Reviewer's report

Title: Schizophrenia susceptibility and NMDA-receptor mediated signalling: an association study involving 47 tagSNPs of DAO, DAOA, PPP3CC, and DTNBP1 genes

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Reviewer: Erik Jönsson

Reviewer's report:

The authors have analysed the putative association between four N-methyl-D-aspartate receptor (NMDAR)-mediated signalling genes (D-amino acid oxidase (DAO), D-amino acid oxidase activator (DAOA), protein phosphatise 3 catalytic subunit gamma isoform (PPP3CCC) and dystrobrevin-binding protein 1 (DTNBP1)) in schizophrenia. The sample consisted of Italian patients with DSM-IV schizophrenia (n=391, 68% men, mean age 42 years, age of onset 24 years, 60% paranoid subtype) and control subjects (n=488, 44% men, mean age 45 years). The authors genotyped 47 tag single nucleotide polymorphisms (tagSNPs), of which 32 (six, six, eight and twelve in the DAO, DAOA, PPP3CC and DTNBP1 genes, respectively) fulfilled inclusion criteria. Four of these 32 SNPs were nominally significant for one or two of the four models analysed in the total case-control material, however none remained significant after correction for multiple testing. The authors then analysed haplotypes (sliding window approach with two or three markers) versus the total case-control sample, without evidence for association in the DAO, DAOA and DTNBP1 genes. However, in the PPP3CC gene there was an association between one haplotype consisting of three polymorphisms (rs4872499-rs11780915-rs13271367) where the CAG haplotype were less common among patients (13%) than controls (19%). In additional (“secondary”) analyses the authors related haplotypes to gender, diagnostic subtypes and age of onset and found some additional associations between haplotypes in the DAO, PPP3CC and DTNBP1 genes with regard to gender.

The authors analysed polymorphisms of four potential candidate genes, based on previous results. The major disadvantage is the many calculations and the low sample size. See below more detailed suggestions.

Major compulsory revisions

1. The major limitation of the study is the relatively low number of subjects. Therefore, the authors should emphasize - both in the abstract and in the conclusions - that the study results are preliminary and that there is need for replication.

2. In the first paragraph of page 17, the authors state that the possibility of type I errors is low because there are still significant associations after correction for multiple testing. However, there are plenty of genetic association reports which
never have been replicated irrespective of solid results in single studies. Also, using a strict Bonferroni correction (32 SNPs analysed for total sample, gender, clinical subtype and age of onset + unknown number of haplotypes analysed for the same parameters) would give a substantial number of analyses performed, probably making none of the analyses remaining significant. Therefore, the authors should be more careful in this respect.

3. Please describe more in detail how the correction for multiple testing was performed.

4. The authors should give the power of their sample for an OR of 1.1-1.2, which is more in line with the literature of recent schizophrenia association results of common polymorphisms. The low power to detect differences of this effect size should also be acknowledged among the limitations of the study.

5. Among the limitations of the study the authors should also include the limited covering of some parts of the investigated genes giving that one third of the investigated SNPs was not available for analysis.

6. I suggest that the authors add supplementary figures with the linkage disequilibrium structure including LD blocks for each of the genes. The authors should also state how many haplotype analyses that were performed for each gene.

Minor essential revisions

7. Page 10, 1st row: The authors write: “We considered significant the allele haplotype frequencies <5%”. Do the authors mean that they considered a p-value equal or less than 5% significant in the haplotype analyses? Or do they mean anything else? Please explain.

8. In the Results section (page 13, 3rd paragraph) the authors refers to the analysis of variance (ANOVA). However, I cannot find this analysis mentioned in the Methods section.

9. Gene abbreviations should be in italic font, e.g. DAO, DAOA, PPP3CC and DTNBP1.

10. Abbreviations should be explained at their first appearance. This refers to e.g. NMDA, DAO, DAOA, PPP3CC, DTNBP1, SNP, RRR, OR, and CI in the abstract and DSM-IV-TR, OR, and CI in the main text.

11. The authors should give references for DSM-IV-TR, SCID-CV, MINI and MMSE.

12. Page 12, last paragraph, row 5 from below: it is written: “Concerning the haplotype analysis, a significant effect of the estimated CAG...” I suggest: “Concerning the haplotype analysis, there was a significant effect of the estimated CAG...”

13. Table 1. The legend could be simplified, e.g.: “Estimated haplotype distributions for selected D-amino acid oxidase (DAO), protein phosphatise 3 catalytic subunit gamma isoform (PPP3CCC) and dystrobrevin-binding protein 1 (DTNBP1) single nucleotide polymorphisms in patients with schizophrenia and
control subjects." It is not necessary to state the specific polymorphisms in the legend, because they are given in the table.

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

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.