Author’s response to reviews

Title: The association between BDNF Val66Met polymorphism and emotional symptoms after mild traumatic brain injury

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Author’s response to reviews:

Dear Editor,

We truly appreciate for your notice regarding the valuable suggestions from reviewers on our manuscript entitled “The association between BDNF Val66Met polymorphism and emotional symptoms after mild traumatic brain injury”. The content of this manuscript is unpublished and will not be considered to publish elsewhere.
In addition, we have corrected the format of the manuscript and the typographical errors in this manuscript carefully. The replies for the reviewers are as follows. We replied the comments and questions in a point-by-point fashion. The major changes have been highlighted in red in the revised manuscript.

The authors have declared that no competing interests exist.

Sincerely yours,
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Leigh Christopher (Reviewer 1):
Wang et al. show an interesting effect of genotype on BDI scores post TBI whereby TT genotypes show higher scores compared to CC and CT genotypes (recessive effect). This is an interesting study, but there some methodological concerns. The lack of covariate inclusion to remove potential confounding effects is concerning, especially given that the p value is marginally significant. Analyses including covariates mentioned below would need to be carried out to draw conclusions from this study. The statistical section of methods is unclear/too brief and tests used need to be clarified.
Grammar

Abstract

* "But BAI scores show no significant associations."

Ans: Thanks for reviewer’s comments. We have corrected the grammatical errors in the revised manuscript carefully.

Introduction

* What is the rationale for the investigation of BDNF in emotional symptoms of TBA? Are there any studies that show that BDNF may be associated with emotional disorders/problems. These should be cited to strengthen the rationale of this study.

Ans: Thanks for reviewer’s comments. Brain-derived neurotrophic factor (BDNF) is one of the most abundant neurotrophins in the central nervous system. BDNF has been found to associate with psychiatric disorders. However, the role of BDNF in TBI-related emotional symptoms has not been characterized.

According to your suggestions, we have appended the following paragraph in the part of introduction (page 5, line 10) : "Accumulating evidence indicated the involvement of BDNF in the pathophysiology of mood disorders [22-25]. Previous animal study showed that BDNF knockdown in the hippocampal subregion produced depression-like behaviors in rats [26]. In addition, the decreased BDNF signaling was observed in the subgenual anterior cingulate cortex in major depressive patients [27]. Furthermore, the recent meta-analysis study revealed that the BDNF blood level had significantly difference between the healthy subjects and patients with major depression [28]".
The articles are as follows:


Results

* What was the goal of assessing patients at 1 and 6 weeks? This should be explained

Ans: Thanks for reviewer’s comments. In this study, we collected the clinical data at multiple time points (at 1, 6 weeks and 6 months) following mTBI for covering short-term and long-term outcomes after brain injury. The original idea is to understand the time-dependent effects after the brain injury. We are particularly interested in the mood disorders such as anxiety and depressive symptoms. However, the dropout rates in the 6 months group is very high (> 70%). It’s almost impossible to do genetic association study in such as a small sample size. That’s the reason we only analyzed the data from the 1 and 6 weeks groups.

* Was the goal to test for a recessive effect of genotype? The BDI scores were higher in the TT genotype group but it is not clear what test was used. There are 3 p values reported in the table, however, this should be clarified in the methods section as well as the results text. Was it a regression using an additive dosage effect for genotype and t tests for recessive and dominant tests? This needs to be clearly stated. In week 6 were the BDI scores still significantly higher? This should also be stated along with the p value in the text.

Ans: Thanks for reviewer’s comments. Following your suggestions, statistical evaluation of our data has been redone using r-project. In addition, we have appended the following paragraph in part of methods (page 8, line 18) : “R 3.2.0 (http://www.r-project.org/ and http://cran.r-project.org/) was used for the statistical analyses. Linear regression model was performed for patient characteristics to define the possible confounding factors, including age, gender, Glasgow Coma Scale (GCS), Extended Glasgow Outcome Scale (GOSE), mechanism of injury and current medication use. We analyzed the magnitude of the association between the different genotypes of rs6265 and BAI, BDI scores through a likelihood ratio test in four models (including codominant, dominant, recessive and log-additive model) that implemented in SNPassoc package. BAI and BDI scores of each genotype group were presented with mean ± standard error (s.e.). Patients with missing or incomplete data of BAI, BDI and baseline covariates were excluded from the analyses. Statistical significance was considered at p < 0.05”.

* What covariates were controlled for? This is not clear. Sex, education, age and severity of TBI should all be controlled for in these analyses to remove confounding effects.

