30, df = 154, $P = 0.02$). The BDNF levels in female GAD patients (1226.78 ± 363.07 μg/L) were significantly higher than in male GAD patients (959.70 ± 334.78 μg/L) (t = 2.30, df = 83, $P = 0.02$). The GDNF levels in female GAD patients (9.27 ± 8.82 μg/L) were also significantly higher than in male patients (5.42 ± 4.19 μg/L) ($t = 2.37$, df = 26, $P = 0.02$).

The BDNF level was negatively correlated with baseline HAMA score ($r = -0.30$, $P = 0.006$). The GDNF level was negatively correlated with the baseline HAMA score ($r = -0.27$, $P = 0.01$). The BDNF level was positively correlated with the GDNF level ($r = 0.36$, $P = 0.001$).

Table 1
Demographic and clinical characteristics of GAD patients and control subjects.

Characteristics	GAD cases ($n = 85$)	Healthy controls ($n = 73$)	Statistics	
			χ^2/t	*P* value
Sex (male/female)	11/74	16/57	2.23	0.14
Age (years)	45.4 ± 11.6	42.7 ± 10.6	1.52	0.13
Education (years)	9.8 ± 4.2	10.9 ± 5.3	1.46	0.15
Family history (yes/no)	8/77	1/72	3.35	0.07

Table 2
Demographic and clinical characteristics of remission and nonremission.

Characteristics	remission ($n = 43$)	nonremission ($n = 42$)	Statistics	
			χ^2/t	*P* value
Sex (male/female)	5/38	6/36	0.13	0.71
Age (years)	45.6 ± 11.3	45.2 ± 12.1	0.15	0.88
Duration of illness (months)	85.9 ± 119.6	75.2 ± 109.4	0.43	0.67
Family history (yes/no)	5/38	3/39	0.11	0.74
Baseline HAMA	20.5 ± 3.1	23.2 ± 3.3	3.88	<0.001
Baseline HAMD	10.1 ± 3.7	11.2 ± 4.1	1.30	0.20

3.3. Demographic and clinical characteristics of remissions and nonremissions

By the end of the study, 43 subjects achieved remission, while 42 subjects were in nonremission. There were no significant differences in the male/female ratio ($\chi^2 = 0.13$, $P = 0.72$), age ($t = 0.15$, $P = 0.88$), family history ($\chi^2 = 0.11$, $P = 0.74$), illness duration ($t = 0.43$, $P = 0.67$), and baseline HAMD between remission and nonremission ($t = 1.30$, $P = 0.20$), but the baseline HAMA in remission was lower than that in nonremission ($t = 3.88$, $P < 0.001$) (see Table 2).

3.4. Comparison of serum BDNF/GDNF levels in remission and nonremission

The baseline BDNF levels in remission were (1261.40 ± 346.30 μg/L) and (1129.40 ± 381.70 μg/L) in nonremission, which was not a statistically significant difference ($t = 1.77$, df = 83, $P = 0.08$). The baseline GDNF levels in remission were (9.02 ± 9.86 μg/L) and (8.53 ± 10.90 μg/L) in nonremission, which was also not a statistically significant difference ($t = 0.22$, df = 83, $P = 0.83$).

The BDNF levels in female patients (1281.46 ± 348.17 μg/L) and male patients (1108.90 ± 324.07 μg/L) showed no significant difference ($t = 1.05$, df = 41, $P = 0.30$) in the remission group. The GDNF levels in female patients (9.29 ± 7.18 μg/L) and male patients (6.95 ± 5.50 μg/L) also showed no significant difference ($t = 0.70$, df = 41, $P = 0.49$) in the remission group.

The BDNF levels in female patients (1169.07 ± 374.33 μg/L) were significantly higher than in male patients (835.35 ± 315.13 μg/L), with no significant difference ($t = 2.06$, df = 40, $P = 0.046$) in the nonremission group. The GDNF levels in female patients (9.26 ± 8.61 μg/L) were significantly higher than those in male patients (4.14 ± 3.14 μg/L), with no significant difference ($t = 2.66$, df = 40, $P = 0.01$) in the remission group.

No significant correlation was found between baseline BDNF levels and the HAMA reduction rate ($r = 0.18$, $P = 0.09$) or between baseline GDNF levels and the HAMA reduction rate ($r = 0.10$, $P = 0.39$).

4. Discussion

Both BDNF and GDNF play important roles in the neuropathology and the treatment response of GAD and depression through their effects on the plasticity in the nervous system (Carlino et al., 2015; Golden et al., 2015; Takebayashi et al., 2006; Qian et al., 2017; Yoshii et al., 2010).This study was one of the first to combine BDNF and GDNF to explore their relationship with GAD, investigating the characteristics of serum BDNF/GDNF in GAD patients and their roles in predicting treatment remission in GAD patients. In this study, we found that serum

BDNF/GDNF levels were lower in GAD patients than in healthy controls. The BDNF/GDNF levels in female GAD patients were higher than those in male patients. BDNF/GDNF levels were negatively correlated with baseline HAMA score. The BDNF level was positively correlated with the GDNF level. Both baseline BDNF/GDNF levels in remission and nonremission showed no statistically significant difference. No significant correlation was found between baseline BDNF level and the HAMA reduction rate or between baseline GDNF levels and the HAMA reduction rate.

