Author's response to reviews

Title: No Association Between the Sigma Receptor Type 1 Gene and Schizophrenia, Results of Analysis and Meta-analysis

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Emma Veitch
Assistant Editor
BMC Psychiatry

Dear Veitch

Thank you for your helpful letter and referees’ comments. I have sent a revised version of the manuscript "No Association Between the Sigma Receptor Type 1 Gene and Schizophrenia, Results of Analysis and Meta-analysis". I hope the following corrections will meet your requirements for final acceptance to be published in "BMC Psychiatry".

Reviewer I
1) Subheading of “DNA samples” was corrected as “Subjects” as suggested. That in authors’ contribution section was also corrected.

2) Method of mutation screening
Sequence of PCR primer pairs for 5’ flanking to exon3 and exon 4 were provided in line3 of mutation screening nd genotyping section as follows;
“primer pairs (for 5’ flanking to exon3; 5’-TGTTGAAGGTGCCAGAGTGA-3’ (position of NT 8413 is 34626978-99), 5’-GCTCCCCTCCTCACTTGACAGTCC 3’ (34628383-60), for exon 4;
5’-GAGACGGTAGTACACGGGCCTGGTG-3’ (34625854-30),
5’-GTAGTGATGTCAGCTGATGTC-3’ (34625318-42).”
Infromation of sequence primer is also provided in line 12 as follows;
“sequence primer for 5’ flanking to exon3, 5’-TCCCTCCACTCGACAGTCTGTC-3’ (34628379-56), 5’-TGACATCTGCCGCTGGGCAGTCTGTC-3’ (34627903-880),
5’-CGAAGGCGCATCCCGGACCTAG-3’ (34627428-405), and for exon 4,
5’-GTCAAGCTGTGATGTGTGTCTG-3’ (34625326-49),
5’-GGTAGTACACGGGCTGGTGAGGC-3’ (34625849-26)).”

3) In the “Statistica Analysis” section, “Fisher’s exact test” was replaced by “Chi square test” as pointed out.

4) I added method of meta-analysis in the section of statistical analysis of methods as follows, “For meta-analysis, numbers of each genotype or each allele of three studies by Ishiguro et al.[14], Ohmori et al. [15] and us were added, and were analysed by by Chi square test.”

5) Statistical power analysis was done using software of G*power v2.1.2. This information was added in the last of statistical analysis section, and one reference for the program was added as ref 16 (E Erdfelder, F Faul, A Buchner: G POWER: A general power analysis program. Behavior Research Methods, Instruments, and Computers 1996, 28: 1-11.)

6) The reviewer 1 required soft conclusion because p value was marginally negative as p=0.06. I changed some sentence in abstract and discussion and conclusion as “it is likely that”, and that in conclusion as “it is suggested that”.

7) The reviewer 1 pointed that allele and genotypic frequencies of the study by Ohmori et al (the reviewer stated as Ohara et al., but it should be a mistake) seems to be similar to those by Ishiguro et al. He speculated that no significance by the Ohmori’s study may result from a lack of power. He required to comment upon this. I added following discussion in the second paragraph of the section of results and discussion.

“Table 2 showed comparison among the present study and the two previous studies of case-control association with the SIGMAR1 gene. Values of allelic and genotypic distribution of Gln2Pro polymorphism were almost similar among the three studies. However, a difference of allelic frequencies between controls and schizophrenics in Ishiguro’s and Ohmori’s study was 4.8 and 4.6 point, respectively, and that of the present study was only 0.6 point. Ishiguro’s study revealed a marginal significant difference between the groups, but Ohmori’s one failed, suggesting a negative result by the latter study may result from lack of power to detect significance difference. However, it is possible that Ishiguro’s result may be a false positive due to population stratification, which is considered as unavoidable in such case-control association design studies. To reduce possible population stratification and (to verify the negative results of association ….)

