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.

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?

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
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?

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). 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.

**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