BDNF has been well studied in depression and consistent results found that the BDNF level was lower in depression than in healthy controls and will be elevated after anti-depression treatment, so BDNF could represent a useful marker in depression (Colle et al., 2017; Gulyaeva, 2017). However, when it comes to GAD, the results are controversial (Carlino et al., 2015; Masatake et al., 2012; Pallanti et al., 2014; Yuan et al., 2015). Moreira et al. (2015) found no difference between GAD and healthy controls, and the Met allele was significantly associated with an increase in serum BDNF levels compared with the Val/Val genotype in GAD participants. No significant difference was found in serum BDNF levels for GAD compared to another study (Carlino et al., 2015). However, the results of Pallanti et al. (2014) showed an unexpected increase in levels of both BDNF and ARTN in patients with GAD. Our study showed that serum BDNF level was significantly decreased in GAD, which is accordance with the results disclosed by Yuan et al. (2015). They also found that BDNF plasma levels in GAD patients were significantly lower than those in healthy controls. The lowered BDNF levels might be a marker reflecting the impairment of neuronal function and synaptic plasticity in mental disorders. Considering the similarity between depression and GAD (including neuropathology, treatment and high comorbidity), it is more reasonable to find the downregulation of BDNF in GAD. The conflicting results may be explained as follows: (1) the age structure was different in these research samples, the mean age in this study was (48.4 ± 11.6), which was similar to (38.3 ± 12.9) in Yuan Wang's study (Yuan et al., 2015), so we both found a decline of BDNF in GAD. The average age in the study by Moffitt et al. (2007) was (26.71 ± 5.06), so no difference was found in the BDNF levels between GAD and healthy controls in their study. There was an obvious difference in age between the GAD (42.29 ± 2.74) and control groups (34.00 ± 3.10) in the study by Pallanti et al. (2014), so they found an increase in the levels of both BDNF and ARTN in patients with GAD. Considering these results, more larger studies are needed to eliminate the effects of age on BDNF/GDNF. (2) The gender composition was different in these research samples, although no significant difference was found in serum BDNF levels for GAD (Carlino et al., 2015), but in the same study, a comparison showed a statistically significant reduction of serum BDNF level in female GAD subjects with respect to female healthy controls. Our study also found a difference in BDNF levels between female/male GAD patients. In a mouse study, Karisetty et al. (2017) found different BDNF expression in adult male, intact female and ovariectomized female mice in stress conditions. They showed that 17β-Estradiol (E2) administration can increase the expression of BDNF in PFC. Considering that the female ratio was 87% in our study, the gender factor should be considered in the following studies. This point may explain the impact of age composition on the results to some extent because some females had been menopausal in this study. To make all the subjects comparable, it is important for all female subjects to have a normal menstrual cycle. (3) The distribution of gene type and BDNF effects depend on the genotype. Moreira et al. (2015) found no difference between GAD and healthy controls, but the Met allele was significantly associated with an increase in serum BDNF levels compared with the Val/Val genotype in GAD participants. It is a pity that we did no such work in this study.

Compared to BDNF, relatively fewer studies have been conducted on GDNF and mental diseases, especially GAD, but GDNF has also been associated with the modulation of synaptic plasticity and the formation of neural circuits (Skibinska et al., 2017). In their meta-analysis, Lin and Tseng (2015) supported blood GDNF levels as a biomarker of depression. In this study, the results show that the serum GDNF level was downregulated in GAD patients. Although the difference in GDNF between GAD patients and healthy controls was not as significant as that on BDNF, the relatively higher standard deviation of GDNF should be considered. GDNF (Ledda et al., 2007) plays a role in synapse formation through the induction of ectopic presynaptic sites. Evidence suggests that the neuroprotective and neurorestorative effects of exogenous GDNF occur by increasing DA neuron numbers (Allen et al., al.,2013) and enhancing the DA function in the nigrostriatal system. GDNF has an effect on the 5-HT system, and GDNF increases the 5-HT neuron soma size, number of primary neurites/neurons, and neurite/neuron length (Ducray et al., 2006). GDNF increases 5-HT 2A receptor gene expression in the frontal cortex and decreases it in the hippocampus (Tsybko et al., 2014). The dysregulation of the neurotransmitters serotonin (5-HT), noradrenaline (NA), dopamine (DA) and γ-aminobutyric acid (GABA) are commonly involved in the etiology of anxiety. Therefore, our findings on GDNF may have certain clinical and neurobiological implications in the diagnosis of GAD.

