Author's response to reviews

Title: ADHD and Disruptive behavior scores - associations with MAO-A and 5-HTT genes and with platelet MAO-B activity in adolescents

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Author's response to reviews: see over
Re MS: 4344947721441078 - ADHD and Disruptive behavior scores -
associations with MAO-A and 5-HTT genes and with platelet MAO-B
activity in adolescents

Dear Editor,

On behalf of the co-authors and ourselves, we hereby resubmit our paper
“ADHD and Disruptive behavior scores - associations with MAO-A and 5-
HTT genes and with platelet MAO-B activity in adolescents”.

We hope that we have satisfactorily dealt with the comments and feel
confident that the MS has been improved.

In conclusion; we have now changed the paper according to the suggestion
from the reviewers; all results reported on genetic associations are the results
obtained using all the DZ and one randomly chosen MZ twin of each MZ
pair.

Yours Sincerely,

Dr. Kerstin Malmberg and Hanna-Linn Wargelius
Answer to reviewers

We thank the reviewers for valuable and constructive comments.

We hope that we have adequately dealt with all the comments and feel confident that the MS has been improved.

1. I remain concerned about the analyses but this really needs a statistician's advice. I agree that one can use each individual twin in population type analyses using STATA to adjust standard errors and that approach has been used in many standard analyses. However I am uncertain as to whether this is valid when undertaking genetic association analysis because some of the pairs share all their genetic risk factors in common (MZ twins). Kim-Cohen's paper was surely not on twins—they used other types of samples including the Dunedin sample. At the very least, if I have doubts, then the readership may do so, so there needs to be further convincing here really!

We agree that there could be doubts concerning how to handle the MZ twins in the genetic association analyses. Therefore, in the present revised version of the manuscript one of the children in each pair of the MZ twins has been randomly excluded from the statistical analyses (n=51). Because the MZ twins share all their genetic risk factors in common this makes somewhat uncertain whether the adjustment of standard errors, as described in the statistical analysis section, is correct for MZ twins. The main findings in our paper were not changed using this new approach excluding one of the MZ twins (i.e. MAOA short allele was still associated with disruptive behavior and MAOB was still lower in girls with disruptive behavior symptoms). With regard to the 5HTT LPR, the associations for heterozygozity and DBD in boys still holds, but also a very weak association with CD in girls show up. However, the previous statistically significant associations with ADHD symptoms and 5HTT LPR “disappear”.

In conclusion; we have now changed the paper according to the suggestion from the reviewers; all results reported on genetic associations are the results obtained using all the DZ and one randomly chosen MZ twin of each MZ pair.

2. Conclusions—the authors now mention some literature on MAOA but the meta-analyses for all the candidate genes tested in this study are not mentioned (Nb findings from Faraone et al, 2005 summary) and the findings not put in context of these (that the meta-analyses did not find association). The possibility of false positives is not considered and this is surely important given the previous lack of findings. Or at least the authors should tell us why they are found here but not in previous larger studies.

We have now presented our results in the context of the very conflicting literature of candidate gene studies on page 14 and 15 in the discussion section according to the following:

“... However, there are also studies reporting an association with ADHD related behaviors and the long MAO-A allele [Kim-Cohen], as well as studies in which no association were found [see Faraone 2005]. Further research is needed to elucidate the direction of gene effects on ADHD related behaviors concerning both the MAO-A and the 5-HTT gene polymorphisms.
Also with regard to the 5-HTT polymorphism, there are conflicting results with studies showing associations between an ADHD phenotype and both the long and the short allele as well as no association at all [Thapar 2006, Faraone 2006]. We found associations between high dimensions of DBD phenotype and 5-HTT LPR heterozygosity in boys and girls.”

In the very last part of the discussion - limitations section, we are now pointing out the possibility/risk of the present positive associations could be false positives/random findings.