Author's response to reviews

Title: Sleep quality, BDNF genotype and gene expression in individuals with chronic abdominal pain

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Author's response to reviews: see over
Response to Reviewer 1

Reviewer's report

Title: Sleep quality, BDNF genotype and gene expression in individuals with chronic abdominal pain

Version: 3  Date: 5 August 2014

Reviewer: Yvette Conley

Reviewer's report:

Some major issues still exist. I’ve pasted the initial reviewer comment, the author’s response, and then my follow up reviewer comment to the 3 most important issues that still exist with this paper and require compulsory revisions.

1. Initial Reviewer comment: The manuscript is written with the cohort of 59 subjects in mind; however microarray gene expression data (a major data element for the project) was conducted in less than half of this cohort (n=26). We are never told what this subset looks like? Are they different in any manner from the larger cohort that is defined? How many controls were included in that n=26? How was this subset selected and why?

Response: The microarray cohort was limited due to cost and control of sample. The sample included only the Caucasian participants information for 26 samples used for microarray data is- Pain: CAP (11) vs healthy control (15), Sleep quality: poor sleep quality (7) vs good sleep quality (19) and BDNF: homozygous (17) vs heterozygous (9). The information is included in the revised manuscript, Methods, Page 14, 3rd paragraph, Line 3. Per quality measures of microarray processing only samples from 26 Caucasian participants were analyzed.
**Follow up Reviewer comment:** This issue is not adequately addressed. It is still unclear what the microarray subset looks like from the perspective of cases and controls. Readers will want to know demographics for the n=26 for cases as well as controls. Also, was this subset different in any way from the full cohort? How was the subset selected – did they randomly select the subset for microarray evaluation, limit it only to Caucasians, or what criteria was used?

**Follow up Response:** Thank you for your recommendation to further clarify the microarray cohort. The microarray cohort was all Caucasians to control for the genetic effects of race. We have now included an additional table (See Table 5) as recommended. The added table provides details of the cases and healthy controls used for microarray experiments. Details of the cases and healthy controls of the sample cohort used for microarray experiments are given in Table 4. Also, the demographics of the microarray sample cohort are provided in Supplemental Table S2. There were no statistically significant differences between the microarray cohort and the full cohort with regard to gender, age, or BMI, however; only samples from Caucasians with available SNP data were included. The requested information is now included in the revised manuscript, Page 4 and 3rd Paragraph, and Page 12, 2nd Paragraph and Lines 1-2). As mentioned earlier, the samples for microarrays were limited due to cost.

**2. Initial Reviewer comment:** The controls for this study are important since most of the evaluations are relative comparisons with controls; however we are given no information about the controls in the manuscript? How was a control defined? Were
they recruited in the same manner as the cases? Were they just as rigorously evaluated for sleep and CAP phenotypes as the cases?

**Response:** The procedure followed to determine categories of Pain and Sleep quality groups are mentioned prior in ‘Methods’ section of the manuscript (Pages 9#10). All study participants (cases and controls) were recruited per protocol now specified in more detail as requested and included in a supplemental file. The participants were grouped based in the pain group if they had a history of abdominal pain for greater than 6 months. Controls had no history of abdominal pain and no other organic disorders or GI diseases (e.g., inflammatory bowel disease, celiac disease, biliary disorders, bowel resection) and had no cardiac, pulmonary, neurologic, renal, endocrine, or gynecological pathology. This added information is as now specified in the added supplemental material. Sleep groups were based on PSQI scores as noted in the manuscript. All participants had the PSQI administered regardless of pain group. All participants had the same assessments.

**Follow up Reviewer comment:** *It is still not clear how controls were defined. The response indicates that cases and controls were recruited per protocol that is now included in the supplemental file. If one looks at the supplementary data on recruitment and inclusion/exclusion criteria one sees that an inclusion criteria to be included in the study is “Have a history of abdominal pain for greater than 6 months”…but then the response mentions that “participants were grouped based in the pain group if they had a history of abdominal pain for greater than 6 months”…based on the inclusion criteria they should all have a history of abdominal pain for greater than 6 months. Additionally,*
if “controls had no history of abdominal pain" then how could the same protocol been used to recruit cases and controls?