It is worth noting that BDNF level was closely correlated with GDNF level, and BDNF expression was much higher than that for GDNF. BDNF highest level was observed in the hippocampus and cerebral cortex, and BDNF administration reduced the gene expression of 5-HT2A receptors in the frontal cortex, increased it in the hippocampus, and increased the functional activity of 5-HT2A receptors. GDNF expression occurs in the dopaminergic structure, the striatum. GDNF increased the expression of 5-HT2A receptors in the frontal cortex, and decreased it in the hippocampus. Thus, there may be an interaction between BDNF and GDNF (Popova et al., 2017). Considering the dysregulation and imbalance of 5-HT and DA in GAD, these results show that GDNF can be a potential factor in serving as a biomarker of GAD combined with BDNF considering their different effects on the 5-HT and DA system.

GAD patients always respond to anti-anxiety drugs slowly, and the treatment outcome is unsatisfactory (Qian et al., 2017). Kurita et al. (2012) reported that patients with depressive syndrome who underwent remission had significantly higher plasma BDNF levels, and they found a significant negative correlation between MADRS scores and plasma BDNF levels. Wolkowitz et al. (2011) also reported that responders to treatment (≥ 50% improvement in depression ratings) had higher pretreatment BDNF levels than non-responders. BDNF is the dominant factor (compared to other neurotrophic factors) in the brain. BDNF is necessary for the normal development and functioning of the 5-HT system, but brain 5-HT also affects BDNF (Klein et al., 2010). GDNF is predominantly considered to be a regulator of the DA system and also interacts with brain 5-HT. It stimulates the growth of 5-HT brain neurons and the expression of the 5-HT-system, but excess 5-HT concentrations decrease the expression of GDNF (Golan 2011; Menegola et al., 2004). The results in this study show that both baseline BDNF and GDNF levels in remission were higher than those in nonremission, but had no significant differences. However, the p-value for this difference between remissions and nonremissions on BDNF levels was 0.080, so the relatively small sample size may explain the negative results. If the sample size is enlarged in future studies, there may be a significant difference between remissions and nonremissions. Considering the sophisticated interaction between BDNF/GDNF and the 5HT/DA system, it may be difficult to use single factors to predict the treatment remission on GAD. No significant correlation was found between baseline BDNF level and the HAMA reduction rate or between baseline GDNF levels and the HAMA reduction rate. However, we found that both BDNF and GDNF levels were negatively correlated with baseline HAMA score. Antidepressants are now the most used drugs in the clinical treatment of GAD. 5-HT is involved in the mechanism of action of all currently used groups of antidepressants. Antidepressant drugs rapidly activate TrkB signaling and gradually increase BDNF expression, and the behavioral effects of antidepressants are mediated by and are dependent on BDNF signaling through TrkB (Castrén and

Antila, 2017). Antidepressants increase the 5-HT level in the synaptic cleft, and 5-HT specifically increases extracellular signal-regulated kinase (ERK) activation and GDNF mRNA expression and is released in a dose- and time-dependent manner (Tsybko et al., 2014). Antidepressants increase the expression and secretion of BDNF as well as GDNF, so both BDNF and GDNF play important roles in the pathway by which antidepressants take effect. Positive results may be observed if the sample size is enlarged in future studies.

We also found that the baseline HAMA scores in the remission group were lower than those in the nonremission group. It seems that mild GAD cases have a faster improvement and a better outcome than more severe cases. Baseline anxiety/depression severity was the most frequently reported statistically significant predictor of anti-depressant treatment outcomes, and many studies reported a negative prediction relationship between baseline anxiety/depression severity and treatment outcomes (the higher the baseline severity, the poorer the outcome) (Ginsburg et al., 2011; Ghesquiere et al., 2014).

In conclusion, both serum BDNF and GDNF were demonstrated to be potential biomarkers of GAD. It seems that serum BDNF and GDNF levels can be used to assess the baseline anxiety severity of GAD, but cannot serve as factors to predict treatment remission.

There are a few limitations to our study. (1) The sample size is too small to prove that BDNF/GDNF is a biomarker of GAD. A larger sample size and longer study duration could also potentially reveal more significant differences in BDNF/GDNF levels between remission and nonremission.(2) It would have been better to analyze the results in subgroups (sex and age), but the power would have decreased. Samples well-matched in age and sex would be ideal for subsequent studies.(3) Although we tried to eliminate the effects of depression syndrome on BDNF/GDNF levels, GAD has a high comorbidity with MDD, and stricter inclusion criteria could be helpful in future studies.(4) If we can acquire dynamic changes of BDNF/GDNF levels in the treatment (we did not collect blood sample postintervention), the relationship between BDNF/GDNF and GAD will be more clear.(5) We also did not directly analyze the effects of drugs on BDNF/GDNF levels.

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Conflict of interest

No conflicts of interest are declared.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2018.08.111.

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