Ans: Thanks for reviewer’s comments. Following your suggestions, we analyzed the correlation between patient characteristics and BAI, BDI scores using linear regression model. In addition, we have appended the following paragraph in the part of results (page 10, line 11) : ”The correlation between patient characteristics and BAI, BDI scores were analyzed using linear
regression model to evaluate potential confounding factors. BAI and BDI scores showed moderate correlation with each other in the first and sixth weeks following mTBI (Supplemental figure 1 and 2). The variants including GCS, GOSE, injuries caused by traffic accidents or falls, and current antidepressant or hypnotic medication use were correlated with BAI and BDI (Supplemental table 1). These variants were adjusted for potential confounding effects.

* Was a history of previous anxiety or depression considered?

Ans: Thanks for reviewer’s comments. The psychiatric history of patients was not collected in this study. However, we had recorded the information of patient’s current medications, including antidepressant, anti-anxiety medication and hypnotic medication (table 1). The correlation between these covariates and BAI, BDI scores were analyzed using linear regression model to evaluate potential confounding factors.

Discussion

* The conclusion of this study is overstated given that there is an unclear understanding of the methods and covariates used in the analysis. Although the ideas are interesting, a stronger methodological approach is needed

Ans: Thanks for reviewer’s comments. Following your suggestions, the statistical evaluation of our study has been redone with covariates adjustment to improve our methodology. Meanwhile, we have modified the description of the results and tables in the revised manuscript.

The following paragraph has been appended in the part of methods to clearly express the statistical analysis in the revised manuscript (page 8, line 18):” R 3.2.0 (http://www.r-project.org/ and http://cran.r-project.org/) was used for the statistical analyses. Linear regression model was performed for patient characteristics to define the possible confounding factors, including age, gender, Glasgow Coma Scale (GCS), Extended Glasgow Outcome Scale (GOSE), mechanism of injury and current medication use. We analyzed the magnitude of the association between the different genotypes of rs6265 and BAI, BDI scores through a likelihood ratio test in four models (including codominant, dominant, recessive and log-additive model) that implemented in SNPassoc package. BAI and BDI scores of each genotype group were presented with mean ± standard error (s.e.). Patients with missing or incomplete data of BAI, BDI and baseline covariates were excluded from the analyses. Statistical significance was considered at p < 0.05.”
To avoid the overstated conclusions of our findings, the following paragraphs has also been modified from “BDNF might be involved in the pathophysiology of TBI-related emotional symptoms. We concluded that rs6265 of BDNF may be a helpful predictor and therapeutic target for depressive symptoms following mTBI” to “Our study provides evidence for the correlation between BDNF rs6265 genetic polymorphism and emotional symptoms following mTBI”. (page 14, line 8)

Francesco Carpi (Reviewer 2):

The study by Wang et al. describes the correlation between BDNF rs6265 and depressive symptoms following mTBI, showing a higher risk of depression for female patients with the TT genotype. Although the manuscript is well written, there are several limitations to this study, as the authors state, and there are some points that need to be addressed.

Critical comment:

*The authors should specify other characteristics of the recruited patients not only age and gender (e.g. ethnicity, health condition before TBI) because these could strongly influence the findings.

Ans: Thanks for reviewer’s comments. According to your suggestions, the patient characteristics (Table 1) has been modified with the following information: Gender, Age, Injury mechanism, Glasgow Coma Score (GCS) at emergency department, Extended Glasgow Outcome Scale (GOSE), BAI scores, BDI scores, current antidepressant medication use, anti-anxiety medication use and hypnotic medication use in the revised manuscript. All subjects in our study were Han Chinese in Taiwan. The association analyses of our data has been redone with covariates adjustment to improve our methodologic approach.

The authors recruited 192 patients, 131 females and 61 males, as described in table 1. These numbers do not match with the ones evaluated at the first week in tables 2,3,4 and 5, in particular:

Table 2, 185 patients evaluated at the first week instead of 192.

Table 3, 187 patients evaluated at the first week instead of 192
Table 4, 127 female patients evaluated at the first week instead of 131

Table 5, 126 female patients evaluated at the first week instead of 131

*The authors should explain why they exclude these missing patients.

Ans: Thanks for reviewer’s comments. These unmatched numbers between tables were due to the missing or incomplete data for BAI and BDI scores. Following your suggestions, we have modified all tables and appended the following sentence in part of methods to clearly describe this exclusion in the revised manuscript (page 9, line 7): “Patients with missing or incomplete data of BAI, BDI and baseline covariates were excluded from the analyses”.