Author’s response to reviews

Title: Decreased plasma neuroactive amino acids and increased nitric oxide levels in melancholic major depressive disorder

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Author’s response to reviews: see over
Re: Ms. ID 1651945282116766

Dear Mr. Carlo Rye Chua and Prof. Markus Donix,

Thank you very much for your letter of March 29, 2014 concerning our manuscript (Ms. ID 1651945282116766) entitled ‘Decreased plasma neuroactive amino acids and increased nitric oxide levels: a possible trait-marker for melancholic major depressive disorder’.

We were pleased to have the chance to address the comments from the reviewers in our revised manuscript. In the attachment of this letter, we have responded to each of the reviewers’ points of concern, giving point by point our responses (R) to the reviewers’ questions (Q). Please find in the website of BMC Psychiatry our uploaded revised manuscript, in which we have highlighted the changes. In addition, the paper has been copyedited to improve the style of written English by the professional English secretary, i.e. Ms. Wilma Verweij of the Netherlands Institute for Neuroscience.

We hope that our modifications are satisfactory, and that they make our paper acceptable for publication in BMC Psychiatry.

Thanks for your kind consideration.

Sincerely yours,

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Reviewer #1

Reviewer's report:
This is an interesting study. To explore the possibility, which neuroactive amino acids changes in patients with naive first episode work as the biomarkers of MDD. The approaches are sound and manuscript well written. I have two major concerns as well as a few minor points.

Authors’ response:
We want to thank this reviewer for the positive remarks on our paper.

Major concerns

Q1. The three groups were not comparable. The key question in this study is “can plasma level of amino acids neurotransmitter equate the CSF level? ” The aim of this study is to establish whether changes in the plasma level of amino acids may serve as biomarker for the melancholic subtype of MDD. But the answer from the second control, the 10 non-depressed subjects, was no. There was no significant correlation between the plasma and CSF levels of amino acids. The conclusion can’t be drawn, because this control was not suitable as the control, they had the medical conditions, mainly the brain diseases, which may cause the BBB abnormal, the results from this control were no credible.

R1: It is, because of ethical concerns, not possible to obtain both plasma and CSF samples from MDD patients or from the healthy control subjects. We tried however to come as close as possible to this situation by recruiting the 10 patients with a range of medical conditions, including stroke, uterus with scar, leukemia, brainstem hemorrhage, and subarachnoid hemorrhage from which plasma and CSF samples both available, and who voluntarily participated in the present study. They were all during their clinical recovery phase (we have now added this information to the text, see page 6 line 5 to 6). We have also described in the Discussion a previous study which might support our findings in this group, that ‘the absence of correlations that we found between plasma and CSF amino acid levels is in agreement with the report that
there were no significant correlations between venous blood and CSF levels of Asp or Gly in healthy subjects (see ref. 27 in the text). This implies that the 2 compartments, i.e. the blood and the CSF, do not seem to have an equilibrium mechanism for the transportation of these neuroactive amino acids.’ (see now page 12 line 10 to 15). We have also explicitly added your concern to the Discussion: ‘The study on the relationship between plasma and CSF levels of amino acids was carried out in a group of non-depressed individuals with different diseases who were during their clinical recovery phases. It is a limitation of present study that, for ethical reasons, we could not study CSF from MDD patients or healthy control subjects. It cannot be excluded that the medical conditions from which these patients were recovering may have affected BBB function. (page 12 line 5 to 10)

Q2: The follow up protocol and process was not clear, only 7 patients were followed up, and the results showed no significant changes before and after treatment (Figure 3). The conclusion was not drawn, because the treatment process was not clear, and the sample size was too small.