**Follow up Response:** Healthy controls are those who meet the inclusion and exclusion criteria and who do not have abdominal pain. Our apologies as the reviewer is correct, we should have noted more clearly that the control group are the healthy volunteers. We follow a particular format at our institution for participant recruitment as given by the link below:

http://www.clinicaltrials.gov/ct2/show/NCT00824941?term=Brain+gut&rank=1

Healthy controls met the inclusion criteria for age, menses (females) and had no history of abdominal pain. Also, healthy controls had no other organic disorders or GI diseases (e.g., inflammatory bowel disease, celiac disease, biliary disorders, bowel resection) and had no cardiac, pulmonary, neurologic, renal, endocrine, or gynecological pathology and had no other exclusions (see Supplemental Information). Now we have included the information in ‘Methods’ of the revised manuscript (Page 4, 3rd paragraph, Lines 2-7).

**3. Initial Reviewer comment:** Evaluation of the BDNF polymorphism data was conducted by comparing homozygotes vs heterozygotes and it is not clear why this was done. Given the small sample size it is understandable that they would dichotomize genotypes particularly if the number of homozygote variants were small, however most studies would have dichotomized into presence/absence of the variant allele and not combined the two homozygote groups for comparison. The authors should clearly state why they did the groupings that they did for this analysis.
**Response:** Based on TaqMan assay of BDNF (rs6265) SNP genotyping results and ‘Quality value’ threshold, samples were classified for BDNF: homozygous (samples having only allele X or allele Y) and heterozygous (samples having both allele X and allele Y). The mutants were batched for heterozygous group categorizing BDNF into two levels for data analysis. Information of the categories of the BDNF group is given in Table 3, and in revised manuscript (Page 5, 2nd paragraph and Page 14, 2nd paragraph).

**Follow up Reviewer comment:** *The response does not address the reviewer's concern. The response gives information on how the genotypes were generated and that the data was dichotomized – but the concern was WHY did they group the genotypes the way they did? It is not the norm to group homozygotes for both alleles and compare them to heterozygotes when dichotomizing genotypes so the authors need to put into the paper WHY they decided to group the genotypes the way they did. Is there a biological rationale that they can give to support the dichotomization that they used because this is not readily apparent.*

**Follow up Response:** We very much appreciate the reviewers comment and appreciate the opportunity to further clarify the genotyping. We are interested in the effects and associations of the Met allele. Previous studies report associations between the Met allele, regardless of whether the genotype is homogeneous or heterogeneous for the Met allele, with depression, cognitive function and molecular expression. The distribution of BDNF genotypes are known to vary among races. Our study included a substantial Caucasian cohort which was selected as a statistically viable group for an analysis of both SNP and gene expression data while controlling for the possible effects
of race (See revised language Page 12, 2nd paragraph). Additionally, in order to assess the associations of the Met allele with the sleep, pain and gene expression data, heterozygous and homozygous Met genotypes were grouped together into a Met carrier group for comparison with the homozygous Val genotype (or non-Met carrier group) as is typically done in the study of these alleles due to the low frequency of the homozygous Met genotype (See revised language Page 9, last Paragraph). In keeping with these trends, and due to the limitations of our particular sample, we use a dominant model of Met expression to analyze our data, which dichotomizes the data and results in increased statistical power. There is precedent in the literature for grouping these data as Val/Val homozygotes and Met carriers (Val/Met and Met/Met). Given the nature and limitations of our data and the aims of the research we feel this categorization is appropriate and makes good use of the available data. As recommended, we have now provided a more detailed explanation for our grouping rational with supporting references (See Page 9, last paragraph). We have also adjusted our language to avoid confusion by hence forth, and more appropriately, referring to the group containing both Val/Met and Met/Met genotypes as the “Met carrier group” (added revisions Abstract, Page 10, line 8; Page 12, 1st and 2nd paragraphs; Page 13, 2nd paragraph 1st and 2nd line; page 15, 3rd paragraph lines 2 and 4.

Thank you again for your consideration. We hope you find our revisions suitable.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable
**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests

**Response to Reviewer 2**

Thank you for indicating that we fully addressed your prior concerns. We have revised the language and formatting of the manuscript as recommended conforming to the style of the journal.