Author's response to reviews

Title: Association study of polymorphisms in the excitatory amino acid transporter 2 gene (SLC1A2) with schizophrenia

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Dear Editor,

Thank you kindly for your e-mail received, Jun 23, 2004. We greatly appreciate your consideration of our paper, MS: 1860571476315515: Association study of polymorphisms in the excitatory amino acid transporter 2 gene (SLC1A2) with schizophrenia. All comments sent to us are very valuable. We revised the manuscript, based on the comments. Uploaded please find the revised manuscript. The reversions and responses to reviewer’s comments we made in the manuscript are as follows,

Reviewer: Evgeny Rogaev

1. Minor point: In introduction authors review the data supporting glutamatergic hypothesis. It might be useful to indicate recent data for positional cloning of G72 and neuregulin genes possibly linked to schizophrenia and glutamatergic dysfunction.

Response: We have mentioned G72 and NRG1 on lines 7-10 of page 3 of the revised manuscript by citing a recent review paper concerned with schizophrenia as reference 10 (Owen MJ et al., 2004).
Reviewer: Massimo Gennarelli

2. Major Compulsory Revisions: If the main goal of the paper is to perform an association study of SLC1A2 polymorphisms and schizophrenia in Japanese, the first analysis should include all people recruited (200 patients and 200 controls). a) If cumulative analysis confirm a negative result (either genotype or haplotype analysis), the authors could discuss the results about the Kyushu and Aichi samples in relation to their possible differences (ethnical origin, inpatients/outpatients, age...) In this respect, a synthetic table with demographic and clinical characteristics of the two samples should be added. b) If the cumulative analysis evidences positive results, the main finding of the paper will change completely.

Response: We have done the cumulative analysis including the two sample sets recruited from the Kyushu and the Aichi area, respectively. The significant haplotype association of SNP2-SNP7 with schizophrenia observed in the Kyushu samples was replicated in the cumulative analysis. According to the positive result, we revised the manuscript on results, discussion and conclusion as follows,

In the “Results” section on lines 12-17 of page 8, “Although significant association of the disease was observed with neither genotype, allele frequencies of SNP2 (P= 0.195, P = 0.178, respectively), nor haplotypes of SNP2-SNP7 (P = 0.084) in the second sample set, the significant haplotype association of SNP2-SNP7 was replicated in the cumulative analysis including the two sample sets (P= 5.0 × 10⁻⁴) (Table 4).”

In the “Discussion” section on lines 6 -19 of page 10, “In our second sample set, the Aichi sample, no significant association of SNP2 was observed in any of the analyses of genotypes, alleles and haplotypes. Cumulative analyses of the two sample sets,
however, provide the replication of the significant haplotype association of SNP2-SNP7 with schizophrenia \( (P = 5.0 \times 10^{-4}) \). The frequency of the G-C haplotype in schizophrenics (26.6%) was notably higher than in controls (5.6%), suggesting that the G-C haplotype may be a risk haplotype for schizophrenia. We observed that the G-C haplotype frequency of schizophrenics (20.0%) was only slightly higher than controls (14.2%) in the Aichi sample, suggesting a less contribution of this locus on schizophrenia pathogenesis in the Aichi sample, although no apparent difference in clinical subtypes between both sample sets studied in this paper. The positive association reported here needs to be validated in larger sample sets, and it would also be worthwhile to search for functional SNPs in the region spanning SNP2-SNP7.

In the “Conclusion” section on lines 1 and 2 from the bottom of the page 10, “We concluded that at least one susceptibility locus for schizophrenia is probably located within or nearby \textit{SLC1A2} in the Japanese population.”.

3. Minor Essential Revisions: (1). The “Methods” section in the Abstract lacks the description of both the samples analyzed in the paper.

Response: We have inserted a following sentence on lines 8-10, page 2, “The positive finding observed in the Kyushu samples was re-examined using 100 Japanese schizophrenics and 100 controls recruited from the Aichi area.” in the “Methods” section in the “Abstract”.

(2). In the Introduction, the authors should report the results of Catalano et al. on EEAT2 and cite other association studies on glutamate receptor genes (Begni S et al., 2003; Hung CC et al., 2002; Rice SR et al., 2001; etc...)

Response: As the results of Catalano et al. on EEAT2 have been described in the
discussion of our manuscript, we did not mention them in the introduction. We have cited two other papers of positive association of glutamate receptor genes with schizophrenia, Begni S et al., 2002 and Begni S et al., 2003 as references 5 and 6, respectively, on line 10 of page 3. We did not refer to the other two papers suggested by the reviewer (Hung CC et al., 2002 and Rice SR et al., 2001) because these reports have not supported the association of glutamate receptor genes with schizophrenia.

Reviewer: Dieter Bernd Wildenauer

4. Discretionary Revisions: The only suggestion I have concerns the sample size. It should be concluded that 100 individuals are on the lower limit for an association study. Therefore, and also because the gene could be considered as a plausible candidate gene for schizophrenia, it would be desirable to test the findings in a larger sample.

Response: Though the cumulative analysis including our two sample sets, the significant association was replicated, suggesting the probability that $SLC1A2$ contributes to the schizophrenia pathogenesis. On line 4 from the bottom of page 10, we have declared the necessity of testing the association in larger sample sets as follows “The positive association reported here needs to be validated in larger sample sets.”.

I would greatly appreciate it if you would kindly consider this revised manuscript for publication in BMC Psychiatry.

Yours sincerely,
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