R2: We have now made the follow up protocol and process clear by stating ‘…after 2 months of antidepressant treatment (fluoxetine 20-40 mg/day) the patients came back to the clinic and their symptoms and HAMD scores were evaluated again, together with their blood samples being taken.’ (page 5 line 13 to 16) Indeed only 7 MDD patients completely finished such a follow-up (page 5 line 16 to 19), as we indicated in the Discussion as a limitation of this study (page 12 line 15 to 17). We have now added your concern as follows: ‘Although the number of followed-up patients is relatively small, the Wilcoxon test for these 7 patients, that showed significant clinical improvement after 2 months antidepressant treatment, did not show significant changes in these amino acids or NO plasma levels before and after treatment, which supports the suggestion that these parameters might serve as trait-markers for melancholic subtype of MDD’ (page 12 line 17 to 22).

Minor points:
Q1: How many item of HAMD was used in this study? Please clarify it.
R1: We used the 24-item Hamilton Depression Scale (HAMD) and we have now added this information to the revised manuscript (page 5 line 5).

Q2: Please introduce the demographic data of subjects? For example, how long the subjects suffered from the MDD.
R2: We have given those demographic data which might have impact on the study results (gender, age, subtypes of depression, major symptoms etc) at page 4 line 22 to 23, and page 5 line 1 to 2, and we have indicated that all these patients were in their first depressive episode (page 5 line 1).

Q3: The author mentioned one of the subject actually committed suicide later, what is the later? How long the subjects were followed up? What are these suicide symptoms outcomes?
R3: We have indicated now that this patient committed suicide after one month of the treatment (page 5, line 7 to 9). For the present study we only got 7 patients who were followed-up according to our protocol (see above), with their blood samples available after 2 months of treatment. For the other patients we indeed made phone call follow-up, and we know that they did not commit suicide but we did not want to include information on the other outcomes in the paper without seeing the patients ourselves.

Q4: The Table 1 is too complicate to understand well.
R4: This Table shows the relationship between these neuroactive compounds and the clinical symptoms. We have now better explained in the legend that Comparison of plasma levels of the 4 amino acids or NO levels among different sub-phenotype groups, based upon the 9 symptoms of major depressive disorder according to the DSM-IV. (page 20 line 2 to 4).

Q5: The Figure 1 is redundant.
**R5:** We have removed this figure.

**Reviewer #2**

**Reviewer’s reports:**

…The authors present interesting data, … This study offers new clinical data on these compound changes in a well defined subtype of major depression.

**Authors’ response:**

We want to thank this reviewer for the positive remarks on our paper.

**Q1:** It is too early to draw the conclusion that changes of amino acids and nitric oxide in plasma as a trait-marker for melancholic major depression before other type of major depression are investigated. The title of the manuscript may better be changed to a more neutral one.

**R1:** We followed your advice and changed the title into ‘Decreased plasma neuroactive amino acids and increased nitric oxide levels in melancholic major depressive disorder’. In addition, we added to the Discussion as the warning ‘Larger patient groups and other types of MDD should be investigated before we can conclude that indeed the changes in the plasma neuroactive amino acids and NO are trait-, rather than state-, marker for melancholic MDD’ (page 13 line 2 to 5).

**Q2:** Since the plasma levels of these amino acids may be influenced by many factors, were there any potential confounding variables (diet, lifestyle etc) that might be contributing to the observed group differences.

**R2:** We have indeed taken such confounding variables (diet, lifestyle etc) into consideration. In the Discussion, we mentioned that these neuroactive amino acids are non-essential amino acids that the body could synthesize by itself. Melancholic MDD patients may have a decreased appetite, and thus also decreased food intake, which may lead to a reduced metabolism and decreased synthesis of these amino acids. This is also the reason why we limited the range of BMIs in the present study (page 5 line
13) that was, however not different from that of the controls. In addition, we also mentioned in the ‘Materials and Methods’ inclusion and exclusion criteria for BMIs when we recruited subjects (page 6 line 8).

Q3: Limitations of the works should be further stated as the study was based on the limited sample size. The authors have already indicated the limitations in specificity and selectivity.

R3: We have now in the Discussion stated as a limitation that the study was based upon a relatively limited sample size (page 12 line 15 to 17). See also R1.