Discretionary revisions

8) The reviewer suggested to divide the “results and discussion” separately. But, this simple paper has a relatively compact results and discussion. I think combined description is easier to understand for readers than separate one. Therefore, I did not separate a section of “results and discussion”.

9) Two psychiatrists who performed diagnosis of each patient were his/her attending physician and one of authors. I added “made a diagnosis” in contribution for all authors as suggested.

10) Spelling errors. “contro” in the table 2 was displaced by “control”. Comma in the title was replaced by colon as suggested.
Discretionary points;
The reviewer 2 recommended mutation search of whole genome of the SR-BP-1 gene not only all exons, exon-intron boundaries and promoter region, which have examined in the present study, but also all deep introns and 3’ flanking region to cover the entire gene. However, as a view point of physiological significance of mutations, those in deep introns or in the 3’ flanking region may seem to have quite less significance than those in exons or promoter region. Therefore, I do not think to need further mutation search in deep introns or 3’ flanking. I think this gene does not confer much to susceptibility of schizophrenia, but may do to individual difference in efficacy or adverse effects of neuroleptics. I want to try to investigate the latter possibility in the next project.

Major points
1) As reviewer 1 required, the reviewer 2 also required method of power analysis. It was added as above-mentioned (5 for reviewer 1).
2) The PCR primer sets used in the mutation search were as explained as 2) for the reviewer 1.
3) Each p value of studies in the table 2 was added.

Minor points
1) The reviewer 2 asked to change title to be more explanatory. “case-control studies” was added after “Results of Analysis and Meta-analysis”
2) The reviewer 2 recommended to use “SR–BP1” as a gene symbol. However, “SIGMAR1”, which was used in the present paper, is considered as an approved symbol in OMIM list rather than “SR-BP1”. In the past, it was used as this symbol once. So, I added this old name in the background section, the second paragraph, line 2.
3) Rs-numbers for the both polymorphisms examined in this study were provided in the second paragraph, line 3 of Background section as follows; “G-241T/C-240T (rs1799729) and Gln2Pro (A61C, rs1800866)”.
4) Names of genotype and allele were corrected by amino acid as Gln and Pro, and order was changed from major one to minor one order as suggested.
5) The reviewer 2 asked to change $x^2$ to $\chi^2$. But, value of chi-square is usually expressed as $x^2$. I think this is valid. Therefore I did not change this term.
6) The reviewer 2 pointed that some words are missing. For example, “Japanese population” in abstract, “sigma receptor type 1 gene” in background section, and ”5’ flanking region” in mutation screening and genotyping section. However, these words are present in my manuscript. I can not understand what the reviewer 2 wanted to pointed out. And the reviewer 2 required overlook my manuscript by native speaker. However, my previous manuscript has been reviewed already by a native English speaker.

Reviewer 3
1) Explanation for methods of statistical power analysis was provided as 5) for the reviewer 1. In the second paragraph, line 7 of results and discussion section, “a power of 1.00” was replaced by “a power of $>0.9999$”.
2) In legend of table 1, reviewer 3 asked to add explanation for relationship A61C and
G-241T/C-240T. I changed name of genotype and allele from A61C to Gln 2Pro as same as table 2 (This is described in 4) of reviewer 2’s request. Now, table 1 is easy to understand for readers.

3) Some errors were corrected as pointed out as follows; page 2, 1st line of background of abstract “role of receptors in etiology” was rewrote by “role of receptors in the etiology”, page 3 line 3” it has been defined” was rewrote by “they have been defined”, page 4, method section “each 4 exon” was rewrote by “each of the four exons”, result section in page 5 ”SIGMAR1 dose not associated” was corrected as “SIGMAR1 is not associated”, in authors’ contribution “DNA samples” was replaced by “subjects”, in table 1, data of catatonic patients was deleted as suggested, and “Contro” in table 2 was corrected as “Control”.

Sincerely,
Hiroshi UJIKE, M.D., Ph.D.