Author's response to reviews

Title: The Estrogen Hypothesis of Schizophrenia Implicates Glucose Metabolism: Association study in three independent samples

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Version: 2 Date: 11 February 2008

Author's response to reviews: see over
Dear Dr. Bucceri

Please find attached the revised manuscript by Olsen et al. entitled

*The Estrogen Hypothesis of Schizophrenia: Association of Candidate Genes in Glucose Metabolism in Three Independent Samples*

We have taken great care to revise the manuscript according to the directions of the referees and have provided a point-to-point account of the revisions made. We hope that you will find the revised manuscript suitable to your readers.

Yours sincerely

Thomas Werge
Director
PI, Danish Psychiatric Biobank
Reviewer's report

Title: The Estrogen Hypothesis of Schizophrenia Implicates Glucose Metabolism: Association study in three independent samples

Version: 1 Date: 10 December 2007

Reviewer: Frank Dudbridge

Reviewer's report:

This paper reports nominal associations of five SNPs in three genes, found in a candidate gene study of ten genes selected for their relationship to the estrogen hypothesis, differential expression and/or genetic linkage to schizophrenia. None of the associations withstand correction for multiple testing, which is acknowledged by the authors.

However the study was well conducted and reported, and these results may prove useful in a future meta-analysis. This report may therefore prove useful, and I have only a few minor questions to raise.

1. Selection of genes: how many genes involved in the estrogen pathway could have been excluded in step 1? I am particularly concerned that the linkage scans have been so inconclusive that step 1(b) is little more than a random filter.

Answer: We agree that details on the numbers of genes excluded in each step of the selection procedure were not given in the original manuscript. This is now corrected.

2. Multiple testing: the authors were appropriately modest in their claims. However they might have tried to establish their hypothesis by combining evidence across genes. This could be done for example by
   - counting the number of nominally significant genes, or
   - using the truncated product of P-values methods.

Answer: We agree that both methods are highly appropriate and have now performed the suggested analyses, as now stated in the section ‘Results’.

3. As it is, there is little reason to focus on the nominally associated genes rather than the rest. Put another way, why not report the full set of association tests, for future use in meta-analysis?

Answer: We agree that reporting the full data set is appropriate for subsequent meta-analysis and have now listed data for all analysed SNPs in ‘Supplementary table 1’

4. It’s unclear why association tests were adjusted for confounding. No stratification was found between populations using Fst, and there is little reason to expect different allele frequencies between males/females. It would have been more powerful to perform a single unadjusted analysis, with secondary analyses looking for effect modification in different populations/genders.

Answer: We understand the comment made by the referee, which from a purely statistical point of view is correct. However, population and gender may be proxies also for non-genetic differences relating to recruitment and treatment of patients, which is why we chose a conservative approach and used the confounders in the primary analysis. However, analysing data without the confounders do not change the result, which is now stated in the section ‘Methods’.

5. Hardy-Weinberg testing: unclear why 8 markers out of (185-21) were excluded for HWE P<0.05, when this is exactly the number that would be expected by chance.

Answer: We agree that none of the markers would have been excluded had a corrected p-value (e.g. Bonferroni) been used. However, we chose to be conservative, which is now mentioned in the section ‘Methods’. We can also confirm that none of the excluded markers were significantly associated with disease.

6. Could the authors comment on the power of their study, and otherwise why the sample sizes were what they were.

Answer: We now state and discuss the sizes of ORs that this study has power>0.8 to detect using the minimum and maximum MAF as examples.

What next?: Accept after minor essential revisions

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

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests: I declare that I have no competing interests
Reviewer's report
Title: The Estrogen Hypothesis of Schizophrenia Implicates Glucose Metabolism: Association study in three independent samples
Version: 1 Date: 14 December 2007
Reviewer: Jubao Duan
Reviewer's report:
The authors aimed to test the hypothesis of association between estrogen pathway and schizophrenia and they bioinformatically selected a panel of 10 genes relevant to glucose metabolisms. They genotyped 185 SNPs in three independent case-control samples of Scandinavian origin (a total of 765 patients and 1274 control subjects). Among the 156 analyzed SNPs, 5 SNPs in three genes showed nominally significant association with disease (p<0.05). Rare haplotypes (frequency =3% in control) in ENO2 gene were found associated with decreased risk of schizophrenia. These genes have not been previously tested for association with schizophrenia. Overall, the paper is well written and the result has been accurately presented.

Comments for Minor essential revisions:

1. I would suggest that authors change the tone of their conclusion to a negative statement, i.e., no supportive evidence for the association with schizophrenia was found with the ten tested glucose metabolism genes. This is because (1) none of the associations could survive the correction for multiple testing; (2) The number of the SNPs showing p<0.05 (n=5) is even less than expected from testing 156 SNPs; and (3) the more significant haplotype in ENO2 is actually very rare, which may due to unreliable haplotyping.

Answer: We agree with the referee and have changed the tone in the conclusion accordingly.

2. HapMap release # should be mentioned: â##No or limited HapMap data were availableâ## seems not true in Phase II data (release #21a). Also, the most important parameter, r-2, for selecting tag SNP using tagger was not mentioned, is it 0.8 or else?

Answer 1: The HapMap release # is now mentioned in the section ‘Method’.
Answer 2: R2>0.8 was used to select tag SNPs and this is now mentioned in the section ‘Method’.

3. QC: what is call rate cut-off value for SNPs? In particularly the call rate for those SNPs that are nominally significant should be stated. Low genotyping call rate could confound the association in particularly when the association strength is weak.

Answer: We agree and have now mentioned the call-rate cut-off value and commented on the values of the nominally significant SNPs.

4. Some QC metrics should be moved to method part, e.g., the part if ‘Hardy-Weinberg proportions’ in result.

Answer: We agree and have now changed the manuscript accordingly.

5. In page 5, result of single SNP association analysis: the sentence of “Also, one SNP in each of the FBP1 and the PCK1 gene (table 3) showed association in the allelic comparisons with elevated risk for schizophrenia of the C alleles of both the rs4129219 and rs1040566 loci.” is not clear, I would change it to “The C alleles of both rs4129219 and rs1040566 in each of the FBP1 and the PCK1 gene showed nominal association with elevated risk for schizophrenia (Table 3)”.

Answer: We agree and have now changed the manuscript accordingly.

6. The authors aimed to provide a high coverage for the ten selected genes, however, in some of the genes there was only one tag SNP. Can the authors estimate the map coverage of their SNP panel (i.e., how many common HapMap Phase II SNPs can be tagged by the selected SNPs)? This can be done by using Tagger.

Answer: We agree and have now estimated the number of SNPs that was tagged (R2>0.8) by the SNPs analysed in this study. This is mentioned in the section ‘Methods’ and commented on in the section ‘Discussion’.

7. Page 11, English needs to be corrected in the sentence: â##Indeed, evidence form a resent study by Bahn and colleagues found elevated glucose levels in cerebrospinal fluid in drug-naÅ¬ve patients that was normalize upon antipsychotic medicatiionâ##. Form (?) from, Resent? (recent), was (?) were, normalize (?) normalized.

Answer: We agree and have now corrected the manuscript.

8. Some references (38,39,40) seems redundant and they appeared inconsistent with the description in the main text:

Answer: We agree and apologise for this mistake. The manuscript has now been corrected.