Author's response to reviews

Title: Associations between Serotonin Transporter Gene Polymorphisms and Heat Pain Perception in Adults with Chronic Pain

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Author's response to reviews: see over
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Re: Revisions to manuscript # 3563893468040814

Professor Thomas Klopstock
BMC Medical Genetics

Dear Prof. Klopstock:

We would like to thank the editorial staff for the thoughtful review of our manuscript. Below, each reviewers’ comments have been placed in italic followed by our response. Revisions made to the manuscript have been highlighted in red for easy identification.

**Reviewer #1**

1. *How much is the correlation between genotypes and protein expression?*

   This important information has been included in the Introduction section as follows (page 3, first paragraph):
   
   “Previous investigators have suggested that 5-HTTLPR polymorphisms may account for a two-fold difference in mRNA levels [6, 7].”

2. *How was the sample size calculated? Did the sample size have adequate power for subgroup analysis?*

   We strongly agree with this important comment. Unfortunately, the study cohort was comprised of consecutive patients admitted to our pain rehabilitation program from March 2009 to March 2010 who met inclusion criteria (please see Methods section, Participants sub-section).

3. *One of the major limitations of the study is lack of a control group.*

   We agree that this is an important limitation. The lack of a control group has been included as a limitation as follows (page 16, last paragraph):
   
   “Although significant differences in thermal pain perception were found among the three 5-HTTLPR genotypes in adults with chronic pain, the lack of a control group could also limit the generalization of our study findings.”

4. *In this study that involved adults with chronic pain, the intermediate triallelic 5-HTTLPR expressing group was associated with greater HP thresholds compared to the high expressing group* — what about comparison with low
expressing group?

We agree with this suggested revision, and the manuscript has been revised throughout to emphasize this important point.

Please see the Results section of the abstract:

“Individual group comparisons showed that values of HP threshold were significantly greater in the intermediate compared to the high expressing group ($P = 0.009$), but not the low expressing group ($P > 0.1$).”

Please see the Conclusions section of the abstract:

“In this study that involved adults with chronic pain, the intermediate triallelic 5-HTTLPR expressing group, but not the low expressing group, was associated with greater HP thresholds compared to the high expressing group.”

The Results section has been revised as follows (page 10, third paragraph):

“Individual group comparisons showed that HP 0.5 was significantly greater in the intermediate expressing group compared to the high expressing group (Mann Whitney $U = 3477.00, Z = -2.67, P = 0.009$), but no significant differences were found between the intermediate and low expressing groups ($P > 0.10$) (supplementary figure 3).”

Please see the legend of supplementary figure 3:

Median value of HP 0.5 and 95% confidence interval for the 5-HTTLPR genotype groups in units of just noticeable difference (JND). Individual group comparisons showed that HP 0.5 was significantly greater in the intermediate expressing group compared to the high expressing group ($*P = 0.009$), but not the low expressing group.

Please see the Discussion section (page 11, first paragraph):

“No significant difference in HP 0.5 was observed between the intermediate and low expressing groups.”

5. “heterogeneous cohort of adults with chronic pain” – what do the authors mean by heterogeneous cohort of adults with chronic pain? What are these conditions? Authors should describe these patients population more clearly.
After careful review, we agree that the term “heterogeneous” is not an accurate descriptor of our study cohort. Therefore, use of this term has been omitted throughout the manuscript.

The description of our patients has been further clarified as follows (page 5, second paragraph):

“The majority of patients have previously used multiple trials of analgesic medications, undergone surgeries, and utilized various interventional pain procedures with incomplete pain relief. Examples of common admitting diagnoses include low back pain, fibromyalgia and chronic headache.”

6. Were some of these patients opiate dependent?

The proportion of patients using daily prescription opioids has been highlighted in the results section as follows (page 10, first paragraph):

“Fifty-two percent (n = 143) of the cohort used daily opioids, and the mean morphine equivalent dose was 46.1 ± 77.9 mg/day.”

7. Give representative gel picture of different polymorphisms.

We agree with this recommendation; unfortunately, the gel pictures are not of suitable for reproduction.

8. Some editing of English are needed.

We have edited the text throughout.

Reviewer #2

1. I would...suggest that the tables should be quoted in the text as “supplementary table x”.

All references to the tables and figures have been revised as suggested (e.g., supplementary table 1, supplementary figure 1).

2. Moreover I would like to include a broader discussion on the role of the serotonergic system in pain management, e.g. by including the results of Treister R, et al. Association between polymorphisms in serotonin and dopamine-related genes and endogenous pain modulation. J Pain. 2011 (8):875-83, into the discussion.

We have included the findings from this study in the Discussion section as follows (page 12, final paragraph):
“In a final study that involved 529 healthy subjects (72% Ashkenazi Jews of Eastern European “origin”, 20% Sephardic Jews of North African or Asian “origin”), the low expressing 5-HTTLPR genotype was associated with decreased pain inhibition compared to the high and intermediate groups [12].”

Please also see the following (page 14, final paragraph):

“The rate of rise of the heat stimuli was 0.3°C/s in the Potvin et al. study [11] in which 3 tests were performed, and the rate of rise in the Treister et al. study [12] was 10°C/s in which 5 tests were performed.”

Reviewer #3

1. Please show a figure depicting genotype vs phenotype.

We agree with this recommendation, and a third figure has been developed depicting the triallelic 5-HTTLPR genotype and the heat pain phenotype.

2. When discussing findings by others please give some post-hoc power for those studies to find an effect of the magnitude found in this manuscript.

We agree with this recommendation, and the effect sizes of 3 previous studies were calculated and compared to the effect size observed in our study (page 15, first paragraph):

“However, despite these methodological variations, the effect size of the difference in thermal pain threshold between the low and high expressing 5-HTTLPR groups ranged from 0.60 to 0.32 in 3 studies that reported mean data. This range is comparable to the effect size of 0.37 for the difference in HP threshold between the intermediate and high expressing groups as observed in our current study.”

Again, we would like to thank the editorial staff for their thoughtful review of our manuscript.

Sincerely,

W. Michael Hooten, MD