Author's response to reviews

Title: Polymorphisms in Nitric Oxide Synthase And Endothelin Genes Among Children With Obstructive Sleep Apnea

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Version: 3 Date: 27 August 2013

Author's response to reviewers:

Response to Reviewers

We would like to thank the Reviewers for their insightful comments and critiques. We have incorporated their suggestions and revised the manuscript accordingly. We believe that their input has substantially improved our manuscript. Below is a point-by-point response to each reviewer’s critique and they are highlighted RED COLOR in the text.

Reviewer #1: Pawel Krawczyk

Chatsuriyawong et al. showed associations between risk of obstructive sleep apnea (OSA) in children and 19 single nucleotide polymorphisms (SNPs) in nitric oxide synthase (NOS) as well as endothelin (EDN) genes. The authors included 608 children in the study and identified 128 patients with OSA. Moreover, they examined 381 SNPs in the family of NOS genes and 155 SNPs in the family of EDN genes as the candidates for OSA risk. Authors also tried to assess endothelial function using measurement of time to peak reperfusion (Tmax) and EDN1 mRNA expression. Even though the study is one of the biggest, it is not without some significant weaknesses.

Major revisions:

1. There are no results in the abstract. “Result” section in the abstract included only methodological aspects of the study and should be redrafted.

Response: We edited and modified both the methods and results sections in the abstract. In addition, we modified our conclusion in the discussion section, last paragraph.

2. Positive value of the article is an attempt to search of relation between large number of SNPs and risk of OSA in children. However, analysis of endothelial
function is of low value due to small sample size. Tmax was calculated only for 23 patients and EDN1 mRNA expression was estimated only in 9 patients with OSA. Because of the inability to examine the relationship between endothelial function and genotypes of NOS and EDN gene families, these results may be omitted and article should be shortened.

Response: We thank the reviewer for his remark and we definitely share the same concern on these data. However, we opted to retain these results because the functional measures obtained are critically relevant to the genes under scrutiny in this paper, and also because the subjects were closely matched for age, gender and ethnicity. Accordingly, the inclusion of the data is not to make inferences on the association with specific SNPs but rather to inform the readership on potential research directions in the future. This is now mentioned in the discussion section.

3. Authors showed associations between single SNPs and risk of OSA (Table 2). Moreover, linkage disequilibrium (LD) analysis of the SNPs in the NOS and EDN gene families was assessed for OSA and for healthy subjects (Figures).

Unfortunately, authors did not present results of step-by-step multiparameter logistic regression analysis with receiver operator characteristic for estimation of impact of different examined genotypes on risk of OSA.

Response: We thank the Reviewer, but will point out that due to cohort size limitations, inclusion of a stepwise logistic regression would necessitate a much larger sample size that the one afforded by our study.

4. In Table 2, authors estimated risk of OSA in patients with different genotypes and in carriers of different alleles. However, only one p-value was presented. Were statistical significances calculated for genotypes or for alleles positivity?

Response: We agree with the Reviewer that only one p-value is presented in Table 2 and this p-value is calculated from the adjusted chi-square test. We calculated the p-value in OSA vs. NOSA in association with each SNP. We believe it would be difficult and confusing to include a p-value for each allele, particularly since adjustments for multiple comparisons would necessitate a more expansive sample size.

Minor revisions:
1. Not all abbreviations are explained in the text.
Response: We inserted and edited all abbreviations in the manuscript.

2. Genes spelling should be in italics.
Response: We changed all genes to be in italics.

3. Information on the Figures 1a and 1b are duplicated. These figures should be joined.
Response: Actually, Figure 1 is not two Panels, it is only one Panel. Now, Figure 1 has been modified to be only one Panel.

4. Article includes as much as 69 references. The literature should be up to date.

Response: We have done every effort to provide an up to date reference section. We have now inserted new references (# 3, 17, 21, 60, and 61).

Reviewer #2: Elias Zintzaras
- The paper is well written and it is methodologically sound.
- The matching should be explained.

Response: We thank the reviewer for bringing this issue to our attention. We now emphasize the similarity of the OSA and NOSA groups as far as the recruitment strategy and the similarities in age, gender and ethnicity among the 2 groups.
- An adjustment for multiple corrections should be considered. Otherwise, the lack of the adjustment should be justified.
- Response: We agree with the Reviewer that performing adjustments for multiple comparisons would be highly desirable. However, as the Reviewer well knows considering his stature in the field of statistics and genetics, the cohort size is a marked limitation that can only be overcome if meta-analyses are performed in the future by assembling studies such as the present one. We performed adjustments for age, gender, ethnicity, and BMI z score, but since the ORs were not altered by these adjustments, we prefer not to overburden the Tables and instead make mention of this issue in the text.

- The generalized odds ratio should be calculated.; In the SNP with significant association, the mode of inheritance should be estimated.
- Response: We thank the Reviewer for these comments but for the sake of simplicity we have opted not to include such analyses. This is now addressed in the text.