Author’s response to reviews

Title: Pilot study indicate role of preferentially transmitted Monoamine oxidase gene variants in behavioral problems of male ADHD probands.

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Answers to reviewers comments

Reviewer reports:

Pingping Zheng (Reviewer 1): Summary and Comments:
The manuscript investigated an Indo-Caucasoid cohort of 190 probands with Attention-Deficit/Hyperactivity Disorder(ADHD) and their parents. The ADHD subjects included 166 boys and 24 girls around eight years old. The authors genotyped a total of 58 previous reported variants from two monoamine oxidase genes MAOA and MAOB using PCR and sanger sequencing and they detected one tandem repeats VNTR polymorphism and seven SNPs of MAOA gene, and another seven SNPs from MAOB. With linkage disequilibrium analysis and
association analysis of candidate alleles with ADHD trait scores, the authors stratified the population data by gender and found 3 SNPs of MAOA and 6 SNPs of MAOB significantly associated with maternal transmission to male probands and they also identified maternal transmissions haplotypes of risk alleles. They also found the young maternal age (<=26 yr) was associated with risk variants.

ADHD is much common in boys than in girls. The manuscript reported a family-based association study of Attention-Deficit/Hyperactivity Disorder(ADHD) and genetic polymorphism of two X-linked genes monoamine oxidase A (MAOA) and B(MAOB). The heterogeneity and complexity of ADHD made it difficult to identify the behavior genetic mechanisms underlying the complex disorder. The manuscript focused on analysis of two MAO genes locating in the mitochondrial outer membrane and maternal transmission in male probands. The screening and association studies might provide a few more candidate risk alleles/variants associated with this disorder. It is interesting maternal age was associated with variants from MAOA, especially rs6323 which is also a risk variant.

Minor Questions:
1. Line 55: The conclusion about preferential maternal transmissions of haplotype combinations to male were also noticed, but not observed in female probands.
The female had a relative small sample size (n 24). If the authors increased the size of female cohorts, would that give a different result?

Ans. Majority of our studied ADHD probands were male and hence, stratified analysis based on gender was performed for the male probands mostly. As rightly pointed out, female probands were only 24 and we have already mentioned in the discussion section that further analysis in large cohort of subjects may aid in understanding the actual contribution of these genes in the female probands. It would be interesting to find out whether increasing the number of female subjects would give a different result or not.

2. Sample size of subgroups was not clear in methods/results:
Line 167-176: how many subjects of each group in the following categories?
* The male probands were divided by with or without derived allele;
* The male probands were divided into "early onset" and "late onset" by the age <= 7 or >7 years;

Ans.

* The male probands were divided by with or without derived allele; the number of subjects were different for each variant analyzed for association with individual traits, primarily due to (1) difference in frequency of the alleles and (2) genotyping success rate. Hence, the number of subjects was not incorporated in the table 3.
* The male probands were divided into "early onset" and "late onset" by the age <= 7 or >7 years- The number of subjects in each group is incorporated in the revised Manuscript (line 159-160).
* Maternal age of pregnancy: <=26 or >26- The number of subjects in each group is incorporated in the revised Manuscript (line 163-164).

3. Some sentences from the manuscript were the same or similar to the referenced papers [ref 48 and ref 49, which are the reference numbers in the manuscript]. Would the authors re-write them a little bit?

Ans. We are sorry for the incidence and necessary editing has been done in the revised Manuscript (highlighted in green)

David Comings (Reviewer 2): This is a study of 190 nuclear Indo-Caucasoid families with ADHD to examine the possible role of MAOA and MAOB genes. Of 58 potential variants 15 were found to be polymorphic in this study. This is a well executed and data-rich study. The major finding was that 3 markers of MAOA (p values of 0.4, 0.4 and .0001) and 6 markers of MAOB (p values of .002 to .0001) showed significant maternal over-transmission to male but not female probands. Different behavioral traits of male probands also showed significant associations. The age of the mother (<= 26 yrs versus >26 yrs) also showed an association of several MAOA but not MAOB variants with male probands. This was of interest to this reviewer
who also found a significantly greater association of the DRD1 gene with some behavior traits for probands with a maternal age of \( \leq 26 \) yrs versus \( > 26 \) yrs. (Comings DC, MacMurray JP. 2006. Maternal age at the birth of the first child as an epistatic factor in polygenic disorders. Am J Med Genet B Neuropsychiatr Genet 141B(1): 1-6.)

Comments.

1. I think it is always useful in association studies showing significant p values to calculate \( r^2 \) or other variables showing the percent of the variance due to the gene in question. In most studies of polygenic disorders this is usually 2% or less. This is helpful to the reader to gain an appreciation of the relative importance of the gene in question especially in relation to whether pharmaceutically modifying the gene in question is likely to be helpful.

Ans. We are obliged for the helpful suggestion. Values obtained after calculating Relative Risk are incorporated for statistically significant data obtained after association analysis Table 1 & 4 of the revised Manuscript).

2. In table 1, for MAOB all but one of the 7 loci show a significant degree of preferential transmission of certain alleles to male probands. This is to be expected since these loci are in linkage disequilibrium. However, Also in table 1, the preferential transmission is highly significant for one locus (rs6323), borderline for two (\( p = .04 \)) and not significant for four and yet the loci at MAOA are also in LD similar to MAOB. Any comments on this difference?

Ans. As have been rightly pointed out, the studied loci are in strong LD for both the genes. However, MAOB variants showed significant preferential transmission while expecting for one significant and two borderline significant transmissions, other MAOA variants failed the show any bias. The reason for this probably lies in the difference in the frequency of the MAOA alleles in the maternal population and maternal transmission of any particular allele. Additionally, MAOA rs6323 was not in complete LD with other MAOA loci since the \( r^2 \) value was low and hence its transmission pattern was different from the other variants which showed strong LD due to high \( D' \) as well as \( r^2 \) values. Based on these observations, we have already stated in the Manuscript (discussion) that this data needs to be validated in extended cohort of subjects.